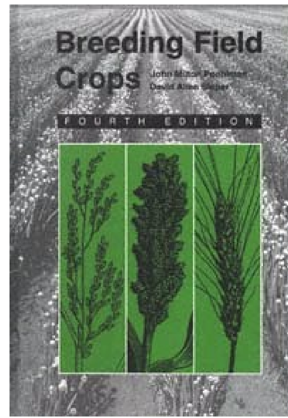
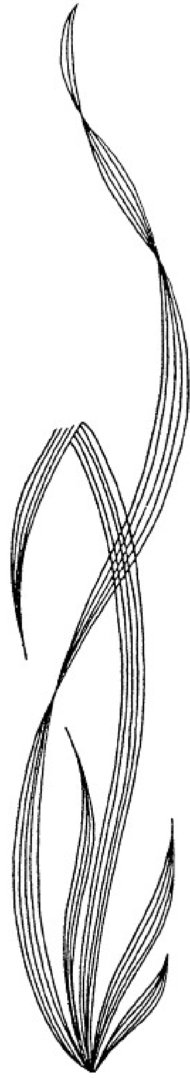

Breeding Field
Crops
FOURTH EDITION

Breeding Field Crops



Breeding Field Crops

Fourth Edition

John Milton Poehlman David Allen Sleper

Iowa State University Press /Ames

JOHN M. POEHLMAN was Professor Emeritus, Department of Agronomy, University of Missouri.

DAVID A. SLEPER is Professor, Department of Agronomy, University of Missouri.

© 1995, 1994 (reprinted third edition), Iowa State University Press, Ames, Iowa 50014. Original © 1959, Henry Holt and Co.; © 1979, AVI Publishing Co.; © 1987 Van Nostrand Reinhold.

All rights reserved

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Iowa State University Press, provided that the base fee of $. 10 per copy is paid directly to the Copyright Clearance Center, 27 Congress Street, Salem, MA 01970. For those organizations that have been granted a photocopy license by CCC, a separate system of payments has been arranged. The fee code for users of the Transactional Reporting Service is 0-8138-2427-3/95 $. 10.

♾ Printed on acid-free paper in the United States of America

Fourth edition, 1995

Parts of chapters 21 and 22 first appeared in chapters 13 and 12 respectively of J. M. Poehlman and D. Borthakur, 1969, *Breeding Asian Field Crops*, New Delhi: Oxford and IBH Publishing Co.

Library of Congress Cataloging-in-Publication Data

Poehlman, John Milton
Breeding field crops / John Milton Poehlman, David Allen Sleper.—4th ed.
p. cm.
Including bibliographical references and index.
ISBN 0-8138-2427-3 (alk. paper)
1. Field crops—Breeding. I. Sleper, D. A. II. Title.
SB185.7.P63 1995
631.5'2—dc20 94-36673

Last digit is the print number: 9 8 7 6 5 4 3

CONTENTS

Preface, xiii

I. What is Plant Breeding?

1. Plant Breeders and Their Work, 3

The Art and Science of Plant Breeding, 3

Why Breed Plants?, 4

The Strategy of Plant Breeding, 4

Education of the Modern Plant Breeder, 6

How Plant Breeding Began, 8

Plant Breeding before Mendel, 9

The Contributions of Gregor Mendel, 10

Plant Breeding after Mendel, 11

Who Are the Modern Plant Breeders?, 12

Study Questions, 15

Further Reading, 15

II. The Genetic Basis of Plant Breeding

2. Reproduction in Crop Plants, 19

Types of Reproduction, 19

Sexual Reproduction in Crop Plants, 20

Nuclear Division and Chromosomes, 24

Chromosome Numbers in Crop Plants, 28

Self- and Cross-Pollination in Crop Plants, 29

Asexual Reproduction in Crop Plants, 34

Study Questions, 37

Further Reading, 37

3. Gene Recombination in Plant Breeding, 38

Variation, the Basis of Plant Breeding, 38

The Mechanism of Mendelian Heredity, 41

Gene Recombination Following Hybridization, 47

Gene Structure and Action, 56

Study Questions, 58

Further Reading, 59

4. Quantitative Inheritance in Plant Breeding, 60

Quantitative Inheritance and Its Measurement, 61

Multiple Alleles, 69

Types of Gene Actions, 70

Heritability, 71

Selection Intensity and Genetic Advance, 75

Gene Frequency and Genetic Equilibrium, 78

Gene Recombination and Plant Breeding, 81

Study Questions, 81

Further Reading, 82

III. Tools of the Plant Breeder

5. Variations in Chromosome Number, 85

Polyploidy, 86

Aneuploidy, 96

Haploidy, 99

Unreduced Gametes, 101

Study Questions, 104

Further Reading, 104

6. Mutation, 106

The Nature of Mutation, 106

Induction of Mutation, 108

Some Useful Mutations in Plant Breeding, 111

Role of Mutation Breeding, 114

Study Questions, 115

Further Reading, 115

7. Fertility-Regulating Mechanisms and Their Manipulation, 116

Incompatibility, 117

Male Sterility, 121

Apomixis, 128

Interspecific Hybridization, 128

Time of Pollen Shed, 130

Study Questions, 131

Further Reading, 131

8. Molecular Biology: Application in Plant Breeding, 132

Plant Cell and Tissue Culture, 132

Clonal Propagation via Tissue Culture, 136

Embryo Culture, Ovule Culture, In Vitro Pollination, 137

Anther Culture and Haploid Plant Production, 140

Genetic Variability from Cell Cultures: Somoclonal Variation, 143

Somatic Cell Hybridization, 145

Plant Genetic Engineering (Transformation), 146

Molecular Markers, 151

Study Questions, 154

Further Reading, 154

IV. Methods in Plant Breeding

9. Breeding Self-Pollinated Crops, 159
What Is a Cultivar?, 159
Genetic Significance of Pollination Method, 160
Breeding Methods in Self-Pollinated Crops, 161
Selection Procedures Following Hybridization, 164
Backcross Breeding, 172
Multiline Breeding, 175
Variety Blend, 176
Non-Traditional Breeding Procedures, 176
Plant Breeding: A Numbers Game?, 178
How Breeding Procedures for Self-Pollinated Crops Are Utilized, 179
Study Questions, 179
Further Reading, 179
10. Breeding Cross-Pollinated and Clonally Propagated Crops, 181
Genetic Structure of Cross-Pollinated Crops, 181
Breeding Seed-Propagated Cross-Pollinated Crops, 184
Breeding Clonally Propagated Crops, 196
How Breeding Procedures Are Utilized, 199
Study Questions, 199
Further Reading, 199
11. Breeding Hybrid Cultivars, 200
The Origin of Hybrid Breeding, 200
Inbreeding in Cross-Pollinated Crops, 202
Hybrid Vigor or Heterosis, 204
Breeding Single-Cross Hybrid Cultivars, 206
Cytoplasmic Male Sterility and Hybrid Seed Production, 209
Alternative Hybrid Procedures, 212
Proprietary Nature of Hybrid Cultivars, 214
How Breeding Procedures Are Utilized, 215
Study Questions, 215
Further Reading, 215
12. Breeding Objectives and Techniques, 216
Yield, 216
Resistance to Lodging and Shattering, 218
Winter Hardiness, 221
Heat and Drought Resistance, 222
Soil Stress, 223
Resistance to Plant Disease Pathogens, 223
Resistance to Insect Pests, 229
Product Quality, 230
Techniques in Plant Hybridization, 231
Conducting Field Trials, 234
Study Questions, 238
Further Reading, 238

V. Germplasm Resources for Breeding Crop Plants

13. Germplasm Resources and Conservation, 243

Germplasm Conservation, 243

Germplasm Resources and Their Maintenance in the United States, 248

Study Questions, 254

Further Reading, 255

VI. Applications: Breeding Field Crops that are Self-Pollinated

14. Breeding Wheat, 259

Origin and Genetics, 259

Biotechnology and Wheat Improvement, 263

Genetic Diversity in Wheat, 264

Flowering and Pollination, 265

Breeding Methods, 266

Breeding Objectives, 269

Study Questions, 276

Further Reading, 276

15. Breeding Rice, 278

Origin, Species, and Types of Rice, 281

Genetics of Rice, 283

Biotechnology of Rice, 284

Flowering and Pollination, 284

Genetic Diversity in Rice, 285

Breeding Rice, 286

Breeding Objectives, 290

Upland Rice, 297

Deep-Water and Floating Rice, 297

Wild Rice, 297

Study Questions, 298

Further Reading, 298

16. Breeding Soybean, 300

Domestication and Species, 301

Soybean Genetics, 303

Biotechnology, 304

Soybean Plant Types, 304

Flowering and Pollination, 305

Breeding Methods, 308

Breeding Objectives, 311

Asian Vegetable Research and Development Center (AVRDC), 317

Study Questions, 317

Further Reading, 317

VII. Applications: Field Crops Utilizing Hybrid Breeding Procedures

17. Breeding Corn (Maize), 321

Origin of Corn, 321

Races of Corn, 322

Genetics and Cytogenetics, 324

Molecular Biology, 324

Flowering and Pollination, 325

Heterozygosity of Open-Pollinated Corn, 327

Breeding Open-Pollinated Corn, 327

Hybrid Corn, 329

Breeding Improved Hybrids, 333

Population Improvement, 336

Breeding Objectives, 337

Special-Purpose Hybrids, 343

International Maize and Wheat Improvement Center, 343

Study Questions, 343

Further Reading, 344

18. Breeding Sorghum, 345

Origin, Species, and Races, 346

Agronomic Groups, 346

Botany, Flowering, and Pollen Control, 348

Genetic Studies, 351

Breeding Methods, 352

Breeding Objectives, 359

International Sorghum Breeding Programs, 365

Study Questions, 366

Further Reading, 366

VIII. Applications: Field Crops with Miscellaneous Breeding Procedures

19. Breeding Cotton, 369

Origin and Species, 369

Flowering and Pollination, 370

Genetics and Cytology, 372

Origin and Diversity of American Upland Cotton, 374

Pima Cotton, 375

Breeding Methods, 376

Cultivar Maintenance, 377

Breeding Objectives, 378

Study Questions, 385

Further Reading, 385

20. Breeding Cross-Pollinated Forage Crops, 387

Why Breeding Cross-Pollinated Forage Species Is Unique, 387

Pollination, Fertilization, and Seed Setting, 388

Vegetative Propagation, 397

Procedures for Breeding Cross-Pollinated Forages Species, 398

Application of Biotechnology in Forage Breeding, 407

Breeding Objectives, 407

Seed Increase of New Cultivars, 414

Study Questions, 415

Further Reading, 415

IX. Applications: Field Crops that are Vegetatively Propagated

21. Breeding Potato, 419

Classification of Potato, 420

Botany of Potato, 421

Genetics of Potato, 424

Breeding Methods for Potato, 426

Breeding Objectives of Potato, 428

True Potato Seed (TPS), 433

Study Questions, 433

Further Reading, 433

22. Breeding Sugarcane, 434

Species of Sugarcane, 434

Botany of Sugarcane, 436

Cytogenetics and Genetics, 441

Biotechnology, 442

Methods of Breeding, 442

Cultivars, 446

Breeding Objectives, 446

Study Questions, 449

Further Reading, 449

X. Maintenance and Seed Production of Improved Cultivars

23. Cultivar Increase, Maintenance, and Seed Production, 453

Public and Private Plant Breeding and Seed Distribution, 453

Classes of Certified Seed, 455

How a New Cultivar Reaches the Farmer, 457

How a Cultivar Is Certified, 458

Agencies Concerned with Seed Certification in the United States, 459

AOSCA, 459

OECD, 460

Intellectual Property Rights, 461

Practical Problems in Seed Production, 462

Vegetatively Propagated Forages, 466

Study Questions, 467

Further Reading, 467

Glossary, 469

Figure Credits, 481

Index, 483

PREFACE

This book is a revision of the popular textbook, *Breeding Field Crops*, written specifically for teaching plant breeding at the introductory level. As with earlier editions, it will provide supplementary reading for the advanced undergraduate or (post) graduate-level student and will be consulted freely by professional plant breeders.

Many advances have been made in plant breeding science and practice since earlier editions of this textbook. New knowledge in genetics and plant breeding has contributed to the sophistication and precision with which new crop cultivars can be developed. Exciting developments in the field of molecular biology are bringing powerful new tools to supplement, although they will not replace, the arsenal of the plant breeder. Expanded knowledge in quantitative genetic theory, heterosis, cytoplasmic-male sterility, mutation induction, evolution of polyploid species, nature and inheritance of stress resistance, genetic control of disease and insect resistance, and genetic influence on components of product quality are contributing to the plant breeder's success in improving cultivar performance and stability.

Plant breeding research and practice has expanded from traditional national research programs that focused on local breeding problems to become an integrated international activity. International Research Centers have been created that contribute to the development and spread of new high-yielding cultivars in the lesser developed countries. An international network of genebanks has been organized to collect, maintain, and distribute diverse germplasm resources of economically important field and vegetable crops to plant breeders everywhere. Privately organized and supported plant breeding research is contributing an increasingly larger commitment to cultivar development. Perhaps the greatest change is the increased knowledge and sophistication with which the breeders conduct the breeding research.

These developments have had important implications on the way this fourth edition has been written. Teachers of plant breeding, worldwide, are better informed than ever before. There is a greater dissemination of plant breeding knowledge through books, journals, international symposia, foreign study programs, and personal travel. Due to the extensive database of current plant breeding literature, which may be accessed by the student through computer programs such as AGRICOLA (AGRICultural OnLine Access), the extensive bibliographies included in earlier editions have been reduced to some key references designated "Further Reading." With the

increased information flow, teaching in plant breeding has improved and become less provincial. The basic tenets of breeding field crops introduced to the student through this textbook will be applicable wherever the textbook may be used.

The general format and structure of earlier editions have been continued in this edition. The basic objectives of the textbook are: (1) to review essential features in plant reproduction, Mendelian genetic principles, and related genetic phenomena that contribute to plant breeding practices; (2) to describe and explain basic plant breeding methods and techniques; (3) to emphasize the importance of selecting the breeding objectives whose improvement will contribute the greatest economic benefit to the farmer growing the new cultivar; and (4) to describe procedures for the increase, maintenance, and distribution of seeds or vegetative propagules of new crop cultivars.

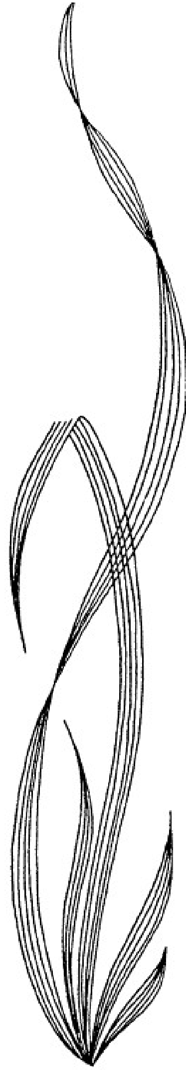
Plant breeding activities are normally organized around a particular crop species. A trademark of this textbook has been the chapters devoted to procedures and objectives for breeding selected crop species. These chapters take plant breeding out of the realm of the theoretical and bring the student face-to-face with the practical decisions that the plant breeder makes in the course of developing a plant breeding program, an approach to teaching plant breeding that is continued here. The crop species have been selected to provide diversity in reproductive system and breeding procedure.

Throughout the textbook a balance has been sought between completeness and unessential detail. The simplest terms were used when there were alternatives. The criterion for inclusion of information has been whether it will facilitate student learning at the introductory level of instruction. Photographs and line drawings are used freely to illustrate particular points. A list of study questions and a short list of references for further reading will be found at the end of each chapter.

The authors wish to express their sincere appreciation to all who have contributed to the development of this edition. We are particularly indebted to Lillie Cuppy for transferring the third edition to diskettes and to the following for reading one or more chapters and offering useful suggestions: S.C. Anand, R.L. Arnett, T.C. Barker, J.E. Berg, T.T. Chang, C.F. Crane, L.L. Darrah, G.L. Davis, S.R. Eberhart, G.C. Eizenga, C.V. Feaster, B. Gebrekidan, K.D. Kephart, G. Kimber, M.H. McCaslin, A.L. McKendry, J.D. Miller, H.C. Minor, J.F. Pedersen, S.C. Pueppke, A.P. Rao-Arelli, H.L. Shands, W.D. Smashey, D.M. Stelly, D. Tague, P.Y.P. Tai, M. Wanous, W.W. Xu, J.M. Yohe. Additionally, many colleagues and support staff at the University of Missouri, as well as professional colleagues at other institutions, have given freely of their time and counsel for which we are extremely grateful and express our thanks. However, we assume full responsibility for the writing, and hope that errors or misstatements will be called to our attention so that they may be corrected.

Finally, special thanks are expressed to our wives, Rose Kentner

Poehlman and Elaine Mae Sleper for their patience, understanding, and assistance during the preparation of the manuscript.



I
WHAT IS PLANT BREEDING?

1. Plant Breeders and Their Work

Plant breeding is the art and the science of improving the heredity of plants for the benefit of humankind.

The Art and Science of Plant Breeding

The art of plant breeding lies in the breeder's skill in observing plants with unique economic, environmental, nutritional, or aesthetic characteristics. Before plant breeders possessed the scientific knowledge that is available to them today, they relied solely on skill and judgement in selecting novel plants which could be propagated through seeds or vegetative parts. Thus, *selection became the earliest form of plant breeding*. The successful plant breeders were keen observers, quick to recognize variant plants of the same species which would improve performance in the field or garden. For them, plant breeding was purely an art. Many of the early breeders were amateurs—a cultivator who found an "off-type" plant in the field or a gardener who found a "sport" in the bed. Some, like Luther Burbank, were professionals who searched far and wide for unusual plant types that could be propagated and exploited for commercial gain.

Plant breeding developed into a science as knowledge progressed in classical genetics and related plant sciences. The foundation of plant breeding was based on recognition of the gene as the unit of heredity, on procedures for gene manipulation, and on rules of genetic behavior that permitted accurate prediction of the results from gene manipulations. The genes were identified by their effects on the visible expression of plant traits, such as whether a plant was tall or dwarf, or the flower color was white or pink. Through systematic cross-pollination, particular combinations of genes for different meritorious traits could be combined into a single plant cultivar. *Hybridization then became the principal plant breeding procedure*. It was no longer necessary for the breeder to rely so completely on skill in finding chance variants with which to establish new cultivars. It now became possible to plan and synthesize new plant types more or less at will. Plant breeding became more of a science and less of an art.

More recently, the science of *molecular genetics* proposes to advance plant breeding to an

even higher level of sophistication. Molecular genetics was ushered in with the description of the chemical structure of deoxyribonucleic acid (DNA), the material that constitutes the gene. DNA carries the instructions for synthesis of specific enzymes, proteins that determine the visible expression of particular plant traits. According to the new technology, the DNA (gene) encoding for a desired trait would be identified, cloned, and inserted into the DNA of a plant reproductive cell line. There it would replicate and express its unique character in the transformed plant. As the new technology becomes routinely operational, it offers an opportunity to enhance performance of crop cultivars through the introduction of foreign genes from almost limitless sources—genes not previously accessible through traditional hybridization breeding procedures.

The enhancement of cultivar performance through transformation with single units of DNA (genes), that encode for proteins that determine exotic plant characters, has exciting implications for plant breeding. But the new technology does not supplant nor diminish the importance or need for the traditional selection and hybridization procedures in the breeding of improved cultivars. Present cultivars have reached high levels of performance through refined selection and hybridization breeding systems that bring together multigenic combinations for performance of the whole plant. These breeding procedures will continue to be the basic source of high performing cultivars, albeit performance that may be further enriched through transformation by new biotechnology procedures.

Why Breed Plants?

The goal of plant breeding is to change the plant's heredity in ways that will improve plant performance. Improved plant performance may be manifested in many ways. Improved yield and quality are usually the primary breeding goals, whether the product harvested is seed, forage, fiber, fruit, tubers, flowers, or other plant parts. Plants are the basic source of food for the world's people (Fig. 1.1). Higher yields of food plants contribute to a more abundant food supply, a more profitable agriculture, and a lower cost of food products for the consumer. Yields of the major food grains increased rapidly in the United States over the fifty-year period, 1941-1990 (Fig. 1.2). The yield increase resulted from improving the cultural environment in which the crops were grown and the genetic potential of new cultivars to produce in more favorable cultural environments. Breeding for improved quality in food plants may make the product more nutritious, increase the ease of processing, or reduce presence of toxic compounds. Improving health of the plant by breeding for disease or insect resistance increases the yield and quality of the product and is an environmentally sound practice as fewer protective chemicals will be utilized in the culture of the resistant plants. Plants may be adapted to a wider range of production areas by breeding for increased tolerance to drought, extremes of temperature, salinity, or other adverse environmental production hazards.

The Strategy of Plant Breeding

The strategy of plant breeding is relatively straightforward. The basic elements of this strategy are

• to identify the morphological, physiological, and pathological traits in a cultivated plant

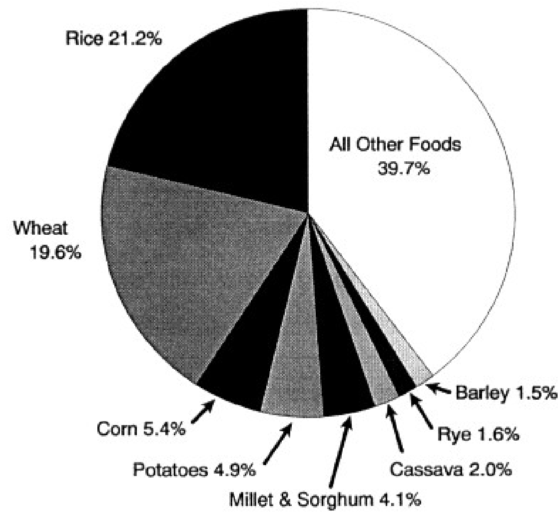


Fig. 1.1.
Sources of food for the world's people. Over 50% of the food supply comes from seven cereal grains, over 40% from rice and wheat. Plants are the original source of the food supplied by animal products.

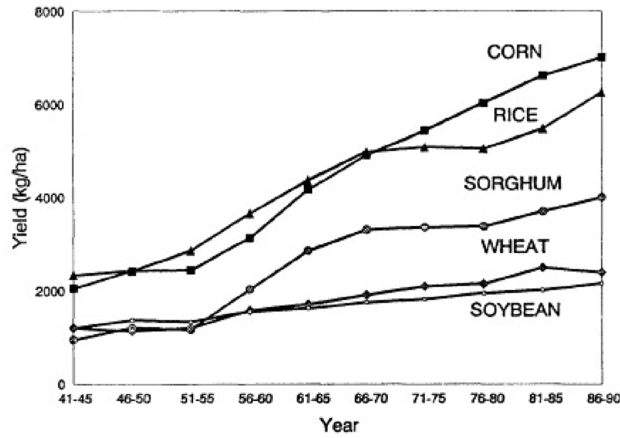


Fig. 1.2.
Yields of five major field crops grown in the United States, 1941 to 1990. The yield increases resulted from providing (1) more favorable cultural environments in which the plants were grown (soil nutrients, soil water supply, tillage, pest control), and (2) improved crop cultivars with genetic potential for utilizing the more favorable cultural environment.

species that contributes to its adaptation, health, productivity, and suitability for food, fiber, or industrial products;

• to search out new genes that encode for desired traits in different strains of the cultivated species and their close relatives;

• to combine genes for the desired traits into an improved cultivar through traditional breeding or new biotechnology procedures;

• to assess performance of the improved breeding lines in the local environment in comparison with present cultivars; and

• to distribute as new cultivars breeding lines superior to cultivars currently grown.

How this strategy is presently accomplished will be discussed in succeeding chapters.

Education of the Modern Plant Breeder

The student may ask, "What do I study to become a plant breeder?" The simplest answer that can be given is, "You need to study plants," but the study of plants involves study in many disciplines. Knowledge in numerous fields of plant science is essential in the education of the modem plant breeder (Fig. 1.3). These include:

BOTANY. Plant breeders should be accomplished biologists with a broad understanding of the taxonomic classification, anatomy, morphology, reproductive mechanism, and cellular structure of the crop plants with which they work.

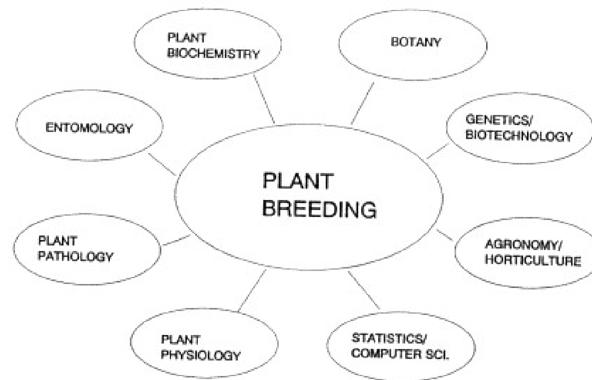


Fig. 1.3.
Overlapping relationship of plant breeding with other plant science disciplines. In breeding an improved crop cultivar, the breeder needs a working knowledge in many plant science disciplines. The spectacular accomplishments in plant breeding are usually the result of cooperation of the breeder with specialists in these disciplines.

GENETICS. The plant breeder needs a thorough understanding of the mechanism of heredity in plants as modern plant-breeding methods are based on a knowledge of the gene and its inheritance. With the advances in molecular genetics, the knowledge of genes has been extended to the molecular level.

PLANT PHYSIOLOGY. Cultivar adaptation is influenced by the response of plants to environmental stresses, such as extremes in temperature, light, soil moisture, and soil nutrients. The plant breeder strives to modify the plant's physiological processes that will enable it to function more efficiently in the environment in which it is grown.

PLANT PATHOLOGY. Healthy plants are essential for good crop performance. The plant breeder cooperates with the plant pathologist in identification of genes for resistance to plant disease pathogens. Incorporation of genes for resistance to disease into cultivars improves plant performance and reduces the need for chemical disease control.

ENTOMOLOGY. Breeding for insect resistance is an economical and an environmentally sound means for avoiding insect damage while reducing the use of pest control chemicals in field and horticultural crops.

PLANT BIOCHEMISTRY. The inherent nutritional value of a crop cultivar for food or for livestock feed, or for utilization by industry, often may be improved by plant breeding. Examples are texture and flavor in tomato, increased lysine content in feed grains, milling and baking quality in wheat, or fiber fineness and strength in cotton. Molecular genetics has contributed toward a better understanding of the chemical structure and function of the genetic material.

STATISTICS. The performance of genetically similar strains are compared in the breeding nursery. The breeder needs to be familiar with field plot evaluation techniques that will generate reliable data and statistical procedures to interpret the data accurately. Analytical statistical procedures provide a better understanding of quantitative genetics and its utilization in breeding for improved plant performance.

COMPUTER SCIENCE. The computer has become an essential tool for systematic planning of the breeding nursery, recording observations, and rapid analysis and interpretation of the data.

AGRONOMY/HORTICULTURE. Breeders need to know crops and how to produce them. They should understand the grower's needs in new cultivars of field or horticultural crops in order to evaluate available breeding materials, plan efficient breeding procedures, and direct breeding efforts toward important breeding goals.

A plant breeder cannot be a specialist in all of these fields of plant science. In the practice of plant breeding, the breeder is not working exclusively in any one of them. The task of the plant breeder is to apply the whole of his knowledge and experience with plants toward the development of superior cultivars. If additional information is needed about the inheritance of a plant character, or about a procedure for measuring the comparative tolerance of different cultivars in a particular environment, the breeder may initiate research to study those particular problems. Joint research efforts between the breeder and research specialists in related

disciplines to solve common problems are desirable activities to carry out in conjunction with a breeding program. Because genetic improvement of plant species involves research in several fields of science, the most rapid advance is made when a team of geneticists, physiologists, pathologists, entomologists, or biochemists work cooperatively with the plant breeder. Spectacular accomplishments in plant breeding are often the result of such teamwork.

How Plant Breeding Began

Plant breeding began when prehistoric man and woman learned to look for superior plants to harvest. With few exceptions, the species of agricultural plants cultivated today evolved over many centuries from wild ancestors. The domestication of wild species was hastened by the early practice of harvesting mutant plants with useful traits. A good example is cultivated wheat as it descended from wild ancestors that differed markedly from modem cultivars. The seed-bearing spikes of the primitive wheats were brittle and easily broken apart, causing seeds to shatter and fall to the soil surface as they ripened. Seeds were covered with hulls that adhered tightly and were difficult to remove before seeds could be eaten. Over long periods of time, perhaps 5,000 to 10,000 years, mutant forms of wheat gradually emerged in which seeds were held firmly until harvest, yet could be separated cleanly from the hulls. These new forms of wheat were gathered as they were easier to harvest and to prepare for food. Eventually, they became the wheats cultivated by prehistoric people.

The early Native Americans accomplished remarkable feats in domesticating Indian corn (maize). There is less certainty about the ancestry of Indian corn than the ancestry of wheat, but it seems relatively certain that the prehistoric corn had small and flinty kernels, more like our current popcorn. As evolution and domestication of corn progressed, Native American tribes living in different climatic regions selected vastly different plant and kernel types. The "races" of corn that evolved range in kernel structure from the small-seeded, flinty endosperm types grown in the central plains of North America to the large-seeded, floury endosperm types grown in Central and South America.

Domestication of the potato may be credited to Native Americans living on the high plateaus of Bolivia and Peru. Tubers of wild potato often contained alkaloids that made them bitter to the taste and somewhat toxic if eaten. As mutant forms less bitter and less toxic were discovered, they were collected and became the progenitors of the current cultivated potato. Similar origin and domestication from wild species by early man have been described for other cultivated crops: barley, oat, and chickpea in the Middle East; rice and sugarcane in southeast Asia and adjacent islands in the South Pacific; soybean in China; sorghum and millet in Africa and India; cotton, tobacco, tomato, and capsicum pepper in the Americas; and many others.

Wild species are still being domesticated for cultivation, usually with a skilled plant breeder genetically manipulating the wild plants to improve productivity and utility. The sunflower and sugarbeet were upgraded from wild weeds to cultivated crops through planned plant breeding programs. In early sugarbeet cultivars clusters of flowers borne in a single axil fused without cleavage wails and developed into a *multigerm seedball*. When the seedball germinated, a cluster of seedlings emerged necessitating expensive hand thinning of seedling plants to obtain uniform stands of beets and development of beets with uniform conformation (Fig. 1.4A and B). By breeding new cultivars with a single flower in the axil of the bract, a *monogerm seedball* is formed which gives rise to a single seedling, eliminating the need for hand thinning to obtain uniform spacing of sugarbeet plants (Fig. 1.4C and D).

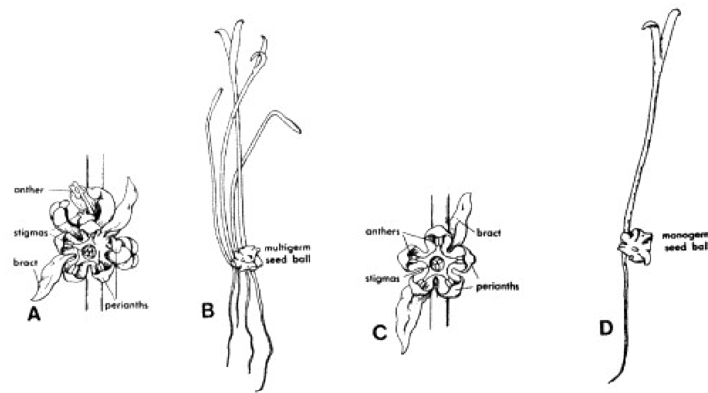


Fig. 1.4.
(A) Cluster of sugarbeet flowers in axil of a flowering bract.
(B) The flower cluster gives rise to a multigerm seed ball and a cluster of seedling plants necessitating expensive hand thinning to obtain uniform stands and development of beets with uniform conformation.
(C) Single sugarbeet flower in axil of a flowering bract.
(D) Single flowers give rise to monogerm seedballs which may be uniformly spaced by machines, eliminating the necessity of expensive hand thinning.

The domestication of wild rice (*Zizania palustris*) began only recently in northern United States as the direct result of planned plant breeding activities. For countless generations seeds of wild rice had been harvested for food by Native Americans from wild plants growing in shallow lakes in northern Minnesota and adjacent areas of Canada. The mature seeds shatter when ripe, fall to the bottom of the lake where they overwinter, and then germinate to start the next season's crop. The Native Americans harvested the seeds by shaking them into their canoes as they floated daily among the standing plants. In recent decades the popularity of wild rice as a gourmet food increased until the demand could not be met with the meager harvest by primitive methods from native stands. Through a plant breeding program, nonshattering cultivars were developed that are adapted to mechanical harvesting. In the future, genetic improvements will be made in other wild species and they will become adapted for commercial production and new uses.

Plant Breeding Before Mendel

Establishing a date when man consciously began to breed plants would be difficult, but some important early landmarks regarding selection and hybridization can be identified. In 1856, a Frenchman, Louis Leveque de Vilmorin, utilized the progeny test to increase the sugar content in the wild sugarbeet. The *progeny test* evaluates the breeding value of a single plant by the performance of its progeny. The efficacy of developing cultivars from progenies of single plants in self-pollinated species was verified at the plant breeding station of the Swedish

Seed Association, Svalöf, shortly before 1900. Professor Hjalmar Nilsson demonstrated that *the plant is the correct unit for selection*, not a single flowering structure on the plant, as was advocated by some breeders in that period. The correctness of using the plant as the unit of selection to produce uniform, tree-breeding cultivars was confirmed independently in 1903 by a Danish botanist, Wilhelm Johannsen, through research on garden beans, and by Willet M. Hays with wheat in the United States.

Pioneering accomplishments in breeding for disease resistance were reported by W.A. Orton in 1898, who was breeding for wilt resistance in cotton, and by H.L. Bolley in 1901, who was breeding for wilt resistance in flax. Both subjected mixed populations of plants to a natural epidemic of the disease by growing the plants in soil infested with the wilt organism and then selecting surviving plants. The principle of survival is basic today in breeding for resistance to pathogens that incite plant disease (Fig. 1.5). Knowledge of the function of pollen in the fertilization of plants and an understanding of the plant's reproductive system are essential for comprehension of basic hereditary principles and their application in the rational development of hybridization as a plant breeding procedure. Although the fact of sex in plants was established with certainty as early as 1694 through research by the German botanist, R.J. Camerarius, the details of gamete fusion and double fertilization were not known until the late 1800s.

The Contributions of Gregor Mendel

Many plant hybridizers were active prior to 1900, but none made as important a contribution as the Augustinian monk, Gregor Mendel (Fig. 1.6). Working in the garden of a monastery in Brünn, Bohemia, Mendel cross-pollinated alternative forms of the common garden pea and studied the ratios of the different forms through successive generations. Through keen observation and clear reasoning, Mendel established a few fundamental "Laws of Inheritance" which remain valid today and form the basic rules that plant breeders follow. Mendel's research was first reported in 1865, but it went unnoticed until independently discovered by three plant scientists in 1900. Since then the "Laws of Inheritance" that Mendel established have been enlarged and supplemented by a wealth of additional knowledge. Collectively, this information comprises the large and important branch of science known as

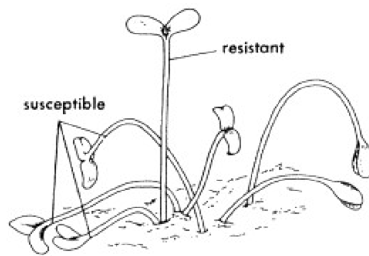


Fig. 1.5.
Seedling plants of flax grown in "flax-sick" soil in which the flax wilt pathogen is present. The principle of survival when exposed to the pathogen is basic for identification of resistant plants in disease resistance breeding.

genetics. Genetics, more than any other science, provides the foundation upon which plant breeding has developed.

Plant Breeding After Mendel

Mendel's experiments stimulated research by many plant scientists dedicated to improving crop production through plant breeding. One of the studies led to the discovery of the method for breeding hybrid corn. The study began in 1904 when G.H. Shull started to inbreed open-pollinated corn at the Station for Experimental Evolution, Cold Spring Harbor, New York. Hybrid corn proved to be so successful that it replaced the "open-pollinated" corn then grown. Hybrid vigor has since been utilized in the breeding of onion, pearlmillet, sorghum, sugarbeet, sunflower, tobacco, tomato, and a host of other field and horticultural crops.

The practice of combining genes from diverse sources of germplasm frequently leads to a high level of cultivar performance. Crosses between short "semidwarf" wheat from Italy and Japan and locally cultivated wheats subsequently led to the development of short, profusely tillering, and highly productive wheat cultivars that lifted wheat yields to a new high. Norman E. Borlaug (Fig. 1.7) internationalized wheat breeding by utilizing the new germplasm to produce a series of short-statured, highly productive cultivars that became the basis for a "green-revolution" in wheat production when introduced into subtropical areas of the world. For his contribution to world food production through the high-yielding wheats, Borlaug was awarded the 1970 Nobel Peace Prize.

Fig. 1.6.
Gregor Mendel (1822-84), the Augustinian monk who studied inheritance in the garden pea and established the "Laws of Inheritance." These basic tenets gave rise to the present science of genetics.

Short-statured rice cultivars developed under the guidance of Dr. T.T. Chang (Fig. 1.8) at the International Rice Research Institute, Los Baños, Laguna, Philippines, became the basis for the "green revolution" in rice production. These cultivars increased rice yields by more than 40% in tropical Asia.

Important advances in forage crop breeding procedures were made by T.J. Jenkin, whose concept of strain-building was developed at the Welsh Plant Breeding Station, Aberystwyth, beginning in 1919. Strain-building is a system of breeding in which individual plants of a cross-pollinated species are selected and combined to produce a synthetic cultivar. The procedure is widely used today in the improvement of alfalfa and many other forage species. At the Coastal Plains Experiment Station in Tifton, Georgia, Glen W. Burton (Fig. 1.9) demonstrated that it was possible to select a productive

Fig. 1.7.
Norman E. Borlaug, recipient of the 1970 Nobel Peace Prize for his contribution to peace and humanity through the breeding of the "high-yielding" wheats, discusses wheat breeding with a group of trainees at the International Maize and Wheat Improvement Center in Mexico.

hybrid plant of bermudagrass, a species that produces seed sparingly, and propagate the hybrid cultivar by planting vegetative sprigs. Burton's innovative ideas made many other contributions to forage breeding. He identified cytoplasmic male sterility and fertility-restorer genes in the tropical plant, pearlmillet, and established a procedure for hybrid production in that species.

At Stoneville, Mississippi, Edgar E. Hartwig has devoted half a century to breeding improved cultivars of soybean (Fig. 1.10). The cultivars he developed are noted for their productivity and resistance to disease, nematodes, and insects.

These and many other examples of accomplishments in breeding field crop plants will be described in succeeding chapters.

Who Are the Modern Plant Breeders?

Plant breeding is conducted principally by:

• publicly supported state (or provincial) and national agricultural research agencies,

Fig. 1.8.
T.T. Chang. As rice breeder and head of the rice germplasm center at the International Rice Research Institute, Chang contributed to the development of the high-yielding semidwarf rice cultivars that increased average rice yields in tropical Asia by 42%. Chang is shown here in the rice germplasm, long-term storage room, where 80,000 varieties of rice seeds are maintained for use by plant breeders.

• privately owned seed companies or institutes, and

• international agricultural research institutes.

The role of each of these agencies in breeding field and horticultural crops differs with the plant species and the country.

In the United States, plant breeding research and cultivar development began largely as activities of the State Agricultural Experiment Stations in cooperation with the U.S. Department of Agriculture (USDA). Prior to about 1950, most cultivars of wheat, barley, oat, soybean, tobacco, alfalfa, sorghum, sugarbeet, and the hybrids of corn and sorghum were developed by representatives from these agencies. The responsibility for breeding hybrid corn and sorghum passed to private companies as the acreage of hybrids increased because the private seed companies could pursue seed production and marketing activities more efficiently than publicly financed agricultural experiment stations. The private hybrid seed companies soon began breeding programs and now do substantially all of the breeding for hybrid corn and hybrid sorghum in the United States. Cotton emerged as a plantation crop with breeding conducted largely by private seed companies. A Plant Variety Protection Act enacted in 1970 gave intellectual property right protection to developers of sexually produced plants. This Act encouraged private seed companies to increase breeding programs in field and horticultural crops that have large seed requirements, such as wheat, soybean, alfalfa, sugarbeet, turfgrasses, and numerous other crops. The Plant Patent Act of 1930 had provided similar protection to breeders of asexually reproduced crops, encouraging greater participation in the

breeding of those crops by private companies and nurseries. The modem era of biotechnology has potential for producing novel plant cultivars and hybrids in sexually reproducing plants and sexually reproducing plants have been patented since 1985. Research to develop new plant breeding methods is conducted principally by the State Agricultural Experiment Stations and the U.S. Department of Agriculture, who also have primary responsibility for introduction and maintenance of germplasm from foreign sources.

Fig. 1.9.
Glen W. Burton, distinguished forage crop breeder for the U.S. Department of Agriculture and the Georgia Coastal Plains Experiment Station, Tifton, Georgia, where he made many contributions to forage improvement breeding in the southern United States.

In Western Europe, research on plant breeding and plant genetics is conducted by both publicly and privately supported institutions, but a substantially larger portion of the breeding has always been done by private breeders than in the United States. In Asian and African countries, most plant breeding programs are conducted by provincial or national governmental agencies.

The International Research Institutes have emerged with extensive multidisciplinary breeding programs for selected crops. Their breeding programs are designed to contribute high yielding cultivars for distribution to lesser developed countries, most of whom are located in the tropics or subtropics. The International Research Institutes receive multinational monetary support from the United Nations, individual donor countries, and private donor organizations. The International Research Centers, their locations, and the field or vegetable crops on which they conduct breeding research are as follows:

Asian Vegetable Research and Development Center (AVRDC), Shanhua, Taiwan (Chinese cabbage, mungbean, pepper, tomato, soybean).

International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria (barley, chickpea, faba bean, tropical forages, lentil, wheat).

International Center for Maize and Wheat Improvement (CIMMYT), Mexico, D. F. Mexico (maize, triticale, wheat).

International Center for Tropical Agriculture (CIAT), Cali, Colombia (dry bean, cassava, rice, tropical forages).

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India (chickpea, millet, peanut (groundnut), pigeonpea, sorghum).

Fig. 1.10.
Edgar E. Hartwig, soybean breeder for the United States Department of Agriculture and the Delta Branch Experiment Station, Stoneville, Mississippi. Hartwig has devoted half a century to improving the soybean for the southern United States. His cultivars are noted for their productivity and resistance to disease, nematodes, and insects.

International Institute of Tropical Agriculture (IITA), Ibaban, Nigeria (cassava, cocoyam, cowpea, lima bean, maize, pigeonpea, rice, soybean, sweet potato, winged bean, and yam).

International Potato Center (CIP), Lima, Peru (potato, sweet potato).

International Rice Research Institute (IRRI). Los Baños, Laguna, Philippines (rice).

Study Questions

1. What contributions has plant breeding made to society?

2. How would you contrast plant breeding prior to Mendel's time versus after Mendel's time?

3. What role does plant breeding play in domesticating a plant species?

Further Reading

Borlaug, N.E. 1983. Contributions of conventional plant breeding to food production. Sci. 219:689-93.

Brooks, H.J., and G. Vest. 1985. Public programs on genetics and breeding of horticultural crops in the United States. HortScience 20:826-30.

Ceccarelli, S., and S. Grando. 1993. From conventional plant breeding to molecular biology. p. 533-37. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson

(eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Duvick, D.N. 1986. Plant breeding: Past achievements and expectations for the future. Econ. Bot. 40: 289-97.

Fehr, W.R. (ed.). 1984. Genetic contributions to yield gains of five major crop plants. Crop Sci. Soc. Am. Spec. Publ. No. 7. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

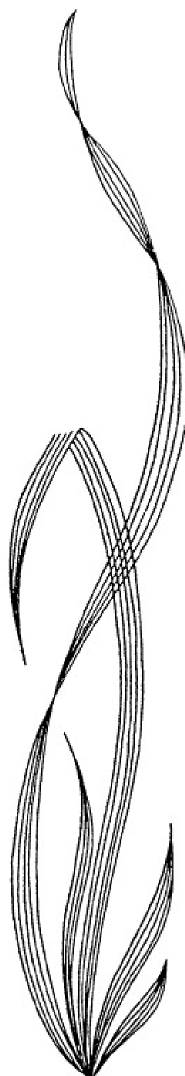
Harlan, J.R. 1992. Crops and man. 2nd ed. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Hayes, H.K. 1957. A half-century of crop breeding research. Agron. J. 49:626-33.

Johnson, V.A. 1981. What makes a successful plant breeder? J. Agron. Educ. 10:85-86.

Poehlman, J.M., and J.S. Quick. 1983. Crop breeding in a hungry world. p. 1-19. *In* D.R. Wood (ed.) Crop breeding. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Roll-Hansen, N. 1986. Svalöf and the origins of classical genetics. p. 35-43. *In* G. Olsson (ed.) Svalöf, 1886-1986, research and results in plant breeding. LtsFörlag, Stockholm.



II
THE GENETIC BASIS OF PLANT BREEDING

2. Reproduction in Crop Plants

Breeding procedures that are used to improve a particular field or horticultural crop are determined by it's mode of reproduction. This relationship will become clearer as the breeding methods used with the various crop species are studied in more detail. It may be illustrated here by comparing two common field crop plants, wheat and corn, which differ in pollination method. In wheat, a self-pollinating crop, pollen normally fertilizes the flower in which it is borne; new genetic material (DNA) that may affect the purity of a cultivar is not introduced during pollination. The seeds of an improved cultivar of wheat may be harvested and planted over and over again without genetic change if care is exercised during its production to prevent mixture with seeds from another cultivar and recognizable mutant forms are not observed. Contrast this with corn, a cross-pollinated crop. In corn the pollen-bearing organ is borne in a tassel at the top of the plant and the seed-bearing organ is borne in a shoot located below the tassel. Pollen is transported through the air from the tassel to the portion of the silk extending from the shoot on the same or a different plant before pollination and seed production are consummated. The pollen may be carried by the wind for large distances. Seeds borne on a single ear of corn may arise by pollination with pollen grains originating from many genetically different plants, this results in a redistribution of contrasting genes for particular traits of the corn plant and each new plant in the population differing from its parents.

An understanding of the details of pollination, fertilization, and seed development for a crop species is necessary to understand the hereditary mechanism in that species. It is from this knowledge that efficient breeding procedures for a particular crop species are devised.

Types of Reproduction

Reproduction in crop plants may be by seeds, *sexual*, or by vegetative parts, *asexual*. With sexual reproduction specialized reproductive cells called *gametes* are formed, a process known as *garnetogenesis*. Fusion of the male and female gametes leads to the development of an embryo and eventually the seed. In asexual reproduction new plants arise from specialized

vegetative organs such as tubers, rhizomes, runners, bulbs, corms, or by various artificial means of propagation such as rooting of plant cuttings, grafting, layering, or tissue culturing. Crop plants such as corn, wheat, rice, soybean, tomato, or common bean normally reproduce sexually and are multiplied from seeds; other crops such as sugarcane, potato, bermudagrass, or cassava may reproduce sexually but are normally propagated asexually for commercial use.

Sexual Reproduction in Crop Plants

In conventional plant breeding procedures, genetic variability is identified, increased, and exploited through sexual reproduction. A knowledge of the plant reproductive process is so important to the breeder that the essential features are reviewed here. Additional details may be found in textbooks of biology and botany.

Parts of the Flower

The flower contains the sexual reproductive structures of the plant. It commonly consists of four floral organs: *sepals, petals, stamens, and pistil* (Fig. 2.1). Typically, petals are large and brightly colored; sepals are small and inconspicuous. Neither petals nor sepals are necessary for reproduction as only the stamens and the pistil function in production of seeds. The stamen usually consists of a slender stalk or *filament*, which supports an *anther*, within which the pollen grains develop. The pistil commonly consists of an enlarged base or *ovary* in which the seeds are formed, an elongated stalk or *style, and a stigma*, which is receptive to pollen deposited on it. Within the ovary are found the ovules, which, after embryo formation within, develop into mature seeds. The number of ovules within an ovary may vary from one, as in wheat or barley, to several hundred, as in tobacco.

Kinds of Flowers

Flowers may be *complete* containing all four floral organs (sepals, petals, stamens, pistil), or *incomplete*, lacking one or more of these floral organs. Complete flowers are borne on cotton, tobacco, rape, potato, cowpea, soybean, common bean, tomato, clovers, alfalfa, cabbage, and many other field and vegetable crop plants (Fig. 2.2A, B, C, and D). Flowers of buckwheat and sugarbeet are incomplete lacking petals and sepals (Fig. 2.3A). Crops belonging to the grass family, including corn, sorghum, millet, wheat, triticale, barley, oat, sugarcane, rice, forage grasses, and turf grasses, have incomplete flowers in which petals and sepals are lacking (Fig. 2.3B). In lespedeza, *cleistogamous* flowers, inconspicuous flowers that do not open, are frequently produced on the same plant with normal, showy flowers.

Perfect flowers are bisexual, bearing stamens and a pistil in the same flower structure, but one of these essential organs is absent in *imperfect* or unisexual flowers. Most crop plants have perfect flowers, for example, wheat, oat, barley, rye, rice, sorghum, cotton, flax, potato, tobacco, sugar beet, sugarcane, soybean, common bean, tomato, common forage and turf grasses, and forage legumes. Imperfect flowers may be *staminate*, bearing stamens but no pistil, or *pistillate*, bearing a pistil but without stamens. The corn plant has staminate flowers in the tassel and pistillate flowers on the shoot. In castor and wild rice, pistillate flowers are commonly borne in the upper portion of the floral structure and staminate flowers in the lower portion. Crop plants in which staminate and pistillate flowers are borne on the same plant, as

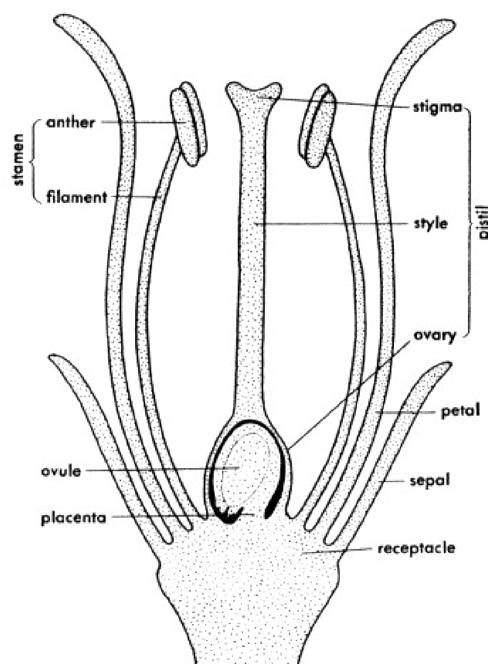


Fig. 2.1.
Parts of the flower.

in corn, cassava, squash, or castor, are *monoecious* (Fig. 2.4); plants in which the staminate and pistillate flowers are borne on different plants are *dioecious* (Fig. 2.5A and B). Hemp, hops, buffalograss, papaya, and asparagus are species with dioecious flowers, although occasional hemp or papaya plants may produce monoecious flowers. Imperfect flowers are always incomplete. Some incomplete flowers, such as occur in the grasses or in buckwheat, are perfect because both the stamens and a pistil are present in the same flower although petals or sepals may be missing.

Pollination and Fertilization

Seeds are formed within a plant by a succession of steps in the reproductive cycle as illustrated in Fig. 2.6. Within the immature anther are four cavities containing many *microspore* or *pollen mother cells*. Each mother cell undergoes two successive nuclear divisions and forms a tetrad of four *microspores*, each with potential for developing into a mature *pollen grain*. The microspore is transformed into a mature pollen grain by a thickening of the spore

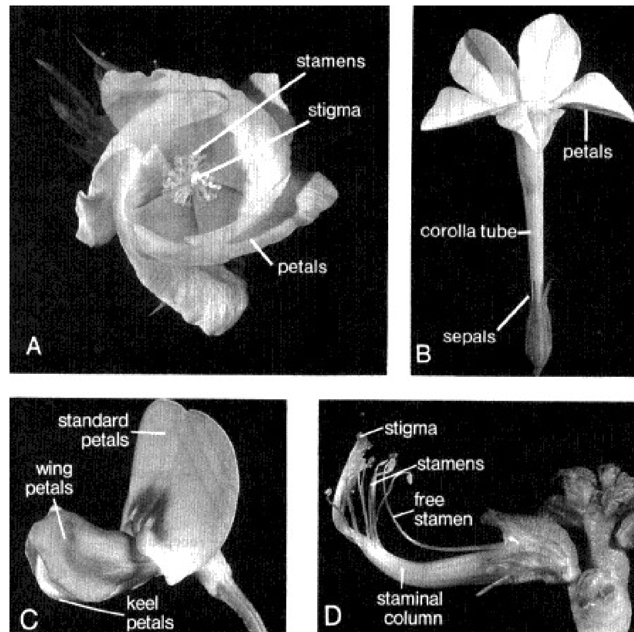


Fig. 2.2.
Complete flowers. (A) Flower of cotton showing whorl of five petals, stamens, and stigma. The sepals are hidden by the petals. (B) Flower of tobacco showing sepals and five petals. The petals are fused and form a corolla tube surrounding the stamens and the pistil. (C) Flower of cowpea, typical of the flowers in the legume family. The corolla is composed of one standard, two wing, and two keel petals. (D) Flower of cowpea with all petals removed. In the typical legume flower, nine stamens form a staminal column, which surround the stigma. The tenth stamen remains free.

wall and a division of the microspore nucleus to form a *tube cell nucleus and a generative nucleus*. As the anther matures, the pollen sacs open and the pollen grains are dispersed. The pollen grains are produced in great numbers; from 20 to 50 million may be produced on the tassel of a single plant of corn.

Pollination is consummated by the transfer of pollen grains from the anther to the stigma. The means of transfer varies with different crops; the pollen from the anther of the corn plant is distributed by the wind. Some of it may pollinate stigmas on the same plant, although it is more likely that the stigmas will be pollinated with pollen from surrounding plants. Forage grasses, rye, sugarcane, and sugar beets are also pollinated by wind-borne pollen. In legumes, such as red clover and alfalfa, pollen is carried from one flower to another by insects. In self-pollinated crops such as soybean, wheat, tomato, or bean, the pollen usually is transferred

Fig. 2.3.
Perfect and incomplete flowers. (A) Flowers of buckwheat lack petals but bear white to greenish white sepals. (B) The flower of grasses lacks petals and sepals. In the oat flower shown here, the lemma is pulled down to expose the stigma and the anthers. The lemma and palea form the hull that covers the mature oat kernel.

directly upon the stigma within the same flower as the anthers open. In wheat, oat, barley, and rice, the stamens and the pistil are enclosed by floral bracts (*lemma* and *palea*), which tend to prevent foreign pollen from entering the floret.

The stigma is the portion of the pistil that is receptive to the pollen. It may be branched or feathery, so that it catches the pollen grains in its branches, or it may secrete a sticky stigmatic fluid to which the pollen grains adhere. Mature pollen grains germinate on the stigma and a slender *pollen tube* grows through the style and enters the tip of the ovule through an opening known as the *micropyle*. Two male germ cells or *gametes*, also called *sperms*, are formed by division of a generative nucleus within the pollen grain (Fig. 2.6). As the pollen grain matures, the sperms move through the pollen tube and are emptied into the embryo sac.

The female germ cell or gamete, also called an *egg*, is produced within the ovule by a succession of events similar to those which led to the production of the sperm (Fig 2.6). Within each ovule is a single *megaspore mother cell*, which, like each of the microspore mother cells, undergoes two successive nuclear divisions and produces a tetrad of four megaspores. Three of the megaspores disintegrate, the fourth megaspore, usually the one farthest from the micropyle, continues to undergo mitotic nuclear divisions and forms an ovoid, eight-nucleate *embryo sac*. The mature embryo sac includes the female gamete or egg and two additional nuclei (*synergids*) which lie near the micropyle; three nuclei (*antipodals*) lie in the opposite end of the embryo sac; the two remaining nuclei, termed *polar nuclei*, lie in the central area. After the two sperms are emptied from the pollen tube into the embryo sac, one sperm fuses with the egg to form a *zygote*, a process known as *fertilization*. The second sperm unites with a diploid nucleus that was formed by the earlier fusion of the two polar nuclei, or all three of these nuclei may fuse simultaneously. The nucleus resulting from this *triple fusion* is called the *primary endosperm nucleus*. These processes, in which both sperm nuclei function, are referred to as *double fertilization*.

The seed has its beginning with the formation of the fertilized egg (zygote) and the endosperm nucleus. The fertilized egg develops into the *embryo*, which, on germination of the seed, grows into the new plant. The primary endosperm nucleus divides many times to form numerous nuclei that become enclosed by cell walls to form the *endosperm*, a tissue in which starch, oil, or protein is stored. This stored food supplies the germinating embryo and the early stages of seedling growth. In the cereals the larger part of the seed is the endosperm. In seeds of soybean, peanut, and other legumes, the endosperm is absorbed by the developing embryo and the food materials are stored in structures called *cotyledons*. The *seed coat* develops from integuments surrounding the ovule.

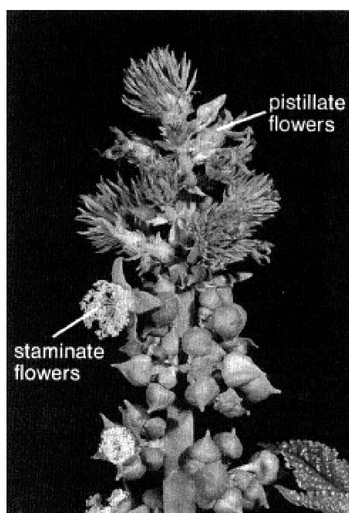


Fig. 2.4.
Monoecious flowers of the castor bean.

Nuclear Division and Chromosomes

A plant is composed of small structural and functional units, the *cells. A* typical cell contains a viscous material known as *cytoplasrn* enclosed in a membrane, a *nucleus*, and a rigid *cell wall*. Enclosed within the cytoplasm are various *organelles* (chloroplasts, endoplasmic reticulum, Golgi bodies, microtubules, mitochondria, ribosomes) and *enzymes* that function in cell metabolism. The nucleus contains the genetic material and functions in cell division and reproduction. With a light microscope and selective staining, shortened rodlike chromosomes may be observed within the nucleus during nuclear divisions. Each chromosome forms one long DNA molecule complexed with enzymes capable of reproducing DNA and RNA copies.

Mitosis

Two types of nuclear division occur (Fig. 2.7). One form of division, *mitosis* (equational division), is normally characterized by the:

• lengthwise replication of the DNA strands comprising each chromosome to form two identical sister chromatids that remain attached at the centromere;

• disappearance of the nuclear membrane and the formation of a spindle of fibers;

• movement of chromosomes with chromatids still attached to the equator of the spindle;

• physical separation of chromatids and migration to opposite poles of the spindle;

• formation of two daughter nuclei, each with a complement of chromosomes identical to

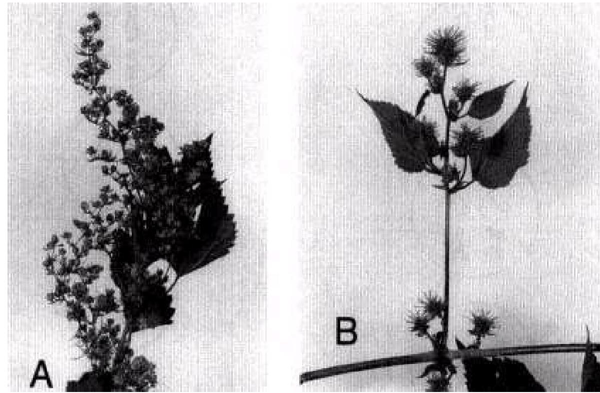


Fig. 2.5.
Dioecious flowers of hops. (A) Staminate inflorescence.
(B) Pistillate inflorescence—the hops used commercially.

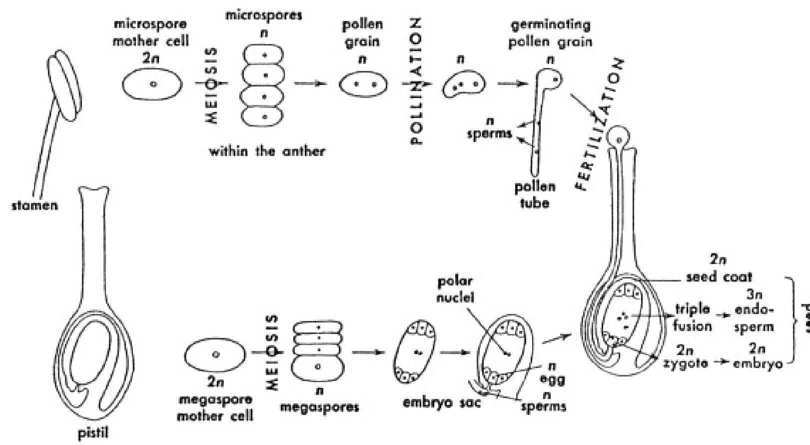


Fig. 2.6.
Steps in the reproduction of a seed plant. Starting with the spore mother cells in the anthers and in the ovules, a succession of events takes place that leads to fertilization and eventual formation of a seed.

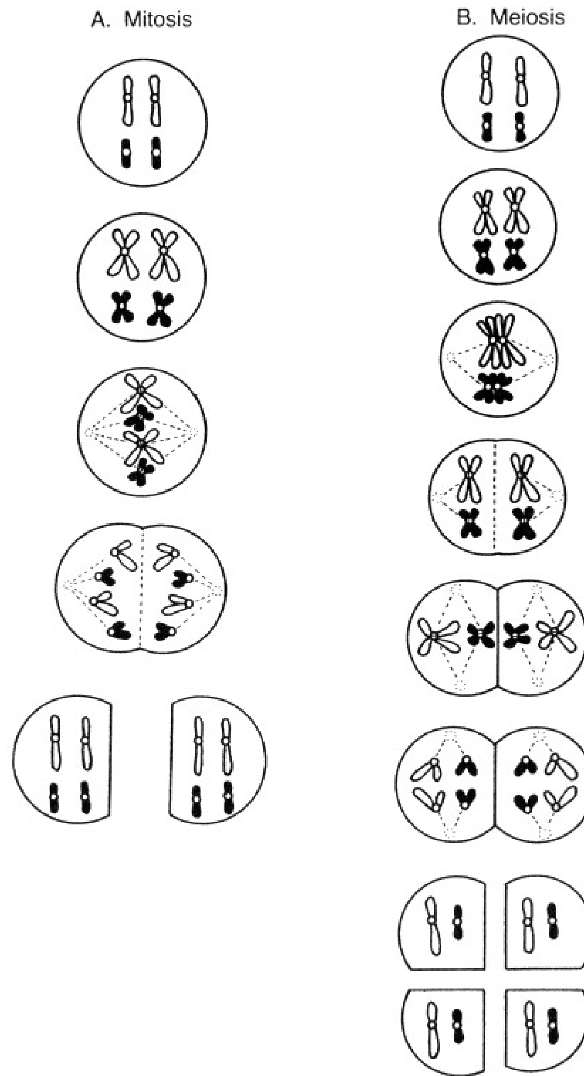


Fig. 2.7.
Comparison of nuclear divisions by mitosis and meiosis. (A) In mitosis, the parent cell may be haploid or diploid; two daughter cells are formed, each with chromosome content identical to that of the parent cell. (B) In meiosis, the parent cell is diploid; four haploid daughter cells are formed.

those present in the parent nuclei; and

• formation of partitioning cell walls between these daughter nuclei.

Biologists recognize several phases of the mitotic division process, but the transition from one phase to the next is a gradual and continuous process. *With mitosis the daughter nuclei normally receive an exact copy of each chromosome originally present in the nucleus of the parent cell*. Mitosis is the method of division by which new cells are formed in the normal growth and development of a plant. It is the only form of cell division associated with asexual reproduction.

Meiosis

The second form of nuclear division, *meiosis*, is associated with sexual reproduction in higher plants. Meiosis occurs when the megaspore mother cell divides to form the spores. It consists of two successive divisions, the first reductional (*meiosis I*) and the second equational (*meiosis II*). Meiosis is characterized by:

• replication of each chromosome lengthwise to form two identical *sister chromatids*, that are joined at the *centromere*;

• pairing (synapsis) of *homologous chromosomes*, i.e., those which have genes at corresponding loci controlling a common hereditary characteristic, and exchange of chromatid segments, a process known as *crossing over*;

• movement of the homologous chromosomes to the equator of the spindle with the chromatids still joined together at *centromeres*;

• random reassortment of the homologous chromosomes with the paired chromosomes separating and moving to opposite poles while the chromatids are still joined (*reductional division*);

• the formation of new spindles in opposite ends of the cell with the joined chromatids becoming arranged at the equator of the new spindles;

• the separation of the chromatids at the centromeres (*equational division*) and the migration of the chromatids to the poles of their respective spindles; and

• the formation of cell walls to form four spores, each with one-half as many chromosomes as the parent spore mother cell (one chromosome from each pair in the parent spore mother cell).

As with mitosis, biologists recognize several phases of meiosis although the process proceeds in a gradual and continuous sequence. *An essential feature of meiosis is the reduction of the chromosome number from the diploid* ($2n$) *number in the megaspore mother cell to the haploid (n) number in the spores (gametes)*. Because gametes are formed from spores by successive mitotic divisions, gametes will contain the haploid chromosome number. The fusion of a sperm and an egg at fertilization brings two haploid sets of chromosomes together and restores the diploid chromosome number in the zygote. The endosperm nucleus is formed by the triple fusion of a haploid sperm with the diploid polar nuclei and contains a triploid ($3n$) chromosome number. Meiosis is important in maintaining a stable chromosome number in a species; otherwise the chromosome number would be doubled with each successive generation due to fusion of the two gametes.

Meiosis has significance for the plant breeder because (1) it permits the maintenance of

a level chromosome number in the plant, (2) the segregation of contrasting alleles leads to their subsequent recombination in the following generation, and (3) the exchange of chromatid segments of homologous chromosomes as the result of chiasmata formation provides the mechanism for recombinations of linked genes and hence new genetic variation. These phenomena will be discussed in more detail in Chapter 3.

Chromosome Numbers in Crop Plants

The diploid chromosome numbers of some commonly cultivated crop species are listed in Table 2.1. The haploid-diploid chromosome number is normally constant for any plant species. It may be noted that the cultivated species of wheat, *Triticum turgidum* and *T. aestivum*, have chromosome numbers of $2n=4x=28$ and $2n=6x=42$, respectively. In the genomic formula, $2n$ represents the somatic chromosome number and x represents the basic chromosome number. The basic chromosome number x is 7 for *T. turgidum* and *T. aestivum*. Certain wild species of *Triticum (T. monococcum*, for example) have chromosome numbers of $2n=2x=14$. Thus the haploid chromosome numbers of the three species, *T. monococcum, T. turgidum*, and *T. aestivum*, are 7, 14, and 21, respectively. A group of closely related species like these in which the chromosome number is increased in an arithmetic ratio constitutes a *polyploid series*. In a naturally occurring polyploid series, the species with the higher chromosome number generally will have larger cell size and be more vigorous and productive. It is sometimes possible to create polyploid plants by bringing together the chromosome complements of two different species, or by duplicating chromosome complements within a plant. The latter is normally accomplished by application of an organic chemical, *colchicine*, to the actively dividing cells in the growing tip, or by other means. These techniques have been used to produce improved strains of sugarbeet, giant cultivars of some common flowers, and a new species, *Triticosecale* sp.

Table 2.1.
Diploid chromosome number of some cultivated crop species

Crop name	Species name	Diploid (2*n*) chromosome number
Cereal crops		
Barley	*Hordeurn vulgare* L.	14
Corn (maize)	*Zea mays* L.	20
Millet, finger (ragi)	*Eleusine coracana* (L.) Gaertn.	36
Millet, pearl (bajra)	*Pennisetum glacum* (L.) R.Br.	14
Oat	*Avena sativa* L.	42
Rice	*Oryza sativa* L.	24
Rye	*Secale cereale* L.	14
Sorghum	*Sorghum bicolor* (L.) Moench	20
Triticale	*X Triticosecale* Wittmack	42, 56
Wheat, durum	*Triticum turgidum* L.	28
Wheat, bread	*Triticum aestivum* L.	42
Fiber crops		
Cotton, Asiatic	*Gossypium arboreum* L.	26
Cotton, Egyptian (Pima)	*Gossypium barbadense* L.	52
Cotton, American upland	*Gossypium hirsutum* L.	52
Jute	*Corchorus capsularis* L., *C. olitorius* L.	14

(Table continued on next page)

Table 2.1. (*continued*)

Crop name	Species name	Diploid ($2n$) chromosome number
Forage crops		
Alfalfa (lucerne)	*Medicago sativa* L.	32
Bahiagrass	*Paspalum notatum* Flügge	40
Bermudagrass	*Cynodon dactylon* (L.) Pers.	18, 36
Birdsfoot trefoil	*Lotus corniculatus* L.	12, 24
Bluegrass	*Poa pratensis* L.	28, 56, 70
Bromegrass	*Bromus inermis* Leyss.	56
Clover, red	*Trifolium pratense* L.	14
Clover, sweet, white	*Melilotus alba* Desr.	16
Clover, white	*Trifolium repens* L.	32
Fescue, tall	*Festuca arundinacea* Schreb.	42
Orchardgrass (cocksfoot)	*Dactylis glomerata* L.	28
Ryegrass, perennial	*Lolium perenne* L.	14
Timothy	*Phleum pratense* L.	42
Oilseed crops		
Canola, Rape	*Brassica napus* L.	38
Flax (linseed)	*Linum usitatissimurn* L.	30
Mustard	*Brassica juncea* (L.) Coss.	36
Peanut (groundnut)	*Arachis hypogaea* L.	40
Safflower	*Carthamus tinctorius* L.	24
Soybean	*Glycine max* (L.) Merr.	40
Sunflower	*Helianthus annus* L.	34
Pulse crops		
Beans, common, navy, snap	*Phaseolus vulgaris* L.	22
Beans, lima	*Phaseolus lunatus* L.	22
Broadbean	*Vicia faba* L.	12
Chickpea (gram, garbanzo)	*Cicer arietinum* L.	16
Cowpea	*Vigna unguiculata* (L.) Walp.	22
Mungbean (green gram)	*Vigna radiata* (L.) Wilczek	22
Root crops		
Cassava	*Manihot esculenta* Crantz.	36
Potato	*Solanum tuberosum* L.	48
Stimulant crops		
Tobacco	*Nicotiana tabacum* L.	48
Sugar crops		
Sugar beet	*Beta vulgaris* L.	18
Sugarcane	*Saccharum officinarum* L.	80
Vegetable crops		
Cabbage	*Brassica oleracea* L.	18
Carrot	*Daucus carorta* L.	18
Cucumber	*Cucumis sativa* L.	14
Lettuce	*Latuca sativa* L.	18
Onion	*Allium cepa* L.	16
Pepper	*Capsicum annuum* L.	24
Tomato	*Lycopersicum esculentum* Mill.	24

Self- and Cross-Pollination in Crop Plants

Self-pollination is the transfer of pollen from an anther to a stigma within the same flower or to a stigma of another flower on the same plant or within the same clone. Pollination among

plants of the same clone, i.e., plants that have been asexually propagated from the same source plant, or pollination among genetically identical plants of an inbred line will have the same effect as pollinating stigmas of flowers on the same plant. Cereal crops, such as wheat or barley, in which the flower is enclosed by floral bracts, are seldom pollinated except from pollen originating in an anther within the same flower. This is in contrast to the cross-pollination in the corn plant, in which pollen is carried by the wind to silks of other plants, or to the pollination in red clover by insects that carry pollen from one plant to the stigma in a flower on a different plant. Fertilization resulting from the union of a sperm and an egg (gametes) produced on the same plant is *self-fertilization* (also called *autogamy*). The union of a sperm and an egg (gametes) produced on different plants is *cross-fertilization* (also called *allogamy*).

From the breeding standpoint, most crop species that reproduce by sexual means may be grouped according to their usual method of pollination as *normally self-pollinated* or *normally cross-pollinated*. These groups are not exclusive, because slight cross-pollinationusually occurs in crops normally classified as self-pollinated, and some self-pollination usually occurs within the normally cross-pollinated crops. A few have large amounts of both self- and cross-pollination. The amount of natural crossing or natural selfing within these crops will vary with the (1) cultivar or strain of the crop, (2) environment (temperature, humidity, precipitation), (3) velocity and direction of the wind at the time of pollination, and (4) insect populations present.

Crops Normally Self-Pollinated

Some common crop plants that are normally self-pollinated are:

barley	millet, finger	sesame
bean, dry	millet, foxtail	soybean
chickpea	mungbean	tobacco
cowpea	oat	tomato
crambe	peanut (groundnut)	triticale
flax (linseed)	pea	vetch
jute	potato	wheat
lentil	rice	wheatgrass, slender

The amount of natural cross-pollination that may occur within crops of this group may normally vary from none up to 4 or 5%. The plant breeder working with self-pollinated crops should be familiar with the extent of natural cross-pollination in the breeding nursery. The percentage of natural cross-pollination in a crop may be estimated by planting two cultivars pure for different forms of an easily recognized and simply inherited character. Individual plants of the cultivar recessive for the character are completely surrounded by plants dominant for the character. Seeds harvested from the plants recessive for the character are grown and the percentage of plants that exhibit the dominant character is determined.

A number of floral mechanisms in plants may exclude cross-pollination and result in a particular species being normally self-pollinated, for example the:

• flowers may not open,

• pollen grains may be shed before flowers open,

• stigma and stamens may be hidden by floral organs after flowers open, or

• stigma may elongate through a staminal column shortly after the anthers open.

The process of flowering is called *anthesis* and is described here for the barley plant, a typical self-pollinating cereal crop. Each barley flower is enclosed by two floral bracts, an outer glume or *lemma*, and an inner glume or *palea*. Sexual organs of the barley flower consist of three stamens and a pistil, the latter having two styles with many stigmatic branches that give it a feathery appearance (Fig. 2.8). As the stigma matures and is receptive to the pollen, two small saclike organs at the base of the ovary, known as the *lodicules*, swell and cause the flower to open. At anthesis (flowering) the stamen filaments elongate, the flower opens, and anthers push out of the glumes. The anthers rupture and are emptied of their pollen, part of the pollen falling inside, and the remainder outside of the flower (Fig. 2.8). Pollen grains usually germinate within a few minutes after falling on the stigma hair and the pollen tube starts growing in the style immediately (Fig. 2.9). The time required for the pollen tube to reach the embryo sac may vary from 20 minutes to 2 hours, depending upon temperature. If the anthers do not produce viable pollen, or if the flower opens before anthers are extruded or burst, it is possible that foreign pollen reaching the stigma will bring about cross-pollination. In barley and soybean, cross-pollination normally amounts to less than 0.5%, although in wheat, oat, rice, and tobacco, cross-pollination may sometimes reach as much as 2 to 3%. Any condition, environmental or otherwise, that disrupts the normal process of anthesis may increase the proportion of cross-pollination in a normally self-pollinated species.

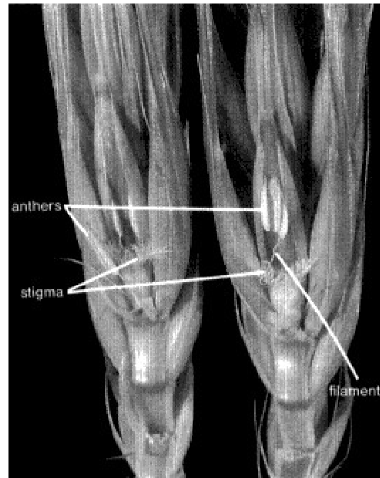


Fig. 2.8.
Flowers of barley. The lemma of the central spikelet has been removed to show the reproductive organs. Left: The anthers have not yet shed pollen. Right: The filaments have elongated, and the anthers have burst and emptied pollen on the stigma.

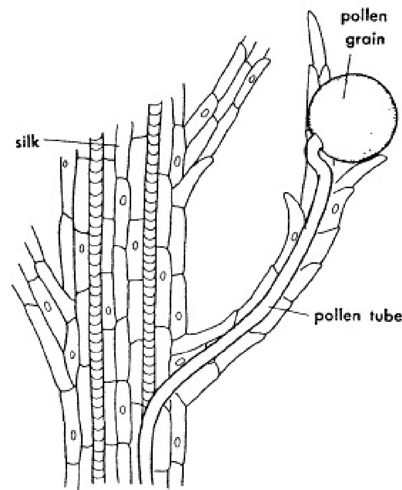


Fig. 2.9.
Germinating pollen on silk of corn.

Crops Normally Cross-Pollinated

Some crop plants that are normally cross-pollinated are:

alfalfa (lucerne)	crested wheatgrass	reed canarygrass
bahiagrass	cucumber	rye
bermudagrass	hemp	safflower
birdsfoot trefoil	hops	squash
bromegrass	johnsongrass	sugar beet
cabbage	meadow fescue	sugarcane
carrot	mustard	sunflower
castor	onion	sweetclover
cassava	orchardgrass	sweet potato
clovers	pearl millet	tall fescue
colocasia	pepper	timothy
corn (maize)	redtop	wild rice

There are mechanisms that may exclude self-pollination in plants and result in normal cross-pollination. These include:

• mechanical obstruction to self-pollination,

• different periods of maturity in the pollen and the stigma,

• self-sterility or self-incompatibility, and

• presence of monoecious or dioecious flowers.

In rye, anthers normally extrude from the flower and spill the pollen outside as they burst. The flowers of rye usually remain open for long periods of time facilitating entry of foreign pollen. Many florets fail to produce seed when flowers of rye are pollinated solely with self-pollen. Most forage grasses are pollinated from windblown pollen and have a high percentage of cross-pollination. Self-incompatibility is commonly found after self-pollination in red clover, white clover, alfalfa, buckwheat, grasses, and certain other plant species. Following self-pollination in self-incompatible crops, the pollen tubes often grow down the style so slowly that the ovules disintegrate before fertilization is completed, or the pollen tubes may fail to penetrate the stigmatic surface of the style. In alfalfa and other legumes the embryo may abort after self-fertilization yet develop normally after cross-fertilization.

Corn is a typical monoecious plant bearing staminate flowers in the tassel and pistillate flowers in the shoot. The pollen is wind-borne with cross-pollination as the rule, although self-pollination may reach 5% or more. Other species with monoecious flowers include cassava, castor bean, and wild rice. Dioecious crops, such as hemp, hops, yams, asparagus, or buffalograss, are cross-pollinated because the staminate and pistillate flowers are borne on separate plants. In breeding dioecious crops both plants with staminate flowers and plants with pistillate flowers must be maintained in the population.

Crops Both Self- and Cross-Pollinated

Some crops do not fit neatly in either the self- or cross-pollinated categories. Examples are broadbean, cotton, pigeonpea, and sorghum. Usually the amount of cross-pollination exceeds that of the normally self-pollinated crops, yet does not reach that of the normally cross-pollinated. In these crops breeding procedures commonly used for self-pollinated crops are modified to accommodate the larger amount of cross-pollination.

Cotton is one of the principal crops in this group. Cotton is largely self-pollinated but cross-pollination may range from 5 to 25% or more. In the flower of the cotton plant, the stigma is exposed and cross-pollination may occur when insects carrying foreign pollen come into contact with an unpollinated stigma. In a few cases where insect populations are abundant, cross-pollination in cotton in amounts up to 50% has been reported. Pollen from the cotton plant is heavy and sticky and is seldom wind-borne. Cross-pollination in sorghum by wind-borne pollen, averages about 6%; it occurs when a flower opens exposing the stigma before pollen is shed. Cross-pollination in sorghum may be prevented by bagging heads before flowering to exclude foreign pollen. Breeding procedures utilized for predominantly self-pollinating species may then be practiced.

Pigeonpea, a grain legume crop grown for food in tropical and subtropical regions, averages about 20% cross-pollination, but may range from 5 to 40% with different cultivars and environments. Sericea lespedeza, utilized in soil conservation practices, produces both *apetalous*, cleistogamous flowers, that fertilize in the bud and are always self-pollinated, and showy, *petaliferous* flowers, that are largely cross-pollinated.

Asexual Reproduction in Crop Plants

Most field and vegetable crop species are multiplied from seeds, but in some species vegetative propagation is utilized because seeds are set poorly, or the crop may exhibit unwanted genetic variability if multiplied through seeds. In other species, seeds are borne without the normal sequence of steps for meiosis and fertilization to take place, a process known as *apomixis*. Apomixis has the same outcome as vegetative propagation because there is no union of sexual gametes.

Vegetative Propagation

Vegetative propagation may be by roots, tubers, stolons, rhizomes, sprigs, stem or leaf cuttings, or by tissue culture. A group of plants that have been propagated vegetatively from a single plant constitutes a *clone*. Plants of the same clone, barring genetic mutation, are identical genetically and bear the characteristics of the parent plant. Plant breeders may use vegetative propagation to establish breeding lines in certain species when seed production is inadequate, or when a genetically uniform population is desired. Seed production fields of particular forage crops are sometimes established by vegetative propagation to maintain genetic purity.

Many root crops are propagated commercially by roots or tubers. Roots are used in propagation of cassava and sweet potato, and tubers for potatoes and most species of yams. Particular stoloniferous grasses, such as bermudagrass, are commonly propagated by vegetative sprigs obtained by breaking apart pieces of sod. The sprigs are composed of rootstocks, stolons, crowns, and pieces of leafy stems.

Stem sections are used in commercial propagation of sugarcane (Fig 2.10). Sugarcane

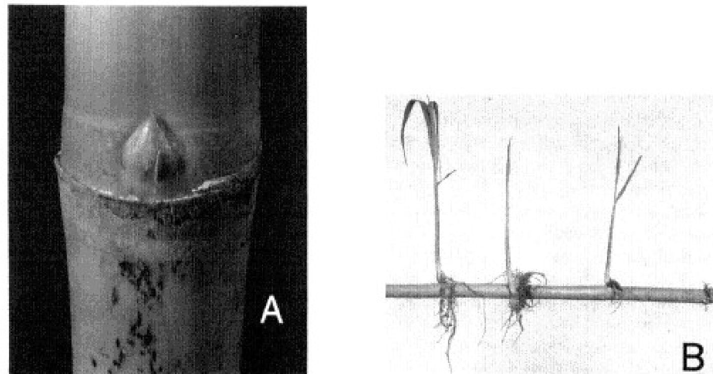


Fig. 2.10.
Clonal reproduction in sugarcane. (A) Bud or eye at node on stem of sugarcane. (B) Section from stem of sugarcane with shoots and roots developing from nodal buds. Stem sections like these are used to propagate sugarcane in the field.

produces flowers sparsely unless critical photoperiod and temperature requirements are met. In vitro methods are commonly used for propagating orchids and are receiving increased attention with other horticultural crops.

Apomixis

Apomixis (= apo, without + mixis, mixing) is an asexual process that substitutes for sexual reproduction in certain flowering plants. In practical usage, there are two types of apomixis.

VIVIPARY. *Vivipary* is the formation of plantlets or bulbils from floral primordia in place of flowers. Vivipary has also been used to describe seed germination on the parent plant, regardless of sexuality. Viviparous apomixis has little use in plant breeding because seeds are not formed.

AGAMOSPERMY. *Agamospermy* is the formation of seeds without the union of egg and sperm nuclei. This type of apomixis may be utilized in crop species where it occurs naturally and can be genetically manipulated. Agamospermous plants can be *obligate*, i.e. reproducing only by apomixis, or *facultative*, i.e. producing some variable and some strictly maternal offspring from the same individual.

Agamospermy can occur by various processes, which can be grouped in a hierarchy according to the source of the embryo. At the top level of the hierarchy, *adventitious embryony* is the development of the embryo directly from a nucellar cell, with or without benefit of an endosperm, while *gametophytic apomixis* is the production of the embryo from the egg cell of a chromosomally unreduced embryo sac. At the second level of the hierarchy, unreduced embryo sacs can arise from the megaspore mother cell (i.e. by *diplospory*) or from a nucellar cell (i.e. by *apospory*), and the endosperm can arise autonomously (*autonomous apomixis*) or after fertilization (*pseudogamy*). All of the preceding processes can be obligate or facultative. At the third, lowest level, the various specific processes that subvert meiosis and fertilization can be recognized and regrouped in a more natural way, but because most of them produce the same totally maternal result, their differences usually do not affect plant breeding.

Different degrees (obligate vs. facultative) and types (pseudogamous vs. autonomous) of apomixis present different problems and opportunities to the plant breeder. Obligate apomixis makes the plant breed true from seed all the time and thus best stabilizes an agronomic cultivar, but it also precludes the breeding of a new cultivar unless compatible sexual germplasm can be found. Facultative apomixis permits selection and breeding of new genotypes, but these might be inadequately stable as crop cultivars. Autonomous apomicts frequently lack viable pollen and, if obligate, resist improvement by traditional breeding methods. In contrast, pollen development is normal in most pseudogamous apomicts, which thus can be used as males in conventional breeding schemes.

Why should a plant breeder even be interested in apomixis? The answer is twofold: first, apomixis allows the plant breeder to fix heterosis upon seed reproduction, and second, apomixis is not all that rare in several economically significant plants. Examples include many lawn and forage grasses, such as Kentucky bluegrass, dallisgrass, buffelgrass, Bahia grass, Guinea grass, and weeping lovegrass. Apomixis is also present in sorghum (where it is rare), mangos, and many citrus fruits (where it is very frequent). An unusually vigorous, disease-resistant or high-quality plant, if apomictic, can be cloned by seed to rapidly produce a new

cultivar. The idealists among us could imagine a big market for apomictic cultivars in third-world countries where the farmer could save seed from year to year without loss of hybrid vigor. Several important crops (Table 2.2) are related to apomictic wild species that could possibly donate apomixis genes. Finally, breeders in blackberries, mangoes, citrus fruits, guayule, bluegrasses, and several subtropical and tropical forage grasses have no choice but to deal with the apomixis naturally present in the species with which they are working.

Table 2.2.
Examples of field and horticultural crops with related apomictic species that could serve as a potential donor of agamospermy

Crop	Potential donor of agamospermy
Corn	*Tripsacura dactyloides*
Cultivated *Triticeae* (wheat, rye, barley, and wheatgrasses)	*Agropyron scabrum*
Oat	*Hierochloe odorata*
Sorghum	Facultative within S. *bicolor*
Bluegrasses	Numerous apomicts within *Poa*
Bentgrasses	*Calamagrostis purpurea, C. inexpausa*, etc.
Timothy	*Alopecurus alpinus*
Pearl millet	*Pennisetum setaceum*
Buffelgrass	Already apomictic
Guinea grass	Already apomictic
Bahia grass	Already apomictic
Bluestems	*Bothriochloa ischaemum, songarica, Dichanthium spp*., etc.
Onion, chives, garlic	*Allium odorum, A. nutans, A. tuberosum*
Rhubarb, buckwheat	*Oxyria digyna*
Beet	*Beta lomatogona, B. trigyna*
Apple, pear	*Malus hupehensis, M. sieboldii*
Strawberry	Numerous apomicts in *Potentilia* spp.
Mango	Already apomictic
Citrus	Apomixis already frequent
Sunflower	*Rudbeckia fulgida, R. triloba*
Guayule	Apomixis already frequent
Tef	*Eragrostis curvula, E. lehmaniana*, etc.

How does the plant breeder recognize apomixis? Apomixis may be suspected when all plants in the progeny look identical to the mother plant. To confirm conclusively that apomixis is present, extensive cytological tests, such as *ovule clearings* and *flow cytometry*, are required. A flow cytometer is an instrument that can measure the DNA contents of thousands of nuclei in a short period of time. Once apomixis has been confirmed, the next step is to transfer genes controlling apomixis into desirable plant types by cross-pollination. Cloning of, and *transformation* with apomictic genes might be possible in the future.

Apomixis has been shown to be under genetic control in several plant species. Generally, genetic recombination and segregation are inhibited when apomixis is present. In addition, if apomixis is unstable as is the case for facultative apomicts, the presence of maternal offspring will skew genetic segregation ratios for the progeny at large, possibly resulting in erroneous interpretations. Apomicts usually have more than the diploid number of chromosomes, and are free to have unusual patterns of relationships among chromosome sets that could not be maintained sexually; both of these characteristics also make for complex or unpredictable

segregation ratios. However, if apomixis is stable, this may be a good means of fixing desirable phenotypes.

Study Questions

1. What are the essential features of meiosis and what role do they play in crop improvement?

2. What comprises a mature female and male gametophyte?

3. How do mitosis and meiosis differ?

4. How can you determine whether a crop plant is primarily cross- or self-fertilizing?

5. How does vegetative propagation differ from apomixis?

6. Which types of apomicts would generally be most useful in a crop improvement program, facultative or obligate? Why?

7. Why is it important that a plant breeder be knowledgeable of flower structure?

Further Reading

Berrie, A. 1977. An introduction to the botany of the major crop plants. Heyden & Son, London.

Knox, R.B., and E.G. Williams. 1986. Pollen, pistil, and reproductive function in crop plants. p. 9-79. *In* J. Janick (ed.) Plant breeding reviews. AVI Publishing Co., Inc., Westport, CT.

Lersten, N.R. 1980. Reproduction and seed development. p. 17-41. *In* W.R. Fehr and H.H. Hadley (eds.) Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

McGregor, S.E. 1976. Insect pollination of cultivated crop plants. U.S. Dep. Agric. Handb. 496. Gov. Print. Office, Washington, D.C.

Purseglove, J.W. 1986. Tropical crops: Monocotyledons. John Wiley & Sons, New York.

———. 1986. Tropical crops: Dicotyledons. John Wiley & Sons, New York.

Raven, P.H., and G.B. Johnson. 1991. Understanding biology. 2d ed. Mosby Yearbook, Inc., St. Louis.

Simmonds, N.W. (ed.) 1976. Evolution of crop plants. Longman, London and New York.

3. Gene Recombination in Plant Breeding

This chapter is about gene recombinations and how they occur during plant reproduction. In the genetic improvement of field or vegetable crops, gene recombination is the mechanism most commonly utilized to create new genotypes.

Variation, the Basis of Plant Breeding

Plants differ in many ways. It may be safely generalized that no two plants are exactly alike, even though we may limit our observations to a single species like corn. Upon casual examination we may be impressed with the similarity of the plants within a field of hybrid corn (Fig. 3.1). There is a constancy of features among the corn plants: the development of the stalk; the size, shape, and arrangement of the leaves on the stalk; the termination of the stalk in a tassel; the formation of ear shoots at nodes midway of the stalk; and many other developmental features. By these characteristics we recognize the corn plant and distinguish it from a plant of wheat, or cotton, or potato.

However, if we should compare adjacent plants of hybrid corn in minute detail and make precise measurements of the separate plant parts, we would find that individual plants differ in many respects. This would be the case even though the field was planted to a single-cross hybrid as illustrated in Figure 3.1, which would be as nearly uniform genetically as we could obtain within a commercial field of hybrid corn. If we were to examine plants from a group of cross-pollinated cultivars of corn (also referred to as open-pollinated), we would expect to find even greater variations. There would be differences in maturity, height, seed-coat color, endosperm color, sugar content of the kernel, presence of plant pigments, disease pathogen resistance, and many more features. Some of the differences could be observed visually; others would require precise quantitative measurements, or even chemical analyses. A correspondingly wide range of variability may be found within other cross-pollinated species of cultivated crops.

Environmental Versus Heritable Variations

Variations among plants of a particular crop species are of two kinds:

- variations due to *environment*, and

- variations due to *heredity*.

ENVIRONMENTAL VARIATIONS are variations in the size, shape, color, composition, or development among plants responding to different intensities of an environmental stress. Environmental variations may be observed by comparing plants in a genetically uniform population. A corn plant growing in infertile soil will not grow as large and vigorous as a plant of identical heredity in fertile soil, assuming both receive the soil moisture, light, and temperature required for a favorable growth response. If a genetically uniform cultivar of soybean adapted in a latitude with a long photoperiod is grown in a latitude with a short photoperiod, flowering will occur earlier and overall growth may be unsatisfactory. Two genetically identical bean seeds, one large and one small, will produce seeding plants differing in size because the large seed has more stored nutrients to start the seedling plant. Two plants of wheat from the same pure strain will differ in development and yield if one is severely infected with a rust pathogen while the other is free from the pathogen. The variations in growth and development of genetically uniform plants described above are caused by variations in the environmental stresses to which the plants are exposed. Environmental effects on a plant are not transmitted to the plant's progeny, and thus selection in a genetically uniform population does not isolate strains that differ in response to environmental stress.

Fig. 3.1.
Plants of a corn single-cross hybrid. Plants within a single-cross hybrid have identical genotypes. Observed differences in size, shape, or form of the hybrid plants are due to environmental effects on the different plants.

HEREDITARY VARIATIONS are variations in size, shape, color, composition, or development in a genetically mixed population that result from heritable causes and are transmitted to the progeny. Hereditary variations may be simple and easily observed seed or plant characters, such as color in the flower or seed, presence or absence of pubescence on the leaf or stem, presence or absence of awns in grass species, or resistance to a disease pathogen; or the variations may be more complex characteristics, such as variations in vigor of plant growth, tillering capacity, size of plant, or days to maturity. The heritable variations are expressed again in the progenies of the different plants, although the intensity with which they are expressed will vary with the environment. A pure yellow-seeded cultivar of soybean is inherently different from a brown- or black-seeded cultivar. Barring genetic mutation or cross-fertilization with another cultivar with different seed coat color, the yellow-seed cultivar will produce only yellow-seeded progeny. 'Dwarf yellow' milo plants are shorter than 'Standard yellow' milo plants when both cultivars are grown in a similar environment favorable for milo production. If a mixed lot of wheat is grown in an environment suited to the development of stem rust and all plants become infected with the rust disease except one, we may assume that the healthy plant differs from the diseased plants by being inherently resistant or immune to infection by the stem rust pathogen, and that the diseased plants are inheritantly susceptible. The latter assumption may be verified by growing progenies of the diseased and healthy plants in an environment favorable for the development of rust and observing whether the progenies of the diseased plants become diseased and the progeny of the healthy plant remains free of disease.

The environmental and heritable variations in plants frequently interact in their effect on the plant. For example, mutant chlorophyll-deficient seedlings are observed occasionally in corn in contrast to normal green seedlings, but chlorophyll does not function to produce "normal" green corn seedlings unless the seedlings are exposed to light. A rust-resistant cultivar of wheat does not have a yield advantage over a rust-susceptible cultivar unless both are exposed to the rust pathogen and are growing in an environment favorable for development of the rust disease. Inherent differences in the cold-hardiness of winter barley strains cannot be distinguished if the winter season is so mild that hardy and nonhardy strains survive the winter alike. Selection of individual plants of beans for high seed yield may be misleading unless the plants have comparable spacings. Otherwise, the thinly spaced plants will have more plant nutrients and moisture available to them and will set more pods than the plants growing in more crowded conditions.

In the consideration of hereditary variations within a species, we are dealing with the contrasting forms of specific plant characters. The *characters*, or *traits*, in a plant develop as the result of the action of the genes in the chromosomes and the interaction of the plant with the environment during its development. The *gene* is a particular nucleotide sequence of DNA that encodes for a specific protein or protein subunit and constitutes the hereditary unit that is passed on from one generation to the next. The genes direct the course of the plant's development and, combined with the effects of the environment, determine the traits of the plant as observed or measured.

Heritable variations are essential to the plant breeder; without them there could be no permanent genetic improvements. The breeder's particular task is to identify those plant characteristics that contribute to improved yield or quality, and to assemble genes for the desirable characteristics into the cultivar. The economic improvement of agricultural plants requires consideration of a wide range of plant characters. Some characters affect morphological or structural features of the plant, such as those related to strength of the stem. Other characters are concerned with physiological processes such as transport and assimilation of

nutrients, or resistance to stress from extremes of temperature or drought. Still other characters enable the plant to withstand injury from plant disease pathogens or insect pests. The variations in some plant characters are discrete, easily identified even in variable environments, and simply inherited. These are referred to as *qualitative characters*. Other plant characters, being easily modified by the environment, are expressed in a continuous range of variations. These characters have a complex inheritance and are referred to as *quantitative characters*.

One of the problems of the breeder is to determine to what extent the observed variations in a plant character are heritable and the result of gene action, and to what extent they may be the result of favorable or unfavorable influences of the environment. This distinction is usually more difficult if the variation in the character is measured by minute quantitative units, which are affected to a greater extent by environmental stresses than are the simple and qualitatively measured characters of a plant. *Seed color* is an example of a simply inherited qualitative character, whereas plant *yield* is a character with complex inheritance and is expressed in quantitative units, kilos/hectare for example. The comparative yielding ability of two cultivars is evaluated by growing the two cultivars in similar environments and measuring the production of each. For this purpose *progeny tests are* commonly conducted by plant breeders to provide further evidence on the breeding behavior of particular plants or cultivars.

How Heritable Variations Originate in Nature

Heritable variations in plants originate in nature from

• *gene recombinations*,

• *variations in chromosome number, and*

• *mutations*.

Plant species have evolved in nature and reached their present stage of development through these processes. If breeders cannot isolate the plant types they desire by selection from genetically mixed natural populations of a plant species, they may employ the above forces to create new populations from which to select improved cultivars. In this textbook we are primarily concerned with how the above forces are utilized by plant breeders, to obtain genetic improvement in crop plants. This requires an understanding of the basic mechanism of Mendelian heredity and the principles upon which it operates. A comprehensive review of the genetic material (DNA) and its operative mechanisms is beyond the scope of this text, but it may be found in many excellent textbooks in biology and genetics.

The Mechanism of Mendelian Heredity

The mechanism of Mendelian heredity is dependent upon the behavior of chromosomes and the genes they carry. The biological mechanisms by which chromosomes and genes are transmitted from parent to progeny are summarized in the following:

(**1**). A mixed population of a plant species will exhibit many heritable variations. From this assortment the breeder selects plants with *traits* or *characters* important for the development of an improved cultivar. Examples of such traits are seed size and color, plant height, earliness of flowering and maturity, disease or insect resistance, chemical composition of the seed or plant part, and many other. Heritable variations may be identified when different plants of the same species growing in uniform environments exhibit contrasting forms of specific traits or

characters. The contrasting forms of the traits are controlled by alternative (contrasting) *alleles* at the same locus in *homologous chromosomes* and the interaction of the trait with the environment during growth and development of the plant. Plant breeders normally focus on traits or characters with economic importance in the crop species, and on the breeding methods by which they can combine genes for desirable traits into a superior plant cultivar. This is in contrast to the geneticist, whose primary focus is on the chemical nature of the genetic material (DNA) and the pathways by which genes function in the development of plant characters.

(**2**). The genes are complex functional or operational units of DNA located in the *chromosomes*. In the classical conception they determine the pathways by which the traits or characters of a plant are formed. Biochemically, the gene is a complex unit of *deoxyribose nucleic acid* (DNA) encoded to direct the synthesis of a specific enzyme that functions in character formation. The influence of each gene may be exerted individually or in combination with other genes and in conjunction with the environment. The action of a gene is specific for the character or characters that it influences. Traditionally, each gene occupies a particular position, or *locus*, in a specific chromosome and is replicated when the chromosome divides. The contrasting forms, called *alleles* encode for contrasting forms of the character.

A gene that expresses itself to the exclusion of its allele is referred to as *dominant. The* contrasting form of a gene, which is not expressed in the presence of the dominant, is referred to as the *recessive*. Genes are commonly represented by a letter or combination of letters, a capital for the dominant allele (*A*) and a lowercase letter for the recessive allele (*a*). The breeding behavior of a plant is determined by the particular combination of alleles for the different genes that it possesses. A plant with like alleles at a given locus in *homologous chromosomes* (chromosomes that pair and form bivalents at meiosis) is *homozygous (AA* or *aa*) for the gene concerned; a plant with unlike alleles at a given locus is *heterozygous (Aa*) for that gene. The exact composite of genes in a plant determines its *genotype*. The visible appearance of the plant, i.e., whether it exhibits the dominant (*A*) or the recessive (*a*) form of the trait for particular characters, determines the *phenotype* of the plant. In some instances the heterozygotes (*Aa*) may be intermediate to the homozygotes (*AA* or *aa*), a condition known as *partial dominance*. For some characters there may be more than two forms of the gene. These are designated *multiple alleles* (A^a, A^b, A^c, ..., A^n). Not more than two contrasting alleles will be present in a diploid cell, one allele at each of corresponding loci in the homologous chromosomes, or the two alleles may be identical. Gene structure and function may change in nature so that the affected trait exhibits a different form, the new form being reproduced in succeeding generations. Such a change in a gene is known as a *mutation*.

(**3**). The *chromosomes are* rod-shaped structures in the cell nucleus, and with proper staining may be observed at the time of cell division. The chromosome functions in storage of genetic information. Structurally, the chromosome consists of two identical chromatids joined at the centromere, each chromatid being a DNA double helix and the site of the genes. The distribution of the chromosomes and the alleles for particular genes they carry in the reproductive cells (gametes) determines the specific distribution of the alleles to the progeny. Chromosomes normally exist singly in haploid spores and gametes; in pairs in the diploid somatic cells, mother cells, and the fertilized egg; and in triplicate in the triploid endosperm cells. The haploid-diploid chromosome number is constant for any plant species.

The trait or character with the simplest inheritance is one that develops under the control of alleles for a single gene. However, many characters of agronomic importance with which the plant breeder works, such as size, yielding ability, winter hardiness, lodging resistance, or quality, are each influenced by numerous genes with small, cumulative effects. These genes

are designated *multiple genes* (also *polygenes*) and may be scattered about in several chromosomes. The inheritance of multiple genes is referred to as *quantitative inheritance* because their effect on the expression of the affected character may be measured in quantitative units. This is in contrast to the inheritance of characters controlled by single genes which is referred to as *qualitative inheritance*.

Inheritance of Simple Characters

The mechanism of heredity may be illustrated most simply by cross-pollinating plants that possess contrasting alleles that encode for a single trait and examining the ratios of plants with contrasting forms of the trait in subsequent progenies. A suitable example illustrated here is a cross-pollination between a hooded and an awned cultivar of barley (Fig. 3.2).

Because barley is normally self-pollinated, pollen from one parent must be manually transported from the anthers of one plant to the stigmas of the other parent to obtain cross-pollination. In the parlance of the plant breeder this is making a *cross* and is the procedure the breeder uses to obtain gene recombination between plants. In barley, the presence of hoods is dominant to the presence of awns. During meiosis in the parent plants the homologous chromosomes carrying the allelic genes (designated *KK* in the hooded plant and *kk* in the awned plant) separate, and each germ cell (spore, and egg or sperm) contains only one allele for this character (Fig. 3.3). The egg and the sperm fuse with fertilization and the homologous chromosomes, one containing a dominant allele (K) from the hooded parent and its homolog

Fig. 3.2.
Spikes of barley differing in alternative forms of two simple inherited characters: lemma appendage (hoods and awns) and number of rows of seed. (A) Hooded, six-rowed. (B) Awned, six-rowed. (C) Awned, two-rowed.

containing the recessive allele (k) from the awned parent, are brought together again within the fertilized egg and subsequently in the embryo of the developing seed. The plant that develops from the seed will be heterozygous (Kk) and will exhibit the dominant hooded trait. The heterozygous plants arising from the cross constitute the *first filial generation and are* designated by the symbol $\mathbf{F_1}$. Succeeding generations arising by successive self-fertilizations are designated $\mathbf{F_2}$, $\mathbf{F_3}$, etc. The $\mathbf{F_1}$ plant is a *hybrid* and originated from a cross between parents that are genetically different. Parents in the cross may be designated by the symbols $\mathbf{P_1}$ and $\mathbf{P_2}$. Reciprocal crosses ($\mathbf{P_1} \times \mathbf{P_2}$ or $\mathbf{P_2} \times \mathbf{P_1}$) produce similar offspring as the same gametes are brought together to produce the hybrid $\mathbf{F_1}$ plant regardless of which parent in the cross is used as the pollinator (male parent) and which as the pollinated (female parent).

The $\mathbf{F_2}$ generation is obtained by self-pollination of the $\mathbf{F_1}$ hybrid plant. As barley is self-pollinated, seeds from $\mathbf{F_1}$ plants may be harvested following normal pollination, although protection against natural cross-pollination may be desirable. In the self-pollinated $\mathbf{F_1}$ plant (Fig. 3.3), the reduction of each mother cell results in the production of a tetrad of four spores (Fig. 2.6), two containing dominant and two containing recessive genes for the hooded-awned trait. Eggs and sperms are formed from the spores after successive mitotic divisions. One-half of the eggs and one-half of the sperms will each contain a dominant gene for hoods; the other half of the eggs and the sperms will each contain a recessive gene for awns. Chance fusion of the sperms and eggs at fertilization will bring together dominant and recessive genes in such proportions that $\mathbf{F_2}$ plants will occur in approximate ratios of 3 hooded: 1 awned (Fig. 3.3). This is a *phenotypic ratio* and is identified by the physical appearance of the $\mathbf{F_2}$ plants, in this example whether they have hoods or awns. The approximate ratio in which the different genotypes occur is the *genotypic ratio*. This will be 1*KK:2Kk*:1*kk*. The $\mathbf{F_2}$ plants homozygous for hoods (*KK*) will produce $\mathbf{F_3}$ progenies with hooded plants only. The $\mathbf{F_2}$ plants heterozygous for hoods (*Kk*) have the same genotype as the hybrid F_1 plants and in the F_3 will produce progenies with phenotypic ratios of 3 hooded:1 awned, or genotypic ratios of 1*KK*:2*Kk*:1*kk*. The F_2 plants homozygous for awns (*kk*) will produce F_3 progenies with awned plants only. These are typical F_2 phenotypic and genotypic ratios that may be expected if cultivars are crossed that differ in alternative forms of a trait determined by a single gene. A cross involving only a single pair of allelic genes is called a *monohybrid cross*.

The Progeny Test

The breeding behavior of an individual plant is learned from growing and observing the characteristics of its progeny. By this procedure one can learn whether the alleles for observable characters in a plant are homozygous or heterozygous. In the cross, hooded × awned barley, three out of each four F_2 plants were hooded. To determine which hooded F_2 plant is homozygous (*KK*) and which is heterozygous (*Kk*) the seeds from each hooded F_2 plant are harvested separately and planted in a *progeny test*. In the F_3, progenies of the homozygous F_2 plants will contain only hooded plants, but progenies of heterozygous plants will be segregating for hoods and awns. By making the progeny test, the genotype of specific F_2 hooded plants can be identified.

Progeny testing is a basic procedure in plant breeding. Superior plants are selected from genetically mixed populations based on their appearance or phenotype. The plant breeder may select a shorter plant, a plant with more vigor, or an outstanding plant that survived a severe winter or a heavy disease epidemic. From the progeny test the breeder learns whether the phenotypic differences observed in selected plants are genetic and inherited, or the result of

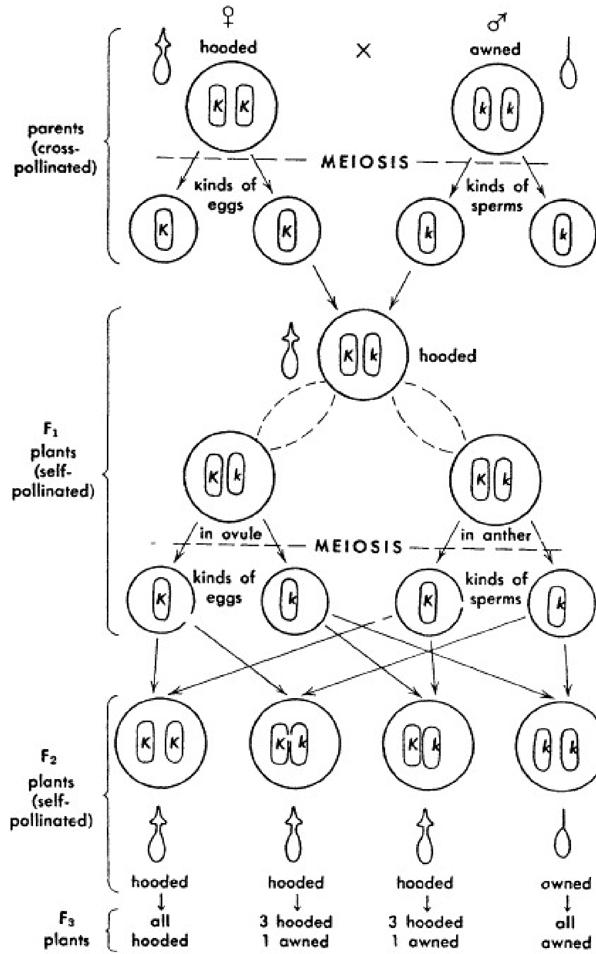


Fig. 3.3.
Distribution of the chromosomes carrying genes for hoods (KK) and awns (kk) in a monohybrid cross in barley. Because hoods are dominant over awns, all heterozygous F_1 plants are hooded. In the F_2, a phenotypic ratio of 3 hooded:l awned and a genotypic ratio of 1KK:2Kk:1kk are expected. Note that the female or seed parent (indicated by symbol ♀) is on the left and the male or pollen parent (indicated by symbol ♂) is on the right, a convention commonly used in writing pedigrees of plant hybrids.

variations in the environment. If the differences are genetic, the breeder may learn whether the selected plant is homozygous and will breed true for the qualitative trait being observed, or whether it is heterozygous and further selection and progeny testing will be needed to identify homozygous plants.

The Testcross

The *testcross* is another procedure for identifying the genotype of a plant. In the testcross the plant in question is crossed with a plant homozygous and recessive for the trait being observed. In the F_2 above, hooded plants were either homozygous (*KK*) or heterozygous (*Kk*). If an F_2 homozygous plant (*KK*) is testcrossed with a recessive awned plant (*kk*), the cross ($KK \times kk$) would produce all hooded offspring (*Kk*) as in the cross between the original hooded and awned parent plants. If an F_2 hooded plant is heterozygous (*Kk*), the cross ($Kk \times kk$) would result in one-half of the offspring plants being hooded (*Kk*) and one-half being awned (*kk*) (Fig. 3.4). In a crop like barley that is self-pollinated, it is easier to harvest F_2 seeds and grow a progeny test of each F_2 plant than to make the testcross and to grow the testcross progeny. If the crop is cross-pollinated, pollination must be controlled to prevent outcrossing before growing a progeny test so it may be as easy to make a testcross as to make a progeny test. In crops that set seed poorly after self-pollination, the testcross may be preferred over the progeny test. In self-incompatible or dioecious crops, in which self-pollination is not possible, the testcross provides the only means of identifying the genotype of particular plants.

The testcross is useful in the study of *linkage*, i.e., the association of genes on the same

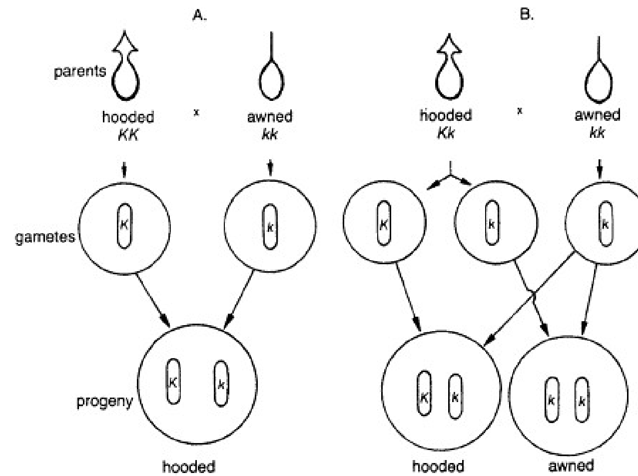


Fig. 3.4.
Segregation in testcrosses. (A) Homozygous dominant (KK), testcrossed to recessive (kk). (B) Heterozygous (Kk), testcrossed to recessive (kk).

chromosome. By testcrossing the F_1 plant to a plant homozygous recessive for the linked genes, the genotype of the F_1 gametes and their proportions may be determined by examination of the testcross progeny.

The Backcross

The *backcross* is a cross of a hybrid to one of its parents. The backcross differs from the testcross in that a testcross is made only to a homozygous recessive parent for the purpose of identifying the genotype. The backcross may be made to either parent. The plant breeder makes a succession of backcrosses to add a gene for a desirable character to an otherwise desirable parent, or the backcross may be made to concentrate genes for a quantitative character. The backcross as a breeding procedure will be discussed in the chapter on breeding self-pollinated crops.

Gene Recombination Following Hybridization

Genes that Assort Independently

The cross between the hooded and awned cultivars of barley used in the preceding illustration was greatly simplified when it was implied that these two cultivars of barley differ only in the alleles for the hooded-awned trait. Actually, barley cultivars may differ in maturity, winter hardiness, disease resistance, height, or many other respects, according to the alleles present for the respective traits. In plant breeding, the usual objective of crossing cultivars is to combine a combination of genes for desirable traits from the parent cultivars into a single genotype. The recombination of genes in this manner may be illustrated with a simple cross involving two pairs of allelic genes positioned in different chromosomes. Because two pairs of allelic genes only are considered, it is called a *dihybrid* cross.

'Oderbrucker' was at one time a leading cultivar of barley in the north central United States, but it possessed an undesirable trait—barbed or rough awns. The 'Lion' cultivar of barley had smooth or barbless awns, but it also had black hulls, which are unattractive and undesirable in barley. From a cross between these two cultivars, a cultivar was developed in which were combined the traits white hulls and smooth awns. Each trait—hull color and barbing of awns—is *monogenic* (determined by one pair of allelic genes). The genes for the two characters are located in different pairs of chromosomes that assort independently at meiosis.

In a dihybrid cross, the distribution of specific genes to the progeny plants will be determined by the distribution of the particular chromosomes that carry the genes. In the parent plants, the homologous chromosomes separate during meiosis; and, following a succession of nuclear divisions, one chromosome from each pair is randomly distributed into each egg or sperm. In the cross outlined here, the parent plants are homozygous for the traits, 'black, smooth awn'(*BBrr*), or 'white, rough awn' (*bbRR*). All of the gametes from the parent plants will carry the genes *B* and *r*, or *b* and *R*. Combining these gametes produces heterozygous $\mathbf{F_1}$ plants (*BbRr*) that will be 'black, rough-awned' in appearance (Fig. 3.5).

During meiosis the chromosome pairs separate, one chromosome from each pair moving to one pole, and the homologue moving to the opposite pole. The specific chromosome of any pair that enters a particular gamete is a matter of chance. Because the $\mathbf{F_1}$ plant is heterozygous

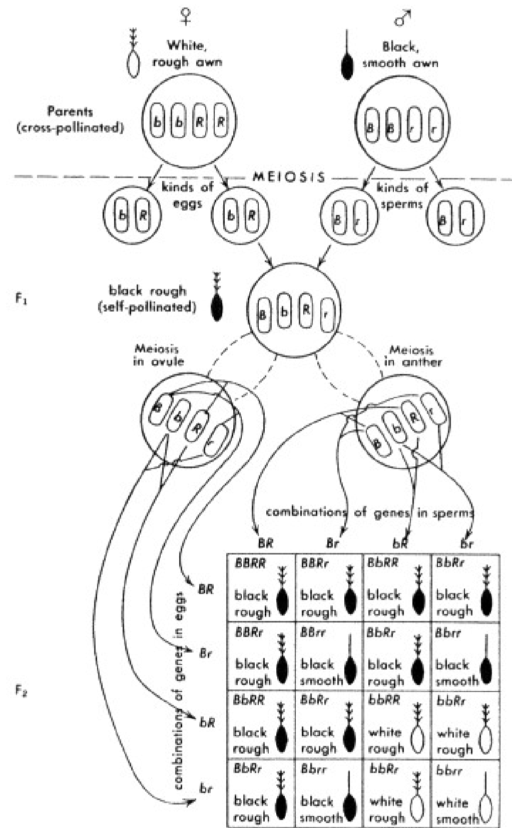


Fig. 3.5.
Distribution to progeny of chromosomes carrying genes for hull color and barbing of awns in a dihybrid cross in barley. The hybrid F_1 plants have one set of chromosomes from each parent. Chromosome assortment to eggs and sperms is shown by the arrows. The possible combinations of eggs and sperms are shown in the checkerboard. The expected F_2 phenotypic ratios from this cross are 9 black-rough:3 black-smooth:3 white-rough:1 white-smooth.

for two pairs of genes (*BbRr*), gametes (eggs and sperms) will be formed with four possible combinations of genes in equal proportions (*BR, Br, bR, br). The* chance recombination of the four kinds of eggs with the four kinds of sperms would produce the genotypes shown by the checkerboard in Fig. 3.5. Combining identical genotypes from the checkerboard, the predicted progeny in the F_2 will be:

Genotypes	Phenotypes
1 *BBRR*	
2 *BBRr*	9 *BR* black, rough awns
1 *BBrr*	
2 *BbRR*	3 *Br* black, smooth awns
4 *BbRr*	
2 *Bbrr*	3 *bR* white, rough awns
1 *bbRR*	
2 *bbRr*	1 *br* white, smooth awn
1 *bbrr*	

Because the combinations of gametes uniting at fertilization are a matter of chance the precise ratios of genotypes shown above may not be obtained. However, due to chance, one out of each 16 plants in the F_2 generation is expected to be 'white, smooth awn', the combination of traits desired in this particular cross. The 'black, rough awn' and the 'white, smooth awn' plants differ from either parent; thus they represent new genotypes arising from a *recombination of genes* for hull color and barbing of awns.

Predicting Genetic Ratios

A checkerboard was constructed to show the genetic combinations arising from the dihybrid cross, 'white, rough awn' × 'black, smooth awn' barley (Fig. 3.5). The checkerboard becomes unwieldy in crosses involving several independently inherited pairs of genes. A simpler procedure is to determine the ratios of each gene separately and then to combine the ratios. For example, a single gene pair has six possible kinds of matings as follows:

Mating	Genotypes	Phenotypes
AA × *AA*	all *AA*	all *A*
AA × *Aa*	1*AA*:1*Aa*	all *A*
AA × *aa*	all *Aa*	all *A*
Aa × *Aa*	1*AA*:2*Aa*:1*aa*	3*A*:1*a*
Aa × *aa*	1*Aa*:1*aa*	1*A*:1*a*
aa × *aa*	all *aa*	all *a*

To show how this system works let us look at the F_2 in the dihybrid cross considered earlier. The genotype of the F_1 was *BbRr*. With self-fertilization of the F_1, the genotypes in the F_2 result from the combined matings of two independent, heterozygous gene pairs (*Bb* × *Bb* and *Rr* × *Rr*). The ratios of genotypes and phenotypes obtained from the matings of each pair separately and combined are shown in Table 3.1. If a cross is segregating for a third pair of genes, the ratios obtained for the third pair are added to all combinations of the first and

second pair in a similar manner. Predictions are only expected ratios based on chance combinations of the gametes. Actual ratios are obtained by making the cross and counting the plants of different kinds in the progeny.

Table 3.1.
Ratios of genotypes and phenotypes in the F_2 generation of the cross 'white, rough awn' × 'black, smooth awn'

Ratios of each character, considered separately		Genotypes when ratios are combined	Phenotypes
1BB	1RR	1BBRR	9BR 'black, rough awn'
	2Rr	2BBRr	9BR 'black, rough awn'
	1rr	1BBrr	3Br 'black, smooth awn'
2Bb	1RR	2BbRR	9BR 'black, rough awn'
	2Rr	4BbRr	9BR 'black, rough awn'
	1rr	2Bbrr	3Br 'black, smooth awn'
1bb	1RR	1bbRR	3bR 'white, rough awn'
	2Rr	2bbRr	3bR 'white, rough awn'
	1rr	1bbrr	1br 'white, smooth awn'

Restrictions with Independent Assortment

By the hybridization procedure, genes for desirable characteristics of parent varieties can be combined into new plant types not present in nature, and thereby increase the heritable variations within that crop. There are, however, restrictions to the recombinations that a breeder can obtain by segregation and independent assortment. For example:

(**1**). Recombinations of genes in separate chromosomes result from the *segregation and recombination* of the chromosomes in which they are carried. Two or more genes in the same chromosomes do not normally assort independently; their distribution to the gametes is influenced by their linkage relations, a topic to be discussed in a later section of this chapter.

(**2**). In a monohybrid cross, a minimum of four F_2 plants is required to recover a proportional representation of all recombinations, but in a dihybrid cross with independent assortment of chromosomes, 16 F_2 plants would be required. As the number of independently assorting genes increases, the minimum population that would permit a proportional representation of all recombinations increases exponentially (Table 3.2). The necessity for growing large F_2 populations if the breeder expects to find plants with desirable combinations of genes in the progeny of a cross from genetically diverse parents becomes apparent. Actually, the possibilities are much better that the desired genotype may be found in later generations, because it may arise by segregation from heterozygous F_2 plants.

Gene Interactions

We have seen that a dominant gene can mask the effect of its recessive allele. This is an example of an interaction between alleles of the same gene. In many instances basic genetic

Table 3.2.
Minimum population to obtain proportional representation of all recombinations with various numbers of independently as-sorting genes

Number of gene pairs assorting	Minimum population required
1	4
2	16
3	64
4	256
5	1,024
10	1,048,576
20	1,099,511,627,776

ratios are modified by interactions between nonallelic genes affecting the same phenotypic trait, a phenomenon known as *epistasis*. Examples of some epistatic gene interactions are listed below:

COMPLEMENTARY ACTION. Two nonallelic genes may be required to produce a single effect. An example is found in oat where two dominant genes A and B are required to render the cultivar resistant to certain races of crown rust. For example, *AB* = resistant; *Ab, aB, ab* = susceptible.

MODIFYING ACTION. One gene produces an effect only in the presence of a second gene at another locus. In corn, a dominant gene *Pr* produces purple aleurone color in the presence of dominant R, but expresses no effect in the absence of *R*. For example, *PrR* = purple aleurone; *prR* = red aleurone; *Prr, prr* = colorless aleurone.

INHIBITING ACTION. One gene may act as an inhibitor of the expression of another gene. The dominant gene *R* for red color in corn does not produce an effect in the presence of a dominant "inhibitor" gene *I*. For example, *Ri* = red; *RI*, *rI, ri* = white.

MASKING ACTION. One gene may hide the effect of a second gene when both are present. In oat, a dominant gene *Y* produces yellow seed coat color, and a dominant gene *B* produces black seed coat color. The gene *Y* will have no visible effect in the presence of B because the black seed coat color will mask the yellow color. For example, *BY*, By = black; *bY* = yellow; by = white.

DUPLICATE ACTION. Either of two genes may produce a similar effect; or the same effect is produced by both of them together. The common shepherd's-purse has a triangular-shaped seed capsule that is produced by either of the dominant genes C or D, or by both together, *CD*. With both recessives the seed capsule has an ovoid shape. For example, *Cd, cD, CD* = triangular shape seed capsule; *cd* = ovoid shape seed capsule.

ADDITIVE EFFECT. Two genes may produce the same effect, but the effects are additive if both genes are present. An example has been reported in barley. Either *A* or *B* will

produce medium-length awns, while the two dominant genes together produce long awns. The recessive genes produce awnless plants. For example, *Ab, aB* = medium-length awns; *AB* = long awns; *ab* = awnless.

PLEIOTROPIC GENES. A single gene may have more than one effect, simultaneously influencing size, shape, color, or function of several organs. *Pleiotropic genes are* genes controlling the expression of more than one trait. The gene may have only one function such as the production of a specific enzyme which in turn affects the expression of several traits in the plant. The "uzu" gene in barley in the recessive condition may shorten stem and rachis internodes, reduce seed size, and produce an erect coleoptile leaf. For example, *Uz* = normal in appearance; *uz* = semi-dwarf, dense spike, short awns, small seeds, short erect flag leaf.

Recombinations of Linked Genes

Large numbers of genes have been identified in corn, barley, wheat, and other crop species. Each chromosome is an aggregate of many genes, which tend to be inherited as a group when the chromosomes are distributed to the gametes. The tendency for genes to be inherited in groups is known as *genetic linkage*, and the collection of genes within a single chromosome is a *genetic linkage group*. The number of genetic linkage groups in any species is equal to the haploid chromosome number. If the genes in a chromosome were so completely linked that they would not separate, there could be no recombinations between genes within the same linkage group. This would impose severe restrictions on breeders, for they could not then obtain new genotypes from recombinations of linked genes. Fortunately this condition does not exist. Recombinations of linked genes occur as a result of a process known as *crossing-over*, in which segments of chromatids of homologous chromosomes are exchanged as they synapse during meiosis.

To illustrate how recombinations between linked genes may be obtained, let us consider a cross between two barley strains that differ in the number of rows of seed on the spike and in lemma color. In barley, the two-row trait (Fig. 3.2C) is dominant to six-row (Fig. 3.2A and B), and purple lemma color is dominant to white. The genes for these two characters are located in barley linkage group I, with a recombination value of 19.4%.

In a cross of the homozygous barley strains, 'two-row, purple' (*VVPP*) x 'six-row, white' (*vvpp*), the heterozygous F_1 plants will exhibit the dominant characteristics, 'two-row, purple' (*VvPp*) (Fig. 3.6). If the heterozygous F_1 plant is next testcrossed to a recessive 'six-row, white' plant, testcross progenies are expected in proportions shown in Table 3.3.

The heterozygous F_1 plant received the dominant linked genes (*VP*) in the gamete from the female parent and the recessive alleles (*vp*) in the gamete from the male parent (Fig. 3.6). The paired chromosomes carrying these genes separate at meiosis in the F_1 plant and subsequently enter separate gametes.

In 80.6% of the F_1 gametes, the linked genes are in the same combination as they were received from the original parent plant (40.3% *VP* and 40.3% *vp*). In 19.4% of the gametes, the linked genes are in new combinations (9.7% *Vp* and 9.7 % *vP*). The recombinations of the linked genes occur as a result of the exchange of chromatid segments (crossover) of the homologous chromosomes carrying the genes (Fig. 3.6). The *crossover value* is equal to the percent of recombinations, or 19.4% for the two linked genes considered here. This value is specific for these two linked genes and was determined by making the cross numerous times and averaging the percent of recombinations obtained in the progenies. Other combinations of

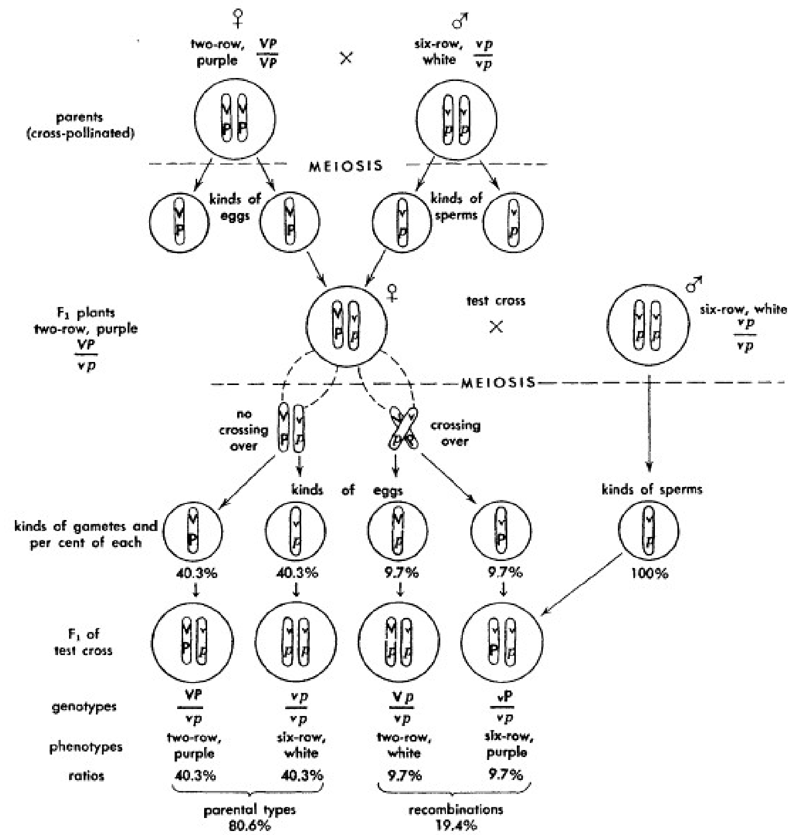


Fig. 3.6.

The distribution of linked genes in a cross. In this cross, a pure strain of barley containing dominant linked genes for two-row and purple-seed coat characters is crossed with a pure strain containing the contrasting recessive alleles (six-row and white-seed coat). The heterozygous F_1 plant is testcrossed to the pure recessive. Four types of gametes are formed in the hybrid plant. Two types of gametes have recombinations of the linked genes (Vp and vP), which originated as a result of an exchange of chromosome segments of homologous chromosomes during meiosis. The exchange of chromosome segments, a process known as crossing over, is the means by which recombinations of linked genes occur.

Table 3.3.
Progeny of heterozygous 'two-row, purple' F_1 plant, from the cross 'two-row, purple' × 'six-row, white,' test-crossed to the recessive 'six-row, white' parent

Genotypes[a]	Phenotypes	Percentage in each class	
$\frac{VP}{vp}$	'Two-row, purple'	40.3	Look like parents
$\frac{vp}{vp}$	'Six-row, white'	40.3	
$\frac{Vp}{vp}$	'Two-row, white'	9.7	Recombinations
$\frac{vP}{vp}$	'Six-row, purple'	9.7	

[a]In this fractional format, the genes above the rule are linked on one chromosome, and the genes below the rule are linked on the homologous chromosome.

linked genes will have different crossover values. The greater the distance between the linked genes, the more frequently will crossovers occur, and the higher will be the crossover value. From crossover percentages, *genetic linkage maps*, which show the relative position of genes in the chromosomes, have been constructed for many crop species.

In the above example the heterozygous F_1 plant received linked dominant alleles from one parent and linked recessive alleles from the other parent. This arrangement of linked genes in the bivalent is referred to as *coupling (cis arrangement*). The condition where the heterozygous F_1 receives a recessive allele and a dominant allele of a linked pair from one parent and the reverse combination from the other parent is referred to as *repulsion (trans arrangement*).

The testcross to a recessive plant was made in the example cited (Fig. 3.6) to identify the proportion of crossovers. In the progeny of the testcross, the genotype of each plant can be identified by the phenotype. Because the breeder generally works with F_2 populations rather than testcross populations, it is of interest to examine the effect of linkage on the F_2 hybrid ratios. Let us consider then what the progeny would be if the F_1 plant in the above cross is self-pollinated to produce an F_2 generation instead of testcrossed to the recessive parent.

In the F_1 plant, four types of gametes, both eggs and sperms, are produced (Fig. 3.6). The gametes and their proportions are *VP*, 40.3%; *vp*, 40.3%; *Vp*, 9.7%; and *vP*, 9.7%. The genotypes in the F_2 progeny and the proportions of each obtained, when all combinations of eggs and sperms are combined, are indicated by the checkerboard in Fig. 3.7. The phenotypes in the progeny and the proportions of each are as follows:

two-row, purple (*VP*) = 66.24%
two-row, white (*Vp*) = 8.76%
two-row, purple (*vP*) = 8.76%
two-row, white (*vp*) = 16.24%

These data show that the parental types *VP and vp* occur with greater frequency than the

Eggs \ Sperms	40.3% VP	40.3% vp	9.7% Vp	9.7% vP
40.3% VP	16.24% VP/VP	16.24% vp/VP	3.91% Vp/VP	3.91% vP/VP
40.3% vp	16.24% VP/vp	16.24% vp/vp	3.91% Vp/vp	3.91% vP/vp
9.7% Vp	3.91% VP/Vp	3.91% vp/Vp	0.94% Vp/Vp	0.94% vP/Vp
9.7% vP	3.91% VP/vP	3.91% vp/vP	0.94% Vp/vP	0.94% vP/vP

Fig. 3.7.
Checkerboard showing expected percentages of F_2 genotypes from cross 'two-row purple' x 'six-row white,' in which genes for row number and hull color are linked with 19.4% crossing over.

recombinations *Vp and vP*, which is a characteristic of linked genes. Without linkage, a dihybrid ratio of *9VP:3Vp:3vP:1*vp would have been obtained (Fig. 3.5).

The principles illustrated by this example of genetic linkage have practical significance to the breeder in the following ways:

(1). The synapse of homologous chromosomes during meiosis, accompanied by a crossing-over and exchange of chromatid segments, provide the mechanism for the recombination of linked genes. The percent of recombinations is fairly constant for any two linked genes.

(2). The exchange of chromosome segments in a *single crossover* occurs only between two of the four strands of the paired chromosomes (Fig. 3.8). The other two strands move intact into the daughter nuclei. As a result the *recombination* or *crossover value* will never exceed 50%.

(3). The proportion of recombinations of two closely linked genes will be smaller than the proportion of recombinations of two genes that assort independently. It will be necessary to grow larger F_2 progenies of crosses involving linked genes than progenies of crosses involving genes that assort independently to obtain similar numbers of recombinations. The smaller the crossover value, the larger the F_2 population that will be needed.

(4). Linkage may be an aid to selection if the desired gene is closely linked with a gene for a character that may be positively identified by visual observation; but may be a handicap if the desired genes are linked with genes for undesirable characters.

(5). The number of recombinations of linked genes observed in a cross is usually a small fraction of those possible. Because the recombinations normally occur prior to selfing, several generations of intercrossing among plants within F_1 or F_2 progenies prior to selfing would facilitate the recovery of a greater proportion of the possible recombinants. This procedure is not normally practiced in self-pollinated crops due to the labor involved in making the crosses, but crossing may be facilitated in some species by utilization of male-sterile genes.

(6). Information on *crossover values* facilitates the genetic mapping of genes in specific chromosomes.

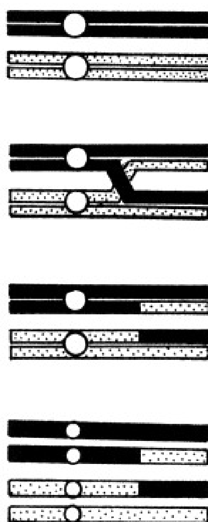


Fig. 3.8.
A single crossover during meiosis
between two of the four strands
of homologous chromosomes.

(7). Individual genes associated with quantitatively inherited characteristics normally produce such small effects that the position of the genes on the chromosome, or even their association with a particular chromosome, cannot be positively identified.

Gene Structure and Action

Thus far we have referred to the gene in the classical sense, as a unit in the structure of the chromosome controlling the inheritance of a particular trait (or group of traits), and it is in this sense that we use the term gene in this textbook. With present knowledge of molecular genetics, information about the structure of the gene and its mode of action has been greatly expanded and clarified. Comprehensive discussions may be found in many excellent textbooks in biology and genetics.

Current knowledge verifies that the genetic information in higher organisms is contained in *deoxyribonucleic acid (DNA*), a compound of high molecular weight and composed of nitrogen bases (*purines and pyrimidines), deoxyribose, and phosphate groups*. The purines include *adenine* (A) and *guanine* (G), while the pyrimidines include *cytosine* (C) and *thyrnine* (T). When a nitrogen base is bound to a deoxyribose sugar and a phosphate group, the resulting molecular structure is called a *nucleotide*. The nucleotides are linked together to form *a polynucleotide chain* in which the sugar of one nucleotide is bound to the phosphate group of another and so on. According to the Watson-Crick model of the structure of DNA, it is made up of a pair of helically coiled chains of polynucleotides with the base pairs connected by hydrogen bonds (Fig. 3.9). The pairing bases are always specific; for example, adenine

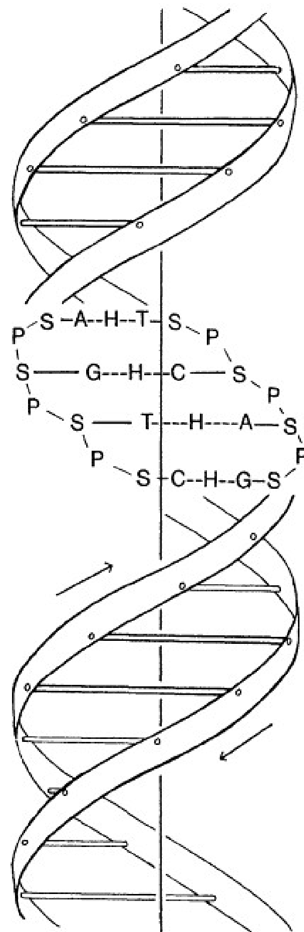


Fig. 3.9.
Structural diagram of a portion of DNA showing the sugar-phosphate linkage between nucleotides and the specific pairing of the nitrogen bases—adenine with thymine and guanine with cytosine—connected by hydrogen bonds.

always pairs with thymine while cytosine pairs with guanine only. A chromosome contains a single molecule of DNA, the molecule extending the entire length of the chromosome.

The DNA molecule replicates prior to cell division, and copies are distributed to daughter nuclei during the process of cell division. Replication of the DNA is *semiconservative*, that is, the strands of the DNA double helix unwind, and each serves as a template for synthesis of a

new complementary strand. A pair of double helixes is formed, each identical to the original and each containing one of the original strands and one new strand. An amino acid is synthesized by bringing together a linear sequence of nucleic acid bases. In turn, a protein is synthesized by bringing together a linear sequence of amino acids. The order of the nucleic acid bases in the DNA strand determines the sequence in which they are lined up in the synthesis of the amino acids and subsequently the protein.

We should now look again at our understanding of the gene. Current perception of the gene restricts its definition to function. The gene is a sector of DNA containing several hundred base pairs. The gene determines the amino acid sequence of a polypeptide, which in turn characterizes the function of a particular protein. The protein may be a structural unit or it may be an enzyme that catalyzes the initiation of some biological activity. A mistake in the process of translation or transcription that changes the grouping of the nucleic acid bases will change the function of the gene and produce a mutation.

Molecular Genetics and Plant Breeding

Plant breeding procedures were developed from Mendelian genetic principles. In contrast to earlier genetic research, which focused on an extension of Mendelian genetic principles, current genetic research is concentrated on the biochemical and molecular aspects of the genetic process. Molecular genetics provides explanations on the molecular level and a more comprehensive understanding of the genetic processes.

Through classical procedures, the plant breeder manipulates alleles so as to obtain recombinations of genes for useful plant characters. This is accomplished largely through hybridization, utilizing normal plant reproductive pathways. Currently, the molecular geneticist is perfecting techniques by which segments of DNA encoded for a novel characteristic may be cloned and integrated into the DNA of a recipient plant. The process, known as *transformation*, bypasses the normal reproductive pathway. This new information and the new molecular techniques bring powerful new tools to the aid of the plant breeder. *Present evidence suggests that these molecular genetic techniques will supplement but will not replace traditional plant breeding procedures. Overall, they do not affect the validity of the Mendelian principles upon which plant breeding is based, nor do they change basic plant breeding procedures*.

Study Questions

1. What two procedures are used to detect linkage? Which do you prefer and why?

2. Assume that two genes A and B are in the repulsion linkage phase. If there is complete linkage, what would be the F_2 phenotypic ratio? What would the F_2 phenotypic ratio be if the genes A and B were in the coupling linkage phase?

3. How can a plant breeder increase genetic variability in crop plants?

4. What does it mean when genes assort independently?

5. What are some of the gene interactions that you are familiar with? Describe these.

6. What is a reciprocal cross? Why might a plant breeder be interested in making a reciprocal cross?

Further Reading

Forbes, J.C., and R.D. Watson. 1992. Plant variation, inheritance and breeding. p. 208-40. *In* J.C. Forbes and R.D. Watson. Plants in agriculture. Cambridge University Press, Cambridge, England.

Gardner, E.J., and D.P. Snustad. 1991. Principles of genetics, 8th ed. John Wiley & Sons, New York.

Morris, R. 1983. Remodeling crop chromosomes. p. 109-29. *In* D.R. Wood (ed.) Crop breeding. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Raven, P.H., and G.B. Johnson. 1991. Understanding biology. 2d ed. Mosby Yearbook, Inc., St. Louis.

Rothwell, N.V. 1988. Understanding genetics, 4th ed. Oxford Univ. Press, New York.

Suzuki, D.T., A.J.F. Griffiths, J.H. Miller, and R.C. Lewontin. 1989. An introduction to genetic analysis. 4th ed. W.H. Freeman Co., New York.

4. Quantitative Inheritance in Plant Breeding

The examples of inheritance already considered have dealt with traits that are simply inherited, mostly by single genes with recognizable effects. For any one trait the phenotypes could be grouped into a small number of easily distinguished, discrete classes. For example, a barley plant may have two or six rows of kernels, black or white hulls, and rough or smooth awns. Characters controlled by few genes which express major phenotypic effects are designated *qualitative characters* and their inheritance is referred to as *qualitative inheritance*. Many traits important in plant breeding are not inherited in simple Mendelian terms. Their inheritance is dependent upon several to many genes at different loci, each gene contributing a small effect to the phenotypic expression of the character. Characters controlled by many genes with small effects are designated *quantitative characters* and their inheritance is referred to as *quantitative inheritance*. The effect of a single gene contributing to the phenotype of a quantitative character is generally too small to be recognized; instead the sum of the effects of all genes contributing to the character is normally measured.

Yield-potential is an important quantitative character in most plant breeding programs. Yield-potential is evaluated by growing the cultivar or breeding line in a yield trial, measuring production (grams\plant, kilograms\hectare, or other units), and comparing that production with standard cultivars. If a large number of plants are randomly chosen and harvested from a genetically mixed population, and their yield-potential compared from progeny tests, the selected plant genotypes could not be grouped into two discrete classes, high vs. low yield-potential, as with a qualitative character. Instead, the yields of the different plant progenies would differ by small amounts and would range continuously from low to high *phenotypic values*, yield being the phenotypic expression of the yield-potential of the different genotypes. If the phenotypic values (yields) are grouped into small classes from low to high, the population would approximate a *normal curve*, with large numbers of classes in the intermediate range, and relatively fewer classes in the high- and low-yielding ranges (Fig.4.1).

Typically, quantitative characters are more influenced by the environment than qualitative

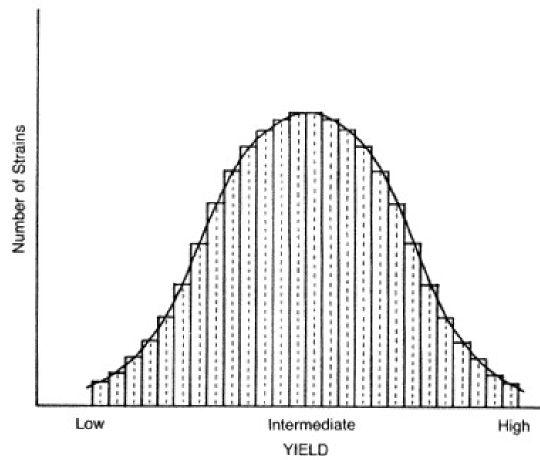


Fig. 4.1.
Normal distribution of a random sample of strains from a genetically mixed population for a quantitatively inherited character. When the observations are grouped into small frequency distribution classes they form a typical bell-shaped curve.

characters. Qualitative characters such as presence of awns on a barley plant, or color of the awns, discussed in Chapter 3, would vary only slightly in different environments. Contrast these characters with yield, a quantitative character, which may be affected greatly by soil fertility, soil moisture, light, temperature, and many other environmental factors. In addition to the *genetic and environmental* components, the yield will be affected by a *genotype x environment interaction* due to the relative performance of the genotypes in different environments, as illustrated in Fig. 4.2.

Quantitative Inheritance and Its Measurement

Classic studies on quantitative inheritance were reported by W. Johannsen, a Danish botanist, in 1903, and by H. Nilsson-Ehle, a Swedish geneticist and wheat breeder, in 1908. By selecting large and small seeds from a genetically mixed lot of beans and comparing the weight of each seed with the average weight of seeds harvested from its progeny, Johannsen established that the seed weight of individual seeds from the mixed lot contained both a genetic and an environmental component (Fig. 4.3). Seeds originally selected from the mixed lot varied in seed weight due to inherent differences for seed size and weight and to environmental factors affecting seed development. In homozygous, pure-breeding lines established by the initial selection, variation for seed weight among seeds from the same plant or plants within the pure line was due to environmental influences only. Variations between the lines were still due to both genetic and environmental influences.

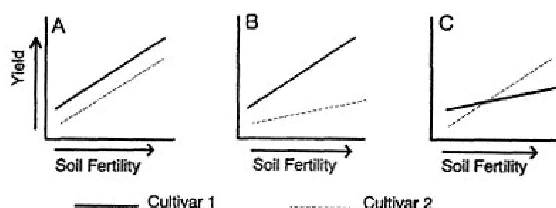


Fig. 4.2.
Comparison of genotype x environment interactions for two cultivars; responses that might be typical for yield of two cultivars as the environment changes from low to high soil fertility. (A) Yields of the two cultivars increase at a similar rate. Note that the lines representing the response of the cultivars are parallel indicating no genotype x environment interaction. (B) Yield of the first cultivar increases at a more rapid rate than the yield of the second cultivar with increases in soil fertility. The divergence between the lines represents a change in magnitude, with the lines not being parallel, which signifies presence of a genotype x environment interaction. (C) Yield of the first cultivar is superior under low fertility, while the opposite is true under high levels of fertility. This is a significant genotype x environment interaction because of a change in ranking of the cultivars under different fertility environments.

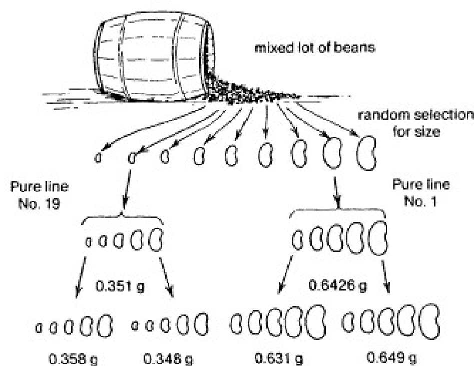


Fig. 4.3.
Pure line selection in beans. From a mixed lot of the 'Princess' bean, a pure line (Pure Line No. 1) was isolated that produced beans averaging 0.64 g in weight. Another pure line (Pure Line No. 19) produced beans averaging 0.35 g in weight. The average seed weights of progenies of beans selected from Pure Line No. 1 were similar to those of the parent line. Likewise, progenies of seeds selected from Pure Line No. 19 were similar to their parent line in average seed weight. Variations in seed weight of beans within a pure line is due to environmental variations on the development of individual seeds. This experiment demonstrated that a mixed population of a self-pollinated crop may be separated into pure lines inherently different, but that further selection within a pure line is ineffective in changing the genotype of the line.

Nilsson-Ehle studied inheritance of color in kernels of wheat. In one experiment, two cultivars of wheat, one with very dark red kernels and one with white kernels, were crossed. The $\mathbf{F}_1$ plants produced kernels that were intermediate in color. In the $\mathbf{F}_2$ generation, plants could be grouped for kernel color into five classes, which ranged from very dark red to white. This distribution was explained on the basis of two pairs of genes segregating independently, with each dominant allele adding to the intensity of the red color. The $\mathbf{F}_2$ genotypes and color classes are illustrated in Table 4.1. Two or more nonallelic genes that affect the development of a quantitative character in a similar way are called *multiple genes* or *polygenes*.

Table 4.1.
F_1 and F_2 progenies from wheat cross, 'very dark red' × 'white' kernel color

Parents	Very dark red $R_1R_1R_2R_2$	×	White $r_1r_1r_2r_2$
F_1		Medium red $R_1r_1R_2r_2$	
F_2 genotypes	Color	Number of dominant genes	Number of plants in 16
$1R_1R_1R_2R_2$	Very dark red	4	1
$2R_1R_1R_2r_2$ $2R_1r_1R_2R_2$	Dark red	3	4
$1R_1R_1r_2r_2$ $4R_1r_1R_2r_2$ $1r_1r_1R_2R_2$	Medium red	2	6
$2R_1r_1r_2r_2$ $2r_1r_1R_2r_2$	Light red	1	4
$1r_1r_1r_2r_2$	White	0	1

In the cross in Table 4.1, approximately 2 of each 16 $\mathbf{F}_2$ plants produced kernels that were as extreme in color as the kernels of the parent plants. The other $\mathbf{F}_2$ plants produced kernels that could be grouped into color classes intermediate to the parents. The distribution of the $\mathbf{F}_2$ plants into color classes, according to the number of dominant genes, illustrates continuous variation in the phenotypic expression of a quantitative character controlled by multiple genes. With only two pairs of genes involved in the cross, a minimum of 16 $\mathbf{F}_2$ plants are required for proportional representation of all genotypes.

In a subsequent experiment, Nilsson-Ehle identified three independent pairs of genes affecting color of kernels in wheat. With three gene pairs segregating independently, the $\mathbf{F}_2$ progeny contained approximately 63 plants with red kernels and 1 plant with white kernels. As larger numbers of independently inherited genes are involved in the expression of a quantitatively inherited character, larger $\mathbf{F}_2$ populations are required for all genotypes to be proportionally represented (Table 3.2). With linkage among the genes, independent assortment is restricted further, so that the required size of the $\mathbf{F}_2$ population becomes even larger.

Transgressive Segregation

Another characteristic in the inheritance of quantitative characters important to the plant breeder is that some of the progeny may fall outside the range of the parents. Consider next

the example of a cross between two cultivars of wheat, each with medium red kernels as presented in Table 4.2. In this cross, plants with kernels both darker and lighter in color than the parent cultivars are obtained in the **F**$_2$ generation. Plants with traits that arise by segregation of genes for a quantitative character that fall outside the range of the parents are known as *transgressire segregates*. Breeders rely on transgressive segregation to obtain segregates that are superior to the parent strains for traits inherited in a quantitative manner. For example, in a cross between two high-yielding cultivars possessing complementary genes for yield-potential, plants may be selected from **F**$_2$ generation progeny with a combination of genes for yield more favorable than that contained in either parent (Fig. 4.4).

Table 4.2.
F_1 and F_2 progenies from wheat cross, 'medium red' × 'medium red' kernel color

Parents	Medium red $R_1R_1r_2r_2$	×	Medium red $r_1r_1R_2R_2$
F_1		Medium red $R_1r_1R_2r_2$	
F_2 genotypes	Color	Number of dominant genes	Number of plants in 16
$1R_1R_1R_2R_2$	Very dark red	4	1
$2R_1R_1R_2r_2$ $2R_1r_1R_2R_2$	Dark red	3	4
$1R_1R_1r_2r_2$ $4R_1r_1R_2r_2$ $1r_1r_1R_2R_2$	Medium red	2	6
$2R_1r_1r_2r_2$ $2r_1r_1R_2r_2$	Light red	1	4
$1r_1r_1r_2r_2$	White	0	1

Transgressive segregation results when progeny plants contain new combinations of multiple genes with more positive effects, or more negative effects, for a quantitative character than were present in either parent. To identify the superior transgressive segregates, it is necessary to grow progenies of selected plants and compare the mean performance of each progeny with the mean performance of parent strains grown in a similar environment. The accuracy with which the superior transgressive segregates can be identified will be increased if environmental variations affecting the expression of the quantitative character in the population are small in relation to the genetic variations.

Characteristics of Multiple Gene Inheritance

Quantitative inheritance is concerned with inheritance of multigenic traits. *Multiple genes* (or *polygenes) are genes at different loci that affect the expression of the same phenotypic character*. The inheritance of multiple genes at each locus follows the same principles of inheritance as with genes for qualitative traits, yet there are characteristic differences in number and expression:

• Each multiple gene expresses a small effect on the phenotype relative to the total

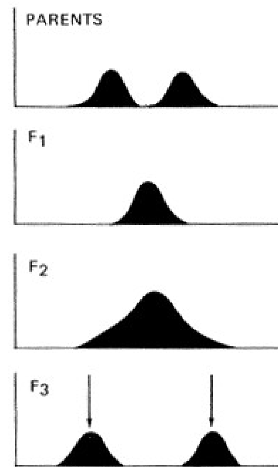


Fig. 4.4.
Transgressive segregation: a portion of the F_3 progeny falls outside the range of the parents.

variation; normally, it is not possible to identify individual gene effects. The example cited, color of wheat kernels, is not typical of quantitative inheritance because the effects of individual genes were of sufficient magnitude that the different phenotypes could be visually identified, however, that fact enabled Nilsson-Ehle to understand and describe how quantitative gene inheritance works.

• Many genes, typically as many as 10 or more at various loci, contribute to the expression of a quantitative character. Distinct segregation ratios are not observed. Recombination and segregation are studied based on the quantitative effects of the genes on the phenotype.

• The individual effects of the genes are cumulative, the net effect enabling measurement of *phenotypic values*. In a random mating population, phenotypic values will exhibit continuous variation from the lowest to the highest value.

• The phenotypic value of a quantitative character includes both a *genetic and an environmental* component and a *genotype x environment interaction*, resulting in an overlapping of genotypic classes, which further accentuates the continuous variation.

• The effects of multiple genes are expressed through different types of gene action: *additive, dominance, epistasis, and overdominance*.

• With multiple-gene inheritance, *transgressire segregates* may arise which fall outside the range of parent values, a phenomenon useful to the plant breeder in obtaining superior cultivars.

Measurement of Continuous Variation

The phenotypic expression of quantitative characters, like yield, height, stalk strength in corn, or baking quality in wheat, is not as easily described and classified as with qualitative

characters, which exhibit alternative dominant or recessive phenotypes. The lemma appendage in barley, discussed in Chapter 3, could be classified into two precise phenotypic classes, hoods and awns. Although individual hoods or awns differ in size and shape, the contrasting forms are readily recognized and can be accurately classified. By contrast, a quantitative character cannot normally be classified into a few easily distinguishable classes, classification being dependent upon measurements of the character taken from a large number of randomly selected plants. These numerical values form a continuous series and are studied by using statistical procedures. Statistics commonly used include the *range, mean, variance, standard deviation, and coefficient of variation*. Of these, the mean and the variance are most important to the plant breeder.

We have noted that quantitative characters are measured and exhibit continuous variation. Phenotypic values for most traits tend to be normally distributed if measured from an infinitely large number of observations; a task hardly practical in a plant breeding program. Instead, a randomly selected sample of the population is examined, randomness being important to ensure independence and eliminate bias in selection of the sample. The sample should be large enough to represent faithfully the range of variability of the character in the population. A larger sample will be needed from a population with a wide range of variability than for a character from a population with a narrow range of variability.

To illustrate how these descriptive values are calculated, a sample data set is presented and analyzed in Table 4.3. The data set contains 100 observations, such as might be made on a quantitative character from a sample of 100 plants. The data are arrayed from low to high for the observed values x, the frequency (f) of each observed value is noted, and the products (fx) obtained. From the sum ( ) of the products (fx) the sample mean (x) is calculated. The *range* is the distance on the scale of measurement from the lowest to the highest observed value. The *mean* is an arithmetic average of the sample measurements for the character being studied.

The dispersion of the observations in the population is described by the *variance*. The variance (σ^2 for the whole population, s^2 and σ^2 for sample-based estimates) is an average of the squared deviations from the mean:

Table 4.3.
Frequency distribution of a 100-sample data set with calculations of descriptive values

Observed value x	Frequency f	Product fx	Deviation of x from mean $(x - x)$	Deviations squared $(x - x)^2$	Product $f(x - x)^2$
8	1	8	-3	9	9
9	7	63	-2	4	28
10	25	250	-1	1	25
11	35	385	0	0	0
12	23	276	+1	1	23
13	8	104	+2	4	32
14	1	14	+3	9	9
Sums ( )	100	1100			126

Number of observations: $n = 100$

Sample mean: $x = \ fx/100 = 11$

Variance: $V = \ [f(x - x^2]/n - 1 = 126/99 = 1.27$

Standard deviation: $s = \sqrt{V} = \sqrt{1.27} = 1.13$

Coefficient of variation: CV = $(s/x) \times 100 = 10.3\%$

$$V = \Sigma[(x - \bar{x})^2]/(n - 1)$$

or (if data are grouped)

$$V = \Sigma[f(x - \bar{x})^2]/(n - 1).$$

Squaring the deviations from the mean eliminates the cancelling effect of negative values and gives greater emphasis to the extreme deviations. The divisor, $n - 1$, is the number of observations, less one, and is used instead of n, where the data are from a sample of an infinite population. The variance is utilized in calculation of a *standard deviation*, in making *heritability estimates*, or it may be broken down into its components in an *analysis of variance*. The analysis of variance is a simple mathematical procedure for measuring the relative importance of two or more known components of variation in an experiment. The residual portion of the variation due to unknown causes is known as the error. It is essential that the plant breeder become familiar with the details for calculation of the analysis of variance. A thorough description can be found in textbooks on statistics.

The standard deviation (σ for population, or s for estimates) is the square root of the variance:

$$s = \sqrt{V}.$$

The standard deviation restores the squared values of the variance to the same terms as the original data set; utilized with the mean it describes the distribution of the observations around the mean. For example, the 100 yield observations from the sample data set are characterized by a mean of 11 with a standard deviation of 1.13. In a normally distributed population (mean = 0.0, variance = 1.0), a range of ±1 standard deviation from the mean will include 68.26% of the observations; a range of +2 standard deviations from the mean will include 95.45 % of the observations (Fig. 4.5). A small variance and a small standard deviation for a population sample indicates that the observations are clustered closely around the population mean; a large variance and standard deviation indicates that the observations are spread widely (Fig. 4.6).

It is often desirable to compare the variability of two or more populations with means that differ widely. In such comparisons the standard deviation may not be suitable because populations with large means tend to have larger standard deviations than populations with small means. For making comparisons of the variability of data groups with quite different means, the coefficient of variation (C.V.) may be used. The *coefficient of variation* expresses the standard deviation as a percentage of the mean and is independent of units measured:

$$\text{C.V.} = (s/\bar{x}) \times 100.$$

In Table 4.3 the coefficient of variation is 10.3%. In biological experiments a coefficient of variation of 10% or less is generally desired.

Observed values in a plant breeding experiment are obtained from measuring the phenotypic expression of the character in each plant in the sample population being studied. If the sample comes from a genetically heterogeneous population, such as an open-pollinated field of corn, then the phenotypic variation from plant to plant will have resulted both from genetic differences among the plants and from the individual plant responses to differences in soil fertility, soil moisture, competition among plants, shading, disease injury, or other

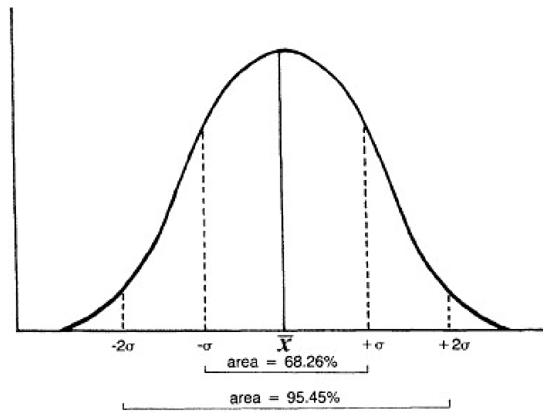


Fig. 4.5.
With a normal distribution, a range ±1 standard deviation from the mean is expected to include 68.26% of the observations; a range of ±2 standard deviations from the mean is expected to include 94.45% of the observations.

environmental factors. If the sample comes from a genetically uniform population, such as a pure-line cultivar of wheat or a single-cross corn hybrid, then all of the phenotypic variation will have been due to varying environmental stresses.

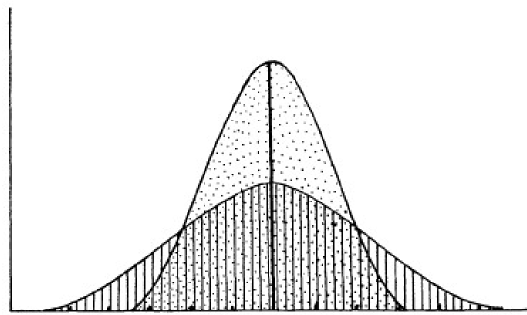


Fig. 4.6.
Populations in which the observations are clustered closely around the mean (dots) have a smaller variance and standard deviation than observations dispersed widely (vertical lines).

Number of Genes Contributing to a Quantitative Character

It is sometimes desirable to have an approximation of the number of genes that contribute to the expression of a quantitative character. A formula has been proposed for estimating the number of genes n involved in the inheritance of a quantitative character:

$$n = (\bar{x}_{P1} - \bar{x}_{P2})^2 / \{8[(\sigma_{F2})^2 - (\sigma_{F1})^2]\}.$$

In the formula, x_{p1} and x_{p2} are the means of inbred parent strains, and σ_{F2} and aσ_{F1} are the standard deviations of the $\mathbf{F_2}$ and $\mathbf{F_1}$ generations, respectively. This method of estimating number of genes is based on the assumptions that the genes have equal effects, no dominance or epistasis is present, and that no two loci are in the same chromosome (hence no linkage). Because these assumptions would seldom hold, the actual number of genes is usually higher than projected. Also, only those genes in which the parents differ would be detected. As a result the method is not very reliable, particularly if more than four or five genes are concerned.

In considering the number of genes affecting a character, it is important to remember that genes may differ in magnitude of their effect, with some genes having a greater effect on a character than other genes. Also, a gene may have a major effect on one character and a minor effect on another character. Then there are the *modifying genes*, which, as their name implies, modify the functions of other genes.

With crosses for breeding purposes, it is impractical to try to estimate the number of genes contributing to a quantitative character. Often the best the breeder can do is to estimate if the quantitative character is governed by a relatively large or a relatively small number of genes. Some predictions may be made from the similarity of the parent cultivars. Parents that are similar in appearance and breeding history, with respect to the quantitative character under consideration, will probably differ by fewer pairs of genes than parents that are dissimilar. The plant breeder may find it easier to select a desirable type if the parent cultivars do not differ greatly in genotype so that fewer genes and less segregation are involved, assuming that both parent cultivars are relatively satisfactory already. For example, a cross between two high-yielding cultivars will normally produce more high-yielding segregates than a cross in which one or both of the cultivars are low yielding. On the other hand, there may be a greater opportunity for obtaining the rare, but greatly superior segregate from crosses between plants with more diverse genotypes.

Multiple Alleles

The genetic component of the phenotypic value of a quantitative character is contributed by many genes. The genes may be groups of multiple genes at different loci or sets of multiple alleles at the same locus.

A diploid plant will carry only two alleles from any set, one at the concerned locus on each of the two homologous chromosomes. If the alleles are identical (A_1A_1 or A_2A_2) the plant will be homozygous at that locus; if the alleles are different (A_1A_2) the plant will be heterozygous for that locus. The number of allelic combinations will be dependent upon the number of different alleles in the populations that are mating. For example, with two alleles at a locus (A_1,A_2), three diploid combinations are possible (A_1A_1, A_1A_2, A_2A_2); with three alleles

(A_1,A_2,A_3), six combinations are possible (A_1A_1,A_1A_2,A_1A_3,A_2A_2,A_2A_3,A_3A_3); with four alleles, 10 combinations are possible, and the number of combinations increases to 21 with six alleles. With *n* alleles, the possible combinations are $n(n + 1)/2$. It can be readily seen that the number of genotype combinations increases rapidly with increases in the number of alleles at a particular locus. It is also necessary to consider that for a particular quantitative character, there may be several loci where sets of multiple alleles are present. The number of genotype combinations then becomes the product of all combinations at all loci, soon reaching astronomical proportions.

What we have referred to above are possible allelic combinations and not the proportions in which they may occur. All alleles at a locus are not necessarily present in the population in the same proportion or frequency. Gene frequency and its relation to genetic equilibrium in populations will be discussed in a later topic.

Types of Gene Actions

Let us now consider how these genes affect the phenotypic expression of a quantitative trait. With multiple genes, four types of gene action are recognized: *additive, dominance, epistasis, and overdominance*.

ADDITIVE EFFECTS refer to the action of genes affecting a genetic trait in such a fashion that each enhances the expression of the trait. If in the expression of a quantitative trait (yield, for example), the effect of a single gene adds one increment of yield, two genes would add two units, and so on, whether they are alleles at one locus or independent genes at several loci. For example, *aabb* = 0, *Aabb* = 1, *AAbb* = 2, *AABb* = 3, and *AABB* = 4.

DOMINANCE EFFECTS are deviations from additivity so that the heterozygote is more like one parent than the other. With complete dominance, the heterozygote and homozygote have equal effects. Using yield, as above, for an example, *aa* = 0, *Aa* = 2, and *AA* = 2.

EPISTASIS EFFECTS are the result of nonallelic gene interactions, i.e., the interaction of genes at different loci. Two genes may have no effect individually, yet have an effect when combined. For example, *AAbb* = 0, *aaBB* = 0, *A B* = 4.

OVERDOMINANCE EFFECTS occur when each allele contributes a separate effect, and the combined alleles contribute an effect greater than that of either allele separately. If the effect of each allele is one, as above, *aa* = 1, *AA* = 1, *and Aa* = 2.

The possible combined effects of the various types of gene action for two genes A and B are illustrated in Table 4.4. It will be noted that with two pairs of genes there are five levels of effects (0 to 4), and that several genotype combinations express each level of effect. In the example here, A and B are shown to have equal effects, but this does not hold for all genes affecting a particular trait because two genes may affect the expression of the trait in different ways. Also, some genes may have pleiotropic effects, affecting different characters in a different manner. From the breeding standpoint, selection may be practiced for additive gene effects with reasonable expectation for success in isolating the superior genotype; selection is less effective in isolating and fixing superior genotypes due to dominance and epistasis.

Although complete dominance is assumed above, partial dominance may be relatively common. Overdominance effects can only be fixed in an F_1 hybrid, through apomixis, or following a wide cross with chromosome doubling.

Table 4.4
Effects of gene action involving alleles of two genes at different loci, assuming that each positive effect adds an increment of one to the expression of a quantitative character[a]

Genotypes	*AA*	*Aa*	*aa*
	Additive gene effects		
BB	+4	+3	+2
Bb	+3	+2	+1
bb	+2	+1	0
	Dominance gene effects		
BB	+4	+4	+2
Bb	+4	+4	+2
bb	+2	+2	0
	Epistatic gene effects		
BB	+4	+4	0
Bb	+4	+4	0
bb	0	0	0
	Overdominance gene effects		
BB	+2	+3	+ 1
Bb	+3	+4	+2
bb	+1	+2	0

[a]Adapted from Comstock (1964). Proc. 19th Annu. Hybrid Corn-Indus. Res. Conf.

Heritability

Individual plants in a mixed population will vary in yield, height, winter hardiness, and other characteristics of a quantitative nature. If two plants selected at random from a mixed population differ in yield, the yield difference may be due to hereditary differences in the plants, differences in the environments in which the plants were grown, or a combination of both. One of the two plants may be inherently more productive, but if it is grown on less fertile soil, its measured yield may barely exceed or may be less than the yield of the genetically inferior plant. If the genetically superior plant is grown on fertile soil, its apparent yield superiority over the genetically inferior plant may be accentuated. The effectiveness of selecting for plants with high yield within a mixed population will depend upon:

• the extent to which the variability in yield of individual plants in the population is the result of genetic factors and is thus transmitted to the progenies of the selected plants, and

• how much the variability in yield among the plants is due to the environment in which the different plants are grown.

Selection of plants for high yield would be ineffective if the environmental variation was so great that it masked the genetic variation. The degree to which the variability of a quantitative character is transmitted to the progeny is referred to as its *heritability*.

Heritability is the proportion of the observed variation in a progeny that is inherited. If the genetic variation in a progeny is large in relation to the environmental variation, then heritability will be high; or if genetic variation is small in relation to the environmental variation, then heritability will be low. Selection is more effective when genetic variation in relation to environmental variation is high than when it is low.

Heritability Estimates

Methods for estimating heritability are based on partitioning observed variation of a quantitative character into genetically and environmentally controlled components. If the quantitative character is height, then all plants in the population sample are measured for height. The height measurements will include variation from all causes. Plants will deviate from the mean height due to the combination of genes they carry affecting height and to gene interactions with environmental factors such as differences in spacing, soil fertility, drought stress, or disease injury.

The statistic that is important here is the variance V, which was discussed earlier. The variance calculated from the observed variations in the quantitative character constitutes the *phenotypic variance* V_P. The phenotypic variance may in turn be divided into three components: *genetic variance* V_G*, nongenetic or environmental variance* V_E*, and variance due to an interaction between genotype and environment* V_{GE}. It follows then that

$$V_P = V_G + V_E + V_{GE} .$$

The genetic variance, V_G, is composed of three major components: *additive genetic variance* (V_A), *dominance variance* (V_D), *and nonallelic interactions or epistasis variance* (V_I). This may be written

$$V_G = V_A + V_D + V_I.$$

The additive component of the genetic variance is the variance contributed by genes having linear additive effects. The resemblance between parents and offspring is largely the result of additive genetic effects and largely determines the response of a population to selection. The dominance component represents the deviation of the heterozygote from the mid-parent or average of the homozygous parents. The dominance effects on the expression of a quantitative character in a population are generally small in comparison with additive effects. The interaction variance results from deviations caused by epistatic effects of nonallelic genes. Examples of epistatic interactions include additive × additive, additive × dominance, dominance × dominance, and additive × additive × additive interactions. The magnitudes of interaction or epistatic effects are difficult to evaluate, but they are generally believed to be small in comparison with additive and dominance effects and they are often ignored in calculating heritability estimates. Procedures by which V_A, V_D, and V_I may be estimated are available in textbooks on statistical or biometrical genetics.

TYPES OF HERITABILITY ESTIMATES. Heritability, the proportion of the phenotypic variance that is due to genetic causes may be given by

$$H = V_G/V_P = V_G/(V_G + V_E + V_{GE}).$$

Heritability may be expressed as a fraction, or may be multiplied by 100 and expressed as a percentage. Heritability percentage estimated from total genetic variance, without taking into consideration the components of genetic variance, is estimated from the formula

$$H = (V_G/V_P) \times 100$$

and is referred to as heritability in the *broad sense* (*H*), because it estimates heritability on the basis of all genetic effects. A more restrictive and often more useful estimate, is obtained if heritability is expressed as a percentage of the additive variance,

$$h^2 = (V_A/V_P) \times 100.$$

The result is represented as heritability in the *narrow sense* (h^2). Narrow-sense heritability cannot exceed, and is usually less than, broad-sense heritability.

In a cross between pure-line parents P_1 and P_2, broad-sense heritability may be estimated by utilizing the variance of the F_2 population as the phenotypic variance $V_{\cdot p}$ The genetic variance is the phenotypic variance minus the environmental variance [$V_G = (V_P, - V_E)$]. An estimate of the environmental variance may be obtained from the variances of genetically uniform populations grown in proximity to the F_2 population, such as the variance of the F_1 (V_{F1}), the mean of the variances of the parents [($VP_1 + V_{P2}$)/2], or the mean of the variances of the F_1 and the parents [$V_{F1}+V_{p1}+V_{p2}/3$]. This procedure estimates heritability in the broad-sense and tends to give too high an estimate.

HERITABILITY FROM VARIANCE COMPONENTS. Heritability can be estimated from variance components, although this assumes that the student is familiar with analysis of variance procedures. The analysis of variance provides a simple statistical procedure for measuring the relative importance of two or more experimental variables that cause variation in an experiment. For example, variables may include replications, plant genotypes, years, locations, and their interactions (of latter three components). If the experiment is designed properly, estimates of variance can be obtained for these variables. Obtaining proper estimates of variance components requires a particular mating design, an experimental design and analysis of variance, and a random model where individuals are chosen at random from a particular breeding population. Details on estimating variance components can be obtained from textbooks on statistics and quantitative genetics.

From an analysis of variance, it is possible to obtain estimates of heritability. Depending upon the mating design, it is possible to obtain estimates of broad- and narrow-sense heritability. The following represents the use of variance components to estimate broad-sense heritability:

$$H = \sigma_g^2/(\sigma_g^2 + \sigma_{gy}^2 + \sigma_{gl}^2 + \sigma_{gyl}^2 + \sigma_e^2)$$

where σ_e^2 = experimental error variance. The experimental design from which these components were estimated included growing plants at more than one location and for more than one year. This takes into account

the fact that certain genotype × environment interactions may be of significant magnitude and removed from the genetic variance. Collectively, $\sigma^2_{gyl} = \sigma^2_{ge}$ (genotype × environment variance).

If the experiment to estimate heritability is evaluated at only one location and one year, it would not be possible to obtain estimates of σ^2_g would be inflated to the extent that the other three variances would be significant biologically. For the plant breeder, this would result in the realized genetic gain from selection falling short of the expected gain. Evaluating the population for more than one year and at more than one location greatly improves upon the accuracy of estimating heritability.

HERITABILITY FROM REGRESSION. Another common procedure for estimating heritability is to use progeny-parent regression. The use of progeny-parent regression is based on several assumptions which include:

• that the trait has diploid Mendelian inheritance,

• the population from which the parents were extracted is in random mating equilibrium,

• no linkage,

• parents are non-inbred, and

• no environmental relationship between the performance of parents and offspring.

The regression of progeny performance on parent performance is based on resemblance between relatives and measures additive variance as a proportion of the phenotypic variance V_A/V_P. This will be recognized as narrow-sense heritability. The regression coefficient (b) of y on × is calculated from

$$b = \Sigma(x - \bar{x})(y - \bar{y})/\Sigma(x - \bar{x})^2$$

with x representing the parent values and y the progeny values. The x and y data for these calculations are obtained from measurements of a quantitative character in the parents and the means of their progenies. The value for x may be the average of the two parents (mid-parent value). If the mid-parent is estimated for all parents, x_1 and x_2, then mid-parent values are $(x_1 + x_2)/2$. If we assume that each parent is independent of the other, i.e., there is no assortative mating then

$$\begin{aligned} V\tfrac{1}{2}(x_1 + x_2) &= \tfrac{1}{4}(V_{x_1} + V_{x_2}) \\ &= \tfrac{1}{2}V_x \\ &= \tfrac{1}{2} \text{ total population variance.} \end{aligned}$$

Therefore, the regression of offspring on mid-parent is $1/2\ V_A/1/2V_P, = V_A/V_P$. If narrow-sense heritability is estimated from the regression of progeny on the mid-parent value, then

$$h^2 = b \times 100$$

where b is the regression coefficient of progeny on mid-parent. In a situation where the pollen parent cannot be identified, such as an open-pollinated cultivar, the value of b is one-half that obtained above and multiplied by 2:

$h^2 = 2b \times 100.$

Heritability and Selection

Quantitatively inherited characters differ in heritability. A character such as yield that is greatly influenced by the environment will have a low heritability. Characters not greatly influenced by environment usually have a high heritability. This may influence the choice of selection procedure used by the plant breeder. Selection in the $\mathbf{F}_2$ of a cross between homozygous parents ($\mathbf{F}_1$ if parents were heterozygous) will not be very effective for characters that have low heritability. Selection in the $\mathbf{F}_2$ is more effective if it is limited to characters that have a high heritability. Selection for characters with low heritability may be made more effectively if based on $\mathbf{F}_2$ progeny performance. The net gain from selection depends upon the combined effect of the heritability, the amount of genetic variation present, and the selection intensity.

A heritability estimate applies only to the particular population sampled and to the environment in which that population was grown. Heritability estimates that are consistently high or consistently low when estimated over a series of populations, environments, and experiments may be considered to be fairly reliable. Examples of characters with relatively high heritability over a range of environments are heading date and kernel size in wheat; ear height, flowering date, and ear characters in corn; and maturity date in soybean. Yield, lodging resistance, winter survival, and protein content generally have low heritability estimates.

The principal uses of heritability estimates are:

- to determine the relative importance of genetic effects which could be transferred from parent to offspring,

- to determine which selection method would be most useful to improve the character, and

- to predict gain from selection (genetic advance).

If meiosis is normal, only additive gene action can be passed from parent to offspring. In this case, the narrow-sense heritability estimate would be particularly useful. However, if a plant breeder is working with clonaly propagated crops such as sugarcane, bananas, rubber, bermudagrass, or if the crop reproduces apomictically, estimates of broad-sense heritability would be appropriate because vegetative propagation and apomixis fix both additive and non-additive (dominance plus epistatic) gene action and transfer it from parent to offspring.

Selection Intensity and Genetic Advance

The plant breeder is continually evaluating mixed populations of breeding materials to identify individual plants or breeding lines with superior genetic potential. The breeding materials may be a population developed through recurrent selection, a segregating $\mathbf{F}_2$ or backcross population, a group of testcross or polycross progenies, or other composite groupings of materials according to the crop species, the breeding procedure, and the objective for which the breeding materials are being evaluated. From the discussion on heritability it should be clear that superior plants or lines in the population may be superior due to genetic potential, favorable environment, or both (positive genotype × environment interaction). The breeder is faced with choosing, based on phenotypic performance, the number that should be

selected without risk of omitting a superior line that did not perform up to its potential because it grew in an unfavorable environment. Also, it would be useful to the breeder to know the progress that can be made with different selection intensities in composite populations. If the breeding objective is high yield, should 10% of the population or breeding lines be kept, or can it be reached by keeping 5% of the population?

Simply, the expected gain or genetic advance with one cycle of selection can be predicted from the population variance and heritability of the quantitative character being studied:

$$G_s = (i)(\sqrt{V_P})(h^2)$$

In this formula, G_s is the predicted genetic advance, *i* a constant based on selection intensity in standard deviation units, $\sqrt{V_P}$ the square root of the phenotypic variance V_P (or standard deviation), and h^2 the narrow-sense heritability of the quantitative character being evaluated. Narrow-sense heritability estimates are used in the genetic advance equation for sexually reproducing crops and broad-sense estimates are used for clonally or apomictically reproducing crops. Values for *i* differ with selection intensities and are available from statistical tables. Representative values of *i* for some selection intensities commonly practiced by plant breeders are:

Selection intensity	
(%)	*i*
1	2.665
5	2.063
10	1.755
20	1.400

For predicting G_s, the phenotypic values should fit a normal curve, or nearly so, and all individuals within the selection intensity group should be included. A hypothetical example of the distribution of the progeny with a selection intensity of 5% is given in Figure 4.7.

The equation for calculating genetic advance may be modified by inserting a *parental control factor* (*c*) that varies with the pollen source. For example,

$$G_s = (c)(i)(\sqrt{V_P})(h^2).$$

If selection is based on the phenotype of the female without regard to pollen source, as in a field of open-pollinated corn, the parental control factor (*c*) is 0.5; if both parents are selected and intermated in isolation to constitute the next cycle of selection, the parental control factor is 1.0. It is evident that the theoretical gain from selection is doubled if both paternal and maternal selection are practiced rather that only maternal selection.

The equation for calculating genetic advance may be modified if different information is desired. For example, assuming one generation is completed per year, the breeder may wish to determine the expected gain per year, over the number of years required to complete a cycle of selection. If so, the gain per year, G_y, would be

$$G_y = [(c)(i)(\sqrt{V_P})(h^2)]/y$$

where y = number of years required to complete a cycle of selection.

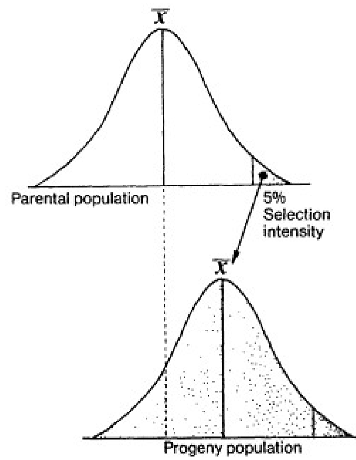


Fig. 4.7.
Distribution of progeny with a selection intensity of 5%. Because the phenotypic values of the selected plants include both a genetic and an environmental component, the progeny mean regresses toward the mean of the parental population.

For an illustration of how heritability and the prediction of genetic advance works, an example may be taken from magnesium concentration in random samples of the outcrossing species, tall fescue. From variance components it was determined that the additive genetic variance was 0.114, the genotype × year variance was 0.015, the genotype × location variance was 0.011, the genotype × location × year variance was 0.009, and the error variance was 0.022 for magnesium concentration. Therefore, the narrow-sense heritability percentage estimate was

$$h^2 = [0.114/(0.114 + 0.015 + 0.011 + 0.009 + 0.022)] \times 100$$
$$= 0.114/0.171 \times 100$$
$$= 67\%.$$

After selecting the top 5% of the plants with the highest magnesium concentration and practicing maternal and paternal selection:

$$G_s = (2.063)(1)(\sqrt{0.171})(0.67) = 0.57.$$

If the mean magnesium concentration is 4.5 mg/g on a dry matter basis in the unselected population, the gain from selection expressed as a percentage of the mean is (0.57/4.5) × 100 = 12.6%. This means that if the top 5% of the tall fescue plants are chosen, the magnesium concentration would be expected to increase by 12.6% after one cycle of selection. With a 10% selection intensity, the predicted advance would decrease to 0.49 mg/g per cycle

of selection. One should not conclude from this that the fewer plants selected, the greater the chance for advance. In practice, the lower the heritability, the larger the number of plants that should be selected to ensure that some of the selected plants are superior due to their inheritance and not to environmental effects alone. If the selection intensity practiced is too low, there is danger of erosion of the genetic base which may have a deleterious impact on future selection. A rule of thumb often practiced by breeders is to keep the best 10%.

Gene Frequency and Genetic Equilibrium

Gene frequency refers to the proportions of the different alleles in the gene pool of a breeding population. *Genotype frequency* refers to the proportions of the various genotypes in the population. In cross-pollinated crops the gene pool is shared by the plants in the population. The relative frequency of a particular gene is determined from the number of individuals in the population and their genotypes. Assuming 100 diploid individuals and two alleles (A_1 or A_2) at a gene locus, a mixed population of 36 A_1A_1 plants, 48 A_1A_2 plants, and 16 A_2A_2 plants would have 120 A_1 alleles [2(36) + 48] and 80 A_2 alleles [48 + 2(16)]. The gene frequency in this population of 100 plants (200 alleles) is 120/200 = 0.6 for allele A_1 and 80/200 = 0.4 for allele A_2. The genotype frequency based on 100 plants is 0.36 A_1A_1, 0.48 A_1A_2, and 0.16 A_2A_2.

If we make the further assumption of an infinitely large population with the same gene frequency and random mating, we can then calculate the frequency of the alleles A_1 and A_2, and the genotypes, in the next generation. Random mating assumes that every male gamete has an equal chance of mating with every female gamete in the population. The random mating frequencies of the genotypes A_1A_1, A_1A_2, and A_2A_2 are recorded in Figure 4.8. The random matings and their frequencies are illustrative of all of the possible matings that could occur among the alleles. The matings, the resulting progenies, and the frequency of the progeny genotypes are shown in Table 4.5. When the proportions of each progeny genotype are totaled it will be seen that the genotypes are in the same frequency as in the preceding generation. Genotypes that maintain the same gene frequency in successive generations are in genetic equilibrium and the frequency will remain constant as long as random mating continues and no factors disturbing the equilibrium are introduced.

The mathematical relationship of genetic equilibrium is stated in the Hardy-Weinberg law, named after the English mathematician Hardy, and the German physician Weinberg, who in 1908 independently recognized the principle underlying the relationship between gene frequency and genotype frequency. According to the Hardy-Weinberg law, the probability of two alleles mating is the product of the frequency of the alleles in the population. If the gene frequencies of two alleles among the parents are p and q, respectively, and $p + q = 1$, then the genotype frequency in the progeny is expressed by expansion of the binomial $(p + q)^2$

$$p^2 + 2pq + q^2$$

Applying this relationship to the example in Table 4.5, if $A_1 = 0.6$ and $A_2 = 0.4$, then A_{12} (0.6 × 0.6) = 0.36, $2A_1A_2$ [2(0.6 × 0.4)] = 0.48, and A_{22} (0.4 × 0.4) = 0.16. It will be noted that these values correspond to the frequencies obtained for A_1A_1, A_1A_2, and A_2A_2 in Table 4.5.

To maintain genetic equilibrium, assumptions are made that there is random mating and that factors disturbing the equilibrium are not introduced into the population. For random mating to occur, an infinitely large population is required to reduce the possibilities that chance

genotypes	A_1A_1 (.36)	A_1A_2 (.48)	A_2A_2 (.16)
A_1A_1 (.36)	.1296	.1728	.0576
A_1A_2 (.48)	.1728	.2304	.0768
A_2A_2 (.16)	.0576	.0768	.0256

Fig. 4.8.
Random mating frequencies of genotypes A_1A_1 (0.36), A_1A_2 (0.48), and A_2A_2 (0.16) and frequency of progeny genotypes with random mating (see Table 4.5.)

deviations in mating will alter the population mix. Certainly, the original mixed population examined ($36A_1A_1$: $48A_1A_2$: $16A_2A_2$) is too small to expect complete random mating to occur. Other factors that may operate to disturb or alter the genetic equilibrium are

• mutation of the A_1 or A_2 allele;

• migration of alleles into or out of the population, such as the introduction of an A_3 allele or loss of A_2 alleles; and

• natural selection that might favor A_1 over the A_2 allele, or vice versa.

These assumptions apply only to the alleles under study, in this example, A_1 and A_2, and not to genes at other loci in the population. In a mixed population it would not generally be possible for the A_1A_2 genotype to be visibly identified from the A_1A_1 genotype as was implied here if the A_1 allele is dominant to A_2.

What happens to a population that is not in genetic equilibrium? If the original mixed population had consisted of 100 plants in the ratio $40A_1A_1$: $40A_1A_2$: $20A_2A_2$, the gene frequency would still be 0.6 for the A_1 allele [(40 + 40 + 40)/200] and 0.4 for the A_2 allele [(40 + 20 + 20)/200]. This population is not in genetic equilibrium but will reach genetic equilibrium for the loci in question after one generation of random mating, which is a general rule for any single independently segregating locus.

The implications of the Hardy-Weinberg equilibrium concept for the plant breeder are as follows:

Table 4.5
Random mating frequencies, genotypes, and genotype frequencies for Figure 4.8

Matings	Frequency of mating	Genotypes of progeny	Frequency of genotypes A_1A_1	A_1A_2	A_2A_2
$A_1A_1 \times A_1A_1$	0.1296	All A_1A_1	0.1296		
$A_1A_1 \times A_1A_2$	0.1728	1/2 A_1A_1,1/2 A_1A_2	0.0864	0.0864	
$A_1A_1 \times A_2A_2$	0.0576	All A_1A_2		0.0576	
$A_1A_2 \times A_1A_1$	0.1728	1/2 A_1A_1,1/2 A_1A_2	0.0864	0.0864	
$A_1A_2 \times A_1A_2$	0.2304	1/4 A_1A_2,1/2 A_1A_2, 1/4A_2A_2	0.0576	0.1152	0.0576
$A_1A_2 \times A_2A_2$	0.0768	1/2A_1A_2,1/2A_2A_2		0.0384	0.0384
$A_2A_2 \times A_1A_1$	0.0576	All A_1A_2		0.0576	
$A_2A_2 \times A_1A_2$	0.0768	1/2 A_1A_2,1/2A_2A_2		0.0384	0.0384
$A_2A_2 \times A_2A_2$	0.0256	All A_2A_2			0.0256
Totals	1.0000		0.3600	0.4800	0.1600

Frequency of allele $A_1 = 0.36 + 0.48/2 = 0.6$

Frequency of allele $A_2 = 0.48/2 + 0.16 = 0.4$

• Random mating in natural plant populations will occur only in cross-pollinated species with populations of infinite size, often referred to as idealized populations. Random mating in natural populations is reduced by such factors as the tendency for cross-pollinations to occur among plants in close proximity, variations in flowering time that prevent early and late plants from mating, variation in vigor of plants so that uneven numbers of flowers and seeds are produced, and other factors.

• Breeding populations are never large enough to obtain natural random mating, but the breeding population needs to be sufficiently large that sampling errors may be disregarded. Sampling errors may be reduced if pollinations arc made with mixtures of pollen to obtain a sampling of pollen gametes. A rule of thumb sometimes followed by corn breeders is to use a sample population of a least 100 plants.

• Random mating does not occur in natural populations of self-pollinated crop species. In self-pollinated species the gene frequency will follow Hardy-Weinberg equilibrium and remain constant if restrictions noted are not violated, but genotype frequency will change, with frequency of homozygous loci increasing and frequency of heterozygous loci decreasing with each generation of inbreeding.

• Selection by the breeder for or against a particular allele or group of alleles contributing to a quantitative character will change the frequency of the allele or alleles in the population and consequently its genotype frequency.

• Selection for a dominant allele in a limited number of generations will not completely eliminate the recessive alleles from the populations, because the homozygous dominant and heterozygous phenotypes cannot be distinguished from each other. Selection for homozygous recessive alleles will eliminate the dominant alleles from the population in one generation.

Gene Recombination and Plant Breeding

This and the preceding chapter have been concerned with gene recombination as the principal tool for creating new genotypes in breeding for improved crop cultivars. Gene recombination plays the major role in the development of present plant-breeding procedures. With gene recombination, the breeder is not creating new hereditary characteristics; rather the breeder is engaged in recombining genes already present, or the incorporation of genes from close relatives into the species. These genes are drawn from nature's gene pools that have evolved over many centuries, as particular species became better adapted to their natural habitats and eventually were domesticated and carried to new and different habitats.

We have seen that some genes produce major effects in the phenotype that are readily identified; others contribute only minor effects, which are identified in total by phenotypic values and evaluated quantitatively by statistical procedures. In a cross between parents with contrasting alleles, inheritance of both major genes and polygenes follows Mendelian principles: segregation of alleles, independent assortment, and linkage. With major genes, progeny plants are normally like one or the other of the parents for specific characters, but with polygenes the progeny plants are generally intermediate to the parents, although there is a potential for obtaining superior or inferior segregates through transgressive segregation.

In a cross between parents, each of which is superior for a different complex quantitative character, it will be extremely difficult to simultaneously recover the maximum number of favorable genes for both characters. In addition to the mathematical odds against recovering a specific recombination of a large number of genes simultaneously for the two characters, complexes of multiple or polygenes in each chromosome are held together more or less strongly by linkage, imposing an additional problem. As a result, the most the breeder can generally hope for is to obtain a favorable assortment of genes for the first quantitative character combined with a favorable assortment of genes for the second character. Several generations of intercrossing among segregates before beginning to self-pollinate may aid in breaking the linkages among the genes and lead to increased gene recombination.

The potential for using gene recombination has in the past depended upon being able to make compatible cross-pollinations so that gametes with contrasting alleles are combined and the resultant zygotes develop into fertile hybrid plants. Through modem molecular genetics, this potential may be expanded by creating transgenic plants with procedures that bypass the normal sexual-reproductive process. For this approach to be efficient, more information about genes that control specific plant biological functions in the various crop species will be needed.

Another aspect of gene recombination, *heterosis* or *hybrid vigor*, will be discussed in Chapter 11.

Study Questions

1. How does quantitative inheritance differ from qualitative inheritance?

2. What is the difference between broad-sense heritability and narrow-sense heritability?

3. If the total genetic variance is 100, the genotype × location variance 10, the genotype × year variance 6, the genotype × location × year variance 3, and the experimental error variance 20, what is the estimate of heritability? Is this broad-sense or narrow-sense heritability? What proportion of the phenotypic variance is composed of genotype × environmental variance?

4. What factors influence gain from selection?

Further Reading

Allard, R.W. 1960. Principles of plant breeding. John Wiley & Sons, New York.

Cook, L.M. 1976. Population genetics. Chapman & Hall, London.

East, E.M. 1910. A Mendelian interpretation of variation that is apparently continuous. Am. Nat. 44:65-82.

Falconer, D.S. 1981. Introduction to quantitative genetics. 2d ed. Longman, London.

Hallauer, A.R., and J.B. Miranda Fo. 1988. Quantitative genetics in maize breeding. 2d ed. Iowa State Univ. Press, Ames, IA.

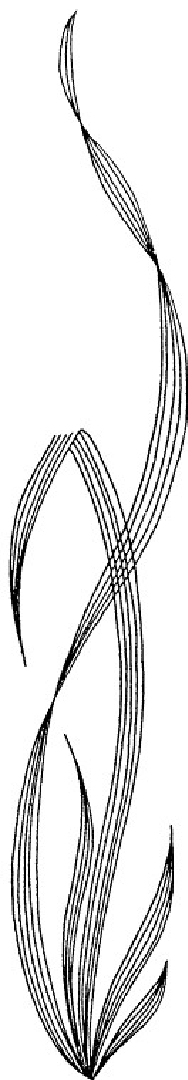
Hartl, D.L. 1988. A primer of population genetics. 2d ed. Sinauer Associates, Inc., Sunderland, MA.

Mayo, O. 1980. The theory of plant breeding. Oxford Univ. Press, New York.

Moll, R.H., and C.W. Stuber. 1974. Quantitative genetics-empirical results relevant to plant breeding. Adv. Agron. 26:277-313.

Scossiroli, R.E. 1982. Consequences of inbreeding and homozygosis in populations. Bol. Genet. 1982(11):3-14.

Wright, S. 1921. Systems of Mating. I. The biometric relations between parent and offspring. II. The effects of inbreeding on the genetic composition of a population. III. Assortive mating based on somatic resemblance. IV. The effects of selection. Genetics 6:111-78.



III
TOOLS OF THE PLANT BREEDER

5. Variations in Chromosome Number

Variability can be created in some crop species by manipulation of the chromosome number. Chromosome numbers may vary in multiples of the complete chromosome set basic to a species, a situation called *euploidy*; or the number may vary by the addition or deletion of specific chromosomes, a condition called *aneuploidy*. Euploid variation (in the number of complete sets) has played an important role in the evolution of many plant species. An understanding of the changes, and their contribution to the evolutionary relationships that exist in the plant kingdom, often enables plant breeders to exploit and utilize genetic variability in species closely related to the crop with which they are working. Important advancements in our understanding of aneuploids and their manipulation have fostered the development of *chromosome engineering* techniques by which individual chromosomes may be moved among cultivars of polyploid species.

In some species of higher plants the haploid and diploid chromosome numbers increase in an arithmetic progression (Table 5.1). The sets of chromosomes contributing to the arithmetic progression are designated *genomes. The* genome is the basic *monoploid* set of chromosomes for the species (or group of related species) and contains only one of each kind of chromosome. The monoploid or basic chromosome number for a species is designated by the symbol x. The *haploid* or gametic chromosome number for a species is designated by the symbol n, and the diploid or *somatic* chromosome number by $2n$. When referring to the number of chromosomes in a species it is desirable to indicate whether reference is made to the monoploid (basic), the haploid (gametic), or the diploid (somatic) number. For example, in corn, the basic and haploid number is 10, and the diploid and somatic number is 20. The haploid number is written $n = x = 10$, and the diploid or somatic number is written $2n = 2x = 20$. In cultivated wheat, the basic chromosome number is 7, the haploid number is 21, and the somatic number is 42; the latter is written as $2n = 6x = 42$.

Table 5.1.
Groups of closely related species in which the haploid and diploid chromosome numbers occur in arithmetic ratios[a]

Species	Gametic (haploid) chromosome number (n)	Basic chromosome number (x)	Somatic (diploid) chromosome number ($2n$)
Avena strigosa	7	7	$2n = 2x = 14$
Avena barbata	14	7	$2n = 4x = 28$
Avena sativa	21	7	$2n = 6x = 42$
Gossypium arboreum	13	13	$2n = 2x = 26$
Gossypium hirsutum	26	13	$2n = 4x = 52$
Nicotiana sylvestris	12	12	$2n = 2x = 24$
Nicotiana tabacum	24	12	$2n = 4x = 48$
Triticum monococcum	7	7	$2n = 2x = 14$
Triticum turgidum	14	7	$2n = 4x = 28$
Triticum aestivum	21	7	$2n = 6x = 42$
Festuca pratensis	7	7	$2n = 2x = 14$
Festuca arundinacea var. glaucescens	14	7	$2n = 4x = 28$
Festuca arundinacea var. genuina	21	7	$2n = 6x = 42$

[a]Species within these groups make up a polyploid series.

Polyploidy

Polyploids are euploids in which the somatic cells possess multiples of complete basic chromosome sets (x) in excess of the diploid number. Polyploids and the number of basic chromosome sets, or genomes, in each are

triploid ⟶ 3x,
tetraploid ⟶ 4x,
pentaploid ⟶ 5x,
hexaploid ⟶ 6x,
septaploid ⟶ 7x,
octoploid ⟶ 8x, and so on.

Euploid plants may arise by duplication of genomes of a single species, *autoploidy* or *autopolyploidy* (auto = same), or by combining genomes from two or more unrelated species, *alloploidy* or *allopolyploidy* (allo = different). An alloploid derived from combining chromosome sets from two different diploid species is called an *allotetraploid* or *amphidiploid*. An autoploid created by duplicating the chromosomes of a diploid species is called an *autotetraploid*.

Autopolyploidy and allopolyploidy are the extremes of a continuum of genomic relationships. Intermediate situations occur, especially in the early generations of allopolyploids, before natural selection for fertility has stabilized bivalent meiotic pairing. Species that

are sufficiently related to cross with one another are likely to have similar genomes, and thus at least limited chromosome pairing is expected between parental genomes in their amphiploids. Perfect autopolyploidy requires homozygosity across all loci, whereas vigor and fertility generally improve with increasing heterozygosity within seemingly autopolyploid species. This heterotic response increases the likelihood of preferential or bivalent chromosome pairing. Although, the terms autopolyploidy and allopolyploidy are used freely by breeders and geneticists, it is necessary to be aware of their limitations.

Polyploidy is of special significance in plant breeding because it permits greater expression of existing genetic diversity. Polyploidy provides the breeder with the opportunity to change the character of a plant by altering the number of genomes and consequently the dosage of allelic genes contributing to particular characters. The effects are varied; some are favorable, others unfavorable. Polyploids with an uneven number of genomes, such as triploids or pentaploids, are generally infertile and can be used to genetically deseed certain plant cultivars. The uneven number of genomes prevents complete bivalent pairing and makes the formation of euploid gametes unlikely which prevents production of seeds. Triploid watermelons and bananas are plants that do not produce seeds because of uneven ploidy levels.

Many commonly cultivated crop species have evolved in nature as polyploids (Table 5.1). It has been estimated that about one-third to one-half of all angiosperms are polyploids. The proportion differs in different families. Approximately 70% of the wild species of the grass family and 23% of the legume family are polyploids. Most of the natural polyploids are considered to be alloploids.

Induction of Polyploids

NATURAL INDUCTION. Examples of naturally occurring allopolyploids include cotton, tall fescue, bread wheat, durum wheat, tobacco, and oat. Examples of naturally occurring autopolyploids include potato, orchardgrass, sweet potato, crested wheatgrass, timothy, and alfalfa.

Autopolyploids and allopolyploids can arise as the result of *unreduced gametes* whereby the chromosome number is not reduced during meiosis and gametes contain the somatic chromosome numbers. Unreduced gametes can occur in the female or male and can be represented as follows:

• $(2n + n)$, female gamete is unreduced and male reduced,

• $(n + 2n)$, female gamete is reduced and male is unreduced, and

• $(2n + 2n)$, both male and female gametes are unreduced.

For example, orchardgrass (*Dactylis glomerata*) is considered to be an autotetraploid. It is well known that the diploid subspecies of *D. glomerata* occasionally form $2n$ eggs and pollen. Thus, the likely origin of orchardgrass and many other polyploid crop species as well is through unreduced gametes.

The classic explanation has been that allopolyploids form naturally as the result of *wide crosses* (from parent plants with different genomes), followed by spontaneous somatic chromosome doubling to restore fertility (Fig. 5.1). Wide crosses do occur in nature, but because spontaneous somatic chromosome doubling is a rare event, it is now believed that formation of allopolyploids through this means is less likely because of the low seed set associated with high levels of meiotic instability. Spontaneous polyploids, both alloploids and autoploids, occurring from crosses between similar or related species through unreduced

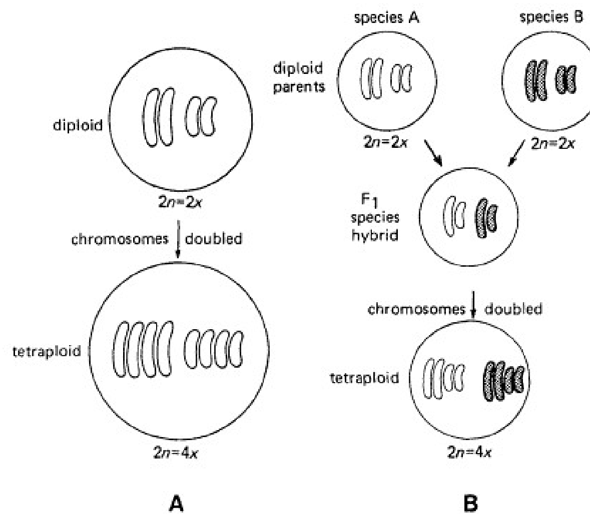


Fig. 5.1.
Origin of polyploids. (A) Autoploids (autopolyploids) arise by duplication of chromosome sets in a single species. (B) Alloploids (allopolyploids) arise by combining sets from two or more species.

gametes is a more likely explanation because of the increased opportunities for higher levels of fertility.

ARTIFICIAL INDUCTION. Artificial induction of polyploidy in a normal diploid plant results in the production of an autopolyploid (autotetraploid to be specific). If the diploid plant was a hybrid that contains unrelated genomes, the resulting polyploid would be an allopolyploid (allotetraploid).

Polyploidy can be induced by environmental shock or with chemicals that disrupt normal chromosome division. Several chemicals will induce polyploidy, but the most widely used has been *colchicine*, an alkaloid extracted from the seeds or corms of the autumn crocus (*Colchicum autumnale*), or *colcemid*, a synthetic equivalent. Colchicine acts by dissociating the spindle and preventing migration of the daughter chromosomes to opposite poles. The resulting nucleus subsequently undergoes normal mitosis, giving rise to polyploid tissue. Colchicine is applied to meristematic regions of the plant by wetting with an aqueous solution, by spraying on in an emulsion, or by rubbing on in a lanolin paste. It is effective when applied to germinating seeds, to young seedlings (Fig. 5.2A), to roots, or to developing meristems. The concentration of the solution, temperature and duration of treatment, and presence of adjuvants such as dimethyl sulfoxide, all affect the outcome and will vary with the species and the part of the plant undergoing treatment. Following induction of polyploidy in diploids, polyploid sectors may be identified, usually on the basis of altered leaf morphology, fertility, or larger

cell size and more chloroplasts in guard cells. Often the sector will be a tiller following treatment of seedlings in grasses (Fig. 5.2B), or a branch following treatment of a bud on a dicotyledonous plant. Adjacent diploid tillers or branches may need to be removed to reduce competition for the often slower developing polyploid tissues. This contrasts with the barley plant illustrated in Figure 5.2B in which the treated plant was haploid and less vigorous than the tillers with doubled chromosomes.

Artificially Induced Autoploids. An induced autoploid is generally stockier and less fertile than its diploid parent. A plant breeder may wish to double the chromosome number of a diploid species, for example, to increase the flower size of an ornamental. Consider a diploid parent species with the genomes *AA*. After doubling, the genomic constitution would be *AAAA*. For this autopolyploid to be fully fertile by seeds, some mechanism must enforce bivalent chromosome pairing or consistently alternate disjunction from multivalents, so that euploid gametes occur. High seed production may not always be necessary, particularly if the plant produced will be propagated vegetatively, which is common in certain ornamentals, forage grasses, and tuber crops.

Fig. 5.2.
Chromosome doubling techniques. (A) Haploid barley seedlings with three to five tillers are partially submersed in a colchicine solution for 5 hr, after which the seedling plants are washed and potted. (B) Barley plant following colchicine treatment. Tillers on left are haploid and sterile, being unaffected by the colchicine treatment. Tillers on right are diploid and fertile, chromosomes having been doubled by the colchicine treatment.

Artificially Induced Alloploids. The genomic relationship of the diploid parents is important to the success of an artificial allopolyploid. Consider, for example, a parent species with the genomes *AA* and a second species with the genomes *BB*. Before an amphiploid of the species can be produced the parent species must be sufficiently related that

• they will cross to produce viable seeds or culturable embryos in the progeny, and

• the resulting diploid (*AB*) hybrid plants are sufficiently normal and viable to reach maturity.

Yet the parent species need to be so distantly related that the A and B genomes from the two parent species are nonhomologous and that chromosomes form only univalents in the diploid hybrid, or some genetic factor preventing pairing needs to be present. After chromosome doubling, each chromosome of genome A should then form a bivalent with its homolog and each chromosome of genome B should form a bivalent with its homolog, with no *homoeologous* pairing between A and B genome chromosomes. Chromosomes originating from different but similar genomes are said to be homoeologous. The genomic dissimilarity required to eliminate homoeologous pairing depends on particular genes. The genetic background of a successful allopolyploid generally eliminates homoeologous pairing more stringently than does the genetic background of related diploids. In natural alloploid species, many homoeologous chromosomes or chromosome segments have genes and arrangements of genes in common because the genomes often evolved from a common ancestral diploid species. Under this circumstance, natural selection favors genotypes with reduced homoeologous pairing.

Wheat exemplifies the genetic suppression of homoeologous pairing. In tetraploid wheat (genomic formula *AABB*), there is considerable homology of chromosomes between the A and *B* genomes. However, a dominant gene called *Ph1* is present in the long arm of chromosome *5B* which suppresses or inhibits pairing of homoeologous chromosomes from the A and B genomes. In the presence of the *Ph1* allele, each chromosome will pair only with its homolog from the same genome. In the absence of the suppressor gene, a chromosome may pair with a homoeologous chromosome from either the A or B genome. Presence of the suppressor gene permits tetraploid, and also hexaploid, wheats to form only bivalents at meiosis, and thus reproduce in the same manner as a diploid species. Similar suppressor genes probably regulate pairing in other naturally occurring allopolyploids, because homeologous genomes would converge by recombination in their absence.

Characteristics of Polyploids

In natural autotetraploids such as potato, orchardgrass, and alfalfa, genetic ratios for simply inherited characteristics are often more complex than in diploids. To accurately determine inheritance of qualitative traits in autotetraploids, more progeny are needed than with diploids. With the alleles *A* and *a*, three genotypes (*AA,Aa,aa*) are possible in a diploid, but five genotypes are possible in the autotetraploid. The genotypes and their designations are

AAAA ⟶ quadruplex,
AAAa ⟶ triplex,
AAaa ⟶ duplex,
Aaaa ⟶ simplex, and
aaaa ⟶ nulliplex.

If the dominance of A is complete, all of the genotypes will exhibit the dominant characteristic except the nulliplex, which will exhibit the recessive characteristic. When a genotype is selfed, the phenotypic ratios of dominant to recessive segregates, assuming random chromosome segregation, will be

$AAAA \longrightarrow 1A{:}0a$,
$AAAa \longrightarrow 1A{:}0a$,
$AAaa \longrightarrow 35A{:}1a$,
$Aaaa \longrightarrow 3A{:}1a$, and
$aaaa \longrightarrow 0A{:}1a$.

With incomplete dominance, the inheritance becomes even more complicated, ranging from five phenotypes if the effect of A is cumulative, to a large assortment of phenotypes if the effect of A is complex. As an example, in alfalfa two or more dominant alleles are required before the dominant character is expressed in the phenotype for some characters. Genetic linkage relationships are exceedingly complex and difficult to identify in autotetraploids. Furthermore, an autotetraploid individual can have up to four alleles per locus, giving a plethora of subtly different possible phenotypes.

Genetic ratios in allopolyploids are often much simpler than for autopolyploids. Strict allopolyploids have bivalent pairing behavior during meiosis and therefore *disomic* inheritance. For example, in wheat, the 42 chromosomes which come from three separate genomes pair as 21 bivalents on the metaphase plate during meiosis. If the heterozygote *Aa* is present in one of the three genomes, upon selfing the genotypic ratio would be 1*AA*:2*Aa*:1*aa*. Having multiple genomes makes it possible to have multiple loci with a corresponding locus in each genome.

In wholly or partial autopolyploid species, plants with recessive characters may appear less frequently in a segregating population than they would in a diploid species. This requires that the breeder grow a larger population of the polyploid to recover a certain number of recessive phenotypes following a cross than would be necessary with an ordinary diploid. Recessive mutations that are deleterious to the parent may be masked by their dominant alleles to a greater extent in polyploids, so that they are not expressed as frequently in the phenotype of the plant. Polyploid species have unique characteristics that make it possible to use relatively simple techniques to identify precisely the chromosome in which a particular gene is located. These techniques will be described later in this chapter.

The effect of polyploidy on the phenotype of the plant is varied and difficult to predict. Breeders of ornamentals have induced chromosome doubling to increase flower size. Autopolyploidy often increases the size of meristematic ceils and guard cells. Often the total number of cells decreases, as does growth rate, which can lead to later flowering. The characteristics of allopolyploids are too variable to generalize.

Autoploidy and Plant Breeding

Not all species have improved vigor after the chromosome number has been increased, leading to the concept of an *optimum ploidy level* for each species. Many species of plants have evolved with maximum performance at only one level of ploidy. For example, corn has maximum vigor at the diploid level. Induced tetraploid corn is inferior to otherwise identical diploid corn in most characteristics of agronomic interest. The banana's optimum ploidy, at least for human consumption, occurs at the triploid level. Diploid bananas have hard seeds which makes them unacceptable commercially while triploid bananas are seedless and

acceptable to consumers. Alfalfa, peanut, potato, coffee, and the easter lily are examples of plants that have maximum vigor at the tetraploid level. The blackberry is insensitive to ploidy levels as vigor is rather constant from 2x to 12x levels.

The simplicity by which chromosome doubling may be accomplished with colchicine increased the interest of plant breeders in utilizing this technique to produce polyploid cultivars with larger yield potential. Relatively few induced polyploids are successful initially, when compared with the parental diploids. From these experiences, three principles have evolved, which serve as guides for the production and utilization of autoploids in a plant breeding program:

• The tendency for autoploids to have greater vegetative growth and reduced seed production suggests that autoploidy would be more useful in breeding crops harvested for vegetative parts (forages, root crops, vegetables, flowers) than in crops harvested for seed.

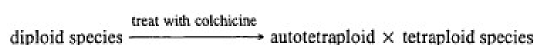
• The greatest successes in obtaining vigorous and fertile autoploids have been achieved from doubling the chromosome content of diploids with low chromosome numbers.

• Autoploids derived from cross-pollinated species may be more successful than autoploids from self-pollinated species because the cross-pollination fosters extensive gene recombination among the polyploids and enhances the chances of obtaining a balanced polyploid genotype.

Diploid species have evolved with chromosome numbers compatible with reproduction and development within the species. Doubling the chromosome number in species that already have large chromosome numbers may lead to misdivisions of the nucleus. Performance of a genotype as a diploid is not a reliable indication of performance after it has been made into a tetraploid. To find a superior genotype at the polyploid level, large numbers of diploid genotypes need to be converted into tetraploids and a new breeding program started at the polyploid level.

Examples in which autoploid cultivars have been successful are few. Considerable success has been realized, however, with root crops and this will serve as one example of using autopolyploidy in cultivar development. Polyploid cultivars of sugar beets, turnips, and fodder beets, are commercially available, principally in Europe (Fig. 5.3 A and B). In sugar beets, both tetraploid and triploid cultivars may be grown with the triploid favored. Earlier most commercially grown polyploid sugar beets were *anisoploids*, i.e. mixtures of triploids, tetraploids, and diploids, because there was no way in which pure populations of triploids could be produced. By pollinating a cytoplasmic male-sterile diploid with a tetraploid, pure triploid strains became possible. The fact that the triploids are highly sterile and do not produce seed is of no consequence in sugar beets because only the roots are harvested for commercial use from the triploid plants.

BRIDGING PLOIDY LEVELS IN INTERSPECIFIC CROSSES. Autoploidy may be used to bridge the ploidy level in intraspecific crosses and to move genes across the interspecific crossing barrier. Crossing between closely related diploid and tetraploid species is often possible by doubling the chromosomes of the diploid species or finding a source of 2*n* gametes in it so that they match those of the tetraploid:



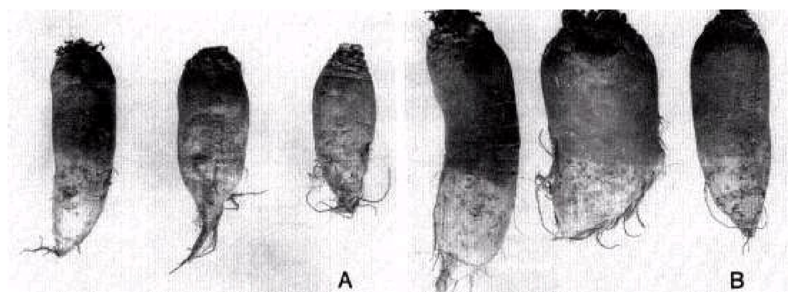


Fig. 5.3.
Turnip roots: (A) Diploid. (B) Tetraploid. Crops grown for vegetative pans usually respond more favorably to polyploidy than crops grown for seed.

Through this procedure genes may be moved from a diploid to a tetraploid species, provided the genomes in the diploid species are compatible with those in the tetraploid species.

'Hycrest' crested wheatgrass is an example of a successful forage grass cultivar developed by using autoploidy as a genetic bridge for gene transfer via interspecific hybridization. The diploid chromosome complement of *Agropyron cristatum* was doubled to induce autotetraploidy which was then crossed to the naturally occurring tetraploid *A. desertorum*. Because the effect of the cytoplasm was unknown, reciprocal crosses were made prior to selection to ensure that both cytoplasms were represented.

Alloploidy and Plant Breeding

Natural alloploids include such economically important crops as wheat, oat, cotton, tobacco, sugarcane, tall rescue, and mustard. The ancestral origin of the genomes is known only for a relative few of the polyploid species. Common wheat (*Triticum aestivum*), American upland cotton(*Gossypium hirsutum*), cultivated tobacco (*Nicotiana tabacum*), and various species of mustard (*Brassica*) are extensively studied polyploids whose ancestors have been identified fairly accurately.

The genomic relationships in the naturally occurring *Brassica* spp. will serve as an example. Three common diploid species of *Brassica, B. campestris, B. nigra, and B. oleracea*, have haploid chromosome numbers of 10, 8, and 9, respectively, which have been assigned the genome designations A, B, and C (Fig. 5.4). *Brassica juncea* (*AABB*) is a natural amphiploid in which are combined the genomes of *B. campestris* (*AA*) and *B. nigra* (*BB*); B. *napus (AACC*) is a natural amphiploid, that contains genomes of *B. campestris (AA*) and *B. oleracea* (*CC*); and *B. carinata (BBCC*) is a natural amphiploid in which are combined the genomes of *B. nigra (BB*) and *B. oleracea (CC*). The origins of the tetraploid *Brassica* species have been demonstrated experimentally by crossing the diploid species suspected as being parents, doubling the chromosome number of the hybrid plant, crossing the experimentally produced amphiploid with the tetraploid species having a corresponding chromosome number,

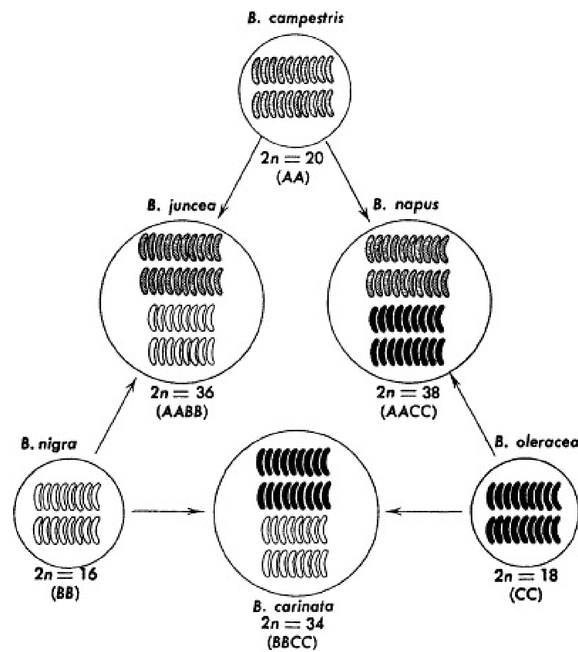


Fig. 5.4.
Diploid and tetraploid species in Brassica. The diploid species B. campestris, B. nigra, and B. oleracea have somatic chromosome numbers 2n = 20, 16, and 18, respectively, to which have been assigned the genome designations AA, BB, and CC. The tetraploid species B. juncea, B. napus, and B. carinata are allotetraploids, originating from the combinations of diploid species, illustrated in this diagram, and have somatic chromosome numbers of 2n = 36, 38, and 34 respectively.

and examining the chromosome homology. Homology of the chromosomes is verified by the extent to which they pair and form bivalents at meiosis, and by the observed fertility of the hybrid plant.

USES OF ALLOPLOIDY. Although natural alloploids are far more common than natural autoploids, the plant breeder has given less attention to the production of induced alloploids than to induced autoploids. Some uses of alloploidy to the breeder are listed here:

Identifying genetic origin of polyploid plant species. Alloploidy has been important in the evolution of plant species. Knowledge of the ploidy relationships between certain plant species

has contributed to understanding the genetic origins of common wheat, tobacco, cotton, and other crops.

Producing new plant genotypes and plant species. Extensive breeding efforts to combine wheat and rye chromosomes to form an artificial alloploid led to the development of a new grain crop, triticale. The many years of research devoted to breeding triticale speaks for the difficulty in attempting to duplicate nature's role in evolving natural alloploids, a role that has extended over centuries or more. Triticale is a self-pollinated crop grown for its seeds, and so triticale breeding defies the general principles stated for working with cross-pollinated species for forage or root production.

The major objective in breeding triticale is to combine the quality of wheat with the hardiness of rye, with other objectives essentially similar to those in breeding wheat. Overcoming infertility and increasing kernel plumpness in triticale have been necessary to improve yield and kernel quality. Shorter and stiffer straw, greater winter hardiness in winter types, photoperiod insensitivity in spring types, yield stability in different environments, and resistance to the ergot disease are all important objectives in breeding triticale. Market quality characteristics desired in triticale remain vague because its utility has not been well established. Until this occurs triticale will likely be used largely for livestock feed.

In the development of triticale, tetraploid ($2n = 4x = 28$), hexaploid ($2n = 6x = 42$), and octoploid ($2n = 8x = 56$) forms have been synthesized. The hexaploid forms of triticale are derived by combining the *AB* genomes of tetraploid wheat ($2n = 4x = 28$) with the R genome of rye ($2n = 2x = 14$), and the octoploid forms by combining the *ABD* genomes of hexaploid wheat ($2n = 6x = 42$) with R genome of rye (Table 5.2). The hexaploid forms have better agronomic traits than the octoploid forms and are generally favored for that reason. Tetraploids are more of a botanical curiosity and have not been considered as agronomic crops.

Table 5.2.
Chromosome number and genomic formula for triticale and its parent species

Species	Chromosome number ($2n$)	Genomes	Common name
Secale cereale	14	*RR*	Rye
Triticum turgidurn	28	*AABB*	Durum wheat
Triticum aestivurn	42	*AABBDD*	Common wheat
Triticosecale (hexaploid)	42	*AABBRR*	Triticale
Triticosecale (octoploid)	56	*AABBDDRR*	Triticale

A fertile wheat x rye hybrid was produced by W. Rimpau in Germany in 1888. Wheat x rye hybrids were later developed in Russia and Sweden, and more recently in the United States, Canada, Mexico, Hungary, and other countries. The plants of triticale are similar to those of wheat, except for having larger spikes and kernels and greater vigor of growth (Fig. 5.5). Triticale may be either spring or winter type, depending on the genes in the parent lines. Some objectionable features have been low fertility, shriveled seeds, and weak straw. Through selection the fertility and seed quality have been improved and by introduction of dwarfing genes, short stiff strains have been developed. Improving these characteristics has increased the acceptance of triticale by farmers as a commercial crop.

In breeding new alloploids, current research suggests that the greatest success will come from working with closely related species at a low ploidy level, such as combining genomes from diploid species.

Facilitating transfer of genes from related species. A knowledge of the genomic relationships is useful to the breeder in bridging crosses between species or between genera. Interspecific crosses may be used to transfer genes for disease resistance, or other characters, from a wild species to a closely related cultivated species. In tobacco, wildfire resistance was transferred from *Nicotiana longiflora* ($4x$) to *N. tabacum* ($4x$) by hybridization. Genes for lint strength in cotton were transferred from the wild diploid species *Gossypium thurberi* ($2n = 2x = 26$, *DD*) to the cultivated tetraploid species *G. hirsutum* ($2n = 4x = 52$, *AADD*). First an alloploid was produced by crossing *G. arboreum* ($2n = 2x = 26$, *AA*) with *G. thurberi. The* resulting allotetraploid or amphidiploid ($2n = 4x = 52$, *AADD*) could then be crossed with the cultivated species. Amphidiploidy in this case and others is used to circumvent sterility in F_1 hybrids and to control chromosome pairing as genes are introgressed into crop species.

Fig. 5.5.
Spikes of triticale. Triticale is a "man-made" alloploid, in which the AB genomes of wheat are combined with the R genome of rye.

Facilitating transfer or substitution of individual chromosomes or pairs of chromosomes. Some polyploids are viable with a chromosome or a pair of chromosomes missing or added. The missing chromosome is compensated by a homoeologous chromosome in another genome. These relationships are discussed more fully below.

Aneuploidy

Aneuploids have incomplete sets of chromosomes. Certain aneuploids can have less than the complete euploid complement of chromosomes. Examples which include the name of the aneuploid and its genomic formula are

nullisomic $\longrightarrow 2n - 2$ (euploid minus one pair of chromosomes),
monosomic $\longrightarrow 2n - 1$ (euploid minus one chromosome),

trisomic ⟶ $2n + 1$ (euploid plus one chromosome), and
tetrasomic ⟶ $2n + 2$ (euploid plus one pair of chromosomes).

Most aneuploids arise as the result of cytological accidents that produce unbalanced gametes. Aneuploids such as a trisomic can occur when an $n + 1$ gamete unites with an n gamete to form a $2n + 1$ plant. Monosomics can arise from plants that produce $n - 1$ gametes after crossing to plants that produce n gametes. One basic cause of these *unbalanced* (aneuploid) *gametes* is *non-disjunction* during meiosis in which unequal sets of chromosomes go to opposite poles. Another cause is failure of an unpaired chromosome to reach either pole at meiosis.

Aneuploids are useful genetic tools for identifying the chromosome in which a particular gene is located, or for facilitating the substitution of a specific chromosome into a genotype. It is more accurate to pinpoint the location of a gene in a specific chromosome, or in an arm of a chromosome, with aneuploid techniques than by ordinary linkage studies. While aneuploid techniques may identify the chromosome in which the gene is located, they do not establish the linkage relationship with other genes in the chromosome. In polyploids, such as tetraploid or hexaploid wheat, inheritance studies by traditional procedures are often complex due to the presence of loci for the character in each genome. The aneuploids utilized to the greatest extent in genetic analyses are trisomics, monosomics, and nullisomics.

Trisomics

Trisomics have been utilized to identify linkage groups in barley, maize, sorghum, tomatoes, and other species. Trisomics may be tolerated in non-polyploid species where nullisomics and monosomics would be inviable. To utilize trisomics for assigning genes to specific chromosomes, it is first necessary to develop a trisomic for each chromosome pair. Then the cultivar with the gene to be tested is crossed successively with each of the possible trisomics to produce trisomic hybrids. Trisomic segregation ratios will differ from diploid segregation ratios. The chromosome in which the gene is located is identified from the particular trisomic hybrid that segregates for the trisomic ratio rather than the normal diploid ratio.

Monosomics

Viable monosomics may be produced in polyploid species where the loss of a chromosome is balanced by genes on homoeologous chromosomes in other genomes. The monosomics may be utilized to identify genes with specific chromosomes in a polyploid species, or to substitute chromosomes containing a specific gene or genes from other cultivars or closely related species into a particular polyploid species. To utilize the monosomic techniques fully, it is necessary to develop a monosomic for each chromosome pair in the species. The 21 possible monosomics for hexaploid wheat, which has 21 chromosome pairs, were first established in the cultivar 'Chinese Spring.' Incomplete monosomic sets have also been established in oat, cotton, tobacco, and a few other polyploid crop species.

Use of the monosomic technique to identify a gene with a specific chromosome, is illustrated by the following analysis of a wheat cultivar to identify the chromosome carrying the gene for resistance to races A and B of the Hessian fly. The hexaploid

wheat cultivar with the gene for resistance was crossed to each of the 21 monosomics of 'Chinese Spring.' The F_1 plants from each cross were examined cytologically to identify plants with the monosomic constitution which lack the 'Chinese Spring' version of the monosomic chromosome. Seeds from the monosomic F_1 plants were planted and the F_2 plants screened for resistance to races A and B of the Hessian fly by an F_3 progeny test. Because 'Chinese Spring' is susceptible to Hessian fly, all crosses gave normal segregation except the cross to the critical monosomic, i.e., the monosomic for the chromosome carrying the gene being tested. With the critical monosomic, segregation was much larger than the expected 3:1 ratio due to reduced functioning of the 20-chromosome pollen. The chromosome in which the gene for fly resistance was located is then identified by the segregation patterns of the different crosses. From these observations chromosome 5A was found to carry a single dominant gene for resistance to races A and B of the Hessian fly.

Monosomic techniques permit exchange of single chromosomes from a donor variety or closely related species for the same chromosome in the recipient cultivar. An exchange between cultivars of the same species is referred to as a *chromosome substitution*, or an *alien chromosome substitution* if it is from another species. The chromosome-substitution procedure permits the introduction of a chromosome carrying a desirable gene, such as one for disease resistance from a cultivar or closely related species, into an otherwise acceptable cultivar. The recipient cultivar would then benefit from the desirable gene (e.g., the gene for disease resistance) without acquiring genes with adverse or deleterious effects from other chromosomes of the donor cultivar or species as would occur with conventional crossing and subsequent segregation. Because the entire chromosome is substituted into the recipient cultivar, undesirable genes on the substituted chromosome would be introduced along with the desirable gene. With the chromosome substitution procedure, a complete set of monosomics is needed for the recipient cultivar, into which the donor cultivar or species is crossed. This particular use of monosomics is becoming obsolete as crop species are more thoroughly mapped with DNA markers. It is possible to mark all chromosome arms with *codominant* (see Chapter 8 for explanation of codominance) loci and recover the heterozygote for (most of) the desired arm from a sufficiently large backcross population. This heterozygote can be selfed to obtain a close approximation of the desired introgressant.

The chromosome substitution method may be used for analysis of quantitative characters as may be shown by the following example. Assume a cultivar of wheat contains a gene or genes for high protein and it is desired to learn which chromosome or chromosomes contain loci associated with the higher protein. Chromosomes from the high-protein cultivar are transferred by a backcross procedure into a cultivar such as 'Chinese Spring,' in which a complete set of monosomics is available. The effect each chromosome from the donor cultivar has on protein content may then be compared with the effect of the corresponding chromosome in the recipient 'Chinese Spring' cultivar. By this procedure the chromosomes with loci contributing to the higher protein content may be identified. As with the monosomic analysis procedure, the substitution line procedure associates a locus affecting the characteristic under study with a specific chromosome, but linkage relations with other loci on the chromosome are not established. Linkage values are relatively easily obtained, however, because there is only a single chromosome difference between the substitution line and the control line, thus eliminating possible confusion due to segregation of genes on the other 20 chromosomes. The cytogeneticist can go further by backcrossing the $\mathbf{F_1}$ to the proper

monosomic, selecting monosomic offspring (each of these monosomes being a potential recombinant from the $\mathbf{F_1}$), and letting each monosomic self for recovery of a disomic. Each of these disomics then is essentially a clone of a chromosome-doubled gamete. It can be multiplied as a pure line and compared with all of its sister lines for quantitative as well as qualitative characters. If enough lines have been obtained, linkage values can be obtained and a linkage map of the particular chromosome constructed. With wheat this technique has been shown to be extremely effective for identifying and mapping genes with quantitative effects.

Nullisomics

In hexaploid wheat, it is possible to have 21 different nullisomics. All have been identified. Hexaploid species have greater genetic redundancy than tetraploids for compensating the loss of a pair of chromosomes. Nullisomics may be used to assign genes to specific chromosomes. For example, if a gene for rust resistance is present in a specific chromosome, and that gene and its allele are eliminated by removing the pair of chromosomes on which they are located, the wheat plant will no longer be resistant to rust. Because nullisomic plants are less vigorous and less fertile than monosomic plants, the nullisomic technique is less practical to use than the monosomic technique.

Haploidy

Haploid plants are sporophytes that contain the gametic chromosome number. *Monoploid* plants are haploid plants produced from a diploid species, such as corn or barley, so that they contain only one genome. In this way monoploids differ from haploids of a polyploid species, which contain two or more genomes and are called *polyhaploids*. Spontaneous haploid plants occur at generally low frequency in many species, both diploid and polyploid. They have been reported in corn, sorghum, wheat, barley, rye, rice, flax, tobacco, cotton, and other crop species. Often, naturally occurring haploid plants may be recognized because they are smaller than diploids. The ploidy level of plants suspected of being haploids may be confirmed most efficiently by flow cytometry, and less efficiently by counting the chromosomes, commonly from root-tip meristems or other embryonic tissue. Haploid plants may be treated with colchicine to produce diploid sectors or plants, usually referred to as *doubled haploids*.

Uses of Haploids

Haploids may be used in breeding crop plants and in genetic studies in various ways. Some of the possibilities include:

•Doubling the chromosomes of the haploid plant will produce a completely homozygous diploid, assuming that no spontaneous mutations occur. The doubled haploid plants produced in one generation will have a higher level of homozygosity than inbred lines developed by a limited number of generations of inbreeding.

• Haploid plants are useful in mutation studies. A recessive mutation will be observed immediately as all loci are in the *hemizygous* (only one allele per locus)

condition and can be identified from the phenotype. In a homozygous diploid plant, a recessive mutation of a dominant gene will be masked by the corresponding dominant allele and the mutation will not be observed until segregation has brought two recessive alleles together in a later generation.

• Selection for dominant alleles is facilitated in haploids. Corresponding recessive alleles in the haploid will not be present if the locus in question is already occupied by the dominant allele. This compares to diploids where selfing would be necessary to eliminate recessive alleles.

• Genetic segregation can be less complex in polyhaploids. For example polyhaploids obtained from autotetraploids could exhibit disomic inheritance and a smaller population is required to generate a particular genotype than with tetraploids. Such polyhaploids can be more easily bred for recessive traits than their polyploid parent can. The selected germplasm can then return to the original polyploid level by use of colchicine or 2*n* gametes.

• Haploids are useful in cytogenetic studies of polyploids, as they provide the genetic material from which a monosomic series may be derived. Analysis of meiotic chromosome association in polyhaploids can illuminate the interrelationship of genomes in their high polyploid parents.

• Polyhaploids (already defined) may be used to transfer genes from the polyploid to a related diploid species.

• In species with self-incompatibility alleles, in which self-pollination is restricted, doubled haploids offer a possible means for producing inbred stocks (completely homozygous plants).

Procedures for Producing Haploids

Before haploids can be utilized successfully in a breeding program, simple and reliable procedures must be available for producing and recognizing the haploids in quantity and for doubling the chromosome number of the haploids to obtain viable diploid plants. Many techniques for producing and evaluating haploids have been proposed with different crops. Some of the more useful techniques for cultivar development include:

• the identification and diploidization of naturally occurring haploids,

• interspecific hybridization followed by elimination of the chromosomes of the wild species, and

• anther- or pollen-culture.

IDENTIFICATION AND DIPLOIDIZATION OF NATURALLY OCCURRING HAPLOIDS IN CORN. In some strains of corn, approximately one kernel out of each 1000 will have an embryo with the haploid or gametic chromosome number of 10 instead of the normal diploid chromosome number of 20 (10 pairs). The haploids arise through development of an unfertilized egg into an embryo by *parthenogenesis. The* naturally occurring haploid plants may be recognized easily by utilization of suitable marker genes. Many of the haploids will grow to maturity, and about one out of each ten can be self-fertilized successfully to give a diploid progeny. The diploid lines developed from the haploids are completely homozygous, whereas inbred lines developed by the conventional method of inbreeding may never quite reach complete homozygosity.

CHROMOSOME ELIMINATION IN BARLEY. In barley, haploids may be obtained following interspecific hybridization of *Hordeum vulgare* ($2n = 2x = 14$) with H. *bulbosum* ($2n = 2x = 14$). The percentage of seed set following the interspecific hybridization is increased by hormone treatment of the emasculated spikes before pollination and again one or two days after crossing. Fertilization occurs normally, but the chromosomes from *H. bulbosum* are progressively lost at mitotic division in the developing embryo, leaving only the haploid chromosome component from the cultivated barley. Within two to five days after hybridization, the haploid embryo is dissected from the endosperm and cultured in vitro (on agar containing a nutrient solution). Haploid plantlets develop from the immature embryo, and the chromosomes of the haploid plantlets are doubled by treatment with colchicine or other suitable means. The doubled haploid is grown to maturity in the greenhouse or in a controlled environmental chamber.

In using the doubled-haploid technique in a breeding program, crosses are made between cultivars as in a conventional hybridization program. The F_1 plants are pollinated with *H. bulbosum* pollen. Haploid plantlets may be expected in 5 to 50% of the rioters pollinated. Using the colchicine technique, chromosome duplication may be expected in 50 to 60% of the haploid plantlets. Alternatively, haploid production might be delayed until the F_3 or F_4 generation, after selection for disease resistance or other characters has been practiced. Doubled haploids may be used for purification of advanced selections that are to be released as new cultivars.

ANOTHER CULTURE. Haploid plantlets may be cultured from anthers or pollen. Ever since methods for generating haploid plants have been known, efforts have been made to utilize this technique as a plant-breeding procedure. Plants have been cultured from anthers with varying degrees of success in tobacco, rice, wheat, alfalfa, barley, triticale, potato, and other crops. Anther culture will be discussed in more detail in Chapter 8.

Unreduced Gametes

The transfer of *exotic germplasm* into cultivated species is often facilitated by using unreduced gametes. Unreduced gametes allow the breeder to manipulate ploidy levels and to create new sources of variation. The transfer of exotic germplasm using unreduced gametes has been reported in a number of crops, including sweet potato, cassava, alfalfa, strawberry, blackberrry, banana, peanuts, sugar cane, and potato. The potato has received the most attention by breeders in this regard.

The cultivated potato is a tetraploid ($2n = 4x = 48$). However, more than 150 species of potato have been identified with a polyploid series ranging from diploid to hexaploid. This extensive range of ploidy levels in potato enhances the opportunity to incorporate exotic germplasm to improve the cultivated tetraploid. Furthermore, crosses between ploidy levels such as $2x \times 4x$ and $4x \times 2x$ are relatively easy to accomplish by the breeder and result in a high frequency of $4x$ progeny. These $4x$ progeny are the result of the formation of unreduced gametes by either the male or female parents (*unilateral sexual polyploidization*). In certain $2x \times 2x$ crosses, $4x$ progeny result because of unreduced gametes occurring simultaneously in the female and male parents

(*bilateral sexual polyploidization*). The $2x$ parents used in these crosses are either polyhaploids of tetraploids or wild diploid relatives.

Triploids are rarely found in the progeny of $2x \times 4x$, $4x \times 2x$, and $2x \times 2x$ crosses. This has given rise to the *endosperm balance number hypothesis*, which assumes that normal endosperm development occurs only when two complete chromosome sets are from the female and one set is from the male. Any deviation from this ratio results in shriveled and hence aborted seeds.

The formation of $2n$ pollen from a diploid potato is under genetic control. Several meiotic mutants have been discovered that lead to the formation of $2n$ pollen with the most useful being the formation of parallel spindles controlled by the recessive *ps* gene. When unreduced gametes are formed from the diploid in the presence of the *ps* allele, it is genetically equivalent to a *first division restitution* mechanism (Fig. 5.6). The

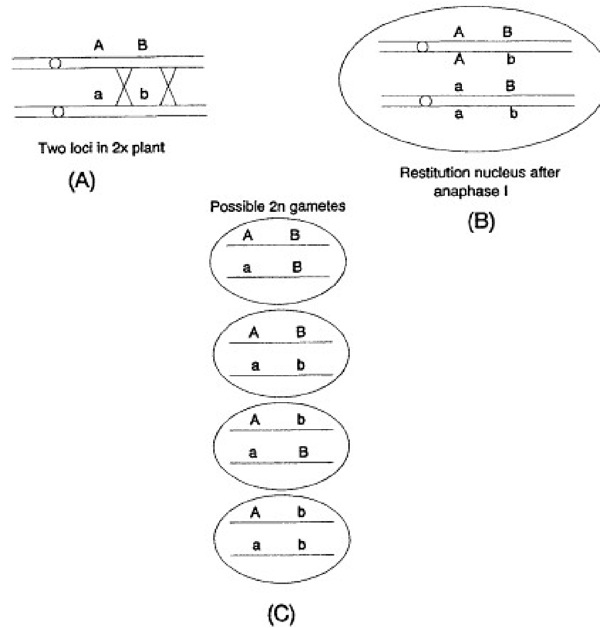


Fig. 5.6.
Formation of unreduced gametes by first division restitution. (A) A pair of homologous chromosomes with two chiasmata in a diploid parent which is heterozygous for all loci. (B) A restitution nucleus after anaphase I when the homologous chromosomes go to the same pole. (C) Unreduced (2n) gametes which are heterozygous from the centromere to the first chiasma and 50% which are heterozygous from the first chiasma to the second chiasma.

potato is an outcrossing species so we can assume that many loci will be heterozygous. If the diploid produces unreduced gametes through a first-division, restitution-like mechanism, all heterozygous loci from the centromere to the first crossover and one-half of the heterozygous loci between the first and second crossover in the $2x$ parent will be heterozygous in the gametes.

Formation of unreduced eggs is most commonly the result of omission of the second meiotic division which is genetically equivalent to a *second division restitution* mechanism (Fig. 5.7). The recessive gene *os* controls formation of unreduced gametes by this mechanism. The genetic significance of second division restitution is that all heterozygous loci from the centromere to the first crossover in the $2x$ parent will be homozygous, and those between the first and second crossover will be heterozygous in the resulting gametes.

Formation of unreduced gametes by first-division restitution and second-division, restitution-like mechanisms is significant because it allows the transfer of large portions of intralocus (*heterozygous*) and interlocus (*epistasic*) interactions from the $2x$ parent to

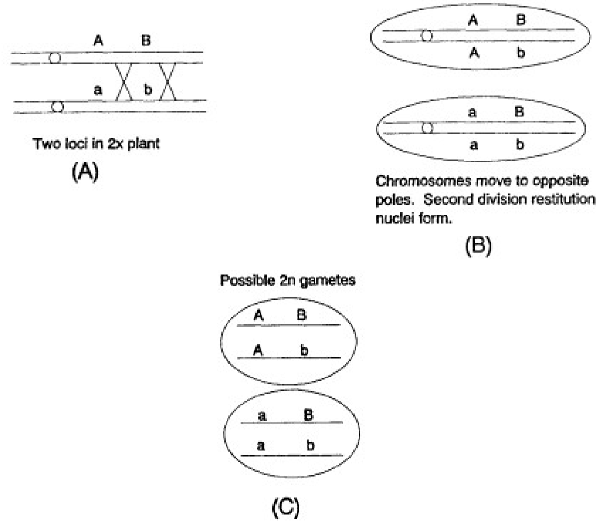


Fig. 5.7.
Formation of unreduced gametes by second division restitution. (A) A pair of homologous chromosomes with two chiasmata in a diploid parent which are heterozygous for all loci. (B) Chromosomes move normally to opposite poles at anaphase I. Second division is suppressed and each gamete will contain the two chromatids that went to the same pole at anaphase I. (C) Unreduced (2n) gametes where all loci are homozygous from the centromere to the first chiasma and all are 100% heterozygous from the first to the second chiasma.

the resulting 4*x* progeny. This is in contrast to normal meiosis in the 2*x* parent which would transfer little or no intralocus and interlocus interactions. It has been estimated that first division restitution can transfer 80% of the heterozygosity and a significant portion of epistasis from parent to progeny. Second division restitution is less efficient and transfers less than 40% of the heterozygosity of the 2*x* female parent to the 4*x* progeny. Both mechanisms allow the breeder to transfer desirable linkage groups and gene interactions intact from parent to offspring without having them broken up through normal meiosis. Several high yielding heterotic 4*x* potato cultivars have been released after manipulating ploidy levels through unreduced gametes from wild 2*x* parents. For a more detailed discussion on how unreduced gametes are used in potato improvement, see Chapter 21.

Study Questions

l. How do polyploids form in nature?

2. Which type of polyploid, autopolyploid, or allopolyploid would likely be most successful in nature? Why?

3. Define monoploid, haploid, and polyhaploid.

4. What are the important items that a breeder should consider before breeding at a higher ploidy level in a particular plant species?

5. What is the genetic significance of first and second division restitution?

6. If you wanted to develop fruits without seeds, could this be accomplished by manipulating ploidy levels? Why or why not?

7. Why do you think so many of our plant species are polyploids?

Further Reading

Asay, K.H., D.R. Dewey, F.B. Gomm, D.A. Johnson, and J.R. Carlson. 1985. Registration of 'Hycrest' crested wheatgrass. Crop Sci. 25:368-69.

Choo, T.M., E. Reinbergs, and K.J. Kasha. 1985. Use of haploids in breeding barley. p. 219-52. *In* J. Janick (ed.) Plant breeding reviews. Vol. 3. AVI Publ. Co., Westport, CT.

Dewey, D.R. 1984. Wide-hybridization and induced-polyploid breeding strategies for perennial grasses of the Triticeae tribe. Iowa State J. Res. 58:383-99.

Forsberg, R.A. (ed.) 1985. Triticale. CSSA Spec. Report No. 9. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Gallun, R.L., and F.L. Patterson. 1977. Monosomic analysis of wheat for resistance to Hessian fly. J. Hered. 68:223-26.

Hadley, H.H., and S.J. Openshaw. 1980. Interspecific and intergeneric hybridization. p. 133-59. *In* W.R. Fehr and H.H. Hadley (eds.) Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Harlan, J.R., and J.M.J. DeWet. 1975. On ö. Winge and a prayer: The origins of polyploidy. The Bot. Rev. 41:361-90.

Hermsen, J.G.Th. 1984. Nature, evolution, and breeding of polyploids. Iowa State J. Res. 58:411-20.

Law, C.N. 1981. Chromosome manipulation in wheat. p. 194-205. *In* M.D. Bennett, M. Bobrow, and G. Hewitt (eds.) Chromosomes today. Vol. 7. George Allen and Unwin, London.

Morris, R. 1983. Remodeling crop chromosomes. p. 109-29. *In* D.R. Wood (ed.) Crop

Breeding. Am. Soc. Agron., Crop Sci. Soc. Am., Soil Sci. Soc. Am., Madison, WI.

Peloquin, S.J., and R. Ortiz. 1992. Techniques for introgressing unadapted germplasm to breeding populations. p. 485-507. *In* H.T. Stalker and J.P. Murphy (eds.) Plant breeding in the 1990s. Redwood Press, Melksham, UK.

Sears, E.R. 1954. The aneuploids of common wheat. Missouri Agric. Exp. Stn. Res. Bull. 572.

Sharp, W.R., S.M. Reed, and D.A. Evans. 1984. Production and application of haploid plants. p. 347-81. *In* P.V. Vose and S.G. Blixt (eds.) Crop breeding, a contemporary basis. Pergamon Press, Oxford, UK.

Singh, A.K., J.P. Moss, and J. Smartt. 1990. Ploidy manipulations for interspecific gene transfer. Adv. Agron 43:199-240.

Thomas, H. 1993. Chromosome manipulation and polyploidy. p. 79-92. *In* M.D. Hayward, N.O. Bosemark, and I. Romagosa (eds.) Plant breeding: Principles and prospects. Chapman & Hall, London.

van Santen, E., P.M. Hugessen, and M.D. Casler. 1991. Sources and frequencies of 2n eggs and 2n pollen in diploid *Dactylis* subspecies. Genome 34:273-78.

6. Mutation

The Nature of Mutation

A mutation is a sudden change in the hereditary material of a cell. Mutation may be genic, involving deletions, or molecular changes within the physical limits of the gene; or chromosomal, involving the rearrangement, loss, or duplication of chromosome segments. In its broadest sense, mutation may include the loss or duplication of entire chromosomes. Most mutations are deleterious and harmful and many are lethal.

For a mutation to be detected some phenotypic change in the plant must occur. A visible change in a morphological characteristic—plant stature, pericarp color, leaf marking, chlorophyll deficiency, vestigial organ, endosperm texture, spike density, etc.—is most easily identified. In crop plants, the most extensive studies of visible mutants have been made in corn (maize) (Fig. 6.1). Mutations causing minute changes in quantitative plant characteristics, such as size, physiological activity, chemical content, or productivity, are more difficult to identify. Their effects may require exacting measurements, often on a population of plants rather than a single plant.

If there is a sudden phenotypic change in the progeny of a normally uniform line, it may be suspected that a spontaneous mutation has occurred. However, the event of finding a plant phenotypically different is not in itself sufficient evidence that there has been a mutation. The new phenotype may have arisen by segregation, in which a recombination of genes has given rise to the phenotypically different plant. In cross-pollinated crops it is exceedingly difficult to distinguish between spontaneous mutation and gene recombination, for recessive genes may be carried along, masked by a dominant allele in heterozygous plants, yet uncovered later by mating with another recessive allele. In self-pollinated crops, natural outcrossing and subsequent gene recombination may give rise to new phenotypes that may appear to be mutations. Procedures that assist in the identification of mutations include growing progeny of suspected mutants and observing whether segregation occurs, or crossing the suspected mutant back to the parent or to a plant with a known genotype, followed by selfing or sibbing of the progeny and observing the segregation. Segregation would not occur in the progeny of a true recessive mutant. In mutation breeding experiments it is essential that the plant materials be genetically uniform for the traits to be examined, and that pollination be rigidly controlled both prior to and during the experiment to prevent outcrossing and subsequent segregation.

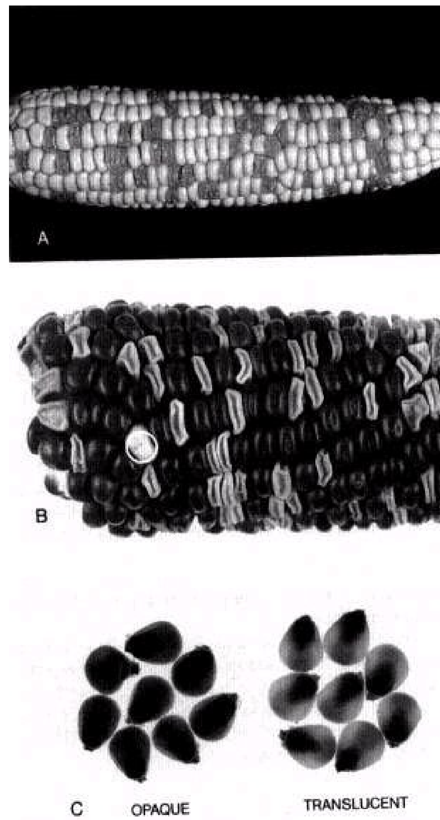


Fig. 6.1.
Mutants of maize. (A) Ear of corn segregating nonsugary, Su-(white, starchy kernels) vs. sugary, susu (wrinkled kernels). (B) Ear of corn segregating for purple-nonshrunken, A-Sh- vs. nonpurple-shrunken, aashsh. The genes for purple and shrunken are closely linked. Circled is a kernel produced from a crossover, nonpurple-nonshrunken, aSh/a-. (C) Kernels of corn with nonopaque (translucid) endosperm, O-, vs.opaque, oo endosperm. The sugary and shrunken kernels, plump, sweet, and watery at milk stage, collapse on drying. The opaque kernels are soft, floury, opaque with transmitted light, and high in lysine content.

Types of Mutations

Gene mutation may be either *recessive* (*A* to a) or *dominant (a* to A). The recessive gene mutation is by far the more common. If the recessive gene mutation occurs in the somatic tissue of a homozygous plant, its effects are not expressed until the following generation from seed produced on the portion of the plant in which the mutant allele is carried. This results because only one gene in the homozygote mutates (*AA* to *Aa*), and the dominant gene remaining in the heterozygote will mask the effect of the mutant recessive allele. After self-fertilization, segregation occurs giving rise to mutant plants (*aa*) in the next generation. A recessive gene mutation would be expressed immediately if both genes mutated simultaneously, but this occurrence would be extremely rare. Effects of a dominant gene mutation (*a* to *A*), also rare, may be observed immediately, because the developing somatic tissue (*Aa*) will express the dominant character. With self-fertilization it would give rise to segregation (1*AA*:2*Aa*:1*aa*) and the production of homozygous mutant plants in the progeny. When mutations occur in somatic tissue, only a small sector (*chimera*) of the plant, such as a tiller or a branch that develops from the cell in which the mutation occurs, will carry the mutant gene. The remainder of the plant will be unaffected. If a recessive mutation occurs in a gamete, and it joins with a gamete carrying a dominant allele, the seed subsequently formed will give rise to a heterozygous plant and segregation will be expressed in that plant's progeny.

Mutations may be identified according to their origin, whether *spontaneous* or *induced*. A spontaneous mutation is one that occurs in nature, while an induced mutation results from the action of a *mutagenic agent*. Spontaneous mutation is the mechanism by which new genetic traits arise in nature. The viable mutant forms then become recombined with existing forms, or they may be duplicated with changes in ploidy, and contribute to the evolutionary process in nature. There is no clear distinction between spontaneous and induced mutation. What appears to be a spontaneous mutation may have been induced, because all plants in nature are subjected to low dosages of natural radiation. Also, a mutation identified following treatment of plant materials with a mutagenic agent may happen to be a spontaneous mutation rather than the result of the mutagenic treatment.

In 1928 L.J. Stadler reported that the mutation rate in barley plants was increased after exposure of seeds to radiations from X rays and radium. In the previous year, H.J. Muller had used X rays to increase the mutation frequency in *Drosophila*, the common fruit fly. These findings immediately suggested that progress in plant breeding might be expedited by using induced mutations to expand the genetic variability of crop species. This approach to plant breeding is often referred to as *mutation breeding*.

Induction of Mutation

Mutagenic Agents

Ionizing radiations and chemical mutagens have been the principal agents employed to increase mutation frequency in plants. The radiations include X rays, neutrons, gamma rays, ultraviolet, and laser beams. X rays were used most extensively in early experiments because X ray equipment was widely available and easily operated, and seeds, plants, or pollen could be treated with fairly accurate doses. Neutron radiation became possible with the development of nuclear reactors. Neutron radiation produces more severe damage to the chromosomes than

X rays and is used principally with seeds. Gamma rays, emitted from radioactive cobalt or radioactive isotopes, cause less injury to the plant cells and are frequently used for radiation of whole plants or plant parts including pollen. The use of laser beams is a more recent event. *All kinds of radiation must be used with extreme caution and with experienced operators handling the equipment*.

The radiation dose is determined by the intensity of the radiations and length of the exposure. It is expressed in Roentgen (r) units, which are a measure of the number of ionizations that occur. If an ionization occurs in or near a chromosome, its force can split chemical bonds, causing various structural changes within the DNA, such as a change in a single nucleotide base of a gene (called a *point mutation*), replacement of one nucleotide base by another, or deletion of one or more bases in the DNA sequence. A gene mutation results from a change in the DNA within the structure of the gene. Chromosome mutations result from deletion of genes, changes in sequence of genes, rejoining of broken chromosome strands in the reverse order, or other aberrations.

Chemical mutagens are often preferred over radiation because they are simpler to apply and produce less damaging effects. The most widely used chemical mutagen is *ethyl methane sulfonate* (EMS), an alkylating agent. *Ethyl methane sulfonate is a powerful carcinogen and must be used with extreme caution*. Seeds, buds, roots, and dormant cuttings can be treated by soaking in a solution of the chemical mutagen, making treatment with EMS relatively simple; expensive X ray or other equipment is not needed. The precise concentration and treatment duration will vary with the plant part being treated.

Chemical mutagens are usually less drastic in their effects than ionizing radiations, producing more gene mutations and fewer chromosome disruptions. However, it is not possible to direct the mutation process so that a specific type of mutation can be produced.

Mutation Induction

For the induction of mutations in mutation-breeding experiments, seeds are commonly treated. Radiation treatment of dormant seeds has several advantages over treatment of other plant parts. Environmental factors such as moisture content, temperature, and oxygen level, which influence the effect of the treatment, are controlled more conveniently with seeds than with living plants. Large numbers of seeds can be treated at one time, and the treated seeds can be stored without injury to the seeds or to those handling the seeds. The mutagen dose administered should be sufficient to kill about 50% of the seeds to obtain the maximum number of mutations. With chemical mutagens, seeds are soaked in a solution of the mutagenic agent and planted immediately.

Various conventions are used to denote the generation following the use of a mutagenic agent. For example, a symbol may denote the specific mutagenic agent used, such as X for X rays, R for ionizing radiations, or C for chemical mutagens. Here, we shall use the broader term M to denote any mutagenic agent. For example, M_1 indicates the first generation after treatment with a mutagen, M_2 the second generation, and so on.

M_1 plants obtained from germinating treated seeds will be reduced in vigor, and many that survive until maturity will be sterile and will not produce seeds. In seeds of cereals like wheat, cells are present in the dormant embryo from which individual tillers originate. If a mutation occurs in one of these cells, the mutant gene will be carried in the tiller developing from that cell and in that tiller only (Fig 6.2). A dominant mutation will be expressed immediately in the tiller. A recessive mutation will not produce a visible effect in the tiller, but the progeny of that tiller will segregate for the mutant character. Because almost all mutations are recessive, the

M_2 generation becomes the segregating generation. Other tillers from the same plant will be unaffected unless another mutation occurs.

In addition to treating seeds, mutagenic agents may be used to treat pollen, living plants, or parts of a plant. If pollen is treated, and a recessive mutation induced, all parts or branches of the M_1 plant will be heterozygous for the induced mutation. This makes it unnecessary to harvest individual sectors of the plant separately, as was illustrated in Fig. 6.2. There are also disadvantages of treating pollen. In some species, it is difficult to obtain a sufficient supply of pollen for treatment, or the longevity of the pollen may be too short to survive the treatment. Pollen may be treated with either radiations or chemical mutagens. The dosage for treating pollen is less than for treating seeds.

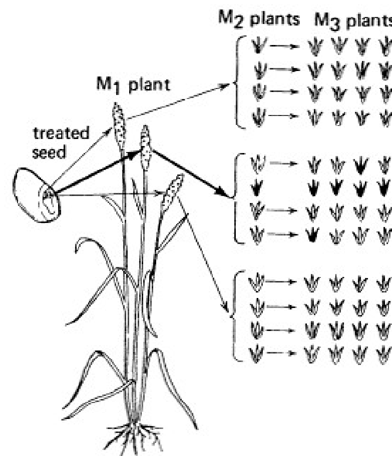


Fig. 6.2.
Identification of recessive mutations induced in seeds of wheat. Treatment of seeds of wheat with a mutagenic agent induces a recessive mutation in an embryo meristemic cell. A tiller that arises from the affected cell is heterozygous for the mutant gene. In the selfed progeny from this tiller (**M_2**), one plant out of four will exhibit the recessive mutant character; and seed from this tiller will produce only mutant plants in the **M_3**. In the same selfed progeny, two plants out of four are heterozygous; segregates from their progeny will exhibit the mutant form in the **M_3**. Other tillers and their progenies may be unaffected. The mutant plants are shown in black.

Mutations may be induced in plants that are vegetatively propagated, such as sugarcane, bananas, potatoes, bermudagrass, fruit crops, and ornamentals. Mutation breeding can be significant in creating new genetic variability for crops that reproduce vegetatively or by apomixis. Radiations are more commonly used with vegetatively propagated species than chemical mutagens. Any portion of the plant that may give rise to a bud may be treated with the mutagen-tubers, rhizomes, cuttings, grafts, shoot apices, or the base of the leaf petiole where adventitious buds arise when a leaf is removed from the plant. All of these plant parts carry multicellular meristematic tissue, which originated from a single meristem cell. A mutation produced in this single cell will normally be carried by all cells in the plant structure arising from that cell, whereas a mutation produced in a cell in the meristematic tissue will normally be propagated in sectors or chimeras. It is essential that treatment with a mutagen be made as early in the formation of the bud as practical, reducing the chances that sectoring will occur. Whole plants are most easily treated in a radiation field, although seedling plants may be treated with a chemical mutagen. In apomictic species, it is common to irradiate seeds.

Somatic Cell Culture Mutations

Somatic cell cultures originating from stems, leaves, floral organs, or meristematic tissue are novel sources of genetic variability. The excised cell tissue is cultured on a sterile nutrient

medium and by appropriate manipulation, the cells are induced to divide and to regenerate plants. Earlier, it was discovered that sugarcane clones regenerated through tissue culture techniques differed from the parental clones in morphological characteristics, disease resistance, and yield. Mutations have since been reported in tissue cultures of barley, wheat, rice, oat, tobacco, corn, and potato. The tissue culture-induced mutations are referred to as *somaclonal variations. The* rate of mutation may be quite high; but unfortunately, many somaclonal variants are not heritable and of little use in mutation breeding.

Some Useful Mutations in Plant Breeding

Mutation-breeding experiments have been conducted on many crop species. Examples cited here illustrate how spontaneous and induced mutants have been utilized successfully.

Mutations in Sorghum

The mutant dwarfing genes in sorghum are examples of spontaneous mutants that have been utilized extensively in plant breeding (Fig. 6.3). A dwarfed mutant plant was found in a farmer's field of 'Standard' milo. Seed from the dwarf plant was increased and gave rise to the 'Dwarf' milo cultivar. A second dwarfed mutant plant was observed in a field of 'Dwarf' milo and from it 'Double Dwarf' milo was developed. These two dwarf mutants, and dwarf mutant plants found later in other cultivars of milo and kafir, were utilized as basic breeding stocks in developing short cultivars that can be machine harvested and hybrids that are grown extensively in the United States. From these breeding stocks, dwarf sorghums have been developed for India, Africa, Central America, Argentina, and other sorghum-growing areas. Mutant dwarf forms of sorghum are exceptional because they are viable and productive, and facilitate mechanized harvesting of the grain sorghum crop.

Mutations in Rice

A successful mutation-breeding program has been conducted with rice, where an interesting and useful series of spontaneous and induced dwarfing mutations

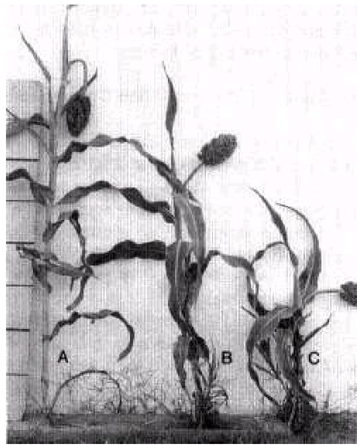


Fig. 6.3.
Mutants in sorghum. Milo cultivars recessive for one, two, and three genes for height. The cultivars and their respective genotypes are (A) 'Tall White Sooner' milo, $Dw_1Dw_1Dw_2Dw_2dw_4dw_4$;
(B) 'Dwarf White Sooner' milo, $dw_1dw_1Dw_2Dw_2dw_4dw_4$;
(C) 'Double Dwarf White Sooner' milo, $dw_1dw_1dw_2dw_2dw_4dw_4$.
A fourth height gene Dw_3 is dominant in the three cultivars. Most commercial grain sorghum hybrids grown in the United States are recessive for two or three of the four height genes.

have occurred. The resulting semi-dwarf mutant plant types are used extensively in plant breeding programs. The modern era of semi-dwarf cultivars in rice began in 1956 with the cultivar 'Taichung Native 1'. One parent of 'Taichung Native 1' was a spontaneous semi-dwarf mutant with a recessive semi-dwarfing gene named sd_1, which was found earlier in a farmer's field in Taiwan and named *Dee-geo-woo-gen(Dee-geo* means short-leg in Chinese). The sd_1 gene has since been used in breeding many semi-dwarf cultivars which contributed to the Green Revolution in the tropical and semitropical rice-growing regions of the world.

'Calrose 76', released in 1976 in California, was the first semi-dwarf cultivar released in the United States. It carries an induced semi-dwarfing gene sd_1 and has served as a donor for the development of other semi-dwarfing cultivars. The induced sd_1 locus, in addition to the semi-dwarfing effect on the rice plant, contributes to higher yields. An interesting feature of the California mutation experience is that a mutant semi-dwarfing gene induced by X rays is allelic to a mutant semi-dwarfing gene that occurred spontaneously in nature. In addition to the induced mutation at the sd_1 locus, non-allelic semi-dwarfing genes sd_2 *and* sd_4 have been discovered and inserted in rice cultivars.

Other important mutations in rice include a partially dominant gene for earliness and an induced mutant gene for waxy endosperm. The first waxy cultivar, 'Calmochi-201', was later recombined with the 'Calrose 76' mutant source of semi-dwarfism to produce the waxy, semi-dwarf cultivar, 'Calmochi-202'. Research is underway to exploit photosensitive genetic-male sterile mutants, other male sterility mutants, and perhaps, mutants for apomixis that would result in true breeding $\mathbf{F_1}$ hybrids.

Gene Substitution from Alien Chromosomes

Interspecific and intergeneric crosses are frequently attempted by the plant breeder to introduce a desirable character from closely related wild species into a cultivated species. Usually, the breeder wishes to transfer only a single gene from a wild species that bears the desired gene into a corresponding segment of a homologous chromosome from the cultivated species. In this exchange it is important that deleterious and undesirable genes should not be brought in with the desired gene, otherwise the yield and quality of the cultivated species may be impaired. A successful exchange might thus be limited to a segment of the chromosome bearing a single gene.

A radiation-induced gene substitution procedure was utilized by E.R. Sears to transfer a gene for leaf rust resistance from a diploid wild grass species (Fig. 6.5A), *Triticum umbellulatum* ($2n = 2x = 14$), to hexaploid bread wheat (Fig. 6.5C), *T. aestivum* ($2n = 6x = 42$) (Fig. 6.4). The wild grass was first crossed to tetraploid emmer (Fig. 6.5B), *T. turgidum* ($2n = 4x = 28$), and chromosomes of the $\mathbf{F_1}$ plant were doubled, and the allohexaploid ($2n = 6x = 42$) was then crossed to 'Chinese Spring', a hexaploid bread wheat. After backcrossing to 'Chinese Spring', leaf rust-resistant plants were identified that contained the 42 wheat chromosomes, plus one additional chromosome. The additional chromosome was suspected to be from *T. umbellulatum* and carrying the leaf rust resistance gene.

To induce a possible chromosome rearrangement, plants with the 43 chromosomes were X-rayed before flowering. The pollen subsequently formed was used to pollinate plants of 'Chinese Spring'. Among the offspring, one plant was found without the undesirable grass plant features, yet it retained the rust resistance. It appears that in this plant a short chromosome segment carrying the gene for rust resistance was transferred to a wheat chromosome (Fig. 6.4). There is no evidence that any of the deleterious genes from the grass chromosome were transferred to the wheat.

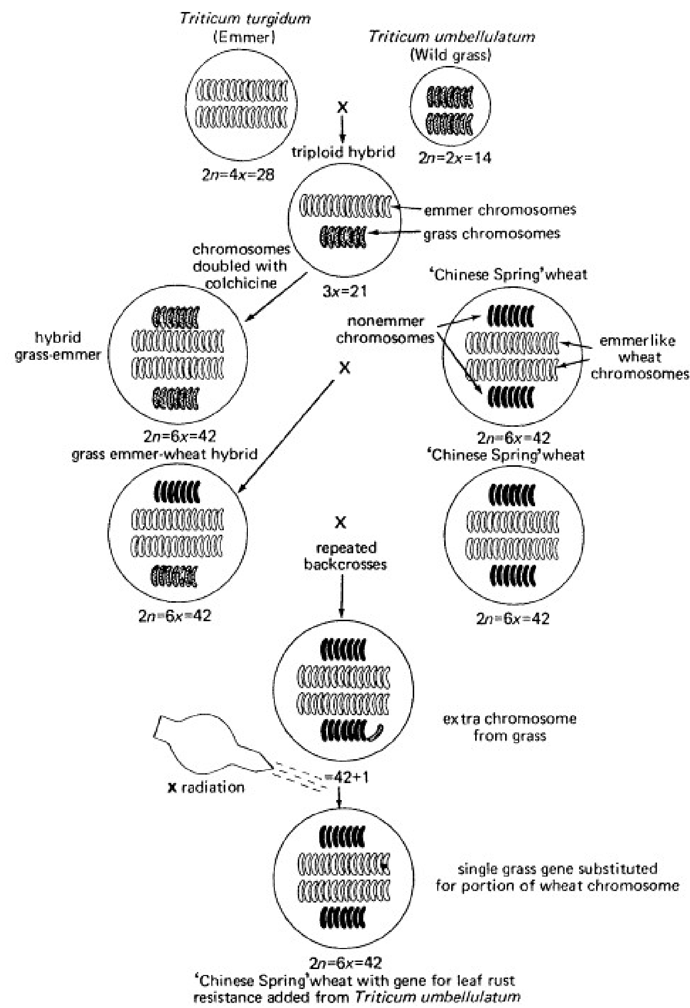


Fig. 6.4.
Radiation-induced gene substitution in interspecific hybridization. The procedure by which a gene for resistance to leaf rust was transferred from a wild grass, Triticum umbellulatum (2n = 2x = 14), to common wheat, Triticum aestivum (2n = 6x = 42). An allohexaploid was made by combining the chromosomes from emmer (2n = 4x = 28), a close relative of common wheat, with those of T. umbellulatum. The resulting allohexaploid (2n = 6x = 42) was crossed with 'Chinese Spring' a cultivar of common wheat. Repeated backcrossing of rust-resistant plants to 'Chinese Spring' gave rise to a plant with the chromosome content of common wheat, plus one chromosome from the wild grass, which carried the gene for rust resistance. By the use of X rays a chromosome rearrangement occurred in which the gene for rust resistance was transferred to a wheat chromosome.

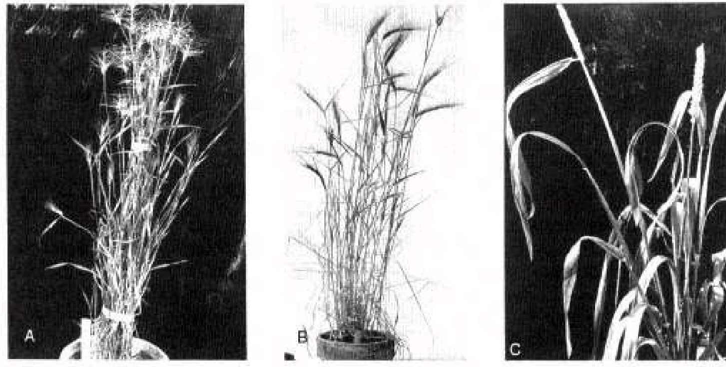


Fig. 6.5.
Parent species used in crosses shown in Fig. 6.4. (A) Wild grass (Triticum umbellulatum). (B) Emmer (Triticum turgidum). (C) 'Chinese Spring' wheat (Triticum aestivum).

The leaf rust-resistant spring wheat cultivar resulting from the X-ray-induced translocation of a gene from *T. umbellulatum* into common wheat, *T. aestivum*, was named 'Transfer' and later used as a source of resistance to leaf rust in the production of several commercial wheat cultivars. Similar techniques have since been used to transfer stem and leaf-rust resistance genes from wild species to common wheat. This experiment represents the transfer of a spontaneous gene for rust resistance already available in nature into a bread wheat chromosome; a new gene was not induced.

Role of Mutation Breeding

The role of mutation breeding as a supplement to conventional recombination breeding procedures has been under consideration since Muller and Stadler presented evidence that heritable changes in animals and plants could be induced. Stadler was less enthusiastic than Muller about the production of mutations useful to the plant breeder, due to the deleterious effects from ionizing radiations that accompany the mutation. According to Stadler (*J. Heredity* 21:3-19, 1930), the use of induced mutations in plant breeding has been much overrated. A more optimistic view of mutation breeding was taken by early Swedish workers at the Svalöf (Sweden) Plant Breeding Research Station. As a breeding procedure, mutation induction has been controversial due to many early extravagant claims.

The primary purpose of mutation breeding is to increase the genetic variability available to the plant breeder, but its practical utilization presents a dilemma. It is well established that exposure to mutagenic agents will increase mutation frequency, but will the heritable changes produced contribute to the breeding of improved crop cultivars? A precise answer is difficult to give because the evidence is not overwhelming. One can only conclude that the results from

mutation breeding in cultivar development have been rather meager in relation to the effort expended.

Mutation has been a dominant force in the evolutionary process. Since prehistoric times, vital mutants have been propagated in nature and exploited. This led to the development of genetically stable and agronomically productive forms of many crop species. Mutation breeding is designed to increase the rate of induction of new desirable mutant forms. In the artificial induction of raw mutants, there is need for rigorous screening comparable to the natural processes that eliminated mutants grossly unfit for survival or those induced in incompatible backgrounds. While choice of mutagenic agent may influence the class of mutations produced, it cannot be directed toward obtaining a specific beneficial mutation. *Success in mutation breeding would appear to be greatest when the breeder is looking for a specific mutant not already available and has screening procedures to identify the mutant plant if the mutation is obtained. Usually, hybridization will be needed to transfer the mutant character into a balanced and stable genotype before it can be utilized in a new cultivar*.

Study Questions

1. Why is it that mutation breeding hasn't led to the development of many more improved cultivars?

2. How does the plant breeder induce mutations? Which method do you prefer and why?

3. If you isolated two mutants with similar phenotypic expressions from two different parental sources, how would you determine if the mutants were the result of modification at the same locus or different loci?

Further Reading

Gustafsson, A. 1986. Mutation and gene recombination—principal tools in plant breeding. p. 76-84. *In* G. Olsoon (ed.) Svalöf 1886-1986: Research and results in plant breeding. Svalöf AB, 268 00 Svalöf, Sweden.

Hu, C.H. 1991. Use of an induced semi-dwarfing gene to alter the rice plant type and cultural breeding practices for sustainable agriculture. Proc. of an Inter. Symp. on Contributions of Plant Mutation Breed. to Crop Imp. Vol. I. IAEA, Vienna, Austria.

Maluszynski, M. 1990. Induced mutations—an integrating tool in genetics and plant breeding. p. 127-62. *In* J.P. Gustafson (ed.) Gene manipulation in plant improvement. II. Plenum Press, New York, NY.

Micke, A., and B. Donini. 1993. Induced mutations. p. 52-62. *In* M.D. Hayward, N.O. Bosemark, and I. Romagosa (ed.) Plant breeding, principles and prospects. Chapman & Hall, New York, NY.

Novak, F.J. 1991. In vitro mutation system for crop improvement. Proc. of Inter. Symp. on Contributions of Plant Mutations Breed. to Crop Imp. Vol. II. IAEA, Vienna, Austria.

Rutger, J.N. 1991. Mutation breeding of rice in California and the United States of America. Proc. of Inter. Symp. on Contributions of Plant Mutations Breed. to Crop Imp. Vol. I. IAEA, Vienna, Austria.

Sears, E.R. 1956. The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp. Biol. 9:1-21.

Sigurbjornsson, B. 1983. Induced mutations. p. 153-76. *In* D.R. Wood (ed.) Crop breeding. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

van Harten, A.M. 1989. Induced mutations in vegetatively propagated crops. p. 55-91. *In* J. Janick (ed.) Plant breeding reviews. Vol. 6. Timber Press, Portland, OR.

7. Fertility-Regulating Mechanisms and Their Manipulation

An important concern of plant breeders is the extent to which a crop species will set seed. Either failure to be self-fertile or failure to be cross-fertile, depending upon the breeding system of the particular species involved, may curtail procedures for obtaining gene recombinations and limit seed production. Self-pollinated species, such as wheat, oat, barley, rice, and soybean, and some cross-pollinated species, such as corn, set seed freely after self-pollination, or after cross-pollination between genotypes within the species. Cross-pollinated species, such as the clovers, alfalfa, sweetclover, rye, sugar beets, and many perennial grasses, have reduced seed set, or fail to set seed, after self-pollination, although they may set seed freely after cross-pollination with other strains within the species. In addition to the problem of obtaining seeds after self- and cross-pollinations within species, the plant breeder is concerned about the extent to which seeds will be set when crosses are made between closely related species, or closely related genera. Amount of fertility in interspecific crosses determines how extensively desirable genes from closely related species may be utilized in a recombination-breeding program.

The term sterility covers those cases of infertility or barrenness resulting from irregularities in the sexual reproductive system. Infertility may be caused by abnormal or imperfect development of the reproductive organs. The stamen or pistil may be realformed, the pollen defective, or the ovules aborted. Infertility may result from failure of viable pollen to function after germination. The pollen tube may not penetrate the stigmatic surface, or pollen tube growth in the style may be reduced so that the sperm cell does not reach the ovule so fertilization can occur. Even though fertilization occurs, the embryo or endosperm may not develop normally to produce a viable seed. After seed formation, infertility in the hybrid plant may result from chromosome irregularities at meiosis.

Regardless of the specific cause, infertility is a hindrance that should be understood and, if possible, overcome if the breeder is to obtain maximum gene recombination through intraspecific and interspecific crosses. Understanding the nature of fertility problems will aid

in devising procedures for (1) overcoming infertility barriers, or (2) manipulating fertility regulating mechanisms and utilizing them to advantage.

Incompatibility

Incompatibility is a form of infertility caused by the failure of plants with normal pollen and ovules to set seed due to some physiological hindrance that prevents fertilization. Incompatibility may be caused by failure of the pollen tube either to penetrate the stigma, or to grow normally the full length of the style, so that fertilization may occur. In the latter, the pollen tube, if formed at all, grows so slowly that it may never reach the ovule; or if it does, it will be so late that the ovule either will have been pollinated by compatible pollen or will have withered. Incompatibility restricts self-fertilization and inbreeding and fosters cross-fertilization and outbreeding.

Incompatibility is widespread and present in species of *Leguminosae, Solanaceae, Cruciferae, Compositae, and Gramineae*. In cultivated crops, incompatibility is found in red clover, alsike clover, white clover, alfalfa, tall fescue, ryegrass, rye, sugar beets, sunflower, pearl millet, tobacco, potatoes, bahiagrass, bermudagrass, and others.

Incompatibility Systems

Incompatibility systems are of two types, *gametophytic and sporophytic*. Gametophytic incompatibility is found in clovers, grasses, sugar beets, potatoes, and tobacco. Rate of pollen tube growth is controlled by a series of multiple alleles in the gametophytic system, which are designated S_1, S_2, S_3, and so on. Because the pollen nucleus is haploid it will contain only one of the incompatibility alleles. The stylar tissue, originating from the mother plant, is diploid and will contain two incompatibility alleles. If the incompatibility allele in the pollen nucleus is identical with either of the alleles present in the stylar tissue, pollen tube growth in the style will be slowed and fertilization will seldom occur. If the incompatibility allele in the pollen nucleus differs from the alleles in the stylar tissue, the pollen tube will grow at a normal rate and fertilization will normally occur (Fig. 7.1). If a plant with the genotype S_1S_2 is self-pollinated or pollinated from another plant with the S_1S_2 genotype, the pollen nucleus will contain either an S_1 or an S_2 allele. Because both alleles match an allele in the stylar tissue, the pollen tube will rarely penetrate the style far enough to reach the ovule in time for fertilization (Fig. 7.1A). If a plant with the S_1S_2 genotype is pollinated with pollen from a plant with an S_1S_3 genotype, only the pollen tubes containing the S_3 alleles will fertilize the ovule (Fig 7.1B). If a plant with the S_1S_2 genotype is pollinated with pollen from a plant with an S_3S_4 genotype, the pollen tubes containing either the S_3 or the S_4 allele may penetrate the style in a normal manner and effect fertilization (Fig.7.1C). A genotype homozygous for the S alleles (for example S_1S_1) would not normally occur because S_1 pollen would not penetrate an S_1 style so that fertilization would be effected.

The effect of the incompatibility alleles is not so great as to prohibit self-fertilization entirely, for most species an occasional seed may set from pollen carrying the same allele that is present in the stylar tissue. This condition is referred to as *pseudo-self compatibility*. The amount of pseudo-self-compatibility may be modified by environmental factors such as temperature, mutation, or perhaps modifying genes. In addition, *self-fertility alleles (S_f)* may be present, which render the alleles for incompatibility ineffective. The S_f allele is a part of the

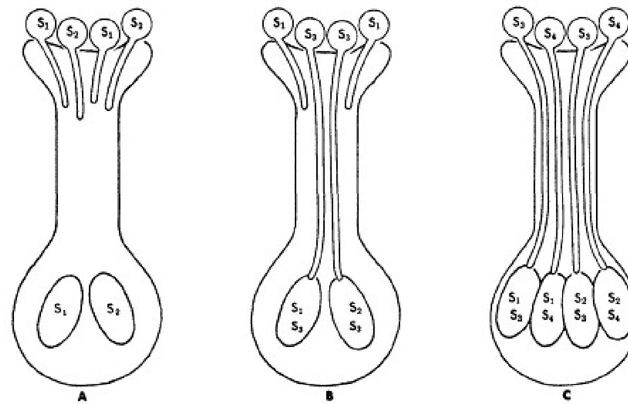


Fig. 7.1.
Gametophytic system of self-incompatibility showing the pollen tube growth in compatible and incompatible pollinations. (A) Pollen tubes do not grow in styles carrying similar alleles for incompatibility. (B) Only pollen grains with different incompatibility alleles from those in the style develop normal pollen tubes. (c) All pollen grains carry different incompatibility alleles from those in the styles and develop normal pollen tubes.

S allele series and may arise by mutation from an S allele. Sometimes, incompatible diploid species become self-compatible with induction of polyploidy, yet some polyploid species, like white clover, possess alleles for self-compatibility.

Because the incompatibility allele in the style opposes the penetration of pollen tubes with the same allele, this explanation of incompatibility was called the *oppositionalfactor hypothesis* by East and Mangelsdorf, who used it to explain self-sterility in tobacco (*Nicotiana* spp.). The genetics of incompatibility on the one-locus system discussed above has been described in detail in tobacco and in red, white, and alsike clovers. The number of incompatibility alleles within a species may be rather large so that cross-pollinations occur freely. In red clover 41 alleles were identified in a sample of 25 plants. In white clover at least 64 alleles at the S locus have been identified.

The genetics of incompatibility in grasses differs by having a two-loci system, each with multiple alleles. The loci involved have been designated *S* and *Z*. If the alleles at both the *S* and Z loci in the pollen are identical with the *S* and *Z* alleles in the style, the mating will be incompatible. If either the *S* or *Z* allele does not have a matching allele in the style, the mating will be compatible. The two-loci system results in about 5 to 10% more compatible matings than the one-locus system in tobacco and clover. The two-loci system has been described in detail for rye and a few pasture grasses, but is believed to be generally distributed throughout the grass family. A four-loci system, working on the same principle as the S and Z system in grasses, has been described in sugar beets.

The sporophytic system of incompatibility is a one-locus system with a large number of multiple S alleles. It differs from the gametophytic system in that the S alleles exhibit dominance, the dominance being determined by the plant producing the pollen. As an example,

if a plant has an S_1S_2 genotype and S_1 is dominant to S_2, then all of the pollen from that plant will function as if it were S_1; and pollen with either S_1 or S_2 alleles will be incompatible in the S_1 style, but will be compatible in an S_2 style. The genetic combinations from the sporophytic system are numerous and complex. In this system hindrance to pollen germination or pollen tube growth is localized in the surface of the stigma, in contrast to the gametophytic system in which the hindrance to pollen tube growth is in the style. Another feature by which the sporophytic system differs from the gametophytic system is that plants may be produced that are homozygous for an *S* allele, either by bypassing the self-incompatibility barrier or through pseudo-self-incompatibility. This feature has been utilized in the production of hybrids in self-incompatible species.

The sporophytic system is found in sunflower, cabbage, broccoli, cacao, buckwheat, and other species of dicotyledons but has not been found in monocotyledons. In several *Brassica* species (cabbage, kale, brussels sprouts), numerous methods have been used to overcome the incompatibility barrier on the stigma surface. These methods include bud pollination, rupturing the stigmatic surface, grafting, electric shock, and increasing the CO_2 concentration. In bud pollination, the barrier is bypassed by placing the pollen on an immature stigma that has not yet developed the incompatibility barrier.

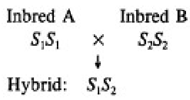
The two incompatibility systems described are *homomorphic*, meaning that the flowering structures are similar in both the pollen-bearing and seed-bearing plants. A *heteromorphic* system, in which the pollen- and seed-bearing flower structures differ has been identified in some species of plants including buckwheat; it is not found in any of the major cultivated crop plants. Seeds are produced only when plants differing in flower structure are crossed.

Incompatibility and Plant Breeding

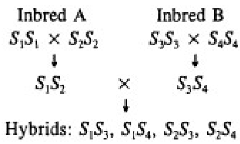
Although incompatibility may hinder the breeder's ability to self-pollinate and produce inbreds in self-incompatible species, it is being utilized to facilitate crossing between self-incompatible lines for the production of hybrid seed. Several systems of utilizing self-incompatibility genes in the production of hybrid seed are being used or have been proposed. The incompatibility system provides a means of controlling pollinations in some species where other means, such as male sterility, are not available. The systems that have received most attention are as follows:

CROSS-POLLINATION OF VEGETATIVELY PROPAGATED, SELF-INCOMPATIBLE CLONES. This is perhaps the simplest procedure and was utilized in the production of 'Tifhi' hybrid bahiagrass. Two clones that were self-incompatible, yet cross-compatible, were established in adjacent strips in the field by vegetative propagation. Seed was produced from cross-pollinating species that had the gametophytic type of incompatibility.

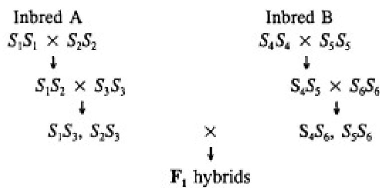
SINGLE-, DOUBLE-, AND TRIPLE-CROSSES. This system has been developed in the *Brassicas* (cabbage, kale, swedes), which have the sporophytic incompatibility system. The dominance feature of that system makes it possible to produce genotypes homozygous for the S alleles (S_1S_1, S_2S_2, etc.). Seed for maintenance of the homozygous genotypes is produced by bud pollination. The single-cross system requires two self-incompatible, cross-compatible inbreds, each homozygous for an *S* allele:



The single-cross system requires that a large amount of inbred seed be produced by bud pollination for commercial production. To overcome this objection, a *double-cross* system may be utilized, which provides one generation of single-cross seed increase. The double-cross system increases the ratio of hybrid seed from a given amount of inbred seed. The double-cross system requires two isogenic, self-incompatible, cross-compatible lines of each inbred, each homozygous for a different incompatibility *S* allele as follows:



To increase hybrid seed production in relation to inbred seed further, a *triple-cross* system was proposed. The triple-cross requires three homozygous genotypes for each inbred and permits one additional generation of seed increase. The procedure follows:



UTILIZATION OF S_f ALLELES AND PSEUDO-SELF-COMPATIBILITY. Self-incompatibility of the gametophytic type has been utilized in breeding hybrid sugar beets and proposed for hybrid red clover. In sugar beets, some seed production is usually obtained on self-incompatible inbreds when grown at high altitudes; or a self-fertility (S_f) allele may be introduced into the inbred to facilitate its maintenance. The inbreds are then utilized in production of either single or three-way crosses. In red clover, hybrid seed production utilizing pseudo-self-compatible inbred lines has been proposed. The procedure is identical to the double-cross system outlined above, except that pseudo-self-compatibility is used to produce the inbred lines. The pseudo-self-compatible inbred lines would be obtained using high temperatures, mutation, or other means not clearly described. The difficulty in a crop like red clover lies in obtaining inbred lines that are both pseudo-self-compatible and vigorous enough to use in commercial seed production fields.

The hybrid breeding systems outlined above were developed to utilize self-incompatibility as a means of managing pollinations for production of hybrid seed. All are difficult to

manipulate and would be abandoned in any crop in which a manageable form of cytoplasmic male sterility could be developed.

Male Sterility

When sterility is due to the failure of functional anthers or pollen, it is termed *male sterility*. *Female sterility* is failure to produce functional ovaries or eggs. Generally, female sterility systems have been less stable and dependable than male sterility systems. In male-sterile plants, flowers do not produce functional anthers or viable pollen, but ovaries function normally. Although the flowers cannot be self-pollinated, they can be cross-pollinated. This makes the male-sterile system useful to the plant breeder. If normally self-pollinated plants are male sterile, cross-pollinations can be made without the laborious task of emasculation.

Genetic Male Sterility

Genetic male sterility is manifested through the action of nuclear genes inhibiting normal development of anthers and pollen (Fig. 7.2). The precise stage at which pollen development is interrupted may differ with the species, or with the specific male sterility gene. Effectiveness of a male-sterile gene may be measured by percentage of pollen grains that are viable, or percentage of seed set. The expression of a particular gene may be complete, so that there will not be any viable pollen or seed set in male-sterile flowers provided they have been protected to exclude pollen from external sources; or the expression of the gene may be partial, permitting small amounts of viable pollen and seed set. The expression of the gene may also vary with the environment. Unless a male-sterile gene will inhibit virtually all seed production and is stable in a wide range of environments, its utility in plant breeding programs would be limited.

MALE-STERILE GENES. Genetic male sterility is predominantly conditioned by a recessive allele, *ms*. The dominant allele, *Ms*, resulted in production of normal anthers and pollen. For the diploid species, the *msms* genotype would be male-sterile while the *MsMs* and *Msms* genotypes would be male-fertile. In tetraploid alfalfa, inheritance of male sterility was reported to be controlled by two independently inherited genes; presumably the genes are present in different genomes.

Maintenance of the male-sterile gene within a population poses some problems. A pure population of genetic male-sterile plants cannot be produced, but male-sterile genes may be carried along at a high frequency in a self-pollinated crop if seeds from the male-sterile plants only are harvested and used to plant the next generation. The seeds harvested from male-sterile plants (rosins) may be pollinated by either homozygous (*MsMs*) or heterozygous (*Msms*) male-fertile plants. If pollination is by the latter, the progeny will segregate 50% *Msms*: 50% *msms* (Fig. 7.3). If a male-sterile plant (*msms*) is pollinated by a homozygous male-fertile plant (*MsMs*), all $\mathbf{F_1}$ plants will be heterozygous and male-fertile (*Msms*) *as* noted above, but the $\mathbf{F_2}$ generation will segregate 25% *MsMs*: 50% *Msms*: 25% *msms*. The proportions of male-fertile and male-sterile plants in succeeding generations can be predicted from the proportions of pollen grains with *Ms* genes vs. those with ms genes. In the $\mathbf{F_2}$, 66.6% of the pollen cells will be *Ms* and 33.3% will be *ms*. Random mating of male gametes in these proportions with ms eggs will result in 66.6% of the $\mathbf{F_3}$ population being heterozygous and 33.3% being

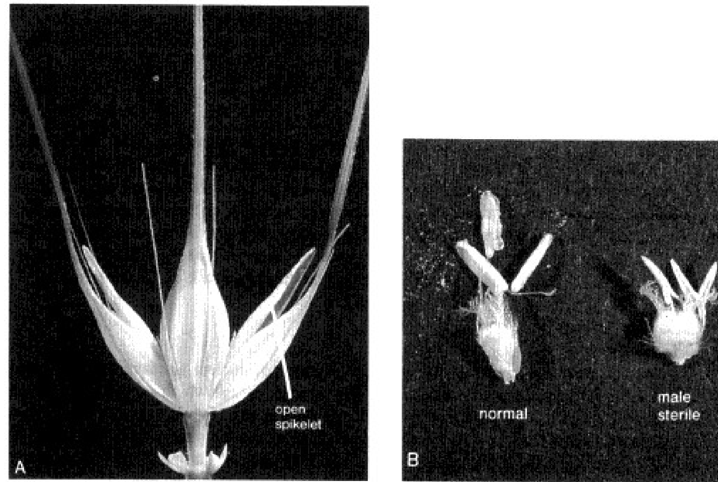


Fig. 7.2.
Male sterility in barley. (A) Open spikelets. In a male-sterile flower of barley, the spikelets remain open unless the stigma is pollinated by foreign pollen. (B) Normal and male-sterile flowers of barley. Anthers of normal flower have dehisced and pollinated the stigma. Anthers of male-sterile flowers do not produce pollen. Note size of sterile anthers in comparison with size of anthers in normal flower.

homozygous recessive and male sterile. Because all of the male-fertile plants in the F_3 will be heterozygous, 50% of the pollen grains will carry the dominant gene and 50% the recessive gene; and this proportion would be maintained in future generations.

UTILIZATION IN A BREEDING PROGRAM. Male sterility genes have been identified in barley, corn, cotton, flax, pearl millet, potato, rice, sorghum, soybean, tobacco, wheat, and other crops. Genetic male sterility is a useful tool for the plant breeder and may be utilized in the following ways:

Eliminate emasculation in hybridization. Elimination of emasculation in self-pollinated crops is the major utility of genetic male sterility. Emasculation in a hybridization breeding program in self-pollinated crops is laborious and time-consuming. If a male-sterile cultivar can be used as the female parent, emasculation is unnecessary.

Male-sterile genes may be transferred to a cultivar by backcrossing. Backcrossing the gene into a cultivar would be practical if the cultivar is to be used in a large number of crosses for several years. Because the backcrossing procedure itself is time-consuming and takes several generations, the additional labor and the delay caused by making the backcross would not be justified unless the cultivar was to serve as a parent in many crosses. In barley, over 100 spring and winter cultivars have been convened to male sterility and are available from the

U.S. Department of Agriculture to breeders who wish to use them. A genetic male-sterile line is maintained through pollination by a line with an identical genotype except for having the dominant allele for male sterility.

Increase natural cross-pollination in self-pollinated crops. Male-sterile genes provide a mechanism for increasing cross-pollination in normally self-pollinated crops. With hand pollinations, the breeder is limited, depending upon the available resources, in the number of cross-pollinations that can be made within a single season. By using male-sterile genes, the potential for obtaining cross combinations is greatly increased, particularly for random crossing among segregating generations. Procedures that have been used in barley in the production of diverse populations will serve as models. Although the procedures have varied, in general they fall into two groups. In the first, a large number of strains are crossed, facilitated by genetic male sterility. The progenies are bulked. Through segregation, recessive male-sterile plants will appear in each succeeding generation and will be naturally cross-pollinated from a wide assortment of genetically different plants. With cross-pollination of the male-plants, a far wider assortment of cross-combinations will be obtained than could have been made by hand-pollination. The second procedure utilizes the male sterility to facilitate recurrent selection. After the original crosses have been completed, *desirable male-sterile plants are selected from the segregating populations and cross-pollinated with pollen from selected male-fertile plants*. Subjecting the segregating populations to environmental stresses such as drought or disease would foster the identification and selection of superior segregates for those characters; and genetic recombination for the character under consideration would be enhanced.

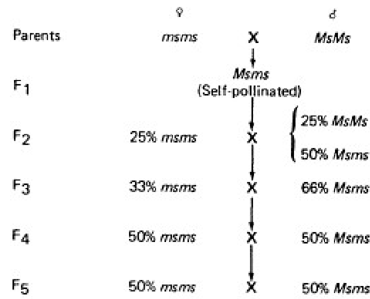


Fig. 7.3.
Proportions of male-sterile (msms) and male-fertile (MsMs, Msms) plants maintained after a cross, with male-sterile plants only being harvested in the F_2 and succeeding generations.

Facilitate commercial hybrid seed production. Hybrid cultivars are grown in corn, sorghum, sugar beets, pearl millet, and other crops. Production of hybrid seeds requires a mechanism for pollination control. Cytoplasmic male sterility has provided the mechanism in many crops where hybrid seeds are produced commercially. Procedures utilizing genetic male sterility have been proposed for several crops in which cytoplasmic sterility is not available, or in which difficulties have arisen with the cytoplasmic sterility procedures. The difficulty with genetic male sterility is that a pure population of male steriles cannot be produced by normal crossing procedures. In sorghum a proposal was made that required roguing out all male-fertile plants before pollen was shed. This system was supplanted when cytoplasmic male sterility became available in sorghum. In corn, barley, and wheat, chromosomal manipulations have been proposed to permit production of a completely sterile population or to carry informational genes that would identify the male-sterile plants. The systems for producing hybrid seed with genetic male sterility are more complicated than systems utilizing cytoplasmic sterility and fertility-restoring genes. This has restricted their utilization.

New hybrid rice cultivars are produced in China by using a photoperiod-sensitive genic male-sterile mutant. Research has shown that the photoperiod-sensitive genic male-sterile

character is controlled by two pairs of major recessive genes and also affected slightly by the presence of other recessive genes. The rice plant is a short-day plant. When the mutant is present, male-sterility occurs under a long-day photoperiod whereas normal fertility occurs under a short-day photoperiod. The original mutant was discovered in the cultivar 'Nongken 58' which is a late japonica rice.

Cytoplasmic Male Sterility

Cytoplasmic male sterility is controlled by the cytoplasm, but may be influenced by genes in chromosomes. Like genetic male sterility, it results in the production of flowers with nonfunctional anthers or pollen (Fig. 7.4). Cytoplasm that causes an organism to be male sterile may be referred to as *sterile cytoplasm* (S) or (**CMS**), in contrast to *normal cytoplasm* (**N**), which permits normal development of functional anthers and pollen. The sterile cytoplasm often results from the introduction of nuclear chromosomes into a foreign cytoplasm. For example, cytoplasmic male sterility in sorghum was obtained by transferring kafir chromosomes into cytoplasm of milo. Male sterility is not expressed when the milo chromosomes are in milo cytoplasm. The kafir chromosomes were introduced into milo cytoplasm by pollination of a milo plant with kafir pollen, and successively crossing the progeny as the female back to kafir as the male, until the entire set of kafir chromosomes were recovered. Similar procedures

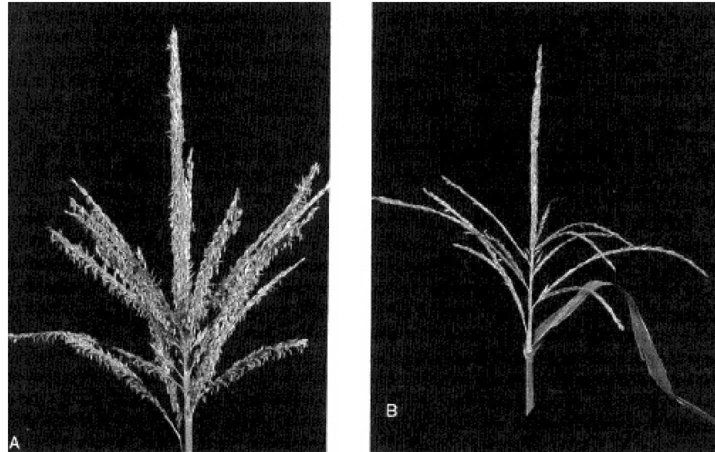


Fig. 7.4.
Cytoplasmic male sterility in corn. (A) Tassel of male-fertile corn plant. Note anthers exerted from staminate flowers on tassel. (B) Tassel of male-sterile corn plant. Cytoplasmic male sterility is used in hybrid seed corn production.

for transferring chromosomes into foreign cytoplasm have been used to obtain cytoplasmic male-sterile wheat and other crops. Because the cytoplasm is transferred through the egg only, the sperm contributing an insignificantly small bit of cytoplasm to the zygote, cytoplasmic male sterility is transmitted only through the female plant. In addition to the procedure of transferring chromosomes into foreign cytoplasm, cytoplasmic male sterility has been induced in pearl millet by soaking seeds in solutions of the antibiotics metamycin and streptomycin.

HOW CYTOPLASMIC STERILITY WORKS. The action of cytoplasmically controlled male sterility may be modified by the action of *fertility-restoring genes* located in the chromosomes. In the presence of a dominant fertility-restoring allele, the sterile cytoplasm becomes inoperative and the anther produces normal pollen; in the presence of the contrasting recessive alleles, male sterility is expressed. In practice, the parent with the sterile cytoplasm necessarily is used as the female and the fertility-restoring genes are contributed by the male parent. The fertility-restoring alleles have been variously indicated by the symbols *Rf* (fertility-restoring) in wheat, corn, and sunflower, and *Ms* (male-sterile) in onions, sorghum, and pearl millet.

The nuclear genes and cytoplasm interact to produce male-sterile and male-fertile plants (Fig. 7.5). Plants with sterile cytoplasm and recessive fertility-restoring genes (CMS, *rfrf*) are male sterile. Plants with sterile cytoplasm and dominant fertility-restoring genes (CMS, *RfRf* or CMS, *Rfrf*), or normal cytoplasm and either dominant or recessive fertility-restoring genes (N, *RfRf*; N, *Rfrf*; or N, *rfrf*) are male fertile. Assuming that one fertility-restoring gene will function to restore fertility, male-sterile plants may have three kinds of progeny, according to the genotype of the pollinator.

CMS, *rfrf* × **N** or **CMS**, *RfRf* ⟶ **CMS**, *Rfrf* (all male fertile)

CMS, *rfrf* × **N** or **CMS**, *Rfrf* ⟶ { 50% **CMS**, *Rfrf* (male fertile); 50% **CMS**, *rfrf* (male sterile) }

CMS, *rfrf* × **N**, *rfrf* ⟶ **CMS**, *rfrf* (all male sterile)

Although one dominant fertility-restoring gene was used in the above example, two dominant genes are required to restore fertility to cytoplasmic male-sterile corn and wheat, and modifier genes are commonly necessary in addition to obtain complete fertility restoration in a wide range of environments.

The interaction of nuclear genes and cytoplasm was first described for the 'Italian Red' 13-53 cultivar of onion and utilized in the production of hybrid onion seed (Fig. 7.6). The original 'Italian Red' 13-53 male-sterile line could be propagated from head sets or bulbils, or by backcrossing to an identical genotype that has normal cytoplasm and recessive fertility-restoring genes. In production of the hybrid onion it was unnecessary to restore fertility to the hybrid because the onion bulb rather than seed was being harvested. In wheat, cytoplasmic sterility was obtained by introducing genes of bread wheat, *Triticum aestivum*, into cytoplasm of *T. timopheevii*. Several sources of cytoplasmic male sterility and fertility-restoring genes are known for maize and wheat. Most cytogenetically distinct species of *Triticum* have different cytoplasms. In maize, a sterile cytoplasm (designated S-cytoplasm) has been identified in which the recessive (*rf*) genes are not transmitted through the pollen. The male-sterile maize with this particular cytoplasm is pollinated with pollen from *Rfrf* plants; only pollen with *Rf* genes will

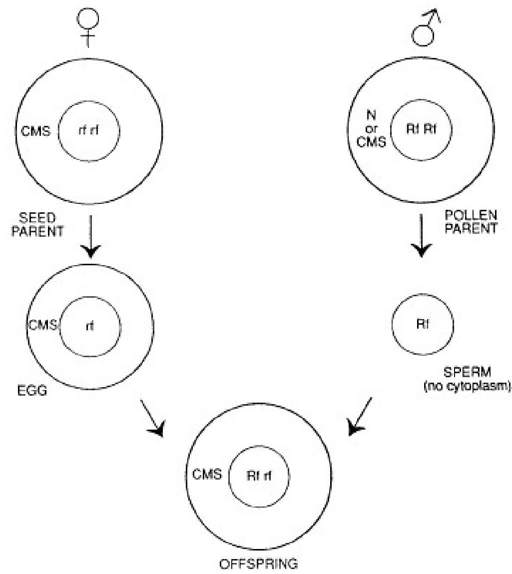


Fig. 7.5.
Cross to restore fertility to offspring of a cytoplasmic male sterile (CMS) seed parent. CMS is transmitted only through the egg; fertility is restored by dominant fertility-restoring genes transmitted through the pollen gametes. The pollen parent may have either CMS or normal (IN) cytoplasm.

function and the progeny will be 100% male fertile instead of 50% male fertile and 50% male sterile as indicated above.

UTILIZATION OF CYTOPLASMIC MALE STERILITY. Cytoplasmic male sterility has been utilized extensively in the production of hybrid seed in several crop plants. Most extensive use has been in corn, sorghum, pearl millet, sunflower, and sugar beets. In corn, cytoplasmic male sterility supplanted the system of detasseling the female or seed-bearing plants in the production of hybrid seed. Later, all hybrids with cytoplasm from a particular source known as the Texas type, because it originated from research conducted in Texas, were found to be susceptible to a leaf blight disease. The use of cytoplasmic sterility in hybrid seed production in corn was then discontinued and detasseling was again practiced. New sources of cytoplasmic male sterility in corn are being studied. These new sources will not be utilized until the breeder is sure that they don't provide undesirable characteristics to the hybrid cultivar such as susceptibility to a disease. Cytoplasmic male sterility is used exclusively at present for producing hybrids of sorghum, sunflower, pearl millet, and sugar beets. In alfalfa which is pollinated by bees, production of hybrids using cytoplasmic male sterility has been unsatisfactory because bees do not freely visit flowers without pollen or nectar.

Fig. 7.6.
Male sterility is used in this commercial onion seed production field in California. The light rows are male sterile, i.e., they do not produce functional pollen. The dark rows produce normal pollen. The male sterile flowers are pollinated by pollen carried to them by insects from the normal rows. Thus only hybrid seed is produced on the male-sterile rows.

Chemically Induced Male Sterility

Chemically induced male sterility offers the breeder an alternative to the use of genetic or cytoplasmic male sterility in the production of hybrid seed. Chemicals that induce male sterility have been variously referred to as *gametocides, pollen suppressants, and chemical hybridizing agents*. The latter is now preferred because the chemicals are potentially useful for pollen control in the commercial production of hybrid seed. In addition, they are useful in eliminating the emasculation procedure before making hand pollinations in a hybridization program of breeding. The general procedure is to use a foliar spray prior to flowering, which inhibits production of viable pollen, but does not injure the pistillate reproductive organs or affect seed development. If the treatment is successful and all of the pollen killed, self-pollination will not occur in the treated plants, but the flowers will set seed freely from cross-pollinations.

Research on the use of chemical hybridizing agents has been conducted on cotton, corn, wheat, sorghum, and other crops, including vegetables, with varying degrees of success. A

major problem has been failure to obtain complete pollen sterility, due to variations in response with different genotypes of the crop, environmental effects on the action of the chemical, or different effects of the chemical itself. The nature of the chemical must be such that it will be absorbed and translocated to the floral meristems at the correct time and in the most effective dosages to inhibit pollen development. The problem is greater in crop species that flower over an extended period of time as compared to species in which the time span for flowering is relatively short, because precise concentrations of the chemical are difficult to maintain within the susceptible plant organs over a long period of floral development.

Research on chemicals that induce male sterility in wheat, cotton, corn, and sorghum is being conducted by several commercial chemical companies. Chemical hybridizing agents are mostly in the experimental stages of development. The advantage of chemically induced male sterility in hybrid seed production is that development and maintenance of cytoplasmic male-sterile and fertility-restoring parent lines are unnecessary.

Apomixis

An asexual form of reproduction in which seeds are formed without union of the egg and the sperm, *apomixis*, was described in Chapter 2. Apomixis is a barrier to cross-fertilization in those species in which it occurs. In the *obligate apomicts*, plants reproduce only by apomixis; but in the *facultative apomicts*, sexual and apomictic embryos may occur in the same ovule or in the same plant as in Kentucky bluegrass. The apomictic mechanism is one that needs to be understood by the breeder working with a species in which apomixis occurs. In true obligate apomicts there would not be opportunity for gene recombination because cross-pollination would result in reproducing the genotype of the mother plant only. However, genetic control of the obligate apomictic mechanism has been found in several species and probably exists in others. In buffelgrass, formerly thought to be an obligate apomict, a sexual plant was discovered. Subsequently, it was learned that reproduction in buffelgrass is determined by two gene pairs. One pair controls apomixis, and the other pair controls sexual reproduction. By manipulation of the genes controlling apomixis and sexual reproduction, it is virtually possible to turn the apomictic process off and on. With sexual reproduction, cross-fertilizations and gene recombinations are possible. If a superior hybrid plant is identified, it can then be converted into an obligate apomict to perpetuate that particular genotype. Utilization of the apomictic mechanism in breeding has been successful in forage grasses.

Interspecific Hybridization

The plant breeder's purpose in making *interspecific* or *intergeneric* crosses is to transfer a gene not available in existing cultivars. The success will be affected by the genetic relationships among the species. The system of classifying plants into species is based on the natural relationships between groups of plants as determined largely by their morphological and physiological characteristics. The classification was worked out to a large extent before the science of genetics was developed and without current information on the relationships existing among chromosomes and genes in different species. As a result it is difficult to make generalizations regarding the breeding behavior in interspecific and intergeneric hybridization.

Cross-Fertility among Related Species

The results of interspecific crossing may range from failure to obtain any seed set upon crossing to complete fertility in the F_1 plant. Some examples of successful interspecies crosses that exhibit varying cross-fertility relationships are as follows:

CROSSES BETWEEN SPECIES THAT ARE HIGHLY CROSS-FERTILE. In some closely related species that have identical chromosome numbers and similar chromosome structure, the chromosomes in the F_1 hybrids pair, regularly forming bivalents at meiosis, and the F_1 plants are self-fertile. Examples of species crosses producing fertile F_1 hybrids that set seed freely are

1. *Glycine max* (cultivated soybean, $2n = 2x = 40$) × *G. soja* (wild soybean, $2n = 2x = 40$).

2. *Gossypium hirsutum* (American Upland cotton, $2n = 4x = 52$) × *G. barbadense* (American Pima cotton, $2n = 4x = 52$)

3. *Zea mays* (Indian corn or maize, $2n = 2x = 20$) × *Z. mexicana* (teosinte, $2n = 2x = 20$).

In reality, these may be variants of the same species rather than different species and may be so designated in the future if sufficient information on the homology of the chromosomes is accumulated to justify the taxonomic change.

CROSSES BETWEEN RELATED SPECIES WITH THE PRODUCTION OF AUTOTETRAPLOIDS OBTAINED BY DUPLICATING THE CHROMOSOMES INC THE PROGENY. Some interspecific crosses produce fertile F_1 plants only if the chromosomes contributed by the parent gametes are duplicated and an amphiploid produced. The amphiploids of *Brassica*, discussed in the chapter on polyploidy, are examples of natural amphiploids. The natural origin of the tetraploid species of *Brassica* was demonstrated experimentally by combining genomes from diploid species (see Fig. 5.4). Where the chromosome architecture permits the experimental production of fertile amphiploids in this manner, the procedure is to cross the species in question and then double the chromosomes of the F_1 hybrid with colchicine, producing an autoalloploid. Not all artificially produced amphiploids will be fertile and set seed. Amphiploids that are fertile and set seed have been produced among species of *Brassica, Triticum, Gossypium, Nicotiana*, and other genera.

CROSSES BETWEEN RELATED DIPLOID AND TETRAPLOID SPECIES BY DOUBLING THE CHROMOSOME NUMBER OF THE DIPLOID PARENT. Certain interspecific crosses may be made, with varying degrees of success, between related species that differ in level of ploidy. For example, crosses may sometimes be made successfully between closely related diploid and tetraploid species by first doubling the chromosome number of the diploid so that it matches the chromosome number of a tetraploid species. This procedure may be utilized to transfer a gene from a wild diploid species to a related tetraploid species.

CROSSES BETWEEN DIHAPLOIDS OF POLYPLOID SPECIES WITH DIPLOID SPECIES. In potato (*Solanum tuberosum*), *an* autotetraploid species, it is possible to produce haploids in the tetraploid species, called *dihaploids*, and then cross with diploid tuber-bearing species.

F_1 hybrid plants from many interspecific crosses are infertile, with lack of chromosome homology being a common cause of infertility. In crops that may be propagated vegetatively, vigorous F_1 hybrids may sometimes be used as the source of new cultivars even though they do not set seed freely. This procedure is used in sugarcane, which is propagated by stem

cuttings, to utilize hybrid vigor from species crosses and to utilize hybrid vigor in some interspecies crosses in forage crops. Interspecific and intergeneric crossing needs to be accompanied by careful cytological studies of parents and hybrid progenies. In *Triticum*, a genus that includes wheat and in which there have been extensive cytological studies, many successful interspecific and related intergeneric crosses have been made.

Time of Pollen Shed

Time of pollen shed has an influence on the mating system of a crop species. For example, it can determine whether a plant will set seed as the result of cross-pollination or self-pollination. There are several mechanisms that can promote cross- or self-pollination. These include:

• *Production of chasmogamous flowers*. Fertilization occurs after the flowers are open which encourages cross-pollination. Examples of crop species having *chasmogamy* include rye, tall fescue, and orchardgrass.

• *Production of cleistogamous flowers*. Pollen is shed within closed flowers which promotes self-fertilization and hence in-breeding. Certain annual fescues, lettuce, certain herbs, some shrubs, Korean lespedeza, and some ornamentals have *cleistogamy*.

• *Production of flowers with and without dichogamy. Dichogamous* flowers are those whose male and female sex organs are active at different times which promotes cross-pollination. Plants such as carrots and rasp-berries are called *protandrous* species because their male sex organs are active before the female organs. Pollen is shed before the egg is ready, thereby eliminating self-pollination. *Protogynous* species, examples which include walnuts, pearl millet (Fig. 7.7), and avocados, have their stigmas receptive before the pollen is shed. Again, this promotes cross-pollination and prevents selfing.

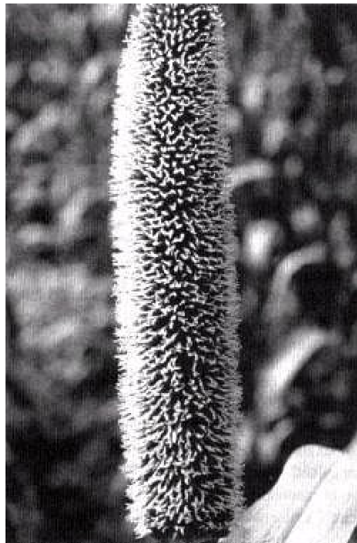


Fig. 7.7.
Spike of pearl millet exhibiting protogynous flowering, a condition in which cross-pollination is promoted by stigmas maturing prior to maturity of the anthers. When this photo was taken only the stigmas were visible with no extrusion of anthers.

Study Questions

1. Characterize gametophytic and sporophytic incompatibility. What are the consequences of these two systems in plant breeding?

2. How does cytoplasmic male sterility differ from genic male sterility? Why are male restorer genes necessary? When might you not need a male restoring mechanism?

3. Can the cytoplasm influence the phenotype of a plant? Give an example.

4. Why are plant breeders interested in fertility regulating mechanisms?

Further Reading

Bashaw, E.C. 1980. Apomixis and its application in crop improvement. p. 45-63. *In* W.R. Fehr and H.H. Hadley (eds.) Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Bashaw, E.C., and K.W. Hignight. 1990. Gene transfer in apomictic buffelgrass through fertilization of an unreduced egg. Crop Sci. 30:571-75.

Burton, G.W., and W.W. Hanna. 1982. Stable cytoplasmic male-sterile mutants induced in Tift $23DB_1$ pearl millet with mitomycin and streptomycin. Crop Sci. 22:651-52.

de Nettancourt, D. 1977. Incompatibility in angiosperms. Springer-Verlag. Berlin.

Edwardson, J.R. 1970. Cytoplasmic male sterility. Bot. Rev. 36:341-420.

Hadley, H.H., and S.J. Openshaw. 1980. Interspecific and intergeneric hybridization. p. 133-42. *In* W.R. Fehr and H.H. Hadley (eds.) Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Hanson, M.R., and M.F. Conde. 1985. Functioning and variation of cytoplasmic genomes: Lessons from cytoplasmic-nuclear interactions affecting male fertility in plants. Int. Rev. Cytol. 94:213-67.

Jones, H.A., and A.E. Clarke. 1943. Inheritance of male sterility in the onion and the production of hybrid seed. Proc. Am. Soc. Hort. Sci. 43:189-94.

Krishna Rao, M., K. Uma Devi, and A. Arundhati. 1990. Application of genic male-sterility in plant breeding. Plant Breed. 105:1-25.

Kumar, K.A., and D.J. Andrews. 1993. Genetics of qualitative traits in pearl millet: A review. Crop Sci. 33:1-20.

Liedl, B.T., and N.G. Anderson. 1992. Reproductive barriers, identification, uses, and circumvention. p. 11-154. *In* J. Janick (ed.) Plant breeding reviews. Vol. 11. John Wiley and *Sons*, New York.

Taliaferro, C.M., and E.C. Bashaw. 1966. Inheritance and control of obligate apomixis in breeding buffelgrass, *Pennisetum ciliare*. Crop Sci. 6:473-76.

Townsend, C.E., and N.L. Taylor. 1985. Incompatibility and plant breeding. p. 365-81. *In* N.L. Taylor (ed.) Clover science and technology. Am. *Soc*. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Yuan, Sheng-Chao, Zi-Guo Zhang, Hao-Hua He, Han-Lai Zen, Kai-Yang Lu, Jian- Hong Lian, and Ben-Xian Wang. 1993. Two photoperiodic-reactions in photoperiod-sensitive genic male-sterile rice. Crop Sci. 33:651-60.

8. Molecular Biology: Application in Plant Breeding

Traditional plant breeding procedures are based on *manipulation of genes and chromosomes through sexual reproduction* in whole plants. These breeding procedures evolved from the principles of Mendelian genetics and were expanded as knowledge increased in quantitative genetics, polyploidy, mutation induction, incompatibility, male sterility, heterosis, and related phenomena. In recent years the science of molecular biology has developed the potential for supplementing traditional plant-breeding procedures *by extending genetic manipulations beyond the level of sexual reproduction*. Applications of molecular techniques in plant breeding and problems associated with their use are discussed in this chapter.

At present, there is much activity in the science of molecular biology. New molecular applications and the technology to implement them are rapidly emerging. Yet, it should be realized that it may take years before some of the molecular technology can be used by plant breeders to supplement current breeding practices. With some exceptions, the new technology is still the product of the research laboratory and has not reached the stage of perfection where it can be routinely implemented by the breeder. Some extravagant claims have been made about the utility of the new molecular biology in plant breeding. *A rational appraisal suggests that molecular genetic techniques will complement, but will not replace, traditional plant breeding practices that are based on Mendelian genetic principles*. Meanwhile, it is essential that plant breeders understand the potentials and limitations of the new technology, and, as it unfolds, that they employ it appropriately for enhancement of existing breeding procedures.

Plant Cell and Tissue Culture

Plant cell and tissue culture includes a wide range of cultural techniques for regeneration of functional plants from embryonic tissues, tissue fragments, calli, isolated cells, or

protoplasts. The regeneration of plantlets through tissue culture techniques is a primary requirement for the utilization of molecular genetic technology in a particular crop species. Unfortunately, crop species often respond differently to specific cell culture techniques. This has hindered the development of culture procedures that can be uniformly applied in plant breeding programs. To fully utilize the molecular genetic technology routinely in a plant breeding program, specific procedures will usually need to be worked out for the genetic material and the culture environment with which the plant breeder is working.

Procedures Utilizing Tissue Culture Techniques

Procedures with potential applications in plant breeding that utilize plant cell or tissue culture techniques to regenerate plants are:

• *clonal propagation*: the rapid multiplication of genetic stocks, through tissue culture, including procedures for isolation of *pathogen-free plant materials and freeze-preservation of germplasm*;

• *embryo and ovule culture*: the rescue and propagation on a sterile nutrient medium of immature embryos from interspecific or intergeneric crosses;

• *anther culture*: the culturing of anthers in vitro for the purpose of generating haploid plantlets;

• *somoclonal variation*: genetic variation induced in somatic cells cultured in vitro;

• *somatic cell hybridization*: the fusion of protoplasts from genetically diverse germplasms; and

• *genetic engineering (transformation*): in plants, the transfer of DNA from a donor species to a recipient species by means of a bacterial plasmid, virus, or other vector, or through microinjection or biolistic device. Plants receiving the new DNA are said to be *transformed*, and are regarded as *transgenic* plants.

Tissue Culture Techniques

Plant cell and tissue culture involves the culture of isolated plant cells or detached fragments of plant tissue on a nutrient medium under aseptic conditions and their subsequent regeneration into functional plants (Fig. 8.1). Undifferentiated plant cells often can be made

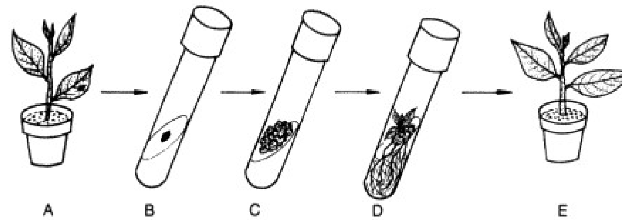


Fig. 8.1.
In Vitro regeneration of plants through tissue culture. (A) Source of tissue explant. (B) Explant culture on agar medium. (C) Callus production. (D) Plantlet regenerated. (E) Plantlet transplanted into sterile soil.

to develop into functional plants when appropriately cultured in vitro. This property is designated *totipotency*. The need to regenerate plants from totipotent cells makes plant cell and tissue culture an essential step in utilization of the new molecular technology. Fairly routine in vitro procedures for plant regeneration have been developed for some model species such as tobacco, potato, alfalfa, sugarcane, rice, and various horticultural species, many of which are readily propagated vegetatively. For other major field crop species such as corn, sorghum, forage grasses, cotton, soybean, and the grain legumes, it has been difficult to develop regeneration procedures that can be employed routinely. Even where procedures are available for a particular crop species, plant regeneration from tissue culture often occurs at a low frequency. Success in plant regeneration from tissue culture varies not only with the species, but also with the genotype within the species, the source of the cultured tissue, the age and health of the donor plant, the nutrient medium, and other factors. Optimal procedures may vary for each crop species and for each specific cultural environment. With the extensive research going forward, it may be expected that some of the current problems will be corrected in the future.

Plant tissue cultures are generally initiated from multicellular tissue fragments, called *explants*, obtained from living plants. Explants may originate from a wide range of plant tissues, including leaf, stem, root, petiole, hypocotyl, cotyledon, embryo, or meristem. Success in plant regeneration with different species often varies with the source of the explant. The explant is commonly cultured on a nutrient medium solidified in agar. Explants from most species of plants may be induced to divide in an unorganized manner on specifically formulated nutrient media. An undifferentiated mass of cells, known as *callus* (plural, *calli*), is formed within 4 to 8 weeks. The callus may be divided, with clusters of cells transferred to fresh agar media to form subcultures. Repeated subculturing of the callus permits rapid multiplication of the cultured material. However, the potential for plant regeneration may decline, and genetic stability of the plant material may be altered, with successive subculturing. Callus cultures are incubated under aseptic conditions, normally in dim light, with temperatures around 25ºC.

Plant cells may also be cultured in liquid media. If pieces of a callus are transferred to a liquid medium and the culture agitated, individual cells or aggregates of cells may be sloughed off. These cells divide to form a cell suspension culture. In addition, cell suspension cultures may be initiated from single cells or clusters of cells mechanically separated from the explant. Plant regeneration from cell suspension cultures is more difficult than plant regeneration from callus tissue.

The components of the nutrient medium are important for success with plant tissue culture. The nutrient medium commonly contains inorganic salts, sugar as a source of carbon, and vitamins to maintain high growth rates. Phytohormones such as auxins and cytokinins may be added to control cell growth and division. The ratio of auxin to cytokinin has an important role in the initiation of shoot and root primordia. Generally, a low auxin:cytokinin ratio stimulates initiation of shoot buds and suppresses root initiation; a high auxin:cytokinin ratio leads to dedifferentiation and favors root initiation; an intermediate ratio favors continued division of cells as undifferentiated callus. The optimum formulation of the culture medium may vary with the species, the genotype within the species, and the origin and age of the cultured tissue. Likewise, the preferred physical state of the culture medium, whether a liquid medium or a solid agar gel, may vary with the species and the culture environment. Refinements of the nutrient medium components and culture conditions, has made it possible to successfully culture plant tissues and regenerate plantlets from an increasingly wider range of crop species.

Plantlet Regeneration

Plantlet formation is initiated through induction of *adventitious shoots, somatic embryos*, or *axillary buds*. Adventitious shoots or somatic embryos may arise either from a callus (Fig. 8.2A,B) or directly from the explant. After adventitious shoots are formed, the culture is transferred to a rooting medium to induce root initiation and subsequently plantlets (Fig. 8.2C). Somatic embryos, like embryos formed following sexual union of gametes, have both root and shoot apices present and can develop directly into plantlets. Adventitious shoot initiation (*organogenesis*) occurs with a wider range of plant species than initiation of somatic embryos; few major field crop species can be routinely induced to form somatic embryos.

Plantlets regenerated through tissue culture techniques are transferred to soil for growth and development. The establishment of a healthy plantlet in soil with minimum mortality is as essential for success in tissue culture propagation as obtaining a high frequency of plantlet regeneration. Species differ in their capability of adjusting to the new environment. During this period the plantlet must change from the heterotrophic state to the autotrophic state, where it synthesizes its own organic food requirements. Water loss from the regenerated plantlet is high, due to inadequacy of the root system formed in culture to maintain the plant in soil, and a reduced presence of epicuticular wax on leaves and stems of regenerated plantlets. The regenerated plantlet must be protected from desiccation and hardened to attain some tolerance to moisture stress. In addition, the new plantlets, which have been developed under aseptic conditions, should be protected from soil pathogens so that they can grow and develop into healthy plants.

Plant materials propagated through tissue culture tend to have a high proportion of genetic variants. Abnormalities that frequently occur are higher levels of ploidy, aneuploidy, point mutations, and chromosome structural changes. Plants regenerated following repeated subculturing of callus cultures are highly variable and frequently exhibit increased levels of polyploidy. It has been proposed that these tissue culture induced variants will provide the

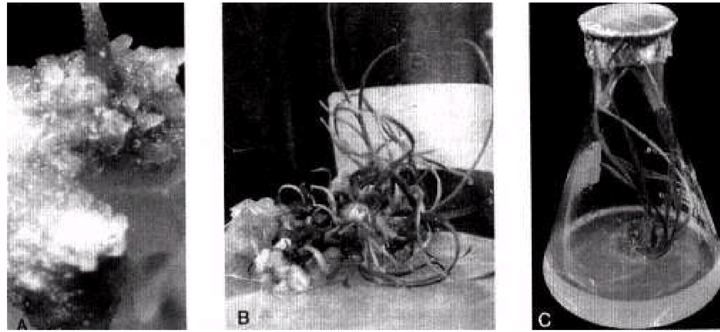


Fig. 8.2.
In vitro plant regeneration in wheat. (A) Emergence of a shoot from callus. (B) Production of shoots from callus. (C) Regenerated wheat plantlet.

breeder with a new source of genetic variation. Like radiation induced mutations, the potential utility of this source of genetic variation in plant breeding seems to have been overrated. For tissue culture propagation of a particular genotype, it is essential that a stable cell line be maintained through repeated cloning without genetic change.

Clonal Propagation Via Tissue Culture

Tissue culture technology may be utilized for rapid regeneration of particular plant genotypes. This technology is referred to as *clonal propagation*, also as *cloning*, or *micropropagation*. Some potential uses of clonal propagation in agronomic crops are:

- large-scale increase of a heterozygous genotype,
- increase of a self-incompatible genotype,
- increase of a male-sterile parent in a hybrid-breeding program,
- propagation of disease-free genetic stocks, and
- preservation and international exchange of germplasm.

Shoot tips cloned from axillary buds or meristem tissue produce fewer genetic variants than cultures from more mature tissues. If, in addition to the meristematic region, one or two leaf-primordia are included in the shoot-tip explant, the explants will be larger, require less time for excision, and have a higher survival rate than the smaller explants cloned without the leaf-primordia. Axillary shoots produced on the shoot-tip explants can be subcultured until the required number of potential plantlets are obtained. The plantlets are transferred to a rooting medium and later transplanted into soil.

Commercial Applications

Clonal propagation has the potential for propagation of thousands of plantlets from a single genetic stock. Extensive commercial application of in vitro propagation is practiced with orchids, but there is widespread potential in other horticultural crops such as pyrethrum, potato, asparagus, strawberry, and various flowers or herbaceous ornamentals that set seed poorly. Tissue culture propagation would not be practical for large-scale increase of field crop species that set seed freely, or where large acreages are to be planted, due to the labor of propagation and transplanting the regenerated plantlets. In vitro propagation may have application for early generation increase of breeding materials in crop species with sparse seed-setting, provided that efficient tissue culture procedures that can be routinely employed have been developed for those species and that genetic identity can be maintained in the plants propagated.

PROPAGATION OF DISEASE-FREE GENETIC STOCKS. Viruses and other plant pathogens may be eliminated from propagating stocks of asexually reproduced plant materials by culturing meristem tips, or by a combination of meristem-tip culture and heat treatment (Fig. 8.3). Viruses present in clones propagated asexually are transmitted to the new plantlets through the infected tissue. The titer of a virus in a plant is highest in the leaves and stems, but is absent or very low in newly developed meristem tissue. By cloning from new meristem-tip cells, virus-free or nearly virus-free explants may be regenerated into new plantlets. The

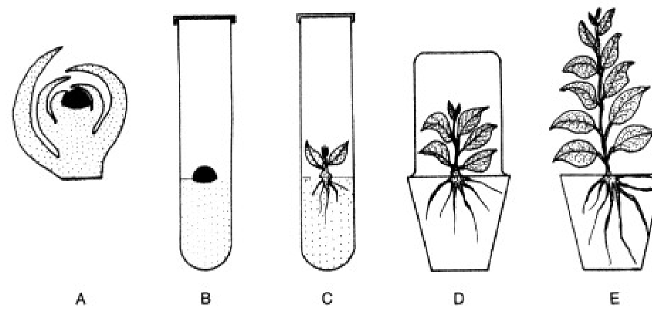


Fig. 8.3.
Meristem-tip culture. (A) Apical meristem showing section to be excised. (B) Excised meristem tip cultured on agar medium. (C) Plantlet regenerated from excised meristem tip. (D) Plantlet transferred to sterile soil. (E) Virus-free plant growing in soil.

smaller the meristem-tip explant cultured, the higher the expected recovery of virus-free plants, but explant survival and plant regeneration will be reduced compared to regeneration from larger and more mature shoot-tip explants. Regenerated plantlets need to be indexed for presence of the virus before they are given a virus-free designation. Meristem-tip culture is used commercially to obtain virus-free foundation seed stocks and breeding lines of potato and cassava, and a virus-free seed nucleus for increases from a virus-infected red clover or grass species.

FREEZE PRESERVATION OF GERMPLASM. Long-term storage of vegetative germplasm may be done by *freeze preservation*, also known as *cyropreservation*. Combining meristem-tip culture with cyropreservation facilitates the international exchange of pathogen-free plant genetic materials.

Embryo Culture, Ovule Culture, in Vitro Pollination

Embryo Culture

Embryo culture is the aseptic excision of an immature embryo from an ovule and its subsequent in vitro culturing on an appropriate nutrient medium. Hybrid embryos from crosses between distantly related species are often weak and inviable due to inadequate nourishment by the endosperm. Such embryos may abort, or collapse shortly following zygote formation, and fail to develop into viable seeds. Often, these immature embryos may be rescued from failure, and their growth, germination, and development into seedling plants secured, through embryo culture procedures. Embryo culturing is not a new technique. The culture of excised radish embryos on a nutrient media was reported in 1904, and embryo culture was utilized in 1925 to rescue hybrid embryos following wide crosses in flax.

In the early studies of embryo culture, the embryo was often more or less mature when excised from the developing seed. Mature or nearly mature embryos are largely autotrophic and have relatively simple nutritional requirements; they are capable of germination and development into plantlets on simple nutrient media. *Proembryos*, embryos in the initial stages of development, have a more complex nutritional requirement and cannot develop independently. In nature, embryos develop inside a sterile ovular cavity and are dependent upon metabolites supplied by surrounding endosperm tissues. An embryo developing as a hybrid between distantly related species may not be properly nourished due to incompatibility between the endosperm and the embryo. To rescue the embryo, the proembryo is excised from the ovule a few days following fertilization and transferred to a solid agar or liquid medium for subsequent growth and germination (Figs. 8.4 and 8.5). As knowledge increased on proembryo nutrient requirements and nutrient media formulation, it became possible to culture progressively younger and smaller embryos and to nurture their growth and germination. After plantlets have developed, they are transferred to sterile vermiculite or soil for growth into mature plants.

Embryo culture is sometimes used to overcome *seed dormancy* barriers in interspecies crosses. With seed dormancy, the hybrid seeds do not germinate until an appropriate period of time has elapsed after pollination of the ovule. Because young embryos do not normally exhibit dormancy, the hybrid embryos can be cultured and germinated shortly after fertilization, thus bypassing the dormancy problem.

Ovule Culture

Fertilized ovules are sometimes cultured to rescue embryos from wide crosses. The ovules containing immature hybrid embryos are excised aseptically shortly after fertilization and cultured on a nutrient medium. Ovules may be excised with the embryo at an earlier stage of development than if the embryo itself is excised. Embryos developing within the ovule may have a more favorable chemical and physical environment for growth and development than embryos cultured outside the ovule.

In Vitro Pollination and Fertilization

In vitro pollination and fertilization involves the culture of unfertilized ovules on a nutrient medium under sterile conditions. The ovules are pollinated by dusting with fresh pollen. Pollen tubes penetrate the ovule wall and the egg is fertilized by a pollen gamete. The procedure has

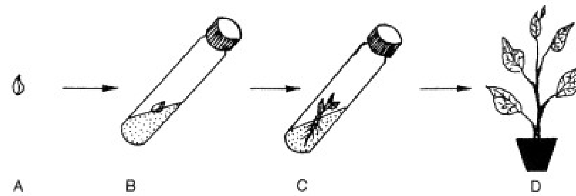


Fig. 8.4.
Embryo culture. (A) Proembryo dissected 3 to 5 days after pollination. (B) Proembryo cultured on solid agar medium. (C) Plantlet developing from embryo. (D) Planter transplanted into soil.

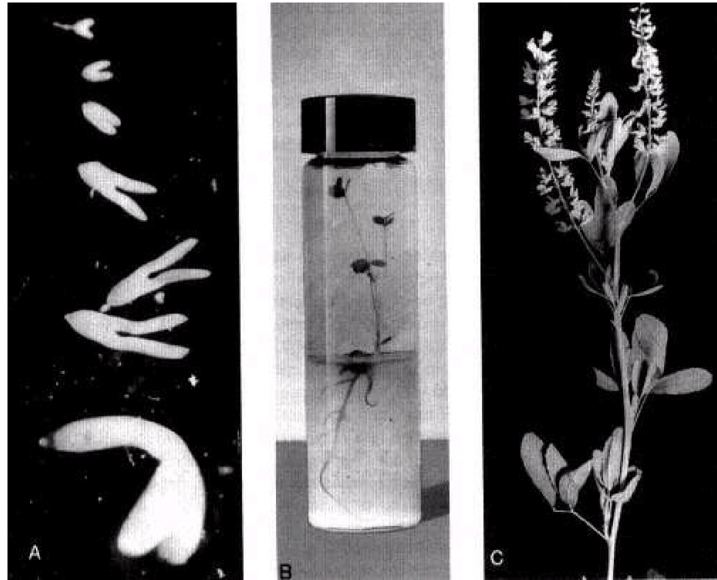


Fig. 8.5.
Embryo culture used to rear embryos of species hybrids in sweet clover. (A) Embryos reared on agar. Starting at the top, the embryos are shown at 7, 8, 9, 10, 11, 12, and 14 days after pollination. (B) Vial in which hybrid embryos from a cross between low coumarin white and common yellow sweetclover were reared in diffused light and controlled temperatures until leaves and roots were well established. The embryos are lying on, or just below, the surface of the agar. (C) Hybrid plant of a sweetclover species cross that was grown to maturity from an embryo reared on agar. Flowers on this plant were selfed or backcrossed to the parents and bore seed.

been utilized to obtain sexual hybridization among species where pollen tubes fail to grow and penetrate the ovule normally following pollination.

Anther Culture and Haploid Plant Production

Anther culture refers to the in vitro culturing of anthers containing microspores or immature pollen grains on a nutrient medium for the purpose of generating haploid plantlets. Haploid plantlets may also be generated from the culture of unfertilized ovules. If the chromosome number of the recovered haploid plantlet is doubled, a completely homozygous diploid plant will be obtained, referred to here as a *doubled haploid*. Anther culture is potentially useful for the plant breeder because the doubled haploids may be increased and utilized immediately as breeding lines or cultivars in self-pollinated crops, or as inbred lines in hybrid breeding. The time required to produce a cultivar or an inbred line by the doubled haploid procedure is reduced by two to three generations, as compared to the conventional procedure of reaching homozygosity by self-pollination (Chapter 9).

Culturing anthers for the purpose of obtaining haploids is not easy with many field crop species, particularly with the cereals, cotton, grasses, and grain legumes. In addition to obtaining haploid plants through anther culture, or culture of unfertilized ovules, haploid plants may *occur naturally* at a low frequency, or they may be produced through the use of *chromosome elimination* techniques (Chapter 5), or by *semigamy breeding* techniques in cotton (Chapter 19).

Anther Culture Procedures

The general procedure for culturing anthers is to select and surface sterilize unopened floral buds or immature florets. Young anthers are dissected and placed on a solid agar medium in petri dishes or glass tubes, or they may be floated on a liquid medium. The cultured anthers give rise to plantlets either directly through *embryos* (Fig. 8.6A), or indirectly and more commonly from *callus* tissue (Figs. 8.6B and 8.7A). *Embryos* commonly arise from division of microspores. If cultured in an appropriate medium, plantlets will develop in 4 to 6 weeks. The plantlets are transferred into an appropriate nutrient medium until healthy roots and leaves develop. After a period of hardening, the plantlets can be transferred to sterile soil. If a callus develops, the callus is transferred into a regeneration medium to stimulate formation of plantlets, which arise from the callus in 4 to 8 weeks. The plantlets are transferred into a new medium to enhance root and shoot development and then transferred to sterile soil. Some anthers may produce only calli and no plantlets; these have no value for the breeder who has the production of haploid plants as his goal.

The chromosome number of haploid plantlets is doubled to produce a diploid plant (Fig. 8.7B). The most common method for diploidization of haploid plants is treatment with colchicine. Not all plantlets generated from anther cultures will be haploids. Anther culture normally produces haploid calli or somatic embryos from gametophytic tissue. However, the cells of gametophytic tissue may become diploid by spontaneous doubling of the chromosomes giving rise to diploid calli or somatic embryos. Diploid calli or somatic embryos may arise also from sporophytic tissue such as the anther wall. The population of plantlets generated may thus contain both haploid and diploid genotypes; even aneuploids or polyploids may sometimes arise.

Factors Affecting Haploid Plant Production Through Anther Culture

Success in production of haploid plants through anther culture is affected by the genetic material and the cultural environment. Some factors affecting haploid plant production are:

• Modifications of the nutrient media and techniques are often necessary to adapt the culture procedures to different plant species. Culture procedures may vary also with different genotypes within particular species.

• For many species, highest plantlet production is obtained by culturing anthers just before or at the time of the first microspore division, with plantlet production declining as anthers approach maturity.

• Strong vigorous plants grown with high light intensity normally produce anthers that respond most favorably to anther culture techniques.

• Pretreatment of donor plants and flower buds, such as chilling of buds at 1 to 4° C for 24 to 48 hrs increases plantlet production. Culturing detached flowering stems in a nutrient medium increases plant-let production in some species.

• The optimum formulation of the nutrient medium varies with the species and sometimes with genotypes within the species.

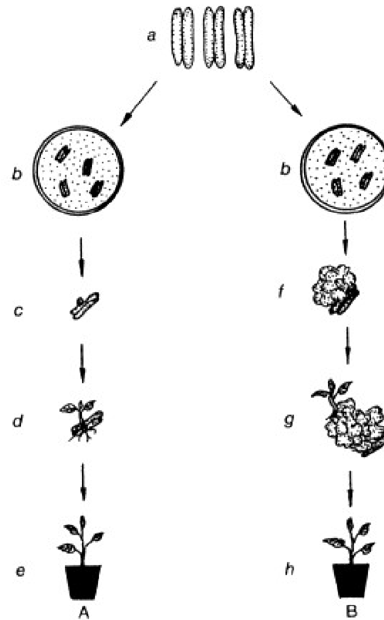


Fig. 8.6.
Anther culture. Immature anthers (a) are dissected from a flower and cultured on agar medium (b). (A) An embryo (c) develops, and a haploid plantlet (d) arises and is later transplanted into sterile soil (e). (B) A callus (f) is formed and a haploid plantlet (g) develops that is later transplanted into sterile soil (h). Formation of cali is more common than formation of embryos.

Solutions to these problems must be worked out specifically for each crop species.

Utilization of Anther Culture Derived Doubled-Haploids in Plant Breeding

In a plant breeding program, anthers would usually be cultured from F_1 or F_2 plants to provide maximum genetic diversity among the recovered haploid plants. The chromosomes in the haploid plants are doubled and the homozygous doubled-haploids obtained are increased and evaluated as breeding lines. The advantages proposed for producing doubled-haploid breeding lines compared to obtaining homozygous lines by inbreeding are:

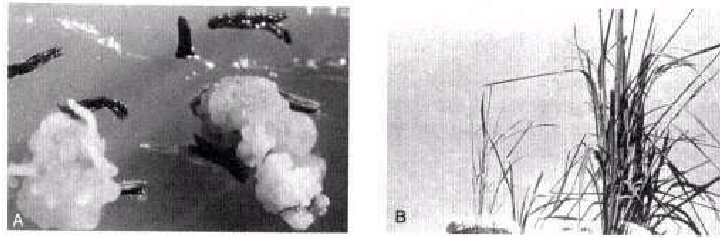


Fig. 8.7.
Anther culture in rice. (A) Callus tissue growing from germinating pollen.
(B) Haploid plant of rice (left) and corresponding diploid plant (right).

• Homozygosity is reached in one generation following hybridization as compared to five or six generations of conventional inbreeding. This should result in savings of two to three generations in the development of a new cultivar (Chapter 9).

• The doubled haploids are completely homozygous at all loci, whereas some residual heterozygosity persists in breeding lines selected after five or six generations of inbreeding.

• Recessive genes that may be masked by presence of a dominant allele in diploid heterozygous breeding lines are uncovered and will be expressed in the phenotype of haploids or doubled haploids.

In spite of these purported advantages, anther culture per se is seldom, if ever, used in plant-breeding programs to generate new genotypes due to problems associated with doubled haploid production and utilization:

• Most cereal and nearly all grain legume species produce haploid plantlets inefficiently. Refinements in anther culture techniques and greater efficiency of plantlet production are needed for doubled haploids to be economically produced.

• Genotypes with low plantlet production will be avoided by the breeder even though the unresponsive genotypes may have genes that will contribute to favorable performance in the farmer's field.

• Genetic variations occur among anther-derived doubled haploid plants leading to unstable breeding lines.

The purpose of hybridization is to obtain a reassortment and recombination of desirable genes from the parent cultivars. The reported advantage of haploid breeding is to obtain rapid homozygosis following hybridization, but the induction of mutations in the segregating progenies confounds the selection process and increases the difficulty of finding the segregants with the desired recombination of genes.

Genetic Variability from Cell Cultures: Somaclonal Variation

Genetic variations are frequently exhibited among plants regenerated from cell cultures (Fig. 8.8). The genetic variations occur naturally in cell cultures at a relatively high frequency and so treatment with a mutagenic agent is unnecessary. Tissue culture-induced variability has been reported among regenerated plants in sugarcane, tobacco, rice, barley, oat, alfalfa, corn, wheat (Fig. 8.9), soybean, and other species. Because the variations originate among cells of somatic origin it has been named *somaclonal variation*.

Genetic variants in somatic cell cultures include a wide mutation spectrum such as point mutations, chromosomal rearrangements, inversions, duplications, polyploidy, aneuploidy, and deletions. Either qualitatively or quantitatively inherited characters may be affected by tissue culture-induced mutations. As with irradiation or chemical mutagen treatments in plants, somaclonal variation cannot be directed toward changing a particular trait. Screening procedures must be relied upon to identify a particular kind of mutant.

Beneficial genetic variations originating in cell cultures may be utilized in crop breeding for the development and release of improved cultivars of field crops. Induction and selection of useful in vitro genetic variants in crop species may be accomplished in two ways:

1. *Crop cultivars or clones deficient in a particular character may be cultured and the regenerated plants screened for mutant forms that would correct or partially correct the deficiency*. The procedure is used in sugarcane breeding, with subclones being generated through tissue culture procedures and examined for genetic variants. Subclones that differ from the original clone in morphological characteristics or disease resistance are propagated and evaluated in field trials. The utility of the procedure in a plant breeding program will be determined by:

• the ease in culturing tissues and regenerating plants from particular species,

• the availability of techniques for screening regenerated plants for the deficiency that needs to be corrected, and

• the genetic stability of the regenerated plants.

A routine procedure for regeneration of large numbers of plants for the species under consideration is essential. Successful identification of beneficial somaclonal variants will be

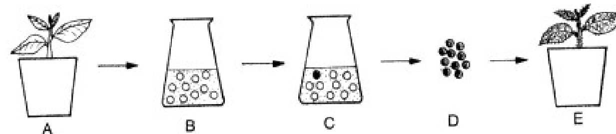


Fig. 8.8.
Somaclonal variation. (A) Haploid plant from which cultured tissue was obtained. (B) Cell suspension culture from haploid plant. (C) Mutant cell in suspension culture. (D) Aggregate of mutant cells. (E) Haploid plantlet that exhibits the mutant character. Culturing cells from haploid plants permits either dominant or recessive mutations to be exhibited in the regenerated plantlet. If tissue from diploid plants is cultured, only dominant mutations will be immediately expressed but recessive mutations will be expressed in some progeny plants as a result of segregation.

Fig. 8.9.
Somaclonal variation in wheat. The parent plants are at the right and left. Between the parents are plants regenerated from tissue cultures that vary in height and presence of awns.

enhanced if the breeder is looking for a mutant to correct a particular deficiency in the cultured genotype for which a reliable screening procedure is available. Genetic stability of the regenerated mutant plant is essential in seed propagated species because the variant character must be expressed unchanged following successive sexual reproductions. In clonally propagated species, stability in sexual reproduction may be unnecessary if the genetic variant is reproducible through vegetative means.

2. *Plant cell cultures may be screened for resistance to stress conditions and plants regenerated from resistant cells*. The advantage of screening at the cellular level is that millions of cells may be grown in a limited space and uniformly screened by applying appropriate stress to the cell culture. The procedure is comparable to the microbial selection techniques successfully utilized in industry. Although screening cell cultures provides a unique opportunity for generating genetic mutants, the process is limited by the kinds of mutants that can be selected in culture and the ability to regenerate plants from mutant cells in the species being studied.

Only traits expressed at the cellular level can be identified by screening cells in culture.

These include traits such as *tolerance to salt, metals such as iron or aluminum, low temperature, or plant nutritional factors; and resistance to herbicides or toxins produced by plant pathogens*. Genotypes isolated with resistance to these types of stresses would have direct utility in a plant-breeding program. Traits such as yield, lodging resistance, or quality, that are inherited in a complex quantitative manner would not be amenable to selection at the cellular level, except as they may be affected by characteristics such as those mentioned above. For most characters, it may be easier to find sources of genes for the desired character already present in available germplasm that could be transferred to the deficient cultivar or clone by proven sexual breeding procedures.

Screening for stress factors at the cellular level involves:

• developing cell cultures of an adapted genotype in a particular species that is deficient in a characteristic and for which a cell culture screening procedure is available,

• applying appropriate stress to the cultured cells in order to inhibit growth of all except the stress-resistant cells, and

• regenerating plants from the surviving cells.

The cells may be subjected to stress by growing cultures in varying concentrations of a herbicide or salt solution, or in the presence of a disease toxin. The stress may be applied more uniformly to cells in a suspension culture than to a callus. Cells surviving the applied stress are transferred to a regeneration medium to initiate shoot and root development. The mutant traits identified in cell culture must be expressed in the regenerated plant and, in seed-propagated crops, expressed unchanged in sexually reproduced progeny of the regenerated plant. Field screening of resistant lines will be required to verify that the resistance identified at the cellular level is heritable and that it is being expressed in the progenies of the regenerated plants under field conditions.

Somatic Cell Hybridization

Somatic cell hybridization, also called *somatic cell fusion or protoplast fusion*, refers to the fusion of plant protoplasts (cells devoid of cell walls) from somatic cells of different species and the subsequent regeneration of hybrid plants from the fused protoplasts (Fig. 8.10). The procedure is proposed for use in plant breeding to form a hybrid by fusing somatic cells where seeds cannot be obtained by sexual hybridization following wide crosses.

Somatic cell hybridization is a multi-stage process involving the isolation of protoplasts from different species, fusion of protoplasts from two different species, identification and cloning of the fused hybrid protoplasts, and regeneration of fertile hybrid plants from the fused protoplasts. Before plant cells will fuse it is necessary to remove the cell walls to produce naked protoplasts. Cells from leaf mesophyll or other plant tissue, callus, or cell suspension cultures are treated with cell wall-degrading enzymes to obtain a suspension of protoplasts. Purified protoplasts of the species to be hybridized are mixed and centrifuged with a fusigenic agent, commonly polyethylene glycol. At this point there is a mixture of parent protoplasts, fused parent protoplasts, and fused hybrid protoplasts. The fused hybrid protoplasts are recovered and plated in microdroplets. After fusion, the hybrid protoplast must regenerate the cell wall, continue dividing, and regenerate roots and shoots. Before somatic cell hybridization becomes a viable technique for the plant breeder, routine techniques for culturing protoplasts

from the parent species need to be developed. Regeneration of plants from protoplasts is easiest in potato, tobacco, alfalfa, and a few other species, and most difficult in the cereal crops and grain legumes. Hybrid cells of distantly related species are often aneuploid and regenerate plants infrequently, or if they regenerate plants, the plants may be infertile.

Plant Genetic Engineering (Transformation)

Plant genetic engineering refers to the transfer of foreign DNA which codes for specific genetic information, from a donor species into a recipient plant species by means of a bacterial plasmid, virus, or other vector. The procedure is also referred to as *transformation*. For the plant breeder, plant genetic engineering has the potential for transferring a desirable foreign gene from a wide range of sources, including non-plant genetic material, into an economic crop species without sexual hybridization. In many respects, plant genetic engineering (transformation) is comparable to the backcross method of breeding in which desirable genes are transferred to a recipient genotype by a succession of crosses. The molecular biologist inserts a segment of DNA that codes for a desirable trait into the plant genotype where it replicates and is expressed in the new plant genotype. The difference is that the plant breeder can employ the backcross only among species that are cross-fertile, whereas the molecular biologist is not limited to obtaining the DNA from a donor plant species that is cross-fertile with the recipient plant species.

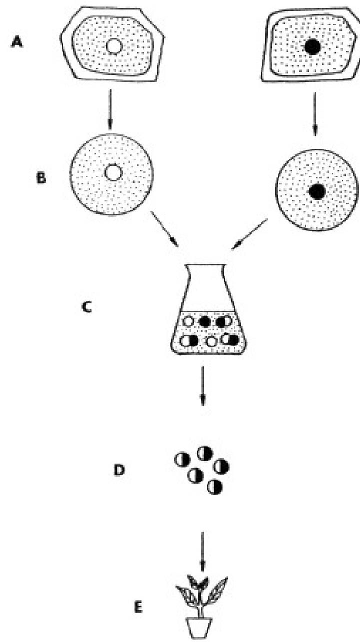


Fig. 8.10.
Somatic cell hybridization. (A) Plants cells from parent species. (B) Plant protoplasts from parent species after cell walls have been removed. (C) Suspension culture of protoplasts from parent species. (D) Hybrid protoplasts following fusion. (E) Hybrid plantlet regenerated from fused protoplasts. Fusion of protoplasts and regeneration of plants would permit hybrids to be obtained between unrelated species that do not produce seeds following cross-pollination.

The crop species that nave been *genetically transformed* with foreign DNA includes corn, alfalfa, orchardgrass, potato (Fig. 8.11), cauliflower, soybean (Fig. 8.12), lettuce, sunflower, tall fescue, carrot, canola, white clover, cotton (Fig. 8.13), tomato, and others; the list continues to grow. Currently, few genetically transformed cultivars have been released. In part, this is due to the rigorous testing procedures mandated before organisms, genetically altered through molecular techniques, can be released.

Fig. 8.11.
Potato plant on right was genetically transformed with a gene from Bacillus thuringiensis var. tenebrionis which gives resistance to the Colorado potato beetle. The potato on the left does not have the gene for resistance to the Colorado potato beetle and was severly damaged by the feeding larvae.

Genetic Transformation

A procedure for transformation through recombinant DNA techniques developed with prokaryotic cells in microorganisms was described by S.N. Cohen in 1975. In the bacterial

Fig. 8.12.
Transgenic soybean on the left was not damaged by the herbicide glyphosate. Nontransgenic soybean on the right does not contain the gene for resistance to glyphosate and was destroyed after application of the herbicide.

Fig. 8.13.
Transgenic cotton on the right with the Bt gene from Bacillus thuringiensis var. kurstaki which gives resistance to Lepidopteran insects. Boll on left is nontransgenic and is susceptible to feeding by Lepidopteran insects.

model, plasmids of the bacterial species *Escherichia coli* are used as vectors. Plasmids are circular, extrachromosomal DNA molecules that replicate independently of the bacterial chromosome. The plasmid molecules are isolated from the bacterial cells and digested with an enzyme, *restriction endonuclease*, that cleaves the molecule at a specific site. A small segment of foreign DNA carrying the desired genetic information is inserted between the broken ends of the plasmid molecule, and another enzyme is used to reform the circle. The vector is then reintroduced into *an E. coli* cell. In addition to the foreign DNA segment, the plasmid vector carries a replicator gene so that it will be reproduced in the *E. coli* cell, and a marker gene so that an *E. coli* cell containing a plasmid vector may be identified and isolated.

The first genetically transformed plants were developed in the early 1980s. One of the first genes transferred to plants was a gene from bacteria called *neo* that codes for antibiotic resistance. When the antibiotic resistant gene *neo* was introduced into plant cells, the genetically transformed cells were easily identified because they grew in the presence of the antibiotic, kanamycin or G-418. This transfer was mediated with the bacterial pathogen *Agrobacterium tumefaciens* which is able to transfer a piece of its DNA (T-DNA) into the DNA of the plant resulting in a new, genetically transformed plant cell. *Agrobacterium rhizogenes* is another bacteria used in transformation but its use is not as frequent as *Agrobacterium tumefaciens*.

Agrobacterium tumefaciens is a pathogenic soil bacteria which causes tumors, called crown galls, in dicotyledonous plants. *Agrobacterium tumefaciens* infects plants by transferring T-

DNA of the Ti-plasmid into plant cells and the T-DNA becomes incorporated into the plant's DNA, hence causing the crown gall disease. The galls or tumors are developed because the T-DNA from the bacteria has genes which regulate the biosynthesis of the plant hormones indoleacetic acid (IAA) and cytokinin. After plants become infected with *A. tumefaciens*, abnormal levels of IAA and cytokinin cause anomalous growth and tumor formation. Mutants of *A. tumefaciens* have been developed in which the T-DNA does not produce IAA or cytokinin. Foreign genes are incorporated into these non-hormonal producing *A. tumefaciens* strains as part of the T-DNA. As a result, the modified *A. tumefaciens* strains serve as a vehicle to introduce the foreign genes into plants. This process now makes it possible to genetically engineer specific crops plants. Utilization of *A. tumefaciens* as a vector in genetic transformation has the limitation that most monocotyledonous species, which include the major cereals, are not easily infected by *A. tumefaciens*.

Adaptation of the bacterial model for foreign gene transfer to crop plants requires these steps:

• introduction of the foreign gene into the T-DNA of the bacteria,

• introduction of the bacteria containing the foreign gene into cells of host plants,

• integration of the foreign gene into the genome of the host cell,

• expression of the foreign gene in the regenerated crop plant, and

• transmission of the foreign gene and its expression through normal sexual processes to plants in succeeding generations in seed reproduced species, or through normal asexual propagation in vegetatively reproduced species.

A simplified model for plant genetic engineering, using a bacterial plasmid as a vector, is illustrated in Fig. 8.14. The *foreign DNA strands* and the *DNA strands of the cloning vehicle*, or bacterial *vector*, are cleaved with a restriction enzyme. In this model, the double strands of DNA are broken, leaving complementary nucleotides. If a fragment of the foreign DNA becomes inserted into a break in the plasmid DNA, the ends of the circular plasmid strands are joined and the break is annealed with the enzyme *DNA ligase*. The formidable step is to obtain insertion of the foreign DNA segment into the plant genome where it will be replicated and expressed. Insertion of the DNA segment into a plant cell or protoplast where it will be replicated requires a suitable vector, but efficient vectors by which this may be routinely accomplished for many crop species have been difficult to identify.

Because many crop plants cannot be genetically transformed by bacterial mediated procedures, other techniques that involve direct DNA uptake by cells or protoplasts have been developed. These techniques include incubation in polyethylene glycol, insertion of DNA using a particle gun, and electroporation (electric shock). Polyethylene glycol and electroporation are used to genetically transform protoplasts. Protoplasts are incubated with the DNA or genes of interest under controlled laboratory conditions. The polyethylene glycol, or an electrical shock, facilitates uptake of the foreign DNA and its incorporation into the protoplast DNA. Once this step is accomplished, it is necessary to regenerate the plant which is often complicated. If protoplasts can't be developed in a particular plant species, DNA coated onto tungsten or gold particles can be projected into target cells using a particle gun. Again, the plant needs to be regenerated, a process that is generally easier in dicots as compared to monocots. But in the later cases, using the particle gun, regeneration is from cells rather than protoplasts which is often easier.

Routine utilization of plant genetic engineering techniques in plant breeding will require the availability of efficient transformation systems, and detailed information on the location,

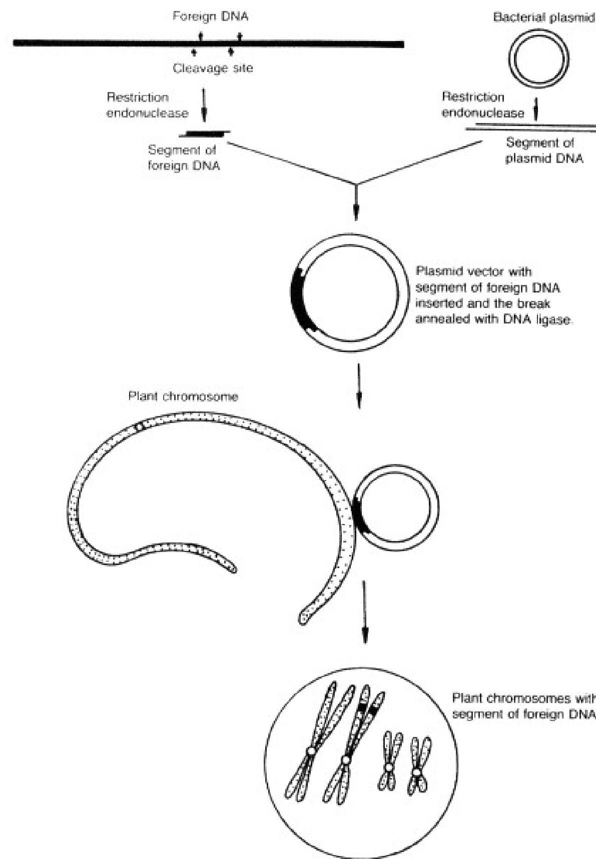


Fig. 8.14.
Model for cleaving foreign DNA and a bacterial plasmid with restriction endonuclease. The foreign DNA is transferred to and inserted in a plant chromosome by means of a bacterial plasmid vector.

structure, and function of the foreign gene to be transformed. Most major plant-breeding problems involve quantitative characters controlled by polygenes and will not be solved by transfer of small, isolated DNA segments or single genes. Developing molecular biological techniques are being used to study quantitative traits at the DNA level. A major contribution from recombinant DNA research may be a better understanding of basic plant genetics and a more complete knowledge of the plant genome, from which plant breeding will benefit.

Molecular Markers

Gene Mapping

In the 1920s, soon after it was discovered that genes reside in chromosomes, techniques were developed for the genetic mapping of individual chromosomes. One of the first organisms used for *genetic mapping* was the fruit fly, *Drosophila melanogaster*. The genetic maps were constructed from observations that genes for particular morphological features were inherited in groups and that independent assortment among them did not occur (Chapter 3). Today, detailed genetic maps on the location of genes, developed from visible plant characters, *morphological markers*, are available for a number of field and horticultural crops including corn, barley, peas, tomato, wheat, and many others. Knowledge of the location of genes in individual chromosomes is important information that has been used by the plant breeder. For example, the breeder may be interested in knowing if a desirable gene might be linked to a gene controlling an undesirable trait. If the desirable gene is tightly linked to the undesirable gene, this will give an indication to the breeder on how much effort will be required to break the linkage, how likely a desirable recombinant will occur among genes of interest after crossing and selection, and how large the selection population needs to be. It is also of interest to know in polyploid species the specific genome in which a desirable gene is located. If the desirable allele is not present in the cultivated polyploid, it may be necessary to search for this allele in the progenitor species.

Molecular Mapping

In addition to utilizing *morphological markers* for the genetic mapping of the chromosomes, maps may be developed using *molecular markers*. The molecular markers being utilized include *isozymes, restriction fragment length polymorphisms* (*RFLPs*), and *random amplified polymorphic DNA* (*RAPDs*). Isozymes are multiple forms of a single enzyme. Chemically, they are complex proteins. Isozymes were the first molecular genetic markers used in plant genetics and breeding. The number and polymorphism level of isozymes are much lower than that of the recently found molecular markers at the DNA level, RFLPs and RAPDs.

Restriction Fragment Length Polymorphisms (RFLPs)

RFLPs are defined as *different fragment lengths of restriction endonuclease digested DNA detected by a defined probe between individuals*. The different fragments of DNA are produced by *restriction enzymes* that recognize and cleave the DNA at specific sequences of *nucleotides* (Fig. 8.15). A typical crop species may have nearly one billion or more *base pairs* per cell. After the restriction enzyme cuts the DNA, many fragments will result which range from a few

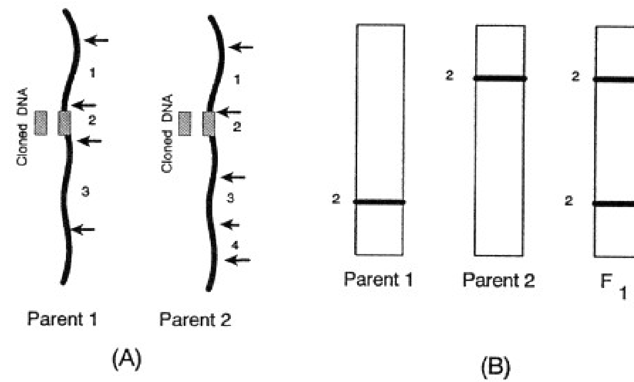


Fig. 8.15.
A generalized procedure for identifying RFLPs from plant chromosomes. (A) Chromosome in parent 1 is cut at four different sites with a restriction enzyme. Parent 2 is cut at five different sites with the same restriction enzyme. Note the different lengths of DNA fragments for each parent. The cloned DNA will hybridize with complementary nucleotide sequences in fragment 2 in both parent 1 and in parent 2 and in a hybrid plant from a cross between them. (B) Audioradiograph showing the migration pattern of the hybridized DNA fragments in an agarose gel. Different size fragments migrate at different rates. Only those fragments that hybridized with the radioactive cloned DNA will be visible. The F_1 shows codominant behavior because fragment 2 from both parents is visible.

hundred base pairs to over several thousand base pairs in length. Not all of these many fragments will be analyzed. Rather, specific DNA sequences that are distributed throughout the genome and complementary to *cloned DNA* will be analyzed to obtain the restriction pattern for a particular plant.

The cloned DNA represents a short segment of the plant's DNA that was incorporated into a vector such as a bacterial plasmid. The vector will multiply these segments many times over so that ample cloned DNA is available to hybridize with complementary segments of DNA cleaved by the restriction enzymes. The cloned DNA will hybridize to the genomic DNA fragment from the plant being analyzed where there are stretches of complementary nucleotides. The cloned DNA is labelled with a radioactive isotope or nonradioactive compound such as biotin, making it possible to detect the hybridization event by autoradiography, thus the cloned DNA is called a *probe*.

The sequence of steps for RFLP analysis from plant tissue includes:

• Isolation of *genomic DNA* where DNA is separated from carbohydrates, proteins, lipids, and other compounds.

• *Restriction digestion*, where one or more restriction enzymes are used to cut the plant's DNA at specific nucleotide sequences.

• *Gel electrophoresis* where the cleaved DNA fragments are separated on an agarose gel by an electrical charge. Different size DNA fragments move at different rates through a gel with smaller fragments moving faster.

• *Southern blotting* where digested plant DNA is denatured (i.e. from doubled-stranded to single-stranded) and transferred onto a nylon membrane. The single-stranded DNA

is tightly bound to the membrane at this point so the single-stranded probe can hybridize to the complementary cleaved DNA by nucleotide base-pairing.

• *Labelling of the probe* with a radioactive isotope, colorimetric label (e.g. biotin), or chemiluminescent label (so probe emits light).

• *Hybridization of the probe* to the DNA on the nylon membrane.

• *Autoradiography* is used so that the hybridized DNA can be visualized on film. The probe will hybridize only to the cleaved DNA that is complementary, that is, has identical nucleotide sequences.

After completion of these steps the amount of hybridization or differences (*polymorphism*) between plants can be determined.

Increased attention is being given to mapping RFLPs because there is potentially an unlimited number of RFLPs in a given plant species and the technology has advanced so that RFLP analysis is a routine procedure in many laboratories. In addition to being abundant, RFLPs are stable, universal, and convenient. RFLPs are expected to show Mendelian *codominant inheritance* (Fig. 8.15), have minimal *pleiotropic effects*, are not affected by the environment, and are detectable in all living tissues and at all stages of development.

How RFLPs Are Used

RFLPs are useful for cultivar identification, genetic mapping, germplasm evaluation, and as indirect selection criteria. Because several unique banding patterns are possible in many crop species, the RFLP technology can potentially be used to distinguish among different cultivars. The amount of polymorphism also gives plant breeders an idea of how much potential genetic variation might be available in a breeding population. Probably the greatest potential use of RFLPs will be their role as an indirect selection criteria (Fig 8.16). If a particular band is linked to a desirable gene, for example a gene for insect resistance, the breeder merely needs to search for that band in a breeding population to identify the insect resistant gene. Once the insect resistance gene is located, it can be transferred into the proper genetic background by conventional plant breeding techniques such as backcrossing. It must be remembered that the RFLP marker is linked to the insect resistant gene unless the RFLP marker itself is complementary to the desirable gene. If the marker is linked to the insect resistant gene it may be separated from it in a recombination event. The probability of this recombination event is proportional to the genetic distance between the gene and the RFLP marker. Therefore, the breeder will need to test the new strain for insect resistance before releasing as a new cultivar.

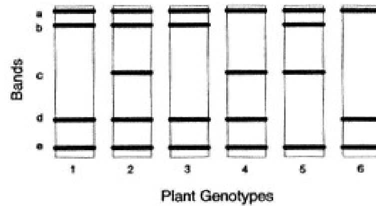


Fig. 8.16.
RFLPs can be used in selection for specific traits in plant breeding. In this hypothetical example, the audioradiograph shows the RFLP banding pattern from six plant genotypes from the same plant species. Of the six genotypes, plant genotypes 2, 4, and 5 only, are known to have resistance to a particular insect pest. Band c is present only in these genotypes suggesting that it may be linked to the gene conferring insect resistance. Six plant genotypes would not provide conclusive evidence that band c is linked to the insect resistant gene. The banding pattern of a sufficient number of plants should be analyzed to give assurance that band c is linked to the proper gene.

The use of RFLP maps in crop improvement is showing promise for identification of genes for disease resistance. Many genes for disease resistance have been linked to RFLP genotypes in several crop species. Examples include Phytophthora rot of soybean, resistance to downy mildew (*Bremia lactuca*) of lettuce, maize dwarf mosaic virus of maize, powdery mildew (*Erysiphe graminis*) of barley, leaf blast (*Magnaporthe grisea*) of rice, powdery mildew (*Erysiphe polygoni*) of mung bean, and others.

Random Amplified Polymorphic DNA (RAPDs)

The RAPD technique was developed in 1990 for rapid detection of polymorphisms among individuals using a single primer of arbitrary sequence (usually 10 nucleotides) and the *PCR* (*polymerase chain reaction*) mediated amplification of random genome DNA fragments. The procedure is rapid, requires small amounts of DNA, and involves no radioactivity. The RAPD markers are usually dominant because the polymorphisms are detected as the presence or absence of bands on ethidium bromide stained agarose gels. Like isozymes and RFLP markers, RAPDs have been used in plants for constructing genetic maps, estimating genetic relationships, and tagging traits such as disease resistance.

Use of Molecular Technology in Plant Breeding

The use of these new biotechnology procedures holds promise to provide tools that will assist the plant breeder in developing new cultivars. Conventional plant breeding procedures will not be replaced by these new biotechnology procedures. If new recombinants or new genes are identified by these novel techniques, it must be remembered that they will be fruitless unless incorporated into the proper parental material. The plant breeder is in the best position to determine which plant material should include these novel genes that would likely lead to a new improved cultivar.

Study Questions

1. What is a restriction fragment length polymorphism?

2. What is somaclonal variation? How might somaclonal variation be used in crop improvement?

3. What are the steps involved in genetic transformation?

4. What potential advantages do the biotechnology procedures discussed in this chapter have for crop improvement?

5. What are some of the potential improvements to crop plants as the result of using biotechnology procedures that would not be likely through conventional plant breeding?

Further Reading

Bajaj, Y. P.S. 1983. In vitro production of haploids. p. 228-87, *In* D.A. Evans, W.R. Sharp, P.V. Ammirato, and Y. Yamada, (eds.) Handbook of plant cell culture. Vol. 1. Techniques for propagation and breeding. Macmillan, New York, NY.

Beckmann, J.S., and T.C. Osborn (eds.) 1992. Plant genomes: Methods for genetic and physical mapping. Kluwer Academic Publishers, Boston, MA.

Blake, N.K., R.L. Ditterline, and R.G. Stout. 1991. Polymerase chain reaction used for monitoring

multiple gene integration in Agrobacterium-mediated transformation. Crop Sci. 31:1686-88.

Day, P. 1993. Integrating plant breeding and molecular biology: Accomplishments and future promise. p. 517-23. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Kung, S-d., and R. Wu. 1993. Transgenic plants. Vol. 2. Present status and social and economic impacts. Academic Press, Inc., San Diego, CA.

Ladd, S.L., and M.R. Paule. 1983. In vitro crop breeding. p. 131-51. *In* D.R. Wood (ed.) Crop breeding. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Lal, R., and S. Lal. 1990. Crop improvement utilizing biotechnology. CRC Press, Inc., Boca Raton, FL.

Lycett, G.W., and D. Grierson (eds.) 1990. Genetic engineering of crop plants. Butterworths, London, UK.

Maretzki, A. 1987. Tissue culture: Its prospects and problems. p. 343-84. *In* D.J. Heinz (ed.) Sugarcane improvement through breeding. Elsevier, Amsterdam, The Netherlands.

Murray, D.R. (ed.) 1991. Advanced methods in plant breeding and biotechnology. Biotech. in Agr. No. 4. CAB International, Wallingford, UK.

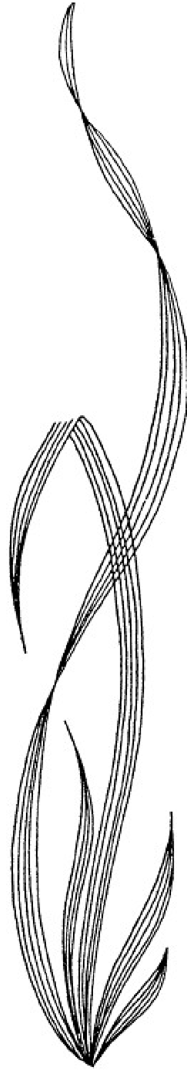
Peacock, W.J. 1993. *Genetic engineering for pastures. p. 19-22. In* M.J. Baker (ed.) Proc. XVII International Grassland Cong. SIR Publishing, Wellington, New Zealand.

Smith, R.H., R.R. Duncan, and S. Bhaskaran. 1993. In vitro selection and somaclonal variation for crop improvement. p. 629-32. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Tanksley, S.D., N.D. Young, A.H. Paterson, and M.W. Bonierbale. 1989. RFLP mapping in plant breeding: New tools for an old science. Bio/Technology 7:257-64.

White, F.F. 1993. Vectors for gene transfer to higher plants. p. 15-48. *In* S-d. Kung and R. Wu (eds.) Transgenic plants: Engineering and utilization. Academic Press, Inc., San Diego, CA.

Young, N.D. 1992. Restriction fragment length polymorphisms (RFLPs) and crop improvement. Expl. Agric. 28:385-97.



IV
METHODS IN PLANT BREEDING

9. Breeding Self-Pollinated Crops

Before discussing the methods by which new cultivars of self-pollinated crops originate, let us consider the question, "What is a cultivar?"

What is a Cultivar?

The *cultivar* (*agricultural variety*) is a group of genetically similar plants, which by structural features and performance may be identified from other groups of genetically similar plants within a species. The cultivar as an agronomic unit is familiar to plant breeders who develop new cultivars and to seedsmen and farmers who multiply the seed and grow the cultivars. But to understand the concept of the cultivar requires a knowledge of the system of plant classification. The plant kingdom is divided into taxonomic groups of similar and closely related plants; in this scheme, *families* of plants are divided into *genera*, which are subdivided into *species*. Within the species, the agronomist and horticulturalist recognize numerous *agricultural varieties*, more commonly referred to as *varieties* or *cultivars*.

This relationship can be clarified by examining the taxonomic classification of a common crop plant, the soybean, a species in the family, Leguminosae:

Family: Leguminosae (subfamily Papilionoideae)
  Genus: *Glycine*
    Species: *max*

The scientific name of the cultivated soybean is *Glycine max*; the first word designates the genus, the second word the species. The species, *G. max*, contains many forms that are genetically different and distinguished from each other by heritable traits such as maturity, seed color, pubescence color, plant type, disease resistance, oil content of seed, and a host of other characteristics.

A population of soybean may be composed of a single genotype or a mixture of genotypes and may be variously referred to as an *experimental strain*, a *strain*, or a *line*. Thousands of

experimental strains are generated in the plant breeder's nursery each year. Once a superior strain is identified, it may be named, the seed increased, and distributed as an 'agricultural variety' or 'cultivar'. Earlier, the term 'variety' was commonly used by farmers and seed producers; later the term 'cultivar' was coined to serve as the international equivalent of a cultivated variety. Variety and cultivar may be used interchangeably, but cultivar is now preferred in scientific literature and is used in this text. *The distinction of being named and distributed commercially serves to set apart the cultivar from the experimental strain or breeding line*. In the United States, the name, description, and developer of new field crop cultivars are registered by the Crop Science Society of America and this information is published in Crop Science.

Two essential characteristics of a cultivar are (1) *identity* and (2) *reproducibility*. Identity is necessary so that the cultivar may be recognized and distinguished from other cultivars within the species. Typically, the distinguishing features may be morphological structures, color markings, physiological response, disease reaction, or performance. Reproducibility is needed so that the characteristics by which the cultivar is identified will be reproduced in the progeny. In self-fertilized crops, a cultivar increased from a single, homozygous genotype will be uniform in appearance, whereas a cultivar increased from a mixture of genotypes will exhibit a range of genetic variability according to that present in the mixture.

Genetic Significance of Pollination Method

Self-pollinated crops differ in genetic make-up from plants in crop species that are normally cross-pollinated. In a crop that is self-pollinated, it is the rule that plants will be homozygous. This assumption may be made because:

- loci with identical genes (*AA* or *aa*) will remain homozygous following self-pollination,

- loci with contrasting genes (*Aa*) will segregate, producing homozygous and heterozygous progeny in equal proportions.

Heterozygosity is reduced by 50% with each successive self-fertilization(Fig. 9.1). After several successive generations of self-pollination, the proportion of heterozygous loci remaining in a population is very small. Although complete homozygosity is theoretically unattainable, plants selected from a mixed population after five to eight generations of selfing will normally have reached a practical state of homozygosity such that their progeny will be uniform in appearance and performance.

Breeding procedures in self-pollinated species are based on the genetic structure of self-pollinated populations. A mixed population of a self-pollinated crop is composed of plants with different homozygous genotypes. If single plants differing in genotype are harvested and the seed increased, each will produce a pure population, although the populations will differ from each other. Heterozygous plants may arise in a population of a self-pollinated crop through (1) *cross-pollination* among plants with different genotypes, or (2) by *mutation*. The progenies of the heterozygous plants will quickly segregate in succeeding generations giving rise to homozygous subpopulations.

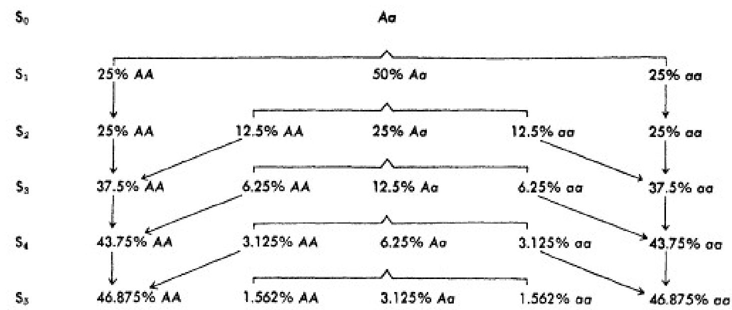


Fig.9.1.
Proportions of homozygous and heterozygous genotypes in a population after successive generations of self-pollination, assuming equal fitness for survival among genotypes. S_0, original selfed plant; S_1, first selfed generation; S_2, second selfed generation; and so on.

Breeding Methods in Self-Pollinated Crops

A new cultivar of a self-pollinated crop normally originates from an increase of:

• a mixture of plants, or a single plant, selected from introduced germplasm,

• a mixture of plants, or a single plant, selected from a local population, or

• a single plant selected from a hybrid population.

Increases from introduced germplasm or local populations involves the identification and increase of established genotypes, whereas selection from a hybrid population involves the creation of new genotypes through cross-pollination and gene recombination. Most new cultivars of self-pollinated crops are developed by the latter procedure. In the breeding of self-pollinated crops, thousands of strains are normally grown in adjacent plots in the breeding nursery without pollination control. Some natural cross-pollination generally occurs, but the amount is usually so small that it is ignored except when extreme purity is essential, as in genetic studies, or when a strain is being increased for final distribution as a new cultivar. If the amount of natural cross-pollination is sufficient to visibly affect uniformity, reselection is practiced to repurify the strain.

Assembly of Germplasm

The initial step in a breeding program is to *assemble a wide assortment of germplasm* (genetic strains of diverse origin) of the desired species, always searching for accessions with genes that will contribute to improved performance. Commercial cultivars are a desirable source of useful germplasm, except where their use is restricted by legal protection. Advanced breeding lines with proven adaptation and productivity are another useful source. Usually, the latter may be assembled from state, national, or international breeding programs or from

genebanks. In earlier years, cultivated native varieties, called *landraces*, could occasionally be introduced and utilized as new cultivars. Today, the landraces have been displaced in cultivation by modem cultivars except in the most primitive areas of the world. Landraces still serve as a source of useful genes in crop breeding programs, but they are seldom available except from genebanks around the world where they have been collected and stored to provide useful genes for future use by plant breeders. Germplasm accessions should be grown initially in the local environment to identify sources of genes for maturity, yield potential, disease resistance, and other desired traits, and to observe inherent weaknesses.

Selection

Selection, as a breeding procedure, involves identification and propagation of individual genotypes or groups of genotypes from mixed populations, or from segregating populations following hybridization. Unless genetic variation can be identified and distinguished from environmentally caused variability within the mixed population, selection may not be effective in isolating the desired genotypes. Selection procedures practiced in mixed populations of self-pollinated crops are *mass selection and pure-line selection*. The populations created are referred to as *mass selections* or *pure lines*, respectively.

MASS SELECTION. In the mass-selection procedure, plants are chosen and harvested on the basis of phenotype and the seeds composited without progeny testing. Cultivars developed by mass selection are normally uniform for qualitative characters with simple genetic inheritance, such as presence of awns, color markings, or maturity, where phenotypic differences can be visibly recognized and utilized as selection criteria. Genetic variations in quantitative traits such as yield, size, or quality, where phenotypic differences are too small to be recognized, or cannot be accurately distinguished from environmentally caused variations, may still be present.

The objectives in mass selection are to:

• purify a mixed cultivar or plant population by selecting and propagating visibly similar plants, or

• develop a new cultivar by improving the average performance of the population.

If a mixed cultivar or plant population is purified by mass selection, testing may be terminated and seed increase started any time after it has been verified that the new strain does not differ in adaptation and performance from the original population, and that it is superior to the original population in uniformity. Some genetic variation within a mass selection may be useful by providing buffering against variations in the environment. With mass selection, it is not possible to distinguish between plants that are homozygous or heterozygous for a qualitative character controlled by a dominant gene. The heterozygous plants will segregate in the following generation and phenotypic selection may need to be repeated. Neither is it possible to distinguish whether a plant owes its superior appearance to hereditary characters or to favorable environmental influences.

PURE-LINE SELECTION. A *pure line* is a progeny descendent solely by self-pollination from a single homozygous plant. *Pure-line selection* refers to the procedure of isolating pure lines from a mixed population. A cultivar developed by pure-line selection is more uniform than a cultivar developed by mass selection, because all of the plants in the cultivar will have

the same genotype. This is assuming that the plant originally selected is homozygous at all loci, an assumption plant breeders often make, but a condition seldom if ever achieved.

In the past, pure-line cultivars were often developed by identifying superior plants in a landrace, or a mixed cultivar. Currently, most new cultivars originate from selections made in segregating progenies following hybridization. In either case, the *progeny test*, as described in Chapter 3, is an essential feature of pure-line selection and serves to evaluate the breeding behavior of the selected plant. Pure-line selection does not create a new genotype, and improvement is limited to the isolation of the best genotype present in the mixed population. Once the superiority of a selected genotype has been proven, the population may be increased, named, and distributed as a new cultivar.

How long does the new cultivar remain pure? That depends upon amount of:

• seed mixtures from other sources,

• natural crossing with other cultivars or breeding lines, and

• mutations.

Harvesting and seed-cleaning equipment are common sources of seed mixtures, either in the breeding nursery, or with commercial lots of seed after cultivar release. Natural crossing may occur between plants of the cultivar and plants occurring as mixtures within the cultivar. The amount of natural crossing will vary with the crop species but rarely exceeds 1 to 2 % in self-pollinated crops. Off-type plants resulting from natural crossing or mutation need to be rogued out to maintain cultivar purity.

THE PURE-LINE THEORY. The theory of the pure-line was established by the Danish botanist, Johannsen, in 1903. Johannsen conducted selection experiments for seed weight in a mixed seed lot of the 'Princess' bean (see Fig. 4.3). Because beans are self-fertilized, the seeds in the original lot were homozygous for genes affecting seed weight. Selection within the original mixed lot of beans was effective in isolating lines that were genetically different. Once the pure line was isolated, further selection within the pure line was ineffective. In Johannsen's original mixed lot of beans, the variations in seed weight were both hereditary and environmental; within the pure lines, the variations were due only to differences in the effects of the environment.

PURE LINES VS. GENETIC DIVERSITY. In the self-pollinated crops, major attention has been given to the development of uniform, pure-line cultivars. Uniformity in appearance has a cosmetic value sometimes referred to as "eye-appeal," uniformity in maturity facilitates harvesting, and uniformity in quality enhances the market value of the product. In recent years the opinion has been growing that cultivars with more genetic variability would produce stable yields over a greater range of environments and seasons, be more widely adapted, and offer broader protection against specialized disease producing organisms than cultivars with limited genetic variability. An objection to the genetic diversity is based on the grounds that cultivar identification in variety protection and seed certification programs becomes more difficult and less accurate.

Hybridization

Hybridization is a breeding method that utilizes cross-pollination between genetically different parents to obtain gene recombination. Following the cross-pollination, segregating

generations are grown, and pure lines selected after homozygosity is reached. The objective is to identify and select lines which combine desirable genes from both parents. The selected lines are evaluated by progeny tests to verify presence of a desirable combination of genes. Lines proven to be superior may be increased as a new cultivar. Hybridization breeding should not be confused with the breeding of hybrid cultivars in which a cross is made and the $\mathbf{F_1}$ generation is grown.

In addition to combining genes for visible traits of the parents, plants may be selected from the segregating progenies that fall outside the range of the parents. Those falling outside the range of the parents are known as *transgressive segregates*. Transgressive segregates with a combination of genes superior to either parent in a quantitative feature, such as yield, seed weight, winter hardiness, or straw stiffness, in which inheritance is determined by multiple genes, may be selected and utilized in the breeding program.

In a cross between pure-line parents, all $\mathbf{F_1}$ plants will have identical genotypes, and be heterozygous at loci where the parents have contrasting alleles. Genetic segregation begins and maximum segregation occurs in the $\mathbf{F_2}$ generation, with heterozygosity reduced by 50 % with each succeeding selfed generation (Fig. 9.1). The number of $\mathbf{F_1}$ plants to grow will depend upon the desired size of the $\mathbf{F_2}$ progeny. Usually a large $\mathbf{F_2}$ population, from 1000 to 10,000 plants, will be needed to provide the desired range of genetic segregation; the number will be affected by how many traits the parents have in common and the number of different genes from each parent that the breeder desires to combine in the progeny. After segregation has virtually ceased, (5th or 6th generation), plants with a superior combination of the desired parent characteristics need to be identified and increased as a pure population. Performance of the new lines are evaluated in field trials in comparison with the parent lines.

Selection Procedures Following Hybridization

Selection procedures that may be used to identify desirable genotypes from segregating progenies, following hybridization in self-pollinated crops, include (1) *pedigree-selection*, (2) *bulk-population*, (3) *single-seed-descent*, and (4) *doubled-haploid*.

Pedigree-Selection

In the pedigree-selection procedure, selection for plants with the desired combination of characters is started in the $\mathbf{F_2}$ generation, and continued in succeeding generations until genetic purity is reached (Fig. 9.2). An example of the pedigree-selection method follows:

Crossing generation. Cross cultivar **A** × cultivar **B**.

F_1 generation. Grow 50 to 100 $\mathbf{F_1}$ plants. Before harvest, eliminate plants that may have arisen from self-pollination.

F_2 generation. Grow 2000 to 3000 $\mathbf{F_2}$ plants. Space plants sufficiently that individual plants may be examined. Select and harvest superior plants in which desired characteristics of the parent cultivars are combined, harvesting the seed separately from each plant.

$F_3$to F_5 generations. Grow progeny rows with seed harvested from superior plants harvested in the previous generation. Space plants in the row so that individual plants may be studied. Identify superior rows, then select and harvest 3 to 5 of the best plants within these rows. Continue selection between and within rows through the $\mathbf{F_5}$ generation. Normally, 25 to

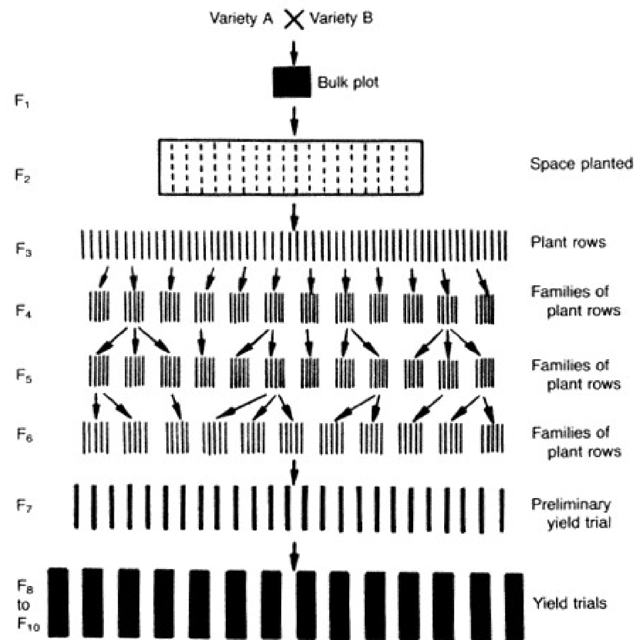


Fig. 9.2.
Pedigree method of selection. From selected F_2 plants, progenies of 25 to 30 plants are grown in plant rows in F_3. Superior plants from the best rows are selected and planted in families of plant rows in F_4 to F_6, with selection being made of best plants, in best rows, of best families. By F_6 families should be relatively uniform. Preliminary yield trials are planted in F_7, and yield trials are continued through F_{10}. Various modifications of this procedure may be made. For example, after plants are selected in F_3 and F_4, remaining plants in row may be bulked and preliminary yield tests started.

50 families may be retained at the end of the $\mathbf{F_5}$ generations. Identity of plant and row is maintained and superior traits of the plants are recorded.

F_6 generation. Grow families of plant rows. Uniform related families may be harvested together and the seed bulked. The separate seed lots are designated experimental lines.

F_7 generation. Grow the experimental lines in a preliminary yield trial in comparison with adapted cultivars.

F_8 to F_{10} generations. Yield trials of superior experimental lines are continued at two or more locations in comparison with adapted commercial cultivars. Only the highest yielding lines are retained for testing in the next yield trial. During the testing period, observations are made on height, tendency to lodge, maturity, disease and insect resistance, quality, and other characteristics as appropriate in the crop being studied. Growing the lines in regional yield

trials in environmentally diverse locations will assist in identifying lines with adaptation to a wide range of environments. If, after 3 to 5 years of yield testing, lines superior to the check cultivars have been identified, one line may be chosen for increase and distributed as a new cultivar.

F_{11} and F_{12} generations. Increase seed and distribute the new cultivar.

Modifications of the pedigree-selection procedure may be employed, such as introducing yield trials as early as the F_3 or F_4 generation. Only the high-yielding lines are then grown in advanced generations. Or, selection may be terminated earlier than indicated if the lines appear to be uniform.

The pedigree-selection method is labor intensive and requires detailed record-keeping during the early segregating generations. It has the advantage that only progeny lines in which plants with genes for the desirable characters have been identified are carried forward to the next generation. This method also permits the collection of genetic information which is not possible with other procedures. The pedigree-selection method of breeding is best suited to crops where individual plants may be examined and harvested separately, as in cereals, garden bean, peanut, soybean, tobacco, or tomato. Although widely used in particular species, the pedigree system is not always pursued as fully as described due to the labor requirements. Resources may be spread by growing more progeny rows, with a relaxation in note-taking and record-keeping. As illustrated, the pedigree-selection procedure requires 12 years to develop a cultivar if one generation only is grown each year. The number of years may be reduced by growing more than one generation per year, either in the greenhouse or by growing winter or off-season nurseries in an area with a favorable climate. The range of natural selection may be increased if the segregating generations are grown in more than one environment.

Bulk-Population

In the bulk-population procedure, seeds harvested in the F_2 and succeeding generations are bulked and grown, with selection delayed until an advanced generation, commonly the F_5 or the F_6, at which time the segregation will have virtually ceased (Fig. 9.3). An example of the bulk-population procedure follows:

Crossing generation. Cross cultivar **A** × cultivar **B**.

F_1 generation. Grow 50 to 100 F_1 plants. Before harvest, eliminate plants that may have arisen from self-pollination. Harvest en masse and bulk seed.

F_2 generation. Grow 2000 to 3000 F_2 plants. Harvest en masse and bulk seed from all plants.

F_3 to F_4 generations. Grow 1/50- to 1/100-hectare plots with bulked seed harvested from the preceding generation.

F_5 generation. Space plant 3000 to 5000 seeds. Select and harvest 300 to 500 superior plants keeping seed separate from each plant.

F_6 generation. Grow progeny rows of selected plants; harvest 30 to 50 progenies in which plants exhibit the desired characteristics of the parents.

F_7 generation. Grow superior progenies harvested in the F_6 in a preliminary yield trial.

F_8 to F_{10} generations. Yield trials are continued in multiple locations as in the pedigree-selection procedure.

F_{11} and F_{12} generations. Increase seed of a superior line and distribute as a new cultivar.

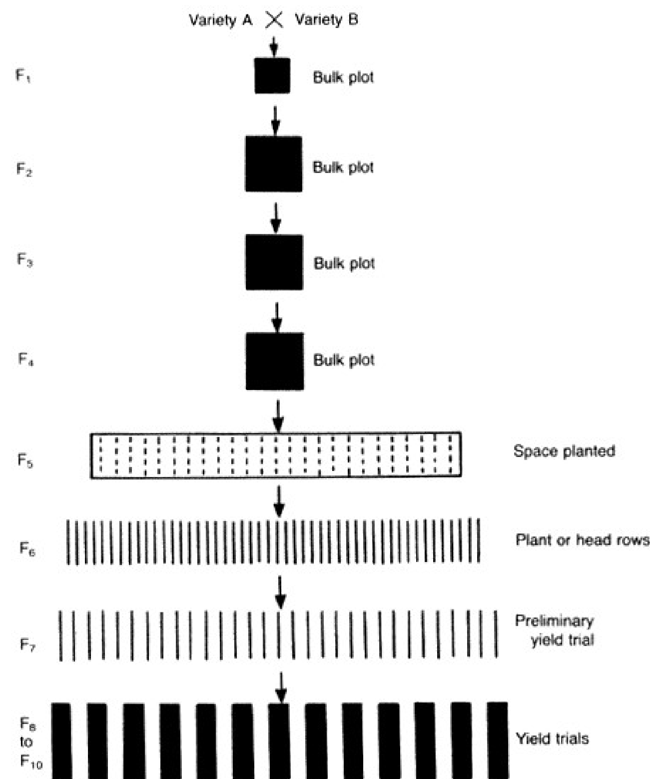


Fig. 9.3.
Bulk-population method of selection. The progeny of the cross is grown in a bulk planting through the F_4 generation. In F_5 the progeny is space planted. Plant or head selections are made and grown in plant or head rows in F_6. Superior rows are selected and grown in a preliminary yield trial in F_7. Superior strains are grown in yield trials in F_8 to F_{10}. Various modifications of this procedure may be made. For example, selection may start as early as F_3 or F_4, with lines having a superior yield being purified in later generations, or bulk plots may be replicated and harvested for yield and entire crosses discarded on the basis of the yield of the bulk plots.

The bulk-population method of breeding is simple, convenient, requires less labor, and is less expensive to conduct during the early segregating generations than the pedigree-selection procedure. It is necessary to grow large populations of spaced plants in the selection generation to have a reasonable chance of finding desirable segregates. Subjecting the bulk-populations to disease epidemics, winter injury, drought, or other adversities during the segregating

generations will foster natural selection for those features. Lines selected from the bulk-population that are still segregating will need to be reselected to establish true-breeding strains. The bulk-population method is suited to crops normally planted in thick spacings, like small grains, in which it is difficult to separate and identify individual plants. In contrast to the pedigree-selection method, no information is obtained during the early generations on inheritance of specific traits or performance of specific lines. During the segregating generations some desirable genotypes may be lost from the population, for example, tall and late plants may suppress short and early plants. The bulk-population selection procedure may be modified by selecting in the $\mathbf{F_3}$ or the $\mathbf{F_4}$ and starting yield trials even though the lines are still segregating. Superior yielding lines may be reselected while yield testing continues.

Single-Seed-Descent

In single-seed-descent the progenies of the $\mathbf{F_2}$ plants are advanced rapidly through succeeding generations from single seeds (Fig. 9.4). An example of the single-seed-descent procedure follows:

Crossing generation. Cross cultivar **A** × cultivar **B**.

F_1 generation. Grow 50 to 100 $\mathbf{F_1}$ plants.

F_2 generation. Grow 2000 to 3000 $\mathbf{F_2}$ plants. Harvest a single seed from each plant. Identity of the $\mathbf{F_2}$ plant is not maintained.

F_3 and F_4 generations. Grow seeds harvested in previous generation. Harvest a single seed from each plant.

F_5 generation. Space plants in field from seeds harvested in previous generation. Select plants superior for desired characteristics and harvest seeds from the selected plants.

F_6 generation. Grow progeny rows from plants harvested in the previous generation. Harvest rows superior for desired characteristics. Each row will have originated from a different $\mathbf{F_2}$ plant.

F_7 generation. Grow preliminary yield trial from rows harvested in the previous generation.

F_8 to F_{10} generations. Continue yield trials in multiple locations as in pedigree-selection and bulk-population procedures.

F_{11} and F_{12} generations. Increase superior line and distribute as a new cultivar.

An alternative procedure would be to space plant the $\mathbf{F_4}$ generation and plant the $\mathbf{F_5}$ in rows, thereby getting lines into yield trials one generation earlier.

The single-seed-descent procedure was proposed as a means of maintaining descendants from the maximum number of $\mathbf{F_2}$ plants, thereby reducing the loss of genotypes during the segregating generations. As currently practiced, single-seed descent is utilized to reduce the time required to grow the segregating generations. Because only one seed is harvested from each plant, optimum plant development in the $\mathbf{F_2}$ to $\mathbf{F_4}$ generations is unnecessary. By thickly planting seeds in a greenhouse bench, growing plants with low soil fertility, and using temperature and lighting regimes that force early flowering, two to three generations are commonly harvested in a 1-year period, and the preliminary yield trial can be reached 1 to 2 years earlier. Species that can be forced to mature rapidly, such as soybean or summer-grown cereals (wheat, oat, barley), are suited for the single-seed-descent procedure. For winter cereals in which a vernalization period is required to obtain flowering, the efficiency of the

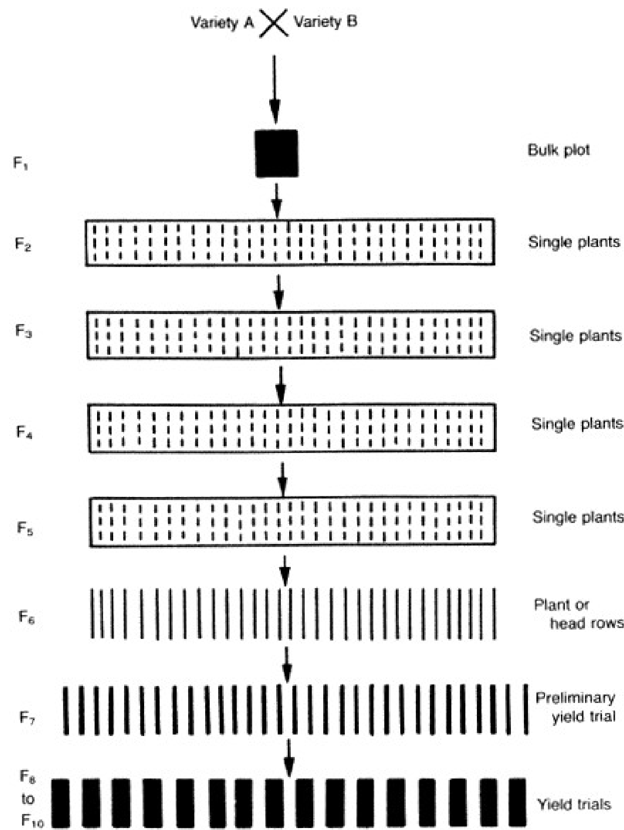


Fig. 9.4.
Single-seed-descent method of selection. Seeds harvested from F_1 plants are space planted in F_2. A single seed harvested from each F_2 plant is used to plant the F_3. Succeeding generations through the F_5 are likewise planted from single seeds harvested from each plant grown in the preceding generation. In the F_5 generation plants are harvested and a progeny row grown in the F_6. A preliminary yield trial is grown in the F_7 and yield trials continued through the F_{10}. Some breeders combine single-seed descent with the pedigree selection procedure, by growing only the F_3 and F_4 by single-seed descent to accelerate the time required to reach a yield trial.

single-seed-descent procedure is reduced.

A modification of the procedure has developed in soybean in which several pods are harvested from each plant instead of a single seed. A two- to three-seed sample from each plant is bulked to grow the next generation. This modified single-seed-descent procedure has become the principal method of advancing early generations in soybean breeding programs. With tropical winter nurseries, two or three generations can be advanced in a year with the modified procedure.

With single-seed-descent, weak plants are not eliminated as in a field-grown nursery, and there is no provision for selection of superior segregates within families descendent from $\mathbf{F_2}$ plants. Modifications to the procedure may be introduced, such as screening for disease resistance or other appropriate characteristics in any generation. No record-keeping is required during the early segregating generations. Final evaluation of progenies and yield trials are conducted in the field.

Doubled-Haploid

In the doubled-haploid procedure, haploid plants are generated from anthers of $\mathbf{F_1}$ plants, or by other means, and the chromosomes of the haploid plants are doubled with colchicine to produce diploid plants (Fig. 9.5). An example of the doubled-haploid procedure using anther culture follows:

Crossing generation. Cross cultivar **A** × cultivar **B**.

F_1 generation. Culture anthers to produce 2000 to 3000 haploid plants.

F_2 generation. Double chromosomes of haploid plants and harvest seeds from the doubled-haploid plants produced.

F_3 generation. Grow progeny rows from doubled-haploid plants and harvest seed from superior rows.

F_4 generation. Grow progeny rows in the field and select superior lines.

F_5 generation. Grow preliminary yield trial.

F_6 to *F_8 generations*. Continue yield trials.

F_9 and F_{10} generations. Increase and distribute superior line as a new cultivar.

Doubled-haploid plants are normally homozygous at all loci and it is unnecessary to grow segregating generations. Lines generated by the doubled-haploid procedure may reach preliminary yield trials two to three generations earlier than with the pedigree-selection or bulk-population procedures. Like the single-seed-descent procedure, early generations are not exposed to environmental stresses in the field, and attrition of lines is greater in initial field evaluation trials than with pedigree-selection or bulk-population procedures, in which the early generations are field grown. For successful use of the doubled-haploid procedure in plant breeding, efficient and reliable techniques for generating haploid and doubled-haploid plants are essential. The doubled-haploids should be vigorous, stable, free from tissue-culture-induced variations, and represent a random selection of the $\mathbf{F_1}$ pollen gametes. Current procedures for production of haploids and doubled-haploids have only been partially successful in attaining these characteristics.

Choice of Procedure

The superiority of a new cultivar is determined by the combination of genes that it contains, not by the procedure by which the cultivar was produced. Choice of procedure should

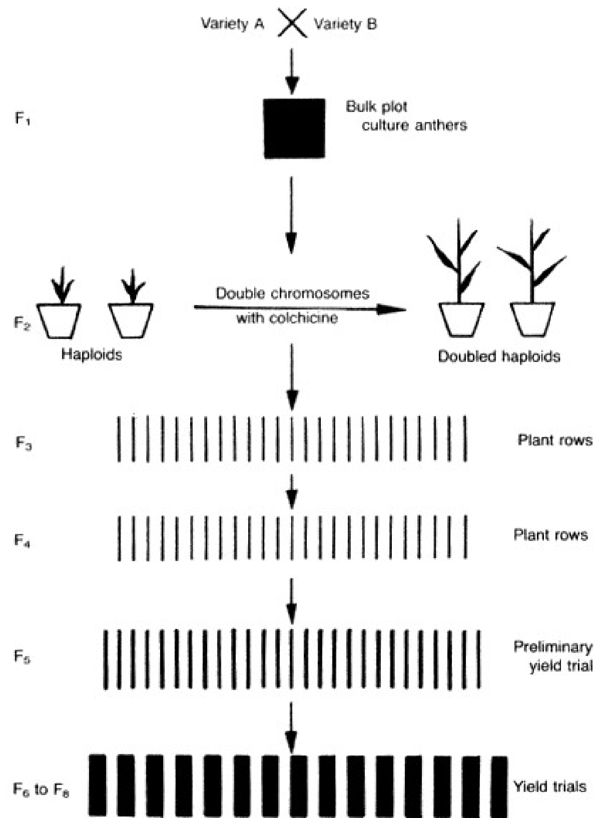


Fig. 9.5.
Doubled-haploid procedure. Crosses are made and F_1 progeny grown as in previous procedures. Anthers from F_1 plants are cultured and chromosome number of haploid plants generated is doubled with colchicine to produce doubled haploids. Progenies of doubled-haploid plants are evaluated in the field in F_3 and F_4 and superior lines tested in yield trials in F_5 to F_5 generations.

be determined by the efficiency with which a superior combination can be assured and will vary with the crop species, the breeding objectives, and the resources available to the breeder. The current trend is to adopt breeding procedures that will reduce the number of years to develop and release a cultivar, and that will enable the breeder to grow and examine the largest number of lines with the resources available. Reduction in years may be accomplished by

growing two or more generations per year through winter nurseries, by single-seed-descent, or by reducing the number of segregating generations as with the doubled-haploid procedure.

The pedigree-selection procedure is certainly the most precise system when the objective is to combine particular parent traits that are simply inherited and easily observed in progeny plants, but it is less precise if the characters to be combined are quantitatively inherited, particularly if the heritability is low. It is labor-intensive in the early generations, due to the extensive seed packaging, planting, note-taking, and record-keeping required. The bulk-population system has gained favor due to the economy of labor and ease of growing large populations in the early generations. Single-seed-descent is suitable for crops that can be grown in a greenhouse environment or in winter nurseries in a semitropical climate. It is economical to pursue, and reduces the time required to grow the early segregating generations. The doubled-haploid procedure is labor intensive in the production of haploids and does not have the proven reliability of the other procedures.

Success in the hybridization method of breeding self-pollinated crops is dependent upon:

• choosing the correct parents, and

• identifying the superior plants from the segregating populations.

The choice of parents will be facilitated by clear and specific breeding objectives and superiority of the parents in characteristics contributing to those objectives. The contributions from the parents should complement each other, so that selected progeny plants will not be lacking in some important agronomic characteristic. Identification of the superior genotypes in the segregating progenies requires exhaustive testing and exposure to many adversities (e.g., disease, drought, or cold), extensive observation in various stages of growth, and accurate recording of the observations. Testing in different seasons at several locations with diverse climatic conditions will aid in identifying genotypes adapted over wide geographic areas. Only those lines that are distinctly superior and fulfill the objectives of the cross should be propagated, with rigorous rejection of mediocre selections or crosses. *The latter requires judgment decisions that can best be made by a skilled and experienced breeder*.

If only one quantitative character is being emphasized in a cross, it should be possible to select transgressive segregates superior to either parent. If two or more quantitative characters are being improved, some compromise may be necessary, because one would seldom find superior transgressive segregation occurring simultaneously for two or more characters. At this point the breeder must choose which line best exemplifies the objectives of the cross and will be increased for further evaluation.

Backcross Breeding

The *backcross* is a form of recurrent hybridization by which a desirable allele for a character is substituted for the alternative allele in an otherwise desirable cultivar. The plan of the backcross is to cross an adapted and productive cultivar, yet one that lacks a desirable allele (or alleles) controlling a superior character, to a breeding line or cultivar in which the desirable allele is present. Beginning in the F_1 and continuing for several generations, hybrid plants containing the dominant allele are selected and successively crossed back to the adapted parent cultivar. The adapted parent, to which the allele is being added, enters into each backcross and is called the *recurrent parent*. The parent with the superior character enters into the initial cross

but does not enter into the backcrosses, and is called the *donor* or *nonrecurrent parent*.

The purpose of the backcross is to recover the genotype of the recurrent parent, except for the substitution of the allele (or alleles) for superior expression of the character being contributed from the donor or nonrecurrent parent. The backcross is a form of inbreeding, and the features of the recurrent parent are automatically recovered after successive backcrosses. The only selection practiced is for the one superior trait contributed by the nonrecurrent parent. The number of backcrosses may vary from two to five, or more, depending upon how completely the breeder wishes to recover the genes from the recurrent parent. The *backcross procedure is most easily carried out if the character being added is simply inherited, dominant, and easily recognized in the hybrid plants*.

The backcross procedure is illustrated in Fig. 9.6 in which the dominant alleles for a gene controlling disease resistance (*RR*) are to be substituted for the recessive alleles in an adapted cultivar. In this cross cultivar **A** is the recurrent parent, and contains the genes for adaptation and yield that the breeder wishes to recover in the new cultivar. Cultivar **B** is the donor parent with a dominant allele for disease resistance that the breeder wishes to add to cultivar **A**. With each successive backcross, the progeny becomes more like the recurrent parent as additional genes for adaptation are recovered. With completion of the fourth backcross, theoretically, 93.75 % of the genes of the adapted parent will have been recovered in the backcross progeny. After each backcross, disease resistant progeny plants (*Rr*) are identified, by artificially inoculating all progeny plants and noting their disease reaction. As many backcrosses may be made as are necessary to obtain plants that are indistinguishable from the recurrent parent except for the substituted allele for disease resistance. The disease-resistant plants in the final backcross progeny will be heterozygous for resistance (*Rr*), and must be selfed for one generation to obtain true breeding resistant plants (*RR*).

If the alleles for disease resistance being transferred should be recessive (*rr*), the progeny of the first backcross would segregate into genotypes (*RR*) and (*Rr*). Because the heterozygous plants that contain the resistance allele (*r*) cannot be identified, it is necessary to self the progeny one generation to find resistant (*rr*) plants before making the next backcross to the recurrent parent. Another procedure would be to backcross both the homozygous (*RR*) and heterozygous (*Rr*) plants to the recurrent parent and simultaneously self each backcross derived plant and test the selfed progenies for resistance. The backcross progenies from the plants that prove to be heterozygous are then kept, and the backcross progenies from the homozygous plants are discarded. If genes for undesirable characters are closely linked with the gene for resistance, they may be added along with the resistance gene. The new cultivar would then differ from the recurrent parent by the genes that were added. If characteristics being added by the backcross procedure are determined by multiple genes, it will be necessary for the backcross progenies to be grown through the F_2 or later generations to obtain plants that exhibit the desired characteristics before proceeding with the next backcross.

One feature of the backcross procedure is that a backcross derived cultivar will be adapted in the same general environment as the recurrent parent, reducing the testing normally necessary to confirm adaptation of the backcross derived cultivar. An additional feature is that it is repeatable. A breeder can recover the same line if the same recurrent and donor parents are used. If two or more characters are to be added to the same recurrent cultivar, separate backcross procedures may be pursued for each character and the backcross-derived lines from each finally merged into a single line.

The backcross procedure is further utilized to transfer entire sets of chromosomes into a foreign cytoplasm to obtain cytoplasmic male sterility for the production of hybrid seed as in

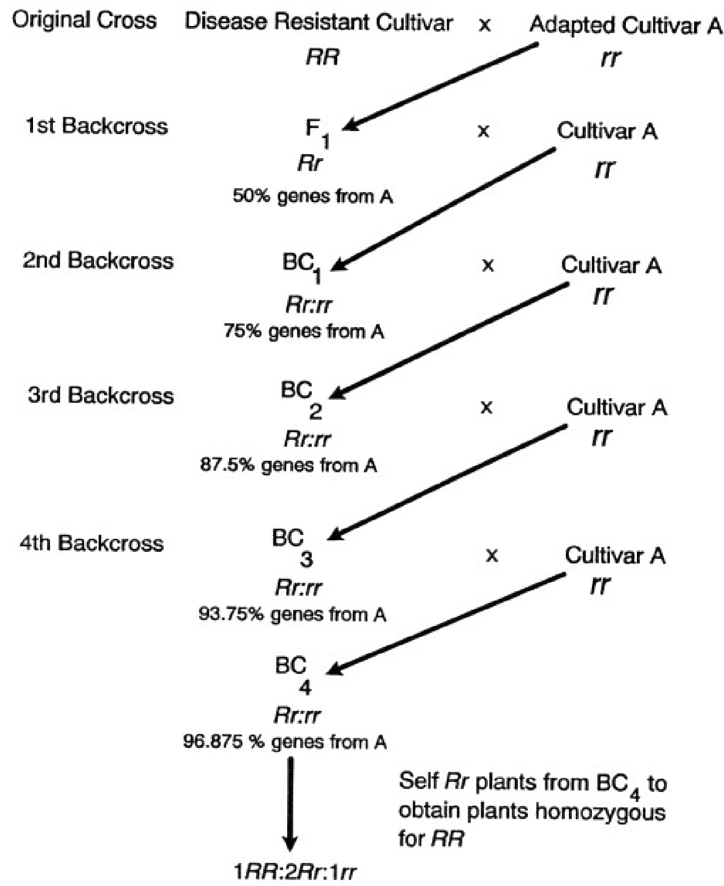


Fig. 9.6.
Procedure for a backcross in which a dominant allele for disease resistance (R) is transferred from a disease resistant cultivar to an adapted cultivar A. The resistant donor cultivar is crossed to the adapted recurrent cultivar A, and the F_1 backcrossed to cultivar A. The BC_1 generation from this cross will be segregating for disease resistance (Rr:rr). The Rr plants may be identified from the rr plants by inoculating the seedling plants with the disease pathogen and observing whether plants exhibit the resistant or the susceptible disease reactions. Only Rr (resistant) plants are backcrossed to A in the second and succeeding backcross generations. After the final backcross, the heterozygous (Rr) plants are selfed one generation to obtain homozygous (RR) and heterozygous (Rr) resistant plants. Progeny tests of the resistant (RR and Rr) plants are grown to identify the homozygous (RR) from the heterozygous (Rr) plants, so that lines pure for resistance may be established.

corn, millet, onion, sorghum, wheat, and other crops. The species or cultivar with the foreign cytoplasm is the female and recurrent parent as cytoplasm is transferred only through the egg. The donor of the chromosomes is crossed as the pollen parent until all donor chromosomes are recovered in the cytoplasm of the recurrent parent. Normally, the original cross and four or five backcrosses are required.

Multiline Breeding

The traditional breeding procedures for self-pollinated crops were developed around the pure-line concept. Pure-line selection was utilized to isolate the superior plant from genetically mixed populations, such as landraces, or from segregating populations following hybridization. Extreme uniformity among the plants within the selected line was stressed. The uniformity often led to cultivars with a single gene for resistance to a particular pathogen being propagated over large geographic areas. If a new race of the pathogen that was virulent on cultivars with the resistance gene arose, widespread disease damage would be caused. The rapidity with which new races of disease pathogens arose sometimes limited the usefulness of cultivars with a particular resistance gene to no more than 5 to 10 years. This condition led to the proposition that greater diversification in resistance genes would provide stronger genetic barriers and spread the risk from disease damage. One solution proposed was the use of multiline cultivars.

As proposed to combat disease resistance, a *multiline cultivar* is a composite of genetically similar lines, except that each line possesses a different gene for resistance to the disease pathogen (Fig. 9.7). Lines that are genetically identical, except for a single gene, are called *isolines*. The procedure for producing a multiline cultivar is to develop isolines of a desirable cultivar, each with a different gene for resistance to a particular disease pathogen. Each gene should contribute resistance to a different physiologic race, or group of races, of the disease pathogen. The backcross-derived isolines are then composited to form the multiline cultivar. As changes occur in the prevalent races of the disease pathogen, isolines with new genes for resistance may be developed. Because the multilines are reconstituted each year, the new genes for resistance may be introduced by changing the mix of the isolines.

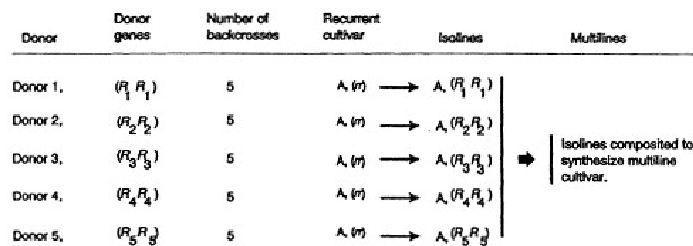


Fig. 9.7.
Procedure for production of a disease resistant, multiline cultivar. Genes for rust resistance, R_1 to R_5, are backcrossed from donor cultivars to a common disease susceptible, recurrent cultivar A. Isolines are generated that differ only in a gene for disease resistance and composited to synthesize the multiline cultivar. The isolines are maintained so that the multiline can be resynthesized as needed. Five crosses (original and four backcrosses) were made to the recurrent cultivar.

The theory of the multiline is to produce a population uniform for height, maturity, and other features, yet mixed in genes for resistance to pathologic races of a virulent disease. The merits of the multiline cultivar are based on the assumption that it will provide partial protection to a broad spectrum of races of a disease-producing pathogen and provide a buffering effect against rapid disease development should a new race of the disease pathogen arise. These assumptions have not yet been fully tested.

The multiline cultivar concept as outlined has distinct disadvantages:

• a multiline has limited utility except in high risk areas where severe disease damage occurs regularly from a highly specialized disease pathogen,

• there is no genetic improvement in yield or other characteristics, except that provided by the disease resistance, as long as the same cultivar is used as the recurrent parent for isolines development, (unless the recurrent parent is improved the multiline may soon become obsolete),

• the labor required to produce and maintain the isolines reduces the resources for improvement in cultivar characteristics other than disease resistance, and

• the release of a multiline cultivar is delayed until all of the isolines are produced and increased.

The term "multiline" is sometimes loosely applied to mixtures of genetically diverse lines combined in various ways to buffer against environmental stresses. More accurately, these mixed populations should be called *composites*.

Variety Blend

A variety blend is a composite cultivar produced by mixing seed of two or more cultivars, the suggestion being that a blend of genotypes will yield consistently higher than the average of the pure component genotypes, due to the buffering effect against genotype × environment interactions, and will be more stable over locations and years than a pure-line cultivar. The advantage of the latter tends to diminish as the number of cultivars in the blend is increased. A variety blend will be less uniform in appearance than a pure-line cultivar. In making a variety blend, cultivars should not be mixed that will adversely affect uniformity in maturity, or features that will reduce the quality of the product. Variety blends need to be reconstituted at regular intervals to maintain stable performance.

Non-Traditional Breeding Procedures

Traditionally, breeding procedures for improving self-pollinated crops involved crosses between two parents, followed by several segregating generations; yield-testing began only after a high degree of purity was reached. When the cycle was completed, superior segregates were crossed to start a new breeding cycle. Opportunities for gene recombination were limited by the narrow, two-parent gene pool, and opportunities to break-up linkage blocks were restricted by a 10 to 15 year breeding cycle. To overcome these deficiencies, breeders sought ways to increase the contributions into the gene pool. This has led to such non-traditional breeding practices as making large numbers of crosses, early generation yield testing and rapid elimination of low-yielding lines, and systems to increase potential gene recombination.

Large Numbers of Crosses

In a hybridization breeding program to improve simply inherited characteristics like seed color or disease resistance, parents with the desired genes may be readily identified. Choosing parents for crosses to improve characters with complex inheritance like yield, is less precise because the complementary genes contributing to yield from a set of parents cannot be identified by examining the parents. Only after the cross has been made and the range of segregation examined can the success of the cross be determined. This has led breeders to make large numbers of crosses to improve the odds that hybrid populations with superior transgressive segregates will have been made. It is also necessary to grow large segregating populations to improve the odds that the plant with the superior genotype will be included. Because resources are never unlimited, the breeder usually must choose between growing moderate populations of a large number of crosses or large populations of a moderate number of crosses. The former is generally favored because it involves use of larger numbers of parent cultivars and increases the odds that cross combinations with greater potential will have been made.

Early Testing

After a cross is made, the progenies from each cross must be evaluated to find the superior segregates, a task that greatly exceeds the labor of making the crosses. One procedure to reduce growth in the testing program is *early testing*, i.e., the evaluation of crosses, or selections from crosses, in yield trials at an early generation. With early testing, inferior segregates, or entire crosses, may be quickly eliminated and only the superior segregates carried to the advanced generations where homozygosity and uniformity are attained. Selection and yield testing, concurrently, in the early generations reduces the population size rapidly so that relatively few strains will be remaining by the F_5 or F_6 generation when a practical state of homozygosity is reached. Superior yielding lines identified in an early generation may immediately be used as parents in new crosses, even though homozygosity has not been fully attained, thus reducing the generations required to complete the crossing cycle and enabling the breeder to make more rapid progress.

Population Improvement

RECUR RENT SELECTION. *Recurrent selection is a population improvement procedure designed to increase the frequency of desirable alleles for a particular quantitative character by frequent intermatings among superior genotypes within the population*. Ideally, superior genotypes are isolated after each cycle of mating and intercrossed to produce the next generation. Considerable success has been achieved with population improvement procedures in cross-pollinated species, where random mating among plants occurs by natural means. Application of population improvement procedures to self-pollinated crops has shown similar success, but is difficult to employ due to the labor involved in making the larger number of hand pollinations required to intermate the selected genotypes.

MULTIPLE CROSS. The *multiple cross*, also called *convergent cross*, is produced by crossing pairs of parents, and then crossing pairs of F_1s until all parents enter into a common progeny according to the following scheme:

A × B		C × D		E × F		G × H
AB	×	CD		EF	×	GH
	ABCD		×		EFGH	
			ABCDEFGH			

With this system, many potential genetic combinations exist, because every seed produced after the initial cross is potentially a new hybrid. A disadvantage is that many undesirable combinations are also brought together. Exceedingly large numbers of hybrid seeds must be obtained in the second and later crosses if the maximum number of genotypes is to be represented in the progenies.

MALE-STERILE-FACILITATED HYBRIDIZATION. Male-sterile genes have been used in barley and other self-pollinated crops to facilitate crossing by eliminating the need for emasculation. A common procedure is to incorporate a male-sterile gene into a few standard cultivars by backcrossing. The male-sterile isolines produced are each pollinated from a group of cultivars and the crossed seeds bulked to plant a composite cross. With uncontrolled pollination, male-sterile flowers will be pollinated from male-fertile plants, thus facilitating gene recombination within the population. To obtain maximum gene recombination, seeds are harvested only from male-sterile plants. During the segregating generations, male-sterile plants may be hand-pollinated from selected male-fertile plants, and the seeds from these crosses used to plant the next generation.

Plant Breeding: A Numbers Game?

To generate the full range of potential genotypes from a cross it is necessary to grow large segregating populations. This concept, combined with the concept that large numbers of crosses among heterozygous genotypes are needed to break linkages, has often led to the popular notion that plant breeding is a numbers game; that chances for success in developing superior cultivars are enhanced by a proliferation in the number of crosses made, by increasing the size of the segregating generations, and by growing a myriad of selections from each cross. The rarity with which really superior genotypes occur provides considerable validity to these concepts.

The danger with the numbers approach is that the breeder may extend crossing and testing activities beyond the resources available needed to evaluate the materials adequately. There appears to be little value in generating the rare genotype unless it can be identified efficiently in the breeding materials being grown. The physical size of the breeding nursery, particularly for yield testing, may be extended by mechanization and standardization of procedures, and by computerization of data collection and processing. But the visual evaluation of the breeding lines, and the final evaluation of the data generated, requires personal judgements, which cannot be wholly mechanized or computerized and are limited in number by the breeder's time. Thus, the breeder needs to compromise between the number of recombinations desired and the number that can be evaluated efficiently and accurately within the resources of the program. While the breeder should select rigorously for the desired genotype and ruthlessly discard those

that are inferior, it is important that there be accurate and dependable information on which to base these judgments.

In traditional breeding procedures, extreme purity was attained before yield testing was initiated. With increased numbers of crosses and early testing, yield testing in the early generations is given more importance in the decision making. Yield testing provides a powerful tool in selection for resistance to environmental stress, or disease, provided that those production hazards are present with sufficient intensity to affect yield each season. On the other hand, too much reliance on yield data, without full consideration of other breeding objectives, often leads to proliferation of undesirable plant characteristics, such as increased height, late maturity, increased disease susceptibility, or inferior quality.

How Breeding Procedures for Self-Pollinated Crops Are Utilized

In this chapter the breeding procedures for self-pollinated crops are discussed. Earlier in this chapter it was stated that the superiority of a new cultivar is determined by the combination of genes that it contains, not by the procedure by which the cultivar was developed. In developing a breeding program for a specific crop, the breeder is faced with many decisions that transcends his decisions on breeding procedures. What are the objectives of the breeding program? How can cultivars already available be improved? What germplasm resources are available to make these improvements? What is the most efficient breeding procedure for accomplishing these objectives?

Wheat, rice, and soybean have been chosen as examples of how breeding programs are developed in self-pollinated crops. These are presented in chapters 14, 15, and 16, respectively.

Study Questions

1. How does the pedigree-selection breeding procedure differ from the bulk-population breeding procedure?

2. In the backcross breeding procedure, does it make a difference whether the donor parent is male or female? Why?

3. How does a multiline cultivar differ from a blend?

4. What are the advantages and disadvantages of early generation testing?

5. Which breeding procedures discussed in this chapter could be used to improve cross-pollinated crops?

Further Reading

Borlaug, N.E. 1959. The use of multilineal or composite varieties to control airborne epidemic diseases of self-pollinated crop plants. Proc. 1st Int. Wheat Genet. Symp., Winnipeg, Canada, 1958. Univ. of Manitoba, Winnipeg.

Borojevic, S. 1990. Principles and methods of plant breeding. Elsevier, Amsterdam.

Briggs, F.N., and R.W. Allard. 1953. The current status of the backcross method of plant breeding. Agron. J. 45:131-38.

Browning, J.A., and K.J. Frey. 1969. Multiline cultivars as a means of disease control. Annu. Rev. Phytopathol. 7:355-82.

Busch, R.H., and D.D. Stuthman. 1990. Self-pollinated crop breeding; concepts and successes. p. 21-37. *In* J.P. Gustafson (ed.) Gene manipulation in plant improvement II. Plenum Press, New York.

Fehr, W.R. 1987. Principles of cultivar development. Vol. 1. Macmillan Publishing Company, New York.

Jensen, N.F. 1952. Intra-varietal diversification in oat breeding. Agron. J. 44:30-34.

McFerson, J.K., and K.J. Frey. 1991. Recurrent selection for protein yield in oat. Crop Sci. 31:1-8.

McProud, W.L. 1979. Repetitive cycling and simple recurrent selection in traditional barley breeding programs. Euphytica 28:473-80.

Olsson, G. (ed.). 1986. Svalöf 1886-1986, research and results in plant breeding. Ltsförlag, Stockholm.

Snape, J.W. 1982. The use of doubled haploids in plant breeding. p. 52-58. *In* C. Broertjes (ed). Induced variability in plant breeding. Centre for Agricultural Publication and Documentation, Wageningen, Netherlands.

Weiss, M.G. 1972. Cultivar versus variety. Crop Sci. 12:551.

10. Breeding Cross-Pollinated and Clonally Propagated Crops

Breeding procedures in crop plants are designed to exploit the reproductive structure of the particular species. Thus, the breeding procedures used with cross-pollinated crops will differ from those used with self-pollinated crops. Furthermore, procedures may differ with different species of cross-pollinated crop plants because the species differ in the structure of the reproductive system. In this chapter models of breeding systems are presented that can be adapted to the breeding of cross-pollinated species. Modifications of the breeding systems that may be necessary to adapt the procedures for particular species, such as corn, potato, sugarcane, or forage crops, are discussed in chapters dealing with those specific crops. Most species of commonly cultivated cross-pollinated crops are seed propagated. Other species, potato and sugarcane for example, that reproduce sexually in their native habitat with normal cross-pollination are propagated asexually as clones when cultivated. A breeding procedure appropriate for asexually propagated species such as sugarcane is also described.

Genetic Structure of Cross-Pollinated Crops

The genetic structure of a population is determined by its gene pool and the opportunity for genetic recombination. It is in these characteristics that cross-pollinated populations differ from self-pollinated populations.

Breeding Cross-Pollinated Versus Self-Pollinated Crops

In the breeding of self-pollinated crops, the homozygous nature of the individual plant is exploited. A population of self-pollinated plants may be composed of either a single,

homozygous genotype, or a mixture of homozygous genotypes. Plant selection is effective only within populations that are mixtures of genotypes. Mass selection for a particular phenotype in a mixed population of a self-pollinated crop will reduce genetic variability and increase the frequency of genes affecting the characters being selected. Pure-line selection in a mixed population isolates single homozygous plants, or plants largely homozygous, which can be increased and reproduced as a breeding line or as a cultivar. *With selection focused on plants in self-pollinated crops, characters with qualitative inheritance tend to receive major attention*. After superior lines are identified, the breeder resorts to artificial hybridization to promote gene recombination and produce hybrid populations in which selection may again be practiced.

In the breeding of cross-pollinated species, the heterozygous nature of the individual plant is exploited. In a population of a cross-pollinated species, open-pollinated corn, red clover, or perennial ryegrass are typical examples, each plant has both homozygous and heterozygous loci, but it is the heterozygous loci that give this group of plants their characteristic genetic structure. As a consequence of natural cross-pollination, the genes are reshuffled each generation and regrouped into new genetic combinations. While theoretically following the Hardy-Weinberg law of genetic equilibrium, with respect to any particular locus, factors upsetting genetic equilibrium—nonrandom mating, mutation, migration, and natural selection—are also operating to restrict the outcome. With an almost limitless number of gene combinations possible within the gene pool, almost never would two plants be found with identical genotypes. Under natural environmental influences, cross-pollinated populations are relatively fluid, in which genes favoring adaptation and increased seed production tend to increase at the expense of genes unfavorable for adaptation or fitness to reproduce. In a breeding population, the shift toward more adapted genotypes may be accelerated by selection, and by environmental stresses to which the breeding population is subjected.

In cross-pollinated crops the focus of the breeder is on populations instead of individual plants, and more emphasis is given to quantitative inheritance in breeding systems than in self-pollinated crops. Due to the extensive heterozygosity in cross-pollinated crops, there is an abundance of phenotypic variation; hence, cultivars of cross-pollinated crops are less uniform than cultivars in self-pollinated crops. Genetic variability for qualitatively inherited characters may be drastically reduced by rigid selection, but genetic variability in quantitatively expressed characters continues to be present, due to inability of the breeder to select accurately for individual gene effects and to the influence of the genotype × environment interactions.

Progeny Test versus Combining Ability Test

An important difference between breeding self-pollinated and cross-pollinated crops is found in the way the breeder evaluates breeding materials. In a self-pollinated crop, in which individual plants tend to be homozygous, the genotype is reproduced in the progeny rather precisely and may be evaluated by progeny tests. In a cross-pollinated crop, individual plants are heterozygous and field grown plants will largely be pollinated by pollen from other heterozygous plants growing in the vicinity. Under these conditions, or even if self-fertilized, the genotype of a heterozygous plant is not faithfully reproduced in its progeny. Thus growing a progeny test of an open-pollinated plant does not provide information comparable to that obtained from growing a progeny test of a self-pollinated plant. What is obtained is an evaluation of the progeny performance of a random selection of gametes from a mother plant combined with a random selection of gametes from pollen plants of unknown origin. A more suitable test would be provided if the plant had been pollinated with a heterogeneous collection of pollen (gametes) of known origin. Performance could then be compared among progenies

of plants pollinated with the same source of pollen. A more precise comparison could be made by pollinating the plants with pollen from an inbred (homozygous) line. Each pollen grain would then be identical and the progeny performance test would measure the result of combining the assortment of gametes from representative mother plants with gametes of identical genotype from the tester line. A test comparing progeny performance of plants or strains pollinated with a known tester line is called a *testcross* and evaluates the *combining ability* of the mother plants or strains with the common tester line. The average or overall performance of a plant or genetic strain in a series of crosses with different tester lines is a measure of its *general combining ability*, whereas the performance of a plant or genetic strain in a specific combination in comparison with the performance of other cross combinations is a measure of its *specific combining ability*. Combining ability tests are used to identify desirable combinations of inbred lines to cross in the breeding of hybrid cultivars, or to identify desirable clones to include in a synthetic cultivar of a forage crop.

Plant Features Promoting Cross-Pollination

Cross-pollination may be promoted by morphological features or physiological characteristics of the plant. The more important of these are:

MONOECY. Monoecy is the separation of pistillate and staminate flowers on the same plant (see Fig. 2.4). Plants with monoecious flowers are normally cross-pollinated but some self-pollination may occur. Corn is an elegant example of an important crop with monoecious flowers. Because the flowers are borne on different parts of the corn plant and a self-incompatibility system is not present, either self- or cross-pollinations are easily made.

DIOECY. Dioecy is the production of pistillate and staminate flowers on different plants (see Fig. 2.5) and promotes cross-pollination. Dioecy is found in various species of field crops (hops, hemp, and buffalograss), vegetable crops (asparagus, spinach), and fruit crops (date palm, papaya). With true dioecy self-pollination is prohibited. However, because some normally dioecious plants produce monoecious flowers in addition, self-pollination on these plants is possible, unless restricted by self-incompatibility systems.

SELF-INCOMPATIBILITY. Self-incompatibility is the failure to become fertilized and set seed following self-pollination (see Fig. 7.1). Self-incompatible species are normally cross-pollinated due to the physiological hindrance of normal fertilization following self-pollination.

MALE OR FEMALE STERILITY. Male sterility, either genetic or cytoplasmic, promotes outcrossing because male-sterile plants do not produce viable pollen (see Figs. 7.2, 7.4, 7.6). With female sterility, the ovule does not function normally and seed production is inhibited. Female sterility is less common in plants than male sterility.

FLORAL DEVICES. Various other floral devices promote cross-pollination, such as failure of male and female sexual organs to emerge and mature at the same time (see Fig. 7.7), or the need for insect manipulation of flowers to effect pollination.

Inbreeding

Self-pollination, or inbreeding, in cross-pollinated crops leads to a decline in vigor and productiveness, a phenomenon observed by many early plant hybridizers who failed to grasp

its significance. Dr. G.H. Shull reported the deteriorating effect of inbreeding open-pollinated corn (maize) in a report to the American Breeders Association in 1908, and suggested that the breeder should strive to maintain superior hybrid combinations. This concept led to the development of procedures for breeding hybrid cultivars in corn and other crops. In forage crops, inbreeding may lead to a reduction in fertility and seed production.

Breeding Seed-Propagated Cross-Pollinated Crops

As in self-pollinated crops, the initial step in a breeding program is to assemble collections of germplasm of the appropriate species from different sources, which we will refer to here as *source populations*. Each source population is a collection of heterozygous plants representative of the population from which it originated. The source and extent of the germplasm collections will be influenced by the particular species with which the breeder is working and the objectives of the breeding program. The germplasm collections may originate from productive commercial cultivars or hybrids, improved breeding populations, introduced germplasm from gene banks or plant introduction centers, or local collections if the species is native to the area or has been grown continuously in the area for a long period of time. The source populations are maintained either as seed or vegetatively propagated materials according to the species involved.

Breeding procedures in cross-pollinated crops are based largely on population improvement principles, i.e., increasing the frequency of genes in the population for the desired breeding objectives. It is imperative for genes that contribute to the enhancement of the desired objectives to be present in the source population. Subjecting the source population and selections from it to environmental stresses, according to the objectives of the breeding program, will assist in identifying the superior genotypes and their progenies. Although *single plant selection* is practiced extensively in the different breeding systems, the cultivar is not established from a single plant in seed-propagated species because segregation and cross-pollination make it impossible to maintain the parent genotype in succeeding generations, and a wider range of genetic diversity than is found in a single plant is needed to maintain a vigorous and productive population. The selection of plants from the source population from visual evaluation is referred to as *phenotypic plant selection*. Except in the mass selection procedure, initial selection is followed by some form of progeny evaluation, either by growing *a progeny test*, or by crossing to a common tester genotype or a combination of testers and growing the *testcross progeny*. Corn, an annual species, is used in the examples of the breeding procedures described here; with biennial or perennial species, the length of the breeding cycles will require a larger number of generations than illustrated here with corn.

The Recurrent-Selection Principle

Recurrent selection is any breeding system designed to increase the frequency of desired alleles for particular quantitatively inherited characters by repeated cycles of selection. A recurrent-selection cycle involves:

• identification in a source population of genotypes superior for the specific quantitative character being improved, and

• the subsequent intermating of the superior genotypes to produce new gene combinations with improved expression of the character.

Selection cycles may be repeated as long as superior genotypes are being generated.

Phenotypic recurrent selection is selection to improve a plant quantitative character based on visual observation or physical measurement of the character. Examples are oil content in corn (Fig. 10.1), fiber strength in cotton, sugar content in sugarbeets, or seed size in wheatgrass. Phenotypic recurrent selection is an appropriate breeding procedure in naturally cross-pollinated species or species where artificial cross-pollinations are made easily.

Genotypic recurrent selection is selection to improve a plant quantitative character based on progeny performance as measured by test crosses, or by other means, and is utilized to improve complex characters such as combining ability in corn inbred lines.

A model for simple phenotypic recurrent selection is illustrated in Fig. 10.2 in which plants are visually selected from the source population and the progenies of the selected plants are grown and intercrossed to obtain new gene combinations. The crossed seed is used to grow a new source population, which starts the next selection cycle. Selection based on phenotype will be effective insofar as the phenotype accurately identifies the superior genotype for the character under consideration. Phenotypic recurrent selection is most effective for characters with low genotype x environment interaction, such as height of ears (in corn), seed size, or disease resistance. For quantitative characters that cannot be selected accurately from the phenotype, breeding procedures have been devised based on progeny or testcross performance that utilize the recurrent selection principle.

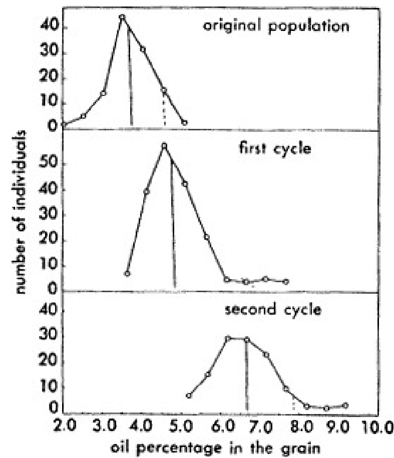


Fig. 10.1.
Comparison of oil percentages in a population of corn after one and two cycles of recurrent selection (Agron. J. 44:329-31).

Mass Selection

Mass selection in cross-pollinated crops is a selection procedure in which:

• individual plants are chosen visually for their desirable traits, and

• the seeds harvested from the selected plants are bulked to grow the following generation without any form of progeny evaluation (Fig. 10.3).

Repeating mass selection utilizes the recurrent selection principle. An example of the procedure follows:

First season: Select 50 to 100 plants with desired features from the source population and harvest open-pollinated seed from each.

Second season: Plant a mixture of the seed harvested in the previous year. From this population, again harvest open-pollinated seed from 50 to 180 plants selected for desired features.

Mass selection was one of the earliest procedures to be used in breeding corn, forages, sugarbeets, cotton, and other cross-pollinated crops. In open-pollinated corn, mass selection was practiced by the farmer each season when he selected seed ears during harvest for planting the next crop. In forage crops, local strains stabilized by natural selection may be harvested en masse and the seed utilized to start a new cultivar. Continuous mass selection for a specific character with high heritability that can be evaluated visually, such as early flowering, will shift the gene frequency of the character in the direction of the selection. Curly-top resistance in sugarbeets was increased by mass selection combined with recurrent selection (Fig. 10.4). Mass selection is simple to conduct; with its one-year cycle, new cultivars can be developed quickly and improvement is continuous. A weakness of mass selection is the lack of control over the pollen source and the gene contribution to the progeny through the pollen gametes. For this reason, heritability estimates are reduced by one-half. Selection for characters with low heritability is relatively ineffective, because plants superior due to phenotype may not be distinguished from plants superior due to environmental influences.

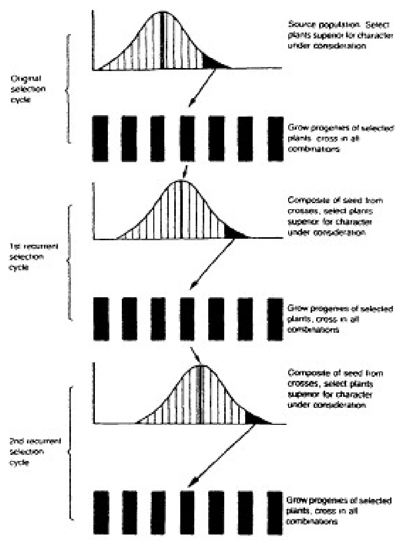


Fig. 10.2.
Model for phenotypic recurrent selection. Note that the mean of the populations has increased following each selection cycle.

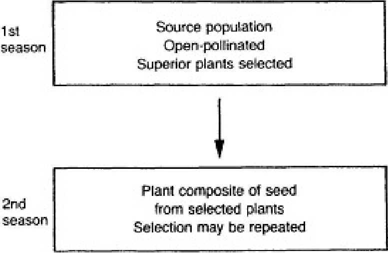


Fig. 10.3.
Mass selection. Individual plants are selected for desirable traits and seed is composited to grow the next generation. The composite may serve as the "source population" for the next selection cycle.

A procedure known as *gridding* may be used to reduce errors in selection caused by uneven environments. The land area on which the source population is grown is divided into small plots or *grids*. Plants are evaluated within each grid and only one superior plant from each grid is harvested. This procedure gives equal representation in the mass selection from all areas of the field regardless of field gradients in soil fertility or moisture supply. The grid system may be utilized when making selections from the source populations in the half-sib, full-sib, and S_1 procedures that follow.

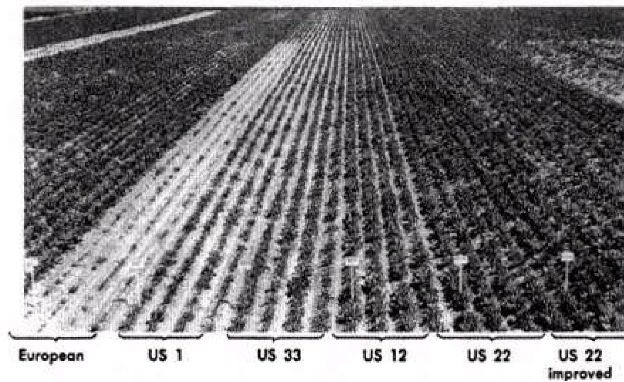


Fig. 10.4.
Progressive improvement in sugar beet for curly-top-virus resistance through a combined mass/recurrent-selection breeding procedure. Improvement was possible because (1) genotypes with genes for resistance were present in the European source population, (2) the severity of the curly-top-virus disease eliminated the susceptible genotypes from the population, and (3) interpollination among the resistant genotypes resulted in sugar beet plants with transgressive segregation for resistance. About 25% of the plants in US 1 were resistant to the curly-top-virus, 40 to 50% of the plants in US 33 were resistant, 75% of the plants in US 12 were resistant, and 85 to 90% of the plants in US 22 were resistant.

Half-Sib Selection with Progeny Test

Half-sib refers to a plant or family of plants with a common parent or pollen source. A half-sib selection procedure based on a progeny test differs from mass selection because the new population is constituted by compositing half-sib lines selected from progeny performance rather than from phenotypic appearance. Progenies of 25 to 50 plants are grown in replicated plots, so that the variance and mean performance may be evaluated. An example of the half-sib selection procedure as used with corn follows (Fig. 10.5):

First season. Select 50 to 100 plants with desired features from an open-pollinated source population, keeping the seed harvested from each plant separate. The seed from each plant will constitute a different breeding line.

Second season. Using seeds harvested from open-pollinated plants in the previous season, grow a progeny test of each line in an isolated area. Retain the remnant seed.

Third season. The population is reconstituted by compositing equal quantities of either (**a**) seed harvested from the 5 to 10 superior progenies, or (**b**) remnant seed from the 5 to 10 lines with superior progeny performance. Grow the composite in isolation with open-pollination to obtain new gene combinations. In (**a**), one-half of the genes come from a random assortment of pollen from the lines in the progeny test, and in (**b**), one-half of the genes come from a random assortment of pollen from the original source nursery. As an alternative to growing the composite in isolation, plants may be hand pollinated from a composite of pollen. Seed harvested in the third season may be:

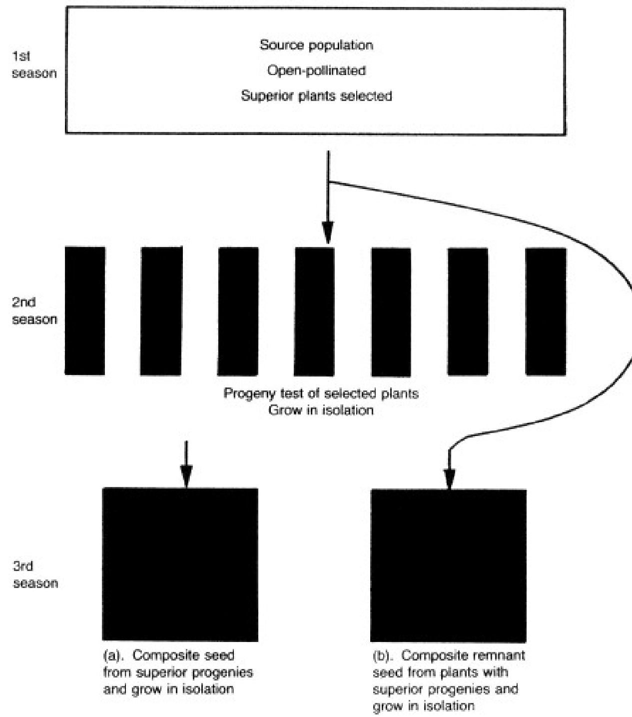


Fig. 10.5.
Half-sib selection based on progeny test performance.

• increased as a new open-pollinated cultivar,

• planted as a source population to start a new selection cycle, or

• planted as a source population for isolation of new inbreds in a hybrid breeding program.

The half-sib selection procedure outlined, or variations of it, was utilized by some corn breeders in the early part of the century. Designated *ear-to-row breeding*, individual ears were selected according to the breeder's fancy, the seed from each ear was planted in a separate row, and superior rows harvested for further selection or seed increase. In corn, the ear-to-row procedure, as with mass selection, was effective in changing gene frequency for highly heritable characters like ear height that could be selected visually, or like oil content of the kernel that could be measured. It was not effective in changing characteristics with low

heritability such as yield. In those days, entries in the progeny test were not replicated and, without replication, variance due to genetic effects could not be separated from variance due to environment. The half-sib procedure is applicable to cross-pollinated crops, like corn or sugarbeets, where sufficient seed can be harvested from a single plant to grow a yield trial, or to cross-pollinated crops where self-pollination cannot be consummated due to self-incompatibility systems. Like mass selection, it is based on maternal plant selection, without pollen control, in which case heritability estimates are reduced by one-half.

Half-Sib Selection with Testcross

In this procedure the selection of the half-sib lines to composite is based on testcross performance rather than progeny performance. An example of the procedure as used with corn follows (Fig. 10.6):

First season. Prior to flowering, select 50 to 100 plants with desired plant characters from a source population: (**a**) pollinate a tester parent plant with pollen from each of the selected plants and harvest crossed seed from the tester parent and open-pollinated seed from the selected plants, keeping identity of each seed lot; or (**b**) with pollen from each selected plant, pollinate a tester plant and self-pollinate the selected plant. Harvest crossed seed from tester parent plants and selfed seed from selected plants, keeping identity of each seed lot.

Second season. Grow testcross progenies.

Third season. Reconstitute the population (**a**) by mixing equal quantities of open-pollinated seed from 5 to 10 selected plants with superior testcross progeny performance; or (**b**) by mixing equal quantities of selfed seed from 5 to 10 selected plants with superior testcross progenies. Grow the seed composite in an isolated seed plot with open-pollination to obtain new gene combinations.

The half-sib testcross procedure permits control over the testcross parents so that a more precise evaluation of the genotype of the selected plant is obtained than from growing a progeny obtained by open-pollination as in the previous procedure. If the tester is an inbred line, plants in each of the lines in the testcross progeny nursery will have one parental gamete in common. Procedure (**b**) would be superior to procedure (**a**) because only genes from the plants with superior testcross progenies enter into the gene pool of the composite, whereas in procedure (**a**) one-half of the genes originate from a random selection of pollen from the source population.

The procedures outlined are applicable to corn and other cross-pollinated crops in which sufficient seed can be produced by crossing to grow a replicated testcross progeny trial. For procedure (**b**), self-pollination is necessary in addition, a requirement that could not be accommodated in self-incompatible species.

Full-Sib Selection

With full-sib selection, crosses are made between selected pairs of plants in the source population, with the crossed seed used for progeny tests and for reconstituting the new population. An example of the procedure follows (Fig. 10.7):

First season. Cross 150 to 200 pairs of plants selected from the source population. Reciprocal crosses may be made to provide a larger quantity of crossed seed.

Second season. Grow a replicated progeny test with seed from each pair of crosses,

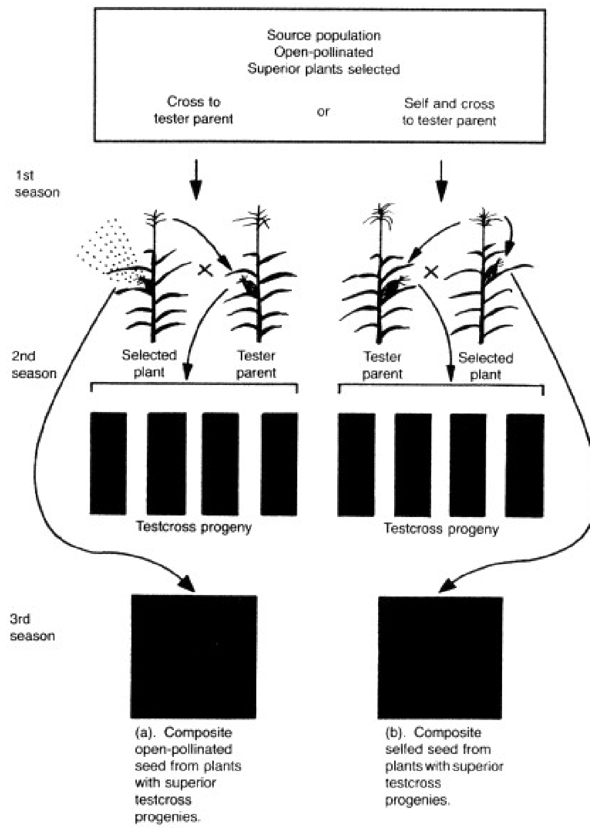


Fig. 10.6.
Half-sib selection based on testcross progeny performance.

keeping the remnant crossed seed.

Third season. Reconstitute the source population by mixing equal quantities of remnant crossed seed from 15 to 20 paired crosses with superior progeny performance, and grow in isolation with open-pollination to obtain new gene combinations.

Full-sib selection measures the combining ability from mating specific pairs of plants and only those pairs with superior progeny performance enter into the composite. The procedure is applicable to many cross-pollinated species, including self-incompatible species.

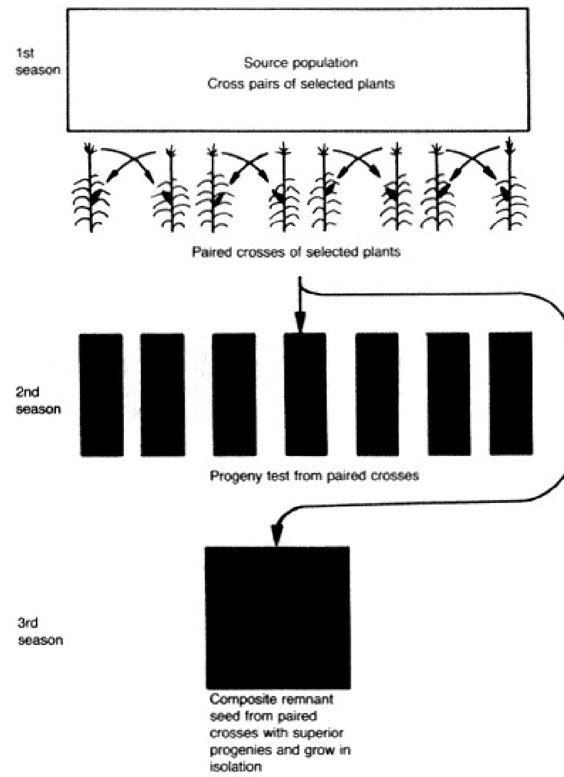


Fig. 10.7.
Full-sib selection based on progeny test performance of paired crosses.

Selection from S_1 Progeny Test

S_1 progeny tests may be utilized to evaluate selected plants from an open-pollinated source nursery. **S_1** refers to the progeny following self-pollination of plants in an open-pollinated population, or in the **F_2** following a cross. The procedure follows (Fig. 10.8):

First season. Select 50 to 100 plants from a source nursery prior to flowering. Self-pollinate and harvest selfed seed from selected So plants.

Second season. Grow replicated **S_1** progeny trial, keeping remnant selfed (**S_0**) seed.

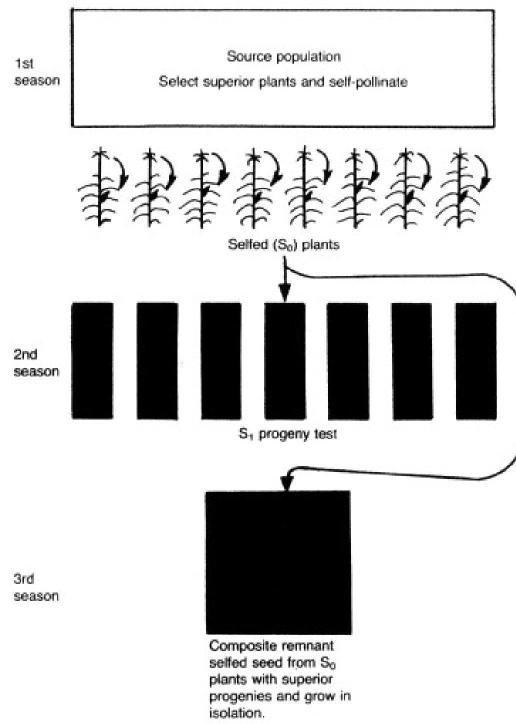


Fig. 10.8.
Selection based on S_1 progeny performance.

Third season. Composite equal quantities of remnant seed from the So plants with superior progenies, and grow the seed composite in isolation to obtain new gene combinations.

Selection is based on performance of $\mathbf{S_1}$ plant progenies. Each $\mathbf{S_1}$ progeny evaluated receives only the genes present in the parent So plant; no genes are introduced into the line from open-pollination or tester parents. The procedure is applicable to corn and other cross-pollinated species in which a quantity of seed sufficient for a replicated progeny trial and remnant seed for making the composite can be obtained by self-pollination. It would not be applicable to self-incompatible species.

Reciprocal Recurrent Selection

Reciprocal recurrent selection is a procedure designed by corn breeders to improve two populations simultaneously for both general and specific combining ability. *Recurrent selection for general combining ability* involves the use of a tester with a broad genetic base and identifies mainly additive genetic effects. *Recurrent selection for specific combining ability* relies on a tester with a narrow genetic base and identifies both additive and nonadditive gene action. With reciprocal recurrent selection, plants are selected in each of two populations, with the selected plants of one population being selfed and outcrossed as the tester to the selected plants in the other population. After the testcross progenies are evaluated, remnant seed from the plants with superior testcross progenies are grown and intercrossed to reconstitute the two populations. This completes the selection cycle.

Synthetic Cultivar

A synthetic cultivar is an advanced generation of a seed mixture of strains, clones, inbreds, or hybrids among them, propagated for a limited number of generations by open-pollination. The word "synthetic" implies a population of plants artificially produced by the breeder. The component strains, clones, or inbreds are maintained, and the synthetic is reconstituted at regular intervals. It is incorrect to apply the term synthetic to populations originating from seed mixtures advanced by open-pollination without periodic reconstitution.

The synthetic procedure is widely used in breeding forage crops. In forage species, the half-sib, full-sib, or $\mathbf{S_1}$ selection procedures are rarely applicable because:

• the quantity of seed produced from a single plant is usually inadequate to grow a progeny test,

• self-incompatibility inhibits production of selfed seed in many forage species, and

• controlled cross-pollinations are difficult to make in most forage species.

In addition to forage crops, synthetic cultivars may be developed in corn, sugarbeets, sunflower, and other cross-pollinated species.

The design of the synthetic cultivar utilizes the partial exploitation of heterosis during a limited number of generations of seed increase. This feature has made the synthetic cultivar popular in breeding forage species where conventional crossing procedures to obtain heterosis are not feasible. The procedure for developing a synthetic cultivar has these essential characteristics:

• The synthetic cultivar is constituted from reproducible units of a cross-pollinated crop (clones in forage species, inbreds in corn or sugarbeets).

• The plant materials entering into the synthetic cultivar are selected from performance in combining ability or progeny tests.

• The synthetic cultivar is constituted by random interpollination of the component units.

• The component units are maintained so that the synthetic may be reconstituted at regular intervals.

A model for the development of a synthetic cultivar in a forage species that embodies these characteristics is illustrated in Fig. 10.9. The procedure involves several distinct populations.

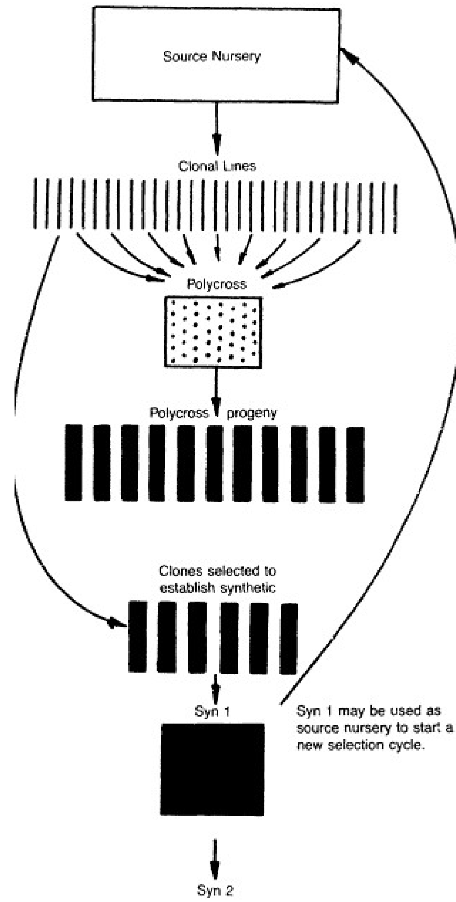


Fig. 10.9.
Procedure for development of a synthetic cultivar
of a forage crop, based on polycross progeny performance.

Source population. Plant selections are assembled from many sources to ensure a broad range of genetic variability. The original plant selections may come from old established pastures or meadows, improved cultivars, introductions, bulked populations after several cycles of recurrent selection for a particular characteristic, or other sources. The clones should be

vigorous and productive so that they can be easily maintained and will produce vigorous and productive progenies.

Clonal line nursery. From the source nursery several hundred superior plants, are chosen and multiplied as clones for establishing a clonal line nursery (Fig. 10.10). Each clonal line in the nursery will be comprised of 20 to 25 plants propagated vegetatively from the original plant. The clonal lines are evaluated for vigor, persistence, and other superior characteristics, depending upon the species and the objectives of the breeding program. Exposure of the clones to adversities, such as severe clipping, disease epidemics, cold, or drought, will aid in identifying clones with superior qualities. Finally, 25 to 50 of the superior clones will be selected for progeny testing in a polycross nursery.

Making the polycross. Seed for growing a progeny performance test is obtained by making a polycross. *The polycross is an isolated group of clonal lines replicated in such a manner that each clone will be pollinated by a random sample of pollen from all of the other clones*. The seed from each clone is harvested separately with the identity of the clone maintained.

Polycross progeny test. The open-pollinated seeds harvested from the clone in the polycross are planted in a progeny test for evaluation of yield and other characters. From the polycross progeny test performance, 5 to 10 of the clones are chosen as components for the synthetic.

Syn 0 generation. The 5 to 10 clones chosen to be utilized in the synthetic are vegetatively propagated and randomly transplanted into an isolated seed field, or, in the case of a legume, the clones may be transplanted into an insect-proof cage and pollinated with bees to obtain seed. This constitutes the **Syn 0** generation. Random cross-pollination among the **Syn 0** clones fosters gene recombination.

Fig. 10.10.
Strains of weeping lovegrass. In the development of a synthetic cultivar in forage crops, the characteristics of the individual breeding lines are evaluated by growing the lines in a field nursery. The combining ability of the lines are evaluated by growing the lines in a polycross yield nursery.

Syn 1 generation. Open-pollinated seed harvested from the **Syn 0** generation is planted in isolation for seed increase. This constitutes the **Syn 1** generation and may be distributed as a synthetic cultivar if seed can be produced in sufficient quantity. Superior plant selections from the **Syn 1** generation may be vegetatively propagated to start a new source nursery.

Syn 2 generation. Open-pollinated seed harvested from the **Syn 1** generation is increased in isolation. This constitutes the **Syn 2** generation.

The purpose of growing the **Syn 2** generation is to increase the quantity of seed that will be available to the farmer. If sufficient seed to meet market demand can be produced in the **Syn 1** generation, it will be unnecessary to grow the **Syn 2** generation. In most instances it is necesary to go to the **Syn 3** or later generations to have adequate seed for sale to farmers. The **Syn 1** and **Syn 2** generations are comparable to the $\mathbf{F_1}$ and $\mathbf{F_2}$ generations, respectively, in conventional hybridization. Each generation the synthetic cultivar is advanced beyond the **Syn 1** there will be successive reductions in vigor. The original clones are maintained so that the synthetic cultivar can be reconstituted when the seed fields need to be renewed. The **Syn 1** generation may be utilized as a source nursery from which to select clones that could be used in breeding future synthetics, thus introducing the recurrent-selection principle. In addition to polycross performance, as illustrated here, to evaluate clones, the clones may be evaluated by $\mathbf{S_1}$ performance trials.

A synthetic cultivar in corn is produced in a similar manner to that illustrated with a forage crop except that the breeder will be working with inbreds instead of clones. The corn inbreds to be used as component lines in the synthetic cultivar are chosen on the basis of combining ability tests, and crossed in all combinations to produce the seed for growing the **Syn 1** generation. The inbreds are maintained so that the synthetic can be reconstituted. Similar procedures may be utilized to produce synthetic cultivars in other cross-pollinated crops. In annual species of plants such as corn, the synthetic will need to be reconstituted each season, otherwise, the population behaves as an open-pollinated cultivar.

Choice of Source Germplasm and Test Environment

With recurrent-selection procedures, the performance level of the source population and the test environment are important. A superior combination of alleles for the character being improved will not be forthcoming if the genes are not in the source population. In choosing component lines for the source population, only those with the highest expression of the desired characters should be included. If the lines have a diverse origin, there may be a greater possibility that they will contain different alleles for the character. When evaluating the populations, they will need to be grown in an environment that fosters expression of the character to be improved, if the superior genotype is to be identified.

Breeding Clonally Propagated Crops

A clone is a vegetatively propagated population of genetically identical plants. In asexually propagated species, the separate genotypes are propagated as clones. Clonal propagation may be practiced with species that produce seeds poorly or that produce seeds only under special conditions. Some crops normally propagated as clones are sugarcane (see Fig. 2.10), potato, sweet potato, cassava, sisal, taro, and some species of perennial grasses, such as bermuda-grass. Asexually propagated species have not normally been subjected to self-pollination and

inbreeding, and individual plants are highly heterozygous, the heterozygosity being maintained through clonal propagation. With potatoes as an exception, most clonally propagated species are perennials. Aneuploid or polyploid chromosome genomes are maintained with clonal propagation, resulting in clones with chromosome numbers that differ from those recorded for the species. Breeding procedures for clonally propagated species may be grouped into:

• germplasm assembly and maintenance,

• clonal selection of natural or induced variants, and

• hybridization followed by selection and propagation of superior clones in the segregating population.

Germplasm Assembly and Maintenance

As with crops that reproduce sexually, the initial step in breeding asexually propagated species is to assemble a germplasm collection that is maintained as clones. The germplasm assembly may include clones selected from local populations if the species is native to the locality, introduced clones from genebanks or other breeders, commercially grown cultivars, or wild relatives introduced from their native habitat. The germplasm collection of clones constitutes the breeder's source nursery. Clones from the source nursery may be propagated and grown directly as cultivars, or the clones may be used as parents in a hybridization program. The germplasm collection is maintained as a collection of living plants in the field; this differs from maintaining a seed collection as in a sexually propagated species. Because vegetative propagation maintains the genotypes without change, except for mutation, large numbers of clones may be grown in the breeding nursery without isolation. In most countries it is mandatory that clones introduced from a foreign source first be grown in isolation to prevent the possible introduction of new species of insects or disease pathogens along with the clone. The hazard may be reduced by introduction of seeds instead of clones, if the species produces viable seeds.

Clonal Selection

In a genetically mixed population of an asexually propagated species such as exists in nature, a superior clone may be isolated and propagated as a cultivar. In a mixed population, progress through clonal selection is limited to the isolation of the best genotype present. Genetic variability may arise in a clone by mutation producing bud sports, chimeras, or genetic mosaics. In species of ornamental plants, variants originating from natural or induced mutations are often utilized as the source of new clones. A high mutation rate has been observed in genotypes of sugarcane maintained through tissue culture techniques, with the mutant plants then propagated as clones.

Hybridization

Gene recombination occurs with sexual reproduction. In a crop species that is normally propagated asexually, sexual reproduction is necessary to create genetic variability through gene recombination. By crossing clones with superior characters, source populations will be created that may be utilized for the selection of new clones as in self-pollinated crops. A typical procedure for developing a cultivar from an asexually propagated species, such as sugarcane, follows (Fig. 10. 11):

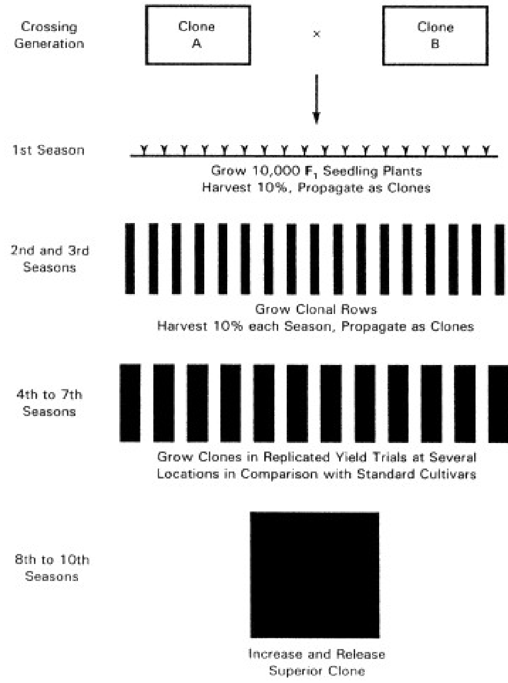


Fig. 10.11.
Hybridization procedure for a clonally propagated species. The seedlings grown in the first season are comparable to an F_2 generation in a conventional hybridization procedure. The genotype of each seedling plant is maintained by vegetative propagation in the first and succeeding seasons.

Crossing generation: Cross Clone A × Clone B.

1st season: Grow 10,000 $\mathbf{F_1}$ seedling plants. Select 1000 vigorous plants and propagate vegetatively.

2nd and 3rd seasons: Grow 1000 clonal rows in 2nd season; select 100 superior clones. Grow 100 clones in 3rd season, preferably at two locations; select 10 superior clones.

4th to 7th seasons: Grow selected clones in replicated field trials at several locations in comparison with standard cultivars or advanced breeding lines.

8th to 10th seasons: Increase propagules of superior new clone and release as a new cultivar.

Number of seedling plants and clones to grow are suggestive only and will vary with the species and resources of the breeding project.

Due to the open-pollination, the parent clones will be heterozygous, segregation occurs in the $\mathbf{F_1}$ generation; each $\mathbf{F_1}$ plant is thus a potential source for a new clone and a new cultivar. Clones propagated from $\mathbf{F_1}$ plants are heterozygous and the heterozygosity of the clone is maintained through asexual propagation. If the breeder does not find a superior genotype in the $\mathbf{F_1}$ generation, the crosses are remade, or different crosses may be made. Self-pollination to produce an $\mathbf{F_2}$ is seldom practiced because self-pollination leads to a reduction in vigor and fertility. If a superior $\mathbf{F_1}$ plant is identified in the hybrid progeny, it is propagated vegetatively to establish a new clone which is evaluated in observation and replicated plot tests.

How Breeding Procedures Are Utilized

In this chapter the breeding procedures for cross-pollinated and clonally propagated species were discussed. For discussion of how the procedures are utilized in particular crops, the student is referred to Chapter 19 on Breeding Cotton and Chapter 20 on Breeding Forage Crops. For discussion of how the procedures are utilized in breeding clonally propagated crops, the student is referred to Chapter 21, Breeding Potato, and Chapter 22, Breeding Sugarcane.

Study Questions

1. What is mass selection? How can the breeder modify mass selection to improve upon its effectiveness as a plant breeding method?

2. What is the difference between half-sib and full-sib recurrent selection?

3. Why is recurrent selection a more efficient breeding method in cross-pollinated crops than self-pollinated?

4. How does the breeding of clonally propagated crops differ from the breeding of non-clonally propagated crops? Why is the first generation in a cross between clonally propagated plants the same as the $\mathbf{F_2}$ in a regular hybridization program?

Further Reading

Abbott, A.J., and R. Atkin. 1988. Improving vegetatively propagated crops. Academic Press, Inc., San Diego, CA.

Cook, R.E. 1983. Clonal plant populations. Am. Sci. 71:244-53.

Fehr, W.R. 1983. Principles of cultivar development, theory and technique. Vol.1. Macmillian Publishing Co., New York.

Frey, K.J. 1983. Plant population management and breeding. p. 55-58. *In* D.R. Wood (ed.) Crop breeding. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Hayes, H.K., E.H. Rinke, and Y.S. Tsiang. 1944. The development of a synthetic variety of corn from inbred lines. J. Am. Soc. Agron. 36:998-1000.

Moreno-González, J., and J.I. Cubero. 1993. Selection strategies and choice of breeding methods. p. 281-313. *In* M.D. Hayward, N.O. Bosemark, and I. Romagosa (eds.) Plant breeding, principles and prospects. Chapman & Hall, London.

Tysdal, H.M., and B.H. Crandall. 1948. The polycross progeny performance as an index of the combining ability of alfalfa clones. J. Am. Soc. Agron. 40:293-306.

Vogel, K.P., and J.F. Pealersen. 1992. Breeding systems for cross-pollinated perennial grasses. p. 251-74. *In* J. Janick (ed.) Plant Breeding Reviews. Vol. 11. John Wiley and Sons, New York.

11. Breeding Hybrid Cultivars

Hybrid cultivars are the first generation offspring of a cross between inbred line parents with different genotypes. In the breeding of hybrid cultivars, homozygous parent lines, generally called *inbred lines, are* first created by *inbreeding* (self-fertilization) in a cross-breeding population. A hybrid cultivar differs from a cultivar produced by hybridization as described for breeding self-pollinated crops in Chapter 9. With hybridization in self-pollinated crops, homozygous parent lines are crossed and the segregating progenies are self-pollinated for several generations for the segregants to reach homozygosity, after which a pure line is selected and grown as the new cultivar. With the hybrid cultivar, homozygous inbred lines (pure lines) are crossed and the heterozygous $\mathbf{F_1}$ generation is grown as the hybrid cultivar.

The hybrid cultivar is produced in three steps:

• the development of inbred lines, normally by several generations of inbreeding in a natural or segregating population of a cross-pollinated species,

• crossing pairs of unrelated inbred lines to produce a *single-cross* $\boldsymbol{F_1}$ hybrid cultivar with many heterozygous loci, and

• producing seed of the single-cross hybrid cultivar for distribution to the grower.

Because the inbred parent lines of the single-cross hybrid are homozygous at all loci, being comparable to a pure line in this respect, single-cross hybrid plants with common parentage will be identical in genotype and uniform in appearance. Not only is the genotype of the superior single-cross plant reproduced in every plant in the farmer's field, but the genotype is reproducible in succeeding years if the purity and identity of the inbred lines used in the cross are maintained.

The Origin of Hybrid Breeding

Hybrid breeding began in 1909 when George H. Shull proposed a method for producing hybrid cultivars of corn (maize). The previous year, Shull had reported that *a field of open-*

pollinated (naturally cross-pollinated) corn is composed of many complex hybrids which decline in vigor with inbreeding, and that *the breeder should strive to maintain the best hybrid combination* (Fig. 11.1). From observations made while inbreeding and crossing in corn, Shull outlined a procedure for developing *inbred lines* (pure lines) in corn, and crossing the inbred lines to produce *single-cross hybrid cultivars*. The proposal completely revolutionized corn breeding, and breeding of hybrids has since been extended to other field and horticultural crops.

Edward M. East, working at the Connecticut Agricultural Experiment Station, also reported on the inbreeding of corn in 1909, but did not describe a clear procedure for utilization of the inbred lines. Later, East and his students, Donald F. Jones and Herbert K. Hayes, made significant contributions to the development of procedures for breeding hybrid corn. It appeared at first that the cost of producing the hybrid seed would limit the use of the system for breeding hybrids, because the seed was being produced on weak and unproductive inbred plants. This problem was solved by Jones who, in 1918, proposed crossing pairs of inbred lines to produce *single-cross* hybrids, and then crossing two unrelated single-cross hybrids to produce a *double-cross hybrid cultivar*, according to the scheme outlined below:

Develop four inbred lines:	**A, B, C, and D**
Cross pairs of inbreds:	**A × B C × D**
Cross single-cross hybrids:	**AB × CD**
Grow double-cross hybrid cultivar:	**ABCD**

The double-cross procedure made the production of hybrid seed corn economically feasible because the final hybrid seed was now produced in quantity on vigorous and productive (female) single-cross hybrid plants, rather than weak and unproductive inbred plants. Pollen was also produced more abundantly by the vigorous single-cross male parent.

Shull was not the first to observe the presence of hybrid vigor. Köelreuter, as early as

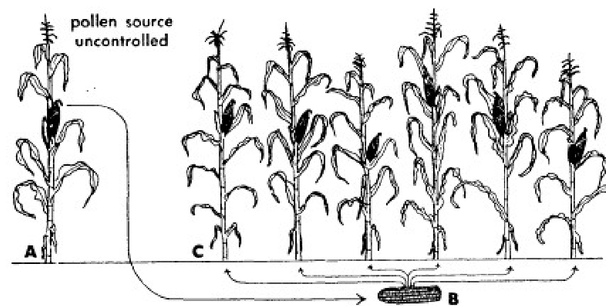


Fig. 11.1.
Open-pollinated plant of corn and its progeny. (A) Plant of open-pollinated corn. (B) Ear from open-pollinated plant: each kernel came from a separate fertilization, the pollen originating from many different plants within the field. (C) Plants grown from the seed of an open-pollinated cultivar of corn. Each plant is a different hybrid and varies in plant and ear characteristics, and in yield. In 1909, Shull proposed that the objective in corn breeding should be to maintain the genotype of the most vigorous plant, a suggestion that led to development of hybrid corn.

1763, had recorded the luxuriant growth of tobacco hybrids; Darwin, in 1877, concluded that the effect of cross-fertilization in plants was generally beneficial, whereas self-fertilization was injurious; and in 1880, W.J. Beal in Michigan reported that *variety hybrids* of corn were more productive than open-pollinated varieties. Beal's procedure for making variety hybrids was to cross open-pollinated varieties by detasseling rows of one variety which were then pollinated by a second variety planted in adjacent rows. The procedure was remarkably similar to today's procedure for producing single-cross hybrid cultivars. But farmers in those days were not ready for such radical innovations.

Before hybrid corn, it was the practice of corn breeders to breed for uniformity by *repeated selection for particular plant, ear, and kernel characteristics*. In practice, the procedure was a mild form of inbreeding that led to a reduction in the heterozygosity of the breeding lines, and gradual loss in vigor and productivity. So, for Shull to recommend growing a hybrid cultivar was revolutionary indeed. Years later (1952), Shull recalled just when he conceived the hybrid concept:

> At the end of 1906, I had only the concept held by Holden, Shamel, East, and all other corn breeders who had experimented with the selfing of maize, that *selfing had deleterious effects, not that crossing has advantageous effects*, other than the simple avoidance of the deleterious effects of selfing.

Quoting from his notebook on his 1907 experiments:

> The obvious results were the same as in 1906, the self-fertilized rows being invariably smaller and weaker than the corresponding cross-fertilized. [But] a very different explanation of the facts was forced upon me .... *It may be assumed that correct field practice in breeding of corn must have as its object the maintenance of such hybrid combinations as prove to be most vigorous and productive and give all desired qualities of ear and grain*.

Shull's concept portended the procedures used today in breeding hybrid cultivars in corn and other crop species.

Inbreeding in Cross-Pollinated Crops

Inbreeding consists of any system of mating that leads to an increase in homozygosity. Inbreeding occurs when individuals are mated that are related by ancestry. The most rapid approach to homozygosity in plants is through self-fertilization; heterozygosity in a population of plants being reduced by one-half with each successive self-fertilization(see Fig. 9.1). With self-fertilization, heterozygous alleles (*Aa*) segregate into the genotype combinations, 1*AA*:2*Aa*:1*aa*; homozygous alleles (*AA* and *aa*) continue to reproduce the same homozygous genotypes. As homozygosity increases in the breeding population, genotype-frequency changes, although gene-frequency remains unchanged (Chapter 4).

Among the naturally cross-pollinated species, corn is a particularly favorable crop in which to study the effects of inbreeding because self-pollinations are easily made and good seed set is normally obtained following self-pollination. In some cross-pollinated crops, red clover for example, seeds are seldom obtained following self-pollination because of self-incompatibility. In plants where the sex organs are on separate plants, or if for other reasons self-pollination does not lead to normal production of seeds, inbreeding may be accomplished through *sib*

matings, the mating of close relatives. A *half-sib mating* is a mating between plants that have one parent or one pollen source in common (see Figs. 10.5, 10.6). A *full-sib mating* is one between plants within the progeny of a single plant (see Fig. 10.7). Sib matings increase homozygosity, but complete homozygosity is reached more slowly than through self-fertilization. Ten generations of full-sib matings are normally required to reach the same level of homozygosity as three generations of self-fertilization (Fig. 11.2).

A major visible consequence of inbreeding in cross-pollinated species is loss in size and vigor in progeny plants as heterozygosity decreases. The decrease in vigor is largest following the first generation of inbreeding and levels off as homozygosity is approached (Fig. 11.3). The decline in vigor with inbreeding is known as *inbreeding depression* and results from increases in the frequency of homozygous loci with deleterious effects. In plants with heterozygous loci, the recessive deleterious allele is not expressed in the plant phenotype due to masking by the favorable, dominant allele. As homozygosity increases, many dominant alleles are lost and the deleterious effects of the recessive alleles on the phenotype are expressed. In forage species such as alfalfa, or various species of grasses, fertility and seed production are frequently suppressed following inbreeding. The decline in fertility often occurs so rapidly that the inbred progenies cannot be maintained by seed propagation, or can be maintained only with great difficulty beyond the first or second selfed generation. With inbreeding in a cross-pollinated species such as corn, many individual plants in the progenies of the selfed plants exhibit serious faults, such as reduction in plant stature, weak stalks and a tendency to lodge, increased susceptibility to disease pathogens, and a wide assortment of other deleterious plant characteristics (Fig. 11.4). In the development of inbred lines, the undesirable plants are discarded, retaining only the most vigorous plants, which are self-pollinated again and the process repeated. The most important trait for an inbred line, its contribution to yield in a hybrid combination, cannot be visually determined. This trait is expressed in the progeny when the inbred line is crossed with another inbred and the hybrid progeny grown in a yield trial. In species of crop plants evolved through natural self-pollination, such as barley, beans, rice,

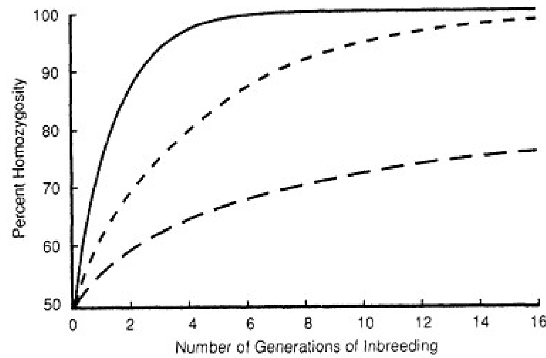


Fig. 11.2.
Comparison of homozygosity reached by self-fertilization
(— —).

Fig. 11.3.
Reduction in vigor in corn with successive generations of inbreeding. S_0 represents the original selfed (inbred) plant and S_1 to S_6, successive selfed (inbred) generations.

soybean, tomato, or wheat, an adverse effect from inbreeding is not generally apparent and these crops can be maintained in a homozygous condition without apparent loss of vigor.

Hybrid Vigor or Heterosis

Hybrid vigor is the increase in size, vigor, or productivity of a hybrid plant over the average or mean of its parents. The latter being referred to as the *midparent value. An* alternative term, *heterosis*, was proposed by Shull to denote the stimulation in size and vigor in a hybrid as an expression of hybrid vigor. The two terms, hybrid vigor and heterosis, are synonymous and may be used interchangeably. To be useful, the hybrid plant needs to exceed the best parent in yield and productivity. Unless a hybrid is superior to its best parent line, it has no advantage for the breeder or the farmer. The effects of hybrid vigor in plants are manifested most often by increased vegetative growth and yield of the harvested product; but hybrid vigor also may be reflected in cell size, plant height, leaf size, root development, ear or head size, grain number, seed size, and other ways. Hybrid vigor is generally greatest following crosses among diverse genotypes of a cross-pollinated species, but also may be expressed following crosses among diverse genotypes in a self-pollinated species.

Explanations of Hybrid Vigor

Two theories are generally offered to explain the phenomenon of hybrid vigor. Neither appears to be wholly adequate. The most widely accepted explanation is based on the assumption that *hybrid vigor results from bringing together an assortment of favorable dominant*

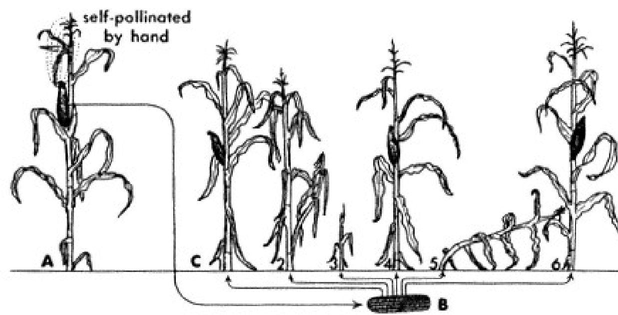


Fig. 11.4.
Self-pollinated plant of corn and its progeny. (A) S_0 plant from open-pollinated cultivar. (B) Ear from S_0 plant. (C) S_1 (first generation selfed) plants. Undesirable plants (2,3,5) are discarded; desirable plants (1,4,6) are again self-pollinated. Selection and self-pollination are continued for five to seven generations to obtain uniform inbred lines.

genes. According to this theory, alleles that contribute to vigor and growth are dominant, whereas the recessive alleles may be neutral, harmful, or deleterious to the individual. If the dominant alleles contributed to the hybrid by one parent complement those contributed by the other parent, the $\mathbf{F_1}$ will then have a more favorable combination than either parent. The theory works like this: for simplicity, let us assume that in corn the dominant genes *ABCDE are* favorable for high yield, that inbred **A** has the genotype *AABBccddEE (ABE* dominant), and inbred **B** has the genotype *aabbCCDDEE (CDE* dominant). Genotypes of the inbreds **A** and **B** and the $\mathbf{F_1}$ hybrid are as follows:

Inbred A	×	**Inbred B**
AABBccddEE		*aabbCCDDEE*
	F_1 Hybrid	
	AaBbCcDdEE	

In this simplified example, the $\mathbf{F_1}$ hybrid contains dominant genes at five loci (*ABCDE*) compared to three loci only in each of the inbred parent lines and would exhibit more vigor than either of the parent inbred lines.

With the favorable-dominant-gene theory of explaining hybrid vigor, the question arose as to why favorable dominant genes could not be concentrated sufficiently in an inbred line so that it would be as productive as a hybrid. In a cross-pollinated population, the deleterious effects of many recessive genes are masked by the presence of dominant alleles; with each self-pollination, approximately one-half of the heterozygous loci become homozygous for recessive genes and contribute to the decline in vigor observed in the inbred line. The theory assumes that in a cross-pollinated crop like corn, there are too many deleterious recessive alleles to recover sufficient loci with homozygous dominants for an inbred plant to exhibit the level of vigor of a hybrid plant. Much progress has been made in obtaining more favorable combinations of the favorable dominant genes since the theory was proposed, especially in corn inbred lines. Inbred lines currently used in hybrid corn production are greatly improved in

vigor and productiveness over those used earlier. Since the early 1960s, farmers have been growing single-cross hybrids from seeds harvested from inbred lines, making the double cross in hybrid seed corn production no longer necessary.

Another theory explains hybrid vigor on the basis that loci that are heterozygous contribute more to productivity than loci that are homozygous; the most vigorous hybrid plant being the one with the greatest number of heterozygous loci. This theory is based on the supposition that there are contrasting alleles, for example, a_1 and a_2, for a single locus. Each allele produces a different favorable effect in the plant. In a heterozygous (a_1a_2) plant, a combination of the effects is produced that is more favorable to the plant than the effect produced by either of the alleles alone. The phenomenon of the heterozygote (a_1a_2) being superior to the homozygote, (a_1a_1) or (a_2a_2), is termed *overdominance* and is based on *interaction of alleles at the same locus. The* theory of explaining hybrid vigor on the basis of heterozygous alleles is defended by results from specific experiments, but neither the *favorable dominant gene* nor the *overdominance* theory satisfactorily explains all cases of hybrid vigor. In general, the favorable dominant gene theory is favored by most experimental evidence.

Utilization in Cultivar Development

The principle of hybrid breeding is to mate homozygous parent genotypes that will combine producing superior $\mathbf{F_1}$ hybrid vigor, and to reproduce the superior $\mathbf{F_1}$ genotype in every plant in the hybrid population. Although hybrid plants are heterozygous at many loci, uniformity is attained, as in an inbred line, or a pure-line cultivar of a self-pollinated crop, or a clonally propagated line, because all hybrid plants have identical genotypes.

For hybrid cultivars of agronomic or horticultural seed-propagated crops to be accepted and used, the $\mathbf{F_1}$ hybrid seed must be produced in sufficient quantity to supply grower demand at an affordable price. This requires a practical form of pollination control in the production of hybrid seed. In corn, a monoecious flowering plant, pollination in seed production fields is controlled by removal of the tassel containing the male floral organs from the seed-producing plants, a process called *detasseling. The* detasseled plants are pollinated from wind-blown pollen from adjacent male-fertile pollinator rows. This procedure is not available in species in which the flower contains both male and female floral organs. In some species, like cucumber, pepper, or tomato, where large numbers of seeds can be produced from pollination of a single floret, *hand emasculation and pollination are* used to produce hybrid seed. But in field crops, hand pollinations are too expensive to produce the needed quantities of hybrid seed. *Cytoplasmic male sterility and fertility-restorer genes are* utilized, or have been proposed for utilization, for controlling pollination in crop species in which usable forms of cytoplasmic sterility and restorer genes are available. Additionally, chemical hybridization agents that prevent pollen formation offer a potential alternative to the use of cytoplasmic male sterility.

Breeding Single-Cross Hybrid Cultivars

The single-cross hybrid cultivar is the product of a cross between two inbred lines or pure-line cultivars. The production of single-cross hybrids began with the research of Shull in corn in 1909 and was an intrinsic part of the double-cross corn breeding procedure because two single crosses were utilized in the production of each double cross. From this background, the single-cross hybrid in corn became the model for breeding single-cross hybrid cultivars in other

field and horticultural crops—onion, pearl millet, sorghum, sunflower, tomato, wheat—as well as in corn.

Inbred Line Development

An inbred line is a homozygous breeding line developed and maintained by self-pollination. When developing inbred lines in a hybrid breeding program in a cross-pollinated species such as corn, breeders normally start by self-pollinating heterozygous plants. The heterozygous plants may have been selected by the breeder from (**a**) a natural population of a cross-pollinated species, or a cross-pollinated population improved through a recurrent selection procedure, or (**b**) the second generation (F_2) progeny from a cross between homozygous parent lines. In breeding hybrid corn the inbred lines were originally developed by selfing selected heterozygous plants from fields with uncontrolled pollination (also referred to as *open-pollination*) as in (**a**) above. In a mature hybrid corn breeding program, inbred lines of corn are more generally developed from the hybrid progeny created by crossing two elite inbred lines as in (**b**). The latter is identical to the hybridization procedure described for self-pollinated crops in Chapter 9. In (**a**), the original heterozygous *selfed plant* is normally referred to as the S_0 plant, and the progeny obtained from selfing this plant as the S_1 (*first-generation selfed*) progeny. The *second-generation selfed progeny are* called the S_2, and so on. If the heterozygous plant originates from a cross between homozygous inbred lines, the F_1, F_2, etc., designation would be used as in self-fertilized species.

The purpose of inbreeding is to reduce the offspring of a heterozygous plant into an array of dissimilar, homozygous, inbred lines. Striking differences are observed between lines with successive generations of inbreeding; within lines, plants become more alike and the individual inbred lines become more distinguishable from each other. With successive generations of inbreeding, homozygosity and uniformity are increased within the progeny lines. The *variance within lines is reduced while the variance between lines is increased*. In a cross-pollinated crop like corn, five to seven generations of self-fertilization and pedigree selection are necessary to obtain inbred lines that are uniform in plant and seed characteristics and that will remain uniform under continued self-fertilization. This procedure is identical to the pedigree-selection procedure utilized in the breeding of self-pollinated crops, where a cross between selected parents is followed with several generations of self-pollination and selection to generate uniform, true-breeding progeny lines (Fig. 9.2). During inbreeding, many undesirable recessive alleles at heterozygous loci will be replaced by dominant alleles and the recessive alleles eliminated from the progeny; at other loci, recessive genes will become homozygous contributing to an overall loss of vigor in the inbred. Greatest vigor is lost during the early generations of inbreeding, the loss in vigor declining as the inbred lines gradually approach homozygosity. After no further loss of vigor is experienced, the genotype of the inbred may be maintained by self-pollination, unless mutations or outcrossing occur.

Selection during the early generations of inbred line development is based largely on visual observations of the inbred plants for characteristics that will affect the suitability of the inbred to be utilized in commercial hybrid production. Are the plants strong and vigorous? Do they stand without lodging? Do they produce seeds in sufficient abundance and quality to assure maintenance of the inbred line? Are the plants free from insects and disease? How do they yield?

During the period of inbreeding and selection, it is desirable to subject the partially inbred lines to adversities, such as high plant density, unfavorable high or low temperatures, drought, lodging, disease, or insect pests, as appropriate for the particular species being inbred and the

objectives of the breeding program. By such tests it is possible to identify the inbred lines that will give the most reliable performance in a wide array of environments. It is also important in inbred line development to find new inbred lines that will contribute to greater productivity when crossed with other inbred lines.

Combining Inbreds into Single Crosses

The utility of the inbred line is determined by its genetic contribution to the hybrid progeny when crossed with another inbred line, not in its production potential per se. However, it needs sufficient production potential so that it can be economically maintained and utilized as a parent line in the production of hybrid seed. Vigor and productiveness that were lost during inbreeding are recovered in the hybrid when the inbred lines are crossed. From experience it has been learned that some inbred lines will combine with a large number of other inbreds to produce high-yielding hybrid progenies; other inbred lines will combine satisfactorily with few or no inbred lines. The ability of the inbred line to transmit desirable performance to the hybrid progeny is referred to as *combining ability*.

GENERAL COMBINING ABILITY. *General combining ability (gca) of an inbred line is the average contribution that the inbred makes to hybrid performance in a series of hybrid combinations in comparison to the contribution of other inbred lines to hybrid performance in the same series of hybrid combinations*. It is not possible from visual observation to predict the contribution of an inbred line to hybrid performance. The **gca** of an inbred line is evaluated by crossing it with other inbred lines and comparing the overall performance of the single-cross progenies. General combining ability evaluates the *additive* portion of the genetic effects. If inbred lines **A, B, C, D**, and E are crossed in all possible combinations (diallel mating) and the single-cross hybrids are grown in a yield trial, the inbred whose single crosses have the highest average yield would have the greatest **gca**. If that inbred is **A**, the implication is that A will contribute to high yield in a wider array of crosses than inbred lines **B, C, D**, or **E**. With large numbers of inbred lines, it is not always feasible to make all possible diallel matings and grow the hybrid progenies in performance trials. The number of possible single-cross combinations that can be made from n inbred lines is equal to **n(n- 1)/2**. With 10 inbreds, the possible number of single-cross combinations is 45; with 100 inbreds, the possible number of single-cross combinations is 4950, an impossible number to produce or grow in a performance trial. These burdensome numbers made it clear to the early breeders of hybrid corn that a simple and efficient system of screening inbred lines for combining ability was needed before pairing the inbred lines in single-cross yield trials.

From testcross experiments (often called topcrosses in corn), it was demonstrated that yields of corn inbred lines pollinated with a mixture of pollen, such as from an open-pollinated cultivar or from a double-cross or single-cross hybrid, were highly correlated with the average performance of the inbred line in a wide array of single-cross combinations. This led to the use of *testcrosses* for preliminary screening for **gca** of large numbers of newly developed inbred lines. As new inbred lines were generated, they were first pollinated with a heterogeneous genotype proven to be an efficient tester, and the testcross progeny evaluated in yield trials. Only inbred lines with superior **gca** were retained for testing in single-cross combinations.

SPECIFIC COMBINING ABILITY. *Specific combining ability (sca) is the contribution of an inbred line to hybrid performance in a cross with a specified inbred line, in relation to its contributions in crosses with an array of specified inbred lines*. Specific combining ability

evaluates nonadditive gene action and is utilized to identify the inbred x inbred cross combination with superior performance. The inbred lines identified as having superior **gca** are crossed in all possible pairs (*diallel crossing*) to create single crosses, which are then evaluated in yield trials for **sca**. For example, if all possible single crosses among inbreds **A, B, C, D**, and **E** are made, and the combination **A** × **E** produces the highest single-cross yield performance, the **A** × **E** cross combination would have superior **sca**. Whether two particular inbreds combine to produce a high-yielding single cross depends upon the extent that the favorable genes for yield from the two parent inbreds complement each other. Experience has shown that inbreds derived from unrelated populations will combine to produce high-yielding single crosses more frequently than inbreds derived from related parent material.

BROAD- VS. NARROW-BASED TESTERS. In early hybrid corn breeding programs, testers with a broad genetic base, such as open-pollinated cultivars, were used to evaluate **gca** of corn inbred lines. As the hybrid breeding programs in particular species, such as corn, became more advanced, changes were made in the way in which the breeder goes about the task of screening inbred lines for combining ability and fitting the lines into hybrid combinations. The **gca** of new corn inbreds is now evaluated more frequently by crossing with narrow-base testers, such as elite inbreds, related inbred lines, or single crosses of related lines, making it unnecessary to conduct preliminary screening tests with heterogeneous testers. This change has evolved because in mature hybrid breeding programs, inbred line development is directed more toward replacement of a specific inbred line in an already established hybrid, rather than toward the development of a group of new inbred lines to be used in the production of completely new hybrids. If an inbred line is sought to replace an inbred in an established single-cross hybrid, the opposite inbred would be the logical tester to use. The testing procedures should be designed so that they will identify whether the new inbred line corrects the weakness of the inbred line being replaced. Growing the testcross progenies at multiple locations is essential to evaluate genstype × environment interactions and to identify inbred lines with stable progeny performance in a broad array of environments.

Cytoplasmic Male Sterility and Hybrid Seed Production

A new era in breeding hybrid cultivars was introduced with the identification of cytoplasmic male sterility (**cms**) and fertility-restoring genes (Chapter 7), and the development of procedures for their utilization in hybrid seed production. It was no longer necessary to detassel the seed parent rows when producing hybrid seed corn, and commercial hybrid seed production became feasible in an assortment of other field and vegetable crops where, previously, restrictions on pollination control made hybrid cultivars impractical.

Utilization of cms in Hybrid Seed Production

Cytoplasmic male sterility as an aid to hybrid seed production was first utilized commercially in the onion in the late 1940s (Figs. 7.5 and 7.6). A male sterile onion strain was planted adjacent to a strain with normal pollen production. The male sterile onion strain was pollinated by wind-blown pollen from an onion strain with fertile pollen. The $\mathbf{F_1}$ hybrid seeds harvested from the male sterile plants were utilized for the commercial production of hybrid onions. Corn and sorghum were the first major field crops to utilize the cytoplasmic-male-

sterility:fertility-restorer-genesystem for commercial production of hybrid seed. In corn, the system offset scarcity of labor required for detasseling and reduced costs associated with the production of hybrid seed. In sorghum, in which male and female floral organs are present in the same flower, the system made hybrid seed production possible.

The A-line, B-line, R-line Model for Hybrid Seed Production

The onion model for producing single-cross hybrid seed was developed utilizing cytoplasmic male-sterile lines called **A-lines**; male-fertile maintainer lines called **B-lines**; and male-fertile, fertility-restoring lines called **R-lines**. The procedure is accomplished in these steps:

• introduction of a male-sterile cytoplasm into the **A-line** by the backcross procedure,

• maintenance of the male-sterile **A-line** by pollination from a male-fertile maintainer line with identical genotype, the **B-line**,

• development of fertility-restorer lines called, **R-lines**, and

• crossing **A-lines** × **R-lines** to produce hybrid seed.

The **A-line, B-line, R-line** procedure for onion is illustrated in Figure 11.5.

Normally two or more nuclear, fertility-restorer genes and some modifying genes are required to restore complete fertility to the cytoplasmic male sterile **A-line**. The cytoplasm and a two-gene (Rf_1, Rf_2) fertility-restorer system are shown for the different breeding lines in the example below:

	♀		♂
Maintenance of A-Line:	**A-Line**	×	**B-Line**
Cytoplasm:	*cms*		*N*
Fertility-restorer genes:	rf_1rf_1, rf_2rf_2		rf_1rf_1, rf_2rf_2
Cross to produce hybrid:	**A-Line**	×	**R-Line**
Cytoplasm:	*cms*		*N*
Fertility-restorer genes:	rf_1rf_1, rf_2rf_2		Rf_1Rf_1, Rf_2Rf_2
Single cross hybrid:		**AR**	
Cytoplasm:		*cms*	
Fertility-restorer genes:		Rf_1rf_1, Rf_2rf_2	

A-LINES AND B-LINES. In the cross to produce hybrid seed, the **A-line** is the female or seed-producing parent line. It contains recessive, nonrestorer (rf_1, rf_2) genes. Inbred lines that do not contain dominant, fertility-restorer genes are crossed to a source of cytoplasmic male sterility and successively backcrossed until the genotype of the **A-line** is recovered in the male sterile cytoplasm. The line with the cytoplasmic male sterility is used as the female parent in the crosses because the cytoplasm is transmitted through the egg, not the pollen. Normally five to seven backcrosses are required to transfer the **A-line** chromosomes into sterile cytoplasm and fully recover the genotype of the recurrent parent. The genotype of the **A-line** must combine with that of an **R-line** to produce a productive hybrid.

The cytoplasmic male sterile **A-line** is maintained by pollination from a male-fertile

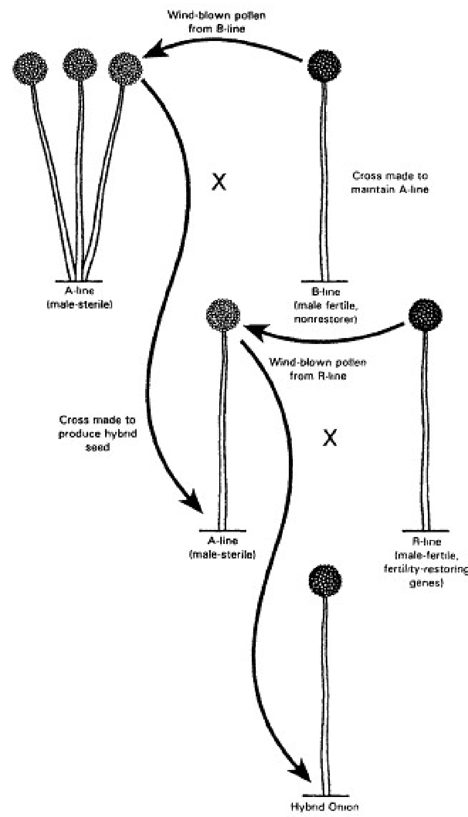


Fig. 11.5.
The A-line, B-line, and R-line model for producing single-cross hybrid seed in onion. The male-sterile A-line is maintained by pollination from a male fertile, genetically identical, nonrestorer B-line. Hybrid seed is produced by pollination of the A-line from the fertility-restoring R-line.

counterpart, called the **B-line**. The **B-line** has the same genotype as the **A-line**, with normal instead of sterile cytoplasm, and recessive, nonrestorer (rf_1, rf_2) genes like the **A-line**.

R-LINES. The **R-line** is the pollen parent in the cross to produce hybrid seed. The function of the **R-line** is to:

• pollinate the **A-line**,

• restore fertility in the hybrid seed, and

• combine with the **A-line** to produce a vigorous and productive hybrid.

Abundant pollen production and complete anther extrusion to aid in pollen dissemination are essential characteristics of the **R-lines**. Because the **R-line** has dominant genes for fertility restoration, it may have either fertile cytoplasm or sterile cytoplasm. It may be advantageous to develop restorer lines with sterile cytoplasm, because presence of restorer genes can then be confirmed without testcrossing to male sterile lines. Two restorer genes are shown in this example, but a third restorer gene and additional modifying genes are often required to completely restore fertility to the hybrid progeny in different species.

Another requisite of the **R-line** is ability to combine with the **A-line** to produce a superior yielding hybrid. The combining ability of potential **R-lines** is evaluated by crossing with **A-line** testers and growing the hybrid progenies in yield trials. If a line with superior combining ability is identified, restorer genes may be added by the backcross procedure. Modifier genes usually are not added with the backcross but must be present in the potential **R-line** for it to be a successful parent line.

CROSSING A- AND R-LINES. The hybrid seed is produced on the **A-line** after pollination from the **R-line**. In commercial seed production, the **A-** and **R-lines** are planted in alternate strips in the hybrid seed production field, with the male sterile **A-line** being pollinated by wind-blown pollen from the **R-line**. In corn, sunflower, and wheat, the symbol R_f is used to designate the fertility-restoring gene. The symbol *Ms* is used for the fertility-restoring gene in onion, millet, and sorghum.

An important feature of the cytoplasmic-male sterility/fertility-restorer gene system is that it made possible the production of hybrid cultivars in crop species in which male and female floral organs are contained in the same floral structures. Hybrid seed could now be produced in crop plants such as faba bean, millet, rice, sorghum, sugarbeet, sunflower, wheat, and other field and vegetable crops that, unlike corn, do not have the luxury of detasseling for pollen control.

Alternative Hybrid Procedures

Hand Emasculation and Pollination

Hand emasculation and hand pollination are normally used to make crosses and obtain hybrid seed for growing an F_1 generation in the breeding nursery. In most field crops, hand emasculations and pollinations are too laborious and expensive to use for commercial production of hybrid seed. But there are a few exceptions in crops where a large number of seeds are obtained from pollinating a single flower, as in cotton and tobacco; or horticultural crops such as tomato, cucumber, melons, and pepper; or flowers where the hybrid seeds are sold at premium prices.

Self-Incompatibility

Various procedures have been proposed for utilization of self-incompatibility in the production of hybrid seed. The simplest procedure is that utilized with cross-pollinated species

that have the gametophytic type of self-incompatibility and that can be clonally propagated. Alternate strips of self-incompatible, yet cross-compatible, clones are transplanted into an isolated field. Cross-pollination among the clones will produce hybrid seeds; selfed and sibbed seed production will be largely prevented by the self-incompatibility of the clones. All plants grown in the isolated crossing field may be harvested for seed. This procedure was used in the production of the 'Tifhi No. 1' cultivar of Pensacola bahiagrass.

Hybrid seed production in plant species having a sporophytic self-incompatibility system is dependent upon the production of inbred lines homozygous for an *S*, self-incompatibility allele. In these species, a protein secretion covers the stigmatic surface just prior to anthesis and acts as a barrier to penetration of the stigma by germinating pollen grains. If buds are opened and the pollen applied before the protein barrier is formed, a procedure called *bud pollination*, seed set will be obtained. Bud pollinations are utilized in maintaining self-incompatible parent lines by self-pollination. None of the major field crop plants have the sporophytic self-incompatibility system, but utilization of the system to produce single-, double-, and three-way crosses in species of *Brassica* was described in Chapter 6.

Clonal Propagation of an F_1 Hybrid

In clonally propagated species, vigorous F_1 hybrid plants may be clonally propagated, faithfully reproducing the genotype of the hybrid parent plant. This procedure is used in breeding sugarcane, potato, forage species such as bermudagrass, and various ornamental, fruit, and forest tree species. Because clonal propagation is expensive, its utilization in field crops is generally limited to perennial or high value crops such as sugarcane or potato.

Hybrids in Dioecious Species

In perennial, dioecious species, hybrids may be produced by crossing pistillate × staminate clones. When a superior hybrid combination has been identified, commercial F_1 hybrid seed may be produced by interplanting the male and female parent clones in an isolated field. The 'Mesa' cultivar of buffalograss was produced in this manner.

Hybrids in Monoecious Species

In castor bean, a monoecious species, a plant was found that produced only pistillate flowers. When cross-pollinated, it serves as the seed parent for production of hybrid seed. The pistillate character is controlled by homozygous recessive genes (*ff*), and when pollinated by a heterozygous monoecious plant (*Ff*), the progeny segregates 50% pistillate:50% bisexual plants. Hybrid seed is produced in isolation on pistillate plants by roguing out the bisexual plants.

Apomictically Propagated F_1 Hybrids

Apomixis is the production of seed without fertilization (union of gametes). *Obligate apomixis* is reproduction solely by apomixis, in contrast to *facultative apomixis*, in which plants reproduce both sexually, by gametes, and by apomixis. If a vigorous F_1 hybrid plant is produced in an apomictic species, and then converted to an obligate apomict, the genotype of that particular hybrid will be uniformly propagated in all of the progeny. This procedure was used in the production of 'Higgins' buffelgrass.

Genetic Male Sterility

Genetic male sterility is controlled by homozygous recessive genes (*msms*) (Chapter 7). The effects vary from reduced anther size and pollen production to complete pollen abortion, according to the species and the specific male sterile gene. Pollen production is restored with the dominant gene, either in the homozygous (*MsMs*) or heterozygous (*Msms*) condition. Producing a completely male sterile population is not feasible, the nearest approach being 50% male sterile and 50% male fertile, obtained by pollinating a male-sterile plant with pollen from a plant heterozygous for the male-sterile gene. This restricts the utilization of genetic male sterility in the commercial production of hybrid seed in crop plants where large quantities of seed are required, because the 50% male-fertile plants would need to be rogued out before they shed pollen, a laborious and expensive hand procedure. Systems for hybrid seed production using genetic male sterility have been proposed for use in barley, corn, sorghum, and wheat, but none has proved practical.

Chemically Induced Male Sterility

Chemical induction of male sterility would eliminate the need for developing cytoplasmic male-sterile and fertility-restoring lines in the commercial production of $\mathbf{F_1}$ hybrid seed. The chemical hybridizing agent is sprayed on the seed production rows in hybrid seed production during a critical stage of flower formation to induce male sterility. The potential exists for efficiently sterilizing large numbers of breeding lines to obtain $\mathbf{F_1}$ seed for yield evaluation trials. Any pair of lines would become potential parents in a hybrid if they combined to produce a high-yielding $\mathbf{F_1}$ hybrid, and one of the lines could be successfully sterilized and used as the seed parent. Consistency in inducing male sterility in widely varying environments is essential. Because the chemical must be applied at a critical stage of floral development, some risk is involved if application of the chemical is interrupted by adverse weather conditions. Chemical sterilization for hybrid seed production has been proposed for use in cotton and wheat.

Proprietary Nature of Hybrid Cultivars

An important consideration in the development of hybrid corn and the extension of hybrid breeding to other crops has been the proprietary nature of the hybrid cultivar. When hybrid corn was first introduced to the U.S. farmer, the inbreds utilized in the early hybrids had been developed by scientists in the state agricultural experiment stations and the U.S. Department of Agriculture. As tax-supported research organizations, it was not appropriate for either one to produce and market hybrid seeds. That activity was left to the private hybrid seed companies that were rapidly emerging. As the resources of the private seed companies increased from sales of hybrid seeds, they developed breeding programs. At that time, legal protection of the intellectual property rights of developers of new cultivars did not exist in the United States, but a hybrid cultivar has a built-in protection for the developer if the developer maintained sole possession of one or more of the inbred lines. This proprietary protection encouraged investment by private seed companies in hybrid corn research and in inbred line development. In the United States hybrid corn is produced and distributed by private seed companies. Hybrid breeding was later extended to sorghum, pearl millet, sunflower, wheat, and other field and horticultural crops.

How Breeding Procedures Are Utilized

In this chapter we have described how hybrid cultivars originated and discussed problems associated with their development and utilization. For a discussion of specific procedures and problems in breeding hybrids, the student is referred to Chapter 17, Breeding Corn, and Chapter 18, Breeding Sorghum.

Study Questions

1. What are the consequences of inbreeding? What are the various methods that the plant breeder can use to obtain inbred plants?

2. What theories are used to explain hybrid vigor? What are the pros and cons for each theory?

3. What is general combining ability and specific combining ability? Why is it necessary to know which type of combining ability is most important for a particular crop species?

4. Why are double crosses no longer used in the breeding of corn?

Further Reading

Hayes, H.K. 1952. Development of the heterosis concept. p. 49-65. *In* J.W. Gowen (ed.) Heterosis. Iowa State Univ. Press, Ames, IA.

Hayes, H.K. 1963. A professor's story of hybrid corn. Burgess Publ. Co., Minneapolis, MN.

Jones, D.F. 1918. The effects of inbreeding and cross-breeding upon development. Conn. Agric. Exp. Stn. Bull. 207.

Jones, H.A., and A.E. Clarke, 1943. Inheritance of male sterility in the onion and the production of hybrid seed. Proc. Am. Soc. Hort. Sci. 43:189-94.

McRae, D.H. 1985. Advances in chemical hybridization. p. 169-91. *In* J. Janick (ed.) Plant Breeding Reviews, Vol. 3. AVI Publ. Co., Westport, CT.

Shull, G.H. 1908. The composition of a field of maize. Rep. Am. Breeders Assoc. 4:296-301.

Shull, G.H. 1909. A pure-line method in corn breeding. Rep. Am. Breeders Assoc. 5:51-59.

Shull, G.H. 1952. Beginnings of the heterosis concept. p. 14-48. *In* J.W. Gowan (ed.) Heterosis. Iowa State Univ. Press, Ames, IA.

Sprague, G.F., and J.W. Dudley (eds.). 1988. Corn and corn improvement. 3rd ed., Agronomy Monograph 18. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Sprague, G.F., and L.A. Tatum. 1942. General vs. specific combining ability in single crosses in corn. J. Am. Soc. Agron. 34:923-32.

Sprague, G.F., and S.A. Eberhart. 1977. Corn breeding. p. 305-63. *In* G.W. Sprague (ed.) Corn and corn improvement, 2nd ed., Agronomy Monograph 18. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Stephens, J.C., and R.F. Holland. 1954. Cytoplasmic male-sterility for hybrid sorghum seed production. Agron. J. 46:20-23.

12. Breeding Objectives and Techniques

The goal of the plant breeder, whether developing a cultivar or a parent for a hybrid, is to create new genotypes improved in one or more important features. The goal is achieved only if the breeding objectives are clearly defined, and carefully planned selection and hybridization procedures leading toward that goal are employed. The *breeder must identify the changes in the cultivar which, if made, will increase yield, stabilize production, or improve quality of the product to be harvested in the crop species with which he is working. He then searches for parent materials superior in those features and combines genes for the desired features into new cultivars to fulfil the breeding objectives*. The objectives in breeding programs will differ with the crop species and the purpose for which the crop is being grown. The objectives for a particular species will differ from program to program because the environmental conditions that affect production and the adversities that limit yield differ from one production area to another. By designing cultivars to fit his particular concept of the ideal cultivar, the experienced breeder leaves a lasting imprint on the cultivars that he develops.

This chapter presents an overview of the major objectives in crop breeding and the breeding techniques by which plant genotypes are screened to identify whether the objective has been accomplished. The relative importance of the objective may differ with the crop species as will be discussed in chapters concerned with the breeding of different species. A prudent choice of objectives is essential for a successful breeding program; since the breeder cannot pursue all possible objectives he must concentrate available resources on those objectives most important in his particular breeding program.

Yield

Yield of grain, forage, fiber, or other plant products has primary importance as a breeding objective because it affects the economic return to the grower. Plant genotypes differ in their inherent *yield potential*. Yield potential is expressed phenotypically through complex plant morphological features and physiological functions, and genetically expressed as a complex

quantitative character that interacts with the environment in which the plant genotype is grown. The plant breeder measures yield potential by the mass or weight of the product produced per unit of land area, through yield trials, in which the harvested yield is compared with that of standard cultivars.

Breeding for *high-yield potential* is normally accomplished by crossing among genotypes with complementary genes contributing to yield potential in order to generate transgressive segregates with superior yield. In the early, segregating generations single plants are selected, which are then evaluated for yield potential by progeny tests. To identify the superior segregates, breeders are faced with the dilemma that for the initial selection, they must rely on visual observation among thousands of spaced plants or progeny rows, yet the critical test is how the genotype performs at normal spacings in yield trials and in farmer's fields, in comparison with the highest yielding commercial cultivars.

Various attempts have been made to find selection criteria that will aid the breeder in selecting for yield during the early selection stage, but none are entirely satisfactory. One approach has been to examine the *components of yield*. For example, yield of wheat is the product of number of spikes per unit of land area, number of grains per spike, and average weight per grain. In theory, an increase in one component with the others held constant would result in an increase in total yield. In practice, as one component of yield is increased, the others tend to decline due to competition for available growth assimilates. Among .the different yield components, kernel weight tends to have the highest heritability coefficient.

With a combination of shorter stature and higher yield in new cereal cultivars, there has been an increase in the ratio of grain weight to total plant weight (grain + straw), the ratio being designated *harvest* index. A higher harvest index is an expression of greater plant efficiency since a higher proportion of the available assimilates is being translocated into the grain rather than being used to produce plant mass. This has led to suggestions for increasing yield by selection for harvest index. The problem here is that harvest index is not a phenotypic plant character that can be visibly selected. Harvest index is a ratio, its calculation requires separate weights of grain and straw, information that is impractical to obtain with modern mechanized harvest of yield trials.

How, then, does the breeder select for high-yield potential in the early segregating generations? Most selection for yield potential at this stage is empirical, based on the breeder's knowledge and experience, and the accuracy of his observations. Uniform guidelines for selection are difficult to establish. They differ with the crop species and its intended use, the breeder's experience, and the local environment. Because the breeder must examine large numbers of plants in the early generations, selection criteria that require detailed measurements are generally impractical. Selection at this stage must be followed by extensive field testing at various locations in the area where the new cultivar will be grown.

All this assumes that the cultivar has a favorable environment in which to grow; that adverse factors such as cold, drought, or disease will not limit the final yield. To find such an environment would be rare indeed. And so in addition to high-yield potential, we breed for *yield stability*, the ability of the plant genotype to produce up to its genetic potential in spite of an adverse environment. We breed for cold resistance in areas where nonhardy plants would be injured, for resistance to drought in the dry areas, for sturdy straw to prevent loss from lodging, for resistance to soil stress in the presence of excess aluminum or toxic salts, or for resistance to disease pathogens and insect pests that affect the plant's health. While constantly striving to improve the potential yielding ability, it is also necessary to stabilize production by breeding for resistance to the adversities that may limit the final harvest.

Substantial increases in grain yields for wheat, rice, sorghum, and hybrid corn have been

recorded over the past 30 to 50 years, particularly in high-yield environments as in the United States, Western Europe, India, or China. The higher yields result from genetic improvement in yield potential; shorter and stronger plants; and resistance to stress, disease pathogens, and insect pests; combined with improved cultural practices such as higher rates of fertilization; increased use of chemicals for control of weeds, disease, and insect pests; and heavier seeding rates to give more plants per unit of land area. The interaction of the new cultivars with the improved cultural practices is complementary. Because the new cultivars have shorter and stronger stems, increased fertilizer applications may be made without lodging; and with larger inherent yield response to increased soil amendments, greater use can be made of irrigation, herbicides, fungicides, and insecticides to prevent yield loss from adverse circumstances.

Maturity

The optimum period for a cultivar to reach maturity will be altered by where the cultivar is grown; its place in the cropping sequence; the importance of maturity in escaping disease, insects, or other natural hazards; and the relation of maturity to yield and product quality. There has been a continuing trend toward earlier maturity in cultivar development. An early maturing cultivar may escape damage from heat, drought, insects, or disease, or permit harvest ahead of damage from rainstorms, hail, floods, or early frosts. Early maturity is advantageous in multiple cropping systems to permit early removal of a crop so that the following crop may be planted. There are also disadvantages in early maturity. Plant size and yield may be reduced in extremely early cultivars of crops such as corn, soybean, or wheat, because the plant has a shorter growth period in which to develop, manufacture, and store nutrient materials. Early maturity may be the result of early flowering or a reduced time period from flowering to ripening. Environmental factors such as photoperiod, temperature, altitude, soil type, and seasonal distribution of moisture affect maturity as well as the plant genotype.

The comparative maturity of crop cultivars are expressed in various ways, some of the more common being days-to-heading (small grains), days-to-silking (corn), or days-to-ripening. In small grains, days-to-heading is influenced less than days-to-ripening by abnormal temperatures, drought, or disease, environmental factors that cause premature ripening. Because all plants within a plot do not flower on the same date, estimates of the date that 75% of the plants flower may be used in comparisons of maturity. The moisture percentage of the grain at time of harvest provides a measure of relative maturity in corn. In cotton, earliness may be measured by days-to-first-flower, length of the boll-forming period, or percentage of lint at first harvest. A standard cultivar is included in all field trials for comparison.

The inheritance of earliness differs with specific crops and cultivars, but is frequently reported as being dominant or partially dominant to late maturity and controlled by a few major and numerous modifier genes. In wheat chromosome substitution studies, seven or more chromosomes are reported to have genes affecting maturity. Maturity is affected by photoperiod response which is controlled by relatively few genes.

Resistance to Lodging and Shattering

Lodging is the bending or breaking over of the plants before harvest. Lodging causes yield losses in small grains, soybean, corn, sorghum, and other crops, with any of the following conditions:

• the plant lodges before it is ripe and the seeds do not fill properly,

• the fallen plant is not picked up in the harvest operation, and

• the fallen plants provide a favorable environment for the development of disease (rust, mildew) or for multiplication of insect pests.

Rain, hail, and windstorms occurring after plants have flowered, but before they ripen, are common causes of lodging in small grains. The plants at this stage are green and heavy and are easily broken over by the added weight of the rain or the force of the wind (Fig. 12.1A). Disease and insect injury that weaken the stem or other causes of lodging in small grains (Fig. 12.1B and C) and other crops.

Breeding to improve resistance to lodging involves changing the architecture of the plant, including the root system, to improve its structural strength and stability, and acquiring resistance to diseases and insects that weaken the plant structure. Resistance to lodging may be improved by the development of cultivars with:

• short, stiff, sturdy stems or stalks,

• vigorous, strong root systems that anchor the plant firmly in the soil,

• more resilient straw that will bend but not break in the wind, and

• resistance to disease and insects that weaken the stem or the root system.

Tall plants with slender or weak stems, as in soybean, or plants that are succulent as a result of excessive nitrogen fertilization or soil moisture, are most susceptible to lodging. In crops such as wheat and rice, dwarfing genes have been utilized to reduce height and increase lodging resistance.

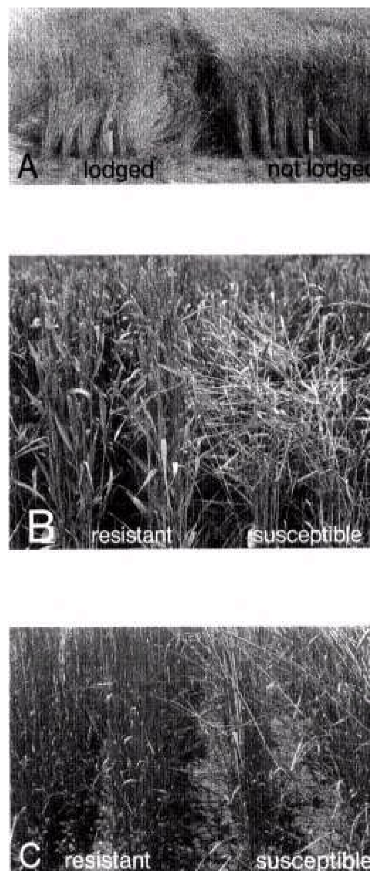


Fig. 12.1.
Comparison of lodging in wheat cultivars. (A) Lodging from wind and rainstorm before the wheat had ripened. (B) Lodging due to disease. Straw of the susceptible cultivar was weakened by stem rust. (C) Lodging from insect injury. The broken and fallen culms of the susceptible cultivar were injured by infestation from the Hessian fly.

Unlike the measurement of yield, in which the quantity is physically measured, evaluation of lodging resistance is almost entirely a visual appraisal. It is obtained by comparing the

relative amounts of bending or breaking over of cultivars growing in adjacent nursery or field plots. It is necessary that all cultivars be grown under as nearly identical conditions as possible, and that a standard cultivar be uniformly included to which experimental strains or new cultivars may be compared. Lodging of an intensity to permit accurate differentiation of cultivars or breeding lines does not occur consistently, making it necessary to replicate field trials at different locations and over a period of seasons. Lodging may be intensified by liberal applications of nitrogen fertilizers, or by letting portions of the yield nursery stand for a period after ripening.

Visual observations on lodging in small grains are normally recorded on a percentage basis (zero to 100), or on a scale of 1 to 10. In com hybrids, lodging may be expressed as percentage of plants with root lodging (leaning more than 30% from the vertical), or with stalk breakage (stalk broken below the ear) (Fig. 12.2). The structural strength of a corn stalk may be measured by the force required to crush a section of the stalk in a hydraulic press. About 50 to 70% of the strength, designated *crushing strength*, is in the outer rind and the remainder is in the pith. Lodging resistance in corn was increased by recurrent selection for increased crushing strength. The firmness with which plants of different corn hybrids are anchored may be measured by the force required to pull the plant, designated the *root pulling force*. Lodging resistance is a quantitative character with complex inheritance, although some of the plant characteristics associated with a reduction in lodging, such as dwarfing genes, or resistance to disease and insect pests, are often simply inherited.

SHATTER RESISTANCE. Shattering refers to seeds that fall out and are lost before harvest or during the harvesting operation. Resistance to shattering is important to prevent loss of yield in small grains, soybean, and some other crops. Visual estimates of loss are commonly made to compare the resistance of cultivars of wheat, soybean, or other crops to shattering. Resistance to shattering is inherited as a complex quantitative character.

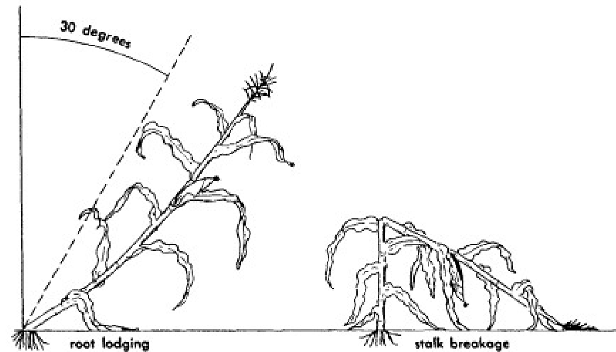


Fig. 12.2.
Types of lodging in corn, Lodging in corn is generally expressed as root lodging, if the stalk leans more than 30 degrees from the vertical, or stalk breakage, if the stalk is broken below the ear.

Winter Hardiness

Winter hardiness refers to the capability of crop plants to survive severe winter stress. The most common types of winter injury to crop plants are:

• *freezing of plant tissues* from the combined effects of low temperature, wind, and insufficient soil moisture, and

• *heaving or uplifting of plants*, shearing off the roots as the plant is torn from the soil, caused by alternate freezing and thawing in water saturated soils.

Plant genotypes differ in resistance to freeze and heaving injury. Winter hardiness is a complex character, the expression of which is affected by environmental and cultural factors such as (**a**) low temperature, (**b**) wind, (**c**) hardening of the plant, (**d**) alternate freezing and thawing, (**e**) moisture content of the soil, (**f**) physical condition and fertility of the soil, (**g**) time and rate of planting, (**h**) disease and insect injury of the plant, and (**i**) snow cover. Field crops most generally subjected to winter injury include the winter cereals (wheat, barley, oat, rye), alfalfa, rape, and various root and forage crops.

Low temperature survival depends upon the plant's inherent ability to harden when exposed to temperatures around 5ºC, and is affected by the rate of freezing and thawing, light, and nutrient status and health of the plant. Low temperature injury is associated with ice formation in the extracellular spaces resulting in freeze-induced dehydration and complex metabolic changes. With low soil moisture and a high wind-chill-index, the plant tissues become desiccated. Cold resistant plants are generally drought resistant, also. Resistance to low temperature injury varies with the species and with genotypes within the species. In the small grains, rye is the most cold hardy species followed by, wheat, barley, and oat in that order. Within a species, the most cold hardy cultivars have generally originated from germplasm that evolved in a severe climatic region. Examples are 'Turkey Hard Red Winter' wheat from the Crimea; and 'Grimm' alfalfa that was subjected to intense natural selection in Minnesota after its introduction from Germany. Cultivars and breeding lines are evaluated for winter hardiness by growing in field trials in areas where they will be subjected to winter injury (Fig. 12.3). Standard cultivars with varying levels of winter hardiness are included for comparison. Survival scores are recorded on a scale of 1 to 100, or a scale of I to 10, from visual observation. Laboratory procedures for measuring cold hardiness are usually of limited utility because they measure only one form of winter stress. Because disease or insect damaged plants are injured more severely than healthy plants, loss from winter injury may be reduced by breeding for resistance to disease pathogens or insect pests causing damage to fall-seeded crops.

Heaving occurs on heavy, moisture saturated soils. Ice crystals accumulate following alternate freezing and thawing, forming successive layers of ice lenses, and uplifting the plant. The heaved plant is frequently uprooted and left on the soil surface where it dies from desiccation. Inherent resistance to heaving is associated with the development of a dense and healthy root system that anchors the plant firmly in the soil. Heaving is more severe in crops with tap roots than with a branching root system.

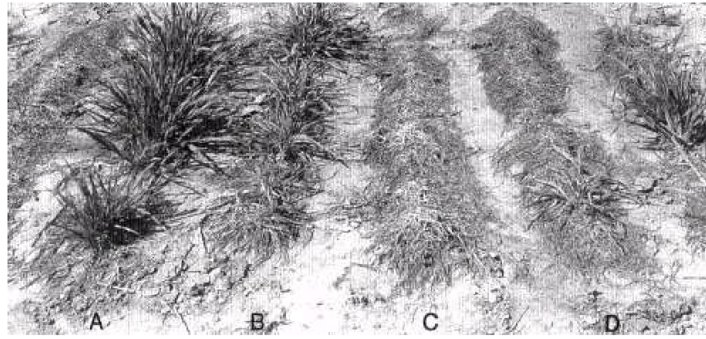


Fig. 12.3.
Comparative winter survival of four strains of winter oat at Columbia, Missouri. Row A is the most hardy. Row B is less hardy than A. Row C is completely killed. Row D has one surviving plant.

Heat and Drought Resistance

Damage and yield loss caused by heat and drought stress are common in high-temperature, low-rainfall areas. The damage may be increased if accompanied by the desiccating effect of strong winds. The effects of heat stress will vary with the crop species, the stage of plant growth, and the duration of the stress and are confounded with the effects of drought stress. Because respiration increases at a faster rate than photosynthesis with increases in temperature, photosynthate supply becomes exhausted, suppressing growth and reducing yield. Injury from heat stress is critical during the flowering period reducing pollen viability, stigma receptivity, and seed-formation. Breeding for earlier maturity may sometimes permit flowering to occur before periods of high temperatures.

Drought resistance mechanisms generally relate to *drought avoidance* or *drought tolerance*. Drought avoidance mechanisms include deep-root systems to acquire soil moisture at lower levels, and plant characteristics to reduce water loss, such as closed stomata, leaf rolling, or waxy substances on the leaf surface. Usually heat and drought tolerance are considered together because high temperatures and drought often accompany each other in the field. Soil moisture gradients from irrigation lines have been used to evaluate strains in different levels of drought stress. Irrigation water supplied will be highest near the irrigation line and will decline outward until a point is reached where no irrigation water is received. Cultivars growing along the gradient are compared for performance.

There is a high correlation between tolerance to heat and tolerance to desiccation. Comparative resistance of plant genotypes may be observed by exposure to high temperature, soil drought, or atmospheric drought. Like winter hardiness, plant genotype resistance to heat and drought stress in the field is determined by complex physiological and morphological characteristics and cannot be accurately evaluated by a single laboratory test. Heat and drought stress resistance are quantitative characters with complex inheritance.

Soil Stress

Soil stress refers to problem soils caused by aluminum or saline toxicity, or various acid or alkaline soil problems. Because many soil problems are related to pH, or other interrelated factors, the precise problem needs to be identified and screening tests devised to evaluate genotype resistance to the specific problem. Screening tests for aluminum or saline tolerance will be more precise if conducted under controlled experiments than when conducted in the field because concentration of the toxic element may be controlled more accurately.

Aluminum Tolerance

Barley and wheat cultivars developed in regions of the world with acid soils and high soluble aluminum usually have higher levels of aluminum tolerance than cultivars developed in regions with nonacid soils. High aluminum soils tend to restrict root and shoot development. Cultivars and breeding lines may be screened for aluminum tolerance in the laboratory by growing seedlings in a nutrient solution containing a high concentration of aluminum ions and selecting for plants with longest root and top growth (Fig. 12.4). When grown in the field on aluminum-toxic soils, aluminum-tolerant cultivars survive winter or drought stress and produce higher yields than aluminum-stressed cultivars, due to the larger root development and top growth.

Salt Tolerance

Crop species are nominally sensitive to high salt concentrations such as is present in sea water. Because large soil areas contain high salt concentrations, efforts have been made to generate crop genotypes with tolerance to this condition. Barley has one of the higher levels of tolerance to salt concentration among the major crop species and studies conducted with this species demonstrated that salt tolerance is a heritable trait. In tomato, salt tolerance was identified in a related wild species and transferred to cultivated tomato.

Resistance to Plant Disease Pathogens

Cultivars of crop plants developed with genetic resistance to destructive disease pathogens are among the foremost contributions in crop breeding. In breeding for host resistance to

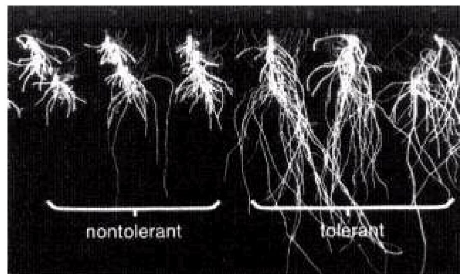


Fig. 12.4.
Effect of aluminum toxicity on root systems of nontolerant and tolerant strains of barley.

different crop diseases each disease is treated as a separate breeding objective. Because crop species are attacked by many disease pathogens, the breeder must establish priorities and concentrate available resources on developing cultivars that are resistant to the most destructive diseases prevalent in the area where the cultivar will be grown. Disease resistant cultivars are developed by identifying genes for resistance in the host species, or related wild species, and transferring the gene or genes into adapted cultivars and breeding lines, normally by hybridization or chromosome engineering techniques. As molecular biology develops, it should become possible to reach out and move genes for resistance from sources not possible through hybridization techniques. Resistance may be controlled by single genes or by polygenes depending on the specialization of the pathogen and the nature of the resistance.

Breeding for host resistance to particular diseases is complicated by the presence of physiologic race specialization in the disease pathogen. If physiologic race specialization is present, various races or biotypes of the pathogen are present in the pathogen population. Resistance in the host genotype to specific races, or blocks of races, of the disease pathogen is conferred by *race-specific genes*. Race-specific genes are simply inherited and confer major resistance effects to particular races or biotypes of the pathogen but not to other races. In highly specialized disease pathogens, many physiologic races may be present, each controlled by a different race-specific gene.

In the absence of race specialization in the pathogen, resistance in the host genotype is conferred by *non-race-specific* polygenes. Non-race-specific polygenes are inherited quantitatively, each contributing a small increment of control of the disease pathogen. Reduction in disease damage may also be achieved by breeding for plant characteristics that enable the plant to escape or avoid disease infection.

Breeding for Race-Specific Resistance

A familiar example of a highly specialized plant pathogen is the pathogen inciting the stem rust disease (*Puccinia graminis* f. sp. *tritici* in wheat *and P. graminis* f. sp. *avenae* in oat). *P. graminis* is composed of numerous specialized biotypes, or physiological races; each race constitutes a population of rust fungi that is genetically different from populations of other races, just like a wheat cultivar is genetically different from other wheat cultivars. The rust fungal race may possess a gene that is *virulent* (capable of infecting) on a specific wheat genotype, yet be *avirulent* (incapable of infecting) on another wheat genotype. The *host genotype* (cultivar or breeding line) may possess a gene, or genes, for resistance to one race of the pathogen, yet be susceptible to another race of the pathogen (Fig. 12.5). The interaction between the gene for virulence in the pathogen and the gene for resistance in the host plant determines whether the reaction is designated *resistance* or *susceptibility*. New physiologic races arise through hybridization among biotypes of the pathogen, or by mutation. If a new cultivar with a gene for resistance to a widespread race of the pathogen is distributed, the new cultivar will screen the avirulent races out of the pathogen population. If a new race of the pathogen arises that is virulent on the new cultivar, the new race may multiply and becomes the prevalent biotype. The breeder must then identify a gene for resistance to the new race and introduce it into the cultivar or breeding lines.

The pathogens inciting the cereal rust diseases are especially notable for their physiologic specialization, but physiological race specialization is found in a host of other disease pathogens and insect pests, including the pathogen inciting powdery mildew, and the insects, Hessian fly and greenbug. Race-specific resistance is normally conferred by a single dominant gene, but may be conferred by two or more genes, or by recessive genes, normally with large effects.

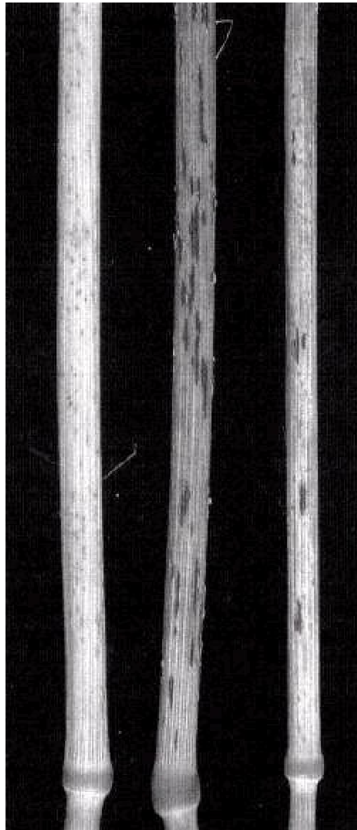


Fig. 12.5.
Reaction of an oat cultivar, 'MO. 0205', to two races of Puccinia graminis avenae. Left: Stem showing resistant reaction to physiologic race no. 7. The resistant-type pustules appear as small black dots. Center: Stem showing susceptible reaction to physiologic race no. 8. The large conspicuous pustules have ruptured the epidermal layer. Right: Stem infected with physiologic races 7 and 8. Both small, resistant-type pustules and large, susceptible-type pustules are present.

Cultivar life is generally thought to be extended by pyramiding genes—the practice of introducing several genes for resistance into a cultivar, each of which would need to be overcome separately by a new race of the pathogen. Reaction to race-specific pathogens may range from *immunity*, with no visible sign of infection, to *hypersensitivity*, in which the host cells immediately around the infection site are killed preventing further spread of the disease pathogen, to *susceptibility*, in which a large spore-producing pustule develops (Fig. 12.6).

Breeding for Non-Race-Specific Resistance

The rapid development and spread of new physiologic races in some disease pathogens and insect pests has necessitated a rapid turnover in cultivars to keep pace with the changes in the parasite. In breeding for resistance to the crown rust pathogen, *Puccinia coronata*, in oat, new cultivars with different genes for resistance were introduced in 1940, 1946, and again in 1953 to replace previously resistant oat cultivars that had succumbed to new races of the crown rust pathogen. Accumulated experiences with specialized pathogens such as the rust fungi have demonstrated that:

• high levels of host resistance to a specific race or a combination of races of the pathogen may be conferred by a single, dominant, race-specific gene, that restricts the infection process as described above,

• the useful life of cultivars with resistance conferred by race-specific genes may be short as new virulent races of the pathogen arise and become widespread, and

• resistance conferred by non-race-

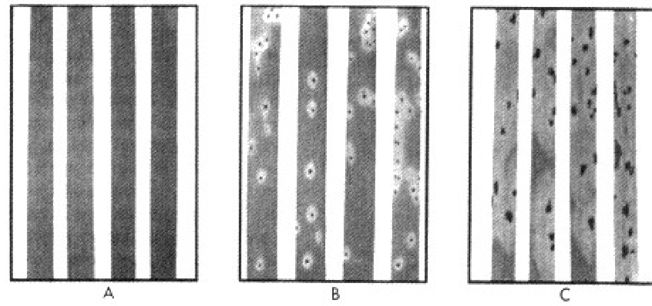


Fig. 12.6.
Types of reaction to the stem rust pathogen, Puccinia graminis tritici, on wheat cultivars. (A) Immune, no visible infection. (B) Hypersensitive, the host cells are killed immediately around the infection site. (C) Susceptible, large spore filled pustules.

specific polygenes that resist colonization and spread of the pathogen would be less vulnerable to damage by new races of the pathogen.

Various non-race-specific systems have been described and designated as *complete resistance, durable resistance, general resistance, partial resistance*, or *slow rusting*. Non-race-specific genes for resistance do not interact differentially with pathogen races, the polygenic resistance extending to all physiologic races. The effects of the polygenes for resistance are small, generally expressed by slowing the rate of disease development through reduced spore production or by other means. The inheritance is complex, improvement being achieved through transgressive segregation.

Avoidance

Avoidance refers to breeding for plant characteristics to escape disease or insect damage. An example is the use of early maturity to ripen wheat cultivars ahead of stem rust epidemics in the U.S. Great Plains.

Techniques for Inducing Disease Epiphytotics

In breeding for disease resistance, exposure to the disease pathogen, either in natural or artificially induced epiphytotics, is necessary to distinguish between resistant and susceptible genotypes. Progeny tests of resistant plants are made to verify the inherent nature of the resistance and to ensure that uninfected plants have not merely escaped infection. A basic problem in breeding for disease resistance is that of providing a disease environment in which to grow the crop so that the resistant plants may be distinguished from the susceptible. Because natural disease epidemics do not occur in the fields every year, it is desirable for the breeder to introduce disease epiphytotics by artificial means, either in the field or in the greenhouse, so as not to be entirely dependent upon the vagaries of nature to provide an adequate disease

environment. Field trials have the advantage that the host genotype will be tested against pathogen races prevalent in the field. But greenhouse tests have the advantage of testing against specific races of the pathogen and provides precise control of temperature and humidity for optimum disease development. Close cooperation between the plant pathologist and the breeder is essential to ensure exposure to the desired biotype of the disease pathogen and selection for both pathogen resistance and agronomic characters. Cultivars with known reaction, both resistant and susceptible, to the biotypes of the pathogen should be included as checks.

INOCULATION TECHNIQUES FOR SOIL-BORNE DISEASE. Diseases incited by soil-borne pathogens that enter the host plant through roots or other underground parts, may be conducted by growing the host genotype in soils in which the disease-inciting pathogen is naturally prevalent, or by growing in controlled environments in sterilized soil which has been inoculated with cultures of the causal organism (Fig. 12.7). The latter differentiates breeding lines more precisely than field tests because temperatures favorable for the growth and development of the disease-inciting organism can be maintained. A cold test for corn has been developed in which seeds are germinated in contact with soil from fields infested with seedling-infesting pathogens. The temperature is maintained near the optimum for the development of the pathogens, which is normally below the optimum for germination of the corn. This simulates early spring planting of corn in cold, wet soils. Mass-screening of seeds for soil-borne organisms may be made by spraying germinating seeds with a suspension of the disease-inciting pathogen. Normal, healthy seedlings are transplanted to soil and grown to maturity; diseased seedlings are discarded.

INOCULATION TECHNIQUES FOR FOLIAGE DISEASES. Many disease-inciting organisms infect the foliage by entering through natural openings, such as stomata or lenticels, or through wounds inflicted during tillage, by insects, or other means. With foliage diseases, such

Fig. 12.7.
Sorghum cultivars tested for resistance to Periconia rootrot or milo disease by germinating seeds in soil infested with the pathogen, Periconia circirata. Lines 2, 4, 6, 8, and 10 are resistant. Lines 1, 3, 5, 7 and 9 are susceptible and have been killed by the disease.

as the cereal rusts, dry spores are collected from disease-infected plants and dusted on the foliage of plants to be inoculated, or a suspension of spores may be sprayed on the foliage (Figs. 12.8 and 12.9). The intensity of the disease infection is increased by growing the host plants in a controlled environment with temperature near optimum for the growth and multiplication of the pathogen and the atmosphere maintained near saturation. Infection may be increased by spraying when the stomata are open, and by reducing the surface tension of the spore suspension with a mild detergent to obtain an even spread on the leaf surface. A power sprayer or a hypodermic syringe may be utilized to apply bacterial inoculum in order to force the inoculum into open stomata, or cause water soaking of the leaves to aid entry of bacteria. Susceptible cultivars planted adjacent to test plants in the field and inoculated with the pathogen serves as a secondary source of infection to the test plants. Host plants may be tested for reaction to two or more diseases simultaneously by sequential inoculations on successive leaves as new leaves emerge.

INOCULATION TECHNIQUES FOR FLORAL-INFECTING DISEASES. Inoculations for floral infecting diseases, such as the loose smut of wheat and barley, are made by injecting ripened spores into the flower during anthesis. Dry spores may be introduced with forceps or a hypodermic needle; or a spore suspension may be injected into the flower with a hypodermic needle, or by means of a vacuum or pressure. Seeds produced in the inoculated flowers are harvested and planted, and the percent of infected plants recorded.

INOCULATION TECHNIQUES FOR SEED-BORNE DISEASES. Inoculation techniques for seedborne diseases are made by applying spores from the pathogen on the seed before seed germination. With common bunt of wheat or covered smut of sorghum, dry spores are dusted on the seeds before planting. If the seed is covered with a hull, as with oat or barley, the seeds are soaked in a spore suspension under a vacuum. The vacuum withdraws the air from under the hull, permitting penetration by the spore suspension.

Fig. 12.8.
Collecting spores from a rust-infected plant of wheat by suction. The rust spores are used to inoculate healthy plants of cultivars and breeding lines to establish their reaction to races of the stem rust pathogen, Puccinia graminis tritici.

Fig. 12.9.
Dusting rust spores on wheat plants in an incubation chamber. The plants are sprayed with water to obtain good dispersal of the spores and kept in a warm humid atmosphere to foster disease development.

Some disease-inciting pathogens, such as *Gibberella* spp. and *Diplodia* spp., that incite root, stalk, and ear rots of corn, are both seed-borne and soil-borne. Inoculation with these pathogens may be made as for soil-borne organisms, or by injecting the pathogen directly into the stalk tissue with a hypodermic needle.

INOCULATION TECHNIQUES FOR VIRUS DISEASES. Virus diseases are transmitted mechanically or by employment of insect vectors, depending on the particular virus. For mechanical transfer of the virus, diseased plant tissue is macerated and the extracted juices are rubbed over the leaves of healthy plants with sufficient force to cause slight mechanical injury. A fine abrasive such as carborundum powder dusted over the leaves first, or mixed with the juices, will aid in obtaining injury and providing avenues for uptake of the virus particles. With insect transmitted viruses, insects of the species specific for natural transmission of the virus feed on virus-infected plants to acquire the virus, and transmit the virus when they feed on a healthy plant. Healthy plants of the genotype to be tested are inoculated by caging with viruliferous insects that feed on and transmit the virus to the test plants. Plants are grown in insect-proof cages to prevent escape of the insects vectoring the virus, or to prevent feeding on healthy plants by naturally occurring vectors (Fig. 12.10).

Resistance to Insect Pests

Host-plant resistance is utilized to control particular insect pests that may be difficult to control through cultural practices or use of pesticides. Breeding for host-plant resistance is

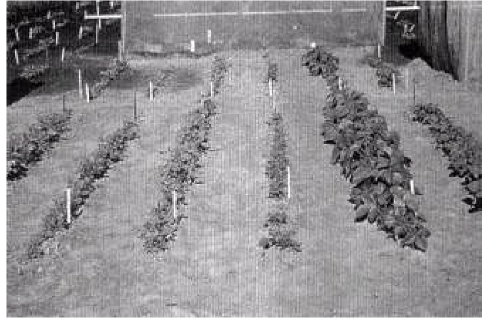


Fig. 12.10.
Comparative reaction of mungbean strains to a strain of cucumber mosaic virus (CMV). The CMV is vectored by naturally occurring cowpea aphids. Mungbean strains grown in insect-proof cages in the rear are virus-free and may be compared with the virus-infected strains.

environmentally safe as it reduces the employment of harmful pesticides. The principles in breeding for resistance to an insect species and the techniques employed are similar to those used in breeding for resistance to disease pathogens. Genes for resistance to the insect species are first identified in crop cultivars or related wild species and transferred to susceptible host genotypes by hybridization. Cultivars and breeding lines are exposed to natural insect populations in the field, or to artificially reared insect populations in controlled environments, in order to distinguish between resistant and susceptible genotypes of the host species (Fig. 12.11).

Biotypes, or races, have been identified in particular insect species that are comparable to biotypes or races in pathogens. The biotypes are identified by reaction to insect feeding, whether resistant or susceptible, on cultivars with known genes for resistance. Insect damage is often related to the stage of growth and development of the plant, so uniform maturity is essential when comparing a series of cultivars or breeding lines for reaction to insect feeding. Cooperation between the entomologist and the plant breeder is essential in development of an insect resistant cultivar.

Product Quality

The objectives for the improvement of crop plants discussed thus far have been directed toward enhancement of *yield potential and yield stability*. In the present market economy, *product quality* has become increasingly important. In breeding for improved quality, consideration is given to the physical and chemical characteristics of the product harvested that affect its

• nutritional value,

• processing, and

• utilization.

The characteristics of product quality will vary with the crop species and its intended use. Wheat, a food crop that is processed for many complex uses will be considered as an example.

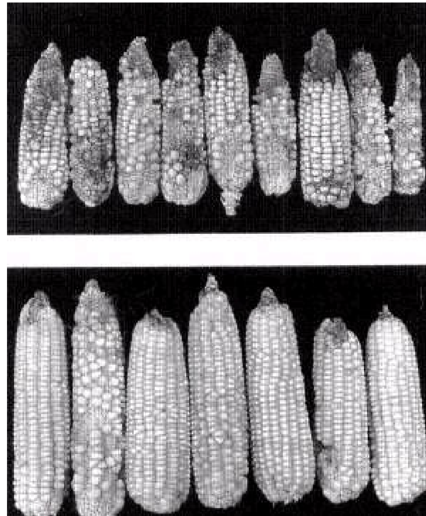


Fig. 12.11.
Comparative damage to corn (maize) inbred lines by the corn earworm, Heliothis zea. (above) Susceptible inbred line. (below) Resistant inbred line.

The principal use of wheat is for milling into flour for making bread, but wheat is also milled into flour for making cakes, cookies, crackers, pretzels, and semolina products. The different wheat products require genetically different kinds of wheat, grown in different climatic areas, and processed by different milling procedures. The milling procedure and the end product of each are affected by the genotype of the wheat cultivar, and are subject to modification in the breeding program. Likewise, other crop species each have distinct quality requirements, that are subject to genetic modification. Developing cultivars with superior quality characteristics, is an important objective in any crop breeding program.

Techniques in Plant Hybridization

Selfing and crossing are essential procedures in breeding crop plants. The exact procedures employed will depend upon the crop species, the structure of the floral organs, and the normal manner of pollination.

Selfing

In self-pollinated species, the plant is permitted to follow its normal mode of pollination, although recognizing that some natural cross-pollination will occur. If slight, the cross-

pollination may be ignored in normal breeding procedures, but if excessive, it may be necessary to protect the flower from foreign pollen by bagging to prevent foreign pollen from reaching the stigma. In selfing or inbreeding a naturally cross-pollinated species, the flower must be covered and protected from foreign pollen. In plants like cotton, with large flowers, the petals may be folded down over the sexual organs and fastened to exclude pollen and pollen-carrying insects. In corn, a paper bag is placed over the tassel to collect pollen, and the shoot is covered to protect it from foreign pollen. Pollen collected in the tassel bag is transferred to receptive silks on the same plant to obtain self-fertilization. Bagging and hand-tripping is necessary in legumes such as alfalfa to obtain self-pollination, or the legume plants may be caged with an insect pollen vector. Many insect-pollinated species are highly self-sterile and selfed seeds are difficult to obtain.

Emasculation Practices

Cross pollinations in species with bisexual flowers are generally accomplished by removing the stamens before pollen is shed from the female parent and transferring pollen collected from the pollen parent to the emasculated florets. The anthers are removed with small, fine-pointed forceps, or by suction, or may be killed by heat, cold, alcohol, or chemical hybridizing agents, and the emasculated flowers are covered with parchment or glassine envelopes, or kraft paper bags, to protect them from stray pollen (Figs. 12.12, 12.13, and 12.14). Emasculation is unnecessary in monoecious or dioecious crops, although the pistillate flower will need to be protected from foreign pollen. In highly self-incompatible plants, the greater compatibility of foreign pollen is usually depended upon to fertilize the ovule. Recessive male-sterile genes may be used to eliminate the emasculation procedure in some species. Cytoplasmic male sterility is utilized for production of hybrid seed without emasculation in onions, corn, pearl millet, sorghum, sugarbeets, sunflower, wheat, and other species.

Pollination Practices

Pollinations are most successful if made within one to three days following emasculation, during the period of maximum stigma receptivity as indicated by the opening of the flower and full development of the stigma. Pollination is carried out by collecting ripe anthers and emptying the pollen directly upon the stigma, or dusting it on with a camel hair brush. In small grains and forage grasses, a spike or panicle of the pollen parent can be bagged with the emasculated flower, a procedure called *approach crossing*. Shaking the bag daily during the period of stigma receptivity will help to disseminate the pollen. Under normal conditions the length of time that pollen remains viable varies from one to two minutes for wheat to several hours for corn. Longevity of the pollen is reduced by high temperatures, but may be extended to several months in some species if dried under vacuum and stored in tight containers at temperatures around 0ºC.

Certain crops, like red clover, alfalfa, and birdsfoot trefoil, are pollinated by insects. In the insect pollinated crops, plants or clones are enclosed in insect proof cages (Fig. 12.15) and the pollinating insect, commonly bees that have been cleansed of pollen, are introduced into the cage. In these species, a high degree of self-incompatibility is depended upon to prevent self- or sib-pollination.

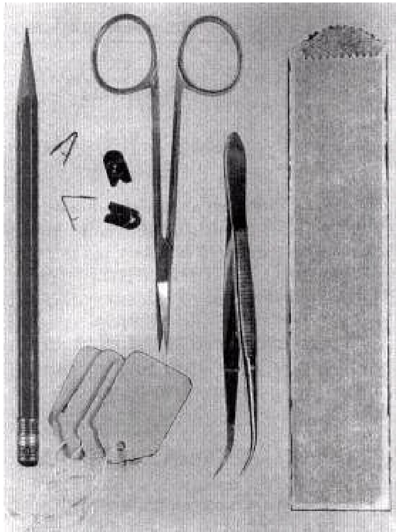


Fig. 12.12.
Equipment commonly used in emasculating and crossing small grains. Either curved or straight-pointed tweezers may be used. The head bags are made of parchment or glassine.

Fig. 12.13.
Barley spikes are covered with head bags after emasculation and pollination. The parents of the cross are recorded on a tag attached to the barley spike.

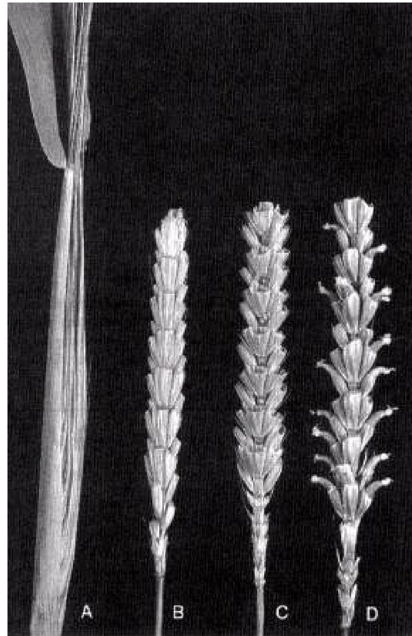


Fig. 12.14.
Barley spikes selected to show successive stages in emasculation and crossing. (**A**) Spike at the stage of development when emasculations are normally made in barley. (**B**) Spike after emasculation. The glumes have been cut back to facilitate emasculation and pollination. Note that the immature florets are closed. (**C**) Spike at desirable stage for pollination. The florets are now open. (**D**) Set of seed obtained from crossing.

Practices to Enhance Flowering and Seed Production

Crossing is often facilitated by growing parent plants in glass houses, screen houses, or environmentally controlled chambers where temperature, photoperiod, and humidity favorable for flowering can be maintained and contamination from wind blown pollen reduced. For plants with winter growth habit, *vernalization* of seeds is needed for flowering to occur. Vernalization of some species is accomplished by exposing germinating seeds to temperatures slightly above freezing. Success in some wide species crosses may be enhanced by excising the immature embryo from potentially viable $\mathbf{F}_1$ seeds a few days after pollination, and culturing the embryo aseptically on an artificial medium.

Conducting Field Trials

New cultivars and breeding lines are evaluated in field trials for yield potential, maturity, lodging resistance, resistance to environmental stress, and naturally occurring disease pathogens

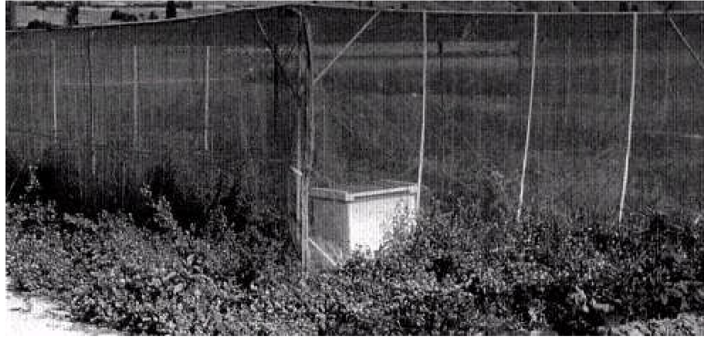


Fig. 12.15.
An insect-proof cage covered with plastic wire in which experimental strains of legumes are grown for controlled pollination studies. A hive of honey bees is placed inside the cage to cross-pollinate the legume strains growing in the cage.

or insect pests (Fig. 12.16). Strains with highest performance in preliminary trials are reevaluated in advanced yield trials at different locations for genotype-environment interactions and yield stability. The practice of conducting yield trials for particular crops with a uniform set of entries over the production area was pioneered by the U.S. Department of Agriculture, and is now widely practiced by the International Research Centers. Additionally, the uniform nurseries serve to disseminate superior germplasm to the plant breeders who conduct the trials.

Principles in Plot Experimentation

Crop cultivars and breeding lines are grown in field performance trials, simulating performance in natural field environments. The field trial is never conducted in an ideal environment, some error in the data is universally present. The error may arise from unavoidable or chance fluctuations among the environments in which the different cultivars are grown, or it may arise from inaccuracy in the conduct of the experiment. If the error is large, the experimenter will not be able to correctly predict cultivar performance. For accurate and trustworthy results, the experimenter must follow careful and proven procedures, which are uniformly conducted with all of the entries included in the trial, and personal bias eliminated in recording notes and in interpreting the data.

SOIL VARIABILITY. Variability in the soil is a universal source of error in field plot trials. Even in small contiguous areas, the soil will vary in fertility, drainage, texture, and productivity; or contain uneven residual effects from previous soil cultural treatments. In selection of the area for field performance trials, consideration should be given for uniformity in topography, drainage, fertility, and previous cropping treatments.

Fig. 12.16.
Wheat breeding nursery conducted by the International Maize and Wheat Improvement Center (CIMMYT) at Ciudad Obregon, Sonora State, Mexico.

COMPETITION AND BORDER EFFECT. Crop plants in adjacent rows compete for the soil moisture and plant nutrients in the space between them. The error resulting from competition between adjacent cultivars may be reduced by planting multiple-row plots, and by grouping together cultivars that are similar in maturity, height, and growth habit. Uniform stands of all cultivars in the trial reduces inter-plant competition and are essential for accurate yield results.

REPLICATION. In cultivar yield trials, the recorded yield for a cultivar harvested from a single plot is always subject to error; the true yield of the cultivar (genotype) being either larger or smaller than the recorded yield. If the yields of several plots of the same cultivars are averaged, the chance fluctuations tend to offset each other and reduce the error. When all entries in an experiment are repeated several times, each repetition is referred to as a *replication*. The number of replications in a field experiment normally ranges from three to five, depending upon the design of the experiment, the accuracy desired in the yield data, and the amount of land and seed available. Replication at a single site is effective in sampling soil variations at that site, and local cultivar × environment interactions. Replication increases precision in the experiment, accuracy in identifying the superior cultivar in that particular experiment, and provides the means for statistical analysis of the experiment and estimation of the magnitude of the experimental error.

LOCATION AND SEASONAL VARIATION. The comparative performance of crop cultivars differ when yield trials are conducted at a particular location in different seasons, and when conducted at different locations in the same or different seasons. Each location and\or season provides a different environment in which to grow the cultivars (genotypes). Replication of an experiment in different seasons and at different locations serves to sample major cultivar × environment interactions (Fig. 4.1), and assists the breeder in identifying cultivars with stable yield performance over a range of environments. It is also important to examine the cultivar × location interactions since cultivars developed with stable yields over a broad range of environments may not be the highest yielding cultivar at a particular location.

Field Plot Design and Techniques

Field experiments are conducted to evaluate comparative cultivar performance and should simulate improved cultural practices as nearly as practical. The design of the experiment will depend upon the particular crop, the number of cultivars to be tested, and the precision desired in the results. The experimental design must include replication and randomization of the treatments (cultivars) within the replications in order to make valid comparisons among the cultivars and calculate accurate estimates of the experimental error. Careful attention to details in planting, harvesting, threshing, and measuring yield are required in order to obtain accurate results. Cultivar yield trials usually consist of complete or incomplete block designs. The statistical analyses of these designs will not be presented here, but may be found in standard textbooks on statistical procedures for agricultural research.

COMPLETE BLOCK DESIGNS. The *randomized complete block* is a simple design in which all treatments (cultivars) are included in each replication of the experiment and are arranged in a random order within the replication. For accuracy, it should be used with small numbers of cultivars only. Replications may be placed end to end or opposite each other, so that the total area covered by the experiment will be as nearly square in shape as possible. Entries with apparent weaknesses may be discarded before harvest, saving the expense of harvest, and the data may still be analyzed by an analysis of variance.

INCOMPLETE BLOCK DESIGNS. With large numbers of entries in yield trials, the size of the experimental area increases, possibly increasing the amount of error due to soil variation. Incomplete block designs are preferable where large numbers of cultivars are compared in a single yield trial. The cultivars in each replication are subdivided into smaller blocks, in a manner designed to reduce the error caused by soil variation. The incomplete block designs, usually referred to as lattice designs, have the restriction that the number of cultivars must be a square of some number. In addition, all cultivars must be harvested; inferior strains cannot be discarded prior to harvest to reduce harvest expense, and still analyze the experiment as a lattice design.

THE ANALYSIS OF VARIANCE. The analysis of variance is a simple mathematical procedure for measuring the relative importance of two or more groups of factors that cause variation within an experiment. The analysis of variance is a standard tool for data analysis by plant breeders and quantitative geneticists. Its applications in experimental design, interpretation of data, and tests of significance are too extensive to be presented here, but may be found in textbooks on statistical analysis and field plot design.

RECORD KEEPING. The plant breeder grows and observes thousands of experimental strains in the breeding nursery, requiring an extensive system of record keeping. An efficient system of record keeping should possess the following requisites:

- completeness,
- accuracy, and
- simplicity.

Modern computers enable the breeder to summarize, statistically analyze, and reproduce data quickly and accurately. Computerized systems are utilized for randomization of entries in the yield trials, printouts of notebook sheets and labels for identification of field plots, instant recording of data, and analysis of data.

Study Questions

1. When is emasculation necessary in plant breeding? Describe how the plant breeder goes about making pollinations. What precautions are necessary when making hand pollinations?

2. How does the plant breeder decide which breeding objectives are important in crop plants?

3. How important are field trials in the overall scheme of plant breeding? What are some of the field experimental designs used in plant breeding?

4. What are some of the techniques used for inducing disease and insect epiphytotics?

Further Reading

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Boca Raton, FL.

Clark, R.B., and R.R. Duncan. 1991. Improvement of plant mineral nutrition through breeding. Field Crops Res. 27:219-40.

Clark, R.B., and R.R. Duncan. 1993. Selection of plants to tolerate soil salinity, acidity, and mineral deficiencies. p. 371-79. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Clarke, J.M., and T.F. Towniey-Smith. 1984. Screening and selection techniques for improving drought resistance. p. 137-62. *In* P.B. Vose and S.G. Blixt (eds.) Crop breeding, a contemporary basis. Pergamon Press, Oxford.

Dyck, P.L., and E.R. Kerber. 1985. Resistance of the race-specific type. p. 469-500. *In* A.P. Roelfs and W.R. Bushnell (eds.) The cereal rusts, Vol. II. Academic Press, Orlando, FL.

Fehr, W.R., and H.H. Hadley. 1980. Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Fowler, D.B., A.E. Limin, A.J. Robertson, and L.V. Gusta. 1993. Breeding for low-temperature tolerance in field crops. p. 357-62. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Gomez, K.A., and A.A. Gomez. 1976. Statistical procedures for agriculture research. The Int. Rice Res. Inst., Los Baños, Philippines.

Harpstead, D.D. 1983. Breeding for improved nutritional quality of crops. p. 255-70. *In* D.R. Wood (ed.) Crop breeding. Amer. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Hooker, A.L. 1983. Breeding to control pests. p. 199-230. *In* D.R. Wood (ed.) Crop breeding. Am.

Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Johnson, R., and G.J. Jellis (eds.) 1993. Breeding for disease resistance. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Parlevliet, J.E. 1985. Resistance of the non-race specific type. p. 501-25. *In* A.P. Roelfs and W.R. Bushnell (eds.) The cereal rusts, Vol. II. Academic Press, Orlando, FL.

Pinthus, M.J. 1973. Lodging in wheat, barley, and oats: The phenomenon, its causes, and preventive measures. Adv. Agron. 25:209-63.

Rasmusson, D.C., and B.G. Gengenbach. 1983. Breeding for physiologic traits. p. 231-54. *In* D.R. Wood (ed.) Crop breeding. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

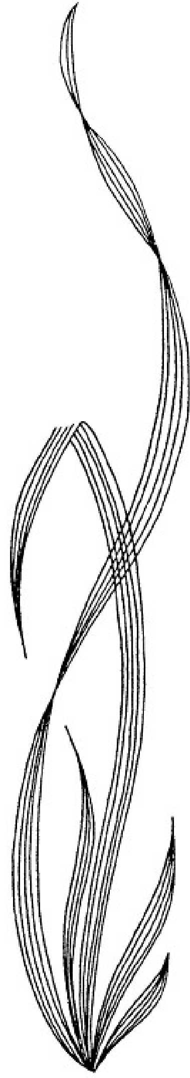
Rowell, J.B. 1984. Controlled infection by *Puccinia graminis* f. sp. *tritici* under artificial conditions. p. 291-332. *In* A.P. Roelfs and W.R. Bushnell (eds.) The cereal rusts, Vol. I. Academic Press, Orlando, FL.

Sleper, D.A., T.C. Barker, and P.J. Bramel-Cox (eds.). 1991. Plant breeding and sustainable agriculture: Considerations for objectives and methods. CSSA special publ. No. 18. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Smith, C.M., Z.R. Khan, and M.D. Pathak. 1993. Techniques for evaluating insect resistance in crop plants. Lewis Publishers, Boca Raton, FL.

Shedecor, G.W., and W.G. Cochran. 1991. Statistical methods. 8th ed. (2nd Printing). Iowa State University Press, Ames, IA.

Sojka, R.E. 1985. Field evaluation of drought response in small-grain cereals. p. 165-91. *In* G.E. Russell (ed.) Progress in plant breeding-I. Butterworths, London.



V
GERMPLASM RESOURCES FOR BREEDING CROP PLANTS

13. Germplasm Resources and Conservation

Plant germplasm is the genetic source material used by plant breeders to develop new cultivars. Germplasm may include seeds or other plant propagules such as a leaf, stem, pollen, or cultured cells that could be grown into mature plants. Seeds may be from new or old cultivars, landraces, special breeding lines or populations developed by the breeder, or special genetic stocks such as mutant lines. Plant germplasm is one of our most important natural resources and must be managed properly if breeders are to continue to develop improved cultivars. Survival of the human race depends upon the proper conservation of these valuable plant germplasm resources.

Germplasm Conservation

Crop plants have been domesticated for a very long period of time, but the germplasm resources accumulated in the process are being eroded very rapidly. Farmer-selected cultivated forms, called *landraces*, originally evolved from wild plant populations. The landraces that did not acquire broad genetic diversity during this evolutionary period eventually succumbed to the ravages of disease, drought, cold, competition with weeds, or other unfavorable environmental stresses. The landraces that survived became modern crop cultivars in the countries of origin and progenitors to modern cultivars in other countries. *Unfortunately, progress in breeding, often by selection and purification of these heterogeneous landraces, inevitably led to more uniformity and less genetic variability within the improved cultivars than was present in the original landraces*.

In addition to the immense genetic diversity in the landraces that emerged as the cultivated cultivars of the last century and the early part of this century, there is tremendous genetic variability in the related wild species for almost all plant characteristics. Studies in California on wild oat (*Avena fatua*), a self-pollinated species, show that samples collected only a few

meters apart, one on a steep slope and the other on a level area above the slope, differed in heading time by 15 days. Differences were observed also in height, number of spikelets, panicle length, and other morphological and physiological characteristics. Obviously, not all of the myriad of plant genotypes found in nature can be conserved in germplasm collections, but those species that may be useful to plant breeders need to be sampled and stored before the natural habitats in which they are now found are destroyed. One suggestion worthy of consideration is that areas be set aside, undisturbed, where the wild species abound, and where they would be conserved in situ in a living collection. *Offsetting the loss of germplasm, modem cultivars tend to have concentrated into them a large assortment of the genes for "fitness" formerly dispersed in many landraces*.

One of the consequences of successful plant breeding is an increased erosion or reduction in genetic variability for the crop undergoing selection. The more successful the selection, the greater the decrease in genetic variation for a particular crop. As a result, breeders need to effectively manage their breeding populations to preserve adequate genetic variation so that future improvements through selection can occur. Proper management of germplasm resources by the breeder includes introducing new germplasm resources on a regular basis to develop new recombinants and hence increase genetic variability. The breeder must carefully evaluate breeding materials being discarded during the selection process so that erosion of the genetic base does not deteriorate beyond recovery. During the selection process, one of the most difficult decisions to be made by the breeder is what material to discard. It is relatively easy to determine what to keep during selection because the breeder will keep those plants with superior performance. The breeder has tremendous responsibility to insure that adequate genetic variability remains available in the crop for use by future generations of humankind.

Proper management of plant germplasm resources is necessary to insure that future improved cultivars can withstand stresses such as caused by insect and disease pests, and climate extremes. In 1970, a disease called Southern corn leaf blight infected much of the corn acreage in the southeastern United States and the Great Plains. Approximately 15% of the corn crop was lost that year. The fungus that caused Southern corn leaf blight (*Bipolaris maydis*) was associated with a particular source of cytoplasm in corn. During the mid-nineteenth century, the Irish potato famine was caused by a fungal (*Phytophthora infestans*) disease of the potato. This disease caused hugh losses in lives and money, and many Irish people relocated in other countries. These examples, and many others, reminds us of how vulnerable our crops can be. It is imperative that a strong genetic resource base be conserved so that the genetic vulnerability of our crops can be reduced.

Centers of Genetic Diversity

Nikolai I. Vavilov, a famous Russian botanist, conceived the idea that vast genetic variations within a particular crop species are generally concentrated within small geographic areas. During the 1920s and 1930s, Vavilov collected plant specimens throughout the world, some from wild populations, but mostly from planted cultivars. His concept of *centers of diversity* was based on the observation that extensive genetic variability of cultivated species could be found in certain restricted areas, while over vast areas relatively little genetic diversity existed. His classical example was wheat. In the Middle East, he found numerous forms of diploid, tetraploid, and hexaploid species of wheat, yet over Europe and Asia, cultivation was restricted to a rather limited group of hexaploid cultivars. Vavilov observed, also, that the centers of diversity for many species occupied the same small geographic area. From these observations he identified eight major centers of diversity and three subcenters (Fig. 13.1). The

centers of diversity and some of the major crops identified with the center are as follows:

1. Chinese center: adzuke bean, millet, naked oat, sesame, soybean

2. Indian center: rice bean, chickpea, aboreum cotton, jute, finger millet, mungbean, rice, sugarcane, taro, yam

2a. Indomalayan center: banana, coconut, sugarcane, yam

3. Central Asiatic center: chickpea, flax, lentil, pea, rye, safflower, sesame, bread wheat

4. Near Eastern center: alfalfa, barley, chickpea, flax, lentil, melon, red oat, pea, rye, sesame

5. Mediterranean center: broad bean, cabbage, lettuce, hulled oat, durum wheat

6. Ethiopian (formerly Abyssinian) center: barley, chickpea, flax, lentil, finger millet, pea, sesame, teff, tetraploid wheat

7. South Mexican and Central American center: common bean, corn, upland cotton, cucurbits (gourd, squash, pumpkin), sisal hemp

8. South American (Peruvian-Ecuadorian-Bolivian) center: lima bean, sea-island cotton, potato, sweet potato, tobacco, tomato

8a. Chiloé center: potato

8b. Brazilian-Paraguayan center: cacao, manioc, peanut, pineapple, rubber tree.

Vavilov suggested that the *center of diversity* for a crop is the *center of origin* for that crop. This view was disputed because some crops had more than one center of diversity, and for some of these crops, one of the centers of diversity was not located in an area that contained wild relatives. This was explained by assuming that the crop had been carried to the latter area in earlier times, and that genetic variation continued to expand, leading to great

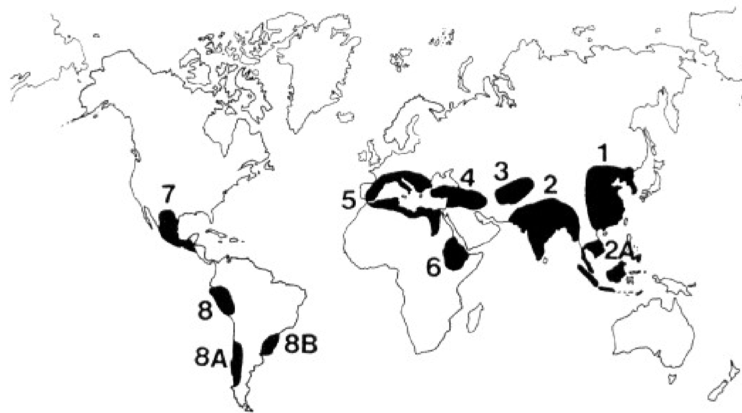


Fig. 13.1.
Centers of origin and diversity for the major crop species, as identified by Nikolai I. Vavilov. (See text for identification of the centers.)

genetic diversity among collections. Vavilov later designated the areas where domestication occurred as *primary centers* and areas where variation continued after domestication as *secondary centers*.

The concept of the centers of diversity is important to plant breeders because the centers represent areas where diverse germplasm may be collected. As an example, recent collections of wild oat in Israel yielded a broad array of genes, including genes for rust resistance and high protein, two economically important characteristics. With advancement in culture and the cultivation of improved cultivars over the entire world, many of these centers of diversity are threatened with extinction. In the future it may not be possible to recover "primitive" species from these areas to find new genes. High-yielding cultivars of wheat are rapidly replacing the landraces of wheat in the Middle East. Reduction in natural habitats with more extensive cultivation and improved cultural practices are eliminating the weedy relatives of wheat. Similar losses of germplasm are occurring with other crops. For example, in northwest Africa, heavy grazing by livestock threatens the extinction of several forage species (Fig. 13.2). It is imperative that a vast array of these diverse germplasm sources and wild relatives be collected before they are lost and that the collections be maintained ex situ indefinitely as sources of germplasm for future use by plant breeders.

International Network for Conservation of Genetic Resources

Past efforts to collect, classify, and conserve genetic resources worldwide, unfortunately, have been too little and too late. A large collection was assembled by Vavilov, at the All-Union

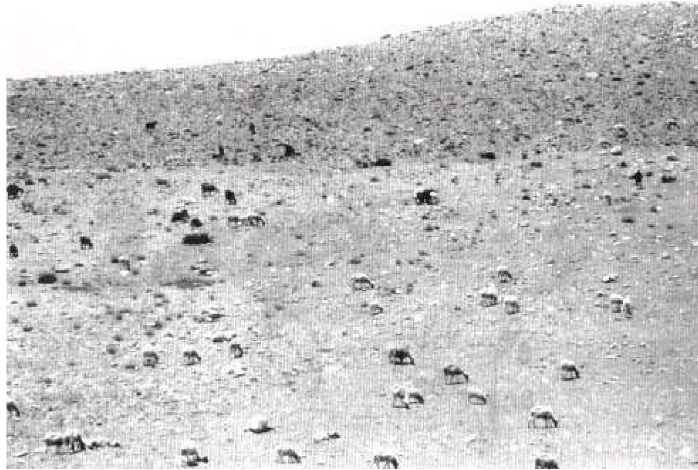


Fig. 13.2.
Heavy grazing by livestock is threatening the existence of many native forage grasses and legumes in Morocco and other countries in northern Africa.

Institute of Plant Industry, St. Petersburg. It consisted of more than 250,000 accessions collected during Vavilov's extensive travels. Protection of this valuable collection of agricultural diversity is essential. In the United States, a comprehensive collection containing more than 400,000 accessions of field and horticultural crops, is maintained by the United States Department of Agriculture (USDA). The organization of this activity and the facilities being utilized will be discussed later in this chapter. Although extensive, the United States collection is deficient in many species and in accessions from many geographic areas. Many early introductions were lost due to breeder evaluation and discard, and the combined effect of inadequate storage facilities, insufficient funding, and a shortage of trained personnel. Important germplasm collections are maintained in many other countries and by many public and private plant breeders, but public information about the accessions in these collections is often difficult to obtain.

In recent years there have been concerted efforts to establish an international network for the conservation of genetic resources. A group of donor countries, developmental banks, and foundations established an *International Board for Plant Genetic Resources* (IBPGR) with headquarters in the *Food and Agriculture Organization* (FAO) of the United Nations in Rome. The functions of the IBPGR are to develop an overview of the worldwide state of genetic resource conservation, to promote and coordinate efforts among countries to conserve germplasm of the major field crops, and to assist appropriate institutions in extending their capabilities for storing and evaluating germplasm collections.

In addition to the various participating countries, whose activities are too extensive to describe here, the network includes the *International Agricultural Research Centers*, which have assumed responsibility for collecting, maintaining, and evaluating germplasm for specific crops with which they are working. Much progress has been made by the *International Rice Research Institute* (IRRI), Los Baños, Philippines, which has collected more than 70,000 accessions of rice. At Los Baños, a modem laboratory to facilitate processing, storing, and distributing seed of the collections has been constructed. Comprehensive evaluations and cataloging of the accessions are being done (Fig. 13.3). Other world collections have also been assembled:

• *International Maize and Wheat Improvement Center* (CIMMYT), Mexico: corn, wheat, and triticale;

• *International Crops Research Institute for the Semi-Arid Tropics* (ICRISAT), Patancheru, A.P., India: sorghum, pearl millet, chickpea, pigeonpea, and peanut (groundnut);

• *International Center of Tropical Agriculture* (CIAT), Cali, Colombia: dry bean, cassava, tropical forages;

• *Asian Vegetable Research and Development Center* (AVRDC), Shanhua, Taiwan: mungbean, soybean, tomato, chinese cabbage, pepper;

• *International Institute of Tropical Agriculture* (IITA), Ibadan, Nigeria: cowpea, cassava, sweet potato, yam;

• *International Potato Center* (CIP), Lima, Peru: potato, sweet potato; and

• *International Center for Agricultural Research in Dry Areas* (ICARDA), Aleppo, Syria: wheat, barley, broad bean, lentils.

Fig. 13.3.
Testing rice germplasm accessions for resistance to the brown plant hopper (Nilaparvata lugens) at the International Rice Research Institute, Los Baños, Philippines. Genes for resistance identified during the screening may be introduced into adapted cultivars by hybridization.

Germplasm Resources and Their Maintenance in the United States

The early immigrants to the United States brought with them seeds of the crops grown in their native lands, or they imported seed after they reached this continent. Without seed and plant introductions, farmers in the United States and Canada would not be growing wheat, oat, barley, rice, sorghum, flax, soybean, alfalfa, clovers, bluegrass, timothy, bromegrass, tall fescue, sugarcane, and numerous other crops. Only a few of the major crops originated in the Americas, most of them outside the borders of the United States or Canada. Of these, corn, tobacco, potatoes, field beans, sunflower, some forms of cotton, and some of the native grasses are the most important. The early farmers came to the United States from widely separated foreign lands and brought with them a diversity of cultivars and strains of different crop species. By trial and error, the cultivars and species with the best ecological adaptation to different crop-producing regions gradually became known and their use extended. Unadapted cultivars and species were dropped from production.

United States National Plant Germplasm System

The importance of introducing plant germplasm was officially recognized early in the United States. In 1819, United States foreign officers were instructed to collect seeds and plants potentially useful for cultivation in the United States and to provide information on the climate and soil conditions to which they were adapted. In 1898, an Office of Foreign Seed and Plant Introduction, later to become the Office of Foreign Plant Introduction, was established in the United States Department of Agriculture. Many early activities in plant introduction were described by David Fairchild, longtime Chief of the Plant Introduction Office, in "The World Was My Garden"; by Nelson Kose in "America's Crop Heritage"; by H.V. Harlan in "My Life with Barley"; among other writers.

The United States program for germplasm conservation evolved into a National Plant Germplasm System (NPGS), now the model system for the United States Department of Agriculture's National Genetic Resources Program. While collection and maintenance of germplasm still play the major roles, more attention is being given to adequate evaluation of the germplasm resources and dissemination of information on the accessions to the plant breeder. If germplasm collections are to be utilized fully, information on the accessions must be documented so that plant breeders can identify potentially useful strains. This requires a computerized information retrieval system. In the United States, the computerized *Germplasm Resources Information Network* (GRIN) is maintained at the United States Department of Agriculture's research center in Beltsville, Maryland, located near Washington, D.C. This system contains computerized information on all of the accessions preserved by the National Plant Germplasm System. While qualitative traits such as morphological features, color markings, or disease reaction may be described with a fairly high degree of accuracy, descriptions of quantitative traits that are subject to genotype × environment interactions are less accurate and often applicable only to the location and season in which the observations were recorded. Without this information a breeder may need to screen thousands of strains to find those with the desired genes.

Seed and Plant Introduction

The soybean is a spectacular example of an immigrant species that has become a major crop in the United States within the past 60 years. As a result of one expedition alone into the Far East, over 3000 soybean accessions were introduced. Plant and seed materials collected and introduced through the United States Department of Agriculture, the state agricultural experiment stations and many private sources are available to plant breeders to augment their individual collections. The value and utility of the worldwide germplasm collections will increase as native and wild germplasm resources are further diminished.

NATIONAL GERMPLASM RESOURCES LABORATORY. The National Germplasm Resources Laboratory, located at Beltsville, Maryland, is the focal point of the United States National Plant Germplasm System (Fig. 13.4). This office receives and records official plant and seed introductions, distributes germplasm to the various collections in the system, and coordinates foreign exchange of germplasms. It also jointly manages along with the USDA's Animal and Plant Health Inspection Service, the *National Plant Germplasm Quarantine Center* located at Beltsville, Maryland. The Quarantine Center tests and certifies that germplasm introductions are free from harmful pests and, for export, assures that quarantine regulations of the United States and importing countries are met.

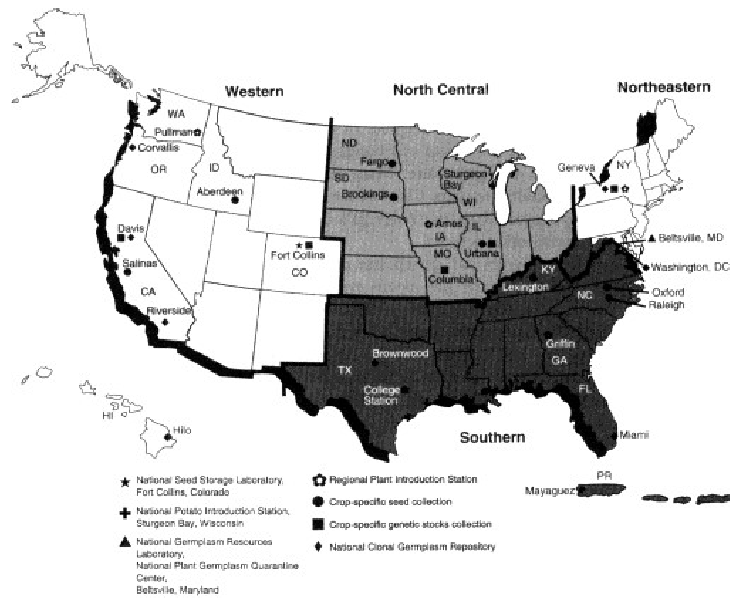


Fig. 13.4.
Plant introduction regions and principal germplasm facilities in the United States.

REGIONAL PLANT INTRODUCTION STATIONS. After seed or plant stocks of a crop are introduced, they must be cataloged, made available to breeders interested in testing them, and maintained in a viable condition so that they may be grown again. Germplasm accessions may be maintained either as seed or as living plants. Because seeds lose their viability in storage, each accession must be grown at regular intervals to renew the seed stocks. Maintaining viable collections of plants propagated vegetatively poses problems that are more difficult than the storage of seeds because living collections must be maintained. To assist in this gigantic task, four Regional Plant Introduction Stations were established in 1946 by the United States Department of Agriculture in different agricultural regions of the United States, in cooperation with state agricultural experiment stations. The Regional Plant Introduction Stations and their locations are Northeast region, Geneva, New York; Southern region, Griffin, Georgia; North Central region, Ames, Iowa; and Western region, Pullman, Washington (Fig 13.4).

The functions of the regional plant introduction stations are to:

• determine germplasm needs within the region,

• assist with foreign explorations to fill regional needs,

• multiply, evaluate, and maintain new plant and seed collections of crops adapted to the region with minimum loss of genetic variability within the strains (Fig. 13.5), and

• distribute the seed and plant accessions to plant scientists worldwide.

Each item received is given a plant introduction (P.I.) number by which it is subsequently identified. Catalogs of available accessions are published by the Regional Plant Introduction Stations, and the information is available on GRIN through a computer and modem.

WHERE GERMPLASM IS CONSERVED. Germplasm is stored in many different regions of the United States (Fig 13.4). At the four Regional Plant Introduction Stations, the following examples of crops are conserved:

• Western station: onion, common bean, garlic, safflower, chickpea, wild rye, horsebean, common vetch, milkvetch, forage grasses, alfalfa, lupin, milkvetch, lentil

• Southern station: sorghum, peanut, forage grasses, peppers, okra, melons, forage legumes, sweet potato, mungbean

• Northeast station: pea, clovers, onion, tomato, birdsfoot trefoil, brassicas

•North Central station: corn, cucumber, pumpkin, beet, carrot, sunflower, millets, canola, mustard, rape, sweet clover, amaranth

In many instances, it may not be possible to store germplasm as seeds. For example, seeds of cocoa and wild rice are damaged when dried and cooled. Therefore, these species must be

Fig. 13.5.
Safflower germplasm collection being increased at the United States Department of Agriculture, Western Regional Plant Introduction Station, Pullman, Washington. Each plant is bagged to exclude foreign sources of pollen and to insure self-pollination.

stored as living plants in the field or greenhouse.

Scientists within the National Plant Germplasm System are researching biotechnology techniques such as tissue culture for use as long-term storage techniques. It has been shown that shoot-tip cultures may be frozen and utilized for long-term storage of germplasm. *Freeze-preservation* or *cryopreservation* of germplasm is also being researched. Cryopreservation involves the conditioning and preservation of cell or tissue cultures in liquid nitrogen at extremely low temperatures (-150° to -196°) for long periods of time and the subsequent regeneration of functional plants (Fig. 13.6). The meristem tissue is precultured before freezing with a cryoprotectant such as a mixture of sugar, polyethylene glycol, and dimethylsulfoxide. Special care must be exercised during both freezing and thawing of the cultures. Freeze-preservation of tissue cultures offers potential for long-term storage of germplasm, particularly for vegetatively propagated species that would need to be maintained as living plants. Combining meristem-tip culture with cryopreservation permits the long-term storage of pathogen-free germplasm and facilitate the international exchange of pathogen-free genetic materials. Perhaps one day, it may be possible to maintain isolated DNA for germplasm conservation.

The location and examples of crop species maintained at national clonal germplasm repositories include:

Fig. 13.6.
Cryotanks are used to preserve seed, pollen, and plant tissues at - 160°C
at the National Seed Storage Laboratory, Fort Collins, Colorado.

• Corvallis, Oregon: pear, strawberry, raspberry, blueberry, mint, hops, filberts, cranberry, blackberry

• Davis, California: stone fruits, almond, pistachio, persimmon, olive, fig, pomegranate, grape, mulberry, kiwi

• Geneva, New York: grape, apple

• Miami, Florida, and Mayaguez, Puerto Rico: mango, coffee, cacao, bamboo, sugarcane, cassava, tropical yam, banana, avocado

• Hilo, Hawaii: papaya, lychee, passion fruit, guava, macadamia, pineapple, Barbados cherry

• Brownwood, Texas: hickory, chestnut, pecan

• Riverside, California: date, citrus

Other storage areas and the crops included are:

• The National Arboretum; Washington D.C.: woody ornamental species

• The National Small Grains Collection; Aberdeen, Idaho: barley, oat, wheat, triticale, rye, rice, *Aegilops* (wild wheat relatives) (Fig. 13.7)

• The Interregional Research Project (NRSP-6); Sturgeon Bay, Wisconsin: potato

• Urbana, Illinois: soybean

• College Station, Texas: cotton

NATIONAL SEED STORAGE LABORATORY. The United States Department of Agriculture's National Seed Storage Laboratory is located at Fort Collins, Colorado (Fig. 13.8). It serves as the base collection repository for long-term storage of seeds in contrast, for example, to the Regional Plant Introduction Stations and the National Small Grains Collection, which maintain active collections to which plant breeders may go for samples of seeds or strains of the species with which they are working. The primary function of the National Seed Storage Laboratory is to provide long-term backup storage for the National Plant Germplasm System. At Fort Collins, over 250,000 accessions are kept, some of which are not yet duplicated at other sites. Samples are distributed from the National Seed Storage Laboratory only when not available from other sources. Storage rooms maintained at - 18ºC provide conditions under which seeds of most species will remain viable for 20 or more years. Seeds are checked at regular intervals for viability, and when germination begins to decline, the seed lot

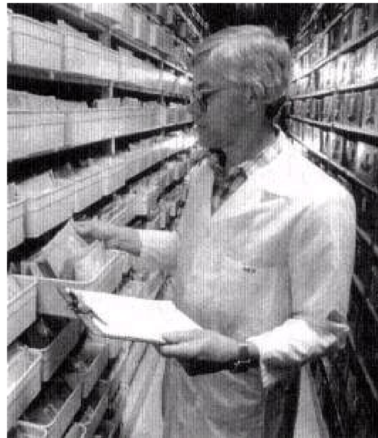


Fig. 13.7.
Harold Bockelman, curator of the United States Department of Agriculture, National Small Grains Collection, examines some of the 43,000 wheat accessions kept in cold storage in the laboratory at Aberdeen, Idaho.

Fig. 13.8.
The United States Department of Agriculture, National Seed Storage Laboratory, Fort Collins, Colorado. The principal missions of the National Seed Storage Laboratory are to preserve the base collection of the National Plant Germplasm System, and to conduct research to develop new and improved technologies for the preservation of seed and other plant propagules.

is regrown at the active collection site to obtain fresh seed.

PLANT QUARANTINE AND PLANT INTRODUCTION. One of the hazards of plant and seed introduction is that plant disease pathogens or insect pests will be introduced with plant or seed materials. To minimize this danger, plant quarantine officers are located at major sea- and airports to intercept and examine plant materials shipped into the United States or carried in by tourists. Many damaging diseases and insect pests have been unwittingly introduced in this way. The European corn borer and the cereal leaf beetle are typical examples. It is believed that the latter was introduced with shipments of grain into ports on the Great Lakes. There are many disease and insect pests that are not present in the United States that could pose a serious threat to United States agriculture if inadvertently introduced. Soybean rust, a disease common in Southeast Asia, is an example. Many countries have similar or even more rigid plant quarantine regulations, requiring that all plant and seed introductions be grown in isolation for one generation before release to the recipient. With present mobility in travel of individuals and increased international shipments of grain, the dissemination of pathogens and insect pests is difficult to control.

Study Questions

1. What is plant germplasm?

2. How important is it that plant germplasm be collected and conserved?

3. What are the components of the National Plant Germplasm System?

4. What is the role of the plant breeder in germplasm conservation?

Further Reading

Day, P.R. (ed.). 1991. Managing global genetic resources: The U.S. National Plant Germplasm System. National Academy Press, Washington, D.C.

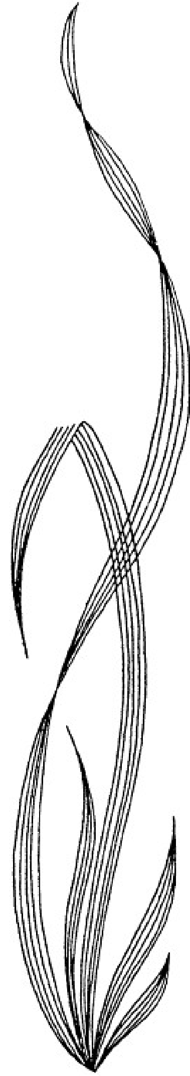
Hancock, J.F. 1992. Plant evolution and the origin of crop species. Prentice Hall, Englewood Cliffs, NJ.

United States Department of Agriculture, Agriculture Research Service. 1990. Seeds for our future: The U.S. National Plant Germplasm System. Program aid 1470. USDA, ARS, Beltsville, MD.

White, G.A., H.L. Shands, and G.R. Lovell. 1989. History and operation of the National Plant Germplasm System. p. 5-56. *In* R.L. Clark, W.W. Roath, and H.L. Shands (eds.) The National Plant Germplasm System of the United States. Plant Breeding Reviews, Vol. 7, Timber Press, Portland, OR.

Wilkes, G. 1993. Germplasm collections: Their use, potential, social responsibility, and genetic vulnerability. p. 445-50. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Williams, J.T., and J.I. Cohen. 1993. Conservation and use of plant genetic resources. p. 423-27. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.



VI
APPLICATIONS:
BREEDING FIELD CROPS THAT ARE SELF-POLLINATED

14. Breeding Wheat

Wheat (*Triticum aestivum* L. em. Thell. and *T. turgidum* L.) is the world's leading cereal grain and most important food crop. Its importance derives from the properties of wheat gluten, a cohesive network of tough endosperm proteins that stretch with the expansion of fermenting dough, yet coagulate and hold together when heated to produce a "risen" loaf of bread. Only wheat, and to a lesser extent rye and triticale, has this property. Wheat is utilized for making bread, flour confectionery products (cakes, cookies, crackers, pretzels), unleavened bread, semolina, bulgar, and breakfast cereals. Its diversity of uses, nutritive content, and storage qualities have made wheat a staple food for more than one-third of the world's population. Wheat has been cultivated in southwestern Asia, its geographic center of origin, for more than 10,000 years. Related wild species still grow in Lebanon, Syria, northern Israel, Iraq, and eastern Turkey. Man began breeding wheat in the early 1800s. Since then there have been improvements in yield and grain quality; modifications in the plant's architecture; and increased resistance to drought, lodging, insect pests, and disease pathogens (Fig. 14.1). The chromosomes of wheat have been combined with the chromosomes of rye to produce a new species, triticale (X *tritosecale* Wittmack). In this chapter we are concerned with breeding methods by which genetic improvements are made in wheat and the nature of the changes.

Origin and Genetics

The genetic origin of wheat is a classic example of how closely related species combine in nature to form a polyploid series (Fig. 14.2). The species of *Triticum are* grouped into three ploidy classes: diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$), and hexaploid ($2n = 6x = 42$). Currently, 11 diploid, 11 (or 12) tetraploid, and 6 hexaploid species of *Triticum are* recognized. The chromosome number and genome formula for selected species of *Triticum*, rye, and triticale are listed in Table 14.1. Only two species of *Triticum are* commercially important: the hexaploid species, *T. aestivum*, the bread wheat and the principal wheat in commerce; and the tetraploid species, *T. turgidum*, the durum wheat that is used for making pasta. The wild tetraploid species, *T. timopheevii*, is the source of the cytoplasmic male sterility utilized in breeding hybrid wheat. Triticale, X *tritosecale*, is a man-made species in which the **AABB** genomes of *T. turgidum* are combined with the **RR** genomes of rye. *T*.

Fig. 14.1.
High-yielding wheat cultivar (HYWV) developed at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. The HYWVs have dwarfing genes, high tillering capability, and a large grain yield-per-spike.

turgidum (**AABB**) evolved as an alloploid combining genomes from the diploid species, *T. monococcum* (**AA**), and an unknown and possibly extinct diploid species containing the **BB** genomes. *T. timopheevii* differs from *T. turgidum* in having the **AA** genomes combined with the **GG** genomes instead of the **BB** genomes. *T. aestivum* (**AABBDD**), is an alloploid combining **AABB** genomes from *T. turgidum* and **DD** genomes from the diploid species, *T. tauschii*. The intrinsic baking qualities that set *T. aestivum* apart from other species of *Triticum* are controlled by genes introduced through the **D** genome.

In the process of becoming an allohexaploid species, chromosomes of the A genome became modified and now are homologous with only six of the seven chromosomes in the ancestral diploid species, *T. monococcum*. The **B** genome, which does not have a known ancestral species, is partially homologous to several closely related diploid species in the genus *Aegilops*. The **D** genome is fully homologous with *T. tauschii*, its presumed progenitor.

The 21 chromosomes (gametic number) of hexaploid wheat have been divided into seven groups, called *homoeologous groups* (Table 14.2). Each homoeologous group contains three partially homologous chromosomes, one chromosome from each of the **A, B**, and **D** genomes. Chromosomes are identified by the homoeologous group number (numbers 1 to 7), and the genome (**A, B**, or **D**) from which the chromosome originated. The three chromosomes within the **ABD** homoeologous group frequently contain loci in common for a particular character. For example, two genes for leaf rust resistance are located on chromosome 2**A**, three genes

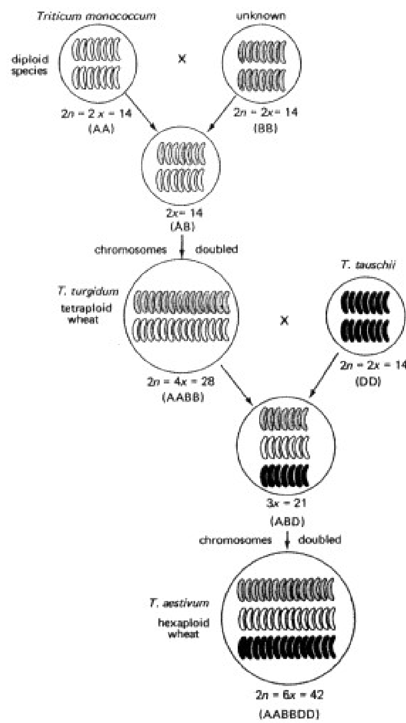


Fig. 14.2.
Origin of tetraploid and hexaploid wheat. The tetraploid species T. turgidum is an alloploid that arose from a combination of the A genome from T. rnonococcum and the B genome from an unknown wild species. The hexaploid species T. aestivum is an alloploid that arose from a combination of the AB genomes from *T. turgidum* and the D genome from *T. tauschii*.

on chromosome 2**B**, and three genes on chromosome 2**D**. The repeated loci suggest that the genomes (**A,B,D**) possibly originated from a common ancestor. Each chromosome exhibits a distinct banding pattern following a "Giemsa" staining technique. The banding pattern makes it possible to identify specific chromosomes from cytological examination.

Polyploid wheats with 28 chromosomes are referred to as tetraploids, and polyploid wheats with 42 chromosomes are referred to as hexaploids. In nature, the tetraploid and hexaploid wheats reproduce as diploids ($2n$ = 28 or $2n$ = 42), having acquired the property of diploid pairing from an allele, *Phl*, in chromosome 5**B**. In the presence of the *Ph1* allele, each chromosome will pair only with its homolog from the same genome. In its absence, a chromosome may pair with a homoeologous chromosome from another genome.

Chromosome Engineering in Wheat

Inheritance analysis and genetic mapping in hexaploid wheat by conventional procedures have proven to be difficult due to the polyploid origin and the repetitive loci for characters in different genomes. The task was made easier with the development of chromosome engineering techniques for the genetic analysis of wheat by E.R. Sears at the University of Missouri. Sears developed a complete set of the possible 21 *monosomics*, aneuploid plants with one chromosome less than normal (see Chapter 5), and sets of related aneuploid forms, in the hexaploid wheat cultivar, 'Chinese Spring.' By manipulation of the aneuploids, loci for a particular character may be identified with a specific chromosome, or with one arm of a specific chromosome, and gene-to-centromere distances may be calculated.

The wheat aneuploids are utilized additionally for substitution of a specific chromosome from a related wild species for a corresponding wheat chromosome in a cultivated species. Many wild species of *Triticum* evolved with genes for resistance to stress from cold, heat, drought, salt, disease, or insects that would be useful to supplement resistance genes in

Table 14.1.
Genome formula for species of *Triticum*, rye, and triticale

Species	Chromosome number	Genome formula	Common name	Domestication
	Diploid species ($2n = 2x = 14$)			
T. monococcum L.	14	**AA**	einkorn	cultivated
Unknown	14	**BB**		
T. tauschii (Coss.) Schmal.	14	**DD**		wild
Secale cereale L.	14	**RR**	rye	cultivated
	Tetraploid species ($2n = 4x = 28$)			
T. turgidum L.	28	**AABB**	emmer, durum	cultivated
T. timopheevii (Zhuk.) Zhuk.	28	**AAGG**		wild
	Hexaploid species ($2n = 6x = 42$)			
T. aestivum L. em. Thell.	42	**AABBDD**	bread wheat	cultivated
X Triticosecale Wittmack	42	**AABBRR**	triticale	cultivated

cultivated wheat. Although some wild species in the genus *Triticum*, and closely related genera, cross readily with cultivated wheat, other wild species are difficult to cross with cultivated wheat. If a desired gene for resistance to a particular stress condition is identified in a related wild species, it may be transferred into cultivated wheat by substitution of the alien chromosome bearing the gene for the homoeologous wheat chromosome. The strain possessing the substituted chromosome is known as an *alien substitution line* and the desired gene as an *alien gene*. To avoid the transfer of unwanted genes along with the useful gene, small segments of the alien chromosome may be transferred, although this process requires elegant cytological techniques. As a result of the application of the aneuploid techniques, the genetic analysis of wheat is now more complete than for any other polyploid crop species. One limitation of the

Table 14.2.
Nomenclature of the 21 chromosomes of hexaploid wheat by homoeologous group and genome

Homoeologous group	Chromosome number and genome		
	A	*B*	*D*
1	1*A*	1*B*	1*D*
2	2*A*	2*B*	2*D*
3	3*A*	3*B*	3*D*
4	4*A*	4*B*	4*D*
5	5*A*	5*B*	5*D*
6	6*A*	6*B*	6*D*
7	7*A*	7*B*	7*D*

aneuploid technique is that the gene must contribute an easily identified phenotypic effect. This restricts its use in the transfer of genes for quantitative characters where individual gene effects cannot be identified.

Genes and Gene Symbols in Wheat

A catalog of gene symbols and genetic linkages in wheat, with recommended rules for gene symbolization, has been published and is available additionally on computer disks. Updates to the catalog are published in the publications *Proceedings of the International Wheat Genetics Symposia, Annual Wheat Newsletter, Cereal Research Communications*, and *Wheat Information Service*. A database is being developed to list pedigrees of wheat cultivars registered with the Crop Science Society of America and to identify alleles associated with each cultivar. Guidelines have been developed for nomenclature of biochemical and molecular markers, including loci that display DNA restriction fragment length polymorphisms (RFLPs).

Genetic Resources

The importance of conserving crop genetic resources was discussed in the preceding chapter. The total number of wheat accessions in national and local gene banks around the world has been estimated to be in excess of 400,000, although many accessions are probably duplicated in the different collections. A major collection was assembled in the N.I. Vavilov All-Union Institute of Plant Industry, St. Petersburg, Russia, although the present status of this collection is not known. Major germplasm collections are held at the United States Department of Agriculture, National Seed Storage Laboratory, Fort Collins, Colorado, and the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. A working collection of 43,000 accessions is stored in Aberdeen, Idaho (Fig. 13.7), as part of the United States National Small Grains Collection. Extensive efforts are underway to inventory the accessions in the different wheat germplasm collections around the world so as to assist the breeder in locating genes for specific breeding programs. The Food and Agricultural Organization of the United Nations (FAO) has developed a set of "descriptors" for wheat to facilitate uniformity in describing wheat accessions in the different gene banks.

Biotechnology and Wheat Improvement

Biotechnology is proposed as an important complement to conventional plant breeding procedures in different crop species. The implementation of molecular biology techniques progresses more slowly in wheat than in some monocotyledonous species such as rice, or in dicotyledonous species such as soybean. A particular problem with wheat was the difficulty of regenerating plants from protoplasts, a procedure first accomplished only recently. Restriction fragment length polymorphism (RFLP) mapping of the wheat genome is hindered by the large number of chromosomes and by the duplication of DNA sequences in polyploid wheat species. As procedures for production of transformed wheat plants are clarified, significant genetic improvements could be achieved through introduction of genes for disease resistance or other characters from wild wheat species. A strong conventional wheat breeding program will need to be maintained to provide recurrent parent material if the genetically engineered genotypes are to be productive.

Genetic Diversity in Wheat

The cultivated bread and durum wheats are exceptionally diverse in (1) the physiological characteristics of the wheat plant that adapts different wheat cultivars for production in a wide range of climatic environments, and (2) the chemical and physical characteristics of the wheat gluten that contributes to the wide use of wheat grain for many different food products.

The physiological characteristics of the wheat plant that adapts different wheat cultivars to different climates are generally related to

• vernalization requirement,

• winter hardiness or cold tolerance, and

• photoperiod response.

The vernalization requirement determines the wheat growth habit, whether spring or winter type, and is a major plant characteristic in determining the kind of wheat that is grown in particular regions of the world. Wheats with winter growth habit may be distinguished from wheats with spring growth habit because a period of vernalization (exposure in the seedling stage to near-freezing temperatures) is required before flowering will occur. Another characteristic of winter-type wheats that distinguish them from spring-type wheats is their ability to harden and withstand freezing temperatures. The vernalization requirement and the freezing stress that can be survived varies with the genotype of the winter wheat plant. Differences between winter and spring types are not always distinct as some facultative winter-type cultivars have a low vernalization requirement and low tolerance to freezing stress and differ only slightly from spring types.

Wheat is grown predominantly in the northern hemisphere, roughly between 25 and 60º North latitude. Winter-type wheats are grown where winter temperatures are sufficiently low to meet vernalization requirements, yet not so low that the wheat will not survive. Spring-type wheats are planted in the spring and summer seasons north of the winter wheat areas where winter temperatures are too cold for the winter wheats to survive. The spring-type cultivars grown in these northern areas are photoperiod sensitive and flower during declining day-lengths. In subtropical climates south of the winter wheat area, where winter temperatures are too warm to meet vernalization requirements for winter wheats, spring-type wheats are grown. There, the spring type wheats are grown during the winter months, when the temperatures are coolest and most favorable for wheat, but not so low that the spring-type wheats will be killed by frost. Similar patterns of planting prevail in the southern hemisphere, except that the seasons are reversed as one goes from north to south. The spring-type cultivars grown in subtropical climates are normally insensitive to photoperiod and may be grown in any season of the year.

The diversity of end products for which wheat is utilized requires the production of cultivars with a wide range of characteristics affecting grain quality. Genetic differences in grain quality vary with the market class of wheat, whether hard, soft, or durum, and with the cultivar within the market class. Before initiating a breeding program, the wheat breeder needs to be familiar with the stress conditions that limit wheat production in the climatic area where the wheat cultivars will be grown and strive to overcome the stress limitations through the breeding program. It is also necessary to identify quality characteristics desired by the wheat market that is being served and design the breeding program to produce cultivars that will meet the highest quality standards for that market.

Market Classes of Wheat Grown in the United States

Wheat in the United States is divided into commercial classes that are grown in the following general areas:

Hard red winter, in the central and southern Great Plains.

Hard red spring, in the northern Great Plains.

Soft red winter, in the eastern and southeastern states.

Soft white, in New York, Michigan, and the Pacific Coast states.

Durum and Red Durum, in North Dakota.

Except for the durum wheats, cultivars of the different classes belong in the hexaploid species, *Triticum aestivum* (**AABBDD** genomes). The durum wheats are tetraploids (**AABB**) in the species *T. turgidum*.

Flowering and Pollination

The wheat inflorescence is a spike bearing spikelets at the nodes. Two to five florets are borne in each spikelet, subtended by a pair of glumes (Fig. 14.3A). Each floret contains three anthers, a feathery stigma, and an ovary in which, upon fertilization, the seed develops (Fig. 14.3B, 14.4). Wheat is a self-pollinating crop with pollen shed directly from the anthers to the stigma. Flowering starts several days after the wheat spike emerges from the boot, the florets on the main culm flowering first and those on the tillers flowering later. Flowering begins in early morning and continues throughout the day, with two to three days required for a spike to finish blooming. Normally, the glumes are open during the flowering process; the anthers protrude from the glumes; and part of the pollen is shed outside of the flowers (Fig. 14.3C). While the flower is open, foreign pollen may enter, normally resulting in about one or two percent cross-pollination. If conditions are unfavorable for the opening of the glumes, the anthers may shed the pollen without being extruded.

Wheat flowers are emasculated in preparation for crossing by clipping back the glumes, removing the anthers with fine-pointed tweezers before pollen is shed, and covering the wheat spike with a plastic bag to protect the flower from foreign pollen (Fig. 14.5). Pollinations

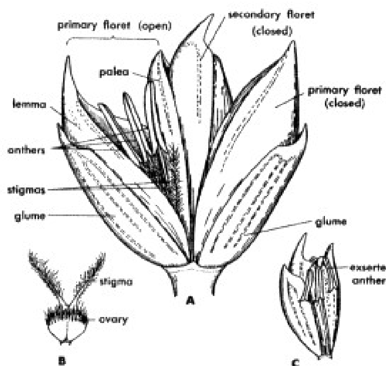


Fig. 14.3.
Spikelet of wheat. (A) The primary floret on the left is open, showing the three anthers and a portion of the feathery stigma. The primary floret on the right and the secondary floret are closed. (B) Pistil of wheat flower showing feathery stigma and ovary. (C) Floret showing anthers exserted after blooming.

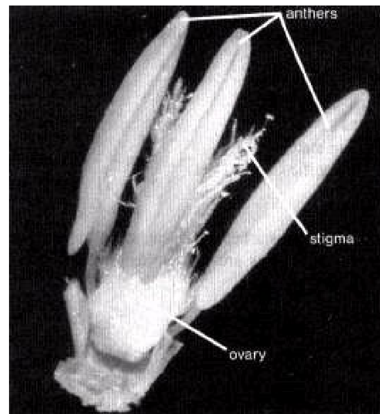


Fig. 14.4.
Organs of wheat floret; three anthers, feathery stigma, and ovary.

are made one to two days later by crushing a ripe anther over the stigma, or by twirling a fresh spike shedding pollen over an emasculated spike with open florets. In an approach method of pollination, spikes of the pollinator parent are bagged with the emasculated spike. The bag is shaken occasionally to assist in dissemination of the pollen. Pollen from wheat remains viable for a very short period, usually not more than one to three minutes. Fresh pollen is essential for obtaining good seed set in crossing.

Breeding Methods

Wheat breeding was put on a scientific basis following the rediscovery of Mendel's laws of inheritance in 1900. Selection and hybridization had been practiced much earlier by farmer/breeders, but scientific information to guide them on how to select and maintain breeding materials was not yet available. The principal breeding procedures include (1) *introduction and collection of germplasm*, (2) *pure line selection*, (3) *hybridization*, and (4) *hybrid cultivar development*. Backcrossing is frequently utilized to add desirable genes to established genotypes. Multiline breeding, first proposed as a means to increase diversity within the cultivar when breeding for rust resistance, has been utilized infrequently. Specific procedures for developing new cultivars by these methods were described in Chapters 9 and 11.

Hybridization is the principal breeding procedure for the development of new cultivars in wheat. The role of selection in wheat breeding has changed as compared to its role in earlier years. When wheat breeding was in its infancy, many cultivars were developed by identifying and increasing superior genotypes selected from mixed cultivars or landraces. With present distribution of improved, high-yielding, pure line cultivars in all of the world's wheat-growing areas, selection from established cultivars would rarely isolate a new genotype. The principal role of selection today is to isolate superior genotypes from segregating populations created by hybridization. The role of hybridization is to cross diverse genotypes and create hybrid populations from which new combinations of genes may be selected. The selected lines are evaluated by growing in field trials across the area of intended use. It is necessary to grow yield trials in several environments to evaluate cultivar × environment interactions. Wheat cultivars developed by hybridization often have complex pedigrees due to the practice of crossing superior selections from previous crosses. The repetitive sequence of crossing, selection, and then intercrossing superior selections as parents in a new generation of crosses constitutes a form of *recurrent selection* in which a selection cycle may take 5 to 8 years.

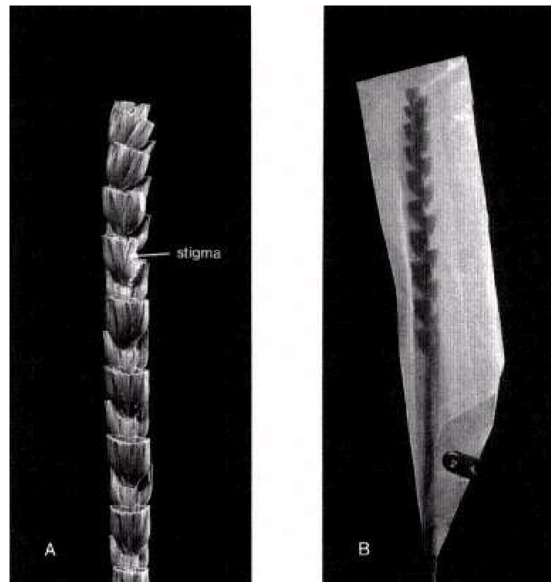


Fig. 14.5.
(A) Wheat spike with florets clipped back to facilitate emasculation and crossing. Glume and lemma have been removed from a floret on the upper right to show development of the stigma at this stage. Anthers have been removed already. (B) Wheat spike covered after emasculation to exclude foreign pollen.

Hybrid Wheat

Development of hybrid wheat first became a possibility after cytoplasmic male sterility and fertility-restoring genes were identified in wheat. The male sterility resulted from the interaction of nuclear genes from *T. aestivum* with cytoplasm from *T. timopheevii*. Fertility is restored by genes from *T. timopheevii* and other sources. Utilization of the system as proposed for the production of hybrid wheat involves development and maintenance of cytoplasmic-male sterile **A-lines** and their maintainer **B-line**s, development and maintenance of fertility-restoring **R-lines**, and crossing **A-lines** × **R-lines** for the commercial production of hybrid seed, as described in Chapter 11 and illustrated here in Figure 14.6. A two-gene model for restoring fertility, the cytoplasm and restorer genes present in the parent lines and the hybrid are listed in Table 14.3. Unfortunately, fertility restoration in wheat is not this simple. It seems that a third major gene and numerous minor genes are required to convey complete restoration in stress environments. The effects of the genes are cumulative, giving various degrees of

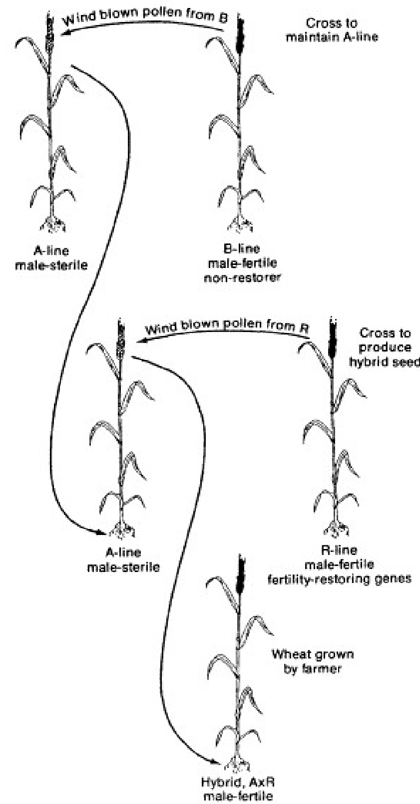


Fig. 14.6.
Procedure for producing hybrid wheat utilizing cytoplasmic male-sterility and fertility-restorer genes. The male-sterile A-line is maintained by pollination from the B-line, which is genetically identical but is in normal cytoplasm. The hybrid seed is produced by pollinating the A-line from the R-line. The R-line has dominant fertility-restorer genes and combines with the A-line to produce a high-yielding hybrid.

restoration depending upon the combination of modifier genes and their interaction with the environment.

An alternative procedure proposed for production of hybrid seed in wheat would utilize chemical hybridizing agents that suppress pollen production in the female parent. The chemical sterilants are sprayed on the wheat plants at a critical stage of floral initiation to suppress anther and pollen development. Hybrid seed production would be simplified compared to the use of cytoplasmic male sterility because only two parent lines are needed, one line that can be sterilized without harmful side effects to serve as the seed production line, and one line to serve as the pollinator. But the use of chemical hybridizing agents for production of hybrid wheat also has unique problems. The application of the chemical hybridizing agent must be timed so that the chemical reaches the immature floret at the proper stage for inhibiting pollen production; the chemical should remain effective for a long enough period of time to inhibit pollen production in late-developing tillers; the effect of the chemical should be unaffected by environmental stress conditions; and the chemical should not produce adverse side effects.

In wheat, a self-pollinated crop, hybrid wheat has not become a viable breeding procedure for two reasons:

• heterosis has not contributed the same yield increases that are obtained in cross-pollinated crops such as in corn or sorghum, and

• low seed set from cross-pollination in seed production fields increases the cost of producing hybrid seed for the farmer.

These problems are common to both the cytoplasmic-male sterile and the chemical sterilant systems of producing hybrid wheat. Another difficulty with the cytoplasmic-male sterility/ fertility-restorer gene system is the longer time required to produce a hybrid cultivar compared

Table 14.3. Origin of cytoplasm, fertility-restoring genes, and pollen fertility of lines utilized in breeding hybrid wheat

Wheat material	Cytoplasm from	Fertility-restoring genes	Pollen fertility
A-line	*Triticum timopheevii* (sterile)	$rf_1rf_1rf_2rf_2$	Male-sterile
B-line	*Triticum aestivum* (fertile)	$rf_1rf_1rf_2rf_2$	Male-fertile
R-line	*Triticum aestivum* (fertile)	$Rf_1Rf_1Rf_2Rf_2$	Male-fertile
	or		
R-line	*Triticum timopheevii* (sterile)	$Rf_1Rf_1Rf_2Rf_2$	Male-fertile
Hybrid	*Triticum timopheevii* (sterile)	$\mathrm{Rf_1Rf_1Rf_2Rf_2}$	Male-fertile

to the conventional cultivar produced by hybridization. This is due to the additional steps of converting potential **A-lines** to cytoplasmic-male sterility and adding fertility restorer genes to potential **R-lines**. Release of higher yielding conventional cultivars during this period reduces the heterosis advantage originally projected for the hybrid.

Breeding Objectives

The goal of the wheat breeder is to create new genotypes improved in features that contribute to greater *yield potential*, increased *yield stability*, and improved *product quality*. Yield potential is important because it affects the amount of the product harvested. Yield stability is important to obtain a uniformly high yield over a wide range of environments and assure broad adaptation of the cultivar. Yield stability is increased with optimum maturity and resistance to lodging, drought, adverse soil factors, disease pathogens, and insect pests. Product quality is important to assure the highest market value for the product harvested.

The breeder needs to examine the wheat cultivars currently in production in the area where he is working, discover their strengths and weaknesses, decide which genetic improvements will contribute the greatest gain to cultivar performance, establish priorities for reaching the breeding goals, and search out sources of genes to accomplish the breeding objectives.

Yield Potential

Yield potential is a complex quantitative character. In wheat, yield potential refers to the ability of the plant to manufacture, translocate, and store food materials in the wheat grain. Each is a complex physiologic process that is affected by the wheat genotype, the environment, and a genotype × environment interaction. Measuring the separate expression of each physiologic process is impractical, so they are measured in total by grain yield. Improvement in yield potential has been obtained traditionally by crossing high-yielding genotypes and selecting in the segregating generations for transgressive segregates with increased yield potential. The breeding procedures to accomplish this goal were described in Chapter 9, Breeding Self-Pollinated Crops.

In many areas of the world, emphasis is now being given to the breeding of what has become known as "high-yielding" wheat cultivars. "High-yielding" here refers to high-yield

potential. Although many new wheat cultivars in the past have been designated as high-yielding when compared to the cultivars they replaced, most cultivars currently described as "high-yielding" have resulted from the exploitation of *short-statured cultivars with dwarfing genes* that are generally referred to as *semidwarfs*. The semidwarf wheats have a long history that is rooted in the development of early, short-strawed cultivars in Japan and Korea during the early years of this century. These wheats were introduced into Italy in 1911, into the United States in 1946, and into the international breeding program of CIMMYT in Mexico in 1953. Semidwarf descendants from crosses with these wheats are reduced in height due to the presence of the dwarfing genes, and high yielding due to a high capability for tillering and increased grain yield per spike (Fig. 14.1). In many wheat breeding programs, selection for shorter stature was accompanied by selection for *photoperiod insensitivity* which enabled the cultivars to be grown in different latitudes and seasons. The utilization of wheats with dwarfing genes spread rapidly, and dwarfs are now grown in wheat production areas around the world.

Yield Stability

Yield stability refers to the ability of the plant genotype to express yield potential over a wide assortment of environments. It is evaluated by growing cultivars and breeding lines in representative climates over several years and in an assortment of locations in order to sample different environments, during which genotypes are selected with low cultivar × environment interactions. Yield stability also implies that there will be a minimal loss from vagaries of climate, stress, or destructive pests. It is the breeder's responsibility to identify the climatic or pest conditions that can cause significant reductions in yield in his region and to develop breeding strategies that will prevent yield losses from those causes.

Maturity

Early maturing cultivars of wheat permit the crop to be harvested before loss from heat, drought, or disease, and facilitates rapid crop succession in multiple cropping systems. Loss from stem rust on wheat is frequently averted in the Western Plains of the United States by cultivars that ripen before the rust epiphytotic develops. There are disadvantages to early maturity. Cultivars that mature extremely early tend to be lower in yield because the wheat plant has a shorter growth period in which to tiller, bloom, and store nutrients in the wheat kernel. Extreme earliness in flowering in winter wheats occasionally results in injury from late spring frosts.

Lodging Resistance

Lodging refers to the bending or breaking-over of the wheat culm. Grain losses occur in lodged wheat from reduction in photosynthate production due to shading, to interruption in translocation of photosynthate, or from disease due to the lodged grain providing a favorable environment for disease development. Lodging in wheat can be caused by damage from wind and rain (Fig. 12.1A), from disease (Fig. 12.1B), or from insect pests that damage the roots, crown, or stem (Fig. 12.1C). Breeding for resistance to lodging involves development of cultivars with a vigorous and healthy root system, short and sturdy straw, resilient straw that does not break in the wind, and resistance to disease pathogens or insects that attack and weaken the straw or the root system.

Shorter straw has been obtained by introduction of dwarfing genes that reduce plant

stature. Genes that reduce height in wheat are given the symbol *Rht*. About 20 *Rht* genes have been identified from different sources, all apparently tracing back to short-strawed introductions from Japan. Genes Rht_1, Rht_2, from Japanese cultivars and Rht_8 from Yugoslav cultivars have been widely used in breeding programs for short-statured wheats. Use of the Rht_8 gene is increasing due to less decline in kernel density and weight. In addition to reducing height, the *Rht* genes increase grain yield due to increased tillering and number of seeds per plant. As yield is increased, seed size and protein content of the seed is normally reduced. Plants with a combination of two dwarfing genes are referred to as double-dwarfs. Double-dwarfs are shorter and increased in tillering as compared to single-dwarfs which have only one *Rht* gene. Through monosomic analysis, the Rht_1 gene was found to be located on chromosome 4**A** and the Rht_2 gene on chromosome 4**D**, thus facilitating their transfer by chromosome substitution procedures.

Winter Hardiness

Winter injury in wheat results *from freezing* to death of plant tissues, or from *heaving* of plants from the soil. Winter injury from freezing is caused by ice crystal formation in intercellular spaces of plant tissues that interrupts normal physiologic processes. Freezing to death usually occurs in association with insufficient soil moisture and results in desiccation of the plant tissues. Cold-hardy wheats are often drought resistant in addition. In the United States, the hard winter-type wheats that originated in the Crimean area of Ukraine and are generally referred to as Turkey Red wheats are best adapted to the dry Western Plains where winter injury results from desiccation of leaf tissues following exposure to exceptionally low windchill temperatures. Heaving is caused by uplifting of wheat plants due to alternate freezing and thawing of heavy, moisture-laden soils, a soil condition commonly found in the higher rainfall areas of eastern United States. The wheat plant is uprooted, the roots being sheared off as the plant is torn from the soil. The uprooted plant is left lying on the soil surface and dies from desiccation. In the United States, the soft red, winter-type wheats possess more extensible root systems that stretch with the heaving stress than the hard red wheats of Turkey Red origin. These characteristics adapt the soft red winter wheat cultivars to the eastern United States where wet soils in winter are subjected to frequent freezing and thawing. Winter hardiness is evaluated by growing the cultivars and breeding lines in comparison with adapted cultivars in climatic areas where winter injury is common. Artificial freezing tests have limited utility because they measure resistance to one form of stress only. Resistance to winter injury is a complex character with quantitative inheritance.

Drought Resistance

Wheat is the major cereal grown in the drier regions of the temperate zone. Plant mechanisms that contribute to drought resistance in wheat are early maturity to ripen the crop ahead of periods of drought stress, vigorous and deep root systems to utilize available soil moisture efficiently, ability to close stomata during periods of drought stress to decrease water loss, and a waxy bloom on the leaf surface to reduce transpiration loss. Wheat cultivars of Crimean origin adapted in drought stress areas tend to have narrower leaves and lower shoot/root ratios than soft wheat cultivars adapted to areas of higher rainfall. In subtropical regions, wheat is grown in the winter season to avoid periods of prolonged summer heat and drought. Durum wheats are noted for their drought resistance and are the principal wheats grown in the drier regions of North Africa and the Middle East. Drought resistance is a

complex quantitative character and is not subject to measurement by a single laboratory procedure.

Aluminum Tolerance

Wheat cultivars adapted in regions of the world with acid soils and high soluble aluminum have higher levels of aluminum tolerance than cultivars adapted in regions with nonacid soils. Aluminum toxicity restricts root and top growth and reduces yield. Wheat genotypes may be screened in the laboratory for aluminum tolerance by growing wheat seedlings in nutrient solutions with a high concentration of aluminum or in aluminum-toxic soils in the field and selecting for plants with largest root and top growth (Fig. 12.4). Inheritance is controlled by both major and minor genes.

Disease Resistance

The development of cultivars of wheat with resistance to destructive disease pathogens that reduce grain yield and quality constitute major wheat breeding objectives. It is essential that the breeder identify the wheat diseases that cause major damage and yield losses in the production area and establish goals for reducing the losses by breeding for resistance. Problems associated with physiologic specialization in the pathogen and inoculation techniques to identify resistant genotypes were discussed in Chapter 12. Wheat diseases with a high degree of pathogen specialization include:

RUSTS. The rust diseases and the causal pathogen include stem rust, *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.; leaf rust, *P. recondita* Rob. ex Desm. f. sp. *tritici*; and stripe rust, *P. striformis* West. Stem rust is one of the most destructive plant diseases. The stem and leaf rust pathogens may spend part of their life cycle on alternate host plants from which wind borne spores are spread to wheat, or the pathogens may overwinter on wheat plants in subtropical areas with spores spread directly to other wheat plants. Numerous physiologic races have been identified in each rust species. Thus far, 37 genes have been identified for resistance to *Puccinia graminis*, 34 genes for resistance to *P. recondita*, and 16 genes for resistance to *P. striiformis*. A continuous succession of resistant cultivars have been developed by wheat breeders as new physiologic races of the rust pathogens emerged. Many of the genes for resistance were recovered from the wild relatives of wheat and breeders are continually searching for new genes.

SMUTS. The major smut diseases that attack wheat and the causal organisms are loose smut, *Ustilago tritici* (Pers.) Rostr.; common bunt *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn.; and dwarf bunt *Tilletia controversa* Kühn. The loose smut disease destroys the entire spike of infected plants. In common and dwarf bunt the kernel is replaced by a ball of smut spores. Physiological specialization is present in the smut pathogens. Four genes have been identified for resistance to *Ustilago tritici*, and 10 genes have been identified for resistance to *Tilletia* species.

POWDERY MILDEW. Colonization of wheat by the powdery mildew fungus, *Erysiphe graminis* DC. ex Merat, became more severe with increases in nitrogen fertilization of the wheat crop. The fungus spreads rapidly with cool temperatures and high humidity, producing a

heavy, white mycelial growth over the surface of the leaves. More than 30 physiologic races of the pathogen and 12 genes for resistance have been identified.

SEPTORIA DISEASES. Two species of *Septoria* cause leaf and head blights of wheat. The diseases, *Septoria tritici* blotch and *Septoria nodorum* blotch, infect wheat leaves early in the season and are spread to the upper leaves and glumes by splashing raindrops as the season advances. Breeding for resistance has been difficult as resistance to each disease is quantitatively inherited.

OTHER DISEASES. Other diseases of wheat include those causing root and crown rots, fungal foliage and head blights, bacterial blights, and virus diseases. As major diseases such as the rusts and smuts were brought under control by breeding resistant cultivars, diseases formerly considered to be of minor importance became widespread and gradually assumed roles of greater importance.

Insect Resistance

Wheat is host to many destructive insect pests, but relatively few cause major damage. Some widely destructive insects are the Hessian fly, greenbug, and cereal leaf beetle. The Hessian fly and the greenbug are highly specialized, with new biotypes arising through hybridization or mutation.

HESSIAN FLY, *MAYETIOLA DESTRUCTOR* SAY. Injury by the Hessian fly causes dwarfing of infested plants, reduction in tillering, increased winter injury in winter types, and breaking of the straw after ripening (Fig. 12.1C). The damage is caused by overwintering of the fly on the wheat plant and injury from larval feeding. Resistance in most wheat cultivars results from *antibiosis*, in which the insect dies after feeding on the plant. In other cultivars, resistance is due to *tolerance*, in which the insect continues to feed, but the plant compensates by growing more tillers. At least eight biotypes of the Hessian fly and 19 genes for resistance have been identified. Major race-specific genes from the cultivars 'W38,' 'PI94587,' and 'Ribeiro' and complex resistance derived from 'Marquillo' and 'Kawvale' are used in breeding resistant cultivars in the United States.

GREENBUG, *SCHIZAPHIS GRAMINUM* RONDANI. The greenbug overwinters on the crowns of wheat plants, the population multiplies rapidly as spring temperatures rise, and the adult greenbugs feed on the wheat leaves. The biotypes A, B, C, D, E, F, G, and I have thus far been described. Resistance to biotypes A to F was conditioned by combinations of five genes. Biotype G, identified in 1988, was virulent on wheat containing previously identified genes, but resistance to race G was later discovered in lines originating from a wheat x rye cross.

CEREAL LEAF BEETLE, *OULEMA MELANOPUS* L. The cereal leaf beetle causes widespread damage to small grains. Both the larvae and the adult feed on the plant, consuming large portions of the leaves with subsequent yield losses up to 25% (Fig. 14.7). The cereal leaf beetle exhibits nonpreference for feeding on wheat cultivars with pubescent leaves. To reduce the damage from cereal leaf beetle feeding, cultivars are being developed with pubescent leaves.

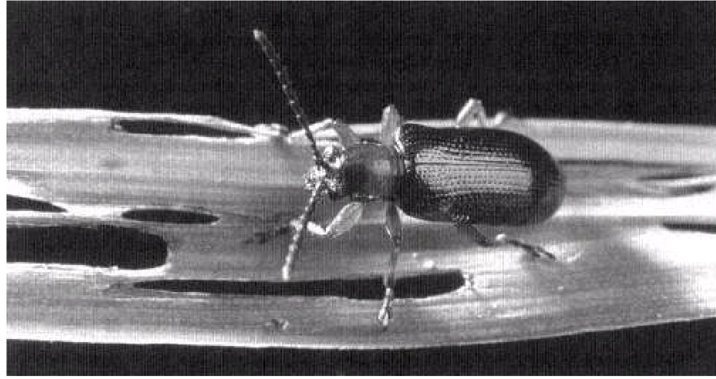


Fig. 14.7.
Cereal leaf beetle and damage from feeding on leaf of wheat. The cereal leaf beetle feeds sparingly on wheat leaves with dense pubescence, so cultivars are being developed with the dense pubescence character.

Quality

Quality in wheat refers to many properties of the wheat kernel that affect its utilization for specific products. The properties are inherent in the cultivar, yet may be strongly influenced by the environment in which the wheat is grown. From the standpoint of utilization, three basic market groups of wheat cultivars are grown, each with specific properties:

• hard (common or bread) wheat of the hexaploid species, *Triticum aestivum*,

• soft wheat of the hexaploid species, *Triticum aestivum*, and

• durum wheat of the tetraploid species, *Triticum turgidum*.

Each market group has distinctive grain properties, inherent in the wheat cultivar, that determines its suitability for producing a high quality baked product.

The hard (common or bread) wheats have "strong" gluten strands and hard kernel texture. Gluten is an indefinite, tough, nitrogenous compound that remains after wheat flour is washed to remove the starch. When made into a dough using yeast as the leavening agent, large amounts of water are absorbed by the gluten and CO_2 is released by chemical action of the leavening agent. As the gluten strands become solidified by baking, the CO_2 becomes trapped in the cellular structure of the gluten, resulting in a large well-piled loaf of bread. Because hard wheats in the United States are generally grown in drier climates than the soft wheats, they normally have a higher grain protein content which complements the greater strength of the gluten strands so that loaf volume becomes an important criterion for measuring quality in hard wheat flours.

Flours from different hard wheat cultivars differ inherently in capacity for flour yield,

water absorption, particle size index, dough mixing time, and other quality components. Laboratory tests have been devised to measure the individual components of hard wheat quality, but the final test is in the loaf of bread that can be baked from the different cultivars and breeding lines (Fig. 14.8). Because only a small quantity of seed of breeding lines is generally available, microtests have been devised that require smaller amounts of grain. For baking tests, these include smaller loaves, known as pup loaves, for comparison of different cultivars and breeding lines (Fig. 14.9).

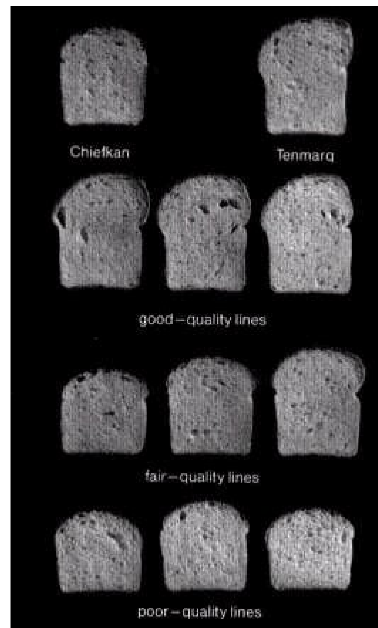


Fig. 14.8.
Loaves of bread baked from a poor quality hard red winter wheat cultivar (left), and a good quality cultivar, and from experimental lines selected from the cross. Large loaf volume indicates superior water absorption and gas retention of the wheat gluten, a property desired in flour used for baking bread. In order to compare baking quality of different cultivars and experimental lines, it is necessary that the cultivars be grown under similar environmental conditions.

The soft wheats mill into a fine, silky flour, with "weak" gluten strength, suited for making confectionery products, such as cakes, cookies, pastries, crackers, or flatbread. The soft wheats are generally adapted in higher rainfall areas than the hard wheats, resulting in lower protein, complementary to the weaker gluten. Flours from different soft wheat cultivars, as in the hard wheats, differ in milling and baking characteristics that are genetically controlled and may be improved by breeding.

Durum wheats have the greatest hardness and are milled to produce a coarse, granular product called semolina, used in making pasta, and durum flour, used for making noodles. Durum wheat cultivars differ in milling and baking characteristics that are genetically controlled and may be improved by breeding.

Within each market class, the breeder must give consideration to development of cultivars with physical and chemical characteristics of the grain that will produce a superior baked product and be acceptable to the trade. Milling characteristics to consider are test weight, kernel hardness, and flour yield. For bread baking, the cultivar should yield flour with high capacity for water absorption, medium-long mixing time, and high loaf volume (Fig. 14.8). Soft wheat quality varies with the intended use, whether for cakes or cookies baked with a chemical leavening agent; for crackers, pretzels, or flatbread made with a yeast leavening agent; or for noodles. Micromilling procedures have been developed for cultivars and breeding lines where only small quantities of seeds are available. In hard wheats the experimentally milled flour is tested for dough mixing and bread baking properties. In soft

Fig. 14.9.
Bread baking tests to evaluate wheat cultivars and breeding lines at the United States Grain Marketing Research Laboratory, Manhattan, Kansas. In the foreground, dough samples are being mixed in preparation for the baking test. In the background, pup loaves are being removed from the oven.

wheats, in which the gluten strands are weaker than in the hard wheats, a cookie test that measures spread of the dough rather than volume is utilized to evaluate baking quality (Fig. 14.10).

Market Quality refers to grain that is plump, heavy, sound, and disease-free. Selection for these characteristics must always be foremost in the breeding program, the breeder ruthlessly discarding strains with light, shriveled, or diseased seeds.

Study Questions

1. What are the different market classes of wheat? What are the uses of each?

2. What are the problems associated with development of hybrid wheat cultivars? What different means are available for developing hybrid wheat cultivars?

3. What is the function of the dominant *Ph* allele in wheat? What is homoeologous chromosome pairing?

Further Reading

Allan, R.E. 1980. Wheat. p. 699-748. *In* W.R. Fehr (ed.) Principles of cultivar development. Vol. 2. Macmillan Publishing Co., New York.

Cantrell, R.G. 1987. Breeding and genetics of durum wheat. p. 11-40. *In* J. Janick (ed.) Plant breeding reviews, Vol. 5. Van Nostrand Reinhold, New York.

Cox, T.S. 1991. The contribution of introduced germplasm to the development of U.S. wheat cultivars. p. 25-47. *In* H.L. Shands and L.E. Wiesner (eds.) Use of plant introductions in cultivar development. Part 1. Crop Sci. Soc. Am., Spec. Publ. No. 17. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Dalrymple, D.G. 1986. Development and spread of high-yielding wheat varieties in developing countries. Bur. Sci. Tech., U.S. Agency for Int. Dev., Washington, D.C.

Gale, M.D., S. Chao, and P.J. Sharp. 1990. RFLP mapping in wheat-progress and problems. p. 353-63. *In* J.P. Gustafson (ed.) Gene manipulation in plant improvement II. Plenum Press, New York.

Gale, M.D., and S. Youssefian. 1985. Dwarfing genes in wheat. p. 1-35. *In* G.E. Russell (ed.) Progress in plant breeding-1. Butterworths, London.

Heyne, E.G. (ed.). 1987. Wheat and wheat improvement. Agron Monograph No. 13. 2nd ed. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

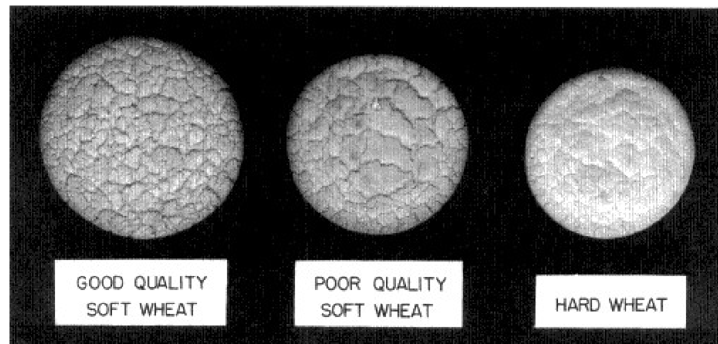


Fig. 14.10.
Microcookie baking test developed at the United States Soft Wheat Quality Laboratory, Wooster, Ohio, to evaluate quality of flour milled from different soft wheat cultivars and breeding lines. The diameter or spread of the cookie is an index to the quality of the flour for baking cakes or cookies. Only 40 g of flour is required, so the test can be used to evaluate experimental lines in early stages of the breeding program when only small amounts of seed are available.

Knott, D.R. 1989. The wheat rusts-breeding for resistance. Springer-Verlag, Berlin.

Lupton, F.G.H. (ed.). 1987. Wheat breeding, its scientific basis. Chapman and Hall, London.

Roelfs, A.P., R.P. Singh, and E.E. Saari. 1992. Rust diseases of wheat. Concepts and methods of disease management. International Maize and Wheat Improvement Center (CIMMYT), Mexico, D.F. Mexico.

Sears, R.G., and T.S. Cox. 1993. Improving milling and baking quality of wheat. p. 665-69. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Srivastava, J.P., and A.B. Damania (eds.). 1990. Wheat genetic resources: meeting diverse needs. John Wiley & Sons, Chichester, England.

15. Breeding Rice

Rice (*Oryza sativa* L.) is the world's second most important cereal crop, grain production of rice being exceeded only by that of wheat. Nearly all of the rice produced is consumed as human food, rice being the staple food for over one-third of the world's people. In contrast to wheat, which is a cool-season crop with production centered in temperate climates and extending into subtropical climates only as a winter crop, rice is a warm-season crop and is grown predominantly in tropical and subtropical climates. In regions with a monsoon climate, in which rainy and dry seasons alternate, rice production is concentrated in the rainy season and is grown in the dry season only if a supplemental water supply is available. In temperate climates or at high elevations, the rice growing season is limited by the frost-free period. More than 90% of the world's rice is produced and consumed in Asia.

Rice is grown in four types of culture (Fig. 15.1):

• *Irrigated* or *flooded* rice is grown in standing water impounded by bunds or levees. Water needed in excess of that supplied by natural rainfall is provided by irrigation. The depth of water in the rice paddy seldom exceeds 15 cm (Fig. 15.2).

• *Rainfed* or *lowland* rice is grown in standing water impounded by bunds or levees from natural rainfall. Supplemental irrigation is not provided. As the monsoon season progresses, depth of the water increases, sometimes reaching 50 to 100 cm.

• *Deep-water* or *floating* rice is grown in low-lying areas that may become submerged to depths of 1 to 5 m during the monsoon or rainy seasons. The stems of floating rice elongate as the water rises and the leaves float on the surface of the water (Fig. 15.3).

• *Upland* or *dryland* rice is grown with natural rainfall, mostly in hilly or newly cleared areas where the topography does not permit impounding water for irrigation (Fig. 15.4).

Irrigated rice is the principal method of growing rice, occupying slightly over one-half of the world's total rice area. The rice receives a continuous supply of water throughout the season from rainfall supplemented by irrigation. Rainfed lowland rice is a common method of growing rice in monsoon climates of Asia and Africa and occupies about one-fourth of the world's total rice area. The water level varies with the topography and the rainfall received. Deep-water and floating rice are grown in low-lying coastal areas or tidal wetlands that are subject to flooding during the monsoon rains and occupy about 11% of the total rice area. Dryland rice is strictly rainfed and is grown without benefit of supplemental irrigation. Rice breeding programs have

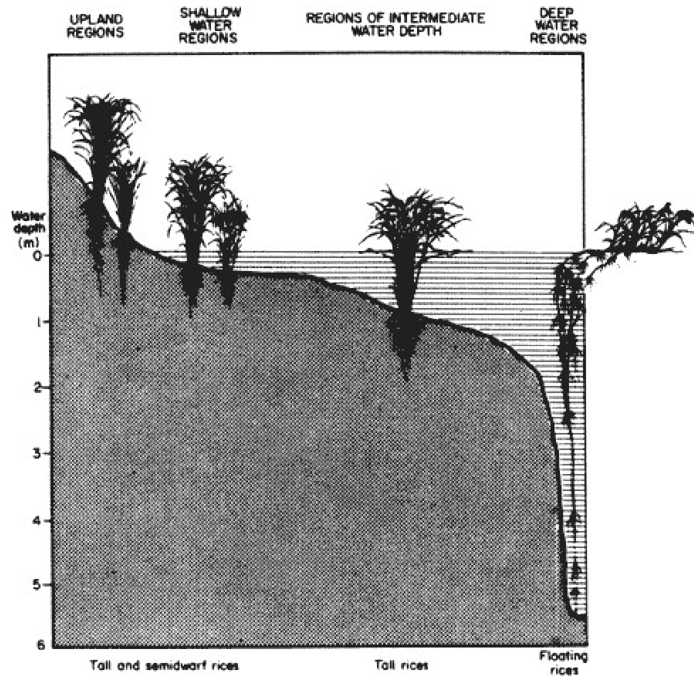


Fig. 15.1.
Comparison of types of rice grown with different depths of water. Tall and semidwarf cultivars are grown in upland and shallow water regions. Tall cultivars are grown in lowland areas where water reaches depths of 1 m. Floating rice, in which the stem elongates as the water increases in depth and the plant floats on the surface, may be grown in water up to 5 m in depth.

Fig. 15.2.
Test plots of irrigated or flooded rice cultivars on the Rice Branch Experiment Station, Stuttgart, Arkanas.

Fig. 15.3.
Lowland strains of rice growing in a breeding nursery in Thailand. The depth of the water is increased daily to test the ability of the rice to elongate.

Fig. 15.4.
Upland rice growing in a breeding nursery at Goiania, Brazil. Upland rice is grown with natural rainfall or supplemental irrigation similar to a crop of wheat.

given major attention to development of cultivars for the irrigated areas and for rainfed areas that have a favorable rainfall pattern.

Origin, Species, and Types of Rice

Oryza sativa L., the principal cultivated species of rice, is believed to have been domesticated nearly 10,000 years ago in an area that includes northeastern India, Bangladesh, Burma, Thailand, Laos, Vietnam, and southern China. The greatest diversity of the primitive cultivated forms and their wild relatives have been found in this broad area. Rice is one of the oldest cultivated crops, having been cultivated in India and China for up to 8,000 years. Rice spread from its area of primary diversity throughout southeast Asia and adjacent islands of the Pacific region. The only other cultivated species of rice, *O. glaberrima* Steud., is indigenous to the upper valley of the Niger river in West Africa. Although still cultivated in small areas of west tropical Africa, *O. glaberrima* is being replaced by improved cultivars of *O. sativa*. It has been suggested that *O. satira* and *O. glaberrima* descended from a common progenitor in the distant past, but evolved through separate pathways following the fracture and drift of the great land masses that led to formation of the Asian and African continents.

The genus *Oryza* contains 20 species with a basic chromosome number of 12. The genus includes both diploid and tetraploid species with six genome groups, **A, B, C, D, E**, and **F**. The cultivated species, *O. sativa* ($2n = 2x = 24$) has the **AA** genome formula. *O. glaberrima* ($2n = 2x = 24$) does not pair well with *O. sativa* and has been given the genome formula $\mathbf{A_gA_g}$. Six of the species of *Oryza* are annuals, the remainder perennial. Two diploid wild species that contain the **AA** genome, *O. nivara* and *O. rufipogon*, are widely distributed throughout southeast Asia and hybridize freely with each other and with cultivated rice.

The species, *O. sativa*, has evolved into three types, or ecographic races, generally characterized as follows:

• *Indica*: the tropical type, typically with tall plants, weak stems, long and droopy leaves, sensitive to low temperature and photoperiod, slender grains that shatter easily and remain dormant for long periods, and the source of dry-cooked rice.

• *Japonica* (or *Sinica*): the temperate type, typically with short leaves and stems, moderate tillering, resistant to low temperature, short rounded grains with low amylose content that makes the grain cohesive or sticky when cooked.

• *Javanica*: characteristically, tall with thick stems and broad stiff leaves, low tillering, long panicles, resistant to shattering, and large bold grains.

The inherent characteristics in the tropical and temperate types can be better understood by considering the environmental conditions under which each developed. The tropical (*indica*) type evolved under the monsoon climates of south and southeast Asia, where soils were infertile, fields were unevenly flooded, solar radiation was low due to heavy cloud formations during the rainy season, temperatures were high at the time of planting, day lengths declined as the season advanced, and weed competition was intense. Seedlings were started in seedbeds before the onset of the monsoon rains and transplanted into the field where they were spaced widely apart, or, in unfavorable areas, seeds were sown by broadcasting into dry soil. With wide spacing, tall plants with heavy tillering and long and droopy leaves made maximum use of the sunlight during early growth and provided greatest competition to the weeds. Sensitivity

to photoperiod delayed maturity until the end of the rainy season when the grain could be harvested under more favorable field conditions. Grain dormancy prevented loss from sprouting before harvest or in lodged plants.

By contrast, the temperate (*japonica* or *sinica*) type arose in the moderate climates of China where temperatures were lower at time of seeding, although total solar radiation was higher due to less cloud cover and longer days in summer, and rainfall was more evenly distributed. Intensive cultural practices provided better weed control, more uniform water depth, closer spacing of plants, and higher soil fertility. Under these climatic conditions and cultural practices, cold tolerance in the seedling stage produced better stands, narrow upright leaves provided better light penetration into the canopy, and short straw helped to reduce lodging. These characteristics were intensified by hybridization and selection under high soil fertility.

The intermediate (*javanica* type), also called *bula*, originated later in Indonesia and hilly areas of southeast Asia from tropical progenitors. Many of its characteristics are intermediate to the tropical and temperate plant types. Each group of rice varieties could be further differentiated into nonglutinous (nonwaxy), containing both amylopectin and amylose in the starch, or glutinous (waxy), containing only amylopectin in the starch.

Hybridization between the tropical and temperate plant types has been increasing in current rice-breeding programs, although partial sterility is often present. As introgression of the traditional *indica* and *japonica* types increased, the distinctiveness of the varietal types has been reduced. This occurred in southern United States, where many rice cultivars originated from crosses between tropical and temperate plant types and is currently occurring in California. In Taiwan, short-statured, photoperiod-insensitive cultivars of both native (*indica*) and introduced (*japonica*) types were developed from breeding stocks obtained from Japan. Emphasis was placed on breeding high-yielding cultivars with year-round cultivation in subtropical climates. The semidwarf *indica* types of Taiwan and mainland China have furnished the world with readily usable germplasm that led to the "Green Revolution" in rice production.

Types of Rice Grown in the United States

Rice production in the United States is concentrated in the states of Arkansas, California, Louisiana, Mississippi, and Texas, with smaller amounts in Missouri and Florida. Culture of rice has flourished in these areas due to favorable climate, soil, topography, and a supply of water for irrigation. Rice culture in the United States is highly mechanized, with precision land leveling to permit accurate control of depth of irrigation water, airplane seeding, chemical weed and pest control, and fertilization carefully timed for maximum grain production. Rice cultivars in the United States are basically of *japonica* and *indica* origin with some introgression of *javanica* germplasm.

Rice cultivars grown in the United States are grouped into three types according to the length and shape of the grain: *long-grain, medium-grain*, and *short-grain* (Figs. 15.5 and 15.6). More than 50% of the rice area in the United States is seeded to long-grain cultivars, about 40% to medium-grain cultivars, and the remainder to short-grain cultivars. The long-grain cultivars are characterized by long, slender grains with vitreous texture. The medium-grain cultivars have shorter and broader grains that are less vitreous than grains of the long-grain cultivars. The short-grain cultivars have short, roundish kernels that are cohesive and sticky when cooked. Short-grain cultivars of *japonica* type were introduced into California because they were earlier in maturity and tolerated the cold irrigation water impounded from melting snow. Grain marketing standards for rice in the United States require exacting grain size

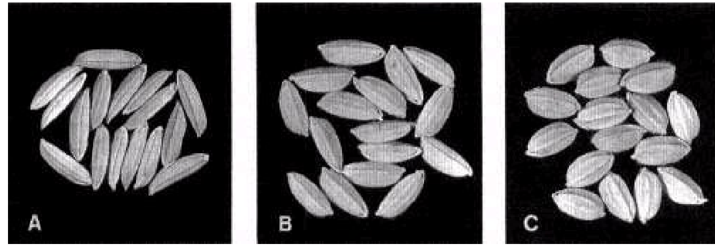


Fig. 15.5.
Unhusked grains of rice, known as rough or paddy rice. (A) Long-grain, (B) medium-grain, and (C) short-grain. Note the short glumes at the base of the grain (1.3x).

measurements to classify as long-, medium-, or short-grain types. The amylose content of the starchy endosperm is roughly proportional to the length of the grain.

Genetics of Rice

Extensive genetic and cytological studies have been made on rice. The early studies were generally concerned with color markings or morphological characters not important in the breeding program. But attention has also been given to inheritance studies dealing with characters of agronomic importance; plant stature, photoperiod sensitivity, maturity, disease and insect resistance, components of quality, and fertility restoration to male-sterile cytoplasms. Relatively few inheritance studies have been conducted involving quantitative characters.

Knowledge of inheritance of plant stature is important in breeding short-strawed, lodging-resistant cultivars in rice. Both qualitative and quantitative inheritance of stature has been reported. Recessive, dwarfing genes have been identified in nature, or induced, (d_1, d_2, etc.), but these are not useful in breeding because they reduce kernel size and grain yield in addition to internode length. Rice cultivars with semidwarf stature (about 1 meter in height) based on

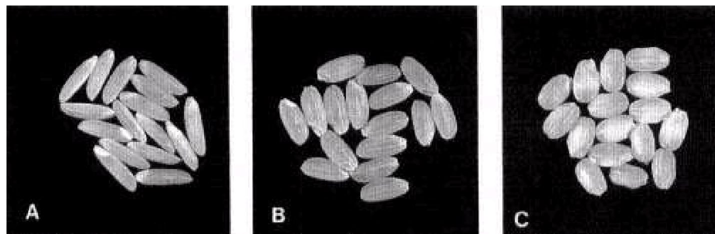


Fig. 15.6.
Grains of brown rice from which husks have been shelled. (A) Long-grain, (B) medium-grain, and (C) short-grain (1.3x).

the recessive gene, *sd*$_1$, have been most successful in breeding high-yielding rice cultivars with reduced height and lodging resistance. The *sd*$_1$ gene is a natural mutant found in a semidwarf cultivar, "Dee-geo-woo-gen," grown by farmers in Taiwan. Cultivars with the *sd*$_1$ semi-dwarfing gene are high yielding due to heavy tillering yet have normal seed size. Expression of the *sd*$_1$ gene is affected by various modifying genes. The *sd*$_1$ allele was also induced by radiation in the California rice cultivar, 'Calrose,' and has been used in breeding short-stature cultivars in the California breeding program. The United States cultivar 'Century Patna 231' has short stature comparable to that conveyed by the *sd*$_1$ gene, but the short stature is quantitatively inherited, with no major genes for stature being involved.

Biotechnology of Rice

Molecular biology techniques advanced rapidly in rice because rice responded favorably with tissue culture techniques and subsequent regeneration into a whole plant. Saturation of the rice genome with restriction fragment length polymorphisms (RFLPs) led to the construction of molecular genetic maps of the twelve haploid chromosomes of rice. Rice genes are "tagged" by identification of an RFLP marker near the gene, thereby increasing the efficiency for manipulation of the tagged genes. Insertion of new genes from wild species for resistance to disease pathogens and insect pests are major targets for production of transgenic plants in rice. For example, the insertion of the *Bacillus thuringiensis (Bt*) gene into the rice plant would result in the production of a toxin that impairs insects feeding on the rice plant. Progress in production of transgenic plants in rice has been aided by the relative ease of transforming rice protoplasts as compared to transformation of protoplasts in other monocotyledonous cereals such as wheat or corn. Addition of useful traits by transformation will complement but does not replace traditional plant breeding procedures in rice because success of the transformed plant genotype is dependent upon performance of the whole plant, not just the introduced gene. So far, no major breakthrough has occurred.

Flowering and Pollination

Rice is a member of the grass (Gramineae) family. The rice inflorescence is a panicle bearing single-flowered spikelets (Fig. 15.7). The rice flower differs from that of other common cereals, such as wheat or barley or corn, in having six stamens instead of three (Fig. 15.8). The flower is surrounded by a lemma and palea, structures that form the hull or husk that enclose the caryoposis in unhusked grain. The hull may be pubescent, but pubescence hulls are irritating to rice grain workers and are generally avoided in cultivar development. The outer glumes are sterile and usually obscure, being only about one-fourth the length of the lemma and palea, except in a few cultivars where they approach the lemma and palea in length. The blooming of rice normally occurs between 10 a.m. and 2 p.m. The flowers in a single panicle bloom over a period of 3 to 7 days, with most of the flowers blooming between 2 and 4 days after emergence of the panicle from the boot-leaf. The time and rate of blooming varies with the cultivar and is affected by environmental factors such as temperature, humidity, and light. The breeder needs to observe the timing of maximum blooming under the particular environment in which the rice plants are growing in order to know when to make emasculations and when viable pollen can be collected most abundantly for crossing. Pollen is generally shed at the time the flower opens with blooming of the spikelet starting at the apex of the panicle

and proceeding downward. The rice flower is normally self-pollinated; the extent of natural crossing varies from none to 3%, depending on the cultivar and the environment, with an average of about 0.5 %.

Crossing Procedures

Rice flowers are emasculated in preparation for crossing by cutting back the tip of the floret and removing the anthers. Clipping back the tip of the lemma and palea (Fig. 15.9) facilitates the removal of the anthers and placement of the pollen on the stigma. The anthers may be removed by tweezers, but the emasculation process will be speeded up using suction. A pipet is attached to a vacuum pump and the tip of the pipet inserted into the floret to collect the anthers. Emasculations performed in the morning may risk anthers opening during their removal, and so it is safer to remove them the afternoon before. After emasculation, flowers are covered to protect them from natural cross-pollination until they open and are ready for pollination.

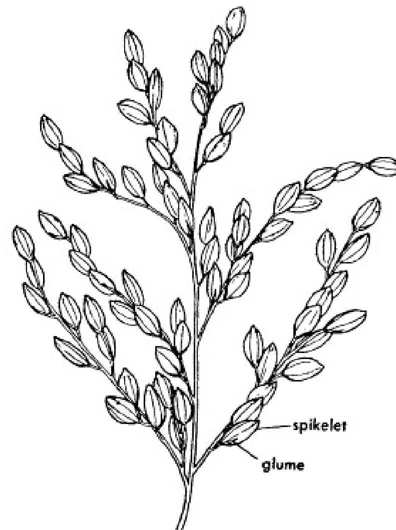


Fig. 15.7.
Branch from a panicle of rice. The rice inflorescence bears single-flowered spikelets.

The rice panicles are pollinated the morning following emasculation. Rice flowers may be pollinated by

• placing ripe anthers in each flower by hand,

• twirling a panicle shedding pollen over the emasculated panicle, or

• utilizing the approach method as described for wheat.

After pollination, the panicles are covered for protection from stray pollen. Pollen of rice is short-lived, only remaining viable for about 5 minutes.

It is physically easier to make crosses on plants in a greenhouse, or a screenhouse, than in standing water in a paddy field. Tall plants used as females may need to be staked, but staking is generally unnecessary when a semidwarf is used as the female parent. In crosses of semidwarf-females × tall-males, the F_1s will be tall; selfs will be dwarf and can be discarded.

Genetic Diversity in Rice

There is great genetic diversity in rice. Natural mutants have occurred with a rather high frequency, enabling *O. sativa* to adapt to a wide range of agroclimates. Tens of thousands of

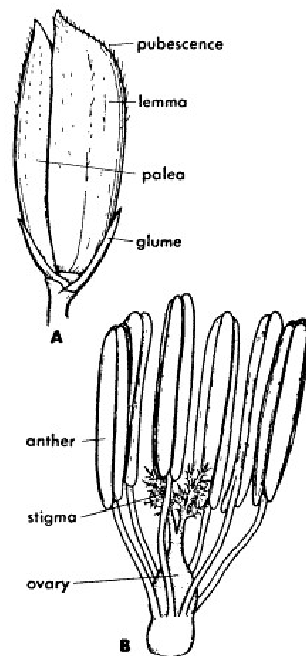


Fig. 15.8.
(A) Spikelet of rice. The lemma and palea form the hull that encloses the rice grain, the glumes are small and inconspicuous.
(B) Flower of rice. The flower differs from that of wheat and other cereals in having six stamens.

native varieties have evolved in the microclimates of traditional rice-growing areas, and more have been added through rice-breeding programs in many countries. Native varieties collected from farmers' fields are first grouped into ecological types based on culture and climate and into subpopulations based on visible characteristics such as pigmentation of the leaf sheaths, resistance to pathogen biotypes, gelatinization temperature of the grain, and many more including variations in quantitative traits. The 18 wild species, and wild relatives in related genera, provide a rich spectrum of useful genes for the breeder. Several wild species hybridize with cultivated rice, leading to development of innumerable hybrid swarms. The number of genetic accessions of rice collected and in storage exceeds those of any other cultivated species. The International Rice Research Institute, Los Baños, Philippines, has the major collection with around 85,000 accessions. The U.S. Department of Agriculture collection contains about 16,000 accessions. The germplasm available in the world at one time probably exceeded 120,000 accessions, but that rich genetic base has been greatly reduced as improved, high-yielding cultivars replaced the native varieties in farmers' fields. The large-scale cultivation of semidwarfs with the *sd* genes, many of which share the same cytoplasm, has heightened the genetic vulnerability of present-day *O. sativa* cultivars to disease pathogens and insect pests.

Breeding Rice

The methods of breeding rice are similar to those utilized in breeding wheat and other self-pollinated crops: (**1**) *introduction based on germplasm collections*, (**2**) *selection*, (**3**) *hybridization*, and (**4**) *development of F_1 hybrid cultivars. Mutation breeding* has contributed mutant genes for specific characters such as short stature, earliness, and waxy endosperm.

Introduction has played an important role in distributing rice germplasm from its center of diversity in Asia to other regions of the world. Although rice was introduced into South

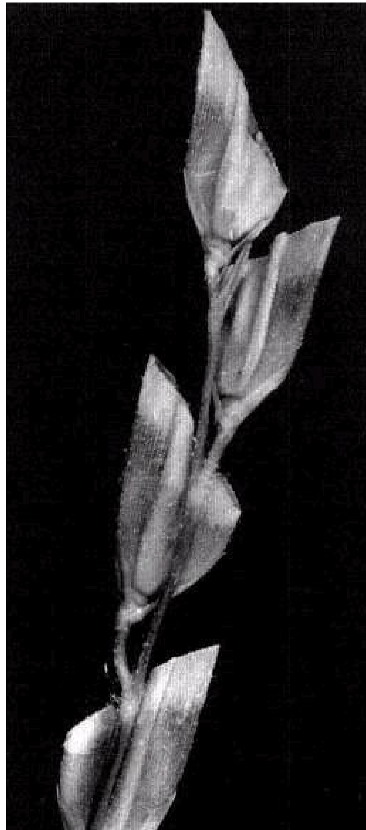


Fig. 15.9.
Branch of rice panicle with individual spikelets clipped back to facilitate emasculation and artificial cross-pollination.

Carolina as early as the seventeenth century, rice breeding was not started in the United States until the beginning of the present century. The International Rice Research Institute (IRRI), located at Los Baños, Philippines, through its extensive breeding program is distributing improved cultivars and breeding lines into less developed countries throughout the world.

In the beginning, *selection* was utilized to isolate pure lines from mixed landraces or natural populations. Now, its major role is isolation of superior genotypes from segregating populations following hybridization.

Hybridization, as in wheat, is currently the principal method for developing improved cultivars. In the tropical and subtropical regions, seeds from crosses are normally germinated in seed beds and the seedlings transplanted into the field. The pedigree method of selection, or modifications of it, is utilized because rice plants can be spaced far enough apart to permit observation of individual plants.

Hybrid Rice

The most recent development in rice breeding has been the production of *hybrid rice*. The procedure for producing hybrid rice uses the **A-line, B-line, R-line** model described in Chapter 11 find again in Chapter 14 for hybrid wheat. The procedure is plagued with low seed set as described for hybrid wheat. In China, where hybrid rice was developed and is now utilized extensively, the problem of low seed set has been overcome, at least in part, by employment of labor-intensive practices that would be impractical in countries with higher labor costs.

Successful production and utilization in rice of $\mathbf{F_1}$ hybrid cultivars requires:

• a cytoplasmic male-sterile restorer-gene system for hybrid seed production,

• sufficient seed set in the seed production field so that the hybrid seed can be economically produced in large quantities, and

• heterosis so that the yield of the hybrid cultivar will exceed the yield of conventional cultivars sufficiently to offset the additional cost of producing the hybrid seed.

Chinese rice scientists started breeding hybrid rice after discovery of a "wild-abortive" (**WA**) source of cytoplasmic male sterility (**cms**). The breakthrough came when a wild rice plant (*O. sativa* f. *spontanea*) with aborted pollen was found on China's Hainan Island in 1970 and fertility-restoring genes were identified in *indica* cultivars. Seed of hybrid rice was first distributed in China in the mid-1970s. By the mid-1980s, several million hectares of hybrid rice were being grown with yield increases of more than 15% above those of the best improved cultivars. The yield of hybrid rice over conventional cultivars has further risen to 20%, and the planted area has risen substantially. Additional cytoplasmic male-sterility systems have been identified in rice, but the **WA** system has proven to be the most stable. Fertility is restored to the sterile cytoplasm by two dominant nuclear genes with additive effects.

Procedures for seed production of hybrid rice are similar to those proposed for seed production of hybrid wheat. In the **A-line** × **B-line** cross, one row of the pollinator **B-line** is planted to six rows of the seed-producing **A-line**. In the **A-line** × **R-line** cross, one row of the pollinator **R-line** is planted to 8 rows of the **A-line**, with rows planted 10 cm apart. Because the **R-line** carries fertility-restoring genes, the hybrid plants will be fertile and will express heterosis commensurate to the combining ability of the two lines.

Production of seed of hybrid rice as practiced in China has particular problems. Seed set on the **A-lines** ranges only from 15 to 40%. The IRRI lines identified as pollinators (**R-lines**) are later in maturity than the **A-lines** (Fig. 15.10), necessitating the planting of pollinator rows about three weeks earlier than the **A-lines**, or planting on several dates to spread out the pollination period. This lack of synchronization in flowering will eventually be overcome by development of earlier maturing pollinator parent lines, but the low seed set from cross-pollination in a normally self-pollinated species presents a more difficult problem and increases the cost associated with hybrid seed production. In China, some labor-intensive procedures are practiced to enhance flowering and cross-pollination, such as cutting back the flag leaves of female plants to facilitate pollen reaching the flowers and dragging a rope or pole across the

Fig. 15.10.
Seed production field of hybrid rice in China. The tall pollinator R-line requires a longer time to mature and was planted earlier than the seed-producing A-line.

field at the level of the panicles each day during the flowering period to assist in pollen dispersal. In countries with high labor costs, such as the United States, the increased yield from hybrids would not be sufficient to offset the additional costs of producing the hybrid seed. Selection for greater pollen production, or more open flowers to improve pollen receptivity, might improve seed set, but finding genotypes with these improved characters may prove to be difficult. Another problem with hybrid rice in the United States relates to the breeding emphasis placed on grain quality, a complex, quantitatively inherited character. It will be difficult to find parent lines sufficiently diverse genetically to obtain maximum heterosis and still maintain a satisfactory level of market quality in the hybrid.

Radiation-induced mutations have been utilized to obtain short stature and early maturity in rice by U.S. Department of Agriculture research workers at the California Agricultural Experiment Station. The mutations were induced following exposure of seeds of the 'Calrose' cultivar to gamma rays emitted from isotopes of radioactive cobalt. The short stature was inherited as a single recessive gene, sd_1, and a semidwarf mutant strain was released as the cultivar 'Calrose 76' (Fig. 15.11). Cultivars with the sd_1 gene in a japonica background average 90 cm in height compared to 120 to 130 cm for tall cultivars.

International Rice Research Institute (IRRI)

The International Rice Research Institute (IRRI) was founded at Los Baños, Laguna, Philippines, in 1960. IRRI is an international research and training center, devoted to the study

Fig. 15.11.
Comparison in height of 'CS-M3,' a tall Calrose-type cultivar, and 'Calrose 76,' a semidwarf cultivar. Calrose 76 is a mutant line containing an induced sd (semidwarfing) gene. It was selected following gamma radiation of a seed lot of tall Calrose.

and improvement of rice for the benefit of the developing countries of the world. In addition to research in the Philippines, cooperative research programs have been established with many countries; the international research centers in Ibadan, Nigeria (IITA), and Cali, Colombia (CIAT); and the West Africa Rice Development Association (WARDA), in Ivory Coast, West Africa.

Scientists at IRRI recognized early the potential of short-statured plant types as found in Taiwan's semidwarfs and nitrogen-responsive plant types as found in United States cultivars. 'Taichung Native 1,' a semidwarf cultivar bred in Taiwan performed well in the tropics as a prototype for short-statured, photoperiod-insensitive, high-yielding rice cultivars (Fig. 15.12). A breeding program at IRRI was built around the Taichung Native 1 plant type. The IRRI program quickly led to the development of 'IR8' and other high-yielding, semidwarf cultivars. Later research programs were designed to further reduce growth duration and to improve disease and insect resistance and quality of the high-yielding, short-statured types of rice. Germplasm with these characteristics was distributed to rice breeders in developing countries throughout the world. IRRI also widely distributes improved germplasm and wild species for research and breeding from its 85,000-accession germplasm collection.

Breeding Objectives

In breeding improved cultivars of rice, the overall objectives are (*1) high-yield potential, (2) yield stability*, and (*3) grain quality*. Additional objectives are important in local areas, for

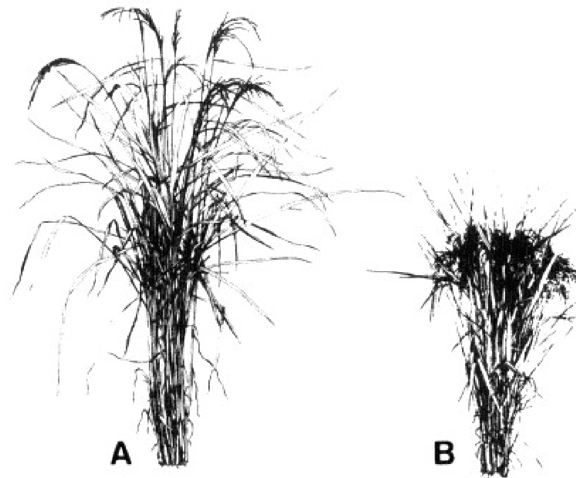


Fig. 15.12.
Rice plant types. (A) Tropical plant type; tall, weak stems, long droopy leaves, and open panicles. (B) Temperate (Taichung Native 1) plant type; short stature, stiff upright stems, compact panicles, and nitrogen responsiveness. Rice breeders at the International Rice Research Institute recognized the potential of the short stature and nitrogen responsiveness and built a breeding program around the Taichung Native plant type.

example, the breeding of deep-water rice for flood-prone areas or upland rice where the contour of the land makes bunding and flooding impractical.

Yield Potential

Rice is a crop with potential for producing high grain yields when water supply is not limited. To reach the high-yield potential requires an inherently high-yielding cultivar and a package of cultural practices—fertilization, water control, pest control, weed control—that permit maximum expression of the inherent yield potential. To identify strains of rice with high-yield potential under optimum cultural conditions, it is necessary to conduct the breeding program in a high-yield environment. As in wheat, a real breakthrough in yield potential in rice yields came with the development of short-statured semidwarf cultivars under irrigation and intensive management.

PLANT TYPE. High yield potential in a rice cultivar is associated with a combination of morphological and physiological characteristics in an efficient architectural plant type. The morphological characteristics include short-stature, high tillering, stiff culms and compact particles to hold the plant erect, erect leaves to reduce shading and utilize solar radiation efficiently, and well-filled heavy panicles. The physiological characteristics include seedling vigor, early maturity to permit growing more than one crop of rice per year, photoperiod insensitivity to permit growing a cultivar in any crop season or in different latitudes, and nitrogen responsiveness—the ability of the rice plant to utilize increased rates of nitrogen fertilizer efficiently in the production of high grain yields.

The plant type described is best embodied in the semidwarf indica rices from Taiwan with the sd_1 gene and its modifiers described earlier. The sd_1 gene in semidwarf parents, such as Dee-geo-woo-gen, was introduced into the high-yielding rice cultivar 'Taichung Native 1' in Taiwan, which produced spectacular yields in Taiwan and India. A cross of 'Taichung Native 1' × 'Peta' at the International Rice Research Institute in the Philippines produced the cultivar 'IR8' which started the Green Revolution in tropical rice production. Other high-yielding semidwarf breeding lines from IRRI have been used extensively in hybridization efforts in various national rice breeding programs. A yield plateau in tropical rice production appeared after the development of 'IR8' and 'IR24.'

In the United States, semidwarf cultivars have gained in use and popularity due to their high yield and improved lodging resistance. Introducing the sd_1 and its modifiers into United States cultivars while maintaining their excellent grain quality was difficult, requiring employment of extensive backcrossing and some three-way crosses. In California, an allele of the sd_1 gene was induced through a mutation breeding program. In other cultivars, short stature was obtained by selection for transgressive segregates for shorter height. In these cultivars the semidwarf character is quantitatively inherited and is not dependent upon the sd_1 gene.

Yield Stability

Yield stability refers to the consistency with which a cultivar is productive in a local climate in which weather and plant disease or insect pests vary from year to year. Yield stability is dependent upon plant characteristics that enable the plant to avoid, tolerate, or resist the debilitating effects and vagaries of adverse climate or disease and insect pests. Screening for stability requires that the cultivars be tested at represenative locations within the production area over several seasons.

EARLY MATURITY. In tropical and subtropical climates, total food production from a given piece of land can be greatly increased by growing multiple crops of early maturing rice or crops of early rice rotated with another short-season crop, such as a pulse crop or a winter crop of wheat. In those climates, the early maturing semidwarf rice cultivars have contributed to the success of multicropping rotations. Additionally, early maturing rice utilizes less irrigation water or permits harvest of a ratoon crop after the initial crop. In the United States, progressively earlier maturing cultivars have been developed through transgressive selections and induced mutations. Most long-duration cultivars grown in the tropics are photoperiod sensitive and are unsuited for growing in another crop season or at different latitudes.

RESISTANCE TO LODGING. Yields are reduced in a lodged crop due to stem breakage and grains not filling properly. The lodged plants create a favorable environment for disease development or grains sprouting in the panicles and may cause loss of yield in harvesting. Milling quality of the rice grain is reduced due to the chalkiness in kernels or sprouted grains that develop in lodged rice plants. Lodging resistance is essential in high-yielding and nitrogen-responsive cultivars, especially in rice grown in the monsoon season. Plant characteristics conferring lodging resistance include short stature (Fig. 15.13), erect leaves which permit light penetration into the canopy, a strong root system, and resistance to diseases and insects that may weaken the culms or the root system. The semidwarf types have been useful in conferring lodging resistance combined with high yield potential. Lodging resistance is a quantitative character with complex inheritance.

RFSISTANCE TO STRESS ENVIRONMENTS. Rice cultivars evolved over long periods of time with varying adaptation to different climatic situations. The resulting diversity in genotypes has provided the rice breeder with a wide range of genetic variability to use in developing cultivars for different stress environments. The *japonica* races that evolved in China, Korea, and Japan have greater tolerance to cool temperatures than the *indica* races that evolved in tropical Asia. The cool temperature tolerance permits rice to be grown at latitudes up to 45 to 50º north, or during the winter season in the subtropics, or at high elevations in tropical and subtropical areas. Cool night temperatures during pollen formation increases sterility and reduces yield. In California, cold tolerance of the *japonica* type cultivars, 'Colusa' and 'Calora,' enabled rice to be grown in cold irrigation water from melting snow.

In rainfed culture, traditional rice cultivars are generally superior to semidwarf cultivars that are subjected to drought in seasons with low rainfall due to deeper root growth and a larger root-to-shoot ratio. The deeper root growth enables the rice plant to obtain water from deeper soil levels and maintain a higher leaf-water potential. On the other hand, the semidwarfs recover more quickly after a short period of water deficiency. High-temperature stress may severely restrict pollen shedding in heat-susceptible cultivars. The increasing use of marginal land for rice production in the developing countries has increased the need for breeding rice with tolerance to soil mineral stresses caused by alkali and salt injury or deficiencies in zinc, iron, and phosphorus.

Disease Resistance

Rice is grown in warm and humid environments that favor intense disease development. These include tropical, semitropical, and warm temperate climates, and irrigation or monsoon rains. Pathogens causing disease in rice include bacteria, fungi, viruses, and nematodes. Chemical control of disease pathogens is expensive, less effective in tropical than in temperate

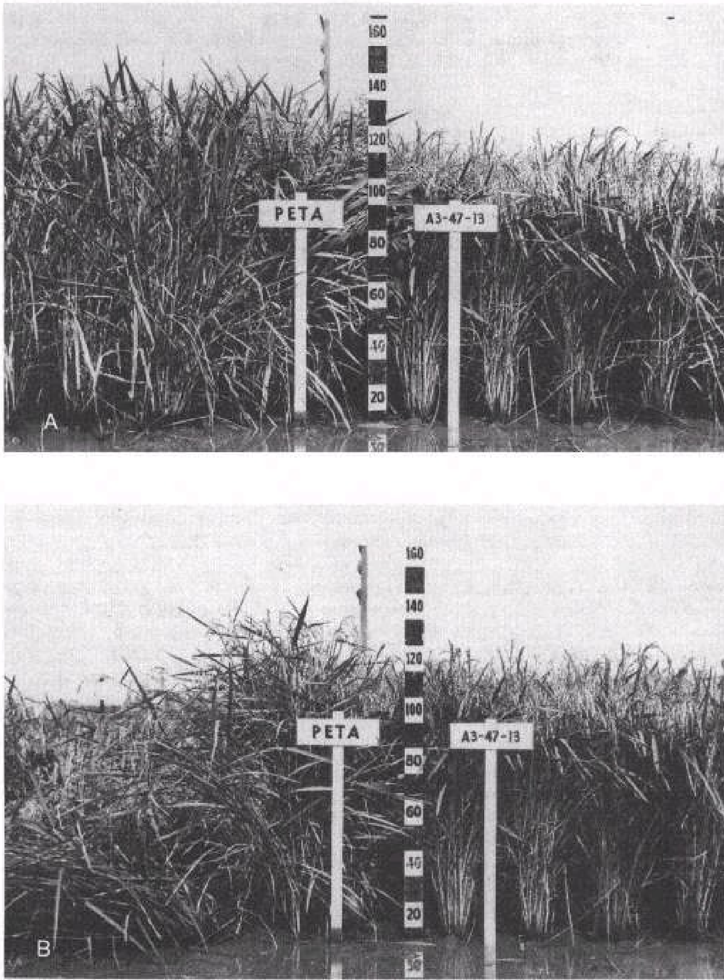


Fig. 15.13.
Lodging before and after a tropical rainstorm in a tall, leafy, tropical rice cultivar, 'Peta,' compared to a short, semidwarf breeding line, A3-47-13. (A) Before a tropical rainstorm. (B) After a tropical rainstorm.

climates, and environmentally less safe than breeding resistant cultivars. When the semidwarf cultivars were first distributed in the tropics and grown year-round, they encountered serious disease problems. Multiple cropping and planting over staggered dates in large production areas added to the severity of disease and insect problems. This was followed by development of new rice cultivars with greater resistance to the major disease pathogens.

BLAST. Blast, incited by *Pyricularia oryzae* Cavara, is the most serious fungal disease of rice, causing damage in many rice-growing areas. Spores of the rice blast disease are airborne and produce lesions on the leaves which spread quickly, blighting the entire leaf or even the entire panicle. The blast pathogen, like the rust pathogens in wheat, is highly specialized with numerous races that change over years and locations. Thirteen or more genes for resistance have been identified. In addition to race-specific resistance induced by major genes, general resistance, which results in the production of fewer and/or smaller lesions, has been identified as a more durable line of defense. New cultivars released in Japan or through the International Rice Research Institute are rigorously screened for resistance to prevalent races of the blast pathogen.

LEAF BLIGHTS, SHEATH AND STEM DISEASES. Leaf-blighting diseases that occur in rice and the causal organism include brown spot (*Cochliobolus miyabeanus* Ito et Kuribayash), narrow brown leaf spot (*Cercospora oryzae* Miyake), and bacterial blight (*Xanthomonas campestris* p.v. *oryzae*). Bacterial leaf blight is a destructive disease in many rice-growing areas in Asia. Disease development is encouraged by application of nitrogenous fertilizers. Sheath blight infects irrigated rice plants at the waterline by floating sclerotia of *Rhizoctonia solani* Kuhn. The change to semidwarf cultivars combined with higher nitrogen fertilization increased the damage from sheath blight in southern United States. Stem rot, caused by *Sclerotium oryzae*, is a rice disease in California and southern United States.

VIRAL DISEASE. Tungro virus, transmitted by the green leafhopper, and grassy stunt virus, transmitted by the brown planthopper, are destructive viral diseases in south and southeast Asia. Two forms of the tungro virus have been identified; one responsible for virus transmission, the other for symptom development. Resistance to grassy stunt virus has been transferred from the wild species O. *nivara* by backcrossing, but a new biotype of the virus evolved rendering the gene from O. *nivara* useless. Hoja blanca is a virus disease of rice in Central and South America.

Insect Resistance

Brown planthoppers, whitebacked planthoppers, leafhoppers, stem borers, and gall midges are major insect pests of the rice crop in Asia. The rice water weevil and the rice stinkbug are major insect pests of rice in the United States. Extensive research on breeding for insect resistance has been conducted at the International Rice Research Institute in the Philippines, in India, and in the United States.

BROWN PLANTHOPPER (***NILAPARVATA LUGENS*** **STÅL**). The brown planthopper feeds on the rice plant with damage ranging from loss in vigor to complete destruction of the crop (Fig. 15.14). The brown planthopper is a vector of the grassy stunt virus. Several biotypes of the brown planthopper and nine genes for resistance, four dominant and five recessive, have been identified. The major genes providing resistance to this insect break down quickly after

Fig. 15.14.
Comparison of damage caused by brown planthoppers on a resistant rice cultivar (left); and susceptible rice cultivars (right).

widespread cultivation of a resistant cultivar. Cultivars and breeding lines are tested for resistance by caging rice plants with the planthopper and observing the damage.

WHITEBACKED PLANTHOPPER (***SOGATELIA FRUCIFERA*** **HORVÁTH**). Likewise, biotypes of the whitebacked planthopper exist and two dominant genes and one recessive gene have been identified for resistance in rice cultivars.

GREEN LEAFHOPPER (***NEPHOTETTIX VIRESCENS*** **DISTANT**). The green leafhopper damages the rice plant by sucking out the sap. Infested rice plants are reduced in vigor and set fewer seeds. The green leafhopper transmits rice tungro and other virus diseases. Both dominant and recessive genes for resistance have been identified in rice cultivars. Genetic resistance to the green leafhopper appears to be more stable than resistance to the brown leafhopper.

STEM BORERS. About 20 species of stem borers attack the rice plant, often with mixed populations in the same field. Both simple and polygenic inheritance has been reported to the different species.

Grain Quality

Rice grain quality is a combination of many characteristics that affect its market value and utilization as food, whether grown for the commercial market or for home consumption. From a breeding standpoint the factors affecting quality in rice may be grouped into four broad areas: (*1) market quality, (2) milling quality, (3) cooking and processing qualities, and (4) nutritional quality*. Quality is affected by the genetic potential of the cultivar and its interaction with the environment in which the rice is grown. Greater attention is given to rice grain quality in United States breeding programs than in breeding programs in other countries.

MARKET QUALITY. Market quality refers to the general appearance and physical properties of the rice grain. It includes such features as size, shape, and uniformity of the grain; awnlessness; hull pubescence; translucency, color, and freedom of chalkiness of the kernel; and seed dormancy to prevent preharvest sprouting. In the United States, rice is classified in the market as *long grain, medium grain*, or *short grain*. Each grain type possesses specific milling, cooking, and eating qualities that adapt it for processing for a particular prepared cereal product or convenience food. In the market, the grain types must conform to exacting grain dimensions with regard to length, width, length/width ratio, thickness, and grain weight. In Asia, cultivars are developed to satisfy personal preferences of people with different cultural and ethnic backgrounds. In India there is demand for scented rices, locally referred to as 'Basmati' rice.

MILLING QUALITY. The unhusked rice grain received by the miller is called *rough rice* or *paddy*. It is converted to *brown rice* by shelling off the hulls and converted to *milled rice* by scouring off the outer bran layers. The value of the rough rice is largely determined by the yield of *head rice* (whole and broken grains three-quarters size or larger) to *total rice*, the total yield of rice recovered after the milling process. Short- and medium-grain cultivars normally give larger mill yields than long-grain cultivars. The milling output of advanced breeding lines needs to be rigidly evaluated to ensure that newly released cultivars will produce high yields of head rice and total milled rice. In the United States, a Regional Rice Quality Laboratory for evaluating milling and processing quality of new rice cultivars and breeding lines is operated by the U.S. Department of Agriculture and the Texas Agricultural Experiment Station at Beaumont, Texas.

COOKING AND PROCESSING QUALITIES. Cooking characteristics of rice vary with the grain type and cultivar. Long-grain cultivars remain dry and flaky when cooked; medium- and short-grain cultivars are sticky or chewy. Intermediate forms are also found. Demand for a particular kind varies with cultural and taste preferences and with the industrial and food uses.

In the United States, specific physical and chemical properties measured by laboratory tests have been established as indices for evaluating rice cooking and processing characteristics of breeders' rice samples. Among the laboratory tests, *water uptake* (Fig. 15.15), *amylose content, and alkali reaction*, which measures *gelatinization temperature, are* rated high as predictors of cooking and processing characteristics. New cultivars and breeding lines are always compared with standard cultivars of acceptable quality. High

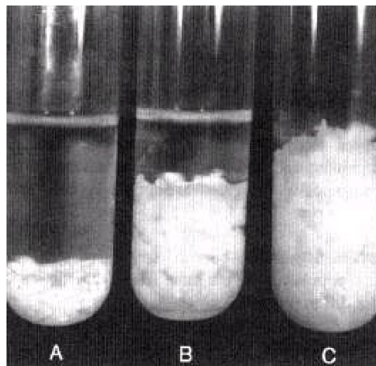


Fig. 15.15.
Quality evaluation in rice. Comparison of water uptake capacity of: (A) Cultivar of the long-grain type; (B) Cultivar of the medium-grain type; and (C) Cultivar of the short-grain type. Water uptake capacity is a measure of the water absorption and swelling of rice during cooking and is smallest in rice of the long-grain type and greatest in rice of the short-grain type.

amylose content, medium gelatinization temperature, and low water absorption characterize United States long-grain cultivars, whereas low amylose content, low gelatinization temperatures, and high water absorption characterize United States medium- and short-grain cultivars.

NUTRITIONAL QUALITY. Because rice is the principal source of protein and calories for about 40% of the world's people, breeding for improved nutritional value would be beneficial if it could be accomplished without loss in total yield. Protein averages about 8 % in brown rice and 7% in milled rice. Although relatively low in protein compared to other cereals, the nutritional value of the rice protein is high due to the high content and favorable balance of the essential amino acids, including lysine. Attempts to improve protein content by breeding have generally resulted in reduced lysine content in the protein and reduced grain yield.

Upland Rice

Upland rice is grown in Asia, Africa, Brazil, and Latin America in strictly rainfed, dryland culture, and constitutes about 13% of the total world area planted to rice. Upland rice generally has tall stature, few tillers, long panicles, and high flower sterility. Genetic improvement has been meager when compared with that of irrigated rice. The objectives in breeding upland rice include drought resistance, weed competitiveness, and disease and insect resistance, traits contributing to yield stability rather than high grain yield.

Deep-Water and Floating Rice

Deep-water rice is grown in low-lying coastal areas of southeast Asia where water cannot be controlled and reaches depths of 1 m or more in rainy seasons. Floating rice, in which the stems elongate as the water becomes deeper and the leaves float on the surface of the water, may be grown in water with a depth of as much as 5m. Floating rice is sensitive to photoperiod, normally flowering after the flood water has receded. The genes for culm elongation are being transferred from floating to semidwarf rice in attempts to increase yield potential. Yield stability is a major breeding objective, sharing importance with yield productivity.

Wild Rice

The Indian wild rice (*Zizania palustris* L.) is a native wild grass that grows in shallow lakes in north central United States and adjacent areas in Canada. The plant has monoecious flowers and is related to *Oryza* (Fig. 15.16). In native ecotypes, seeds shatter and drop to the bottom of the lake, where they remain over winter before germinating to start a new crop. Traditionally, native stands have been harvested by local Native American tribes from canoes. The species has been domesticated and commercial production established. Breeding programs are concentrating on developing nonshattering cultivars, with stronger straw for mechanized harvest, and larger seed size.

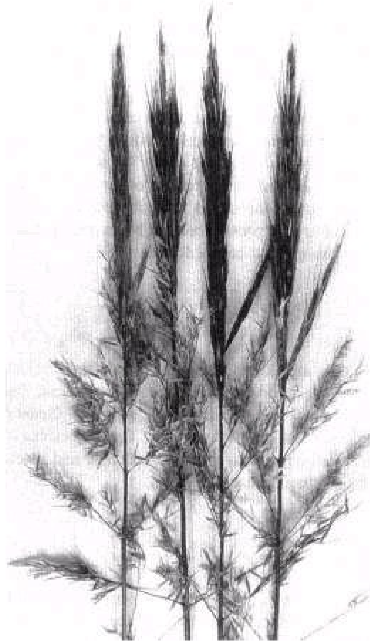


Fig. 15.16.
Monoecious flowers of Indian wild rice (Zizania palustris).
The pistillate flowers are borne in the erect upper branches,
the staminate flowers in the spreading lower branches of the
panicle. The staminate flowers have six stamens as in Oryza.

Study Questions

1. What are the different types of rice? What environments do each of the different types of rice grow best in? What are the types of rice grown in the United States?

2. Describe the procedure for producing hybrid rice cultivars.

3. What role has mutation breeding played in the development of rice cultivars?

Further Reading

Chang. T.T. 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. Euphytica 25:425-41.

Chang, T.T. 1993. The role of germplasm and genetics in meeting global rice needs. p. 25-33. *In* Recent Adv. Bot., Monograph Series No. 13, Academia Sinica, Taipei, Taiwan.

Chang, T.T., and C.C. Li. 1991. Genetics and breeding. p. 23-101. *In* B.S. Luh (ed.) Rice, production. Vol. I. Van Nostrand Reinhold, New York.

Chang, T.T., and D.A. Vaughn. 1991. Conservation and potentials of rice genetic resources. p. 531-52. *In* Y.P.S. Bajaj (ed.) Rice. Biotechnology in agriculture and forestry. Vol. 14. Springer-Verlag, Berlin.

Dalrymple, D.G. 1986. Development and spread of high-yielding rice varieties in developing countries. Bur. Sci. Tech., Agency for Int. Dev., Washington, D.C.

Hayes, P.M., R.E. Stucker, and G.G. Wandrey. 1989. The domestication of American wildrice (*Zizania palustris*, Polaceae). Econ. Bot. 43:203-14. IRRI. 1989. Hybrid rice. International Rice Research Institute, Los Baños, The Philippines.

Juliano, B.O. 1993. Improving food quality of rice. p. 677-81. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Khush, G.S. 1991. Selecting rice for simply inherited resistances. p. 303-22. *In* H.T. Stalker and J.P. Murphy (eds.) Plant breeding in the 1990s. CAB Int., Wallingford, Oxon, U.K.

Khush, G.S, and D.S. Brat. 1991. Genetic resistance to insects in crop plants. p. 223-74. *In* N.C. Brady (ed.). Adv. Agron., Vol. 44. Academic Press, Inc., San Diego, CA.

Khush, G.S, and G.H. Toenniessen (eds.) 1991. Rice biotechnology. CAB Int., Wallingford, Oxon, U.K.

Khush, G.S., and S.S. Virmani. 1985. Breeding rice for disease resistance. p. 239-79. *In* G.E. Russell

(ed.) Progress in plant breeding I. Butterworths, London.

McKenzie, K.S., C.N. Bollich, J.N. Rutger, and K.A.K. Moldenhauer. 1987. Rice. p. 487-532. *In* W.R. Fehr (ed.) Principles of cultivar development. Vol. 2. Macmillan Publ. Co., New York.

Rutger, J.N. 1983. Applications of induced and spontaneous mutation in rice breeding and genetics. p. 383-413. *In* N.C. Brady (ed.) Adv. Agron., Vol. 36. Academic Press, Inc., San Diego, CA.

Rutger, J.N., and C.N. Bollich. 1991. Use of introduced germplasm in U.S. rice improvement. p. 1-13. *In* H.L. Shands and L.E. Wiesner (eds.) Use of plant introductions in cultivar development, Part 1. Crop Sci. Soc. Am., Spec. Publ. No. 17. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Webb, B.D. 1991. Rice quality and grades. p. 89-119. *In* B.S. Luh (ed.) Rice, utilization. Vol. II. Van Nostrand Reinhold, New York.

16. Breeding Soybean

In the United States the soybean, *Glycine max* (L.) Merrill, emerged from a relatively obscure immigrant plant to become a major grain crop (Fig. 16.1). Before 1940, the soybean was cultivated largely for forage; today it is third in production of grain after corn and wheat, and second in value after corn. There are two major areas of soybean production in the United States, the Corn Belt and the lower Mississippi Delta. The United States produces 51% of the world production with three other soybean-producing countries, Brazil, the People's Republic of China, and Argentina, producing another 38%. The remaining production is scattered among various countries in Asia and South America, with only minor production in Africa and Europe.

Why the phenomenal increase in production and value of soybean in the United States since 19407 There are two major contributing factors:

- the soybean seed, averaging 40% protein and 20% oil, is unexcelled in nutritive content among the major grain crops, and

- a major research effort was directed toward *breeding improved cultivars*, disease and insect control, and production technology.

Soybean breeding in the United States developed as an interdisciplinary research activity, cooperative between the United States Department of Agriculture and Agricultural Experiment Stations in the midwestern and southern states. Intensive research on the soybean began in 1936, when a cooperative federal/state research program, currently the *U.S. Regional Soybean Laboratory*, was established at the University of Illinois, Champaign/Urbana. Through the Regional Soybean Laboratory, soybean breeding research was coordinated and regional testing programs were developed among United States and Canadian soybean research workers. The enactment of the United States Plant Variety Protection Act of 1970 provided an incentive for private seed companies to initiate soybean breeding programs. Plant breeding research on cultivar development by private seed companies now exceeds that of the publicly supported programs in the United States.

Fig. 16.1.
Field of soybean. During the past half century, the soybean has become a major grain crop in the United States. The increase in acreage and production of soybean would not have been accomplished without the rapid progress that was made in breeding highly productive, disease-resistant cultivars.

Domestication and Species

The soybean was domesticated in northeastern China about 2500 B.C. From the area of its origin, the soybean spread to southern China, Korea, Japan, and other countries in southeastern Asia. The soybean was introduced intermittently into the United States in the late 1700s, but it remained a very minor crop grown mostly for forage until the 1920s and 1930s. Most of the early introduced cultivars were useful only for forage as the seeds shattered when ripe, making mechanical harvesting impossible. With the development of lodging- and shatter-resistant cultivars, the soybean changed from a forage to an oilseed crop. This change resulted in a rapid rise in area planted to soybean in subsequent years.

Species of Soybean

Taxonomically, the soybean is classified in the legume family, Leguminosae, subfamily Papilionoideae, tribe Phaseoleae, genus *GIycine*. The genus *Glycine* contains two subgenera, *Soja and Glycine*.

The subgenus *Soja* contains two species:

• *Glycine max* (L.) Merrill., ($2n = 2x = 40$), the cultivated soybean.

• *Glycine soja* (L.) Sieb. and Zucc., ($2n = 2x = 40$), a wild species native to the Far East with viny prostrate growth and a tendency to shattering (Fig. 16.2).

Glycine max has never been found growing wild and among the known species *G. soja* is its most probable progenitor. *Glycine max and G. soja are* cross-fertile, but the growth characteristics of *G. soja* make it an undesirable parent in a crossing program except for possible transfer of a particular gene for a desirable trait *in G. soja* to the more productive species. A backcross to the cultivated species will probably be needed to recover a desirable plant type.

The subgenus *Glycine* contains 12 wild, perennial species native to Australia or adjacent areas of the Pacific. Ten species in the subgenus *Glycine are* diploid ($2n = 2x = 40$), and two are mixtures of diploid and tetraploid plants. Cross-fertilizations of G. *max* with species in the subgenus *Glycine* are normally sterile or produce only immature pods, although limited success in cross-fertilization of *G. max* × *G. tomentella* was obtained by using in vitro embryo rescue.

Fig. 16.2.
Plant of wild soybean, *Glycine soja* (left), compared with the cultivated soybean, *Glycine max*.

Soybean Genetics

Considering the economic importance of the soybean crop, genetic and cytogenetic research on soybean was relatively minor until recently in comparison to that on the major cereals such as barley, corn, or wheat. Some genetic research on soybean was reported from the University of Illinois during the 1930s. But intensive research on the soybean did not begin until establishment of the Regional Soybean Laboratory in 1936, and at first the research was concentrated on cultivar development rather than genetic studies.

Soybean introductions from the Far East were utilized as the basic germplasm in the early breeding programs. The germplasm contained many diverse morphological and physiological characters, such as seed size and color, flower color, leaf and stem pubescence, leaf shape, number of seeds per pod, or flowering time and maturity. It was logical then that early inheritance studies would be devoted to these characters as this knowledge would be useful immediately to the soybean breeder. As the soybean crop attained economic stature and soybean breeding expanded, genetic studies were designed to provide answers needed in the expanded breeding programs. These included inheritance of growth type, nitrogen fixation, photoperiod response, disease and insect resistance, and components of seed quality. Quantitative inheritance studies were utilized to evaluate heritability of particular characters, heterosis, and breeding strategy, but in general they have received less attention in the soybean, a self-pollinated species, than qualitative inheritance studies. Recurrent selection as utilized in cross-pollinated species for population improvement has been employed sparingly in the soybean due to the labor required to make cross-pollinations. However, the practice of crossing superior cultivars or breeding lines, followed by selection and crossing of superior segregates from the new generation of crosses, constitutes a long-term *recurrent selection* program.

More than 200 genes for simple qualitative characters have been identified in soybean and the gene symbols published in the *Soybean Genetics Newsletter*. Guidelines for naming genes and coordinating genetic studies is the responsibility of a Soybean Genetics Committee. The genes identified in inheritance studies are conserved in a Genetic Type Collection at the University of Illinois, Agricultural Experiment Station, Urbana. Insofar as possible, the genes in the Genetic Type Collection are maintained in the cultivar in which they were originally identified and, additionally, in isolines of several adapted cultivars. Germplasm containing the genes in the collection are available for study and utilization in breeding programs by soybean research workers. *Linkage groups* have been identified for 13 of the 20 soybean chromosomes, and the linkage combinations are maintained in the soybean genetic collection. A separate collection of cytological materials is maintained at Iowa State University, Ames, Iowa.

Genetic Male Sterility

Four recessive genes for genetic male sterility, ms_1, ms_2, ms_3, and ms_4, have been identified in the soybean. The male-sterile genes inhibit development of viable pollen and prevent normal self-fertilization. In addition, a temperature-sensitive gene, *msp* for partial male sterility, and duplicate recessive genes fs_1 and fs_2 for an abnormal flower structure that prevents normal self-fertilization, have been identified in soybean. Male-sterile plants can be distinguished from normal plants by selecting green plants with partially filled pods in late fall after normal plants have shed their leaves.

Biotechnology

Traditional plant breeding procedures have produced dramatic improvements in soybean cultivars. Molecular biology offers new procedures that permit transfer of DNA from a wider range of organisms because the transfer is not dependent upon cross-fertilization and reproduction. A new class of genetic markers for DNA fingerprinting of soybean germplasms and commercial cultivars is provided by restriction fragment length polymorphisms (RFLPs). For example, RFLP markers linked to a soybean cyst nematode resistance gene, Rhg_4, provide an alternative selection method to the conventional phytopathological assay, although the latter is still the definitive test. Soybean has been transformed to exhibit tolerance to glyphosate, an active ingredient in widely used, foliar-applied, broad-spectrum, nonselective postemergent herbicide. Genetically engineered herbicide tolerance does not result in tolerance to all herbicides, only to the single herbicide or class of herbicides against which the mechanism is designed. Molecular biology techniques supplement but do not replace traditional breeding procedures.

Soybean Plant Types

The plant of the cultivated soybean is an erect, bushy annual that branches profusely if given sufficient space. Breeding efforts have been directed toward the selection of cultivars with short branches to accommodate higher plant populations. Soybean cultivars are grouped by plant growth type as:

- *indeterminate*, in which flowering begins before stem elongation ceases, and flowers are borne in axillary racemes (Fig 16.3), and
- *determinate*, in which flowers are borne in both axillary and terminal racemes, stem elongation ceasing with differentiation of a terminal bud.

The indeterminate type is adapted to short growing seasons, with flower and seed production proceeding before the soybean plant completes its growth, yet seed maturity for all flowers is normally reached simultaneously. The determinate type is adapted to a long growing season, in which the soybean plant completes growth before or shortly after flowering is initi-

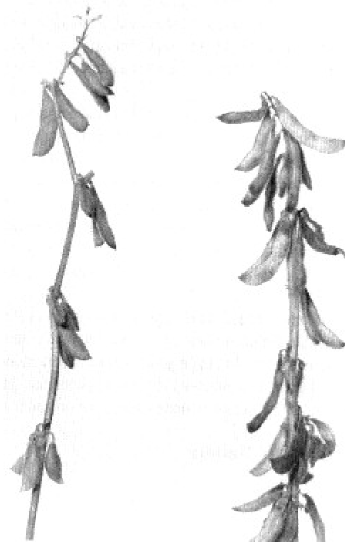


Fig. 16.3.
Indeterminate (left) and determinate (right) stem types in soybean. With the indeterminate type, flowering begins before stem elongation ceases, the flowers being borne on axillary racemes. In the determinate type, the flowers are borne in both axillary and terminal racemes, and stem elongation ceases with differentiation of the terminal bud.

ated. As a general rule in North America, cultivars of indeterminate growth type are grown northward and cultivars of determinate growth type are grown southward from latitudes of about 35º N. The dividing line falls in maturity group IV, which contains both determinate and indeterminate cultivars. The determinate growth habit is controlled by a recessive gene, dt_1. Determinate and semideterminate cultivars have been developed in maturity groups adapted to the northern United States by transferring the dt_1 gene into indeterminate cultivars. These types have shorter and stiffer stems, but many pods are often set so low on the stem that they are lost during harvest. These types also tend to be sensitive to stress in the early growth stages of plant development.

Flowering and Pollination

The cultivated soybean bears flowers in clusters of 3 to 15 blossoms in the axil of a branch (Fig. 16.4). Many flowers are shed without forming pods. The flowers are characteristic of the legume family, the corolla consisting of five petals which enclose a pistil and ten stamens (Fig. 16.5). Nine stamens develop in a tube around the pistil; the tenth stamen remains free. Pollen from the anthers is shed directly on the stigma, resulting in a high degree of self-pollination and less than 1% of natural cross-pollination. The flower normally opens early in the morning, but opening may be delayed in cool, damp weather. In prolonged periods of cool temperatures, or on short days, cleistogamous flowers that do not open may be produced.

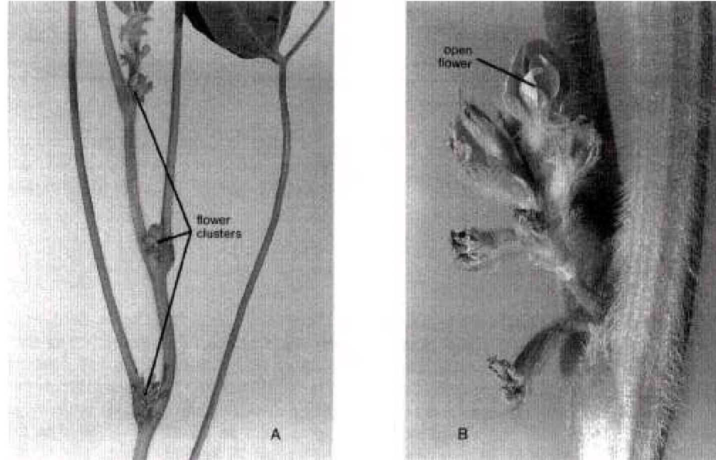


Fig. 16.4.
(A) Soybean flowers are borne in clusters of 3 to 15 blossoms in the axil of a branch.
(B) Single flower cluster of soybean. (Note the pubescence on the stem of the soybean.)

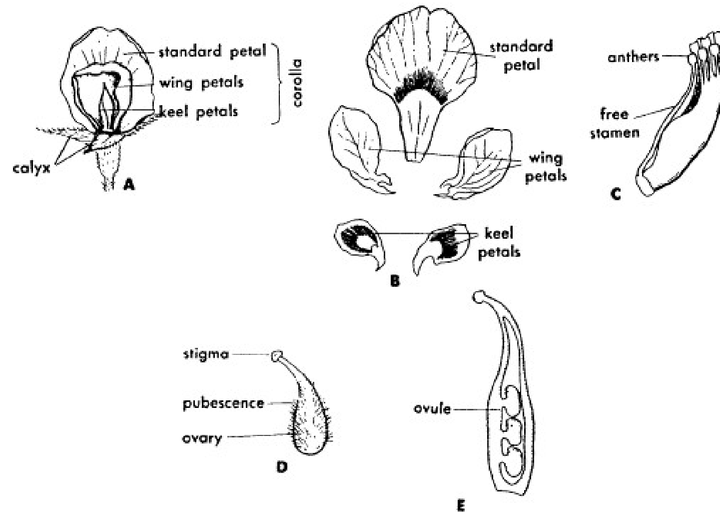


Fig. 16.5.
Flower of soybean. (A) Single open flower showing the corolla and the calyx.
(B) Corolla dismembered to show the standard, two wing, and two keel petals.
(C) Nine stamens develop in a tube around the pistil, the tenth stamen remains free.
(D) Pistil covered with pubescence. (E) Section through the pistil of a
mature flower showing three ovules.

Pollen shedding normally occurs shortly before or shortly after the flower opens but sometimes occurs within the bud. Soybean pollen is heavy and seldom windborne but may be carried from flower to flower by insects. Natural cross-pollinations rarely occur at distances of more than 10 to 15 m.

Crossing Techniques

Artificial cross-pollination is a tedious operation due to the small floral pans. Seed-set is normally lower than with the cereal grains. Flowers are prepared for crossing just as they emerge from the bud and before the petals are visible by removing the sepals and petals to expose the ring of stamens that surround the pistil (Fig. 16.6A and B). The stigma is receptive to pollen on the day before the anthers begin shedding pollen. Some breeders do not remove the stamens. Instead, they use a dominant marker gene, such as purple flower color in the pollinator, that produces a purple hypocotyl in the hybrid $\mathbf{F}_1$ seedling plant. The purple hypocotyl can be observed shortly after emergence of the seedling and is used to distinguish hybrid plants from plants originating from self-pollination. If the stamens are removed, care must be taken to prevent injury to the pistil. Pollinations are made by distributing pollen on the

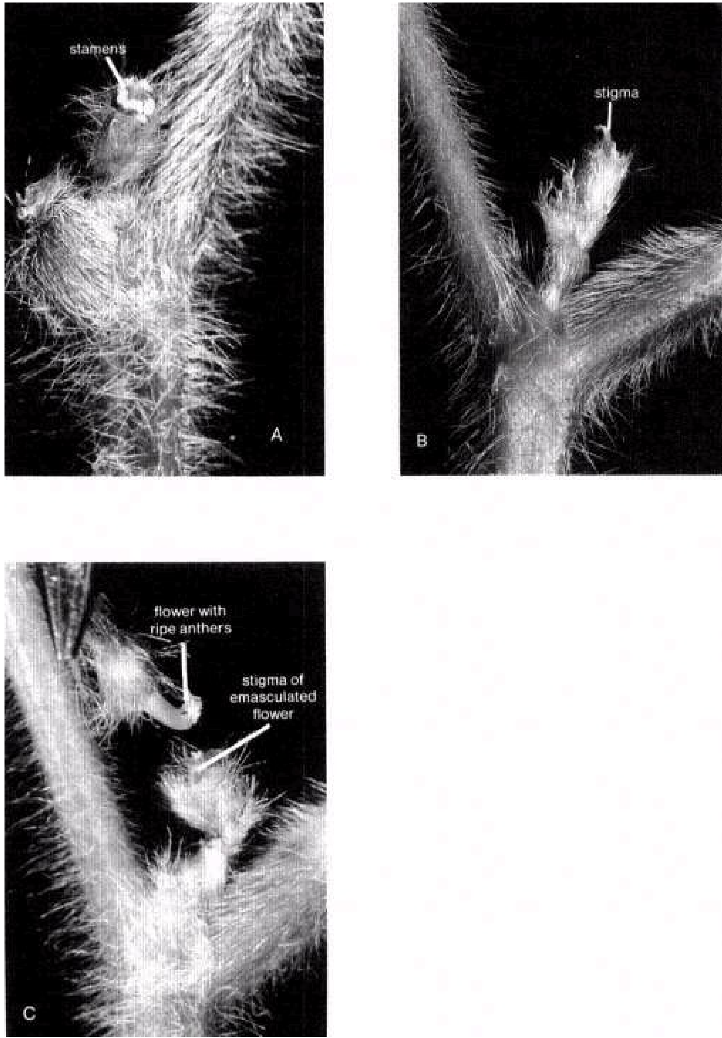


Fig. 16.6.
Steps in crossing soybean. (A) Flower removed exposing a ring of stamens that surround the pistil. (B) Exposed stigma, after the ring of stamens has been removed. (C) Pollinating the flower by rubbing ripe anthers across the stigma. The inverted flower bearing the anthers is being held by fine pointed tweezers (upper left).

stigma immediately after emasculation (Fig. 16.6C). Choosing anthers at the proper stage of development is crucial for obtaining high seed-set. Some breeders select freshly opened flowers in early morning and store the flowers in envelopes in desiccators at 25 to 27ºC to use as a source of pollen later in the day. Flowering of early and late cultivars may be synchronized by a succession of planting dates or by alteration of the photoperiod and temperature.

Effects of Photoperiod and Temperature on Flowering

Soybean is a short day plant, and genotypes differ in photoperiod requirement for flowering. Due to the photoperiod sensitivity, soybean cultivars are adapted for growing in a relatively narrow range in latitude. If soybean cultivars are grown in lower latitudes with summer day-lengths shorter than in the area of their adaptation, flowering is hastened, pods are set lower on the plant, and yield and seed quality are reduced. When cultivars are grown in higher latitudes with summer day lengths longer than in the area of adaptation, vegetative growth will be increased and flowering and seed maturity delayed. In North America, soybean cultivars are classified into *maturity groups*, 000 to X, according to the range in latitudes in which they are adapted and will be most productive (Fig. 16.7). Group 000 is the earliest and group X is the latest maturity group. The largest number of cultivars are found in maturity groups II to VI, corresponding to the latitudes in which there is the most extensive soybean cultivation. Days-to-flowering is also affected by temperature. Within the range of 10 to 30ºC, increasing mean temperature will hasten flowering and reducing mean temperature will delay flowering.

Breeding Methods

The methods used in breeding soybean are those described in Chapter 9 for breeding self-pollinated crops: *introduction and germplasm assembly, selection, and hybridization*. The order listed is a normal sequence for breeding activities in a new crop. Because a major breeding effort on soybean in the United States did not begin until the 1930s, progression through the above sequence of methods was compressed into a period of less than 60 years.

New cultivars and germplasm lines of soybean are registered by the Crop Science Society of America, and descriptions of the cultivars and germplasm lines are published in *Crop Science*.

Introduction and Germplasm Assembly

The soybean is reported to have been first introduced into the United States in 1765. Scattered introductions of soybean cultivars were continued up to the beginning of the present century, although the soybean never became firmly established as a crop during the period. The United States Department of Agriculture began recording introduced soybean lines, generally referred to as *plant introductions*, or more simply, *introductions*, in 1898 and assigning each a permanent P.I. (Plant Introduction) number. In the 30-year period that followed, about 3000 accessions were recorded as introductions. In 1929, the department's Division of Plant Exploration and Introduction sent P.H. Dorsett and W.J. Morse to Japan, Korea, and China to collect soybean seed materials. They returned in 1931 with seeds of 4451 soybean accessions and other propagating materials.

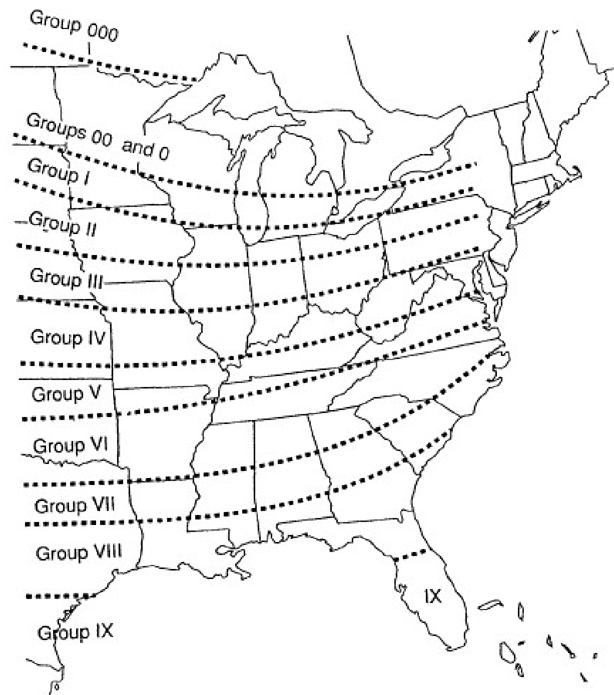


Fig. 16.7.
Map showing approximate areas where different maturity groups of soybean are grown in the United States and Canada. Soybean cultivars are classified into 12 maturity groups, with group 000 cultivars having the earliest maturity and group X the latest maturity. Groups IX and X are grown in semitropical or tropical environments. In the tropics, more than one maturity group can be grown depending upon elevation and season.

Soybean introductions, or selections from introductions, many from the Dorsett-Morse collection, provided the basic germplasm upon which soybean breeding in the United States developed. As late as 1945, 60% of the soybean cultivars grown in the United States were either direct plant introductions or selections from plant introductions. In addition to the introductions, the United States soybean germplasm collection of G. *max*, which now contains about 14,000 accessions, includes cultivars and breeding lines generated from United States breeding programs. Soybean germplasm is maintained at the United States Soybean Research Laboratory, Champaign/Urbana, Illinois.

Soybean cultivars grown in the United States have a relatively narrow genetic base. The ancestry of presently grown cultivars includes not more than 50 of the original introductions,

with 80 to 90% of the gene pool contributed by 10 to 12 introductions in northern United States cultivars, and by seven introductions in southern United States cultivars. This lack of genetic diversity increases the potential vulnerability of the soybean in the United States to outbreaks of plant disease epidemics.

Selection as a Breeding Method

Selection has played an important role in the development of soybean cultivars in the United States. It was utilized in the beginning to purify mixed seed lots of introduced germplasm and currently to isolate pure lines from hybrid populations.

Hybridization as a Breeding Method

After the original soybean plant introductions in the United States collection were purified by selection and superior lines increased and distributed, the next phase in breeding was to combine the superior features of selected cultivars through hybridization. Interestingly, as emphasis shifted from growing soybean for forage to growing soybean with large yellow seeds for industrial uses, seed yields at first declined. But following several generations of crossing and selection in the hybridization breeding programs, the higher seed yields returned and even increased significantly. It proved to be relatively easy to select transgressive segregates for improved seed yield, seed weight, maturity, lodging resistance, or seed quality from hybrid progenies. The improved lines were recycled by intercrossing to create new pools of genetic variability, from which were obtained further increases in yield, adaptation, stronger stems to reduce lodging, or improved seed quality. Addition of genes for resistance to disease pathogens, insect pests, and the cyst nematode by backcrossing reduced yield losses from those hazards and increased yield stability.

Hybrid populations generated from artificial cross-pollinations were advanced through pedigree selection following the early soybean crosses, but single-seed-descent, in conjunction with winter nurseries, has generally supplanted pedigree selection to advance generations rapidly at low cost. Growing a winter nursery in Puerto Rico or a comparable semitropical climate, or in Chile, a temperate climate, during the winter season, is now a common practice with public and private soybean breeders in the United States. Through the winter nursery, hybrid materials can be advanced one generation for determinate growth types, or two generations for early maturing indeterminate growth types. In the single-seed-descent procedure as now generally practiced, two to three pods are harvested from each hybrid plant, and one seed from each pod is placed in a pool to grow the next generation. In winter nurseries, seed set is often improved by artificially increasing day length and light intensity.

Progress in public soybean breeding programs throughout the midwestern and southern soybean regions was hastened by coordination of the state breeding programs through the United States Regional Soybean Laboratory. Annual workshops and planning conferences were held in which both United States Department of Agriculture and State Agricultural Experiment Station breeders participated. Through these planning efforts, research duplication was avoided, comprehensive testing programs developed, and research results rapidly disseminated.

The Backcross

The backcross has been utilized in soybean breeding to:

• add a desirable gene to an otherwise high-yielding cultivar through a succession of backcrosses (Fig. 9.6), or to

• intensify genes for a desirable quantitative character by one or two backcrosses only.

The first procedure was used to add a gene for resistance to the pathogen inciting phytophthora rot disease, or the cyst nematode, to agronomically improved cultivars through a succession of backcrosses to the improved cultivar. The backcross works best when the character being added is controlled by a single dominant gene. The second procedure was used to concentrate genes for a quantitatively inherited character, such as lodging resistance, or high oil content, by crossing an improved cultivar to a cultivar superior for the quantitative character and making one backcross to the cultivar with the superior quantitative character.

Hybrid Soybean

The commercial production of hybrid soybean will require a source of usable cytoplasmic sterility, a chemical hybridizing agent, or other mechanism to facilitate natural cross-pollination; a high percent of seed set from natural cross-pollination; and heterosis sufficient to make growing hybrid soybean profitable. These requisites are currently not available.

Breeding Objectives

Objectives important in breeding soybean are seed yield, maturity for the area of production, resistance to lodging and shattering, tolerance to stress environments, disease and nematode resistance, insect resistance, and seed quality and composition. Much attention has been given to improving the nitrogen-fixing potential of the soybean, but there has been little progress on this objective. In some regions consideration is given to breeding special-purpose soybean to be utilized as vegetables or for culinary purposes.

Seed Yield

The major objective in breeding soybean is to develop cultivars with the potential for high seed yield. This objective is achieved by:

• increasing the genetic potential for yield per se and maximizing regional adaptation, and

• protecting against yield loss from environmental stress, disease, nematodes, or insect pests.

Without the cultivar improvements in yield potential that have been made since the 1930s, the soybean would never have acquired the prominent rank that it now holds as a field crop in the United States, or as the source of protein and oil that it now supplies in the world's markets. Actual yield increases in soybean may seem moderate when compared with the meteoric rise in yields of new short-stature cultivars of wheat or rice, or hybrids of corn and sorghum (Fig. 1.2), all of which benefit from high-yield environments. But a major difference exists between the soybean and the cereals. In seeds of soybean, major storage is in protein (38 to 42%) and oil (18 to 22%), both concentrated food materials, whereas in the cereal grains the major storage is in starch. The pathways for manufacture of oil and protein are more complex than

those for manufacture and storage of starch. It should not be surprising then that increasing yield per se in soybean, although it has been steady and substantial, has not been achieved as rapidly as in the cereal grains. If caloric values of protein and oil are considered when comparisons are made of the soybean with cereals, then the substantial advances in the breeding of soybean are more readily apparent.

Maturity for the Area of Production

The photoperiod response of the soybean cultivar is the major factor affecting the days required to reach flowering and maturity in a particular latitude. Summer photoperiods increase with increases in latitude, and each cultivar has a narrow range of latitude in which it is optimally adapted. The grouping of soybean cultivars in North America into 13 maturity groups, as previously described (Fig. 16.7), is objective in that the classification is determined by growing new cultivars in field trials in particular latitudes and comparing with cultivars with known maturity grouping. Three major dominant genes, E_1, E_2, and E_3, have been identified for late maturity, and a fourth gene, E_4, for sensitivity to long photoperiod.

Resistance to Lodging and Shattering

Although genetically unrelated, resistance to lodging and shattering are both necessary to prevent loss in yield and facilitate machine harvesting of the soybean. A strong stem is the most important character to obtain lodging resistance. The determinate cultivars, described earlier, tend to be shorter and stockier and less likely to lodge on fertile soils than the indeterminate cultivars. In the northern United States Corn Belt, the need for early maturity resulted in breeders selecting for the indeterminate type, but in the southern states the indeterminate types tended to grow to an excessive height, and selection has been for the determinate type. To improve lodging resistance, semideterminate cultivars were produced by transferring the dt_1 gene for determinate growth type into indeterminate cultivars, but the cultivars developed were not competitive in yield.

Pods of the wild soybean dehisce after they are ripe, causing the seeds to shatter. This characteristic tended to be present in some early cultivars of the cultivated soybean. Selection for nonshattering has produced cultivars currently grown that normally stand until harvest without serious seed loss.

Breeding for Stress Environments

Unusual stress environments in which soybean may be subjected include exposure to cold during germination, excessive heat and drought, problem soils, and exposure to injurious herbicides. The soybean is a warm-season crop. Development of strains that germinate, emerge, and resist seedling fungal infection in cool temperatures would permit earlier planting in the higher latitudes of the temperate production areas or during winter seasons in semitropical production areas. Increased heat and drought tolerance could result in improved yield and seed quality and reduced flower shedding in areas where flowering and seed formation occur during periods of high temperature and deficient rainfall (Fig. 16.8). Problem soils on which soybean are grown include calcareous soils on which the soybean exhibits iron deficiency chlorosis, high-aluminum soils in which root growth is restricted, or manganese-toxic soils. Genotype differences to these stress problems has been reported. Soybean may be injured by particular herbicides or from residual effects of herbicides used on the preceding

Fig. 16.8.
The effect of drought stress during flowering of soybean is studied by geneticist Jeffery Tyler, Delta Research Station, Stoneville, Mississippi, by growing soybean cultivars and breeding lines under a shelter that excludes natural rainfall.

crop. Development of transgenic plants with tolerance to widely used herbicides is one of the goals of recent biotechnology research (see Fig. 8.12).

Disease Resistance

In the early years of soybean improvement in the United States, the soybean was relatively disease-free, and disease resistance received less attention in breeding than regional adaptation, lodging and shatter resistance, or high-yield potential. As the soybean became more widely cultivated, diseases became more widespread and destructive. More than 100 pathogens infect the soybean, with about 35 being economically important in the United States. Breeding for disease resistance is a major component in cultivar development research. Due to the number of diseases present, the breeder must choose which disease, or diseases, will receive priority for available resources in the breeding program. Development of inoculation techniques for creating artificial disease epidemics is essential in disease resistance breeding. Breeding problems related to a few of the major diseases are discussed here.

DUNGAL ROOT AND STEM ROTS. A large number of fungal pathogens incite root and stem rots in soybean, but none is more widespread and destructive than that causing phytophthora rot. Phytophthora root and stem rot was first recognized as a disease of soybean

in 1948. Incited by *Phytophthora megasperma* Drechs. f. sp. *glycinea* Kuan and Erwin, phytophthora rot infects soybean at all stages of development. Infected seedling plants die quickly; older plants turn yellow, wilt, and soon die. Entire stands of susceptible cultivars may be wiped out. The phytophthora rot pathogen is highly specialized, with 22 or more identified races. Breeding for resistance has been *race-specific*, using major genes that confer resistance to blocks of several races. Singly, none of the genes control all races, but control is achieved when specific combinations of the genes are pyramided by successively backcrossing them into commercial cultivars or breeding lines. *General* or *non-race-specific* resistance conferred by quantitative genes provides an alternative to using race-specific genes, but incorporation of large numbers of quantitative genes into a cultivar is more difficult because effects of individual genes cannot be recognized.

FUNGAL LEAF SPOTS. Among the fungal leaf spots, soybean rust is a widespread and damaging disease on soybean in tropical Asia, Australia, and Brazil, but is not present in the United States. The casual organism is *Phakopsora pachyrhizi* Syd and P. Syd. Rust pustules develop in chlorotic or brown spots on soybean leaves, petioles, and young stems, followed by premature defoliation and reduction in pod and seed set. Extensive breeding research on soybean rust is in progress at the Asian Vegetable Research and Development Center (AVRDC) in Taiwan and in other South Asian countries.

FUNGAL SEED DISEASES. Purple seed stain, caused by *Cercospora kikuchii* (T. Matsu and Tomoyasu) Gardner, is a fungal disease worldwide that develops on soybean seeds and reduces germination and market value.

BACTERIAL DISEASES. Bacterial blight, the major bacterial disease in soybean, causes widespread damage worldwide, particularly on soybean grown during cool wet weather. The pathogen causing bacterial blight is *Pseudomonas syringae* pv. *glycinea* (Coerper) Young, Dye and Wilkie. Bacterial pustule, caused by *Xanthomonas campestris* pv. *glycines* (Nakano) Dye, develops in warm climates with frequent rain showers and high relative humidity. Disease loss from bacterial blight and bacterial pustule on soybean in southern United States has declined with the development of resistant cultivars. Disease resistance in bacterial pustule is controlled by a single dominant gene from the cultivar 'Clemson Nonshatter.'

VIRAL DISEASES. More than 100 viral diseases are known to infect soybean, of which about one-third have economic importance. General symptoms of viral diseases are yellow/green mottling in leaves, leaf distortion, stunting, and reduced seed production. The viruses are usually seed-borne or insect-transmitted and infect more than one plant species. Virus identification is commonly made through knowledge of the host range, host plant symptoms, means of transmission, and characteristics of the virus particles. Genetic resistance to particular viruses have been identified in cultivars of G. max and in wild species of *Glycine*.

The principal viral disease on soybean is soybean mosaic virus (**SMV**). A member of the virus group, potyvirus, with worldwide distribution, **SMV** is seed-borne and transmitted by numerous aphid species. Strains of the virus have been distinguished that are virus-aphid-specific, meaning that they are transmitted by specific aphid species. Two genes, *Rsv1* and *Rsv2*, have been identified for resistance to specific strains of **SMV**.

NEMATODES. Many species of nematodes feed on the soybean (Fig. 16.9), but the two forms that are most damaging are the soybean cyst nematode, *Heterodera glycines* Ichinohe, and

the root knot nematode, *Meloidogyne* spp. Symptoms of nematode damage are stunting, wilting under moisture or temperature stress, and reduced yields. Nematode damage results from destruction or alteration of root tissues and disruption of the vascular system, limiting nutrient and water uptake by the soybean plant. Injury to roots by nematodes often permits invasion of fungi that further damage the soybean plant.

The soybean cyst nematode (**SCN**) has spread to all soybean production areas in the United States. The eggs of the soybean cyst nematode can remain encased in a cyst in the soil for several years. Sixteen races of the **SCN** organism have currently been described on host differentials. Most nematode populations are mixtures of several races. Inheritance of resistance is complex; three recessive genes rhg_1, rhg_2, *and* rhg_3, and one dominant gene, Rhg_4, have been identified in 'Peking,' and an additional dominant gene has been reported in PI88788. The resistance genes have been introgressed in resistant cultivars conferring resistance to the common SCN races 3, 5, and 14. A new cultivar, 'Hartwig,' has been released that is resistant to all currently known races of SCN in the United States.

Six species of *Meloidogyne*, the root knot nematode, infect soybean, producing galls on roots, stunting, and chlorosis. *Meloidogyne* species are race-specific. Resistant cultivars are grown in the southern United States where root knot nematode is most prevalent.

Insect Resistance

Several hundred species of insects feed on the soybean plant, but most of the damage is caused by a relatively few species. Research on breeding for resistance to insects was started much later than breeding for resistance to diseases because insect damage was less visible than disease damage, and insecticides for control of the major insects were usually available if the damage became severe. Progress has been made in the United States in identifying introduced strains of soybean with multiple resistance to feeding by the Mexican bean beetle, the soybean looper, the bollworm, and the tobacco budworm. The mechanism of resistance involves both nonpreference and antibiosis. Procedures for multiplication of insect populations and infestation of soybean breeding lines are necessary for insect resistance breeding. Because the resistant introductions are often poor

Fig. 16.9.
Roots of soybean infested with female cyst nematodes. The female nematodes appear here on the soybean root as small round cysts, about the size of a pinhead. The cysts are filled with eggs that hatch and develop into adult nematodes in about one month. The young nematodes invade the roots disrupting movement of water and nutrients. Sixteen races of the soybean cyst nematode have been identified. Resistant cultivars have been developed.

agronomic types, resistance genes are transferred into adapted genotypes by backcrossing. Worldwide, the southern green stink bug and related species are major insect pests causing seed damage and pod abortion by piercing young pods and feeding on the plant juices. Azromyzid beanflies cause serious damage to seedling plants in Asia and Africa, often destroying entire stands of soybean.

Seed Quality and Composition

The soybean is the world's leading source of vegetable oil and protein meal. The oil extracted from soybean is used for cooking oil, margarine, and salad dressings. The protein meal remaining is used as feed for poultry and livestock and is processed for industrial products. In Asia, the soybean is utilized for a variety of food products, including fermented products, soy sauce, soybean milk, tofu, and bean sprouts. Breeding for seed quality involves the development of cultivars with attention to:

• marketable seed (in the United States) and resistance to field weathering (in tropical climates),

• protein content and quality,

• oil content and quality, and

• food and industrial uses.

Breeding to improve protein and oil content of soybean seeds has been facilitated by utilization of near-infrared spectroscopy that makes it possible to analyze large numbers of soybean genotypes.

MARKET QUALITY. Breeding for high market quality in the United States involves development of cultivars with bright seed color; freedom from defective, shriveled, or diseased seeds; and high germination. Commercial cultivars have yellow seed coats. Plump seeds are generally associated with high oil content.

RESISTANCE TO FIELD WEATHERING. Preharvest injury by seed pathogens, mechanical damage to harvested seed, or improper storage causes seed germination to deteriorate rapidly. Selection for hard seedcoat and resistance to *Phomopsis* spp. infection reduces field weathering associated with preharvest pathogen injury.

PROTEIN CONTENT AND QUALITY. The protein content of the soybean averages 38 to 42%. After the oil is extracted, the protein fraction remaining in the meal averages around 50%, making the soybean meal a sought-after ingredient in rations for poultry and swine, with a monetary value exceeding that of the oil fraction. Increasing the inherent protein content in the soybean seed is limited by an inverse oil:protein ratio in which an increase in protein content is associated with reduced oil content. Seed protein content is a quantitative character with complex inheritance. The essential amino acid, methionine, is deficient in soybean protein, and supplementation is required for balanced nutrition.

OIL CONTENT AND QUALITY. Soybean oil makes up more than 75% of the market share of edible vegetable oils in the United States. The amount of oil in the seed of the soybean varies from 18 to 22% but accounts for about 40% of the value of the seed. The oil content is influenced by the cultivar and the environment. Increases in oil content are made at the

expense of the protein content. Inheritance of oil content is complex, controlled by additive genetic variance but may be altered through recurrent selection procedures. In soybean used for food, reduced oil content is preferred. Selection for a high level of oleic acid tends to reduce the level of linolenic acid and improves the flavor and stability of the oil.

MISCELLANEOUS FOOD USES. Soybean is widely used in various forms as food in China and other Asian countries, but their use for food in the United States was slow to develop. Numerous cultivars were introduced in the 1920s as "vegetable varieties." Attempts to popularize them were unsuccessful due to an unpleasant or "beany" taste caused by anti-nutritional factors present in raw soybean. Inactivation of the anti-nutritional factors is necessary for efficient utilization of soybean in food products. Progress is being made in inactivating the anti-nutritional factors through breeding.

Asian Vegetable Research and Development Center (AVRDC)

The Asian Vegetable Research and Development Center (AVRDC) is a multinational research institution located at Shanhua (Tainan), Taiwan, Republic of China. The soybean is one of six crops on which breeding research is conducted at AVRDC. Major emphasis is on the breeding and production of soybean for the tropics and subtropics, with special consideration to breeding for resistance to tropical diseases, including soybean rust. AVRDC has joined with the International Institute of Tropical Agriculture (IITA) in Nigeria in developing an international soybean research network to cooperate with national soybean breeding programs in lesser-developed countries.

Study Questions

1. What different plant types of soybean are available commercially? What are the characteristics of these different plant types, and where are they grown?

2. What breeding methods are used to develop improved cultivars of soybean? What other crop plants can these same breeding procedures be used on to develop improved cultivars?

3. What are the breeding objectives for soybean? How do these breeding objectives differ for the different production areas of soybean?

Further Reading

Anand, S.C. 1992. Registration of 'Hartwig' soybean. Crop Sci. 32:1069-70.

Dashiell, K.E., L.L. Bello, and W.R. Root. 1987. Breeding soybeans for the tropics. p. 3-16. *In* S.R. Singh, K.O. Rachie, and K.E. Dashiell (eds.) Soybeans for the tropics, research, production, utilization. John Wiley and Sons, Chichester, England.

Hymowitz, T., R.G. Palmer, and R.J. Singh. 1991. Cytogenetics of the genus *Glycine*. p. 53-63. *In* T. Tsuchiya and P.K. Gupta (eds.) Chromosome engineering in plants: Genetics, breeding, evolution. Part B. Elsevier Science Publishers, Amsterdam.

Jackal, L.E.N., A.R. Panizzi, G.G. Kundu, and K.P. Srivastava. 1990. Insect pests of soybean in the tropics. p. 91-156. *In* S.R. Singh (ed.) Insect pests of tropical food legumes. John Wiley and Sons,

Chichester, England.

Kogan, M. 1980. Insect problems of soybeans in the United States. p. 303-25. *In* F.T. Corbin (ed.) World soybean research conference II: Proceedings. Westview Press, Boulder, CO.

Myers, O., Jr., J.H. Yopp, and M.B.S. Krishnamani. 1986. Breeding soybeans for drought resistance. p. 203-43. *In* J. Janick (ed.) Plant breeding reviews, Vol. 4, AVI Publishing Co., Westport, CT.

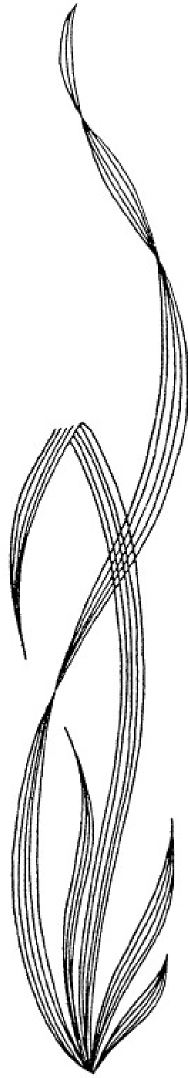
Rao-Arelli, A.P., S.C. Anand, and J.A. Wrather. 1992. Soybean resistance to soybean cyst nematode race 3 is conditioned by an additional dominant gene. Crop Sci. 32:862-64.

Shanmugasundaram, S., G.C. Kuo, and A. Na-Lampang. 1980. Adaptation and utilization of soybeans in different environments and agricultural systems. p. 265-77. *In* R.J. Summerfield and A.H. Bunting (eds.) Advances in legume science. Royal Botanic Gardens, Kew, London.

Shibles, R. (ed.). 1985. World soybean research conference III: Proceedings. Westview Press, Boulder, CO.

Wilcox, J.R. 1983. Breeding soybeans resistant to diseases. p. 183-235. *In* J. Janick (ed.) Plant Breeding Reviews. Vol. 1., AVI Publishing Co., Westport, CT.

Wilcox, J.R. (ed.). 1987. Soybeans: Improvement, production, and uses. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.



VII
APPLICATIONS:
FIELD CROPS UTILIZING HYBRID BREEDING PROCEDURES

17. Breeding Corn (Maize)

Corn, or maize (*Zea mays* L.), is the world's third leading cereal crop, after wheat and rice. The United States produces nearly 40% of the total world production. The next largest corn-producing countries are the People's Republic of China and Brazil. Corn is the leading grain crop in the United States, with average production (metric tons) approximately three times that of wheat, the next leading grain crop. Corn is the primary food grain in Mexico, Central America, and the Andean region of South America, and is important as a food grain in eastern and southern Africa and China. In the United States, corn is used primarily as a feed grain for livestock and for industrial products.

Corn is a naturally cross-pollinated crop, and due to its uncontrolled pollination is often referred to as being *open-pollinated*. The principal contributions to corn improvement during the twentieth century have been:

• a method for breeding hybrid corn and development of the infrastructure for large-scale, commercial production of hybrid seed, and

• genetic improvements in the corn plant that contribute to its increased productivity, earlier maturity, stronger root systems which combined with shorter and stronger stalks reduce lodging, and resistance to destructive disease pathogens and insect pests.

Because of the important place of corn in United States agriculture, it is appropriate that a system for breeding hybrid corn is the foremost contribution of United States scientists in plant breeding.

Origin of Corn

The corn plant is indigenous to the Americas and was the principal food grain of Native Americans. Corn was domesticated about 8000 years ago and is no longer capable of survival in its wild form. During the centuries that corn was cultivated before Europeans came to the Americas, Native Americans accomplished remarkable feats by evolving races of flint, flour,

gourdseed dent, pop, and sweet corn. Building on this legacy, early American farmers evolved high-yielding, open-pollinated dent cultivars adapted to the central Corn Belt and the eastern and southern regions of the United States, and early maturing flint cultivars for northern United States. Modern corn cultivars differ from primitive corn in having more productive plants due to an increased number and weight of individual kernels on a cob of corn.

How corn evolved and its early progenitors have been vexing matters of speculation. It now seems to be generally accepted that corn originated from *teosinte*, the nearest known relative of corn. There is still discussion as to whether corn originated by a single domestication from the basal branching teosinte subspecies *Zea mays* L. spp. *parviglumis*, or from the lateral branching subspecies Z. *mays* L. spp. *mexicana*, or by a dual domestication from the two subspecies (Fig. 17.1). Teosinte is native to Mexico and Guatemala and in its native habitat may be found growing wild in cultivated fields of corn. The wild annual forms of teosinte have the same chromosome number as corn and cross readily with corn to produce fertile hybrids. Teosinte, like corn, is monoecious in flowering habit, with staminate and pistillate flowers borne in separate inflorescences; it differs from corn in that the pistillate spikes bear 6 to 12 kernels in hard triangular, shell-like structures. The teosinte seed structures break apart and shatter when mature, forming a natural means of seed dispersal.

The geographic origin for a crop species is identified by locating areas in which there are large numbers of diverse types. On this basis, corn has two possible centers of origin; the highlands of Peru, Ecuador, and Bolivia; and the region of southern Mexico and Central America.

Races of Corn

Beginning in 1943, several thousand local varieties of corn were collected in Mexico, Peru, Bolivia, Brazil, Guatemala, and other countries of Central and South America by scientists from the Rockefeller Foundation, the United States Department of Agriculture, and the Mexican Ministry of Agriculture. Local varieties from the same geographic area that had similar morphological, physiological, genetic, and cytological characteristics were grouped together into more or less distinct races. The classification of local varieties into races facilitated the breeder's search for germplasm containing particular characters of use in the breeding program. Unfortunately, many of the original collections were lost because a centrally located and adequately equipped storage facility was not available at that time. New collections have been made and over 13,000 germplasm accessions are currently stored in new facilities at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico, with duplicate storage in Colombia, Peru, and the United States National Seed Storage Laboratory at Fort Collins, Colorado.

Varieties of corn indigenous to the United States, except for sweet corn and popcorn, have been classified into 9 or 10 racial groups, the most important races being the *Corn Belt dents*, the *southern dents*, and the *northern flints*. Within these races, early farmer-breeders developed open-pollinated varieties by repeated selection for particular plant and ear characteristics. These open-pollinated varieties later became the germplasm base from which modern hybrid corn cultivars were developed. Open-pollinated varieties are no longer grown by farmers in the United States, having been replaced by hybrid corn, but are still grown in some underdeveloped countries.

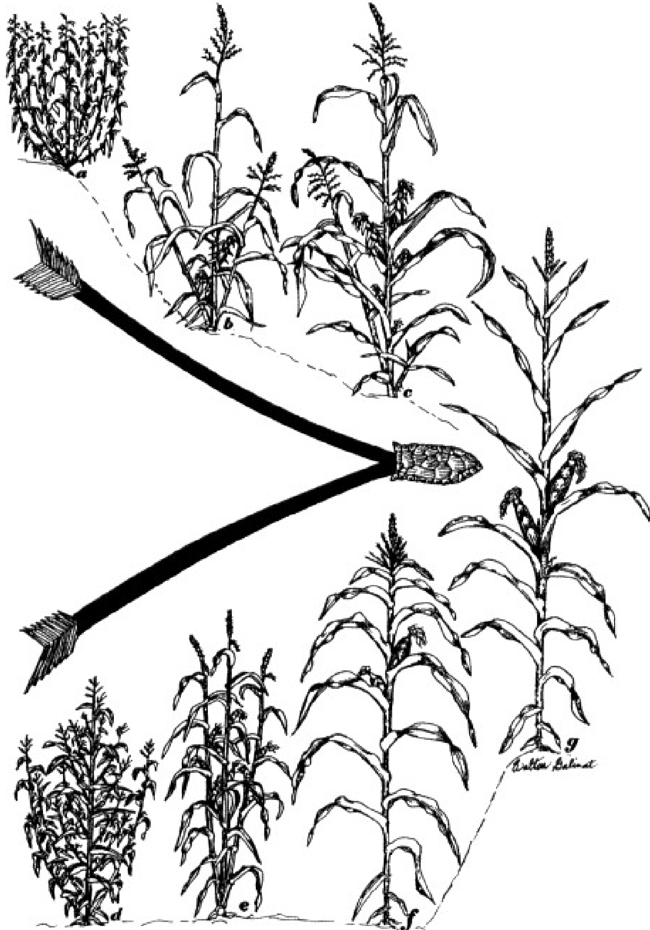


Fig. 17.1.
Proposed pathways for double origin of corn from different subspecies of teosinte.
Upper: Basal branching type from subspecies parviglumis. Note proliferation of
tillers at the base of the plant. Lower: Lateral branching type from subspecies mexicana.
Note that branching is lateral, with each branch terminating in a tassel. (After W.C. Galinat.)

Genetics and Cytogenetics

Corn is a diploid species with chromosome a number of $2n = 2x = 20$. It has been the object of more intensive genetic and cytogenetic studies than any other crop species as the result of:

• the economic importance of corn as a field crop in the United States,

• the ease with which corn can be genetically manipulated by either self- or cross-pollination,

• the large number of seeds obtained from a single pollination,

• the easily observed plant and seed characters available for study,

• the recovery of many recessive alleles by inbreeding or use of mutagenic agents,

• the small number of chromosomes; corn is a diploid species ($n = 10$), and

• the ability to recognize individual chromosomes under the microscope from their length and the presence on them of distinctive knobs.

Genetic studies on corn have contributed substantially to understanding genes and gene action, the mutation process, heterosis, and quantitative genetic theory. In the breeding of corn, a cross-pollinated species, quantitative genetics has a more important role than in the breeding of self-pollinated species. The system of breeding hybrid corn originated from a genetic study, an event that stimulated further genetic investigations in the corn species. In addition to the abundance of natural variation in the species, new mutant forms are readily induced by radiation and chemical mutagens. The genetic mapping of the corn genome is more complete than for any other plant species. More than 1000 loci have been studied, and the positions of more than half of the loci have been established on linkage maps of the 10 chromosomes. The gene symbols, chromosome location, name, and phenotype of the genes studied are recorded annually in a *Maize Genetics Cooperation Newsletter*, published by the United States Department of Agriculture and the Department of Agronomy, University of Missouri, Columbia. Genetic stocks of corn are maintained and made available to research scientists from the Maize Genetic Stock Center, University of Illinois, Urbana-Champaign.

Molecular Biology

The considerable information generated from the genetic mapping of the corn nuclear genes heightened interest in the molecular mapping of genetic markers in the corn genome by restriction fragment length polymorphism (RFLP) techniques and other means and the utilization of the molecular markers as breeding tools. The RFLP technology has the potential for screening inbred lines for specific genetic traits. An RFLP marker associated with a nuclear gene for a character difficult to identify from visual observation of the corn plant would be useful in locating the nuclear gene on the chromosome and in identifying other corn plants possessing the gene.

The insensitivity of monocots such as corn to *Agrobacterium* infection has limited the effectiveness of this procedure for genetically transforming the corn plant, but the particle-gun technique offers a potential, reproducible system by which transformation can be attained in corn. Regenerating plants through cell and tissue culture techniques is a prerequisite for creation of transgenic plants. Transformation in corn will be facilitated by the information

generated by the molecular mapping of the corn chromosomes. Transformation does not replace conventional breeding procedures, but it provides a new breeding tool whereby DNA may be inserted into the corn genome from a wider range of donors than is possible by traditional cross-fertilization procedures.

Flowering and Pollination

The corn plant has *monoecious* flowering structures with *staminate* flowers borne in the tassel and *pistillate* flowers borne on a shoot midway of the stalk. Pollination is consummated by transfer of viable pollen from the staminate flowers in the tassel to the silks, the receptive organs of the pistillate flowers. Wind is the principal agent in the uncontrolled or *open-pollination* of the corn plant. Normally, about 95% of the ovules on a shoot are cross-pollinated and 5% self-pollinated. Most of the pollen that pollinates an ear of corn comes from plants in the immediate vicinity, although the pollen may be carried by the wind for great distances. It is not uncommon to observe occasional yellow grains in ears of white corn even though the nearest field of yellow corn from which the pollen could have originated was 1000 m distant.

The main stem of the corn plant terminates in a tassel which bears two-flowered staminate spikelets, each flower having three anthers (Fig. 17.2). As the tassel flower opens, the anthers are pushed out by elongating filaments (Fig. 17.3), and pollen grains are emptied from the extruded anthers. A single tassel from a normal plant may produce 25,000,000 pollen grains, or an average of over 25,000 pollen grains for each kernel on an ear of corn. Pollen shedding usually begins one to three days before the silks emerge from the shoots of the same plant and continues for three to four days after the silks on the plant become receptive to pollen. The pollen may be killed by temperatures above 35ºC (95ºF) during the pollination period. Considering the large number of pollen grains produced, seed set is normally unaffected if 10% of the pollen grains survive. In the breeding nursery where pollen supply may be limited, high temperatures can severely reduce seed set.

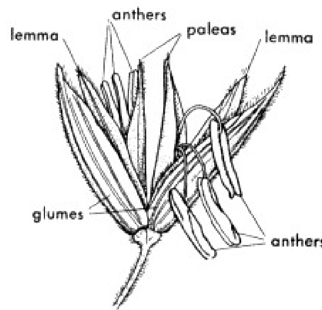


Fig. 17.2.
Staminate spikelet of corn. Staminate spikelets are two-flowered, each with three anthers.

The ear shoots arise as branches from nodes about midway on the stalk. Each shoot is composed of a shank from which the husks arise and terminates in the cob on which the pistillate flowers are borne (Fig. 17.4A). The spikelets are borne in pairs, each spikelet normally containing one fertile and one sterile ovule, resulting in an even number of rows of kernels on the ear. Fertilization of the second ovule produces crowded and irregular rows of kernels on the ear. The silks are attached to the tip of the ovary (Fig. 17.4B). The silks function both as stigma and style and are receptive to fresh pollen throughout their entire length. Fertilization of the ovule usually occurs within 12 to 24 hours after the

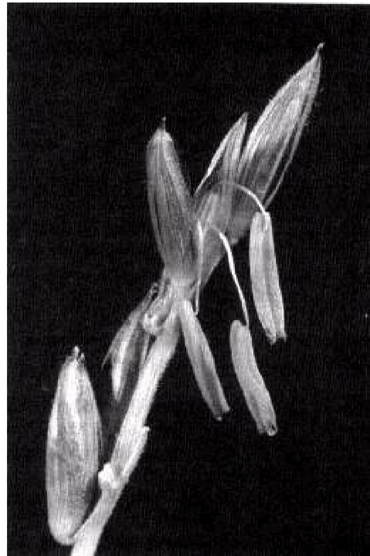


Fig. 17.3.
Tassel branch of corn with anthers exerted from a staminate flower.

silks have been pollinated. Severe drought delays emergence of the shoots, which combined with early termination of pollen shedding, will result in failure to set seed and in the production of barren or partially barren ears.

Xenia

Xenia is the immediate effect of pollen on the developing kernel. When yellow corn pollen fertilizes an ovule of white corn, a light-yellow kernel develops. When white corn pollen fertilizes an ovule of yellow corn, a medium yellow kernel develops. By cutting a kernel of yellow corn lengthwise it will be observed that the yellow color is found only in the vitreous starch of the endosperm, whereas the seedcoat is white or transparent. The endosperm develops from fusion of the second sperm nucleus with the diploid polar nuclei and has a triploid chromosome number. Yellow endosperm color is conditioned by a dominant gene (*Y*); the recessive alleles (*yy*) produce a white endosperm. Because the endosperm receives two sets of chromosomes from the polar nuclei, it will receive two genes for *Y*, or *y*, depending on the character of the mother plant, to one gene for *Y*, or *y*, from the pollen. Table 17.1 lists the possible combinations of endosperm color genes from the polar nuclei with endosperm color genes from the pollen and the xenia effect on endosperm color of the developing kernel. In addition to yellow vs. white endosperm color, endosperm characters that exhibit xenia are purple vs. colorless aleurone (Fig. 17.5A), starchy vs. sugary endosperm (Fig. 17.5B), nonshrunken vs. shrunken endosperm, and nonwaxy vs. waxy endosperm.

Table 17.1.
Endosperm color genes and xenia effect in corn endosperm

Endosperm color genes in polar nuclei		Endosperm color genes in sperm		Endosperm color genes and xenia effect in endosperm
YY	+	*Y*	=	*YYY* (deep yellow)
YY	+	*y*	=	*YYy* (medium yellow)
yy	+	*Y*	=	*Yyy* (light yellow)
yy	+	*y*	=	*yyy* (white)

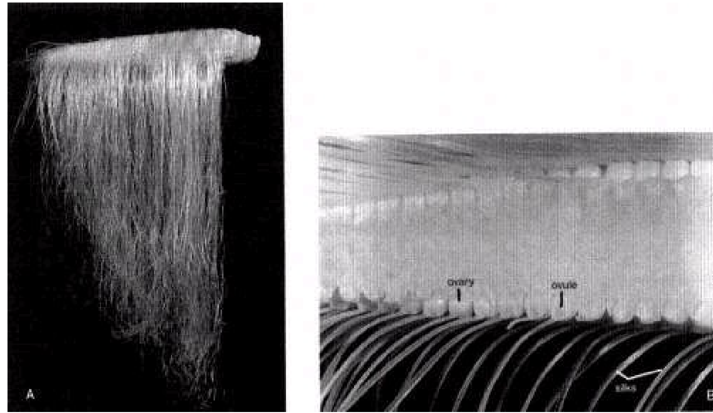


Fig. 17.4.
Ear shoot of corn. (A) With husks removed. A fresh silk is receptive to pollen along its entire length. (B) Cross section of ear shoot showing silks attached to tip of the ovaries.

Heterozygosity of Open-Pollinated Corn

Heterozygosity and genetic variability are characteristics of cross-pollinated crops (Chapter 10). Conceivably, every ovule on an ear of open-pollinated corn could be pollinated by a different pollen parent; this makes it doubtful that any two seeds on an ear of corn, or any two plants in a field of open-pollinated corn, have exactly the same genotype. Each corn plant is a different hybrid genotype, and a field of open-pollinated corn is a mixture of complex hybrid plants with both genotypic and phenotypic variation (Fig. 11.1). With each new generation, there is a reshuffling of the genes, which keeps open-pollinated corn highly heterozygous and maintains its genetic variability.

Breeding Open-Pollinated Corn

Improvement in corn has taken place through selection by Native Americans since its earliest cultivation. Choosing an ear of corn for seed was an obvious practice that had to be repeated each time corn was planted. In the United States, many open-pollinated cultivars of dent corn were developed by farmer-breeders during the latter half of the nineteenth century.

Mass Selection

Mass selection as a breeding procedure is used to maintain existing open-pollinated cultivars and for developing new cultivars. In the mass selection method of breeding open-

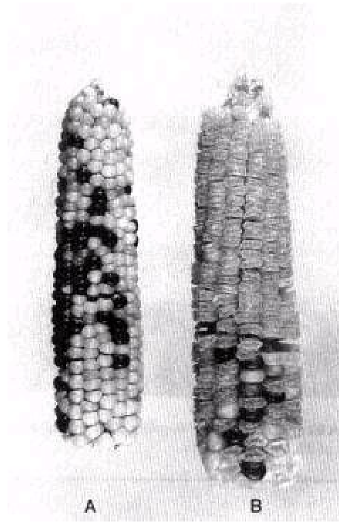


Fig. 17.5.
Xenia in corn. (A) White corn partly pollinated from purple corn. (B) White, sugary endosperm corn partly pollinated from white and purple, nonsugary-endosperm field corn.

pollinated corn, ears are chosen on the basis of visible plant and ear characteristics. Seeds shelled from the ears are mixed and planted en masse. Mass selection is a form of recurrent selection with the plant as the unit of selection and with selection repeated each generation. New variety types of open-pollinated corn were developed for new production areas, such as the early dents for the northern Corn Belt, or for specific purposes, such as large, woody cobs for making cob pipes. Each farmer became a breeder as he selected ears for planting the next year's crop. By repeated selection the farmer-breeder could change the appearance of the corn plant for visible characteristics such as height, maturity, or ear and kernel conformation. Selection was often based on ear and kernel types that would win prizes in a corn show, or just to suit the fancy of the breeder, without knowledge of the effect that the selection would have on performance. Although the physical appearances of the open-pollinated cultivars were changed by mass selection, yield was not significantly improved because:

• selection was based on visible characters of the plant generally unrelated to yield,

• superior plants were pollinated at random with pollen from both superior and inferior yielding plants, and

• rigorous selection for specific plant characteristics often led to inbreeding and a decrease in vigor.

It has since been demonstrated that mass selection can be effective in improving yield if grain weight rather than unrelated visual features is the primary selection objective and if experimental procedures are followed that reduce the effect of the environmental variation on selection. The latter was accomplished by subdividing the experimental area into small *subplots* or *grids*. Each subplot was harvested separately, and only the heaviest ear from each subplot was retained for planting the next generation. Beginning in the 1930s, mass selection was discontinued as a breeding procedure in the United States but is still used in underdeveloped countries.

Ear-to-Row Breeding

The ear-to-row breeding method was first used by C.G. Hopkins at the Illinois Agricultural Experiment Station about 1896 in selection experiments to increase protein and oil content in corn. The essential features of the ear-to-row system of breeding, as it later developed, are as follows:

• fifty to 100 ears are shelled separately with part of the seed from each ear planted, an ear to a row; remnant seed from each ear is labeled and stored separately,

• each row is scored for desirable characters and harvested for yield so that the superior rows may be identified, and

• remnant seed lots from superior rows are mixed and used to plant an open-pollinated plot the second year, from which ears are selected for repeating the process.

Visual plant and kernel characters could be altered with the ear-to-row method of breeding in the same manner as with mass selection, but the changes occurred more rapidly because the pollen source was controlled and limited to selected genotypes. For characters such as yield that are not evaluated accurately by visual observation, the method was ineffective for the same reasons that mass selection was ineffective. By modifying the procedure to include replication, so that environmental effects on yield could be separated from genetic effects, yield could be altered by ear-to-row selection. The ear-to-row procedure is the same as the procedure described in Chapter 10 as 'half-sib with progeny test.'

Variety Hybridization

Hybridization between open-pollinated cultivars of corn, either intentional or accidental, was responsible for the origin of many of the commercial cultivars of open-pollinated corn. In 1880, W.F. Beal at the Michigan Agricultural Experiment Station described a variety hybridization experiment in which a cultivar of open-pollinated corn was detasseled and pollinated by a second cultivar growing in an adjacent row. An increase in yield was obtained when seeds harvested from the detasseled row were planted. Beal described a procedure by which farmers could produce their own crossed seed that closely resembles present hybrid seed production procedures. But the technology was too advanced for that period and variety hybridization never became popular with farmers.

Hybrid Corn

Corn became the model for breeding hybrid cultivars. The double-cross hybrid, proposed by D.F. Jones in 1918, became the model for breeding hybrid corn until replaced by the single-cross hybrid in the 1960s. During the 1920s and 1930s, major efforts in breeding hybrid corn were directed toward development of inbred lines from open-pollinated cultivars and fitting the inbred lines into productive single- and double-cross hybrid combinations adapted to the United States Corn Belt. This effort, led by F.D. Richey for the United States Department of Agriculture, H.K. Hayes in Minnesota, M.T. Jenkins and G.F. Sprague in Iowa, and many other corn breeders, was dedicated to finding the most efficient procedures for breeding hybrids. By the 1940s hybrid corn had replaced most of the open-pollinated corn throughout the United States Corn Belt and was being introduced to other major corn producing areas of the world.

The 1950s and 1960s brought innovations that further changed corn hybrid seed production practices:

- introduction of cytoplasmic male-sterility (**cms**) to eliminate detasseling, and
- replacement of double-cross hybrids with productive single-cross hybrids.

Until the concept of hybrid corn, there was no breeding method by which every plant within a field of corn would be a high-yielding genotype. Additionally, the breeder's ability to identify the high yielding plants was limited because procedures for field-plot testing and data analyses available at that time did not permit separation of genetic and environmental effects on yield. For hybrid corn to be widely grown, it was also necessary that the hybrid seed be available at prices that the farmer could afford. To meet this need private seed companies emerged to produce and market the hybrid seed. Most of the seed companies developed extensive breeding and research programs, and, over time, the breeding of hybrid cultivars of corn passed from the publicly supported breeding programs to the private breeding programs.

What is Hybrid Corn?

Hybrid corn is the first-generation progeny from a cross between inbred lines or hybrids among them. The double-cross hybrid has been replaced by the single-cross hybrid, modified single-cross hybrid, and three-way cross combinations. All are based on the farmer growing F_1 populations of crosses among homozygous inbred lines.

INBRED LINES. Inbred lines of corn are populations of identical (or nearly identical) homozygous plants usually developed by self-pollination. Inbred lines are (*a*) the products from inbreeding heterozygous plants from open-pollinated populations until homozygosity is reached or (*b*) products from inbreeding segregating populations following a cross between two inbred lines. The latter is comparable to the hybridization procedure in breeding self-pollinated species. In producing inbred lines in corn, pollination is controlled, as illustrated in Figure 17.6A to 17.6F.

SINGLE-CROSSES. A single-cross is the hybrid progeny from a pollination between two homozygous inbred lines (Fig. 17.7). Single-cross plants are heterozygous at all loci in which the parent inbreds differ; yet, within the single-cross, plants are genetically identical (or nearly identical). In the farmer's field, the single-cross hybrid is uniform in appearance, maturity, and yield potential; yet it exhibits vigor and productiveness that was lost during inbreeding. The combinations of inbred lines that may be crossed to produce superior-yielding single-crosses are rather rare. So new inbred lines are tested for general and specific combining ability (**gca** and **sca**) to identify productive single-cross combinations (see Chapter 11). Modem inbreds are more vigorous and productive than those developed earlier, and kernel size and shape approach that of hybrids. These improvements made it possible for seed producers to market and the farmer to grow single-cross corn hybrids.

In the commercial production of single-cross hybrid seed, the parent inbreds are planted in separate rows in an isolated field. Choice of inbred to be used as the pollen parent, and inbred to be used as the seed parent, will generally be determined by which inbred produces the most plentiful supply of pollen and which inbred produces the largest yield of seed. A planting pattern in common use for single-cross hybrid seed production is to plant one pollen-parent row to four seed-parent rows (ratio 1:4) (Fig. 17.8). In this arrangement, one-half of

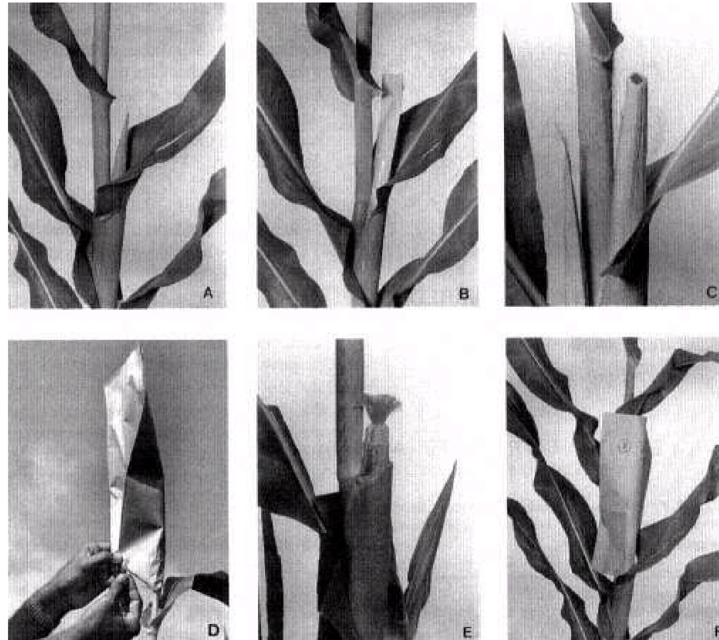


Fig. 17.6.
Steps in selfing and crossing corn. (A) Ear shoot of corn emerging from the leaf sheath. Ear shoots are covered 1 to 2 days before the silks emerge to prevent their being pollinated. (B) A parchment ear shoot bag has been slipped over the ear shoot. (C) The ear shoot is cut back on the day before pollination and the shoot bag replaced immediately. (D) A tassel bag is fastened over the tassel on the day before the pollen is to be collected. (E) Silk brush grown out ready for pollination. The brush provides a uniform growth of fresh silks on which to distribute the pollen. (F) After pollination of the brush, the tassel bag is fastened over the shoot to protect the developing ear.

the seed-parent rows are adjacent to a pollen-parent row, and none are more than two rows from the pollen parent. The seed-parent rows are detasseled and pollinated by wind-blown pollen from the adjacent pollinator parent. The pollen-parent row is destroyed after pollination to prevent seed mixture during harvest. The pedigree of a single-cross made from inbreds **A** and **B** is written **A** × **B** where **A** is the seed parent and **B** is the pollen parent.

MODIFIED SINGLE-CROSSES. A modified single-cross is the hybrid progeny from a three-way cross, which utilizes the single-cross from crossing two related inbreds as the seed parent and an unrelated inbred as the pollen parent (Fig. 17.9). The two related inbred lines (A' and

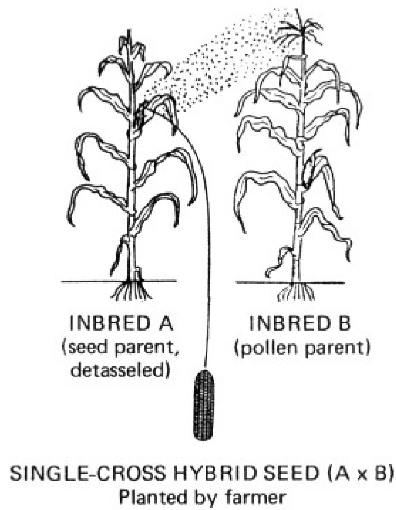


Fig. 17.7.
Procedure for making single-cross hybrid corn. The seed parent, inbred A, is detasseled and pollinated from the pollen parent, inbred B.

A") are genetically similar with respect to plant type but genetically different to the extent that when crossed heterosis for vigor and yield is expressed. This type of hybrid has the advantage of a single-cross hybrid in the farmer's field and that of a three-way cross in seed production. The pedigree of a modified single-cross made with inbreds **A', A**", and **B** is written (**A' × A") × B**.

THREE-WAY CROSSES. A three-way cross is the progeny from a cross between a single-cross hybrid and a third inbred line. The three-way cross differs from the modified single-cross in that all three inbreds are unrelated and the hybrid progeny will be more diverse genetically and less uniform, but it has the advantage that the seed is produced on a single-cross instead of an inbred line. The pedigree of a three-way cross made with the single-cross (**A** × **B**) and the inbred C is written (**A** × **B**) × **C**.

DOUBLE-CROSSES. A double-cross is the hybrid progeny from a cross between four unrelated inbreds. The inbreds are crossed m pairs to produce two single-crosses, which are then crossed to produce the double-cross. The pedigree of a double-cross made with inbreds **A, B, C**, and **D** is written (**A × B**) × (**C × D**).

TOP CROSSES. A *top cross* (also called an *inbred-variety cross*) is made by pollinating an inbred line or single-cross with pollen from a genetically mixed population.

MULTIPLE CROSS. A multiple cross is the product of any combination of crosses using more than four inbreds.

Cytoplasmic Male Sterility in Hybrid Seed Production

Prior to the 1950s, hybrid seed corn was produced by the conventional procedure of alternately planting rows of seed-producing and pollinator parents and detasseling the seedproducing (female) parent rows, which would then be pollinated from the pollinator (male) parent rows. During the 1950s, a cytoplasmic-male sterile, fertility-restorer gene system was introduced that replaced detasseling in the production of hybrid seed corn. The male-sterile cytoplasm then used was obtained from the open-pollinated cultivar 'Mexican June' and became known as the Texas-type cytoplasm, or **cms-T**. Fertility was restored by restorer genes R_{f1} and R_{f2} and additional modifier genes. The **cms** and the fertility restorer genes were introduced into the inbreds by a series of backcrosses (Chapter 11).

Fig. 17.8.
Commercial seed-production field of single-cross hybrid corn planted in a ratio of 1 male pollinator row to 4 female seed-parent (detasseled) rows. With this planting pattern, one-half of the seed-parent rows are separated from a pollen-parent by only one row. Additional pollinator rows are planted at the edge of the seed-production field to provide for pollen saturation and ensure genetic purity. Soybean planted at the left of the field provides additional isolation as required for hybrid seed production.

In 1970, a corn leaf blight disease incited by the pathogen *Bipolaris maydis* (Nisik.) Shoem. (Syn. *Helminthosporium maydis* Nisik. and Miyake) spread through the Corn Belt. The pathogen was virulent on corn hybrids with the **cms-T** cytoplasm. Because more than 90% of the hybrid corn being grown in the United States at that time contained the **cms-T** cytoplasm, damage from the leaf blight disease was extensive. As a result of this experience, the utilization of the cytoplasmic-male sterile:fertility-restorer gene system to eliminate detasseling in corn was discontinued. Producers of hybrid seed corn then returned to detasseling in hybrid seed production.

Breeding Improved Hybrids

A new era in plant breeding was ushered in with G.H. Shull's proposal in 1909 for *A Pure Line Method in Corn Breeding*, in which the object would be "not to find the best pure line, but to find and maintain the best hybrid combination." Discovering efficient procedures for finding and maintaining superior hybrid combinations has occupied the attention of corn breeders ever since. In the beginning, as farmers changed from open-pollinated cultivars to

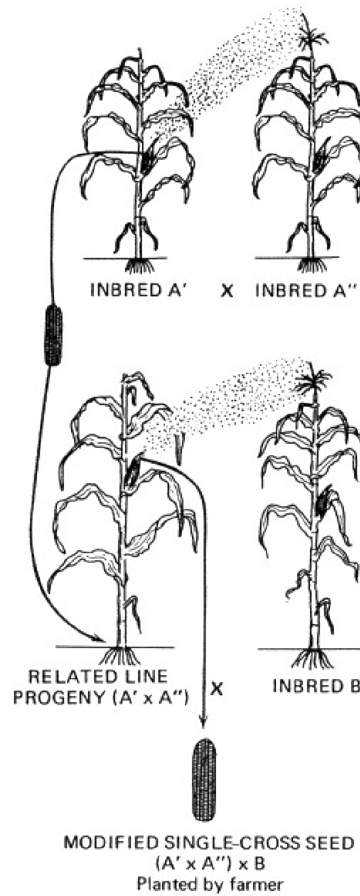


Fig. 17.9.
Procedure for making modified single-cross hybrid corn. A single-cross is made between related inbred lines A' and A". The related line progeny (A' × A") is detasseled and pollinated from inbred B. The seed produced from the latter cross is planted by the farmer. Three-way crosses are made in the same manner except that three unrelated inbreds are used.

hybrids, corn breeders were concerned largely with the development of inbred lines with sufficient vigor that they could be maintained and with fitting the inbred lines into adapted hybrid combinations for commercial production. With the change from double-cross to single-cross hybrids, the breeders' attention became focused more toward improvement of the hybrids already in commercial production by improving the inbreds used in those hybrids.

Development of Inbred Lines

For the development of productive hybrids, corn inbred lines should possess these characteristics:

• the seed-parent inbred must be vigorous and productive of high quality hybrid seed,

• the pollinator inbred must produce an abundance of viable pollen, shed over a long period, and

• the parent inbreds must possess complementary genes that contribute to the productivity and other useful traits in the hybrid.

SOURCES OF INBREDS. New inbred lines are developed by inbreeding selected heterozygous plants until homozygosity is reached. In the beginning, open-pollinated cultivars of corn were the principal source of new inbreds. After the open-pollinated cultivars had been extensively sampled, breeders began inbreeding plants from single-crosses, modified single-crosses, or three-way crosses. Or the backcross was utilized to add a desirable gene to correct a weakness in an established inbred line. A backcross-derived inbred line fits into the same hybrid combination as the recurrent parent inbred line and contributes the effect of the additional gene added through the backcross.

The above sources of new inbreds

were satisfactory for improvement of recognizable qualitative traits but were inadequate to improve complex quantitative traits. Experimental evidence demonstrated that inbred lines superior for a particular quantitative trait could be extracted from recurrent selection populations designed to increase gene frequency of that trait by repeated cycles of selection and intermating. The trait might be grain yield, stalk quality, or quantitatively inherited disease or insect resistance. Recurrent selection populations improved for specific quantitative traits then became popular sources of inbreds. The original population may be a landrace or one that is constructed by intermating inbred lines superior for the quantitative character that is to be improved. Selfing to start inbred line development may begin after any selection cycle in which an increase in gene frequency for the character to be improved has been demonstrated. When generating inbreds from a heterozygous population, the nomenclature and symbols used are S_o for the original self-pollinated plant, and $\mathbf{S_1}$, $\mathbf{S_2}$, and so on, for successive selfed generations.

Currently, in advanced breeding programs, new inbreds are most commonly obtained by crossing inbred lines with superior, complementary traits. This procedure does not differ from the hybridizatrion procedure described for self-pollinated crops (Chapter 9). With this breeding procedure the parent, $\mathbf{F_1}$, $\mathbf{F_2}$, etc., nomenclature and symbols are used in designating generations as in self-pollinating crops.

SELECTION PRACTICES FOR DEVELOPING INBRED LINES. To develop inbred lines in corn, selected S_0 (or $\mathbf{F_1}$) plants are hand-pollinated and pedigree selection is practiced for five to seven generations, or until lines uniform in appearance and performance are obtained. Selection is made both for superior lines and for superior plants within the selected lines. Selection during the early generations is based largely on visual characteristics of the corn plant, but may be supplemented by tests for specific characters-lodging resistance, disease resistances, grain quality, or other—as appropriate for the breeding objective. During each generation of inbreeding, the breeder selects only vigorous plants with suitable maturity, strong roots and stalks, disease and insect resistance, and other desirable traits. Because seed used to plant the single-crosses utilized in the commercial production of hybrid corn is produced on an inbred plant, it is important that the inbred produce a high seed yield. In general, the more vigorous inbreds tend to give the more vigorous hybrid progenies.

Combining Inbreds into Single-Crosses

The single-cross (**A** x **B**) is the simplest hybrid combination. After an inbred line of corn is developed, it must be crossed with another inbred line to produce the single-cross hybrid that is grown by the farmer. It is not possible to predict from visual observation which combinations of inbred lines will produce productive hybrids. For this reason inbred lines are evaluated for general combining ability (**gca**) using testcrosses and for specific combining ability (**sca**) using diallel crosses (Chapter 11).

The experienced breeder will usually have an array of inbred lines that have been proven to be satisfactory testers for identifying new inbred lines with superior **gca**. The new inbred lines identified as having superior **gca** are then crossed in all possible combinations, a *diallel cross*, in order to identify the hybrid combinations with superior **sca**. It is expected that these inbred lines will give superior performance in the field, but actual field tests are required to verify their performance.

If the purpose of developing the new inbred lines is to replace an inbred in an established hybrid, rather than the development of a new single-cross, the opposite inbred would be a logical tester. Similarly, if one inbred in a single-cross is being improved by the backcross

procedure, the other inbred would be used as the tester and the new hybrid compared in yield trials with the original hybrid. If an inbred is being sought to replace an inbred in a three-way cross, (**A** × **B**) × **C**, the tester will depend upon the inbred it is replacing. If inbred **A** or inbred **B** is being replaced, the tester would be the inbred line **C**. If inbred **C** is being replaced, the two inbreds **A** and **B** could be utilized as testers, and the performance of the two testcross progenies averaged, or, better, the **A** × **B** single-cross could be used as the tester.

Test Procedures

Growing the testcross in yield trials at different locations and repeating the yield trials in more than one season permits the evaluation of genotype × environmental effects and identification of hybrid combinations with the widest adaptation and yield stability in a range of environments. The yield trials should be conducted with plant populations and fertility treatments consistent with good production practices in the area where the hybrids are expected to be grown. One or more high-yielding, adapted hybrids should be included in the yield trial as checks to which the performance of the testcross progenies may be compared.

Population Improvement

Recurrent selection is a powerful tool for improving expression of a particular quantitative character in a source population. The population may be improved for a specific quantitative trait, such as resistance to a disease, stronger roots and stalks, earlier maturity, or higher kernel protein content; or it may involve overall improvement in yield, or adaptation in a particular environment. In all instances, the improvement is accomplished by increasing the gene frequency of desirable alleles affecting a quantitatively inherited trait, or for a group of quantitatively inherited traits, if the goal is to improve overall yield and adaptation.

Synthetic Cultivars

A synthetic cultivar of corn is a random-mated increase from a multiple cross (Chapter 10). In the development of a synthetic, six or eight inbred lines are crossed in pairs and the progenies crossed in a systematic scheme until all enter into the final cross in equal frequency. The synthetic cultivar is maintained by open-pollination in an isolated area or by random mating from hand pollinations. The parent inbreds are maintained, and the synthetic may be reconstituted at regular intervals. Various germplasm pools formed by other procedures or from other germplasms are often referred to as synthetics mistakenly because they do not include the feature of systematic reconstitution. The synthetic population may be developed with a specific objective, such as early maturity, stiff stalks, resistance to a particular disease, or high yield. The synthetic may be utilized as an open-pollinated cultivar, or it may serve as a source for inbred line development. Synthetic cultivars have been promoted for use in low-income areas of lesser-developed countries where the organizational structure for production and marketing of hybrid seed may not be available.

Composites and Germplasm Pools

Composites and germplasm pools, as the names imply, are groups of open-pollinated cultivars, inbreds, or other germplasm units, which are pooled in some manner that is less precise than for the production of a synthetic. They also differ from a synthetic in that the

original entries are not maintained for reconstituting the composite, and new entries may be inserted from time to time. As with a synthetic, the composite or germplasm pool may be maintained by open pollination in isolation, or by random mating from hand pollinations. The composite may serve as a source population for inbred line development.

Breeding Objectives

Choice of proper objectives is necessary for the corn breeder to develop hybrids that will be adapted to the area in which they are to be grown and superior to those already in use. The sound choice of objectives must be based on a careful appraisal of the plant characteristics that need to be improved to provide a higher yielding or a more stable yielding hybrid for the farmer to grow, or to produce a novel hybrid with unique characteristics. Breeding objectives need to be reevaluated and updated as changes occur in production practices or in the production environment, such as the result of conservation compliance or no-till practices.

Grain Yield

Corn hybrids replaced open-pollinated cultivars because they produced higher grain yields. Over the next 50 years, development and use of genetically improved hybrids, combined with improved cultural practices, resulted in a 340% increase in corn yields in the United States (Fig. 1.2). The potential for high grain yield is a complex objective, affected by the expression of genes associated with nutrient uptake, photosynthesis, transpiration, translocation, and metabolism of the corn plant, and interaction of the genes in different environments. Corn is a C_4 plant and, along with sorghum, has a higher rate of photosynthesis than a C_3 plant like wheat or soybean. Grain yield is also affected by genes associated with characters that contribute to stability of production, such as optimum maturity, stalk quality, resistance to environmental stresses, or resistance to disease pathogens and insect pests. The potential for high grain yield cannot be evaluated accurately by visual observations; it is measured in yield trials, accurately conducted at several locations and in several seasons so that genotype × environment interactions can be assessed. In the pursuit of higher grain yields in hybrid corn, extensive research has been conducted in related disciplines, such as quantitative genetic theory and its application in development of breeding procedures, improved field plot techniques that enable the breeder to obtain a more accurate evaluation of field performance, and refined statistical techniques which permit a clearer interpretation of research results.

Adaptation

Adaptation, like yield, is a complex objective in hybrid breeding that is directly affected by cultivar maturity; response to soil fertility Ievel and conservation tillage practices; seedling cold tolerance; and resistance to heat and drought. Indirectly, adaptation is affected by other plant characters such as husk covering, root and stalk quality, disease and insect resistance, and endosperm properties that affect seed storage.

MATURITY TO FIT THE AREA OF PRODUCTION. Corn is a short-day plant, with time of flowering influenced by photoperiod and temperature. In tropical and subtropical climates, corn may be grown throughout the year if soil moisture is available; in temperate climates the

corn-growing season is limited to the frost-free period. Generally, corn hybrids that will most completely utilize the full growing season and still safely mature will be the most productive hybrids to grow in a region limited by length of the production season. Yet, early maturing hybrids have certain advantages that foster their use, such as early harvest before damage from rain at time of harvest, or to facilitate planting of the succeeding crop in a rotation system. Early maturing hybrids adapted to the shorter growing seasons in higher latitudes or at higher altitudes will be shorter and have fewer leaves than hybrids adapted to the lower latitudes and longer growing seasons. The shorter growing season is compensated for in part by days with longer photoperiods. Tropical races of corn tend to be taller and have more leaves than the races adapted to temperate climates (Fig. 17.10).

RESPONSE TO SOIL FERTILITY. That certain strains of corn are more productive on fertile soils and that other strains are more productive on poor soils was once a popular belief. Present practice is to correct soil deficiencies rather than to search for genotypes that respond more favorably to fertility differences. At higher fertility levels, planting rates of corn are increased, creating a need for hybrids with smaller plants and shorter and stronger stalks. Short-statured hybrids generally have smaller ears, which are compensated for by higher planting rates.

COLD TOLERANCE. Cold tolerance refers to the ability of a corn hybrid to germinate rapidly at temperatures around 10ºC, while resisting invasion of soil-borne pathogens that incite seedling blights at these low temperatures. Hybrids with cold tolerance are desired for early planting in cold, wet soils, or for no-till corn production. Cold tests of corn inbreds and hybrids are conducted by germinating seeds in contact with pathogen-infested soil at a temperature of 9 to 10ºC for a period of 7 to 10 days and then completing the germination in a higher temperature.

Fig. 17.10.
Field of tropical corn growing in Peru. Note the extreme height of the tropical corn.

RESISTANCE TO HEAT AND DROUGHT. Heat and drought stress reduces yield and quality of corn through restriction of root development, or reduction in leaf area during plant vegetative development, or by associated effects such as poor seed set, ear droppage, higher smut incidence, greater insect damage, or fungal mycotoxin production on diseased ears. Damage from heat and drought stress is most severe if it occurs at flowering causing reduced seed set and barren ears. High-temperature stress, above 38ºC, reduces pollen viability, whereas drought stress reduces the rate of silk elongation so that silk extrusion may not synchronize with pollen shedding. Breeding for resistance involves selection of inbred lines that tolerate to a greater extent the adverse effects of heat and drought stress.

BREEDING FOR CONSERVATION TILLAGE PRACTICES. New tillage practices involving variations of minimum tillage and no-till cultural procedures are aimed at conservation of soil, fuel, and labor in corn production. Changes in cultural practices change the environment in which the corn hybrid is grown, specifically reducing the soil temperature at planting time. These changes need to be assessed in the breeding program.

Stalk Quality

Stalk quality refers to the stalk structural development, maturity, disease resistance, and pest resistance that enable the hybrid plant to stand until mechanically harvested without lodging. Losses in yield due to lodging result from the corn plant leaning over, fostering development of light, chaffy, and immature ears; or the ear touches the ground and is damaged from kernel rots. An inbred line or hybrid is classified as root lodged when it leans more that 30 degrees from the vertical (see Fig. 12.2). Root lodging may be caused by inherently weak root systems, or from roots damaged by disease or insects. A strong root system will enable the corn plant to stand against the buffeting of wind and rain. Progress in resistance to root lodging is made by breeding for shorter plants, plants with low ear placement, increased resistance to root diseases, and resistance to insects that feed on corn roots. The force required to pull corn plants from the soil is used to measure the anchorage and strength of the corn root systems.

A corn plant is classified as stalk lodged if the stalk breaks below the ear (see Fig. 12.2). Stalk breakage may occur either before or after the plant matures. The diameter and thickness or toughness of the hard outer shell or rind affects the inherent stalk strength. Stalk breakage is reduced by breeding for stronger stalks and for resistance to stalk-boring insects, such as the European corn borer (*Ostrinia nubilalis*) and resistance to stalk-rotting diseases. Recurrent selection procedures are effective in developing populations with stronger roots and stalks.

Resistance to Ear Dropping

Resistance to ear dropping is important because ears broken off and dropped to the ground cannot be recovered by a mechanical harvester. Resistance to ear dropping is evaluated by the percentage of ears on the ground at the time of harvest. Hybrids differ in their susceptibility to ear dropping. The amount of ear dropping is affected by the length and strength of the shank, weight of the ear, and disease and insect injury to the shank. The shank supports the ear and is the structure through which photosynthates are conducted as the ear develops. Heavy ears require strong shanks that do not break. Ear droppage is increased with injury to the shank, such as European corn borer tunnels, or subsequent invasion of borer tunnels by corn disease pathogens. Long shanks are structurally weaker and increase the surface available for

European corn borer tunneling. Resistance to ear dropping is increased by selecting for short, strong shanks, and resistance to stalk borers and stalk and ear rots. In selection for short, strong shanks, the shank needs to be long enough for the ears to bend downward when mature to reduce ear rotting from moisture being collected and impounded in the base of the husks.

Husk Covering

The husk protects the ear of corn from weather damage and reduces the injury caused by insects and birds. In the United States Corn Belt, a loose-fitting husk just long enough to cover the end of the ear is desirable to facilitate rapid drying and harvesting. In tropical and subtropical regions, a long husk extending beyond the tip of the ear and remaining tightly closed after maturity is useful in preventing insect and bird injury to the ear.

Rapid Dry-Down

Dry-down refers to the drying of the husk and ear as the corn plant matures. Rapid drying of the husk and ear permits early harvest and grain storage and avoids damage and loss from prolonged standing in the field before harvest.

Disease Resistance

In the beginning of hybrid corn development, progress was made in breeding disease-resistant hybrids by selecting for improvement in plant characters affected by plant disease, such as greater cold resistance, lodging resistance, or higher yield. Inbreds and hybrids susceptible to infection by pathogens inciting root- or stalk-rot disease would be eliminated from the breeding nursery because they lodged or produced unsatisfactory yields due to damage caused by the disease pathogens. Host reactions to many corn disease pathogens, including the complex of *Pythium* spp. and *Fusarium* spp. that incite root, stalk, or ear rots, are polygenicly inherited. Host reaction to other disease pathogens, such as *Puccinia sorghi* Schw. causing common rust, or *Bipolaris maydis* (Nisik.) Shoem. (Syn. *Helminthosporium maydis* Nisik. & Miyake) that incites southern corn leaf blight is race-specific with single-gene resistance to specific races. Progress in disease resistance breeding is facilitated if techniques are available for inoculation of inbred lines and hybrids with disease-producing pathogens.

The resistant reaction of a hybrid is dependent upon the genes for resistance in the inbreds. If resistance is a qualitative character controlled by one or a few genes, having the gene or genes for resistance in one inbred of a single-cross hybrid may be sufficient, although the level of resistance could be affected by presence of modifier genes in the other inbred. If resistance is a quantitative character, the hybrid will generally have greater resistance if both inbreds contain genes for resistance.

A large number of pathogens incite disease in corn. These include: (a) seed rots and seedling blights (*Diplodia maydis, Fusarium moniliforme, Pythium* spp); (b) root rots (*Fusarium* spp., *Pythium* spp.); (c) stalk rots (anthracnose, bacterial stalk rot, charcoal rot, *Diplodia* stalk rot, *Fusarium* stalk rot, *Gibberella* stalk rot); (d) ear rots (*Aspergillus* ear and kernel rot, *Diplodia* ear rot, *Fusarium* kernel and ear rot); (e) foliar disease (anthracnose, *Helminthosporium* leaf spot, northern corn leaf blight, rust, southern corn leaf blight); (f) virus disease (maize dwarf mosaic virus, MDMV, and maize chloric dwarf virus, MCDV); (g) systemic disease (head smut, sorghum downy mildew); and parasitic seed plants (witch weeds). The breeder needs to identify the diseases causing extensive damage in his production region, find

genes for resistance to the pathogen, and incorporate the resistance genes into the inbred lines and hybrids being developed.

Insect Resistance

The principal insect predators in corn are: (a) rootworms (western corn rootworm, northern corn rootworm, and southern corn rootworm); (b) corn earworm (*Hellothis zea*); (c) corn borers (European corn borer, southwestern corn borer, and the southern cornstalk borer); and (d) stored-grain insects (rice weevil, maize weevil, Angoumois grain moth, Indian meal moth). As with corn diseases, the breeder must assess the insect damage in his production region, search out sources of resistance genes to insect species that cause the greatest economic loss, and incorporate the genes for resistance into the inbred lines and hybrids being developed. Resistance to many insect pests is quantitatively inherited, thus eliminating the backcross procedure as a breeding method for transferring resistance genes to a susceptible genotype. For the European corn borer, different genes are involved in resistance to leaf feeding by first generation borers and resistance to sheath-collar feeding by second and later generation borers. Resistance to tunnel boring is evaluated by comparing the length of the borer tunnels in different inbred lines (Fig. 17.11). Recurrent selection may be used to increase the level of resistance for quantitatively inherited characters in a breeding population. Methods for rearing the insect species and their dissemination in breeding populations are requisites for evaluation of resistance.

Quality

Improvement in quality of corn by breeding must take into consideration the use that will be made of the corn. About 90% of the corn utilized in the United States is used as animal feed; the remaining 10% is used in milling and other industrial uses, or for seed. Corn is a high-energy food. It is low in protein and requires supplementation with high-protein feeds to improve its nutritive value for animal or human food. Corn oil is a valuable by-product of milling. Breeding for higher protein or higher oil content has not been given a high priority in the United States because corn marketing practices do not reward growers for producing corn with higher protein or oil.

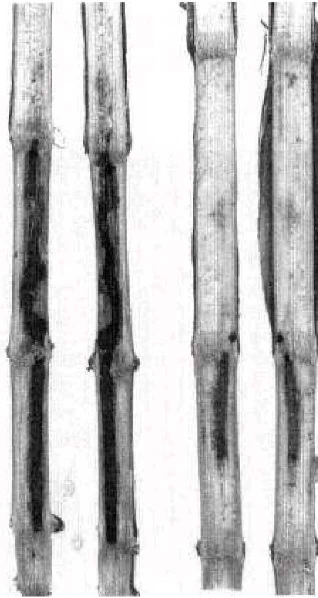


Fig. 17.11.
Comparison of damage from European corn borer in stalks of a susceptible (left), and a resistant (right) corn hybrid. Resistance of inbred lines and hybrids is compared by measuring the length of the borer tunnels.

BREEDING HIGH-PROTEIN CORN. In a selection experiment at the Illinois Agricultural Experiment Station, the protein content of the 'Burr White' cultivar of open-pollinated corn was increased from 10.9 to 26.6% protein after 70 generations of ear-to-row selection (Fig. 17.12A). Increasing the total protein of corn by breeding does not increase the feeding value of the high-protein corn to nonruminant animals in proportion to the increase in the percentage of protein because the increase is in the *zein* fraction of the protein present in the endosperm. The zein fraction composes about 80% of the total kernel protein and is nutritionally deficient in the essential amino acids, lysine and tryptophan. Corn yields are generally reduced as protein content is increased.

IMPROVING PROTEIN QUALITY. The poor quality of corn protein caused by deficiency of lysine in the zein fraction may be improved significantly by addition of the mutant genes opaque-2 or floury-2. Generally, grain yield and kernel weight are reduced by introduction of the opaque-2 or the floury-2 gene, and the corn kernel has a soft, starchy endosperm. A few corn hybrids containing the opaque-2 gene have been developed and have a limited market for feeding nonruminant animals such as swine.

HIGH OIL CONTENT. In an experiment, parallel to the protein selection experiment at the Illinois Agricultural Experiment Station, and conducted with the same Burr White open-pollinated corn, the oil content of corn was increased from 4.7 to 16.6% after 70 generations

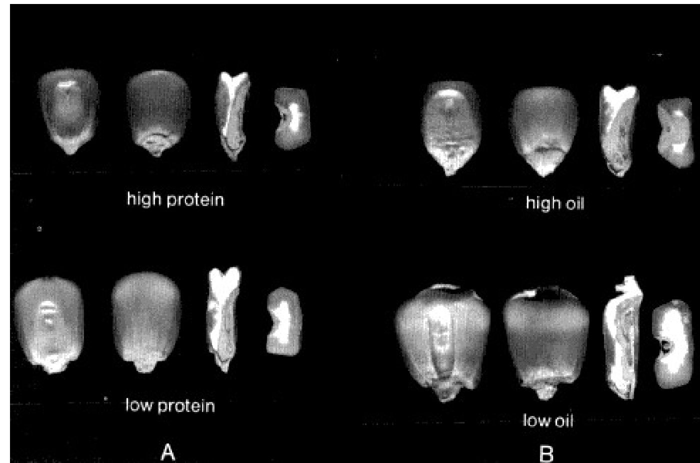


Fig. 17.12.
Kernel characteristics of strains selected for chemical composition.
(A) High- and low-protein strains. High-protein strains have smaller kernels with a larger proportion of horny endosperm than low-protein strains. (B) High- and low-oil strains. High-oil strains have kernels with a larger proportion of germ than low-oil strains.

of ear-to-row selection (Fig. 17.12B). Because most of the oil in corn is in the germ, selection for strains with large germs will increase the percentage of oil. High oil content is valuable to the industrial user of corn who extracts germ oil as a by-product in milling, or for feeding poultry, swine, or dairy cows. Increasing oil content in corn by breeding normally leads to a decrease in grain yield and a decrease in the unsaturated fatty acid, linoleic acid, desirable for low-cholesterol diets.

CORN FOR MILLING. Corn used for milling is processed either by dry milling or wet milling. Corn used in the dry milling process goes into the manufacture of brewers' grits and flakes, hominy grits, corn meal, and other products. The dry miller prefers white endosperm corn with a semihard kernel, moderate indentation, and a white cob. Breeders have developed white corn hybrids with kernel characteristics desired for dry milling. The wet milling process is used to extract starch from the corn kernel for industrial uses, but oil and proteins are recovered as valuable by-products. High kernel weight and resistance to seed molds are important grain quality considerations.

Special-Purpose Hybrids

In addition to the development of corn hybrids for the larger use of the livestock feeder and the corn-milling industry, hybrids have been developed for special purposes, such as *sweet corn, popcorn, wary corn*, or *cob pipe corn*. Sweetcorn hybrids are generally recessive for the endosperm gene sugary (*su*). Sugar content may be increased and period of peak quality extended by presence of the mutant gene, shrunken-2 (sh_2). Sweet corn hybrids with these genes are often referred to as "supersweet" or "extrasweet." Waxy corn contains a special type of endosperm starch, amylopectin, which permits it to be used in the manufacture of adhesives, gums, paper sizing, and puddings. Cob pipe hybrids have large cobs used in the manufacture of corncob pipes.

International Maize and Wheat Improvement Center

The International Maize and Wheat Improvement Center (CIMMYT) is an international research center dedicated to worldwide improvement of corn and wheat. The objective of CIMMYT's corn program is to assist in the development of national and regional corn improvement programs in tropical and subtropical corn-producing countries where corn is utilized as human food. CIMMYT's activities include the maintenance of a germplasm bank; coordination of international corn testing trials; a research program to develop improved corn populations, inbred lines, and hybrids for distribution to corn research workers in cooperating countries; and sponsorship of workshops and training programs for corn research workers.

Study Questions

1. Why has plant breeding been so successful in the development of improved corn cultivars?

2. What plant breeding methods are used to develop improved cultivars of corn? Can these same breeding methods be used to develop new cultivars of other crop plants? If so, which crop plants?

3. Describe how the plant breeder makes self- and cross-pollinations in corn. Are pollinations easier or more difficult to make in corn as compared to other crop plants? Why?

4. Where is the center of origin for corn? What role have humans played in the evolution of corn?

Further Reading

Barry, D., and L.L. Darrah. 1991. Effect of research on commercial hybrid maize resistance to European corn borer (*Lepidoptera:Pyralidae*). J. Econ. Entomol. 76:392-94.

CIMMYT. 1989. Toward insect resistant maize for the third world. Proc. of the Int. Symp. on Methodologies for Developing Host Plant Resistance to Maize Insects. International Maize and Wheat Improvement Center, (CIMMYT) Mexico. D.F., Mexico.

Dudley, J.W., R.J. Lambert, and D.E. Alexander. 1974. Seventy generations of selection for oil and protein concentration in the maize kernel. p. 181-212. *In* J.W. Dudley (ed.) Seventy generations of selection for oil and protein in maize. Crop Sci. Soc. Am., Madison, WI.

Galinat, W.C. 1992. Evolution of corn. Adv. Agron. 47:203-31.

Gordon-Kamm, W.J. 1990. Transformation of maize cells and regeneration of fertile transgenic plants. The Plant Cell 2:603-18.

Guthrie, W.D. 1989. Breeding for insect resistance in maize. p. 209-43. *In* J. Janack (ed.) Plant Breeding Reviews, Vol. 6. Timber Press, Portland, OR.

Hallauer, A.R. 1987. Maize. p. 249-94. *In* W.R. Fehr (ed.) Principles of cultivar development. Vol. 2. Macmillan Publishing Co., New York.

Hallauer, A.R. 1990. Methods used in developing maize inbreds. Maydica 35:1-16.

Hayes, H.K. 1963. A professor's story of hybrid corn. Burgess Publ. Co., Minneapolis, MN.

Magnavaca, R., B.A. Larkins, R.E. Schaffert, and M.A. Lopes. 1993. Improving protein quality of maize and sorghum. p. 649-53. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Pandey, S., and C.O. Gardner. 1992. Recurrent selection for population, variety, and hybrid improvement. Adv. Agron. 46:1-87.

Sprague, G.F., and J.W. Dudley (eds.). 1988. Corn and corn improvement, Third ed. Agronomy Monograph. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Sprague, G.F., and L.A. Tatum. 1942. General vs. specific combining ability in single crosses of corn. J. Am. Soc. Agron. 34:329-31.

Sprague, G.F., and S.A. Eberhart. 1977. Corn breeding. p. 305-63. *In* G.F. Sprague (ed.) Corn and corn improvement. 2nd ed., Agronomy Monograph. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Timothy, D.H., P.H. Harvey, and C.R. Dowswell. 1988. Development and spread of improved maize varieties and hybrids in developing countries. U.S. Agency for Int. Develop., Bur. Sci. and Tech., Washington, D.C.

18. Breeding Sorghum

Sorghum (*Sorghum bicolor* [Linn.] Moench) is the world's fifth leading cereal grain (metric tons), after wheat, rice, corn (maize), and barley, and the third leading cereal grain in the United States, after corn and wheat. Sorghum is a crop with great genetic diversity, but a major characteristic is its mechanisms for tolerating heat and drought. As a result, it is the food grain most widely grown and utilized for human consumption in the semiarid regions of sub-Sahelian Africa and in the dry central peninsular region of India. In the United States, grain sorghum is grown as a feed grain for livestock in areas of the Great Plains that are too hot and dry for growing corn. By growing productive hybrids with high soil fertility and irrigation, the United States harvests about one-fourth of the world production of grain sorghum on 10% of the world's area planted to sorghum.

Genetic improvements in the sorghum crop were orchestrated by a few intensive public breeding programs in the southern and central Great Plains of the United States. Sorghum had become established as a cultivated crop in the United States from plant introductions that were tall, late-maturing, and unsuited to mechanical harvest. Genetic changes in sorghum cultivars began when farmers in Texas discovered mutant plants for dwarfness and early maturity in fields of sorghum. Sorghum breeders utilized the dwarf, mutant types to breed short, erect, open-pollinated cultivars which could be mechanically harvested, and early maturing cultivars that extended the region of sorghum production into the High Plains of Texas and northward into Nebraska and South Dakota. With identification of a cytoplasmic-male-sterility:fertility-restorer-gene system in sorghum, hybrids replaced traditional open-pollinated cultivars. Extensive breeding programs are now conducted in this region by private hybrid-sorghum seed-producing companies. A comprehensive sorghum breeding program for the benefit of the lesser developed countries in the tropics and subtropics has been developed by ICRISAT, the International Crops Research Institute for the Semiarid Tropics, Patancheru, Andhra Pradesh, India.

Origin, Species, and Races

The sorghums originated in Africa about 5000 years ago. The greatest genetic diversity in native sorghums is found in Ethiopia and adjacent areas of northeast Africa. From the area of its origin, sorghum was carried throughout Africa, to India, and to China. Along the way many distinct races evolved. Today, sorghum is an extremely morphologically diverse complex. This diversity has made it difficult to develop a simple system for the taxonomic classification of sorghum.

The genus *Sorghum* Moench is characterized by spikelets borne in pairs: a bisexual and fertile sessile spikelet, and a sterile or occasionally staminate-flowered pedicellate spikelet. The genus is subdivided into five sections, the most important section being section *sorghum* containing three species:

• *Sorghum bicolor* (Linn.) Moench, ($2n = 2x = 20$), the annual wild and domesticated sorghums,

• *Sorghum propinquum* (Kunth) Hitche., ($2n = 2x = 20$), a wild, perennial, diploid, rhizomatous species with small hard seeds, that is cross-fertile with *S. bicolor*, and

• *Sorghum halepense* (Linn.) Pers., ($2n = 4x = 40$), a perennial, tetraploid, rhizomatous species, commonly known as johnsongrass in the United States, where it has become a troublesome weed in the southern states.

The species *S. bicolor* is a very large and complex taxa containing the annual domesticated sorghums, the annual wild sorghums, and hybrid swarms among them. Based on spikelet characteristics, a "simplified classification" of *S. bicolor* was developed by J.R. Harlan and J.M.J. de Wet that recognizes 15 races; five primary races (bicolor, guinea, caudatum, kafir, and durra), and 10 intermediate races originating from the 10 possible hybrid combinations among the primary races (bicolor-guinea, bicolor-caudatum, etc.). The classification of species *S. bicolor* into races has made it possible to assign a sorghum line to a specific race by examination of relatively few morphological characters.

Agronomic Groups

The open-pollinated sorghums introduced into the United States were originally identified according to their potential agronomic use as *grain sorghums, sorgos* or *sweet sorghums, grass sorghums*, and *broomcorn* (Fig. 18.1).

Grain Sorghums

The grain sorghums produce large, palatable seeds that thresh free from the glumes, stalks that are dry to moderately juicy, and juice without sweetness or only slightly sweet. The grain sorghums originally introduced into the United States from different geographic areas of Africa (or India) possessed distinct plant and seed characteristics that identified each as a particular varietal group, of which *milo, kafir, hegari*, and *feterita* were the most important. Although the original types are no longer grown in the United States, these grain sorghum types provided the basic germplasm for development of early sorghum cultivars and present-day sorghum hybrids.

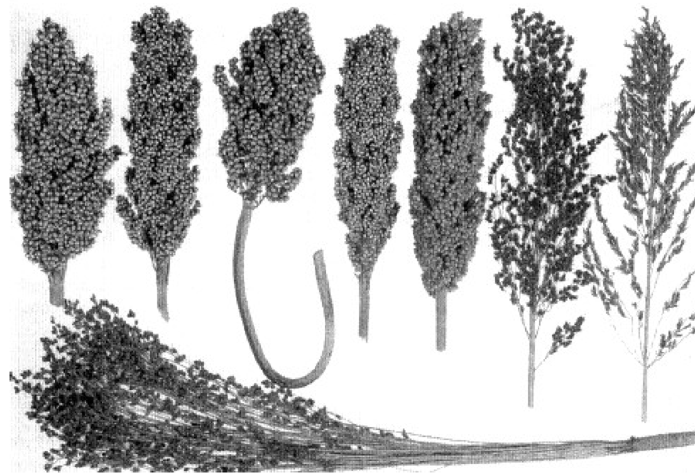


Fig. 18.1.
Heads of sorghum typical of different varietal groups. From left to right: feterita, hegari, milo, kafir, kafir × milo hybrid, sorgo, sudangrass. At bottom, broomcorn.

Milo, *Race durra*, from east-central Africa, is characterized by a compact head borne on a recurved or goose-necked peduncle that makes it unsuited for mechanical harvest. Seeds are large and creamy yellow or white; stalks are slender, dry, pithy, and tiller freely. The original 'Yellow Milo' introductions from east-central Africa averaged 2 to 2.5 m tall, but three successive natural mutations for reduced height led to the shorter varieties: 'Standard Yellow' milo, 'Dwarf Yellow' milo, and 'Double Dwarf Yellow' milo (see Fig. 6.3). There also appeared natural mutant types for early maturity. These dwarf-early mutant types became an important germplasm source for the breeding of the short, early maturing sorghum hybrids grown today. The superior grain quality of milo has been retained in hybrid grain sorghums with milo ancestry, often leading to the crop name being wrongly referred to by farmers as 'milo' rather than 'grain sorghum.' Milo cytoplasm is the source of the cytoplasmic male sterility generally utilized in breeding hybrid sorghum.

The kafirs, *Race kafir*, from South Africa, have strong, stout stalks that are dry to juicy and moderately sweet; long, compact, cylindrical heads that are borne erect; and medium-size seeds that are white, pink, or red. Selections from kafir × milo crosses produced open-pollinated cultivars with stout, strong stalks and erect heads that could be combine-harvested and that possessed milo seed quality. Kafir nuclear genes introduced into milo cytoplasm resulted in cytoplasmic-male sterility which utilized kafir as the seed parent in the production of many early hybrid cultivars.

Hegari, *Race caudatum* from Sudan, is more abundantly leafed, has sweeter juice, tillers more heavily, and has seeds more chalky in appearance than kafir. Hegari was utilized in the

early development of hybrid sorghum. Feterita, also *Race caudatum*, from Sudan, has slender stalks; heads fairly compact and borne erect; and large, white, and chalky seeds that tend to weather easily. Feterita was never grown extensively in the United States but it entered into the parentage of open-pollinated cultivars and hybrids.

Sorgo, Grass Sorghum, and Broomcorn

The sorgos or sweet sorghums, *Race bicolor*, possess an abundance of sweet juice and are used as silage, fodder, and hay; or the juice may be pressed out and used for the production of syrup or sorghum molasses. The panicles range from compact to open. The seeds are small, white, or colored, often bitter and unpalatable, and do not always thresh clean from the hulls. Sudangrass is the principal grass sorghum in the United States where it is utilized as a supplemental pasture crop. Johnsongrass is a perennial grass sorghum that spreads by creeping rhizomes and is a serious weed pest. It is a host and inoculum source for many sorghum diseases and insect pests. The panicle of broomcorn is used in making brooms. Broomcorn originated in Africa and was grown in Europe for several centuries before reaching the United States.

Shattercane

Wild, weedy sorghums similar to 'Black Amber' sorgo are often found in fields of corn, sorghum, or soybean in the United States. Commonly, the plants are tall, slender-stalked, sometimes rhizomatous; the seeds are small, hard, shatter easily due to a deciduous spikelet, and remain dormant for long periods. These sorghums are variously called *shattercane, wild cane, black amber cane*, and *scattercane*. The nonrhizomatous types probably originated as outcrosses of cultivated sorghum to wild or weedy sorghums. They constitute a serious weed pest in many corn and sorghum growing areas in the United States.

Botany, Flowering, and Pollen Control

The sorghum head varies from a compact to an open panicle. The spikelets are borne in pairs, one being sessile, bisexual, and fertile; the other sterile or staminate-flowered and borne on a short pedicel, except for the terminal spikelet, which is borne on a branch and is accompanied by two pedicellate spikelets (Fig. 18.2). The *sessile* spikelet contains two florets, one perfect and fertile, the other

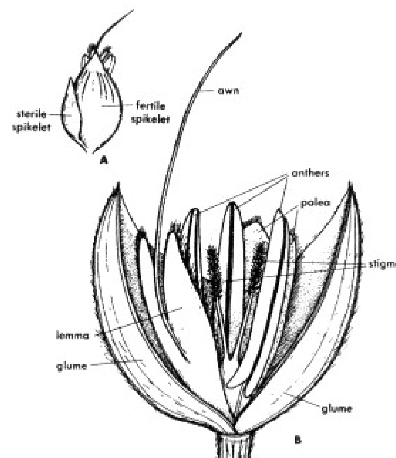


Fig. 18.2.
Spikelets of sorghum. (A) Pair of spikelets.
(B) Fertile spikelet.

sterile. The *pedicellate* spikelet either bears stamens or is sterile. The sorghum flowers start blooming just prior to sunrise and continue in the early morning, but may extend to midday under certain conditions. Blooming starts in the uppermost panicle branch and follows a fairly regular downward progression. From six to nine days are required for all flowers in a panicle to finish blooming. The anthers and stigmas push out as the glumes open (Fig. 18.3). The anthers dehisce as they are exserted, or shortly thereafter, and release a small cloud of pollen. A single panicle of sorghum may produce 24 to 100 million pollen grains. The pollen of sorghum loses its viability within a few hours after being shed. The stigmas are receptive for one or two days before the flower blooms and for several days after blooming.

Sorghum is a short-day plant, and blooming is hastened by short daylight periods and higher temperatures. Sorghums differ in their sensitivity to day length; milo, hegari, and feterita are sensitive to changes in day length, but broomcorn is relatively insensitive.

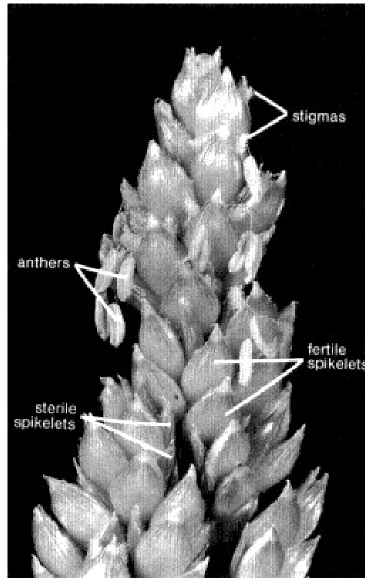


Fig. 18.3.
Panicle branch from sorghum head. Exposed stigmas and exserted anthers are visible. Cross-pollination may occur if the stigma is exposed before being self-pollinated. Sorghum averages about 6% cross-pollination.

Selfing and Crossing Techniques

Stigmas exposed before the anthers dehisce are subject to cross-pollination. The amount of natural cross-pollination averages about 6% in sorghum but may be as high as 30% in sudangrass. To prevent cross-pollination, it is necessary to enclose the sorghum heads in bags during the blooming period (Fig. 18.4).

Artificial cross-pollinations are made by emasculating the seed parent and hand-pollinating it with pollen collected from the pollen parent. Hand emasculations are made by using thin-pointed tweezers, a dissecting needle, a sharp pencil point, or a similar instrument to remove the anthers (Fig. 18.5). Usually, only a small branch of the panicle is emasculated; the remainder of the panicle is clipped away to permit bagging the emasculated portion of the head. Too much trimming may reduce seed set due to drying out of the stigmas. Pollen is collected in bags in the same manner as with hybrid corn and dusted over the exposed stigmas, or a pollen-producing head may be brushed over an emasculated head. Emasculated heads, or heads that have been pollinated, are enclosed in paper bags to protect them from stray pollen.

Mass emasculation is sometimes accomplished by covering the sorghum head with a plastic bag to maintain a high humidity. The anthers will be exserted but do not dehisce and may be

Fig. 18.4.
Sorghum breeding nursery showing method of bagging heads to control pollination.

shaken off by removing the bag and jarring the head sharply. Some selfing may occur, and marker genes are needed to identify the F_1 plants arising from selfed seed.

Genetic Male Sterility

Nuclear genes for male sterility may be used to eliminate the emasculation procedure when crossing sorghum inbred lines or in population improvement schemes involving recurrent selection. Recessive male-sterile nuclear genes have been assigned the symbols ms_1, ms_2, etc. The recessive male sterile gene is backcrossed into the female parent line. Pollination of a genetic male sterile plant (*msms*) with pollen from a plant homozygous for the dominant allele (*MsMs*) will produce heterozygous F_1 plants that upon self-pollination segregate into offspring 50% male fertile and 50% male sterile (see Fig. 7.2).

Cytoplasmic Male Sterility

Hybrid sorghum seed production began in the early 1950s based on a cytoplasmic-male-sterility:fertility-restorer-genesystem that introduces kafir nuclear genes into milo cytoplasm.

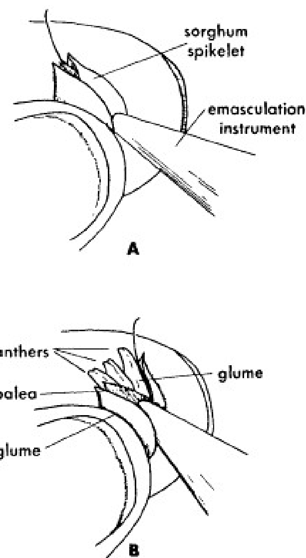


Fig. 18.5.
Emasculating sorghum flowers. (A) Glumes are opened with a medium pointed lead pencil, tweezers, or emasculation instrument while spikelet is held between the thumb and forefinger. (B) Anthers are pushed out by applying pressure through the palea, with a rotating movement.

Fertility is restored by pollinating with sorghum cultivars of milo origin. This system has since been designated the **A1** or milo system. Fertility-restoring genes in sorghum are designated by the symbol *Msc* to identify them from the genes for genetic male sterility. Exclusive use of the **A1** cytoplasm for hybrid sorghum seed production caused concern about the vulnerability of sorghum hybrids should a disease problem arise as occurred with southern leaf blight on corn hybrids possessing T-cytoplasm. Utilization of only one cytoplasm also restricts the germplasm available for use as **A-lines** or alternately as **R-lines**. These concerns led to a search for additional cytoplasms that can be used in hybrid seed production. Four major groups of cytoplasmic-nuclear male sterility have now been identified (**A1, A2, A3**, and **A4**), providing alternative sources of cytoplasmic male sterility. Fertility of milo (**A1**) cytoplasm is restored by a major gene, Msc_1, although a second gene, Msc_2, is required in some backgrounds. The level of fertility restoration is increased in unfavorable environments by additional modifying genes, which are present in some sorghum parent lines.

Genetic Studies

Genetic studies in sorghum generally relate to characters of interest to the breeder, such as male sterility, maturity, stature, grain color, stalk juiciness and sweetness, hydrocyanic (prussic) acid content, endosperm composition, or disease and insect resistance. The genes conditioning maturity and height have had major effects on sorghum cultivar and hybrid development.

Genes Affecting Maturity

In its native habitat, sorghum is a short-day plant, maturity being regulated by genetic response to photoperiod and temperature. In early maturing genotypes, the flowering head is initiated quickly, and the plant has a reduced number of internodes and leaves. Four gene loci, Ma_1, Ma_2, Ma_3, *and* Ma_4, influence time of maturity. Tropical sorghums are dominant at the

Ma_1 locus and do not flower in long summer photoperiods in the United States. Through manipulation of the maturity genes, it is possible to develop sorghum cultivars and hybrids adapted to a wide range of production areas that differ in photoperiod.

Genes Influencing Height

The dwarf milos originated by mutation from taller cultivars. Four independent recessive genes for short stature, dw_1, dw_2, dw_3, *and* dw_4, reduce internode length (Fig. 6.3). Time of blooming and leaf size are not affected. The dwarfing genes have been utilized in the breeding of short-stature sorghum cultivars and hybrids. The recessive dw_3 gene, and one other gene in some hybrids, is unstable and reverts to the dominant form causing tallness, with one tall mutant plant occurring out of approximately each 600 to 1200 plants in the field (Fig. 18.6). Sorghums recessive for the dwarfing genes are called 1-, 2-, 3-, or 4-dwarf, respectively, according to the number of recessive dwarfing genes present. Most commercial hybrids in the United States are 3-dwarf, being recessive at the dw_1 dw_3, *and* dw_4 loci.

Interspecific Crosses

Natural crossing between johnsongrass (*S. halepense*, $2n = 4x = 40$) and cultivated sorghum (*S. bicolor*, $2n = 2x = 20$) is a source of off-type plants in sorghum fields. The hybrid plants are of two types: sterile plants with 30 chromosomes and vigorous rhizomes, and fertile plants with 40 chromosomes and weak rhizomes. The 30-chromosome types are often perennial and persist in mild climates by overwintering as weeds.

Breeding Methods

Before the advent of hybrid sorghum, sorghum cultivars were developed through *introduction, selection, and hybridization* as in self-pollinated crops. Because sorghum averages about 6% natural cross-pollination, bagging sorghum heads before anthesis was necessary to control pollination. Many early cultivars originated by selection of off-type plants in introduced sorghum populations. Hybridization between sorghum cultivars became the next source of improved cultivars. Population improvement procedures have been utilized to concentrate genes for quantitatively inherited characters.

Fig. 18.6.
A rogue in a field of dwarf sorghum. Tall vigorous hybrid plants are commonly found in commercial sorghum fields, either from outcrossing, or mutation of a recessive dwarfing allele to its dominant counterpart.

Introduction and Germplasm Collection

Introduction of sorghum germplasm into the United States began about the middle of the nineteenth century. Eventually, grain sorghums became established as an important crop in the southern Great Plains, where precipitation was insufficient to grow corn. Although the sorghum germplasm base is extremely diverse, the early germplasm utilized in sorghum breeding programs was relatively narrow, originating from about 20 sorgo and 8 or 10 grain sorghum introductions. The present United States germplasm collection is maintained at the Regional Plant Introduction Station, Experiment, Georgia. Duplicate samples are stored in the National Seed Storage Laboratory, Fort Collins, Colorado. A world collection of sorghum germplasm that now exceeds 25,000 accessions has been assembled at ICRISAT, the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, A.P. State, India.

SORGHUM CONVERSION PROGRAM. Sorghum genotypes introduced from tropical climates with short days into temperate climates with longer photoperiods almost always mature too late to produce viable seeds and are too tall to be harvested with modern machinery (Fig. 18.7). Concern about the narrow germplasm base of United States sorghum cultivars led sorghum breeders J.C. Stephens and J.R. Quinby from the United States Department of Agriculture and Texas Agricultural Experiment Station to propose conversion of tall, late-maturing, tropical accessions from the World Sorghum Collection to short, day-neutral genotypes adapted to temperate climates. Tropical cultivars are convened to temperate climate adaptation by substituting two recessive alleles for height for the dominant counterpart alleles in the tropical accessions and the recessive ma_1 maturity allele for dominant Ma_1 through a backcross breeding program. The convened lines provide United States sorghum breeders with germplasm that previously did not flower in temperate climate photoperiods and provides breeders in tropical environments with short-statured, early maturing genotypes adapted to tropical environments. About 1500 sorghum germplasm lines were selected initially for conversion. The program is being continued with new germplasm introductions.

Fig. 18.7.
Tall sorghum, typical of native tropical cultivars.

Hybrid Sorghum

The success attained with hybrid corn stimulated interest in breeding hybrid sorghum. The vigor of F_1 sorghum hy-

brids had been demonstrated many times following varietal crosses in traditional sorghum breeding programs. With the discovery of cytoplasmic-male sterility and fertility-restoring genes in sorghum in the early 1950s, the tools needed for the commercial production of hybrid sorghum became available. In the first sorghum hybrids, pure line cultivars already being grown commercially were utilized as parent lines serving the same function as inbred lines in hybrid corn. In current hybrid breeding programs, new parent lines are developed specifically to be *seed-parent lines* (**A-lines**) or to be *pollen-parent lines* (**R-lines**) depending on whether or not they contain fertility-restoring genes.

CYTOPLASMIC MALE STERILITY:FERTILITY-RESTORER SYSTEM. Cytoplasmic male sterility was obtained in sorghum by backcrossing chromosomes of kafir into the cytoplasm of milo (Figs. 18.8, 18.9). Fertility was restored in the hybrid when the **cms** plants were pollinated

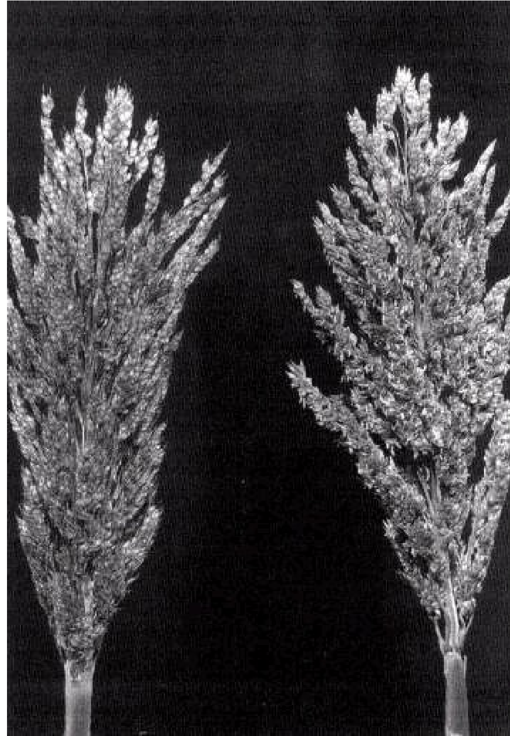


Fig. 18.8.
Cytoplasmic male-sterile (left) and male-fertile (right) heads of sorghum. Note exserted anthers on male-fertile head. The two heads are in a similar stage of blooming.

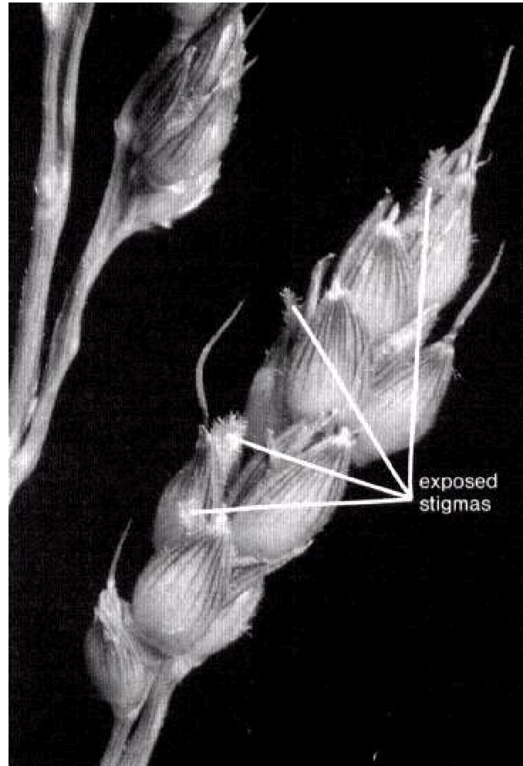


Fig. 18.9.
Panicle branch from cytoplasmic male-sterile sorghum head in Fig. 18.8. Note exposed stigmas, which may be pollinated from windborne pollen. Compare with Fig. 18.3 in which anthers are exserted from male-fertile flowers.

from a milo plant or a milo derivative. Cultivars of kafir origin then in commercial production were readily converted to **cms**, and cultivars of milo origin were used as pollinators.

BREEDING HYBRID SORGHUM. The **A-line, B-line, R-line** model as described in Chapter 11 is used in breeding hybrid sorghum (Fig. 18.10). New parent lines are first evaluated for use as seed-parent lines or as pollen-parent lines. Sorghum lines with fertility-restoring genes cannot be used as **B-lines** or convened into male-sterile **A-lines**. A **B-line** is convened to a male-sterile **A-line** by transferring its chromosomes into sterile cytoplasm by a series of backcrosses in which the line to be sterilized is the recurrent and pollen parent in all crosses. The male sterile line is used as the female line because the cytoplasm is transmitted only

through the egg. After conversion to male sterility, the line is designated an **A-line**. The **A-line** and its **B-line** pollinator are identical in genotype but have different cytoplasms.

Pollen fertility is restored by a dominant gene, *Msc*, originally present in commercially grown cultivars of milo or milo origin. In most cytoplasms, additional modifying genes are required to obtain good seed production in a wide array of environments. In selecting a pollen-restorer line (**R-line**), it is necessary that the pollinator:

• contain a dominant fertility-restoring gene and modifier genes necessary for complete fertility restoration in the $\mathbf{F_1}$ hybrid,

• produce an abundance of viable pollen, and

• combine with the male-sterile seed parent to produce a high-yielding single-cross hybrid with acceptable grain quality.

HYBRID SORGHUM SEED PRODUCTION. In the commercial production of hybrid seed, the **A × R** hybrid is produced by planting alternating rows of A-and **R-lines** in an isolated field, the **A-line** being pollinated by windblown pollen from the **R-line**. Twelve rows of the male-sterile **A-line** are commonly planted to four rows of the pollinator **R-line** (Fig. 18.11). The **B-** and **R-lines** are maintained by bagging heads and harvesting self-pollinated seed or by harvesting seed from an isolated field.

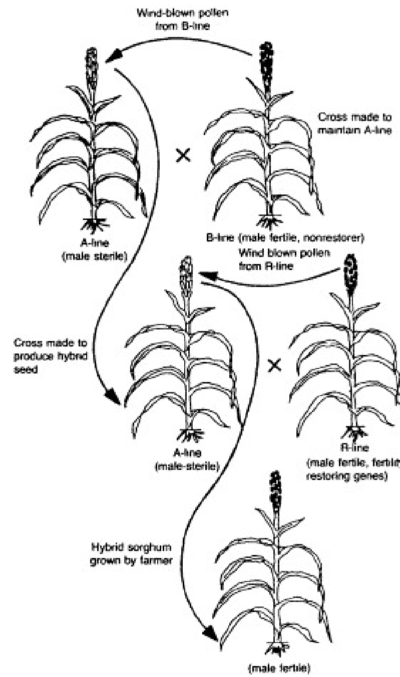


Fig. 18.10.
A-line, B-line, R-line models for producing hybrid sorghum seed using cytoplasmic male sterility and fertility-restoring genes. The A-line in male-sterile cytoplasm is maintained by pollination from a genetically identical B-line in normal cytoplasm. The hybrid seed is produced by pollinating the A-line from the R-line. The R-line has dominant fertility-restorer genes and combines with the A-line to produce a high-yielding hybrid.

The **R-line** is male-fertile, possesses fertility-restoring genes, and combines with the A-line to produce a vigorous single-cross hybrid. The single-cross hybrid seed (**A × R**) is planted by the farmer. The cytoplasm and restorer genes present in sorghum parent lines and the hybrid are shown in Table 18.1. Windblown sorghum pollen may be carried long distances. Isolation of 200 m from other sorghum fields is needed to reduce contamination. Following harvest, it is a common practice for United States seed producers to send seed samples to a tropical location to grow and check for purity before the next crop is planted.

Fig. 18.11.
Hybrid sorghum seed production field. Four male-fertile pollen parent rows are in the center, with male-sterile seed-parent rows on either side.

Table 18.1.
Cytoplasm, fertility-restoring genes, and pollen fertility in hybrid sorghum seed production

Parent or hybrid	Cytoplasm	Genotype for fertility-restoring genes	Pollen fertility
A-line	Male-sterile	$ms_c\ ms_c$	Male-sterile
B-line	Male-fertile	$ms_c\ ms_c$	Male-fertile
R-line	Male-fertile	$Ms_c\ Ms_c$	Male-fertile
	or		
R-line	Male-sterile	$Ms_c\ Ms_c$	Male-fertile
Hybrid, A × R	Male-sterile	$Ms_c ms_c$	Male-fertile

Parent Line Development

Just as hybrid corn is improved by development of superior inbreds, hybrid sorghum is improved by development of superior parent lines, the term parent line here being synonymous with inbred line in hybrid corn. Sorghum breeding lines devoid of restorer genes are grouped as **B-lines** and convened to male-sterile **A-lines**; breeding lines with restorer genes are utilized as **R-lines**. In addition to their **B-line**, or **R-line**, characteristics, parent lines need to be good specific combiners in order that the hybrid combination will be productive. New parent lines may be developed by:

• crossing among **B-lines** and among **R-lines**, followed by selection for superior segregates using the pedigree selection procedure described for self-pollinated crops in Chapter 9,

• addition of genes for desirable characters to an established parent line by the backcross procedure, or

• isolation of new parent lines from populations improved through recurrent selection procedures.

While generating new lines by hybridization and pedigree selection, heads of selected plants are bagged to prevent outcrossing. Emasculation of **B-lines** may be eliminated by using a cytoplasmic male-sterile (**A-line**) counterpart as the seed parent for those **B-lines** that have already been converted to **A-lines**. When adding a gene by the backcross to a **B-line**, the male-sterile **A-line** counterpart may be used as the recurrent parent to eliminate emasculation, with a final cross to the **B-line** to recover the male-fertile cytoplasm. It is important that fertility-restoring genes are not tightly linked to the gene being contributed by the donor parent if the cross is made to the **A-line**.

Population Improvement

Population improvement involves the use of recurrent selection to increase the frequency of genes for a particular quantitatively inherited character or group of characters. Initially it is necessary to synthesize a sorghum random-mating population, using cultivars or breeding lines that possess genes that will contribute to the character to be improved. If lines isolated from the population are to be useful in the breeding programs, the component lines should, in addition, have good agronomic characteristics, dwarfing genes, and disease and insect resistance.

DEVELOPING THE RANDOM-MATING POPULATION. Because natural cross-pollination is limited in sorghum, a male-sterile gene is backcrossed into the component lines to foster random mating. The recessive male-sterile genes ms_3 and ms_7 have been found to express good sterility, do not adversely affect seed set from open-pollination, and may be used in either **B**-or **R**-type populations. The backcross derived lines are blended and planted. The population will be segregating for male sterility, with male-sterile plants randomly pollinated from male-fertile plants. By harvesting seed from 300 to 400 open-pollinated, male-sterile plants, a high level of male sterility will be maintained in the population, reaching a 1 male-sterile: 1 male-fertile ratio in the $\mathbf{F_4}$ generation. Male sterile plants cannot be distinguished from male-fertile plants at harvest, so it is necessary to monitor the plots three or four times weekly during the flowering period and mark the male-sterile plants. From one to three random-mating generations are grown in isolation initially, without selection except for elimination of tall or weak plants, to permit maximum recombination before selection begins for the characteristic under study. A population synthesized in this manner becomes the initial source population for a recurrent selection procedure.

RECURRENT SELECTION PROCEDURES. *Mass selection, half-sib family selection, full-sib family selection*, and S_1 *family selection, as* described in Chapter 10, may be used as recurrent selection procedures for population improvement. Recurrent selection populations may be generated for yield, drought resistance, higher protein, European corn borer resistance, or other characteristics with quantitative inheritance. The recurrent selection population is

utilized as the source population for selection of breeding lines improved for the character under consideration.

Mass selection is the simplest and least expensive recurrent selection procedure. If open-pollinated plants are chosen and propagated, one selection cycle is completed with each generation. With open pollination, there is no control of the male parent, so genetic gain is reduced by one-half. If plants are selected before flowering and self-pollinated, an additional generation is needed for recombination of the selected genotypes so that two generations are required to complete a cycle.

In *half-sib family selection*, male-sterile plants are identified in the population and pollinated from a random selection of male-fertile plants; with *full-sib family selection*, each selected male-sterile plant is pollinated by a single male-fertile plant; and in S_1 *family selection*, selected male-fertile plants are bagged and self-pollinated. Progenies of the respective selected plants are *designated families*. Three generations are required to complete a recurrent selection cycle: *selection generation, evaluation generation, and recombination generation*. Due to the large genotype × environment interactions, evaluations should be made at more than one location and in more than one year.

ISOLATION AND USE OF NEW PARENT LINES. Lines selected from populations improved through recurrent selection may be utilized as parent lines in a hybrid-breeding program. The parent lines are isolated by the pedigree selection procedure outlined in Chapter 9. In hybrid sorghum-breeding programs, it is common to produce two parallel populations, one in fertile cytoplasm without fertility-restoring genes to generate **B-lines**, the other in either fertile or sterile cytoplasm, but including a high frequency of fertility-restoring genes, for generation of **R-lines**. To improve weaknesses in current hybrids, new **B-lines** may be converted to **A-lines** and the new **A-lines** crossed with established **R-lines**, or new **R-lines** may be crossed with established **A-lines**. Preliminary evaluations of new parent lines are made by crossing to established tester lines, or by top-crossing to a heterogeneous population.

Breeding Objectives

Sorghums are grown for the production of grain, fodder, silage, pasture, syrup, and brooms. The sorghum grain is utilized for human food, animal feed, human food, or industrial products. In grain sorghums, the major breeding effort has been directed toward higher grain yields, but other objectives such as earlier maturity, adaptation to mechanized harvesting, resistance to heat and drought stress, disease and insect resistance, resistance to *Striga*, and quality are also important.

Grain Yield

Sorghum grain yields in the United States have increased more rapidly than for any other cereal except corn (Fig. 1.2). Three developments in sorghum breeding made major contributions to the increase in grain yields:

• the development of short-stature, early-maturing sorghum cultivars during the 1920s and 1930s, which adapted sorghum to mechanical harvesting and permitted the production of sorghum in a high-yield environment,

• the development of hybrid sorghum during the 1950s and the rapid replacement of openpollinated cultivars with hybrid sorghum, and

• the development of disease- and insect-resistant cultivars that reduced yield loss from those production hazards.

With the Sorghum Conversion Program, a wealth of new germplasm from tropical sorghums is now available to use in developing higher-yielding temperate sorghum hybrids. The new germplasm will maximize yield gains by increasing the diversity among parent lines.

High yield in sorghum hybrids is correlated with large, heavy panicles, but breeding for high yield also requires that consideration be given to duration of growth, sensitivity to photoperiod, lodging resistance, and resistance to stress, disease, and insect injury. In the harsh environments of many sorghum production areas, stability of yield to adverse climatic conditions is more critical than high yield potential in a sorghum cultivar. Early and medium maturity, stability of seed number, and seed weight are important selection criteria in those environments.

Early Maturity

The development of earlier maturing cultivars and hybrids in the United States has made it possible to extend the production of grain sorghums into regions with higher altitude, shorter summers, and lower rainfall. In low-rainfall areas, early maturity permits sorghum to escape damage from drought, where later maturing cultivars would use available moisture before they mature. Other characteristics that adapt sorghum to shorter growing seasons are ability of the seed to germinate at lower temperatures, permitting earlier planting; photoperiod insensitivity; ability to fill and mature grain under cool nights; an open rather than a compact panicle which hastens dry-down of mature seeds; and panicles that are exserted well from the upper sheath to facilitate rapid drying and earlier harvest.

Adaptation to Mechanized Harvesting

Unimproved tropical cultivars of sorghum introduced into the United States were usually tall with weak stalks that lodged under modern cultivation practices. The short, stiff-stalked, combine-type hybrids currently grown are the culmination of several developments:

• the utilization of dwarfing genes to produce short-statured hybrids;

• development of cultivars with greater stalk strength and erect heads from milo × kafir, milo × hegari, and milo × feterita crosses (Fig. 18.12);

• improved resistance to root and stalk rot diseases;

• stronger stalk structure due to larger stalk diameter and thicker rind (Fig. 18.13); and

• open panicles and greater head exsertion for rapid dry-down and grain maturity.

Selection for short stature was accomplished by utilization of four recessive dwarfing genes—dw_1, dw_2, dw_3, and dw_4—that produce brachytic dwarfs, a form of dwarfing that shortens the internodes but has minor effects on leaf number, leaf size, maturity, and yield. Most combine-type sorghum hybrids grown in the United States have three recessive dwarfing genes and are commonly referred to as 3-dwarfs. Sorghum hybrids with four dwarfing genes, 4-dwarfs, are usually lower in yield than the 3-dwarfs, and sorghums with two dwarfing genes, 2-dwarfs, lodge more readily because they are taller. Parent lines need to be recessive at the same

Fig. 18.12.
Comparison of erect head and stout stalk common to sorghums of milo × kafir parentage (left) with the recurved head and slender stalk of milo cultivars 'Double Dwarf Yellow' (right).

dwarfing gene loci, otherwise the hybrid will be taller than the parents. In addition to the dwarfing genes, genes for early maturity shorten plant height by reducing the number of internodes and the time period for stem elongation. Taller hybrids may be preferred in dry areas, in forage types, or where sorghum is used for ensilage.

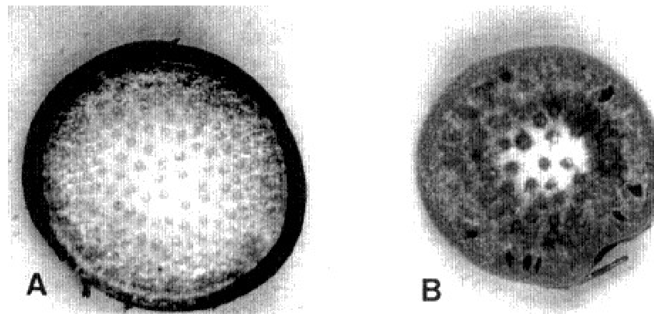


Fig. 18.13.
Cross-section of stem at base of sorghum. (A) A strong, lodging-resistant, breeding line. (B) A weak, lodging-susceptible line. Characteristics shown here associated with lodging resistance are large stalk diameter, thick outer rind, and slow deterioration of the stalk following freeze injury.

Early milo cultivars had recurved or gooseneck peduncles that interfered with machine harvesting. Cultivars with erect heads and improved resistance to lodging were selected from crosses of milo with kafir, hegari, or feterita. Sorghum lines selected from crosses of kafir or hegari with durra or feterita were resistant to shattering, yet they retained the desirable durra or feterita seed characteristics.

Lodging Resistance

Lodging was a serious problem in the tall sorghum introductions grown originally in the United States. Lodging resistance was improved by utilization of dwarfing genes to breed for short stature, by crossing to kafir and hegari to obtain stouter stalks, and by development of cultivars with resistance to the root and stalk rots. Good head exsertion, with the head held erect on a long peduncle permits the head to dry out uniformly as the plant matures and permits early harvest before plants become lodged.

Resistance to Stress from Heat and Drought

Due to its heat and drought tolerance, sorghum is grown in areas of the United States, Africa, Asia, and Australia that are too hot and dry to grow corn. Resistance to stress from heat and drought is provided by mechanisms that permit the plant to remain alive and function normally during adverse heat or drought and to recover sufficiently to produce a harvest. Mechanisms that contribute to stress tolerance include extensive root development to maximize water supply, smaller and fewer stomata to reduce leaf water loss, tolerance to postbloom moisture stress, and tolerance to heat. Drought stressed plants are subject to increased invasion by root and stalk rot pathogens that destroy the stalk's structural framework and cause the plant to lodge.

Resistance to Soil Stress and Tillage Practices

Soil stress may result from aluminum toxicity, iron deficiency, or reduced phosphorus uptake. Aluminum toxicity reduces root development and increases drought injury. Sorghum cultivars with aluminum tolerance have been identified. Sorghum hybrids with small seeds have difficulty in emerging from seedbeds of reduced tillage. Selection for seedling vigor and for cold tolerance during germination are means for overcoming this problem.

Disease Resistance

Sorghum is attacked by a wide array of plant disease pathogens. Disease resistance is necessary in a sorghum hybrid if it is to realize its high yield potential. The sorghum breeder needs to be aware of the most damaging diseases in the production area and concentrate disease resistance breeding efforts on those diseases. In breeding for disease resistance, a screening procedure is needed that includes inoculation of sorghum genotypes with the causal pathogen and evaluation of disease infection either in the field or greenhouse.

ROOT AND STALK ROT DISEASES. Common root and stalk rot diseases of sorghum include *Fusarium* root and stalk rot, charcoal rot, *Periconia* root and crown rot, and red rot. *Fusarium* stalk rot, caused by *Fusarium moniliforme*, is widely distributed and damages the root system, weakens the stalks, causes head blights, and reduces grain filling. The pathogen

survives on plant debris and enters the plant through natural wounds. Selection for seedling resistance is made by germination of seeds at low temperatures in contact with the pathogen. Charcoal rot, incited by *Macrophomina phaseolina*, may cause severe damage to sorghum growing with low moisture and high temperature. Resistance is found in sorgos and forage sorghums and a few lines from the sorghum conversion program. Milo disease, incited by *Periconia circinata*, was a serious disease in milo cultivars at one time. Resistant plants found in a field of Dwarf Yellow milo served as the source of resistance in breeding resistant cultivars. Resistant segregates may be identified by growing sorghum lines in greenhouse flats infested with the *Periconia* pathogen (Fig. 12.7).

LEAF BLIGHTS. Leaf blight incited by *Exserohilum turcicum*, the northern leaf blight, is found in many humid areas of the world. Resistance is present in grain and sweet sorghums, and in sorgo × sudangrass hybrids. The leaf-blighting phase of the disease incited by *Colletotrichum graminicola* may defoliate sorghum plants before they mature. Separate genes control resistance to the leaf blight and the stalk rot phase of the disease.

DOWNY MILDEWS. Crazy top, incited by *Sclerophthora macrospora*, and sorghum downy mildew, incited by *Peronosclerospora sorghi*, are prevalent in many sorghum growing areas of the world causing severe damage to infected sorghum. The downy mildew disease produces a green and white striping on the leaves with infected plants failing to head. Several physiologic races of *P. sorghi* have been identified. Resistance is present in some sorghum genotypes of Ethiopian origin.

VIRUS DISEASES. Maize dwarf mosaic (**MDM**), is a serious virus disease in sorghum throughout the world. MDM symptoms are an irregular mottling on the upper leaves, stunting, delayed flowering or failure to head, and in severe cases, death of the plant. The virus is vectored by the greenbug, corn leaf aphid, and other species of aphids. Many sorghum hybrids possess tolerance but few are highly resistant.

SMUTS. Four smut diseases, long, loose, covered, and head, attack sorghum, but only one, head smut incited by *Sporisorium reilianum*, is dependent upon resistant cultivars for control. Head smut is common in most sorghum-growing areas of the world. Several races of the head smut pathogen have been identified. Resistance has been identified among exotic sorghums convened from tropical to temperate adaptation.

RUST. Rust, incited by *Puccinia purpurea*, is prevalent in many sorghum-growing areas but has seldom been a serious disease in the United States. Resistance is conditioned by a single dominant gene. Slow-rusting types have been identified in the sorghum world collection.

PARASITIC PLANTS. Witchweeds, *Striga hermonthica* and *S. asiatica*, are parasitic on sorghum in semiarid regions of Africa south of the Sahara and in Asia. *Striga* is an extremely devastating weed that depends upon the host plant for survival. Infested plants are greatly reduced in yield and often killed due to loss of water and nutrient supply to the parasite. Resistance to *Striga* is based on finding barriers to establishment of the parasite on the roots of sorghum plants, and sorghum genotypes with a low production of a stimulant, *sorgoleone*, exuded from sorghum roots that is required for germination of *Striga* seeds.

Insect Resistance

Host plant resistance has been important in controlling major insect pests in sorghum. Insect resistance in sorghum is commonly due to nonpreference for insect feeding, or reduced reproductive capability of the insect, known as antibiosis.

GREENBOG. The greenbug, *Schizaphis graminum*, is a destructive insect pest on sorghum. It reproduces by parthenogenesis at a high rate and feeds on the sorghum plant by injecting a toxin into the leaf tissue and sucking out the juice. Resistance is due to antibiosis. Biologic races of the insect have been identified, making it necessary to find new genes for resistance as new races evolve. In addition to the race-specific genes, transgressive segregation for resistance has been reported in particular crosses. Screening procedures for resistance are easy to manipulate because greenbugs multiply quickly on the test plants.

SORGHUM MIDGE. The sorghum midge, *Contarinia sorghicola*, causes severe loss in yield in all areas of the world where sorghum is grown. In the United States the midge is most prevalent in the southern sorghum production areas. Eggs are laid inside the glumes of the flower, and larvae feed on the developing seeds, producing empty spikelets that give the appearance of failure to set seeds. Johnsongrass and wild sorghums provide a reservoir for maintenance of natural midge populations. Progress in breeding resistant hybrids has been made with crosses to resistant germplasm from Ethiopia and Brazil. Resistance, which hinders the deposit of eggs, is quantitatively inherited, so both **A-lines** and **R-lines** need to be resistant.

STALK BORERS. Many species of stalk borers attack sorghum in Asia and Africa. In the United States, the European corn borer, *Ostrinia nubilalis*, and the southwestern corn borer, *Diatraea grandiosella, are* the principal predators, often causing breakage of the peduncle supporting the head. The inheritance of resistance is complex and recurrent selection has been effective in increasing the level of resistance.

SHOOT FLY. The shoot fly, *Antherigona soccata*, is a damaging insect pest of sorghum in the tropics, but is not found in the United States. Eggs are laid on the underside of leaves of seedling plants. The larvae crawl down between the outer and inner leaves and bore into the young shoot, killing the growing point.

Quality

The sorghum grain is utilized for food, livestock feed, and an increasing number of industrial products. The sorghum plant is utilized for forage. This diversity of uses increases the complexity of breeding for improved quality.

SORGHUM FOR FOOD. In Africa and Asia, sorghum grain is used for human food as porridge, leavened and unleavened bread, boiled whole grain, roasting, popping, and for making beer. Traditionally, the grain is tempered and the pericarp removed with a stone mortar and wooden pestle. In the United States, the sorghum grain is processed into flour by a dry-milling process for use in a variety of food products and into starch by a wet-milling process. Color of the sorghum grain and endosperm texture are major characteristics to be considered in breeding for improved food quality. White pericarp color is preferred over pigmented or brown testa seeds. Hard corneous endosperms produce brighter-colored seeds than soft, floury

endosperms. A high proportion of corneous endosperm increases dry-milling yield.

SORGHUM AS A FEED GRAIN. In North and South America, Australia, and Europe, sorghum is widely used as feed for livestock. Improvement of sorghum grain for feed involves elimination of unsatisfactory grain characteristics and increasing the nutritional value of the grain. Grain sorghums with red pericarps weather to a dirty brown color. Sorghums with brown-colored pericarps and testae contain small amounts or tannins or other astringent substances and have a bitter taste. The tannins (polyphenols) form complexes with seed proteins making the protein less available to monogastric animals. Sorghums with tan pericarps and seed coats are generally more resistant to weathering. A white or yellow color is most attractive. Hard or corneous seeds are less digestible than sorghums with softer endosperms.

The nutritional quality of sorghum has been improved by breeding for yellow-endosperm hybrids that contain carotenoid pigments particularly desirable in poultry feeds. The yellow endosperm gene is generally present in at least one inbred in United States hybrids, but the hybrid seed is improved if genes for yellow endosperm are present in both parent lines. High lysine content controlled by a single recessive gene was identified in introductions from Ethiopia. Feeding trials have demonstrated that the high-lysine sorghums have higher nutritive value than normal sorghums, but they are generally lower-yielding.

INDUSTRIAL UTILIZATION. Sorghum grain is utilized for industrial and food products, including starch, flour, grits, breakfast foods, syrups, malt, and fermentation products. In the commercial production of starch from sorghum, the presence of certain red, purplish, or black water-soluble pigments in the plant tissue, the glumes, or the subcoat of the seed may impart an off-color, which the starch absorbs during the steeping process.

FORAGE QUALITY. Sudangrass, the sorgos, and leafy cultivars of grain sorghum are used for forage as fodder, silage, hay, or pasture. Juiciness, sweetness, and leafiness are desirable characteristics in forage sorghums. For silage, a vigorously growing hybrid with a high proportion of grain and leaves to stalk will improve nutritive value. The green leaves of the sorghum plant contain a cyanogenetic glucoside, dhurrin, which on hydrolysis in the rumen releases hydrocyanic or prussic acid. Growth conditions which cause a disruption in leaf tissue growth increases the content of the cyanogenetic glucoside in the sorghum plants to a level that can cause fatal poisoning to ruminants grazing on it. Strains of sorghum and sudangrass differ genetically in the amount of HCN that may be released in similar environments.

International Sorghum Breeding Programs

The International Crops Research Institute for the Semi-Arid Tropics (**ICRISAT**), located at Patancheru, A.P., India, is an international research center with the role of improving the genetic potential for grain yield and nutritional quality in sorghum. Improved breeding materials are distributed to research workers in national sorghum breeding programs in developing countries where sorghum is a major food crop. ICRISAT is the depository for the world collection of sorghums, coordinates an international sorghum testing nursery, and provides training for sorghum research workers from developing countries. Contributions to the international improvement of sorghums are being made by INSORMIL (International Sorghum and Millet), a consortium of United States Land Grant Universities, the United States

Department of Agriculture, and ICRISAT, under sponsorship of the United States Agency for International Development (USAID).

Study Questions

l. How are hybrid sorghum cultivars produced?

2. Where is the center of origin for sorghum? What are the various groups of sorghum and what are their uses?

3. What breeding procedure does the plant breeder use to introduce dwarfing genes into sorghum? Describe the process.

Further Reading

Bennett, W.F., B.B. Tucker, and A.B. Maunder. 1990. Development, selection, and production of hybrid sorghum. p. 39-62. *In* W.F. Bennett, B.B. Tucker, and A.B. Maunder (eds.) Modern grain sorghum production. Iowa State Univ. Press, Ames, IA.

de Wet, J.M.J. 1978. Systematics and evolution of sorghum Sect. Sorghum (Gramineae). Am. J. Bot. 65:477-84.

Dixon, A.G. Olonju, P.J. Bramel-Cox, J.C. Reese, and T.L. Harvey. 1990. Mechanisms of resistance and their interactions in twelve sources of resistance to biotype E greenbug (*Homoptera: Aphididae*) in sorghum. J. Econ. Entomol. 83:234-40.

Doggett, H. 1988. Sorghum. Longman Scientific and Technical, U.K. Group LTD., and John Wiley & Sons, New York.

Duncan, R.R., P.J. Bramel-Cox, and F.R. Miller. 1991. Contributions of introduced sorghum germplasm to hybrid development in the USA. p. 69-102. *In* H.L. Shands and L.E. Wiesner (eds.) Use of plant introductions in cultivar development, Part 1. Crop Sci. Soc. Am. Spec. Publ. 17. Am. Soc. Agron., Crop Sci Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Ejeta, G., and L.G. Butler. 1993. Host plant resistance to *Striga*. p. 561-69. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Harlan, J.R., and J.M.J. de Wet. 1972. A simplified classification of cultivated sorghum. Crop Sci. 12:172-76.

House, L.R. 1985. A guide to sorghum breeding. 2nd ed. Int. Crops Res. Inst. for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India.

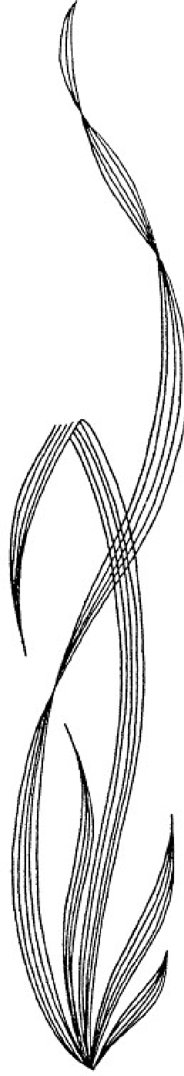
Nath, B. 1982. Population breeding techniques in sorghum. p. 421-34. *In* J.V. Mertin (ed.) Sorghum in the eighties. Vol. 1. Int. Crops Res. Inst. for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India.

Rooney, L.W., F.R. Miller, and D.T. Rosenow. 1989. The use and characteristics of sorghum for human food. p. 31-47. *In* D. Wilkinson (ed.) Proc. 44th Annu. Corn and Sorghum Industry Res. Conf., Chicago, IL, Dec. 6-7. 1989. Am. Seed Trade Assoc., Washington, D.C.

Schertz, K.F., and D.R. Pring. 1982. Cytoplasmic sterility systems in sorghum. p. 373-83. *In* J.V. Mertin. (ed.) Sorghum in the eighties. Int. Crops Res. Inst. for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India.

Stephens, J.C., F.R. Miller, and D.T. Rosenow. 1967. Conversion of alien sorghums to early combine genotypes. Crop Sci. 7:396.

Teetes, G.L. 1980. Breeding sorghums resistant to insects. p. 457-85. *In* F.G. Maxwell and P.R. Jennings (eds.) Breeding plants resistant to insects. John Wiley & Sons, New York.



VIII
APPLICATIONS:
FIELD CROPS WITH MISCELLANEOUS BREEDING PROCEDURES

19. Breeding Cotton

Cotton (*Gossypium* spp.) has been cultivated in tropical and subtropical climates of the world since prehistoric times. Although the wild cotton species are often woody perennials, ranging from shrubs to trees, the domesticated cottons are generally cultivated as herbaceous row crops, or, in a few areas of the world, by ratooning as with the Moco cottons of Brazil. In addition to being the world's most important textile fiber crop, cotton is the world's second-most important oilseed crop after soybean. Cotton, like sorghum, outcrosses freely, the outcrossing being the result of insect pollinations. The natural outcrossing influences the procedures employed in breeding cotton and the practices for maintenance of cotton cultivars.

Origin and Species

The genus *Gossypium* is very large, currently containing 50 species with a basic chromosome number of 13. New species continue to be discovered. Of the known species, 45 are diploid ($2n = 2x = 26$) and are grouped in seven genomes designated **A, B, C, D, E, F**, and **G**. Diploid species with the **A, B, E**, or **F** genome are African or Asian in origin and are referred to as Old World species. These species pair fairly well with each other and are fairly closely related. Diploid species with the **C** or **G** genomes are Australian in origin. Diploid species containing the D genome originated in the Western Hemisphere and are referred to as New World species. Chromosomes in the **D** genome, overall, are smaller than chromosomes in the other genomes. In addition to the 45 diploid species, there are five allotetraploid species ($2n = 4x = 52$). All are New World species, four indigenous to the continental Americas and one to Hawaii. The new world allotetraploids contain the **AADD** genome combination and have 26 large and 26 small chromosomes (Fig. 19.1), although there is some overlapping in size among chromosomes in the **A** and **D** genomes. Their genetic origin was demonstrated experimentally by crossing *G. aboreum*, a cultivated, diploid Indian species (**A** genome), and *G. thurberi*, a wild, diploid American species (**D** genome), and doubling the chromosomes of the sterile hybrid with colchicine. The resulting amphidiploid, **AADD** ($2n = 4x = 52$), produced fertile hybrids when crossed with an American tetraploid cotton. Two diploid and two tetraploid species of *Gossypium* have spinnable seed fibers called *lint*. These became the cultivated cottons:

• *G. herbaceum* L., ($2n = 2x = 26$) **A** genome, large chromosomes,

• *G. aboreum* L., ($2n = 2x = 26$) **A** genome, large chromosomes,

• *G. hirsutum* L., ($2n = 4x = 52$) **AD** genomes, 26 small and 26 large chromosomes, and

• *G. barbadense* L., ($2n = 4x = 52$) **AD** genomes, 26 small and 26 large chromosomes.

G. herbaceum and *G. aboreum* were the original cultivated cottons of India. Now, they have been replaced by *G. hirsutum* and account for less than 1% of the world's cotton. *G. hirsutum and G. barbadense* were growing both wild and as domesticated cottons in the New World in pre-Columbian times. Early colonists cultivated a strain of *G. barbadense* along the coastal islands and lowlands of Georgia and South Carolina, where it became known as Sea Island cotton. Inland from the low coastal areas, at higher elevations, the Sea Island cotton did not mature. In these upland areas the early colonists began growing early strains of G. *hirsutum*, which became known as American Upland cotton, a name that has persisted throughout the years.

G. hirsutum is the principal cultivated cotton and accounts for about 90% of the world's cotton production. The Sea Island form of *G. barbadense* was introduced into the Nile Valley of Egypt, where it became known as Egyptian cotton and was prized for its fine, long, strong fibers. Egyptian cotton was subsequently introduced to Arizona, where it is known as Pima cotton. *G. barbadense* accounts for about 9% of the world's cotton production.

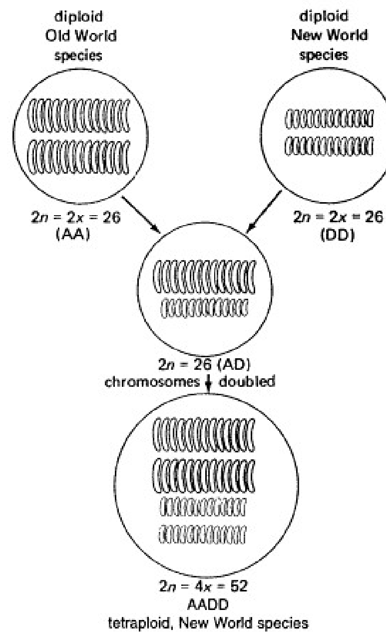


Fig. 19.1.
Genetic origin of tetraploid cotton. The tetraploid New World cottons ($2n = 4x = 52$, genomes AADD) are allotetraploids, in which the AA genomes of the diploid, Old World (Asiatic) species are combined with the DD genomes of diploid, New World (American) species. The chromosomes of the AA genomes are larger than the chromosomes of the DD genomes.

Flowering and Pollination

The cotton flower is surrounded by three triangular bracts forming what is commonly known as squares. The flower contains an open corolla with five petals, a staminal column bearing clusters of stamens and forming a tube that encloses the style. The compound pistil consists of three to five carpels with stigmas protruding above the anthers. The ovary develops

into a three- to five-loculed capsule or boll (Fig. 19.2). From seven to nine seeds are set within each lock or locule.

On the day preceding anthesis, a twisted corolla emerges from the square. On the day of anthesis, the corolla opens and pollen shedding occurs. The corolla turns red the day following anthesis and later falls from the plant (Fig. 19.3).

Pollination occurs with the opening of the anthers and shedding of pollen on the stigma. Pollination is also consummated from pollen deposited on the stigma by insects. Cotton is predominantly self-pollinated, but 5 to 30% cross-pollination may occur from insect pollination. The amount of cross-pollination varies with the kinds and numbers of insect pollinators present in the field. Pollen is windborne only to a very slight extent, if at all, on account of its heavy, sticky nature. Natural cross-pollination in breeding lines can be prevented by covering the cotton bud with a small paper bag or envelope the afternoon before it opens; or fastening together the tips of the corolla with paper clips, robber bands, collodion, or fingernail polish; or by tying them together with a fine copper wire (Fig. 19.4).

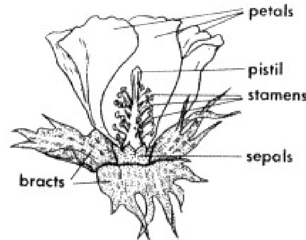


Fig. 19.2.
Cotton flower with petals cut away.

Cotton flowers are emasculated and prepared for crossing by removal of the staminal column on the day before flowering, after which the emasculated flower is covered to protect it from insect pollination. The emasculated flower is pollinated the following day according to procedures illustrated in Fig. 19.5, or by brushing ripe anthers over the stigma of the emasculated flower. Seed-set after artificial crossing is normally about 75% of that from natural pollination. A brightly colored tag attached to the flower stem facilitates identification when the boll is mature.

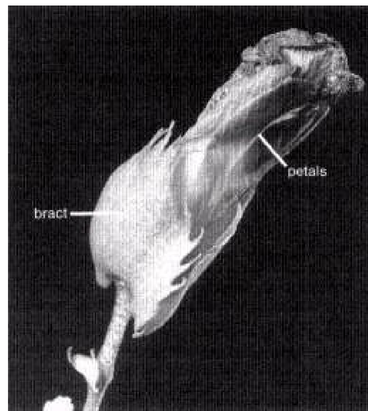


Fig. 19.3.
Cotton flower closed after pollination. The petals turn pink or red the day after pollination and later fall from the plant.

Male Sterility

Both genetic and cytoplasmic male sterility systems have been identified in tetraploid cottons. Genetic male sterility is controlled by single recessive genes, duplicate recessive genes, or a dominant gene. The dominant recessive gene, MS_4, normally gives complete male sterility and may be used to avoid emasculation when crossing cotton. Cytoplasmic male-sterile cotton results from the transfer of *G*.

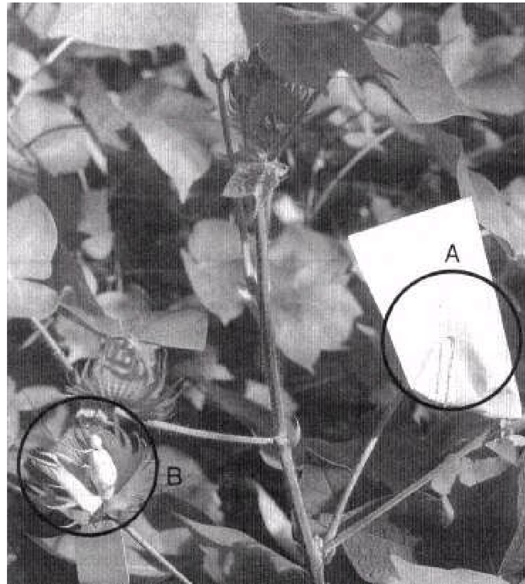


Fig. 19.4.
Methods of preventing cross-pollination in cotton. (A) Flowers covered with paper bag. (B) Corolla tip fastened with a fine copper wire.

hirsutum or *G. barbadense* chromosomes into *G. harknessii* cytoplasm. Fertility restoration is obtained from a single partially dominant gene (*Rf*) transferred from *G. harknessii*. The gene gives good fertility restoration in commercial Upland cultivars when homozygous, but only fair fertility restoration when heterozygous as in $\mathbf{F_1}$ hybrids, thus limiting the potential use of this cytoplasmic:nuclear system. Fertility restoration is improved by a dominant enhancer gene, *E*, from *G. barbadense*.

Genetics and Cytology

Genes for a number of qualitative traits useful for the cotton breeder have been identified. These include genes for disease and insect resistance, leaf pubescence, absence of nectaries, male sterility, and bud gossypol. Genetic maps have been associated with most of the 26 G. *hirsutum* chromosomes. Being a diverse tetraploid species, characters often exhibit dual factor inheritance. Many characters in cotton that have agronomic importance are inherited in a quantitative manner. These include lint yield; percentage of lint; boll size; and fiber length, strength, and fineness.

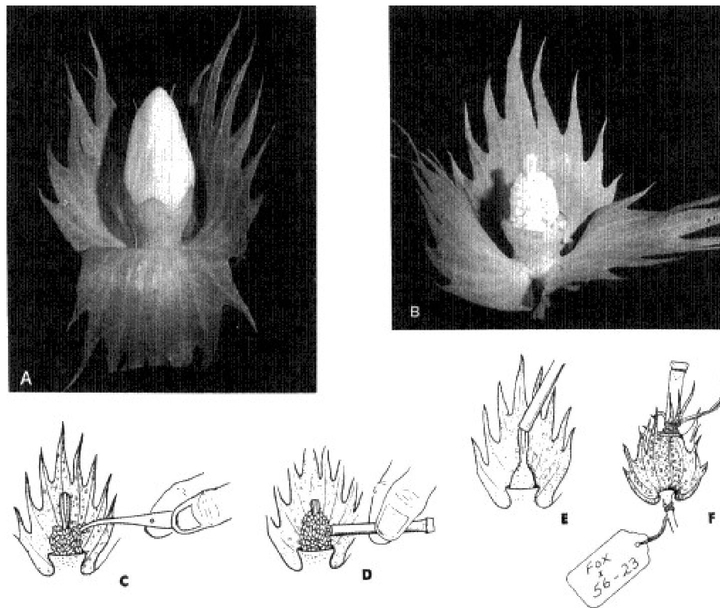


Fig. 19.5.
Steps in crossing cotton. (A) Cotton flower at suitable stage for emasculation and crossing. At this stage the anthers are compressed around the staminal column with the stigma protruding from the tip. (B) Cotton flower with sepals and petals cut away preparatory to emasculation. (C) Removing the stamens with fine pointed tweezers. (D) Collecting ripe anthers from a flower on the pollen parent with a short section of a soda straw. The end of the straw is crimped to hold the anthers. (E) Slipping the soda straw containing the ripe anthers over the stigma of an emasculated flower. (F) Bracts wired around the soda straw, holding it in place over the style, thus protecting the stigma from foreign pollen. The wire also holds a tag on which the cross is recorded.

Research efforts have been directed toward development of a monosomic series in the allotetraploid species of cotton, *G. hirsutum and G. barbadense*, as was done in allotetraploid wheat, but the monosomic series is only partially complete. The monosomics are useful for assigning specific loci to particular chromosomes and for cytogenetic manipulations, such as the synthesis of interspecific chromosome substitution lines. Haploids of cotton appear naturally or they may be induced by *semigamy*, as described in Chapter 5. By doubling the haploid chromosomes, homozygous diploid plants are obtained. Interspecific hybridization has been utilized to transfer genes for the nectariless character, bacterial blight resistance, increased fiber strength, and the $\mathbf{D_2}$ gene for smooth leaf from wild species into cultivated cottons. Crosses of *G. hirsutum* × *G. barbadense* give vigorous $\mathbf{F_1}$ hybrids, but $\mathbf{F_2}$ generations are generally unstable and segregate widely. The United States germplasm collection of cotton and its wild

relatives and the genetic and cytogenetic collections of *Gossypium are* maintained by the United States Department of Agriculture at College Station, Texas, in cooperation with the Texas Agricultural Experiment Station. Collections of *G. barbadense are* maintained at Maricopa, Arizona.

Biotechnology

Molecular genetic maps of the cotton genome using restriction fragment length polymorphisms (RFLPs) are in the process of being developed, and should prove useful for identification and cloning of genes for traits of value in cotton breeding. Tissue culture is being utilized to regenerate cotton, although cotton in the past has proven to be a recalcitrant species for tissue culture regeneration. Transgenic cotton has been reported using the *Agrobacterium-mediated gene transfer system*. More success was obtained with Coker cultivars than cultivars of other origins, pointing to a genotype interaction in cotton transformation. Development of transgenic cotton has concentrated on (1) transfer of the *Bt* gene from *Bacillus thuringiensis* to develop cottons that are resistant to certain *Lepidoptera* insects (Fig. 8.13), and (2) resistance to the herbicide glyphosate. Genetic transformation through biotechnology may be used to supplement but does not replace plant breeding procedures for cotton cultivar improvement.

Origin and Diversity of American Upland Cotton

Gossypium hirsutum cultivars are referred to as American Upland cotton. The early history of American Upland cotton is complex due to the genetic diversity in the early cottons introduced into the southern states; the frequent occurrence of cross-pollination among the different introduced types; and the rapid genetic adjustment in cotton to climatic differences and cultural practices in the southern states in comparison to the tropical climate and primitive cultural practices in the regions where the cottons originated. A major adjustment that had to be made was the adaptation to longer photoperiods. The adjustments were hastened by the contributions of large numbers of early cotton breeders who worked without the genetic guidelines available to cotton breeders today.

Because cotton is partially cross-pollinated, the heterozygosity maintained within a cotton population makes 'variety' a less specific entity than in the self-pollinated crops. The genetic makeup of a cultivar of cotton may change from year to year. Deteriorating purity made it necessary for cotton growers to replace seed stocks frequently with new seed. Plant selections made by seedsmen within the seed fields to meet the demand for pure seed often isolated different genetic types to which new cultivar names were given. Breeding and cultivar development in cotton in the United States differed from that in self-pollinated crops because the breeding was largely done by private breeders and seed companies, rather than the State Agricultural Experiment Stations and the United States Department of Agriculture. It became the custom to identify cotton cultivar names with the name or trademark of the company marketing the new cultivar followed by a number to identify the particular release. The introduction of the new American Upland cultivars to other areas of the world, such as India or the former Soviet Union, changed the types of cotton grown in those countries.

From the early United States cotton breeding programs, four general types of *G. hirsutum* cultivars are now grown in the United States:

• Eastern Upland Type. A medium-size open-boll type that can be spindle harvested, with medium-length staple and resistance to fusarium wilt. The Eastern type is grown in the eastern United States Cotton Belt and is characterized by the cultivars marketed by major seed companies in the region.

• Delta Upland Type. A small- to medium-size open-boll type that can be spindle-harvested, with medium-length staple, grown principally in the Mississippi Delta, southern Texas, and Arizona. The Delta type is characterized by cultivars marketed by Deltapine, Stoneville, and other seed companies in the Mississippi Delta region.

• Plains or Storm-proof Type. These cottons have large bolls, short staple length, and medium fiber strength. The Plains type is a closed-boll, storm-proof type that is harvested by stripping unopened or partially opened bolls from the cotton plant. The Storm-proof type of cotton is adapted to the Western Plains, chiefly in Texas and Oklahoma, where fiber from open-boll cultivars would be blown from the bolls by high winds.

• Acala Type. The 'Acala' type is characterized by medium to large open-bolls, medium to long staple, strong fiber, and a long fruiting period. Acala is the principal type in California.

A comparison of staple length in Plains type, Acala, Pima, and Sea Island cottons is shown in Figure 19.6.

Pima Cotton

Cultivars of *G. barbadense* grown in the United States are generally referred to as 'Pima' cotton. The name Pima has become associated with superior fiber quality by the cotton trade. In 1903, introductions of Egyptian cotton were planted in Arizona and southern California because the climate of that area was similar to the climate of the Nile Valley in Egypt. Original

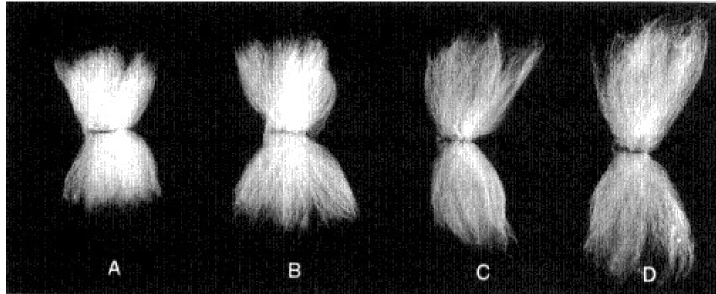


Fig. 19.6.
Comparison of staple length in cotton. (A) A short- to medium-staple storm-proof Plains type. (B) A medium- to long-staple Acala type. (C) An extra-long-staple Pima type. (D) Extra-long staple of Sea Island, a type no longer grown in the United States. A and B are *G. hirsutum*; C and D are *G. barbadense*.

Pima cotton cultivars were direct selections from the Egyptian introductions. Subsequent cultivars involved hybridization followed by selection. Pima cotton is characterized by small open bolls; long, strong fibers; and great yam strength. Production of Pima is concentrated in irrigated areas of Arizona, New Mexico, the upper Rio Grande Valley in Texas, and the San Joaquin Valley of California. New Pima cultivars are identified by a number, for example, 'Pima S-l,' 'Pima S-2,' or 'Pima S-6.' Pima cotton is grown on less than 1% of the total area planted to cotton in the United States.

Breeding Methods

The methods used in breeding cotton differ from the methods used with self-pollinated crops such as wheat or soybean due to the partial cross-pollination and its effect on the genetic makeup of a cotton population. Cultivars of cotton are rarely if ever established as pure lines as in small grains or soybean. A moderate degree of heterogeneity and heterozygosity is desired in a cotton cultivar to provide some heterosis and maintain maximum yield potential. The aim of the cotton breeder is to purify the lines sufficiently to obtain uniformity, yet maintain adequate heterozygosity for the cultivars to be vigorous and productive. This was accomplished by final selection of lines in an early generation while heterozygosity was still present, or by blending related lines or families of lines in the final development and release of the cultivar. The lines are uniform for plant morphological characteristics, disease and insect resistance, and lint quality, yet sufficiently heterogeneous that a degree of heterozygosity and hybrid vigor, and a broad ecological adaptation, will be expressed.

Deterioration in Cultivar Purity

Segregation in advanced generations leads to divergence from the original cultivar type and deterioration in cultivar purity, forcing the cotton grower to procure new seed stocks from the cotton breeder. The strategy of the cotton seeds dealers and breeders is to maintain uniformity in plant type, disease and insect resistance, and desirable fiber characteristics, while exploiting heterosis for fiber yield through cross-pollination among genetically diverse genotypes. As in self-pollinated crops, hybridization is used to develop new genetic combinations, and the backcross is utilized to add desirable alleles to already established cultivars. Recurrent selection procedures may be utilized to concentrate genes for specific quantitatively inherited characters.

Introduction, Acclimatization, and Germplasm Utilization

Acclimatization played a much greater role in the development of introduced cotton germplasm than in the introduced germplasm of self-pollinated cereals or soybean. The early-introduced cotton stocks were largely mixed populations with varying amounts of cross-pollination and heterozygosity that gave them plasticity and potential for genetic change. They were tropical in origin, perennial, photoperiod sensitive, and did not flower under the long days of the United States Cotton Belt. Yet, following generations of repeated selection, these introductions were molded into early maturing, photoperiod-insensitive cultivars adapted for production in the southern United States Cotton Belt. Introduced germplasm continues to serve as a source of specific genes useful in the breeding program.

Selection in Cotton Breeding

Pure-line selection is not practiced in cotton breeding as it leads to homozygosity, reduced vigor, and lower yield. Instead, seed producers normally practice some form of *progeny selection* to maintain purity of existing cultivars, to progressively improve the cultivar, and to generate new lines in a segregating population following a cross in a hybridization program. *Recurrent selection* is used to intensify genes for a quantitatively inherited character. Both genetic and cytoplasmic male-sterile systems are available that can simplify recurrent selection procedures.

Hybridization in Cotton Breeding

Hybridization is the most common breeding procedure for producing new cotton cultivars. Hybridization is utilized to combine genes for desirable characters, to add a gene for a desirable character through a backcross, or to intensify genes for a quantitative character in a recurrent selection program. A pedigree selection procedure is generally followed during the segregating generations. Selection is generally terminated at an early generation while some heterozygosity still remains, rarely being pursued until homozygosity is reached. Lines genetically different, yet uniform for plant type, disease and insect resistance, and fiber properties, are pooled to form the new cultivar.

Hybrid Cotton

Interest in the utilization of hybrid vigor in cotton by growing first-generation hybrids increased following the discovery of cytoplasmic male sterility in cotton which was obtained by transferring *G. hirsutum* chromosomes into *G. harknessii* cytoplasm. Fertility is restored by a fertility restorer (*Rf*) gene from *G. harknessii* and a gene from Pima cotton that enhances fertility. An obstacle not overcome is that of obtaining sufficient cross-pollination by bees and other insects in the seed production field to make hybrid cotton economically feasible. This led to proposals to increase and use $\mathbf{F_2}$ seed. Certain $\mathbf{F_2}$ populations may have sufficient residual heterosis to be an economically competitive cultivar, but overall yield advances have not been sufficient to offset increased costs.

In India, seeds of hybrid cotton are commercially produced by hand emasculation and pollination, or by hand pollination of genetic male-sterile cotton. The labor required for hand pollinations makes this procedure economically unfeasible in countries with high labor costs. The system for producing hybrid cotton is the same as that described for wheat and sorghum, utilizing **A-, B-**, and **R-lines**.

Cultivar Maintenance

Deterioration in cotton cultivars makes it necessary for the cotton grower to obtain new stocks of pure seed on a more or less regular basis. Cultivar maintenance may be carried out by (1) *roguing-out off-type plants*, (2) *mass selection*, (3) *progeny-selection*, and (4) *keeping seed stocks of the original seed increase*. Roguing-out off-type plants reduces mixture but is limited to eliminating plants that can be visually identified as being different from the cultivar type. Off-type plants need to be removed before flowering to prevent cross-pollinations with

normal plants. Mass selection also depends on visual selection to identify plants characteristic of the cotton cultivar. Mass selection may actually increase variability by inadvertent inclusion of off-type plants in higher frequency than they were present in the original population. Progeny-selection is the most widely used procedure for cultivar maintenance. Plants typical of the cultivar are harvested from a seed increase plot and grown in progeny rows. Progeny rows conforming closely to the cultivar type are harvested and the seeds pooled to provide the nucleus for a new pure-seed increase. In progeny-selection, selection is based on a row of plants rather than a single plant as in mass selection. Recent practice is to produce several tons of the original seed stocks of a new cultivar and store in an environmentally controlled cold-room to maintain germination over a period of years. Each year sufficient seed is removed from the reserve supply to provide the nucleus for a new seed increase. Genetic changes in the nuclear seed are thus minimized during the expected life of the cultivar.

Breeding Objectives

Principal objectives in breeding cotton are high production of lint fiber, early maturity, adaptation to mechanical harvesting, resistance to stress environments, resistance to disease and insect injury, and improvement in fiber and seed quality. Other considerations are important in local areas.

Yield of Fiber

High yield of high-quality lint fiber is the ultimate objective in the breeding of cotton. The yield of a cotton plant is determined by (1) *number of bolls*, (2) *size of the bolls*, and (3) *percentage of lint*. The characteristic contributing most to yield is number of bolls. For plants to be high-yielding, they must be prolific and set a large number of bolls. Cotton cultivars differ in size of boll (Fig. 19.7). Boll size is expressed as the weight in grams of seedcotton (lint + seeds) per boll. Normally, cultivars that set a high percentage of five-lock bolls are superior in yielding ability to cultivars with four-lock bolls. Lint production is affected by seed-set because lint is produced on the surface of the seed and by the density of the lint on the seed. The percentage of lint is determined from the weight of the lint cotton that may be obtained from a given weight of seed cotton. Selection for improved yield of lint often results in a reduction in fiber quality. In temperate climates it is important that the bolls be set early enough that most will mature and that few immature bolls remain on the plant when it is killed by frost.

Rapid Fruiting and Early Maturity

Flowering of the cotton plant is indeterminate with bolls set over a period of time. Earliness is influenced by (1) how early the cotton plant begins to set squares and to flower, (2) how rapidly the new flowers develop, and (3) the length of time required for the bolls to mature. Rapid fruiting and early maturity reduce losses to disease and insects, facilitates harvesting with a mechanical picker, and increases production efficiency by reducing inputs of fertilizer, protective chemicals, or irrigation water. Small compact plants and small bolls and seeds are generally associated with earliness in a cotton cultivar. In recent years it was demonstrated that early maturity was compatible with high production and quality, and cotton cultivars in the southern Delta area have been converted to rapid-fruiting, early-maturity types.

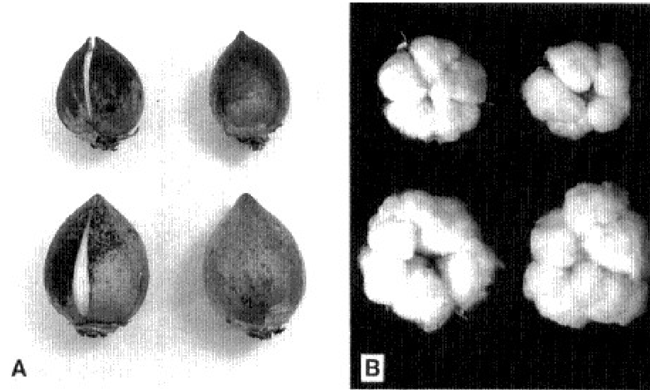


Fig. 19.7.
Comparison of small boll Delta type (upper), with large boll Acala type (lower). (A) Unopened bolls. (B) Mature bolls.

Adaptation to Mechanical Harvesting

In the United States, the eastern, southern, and Acala types of Upland cotton and Pima cotton are harvested with a spindle harvester. Boll size and opening are indexes of picking efficiency. Bolls need to open sufficiently to permit the cotton to fluff and be caught by the spindles. Yet they must have sufficient storm resistance for the fiber to remain in the burr and not be blown or rained out and lost before harvest (Fig. 19.8). A compact, rapid-fruiting plant that does not lodge on fertile soils, with bolls spaced along the main stems and set high enough off the ground that they are not lost in spindle harvesting, is desired. A natural tendency to shed leaves upon maturation of the bolls, or ease of defoliation; small or deciduous bracts; and smooth leaves free of hairs will reduce the amount of leaves and trash in the seed cotton. The storm-proof cotton grown in the high plains is harvested by stripping whole bolls from the plant. Short plants with short fruiting branches, bolls borne singly, early fruiting and early maturity, and seedcotton that adheres tightly in the boll at maturity are characteristics desired in storm-proof cotton cultivars.

Resistance to Stress Environments

DROUGHT AND HEAT TOLERANCE. Water is often a limiting resource for cotton production in dry areas of the world. Limited sources of irrigation water and higher fuel costs for pumping is causing breeders to look for cotton strains with more efficient water use under drought conditions. Genetic variability for root growth and dry matter accumulation has been demonstrated among exotic strains and selections from breeding populations growing in drought environments. Recurrent selection to improve drought tolerance would involve crossing among drought-tolerant strains to form a source population from which selections are made under drought stress conditions. The superior selections are crossed in all combinations to start the

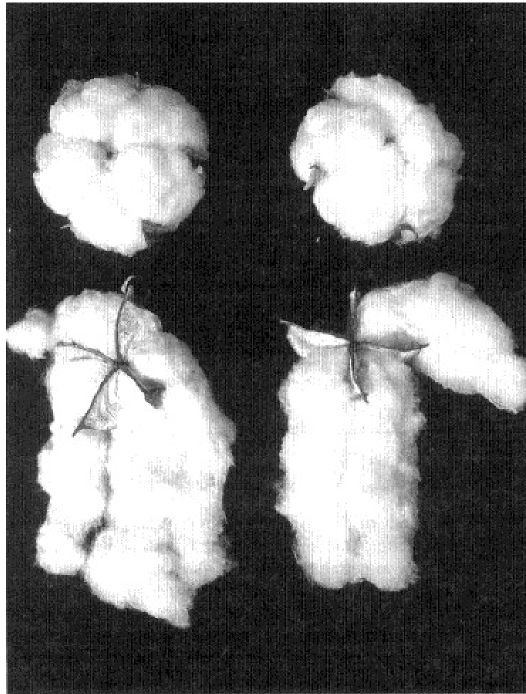


Fig. 19.8.
Comparison of mature bolls of storm-proof (upper) and open boll cottons (lower). In the storm-proof, Plains type, the cotton remains in the boll and is harvested by stripping boils from the plant. In the open boll, Eastern and Delta types, the cotton is harvested by mechanical pickers using spindles. When open boll cottons are subjected to high winds, the lint strings out as shown here with some of the lint being lost.

next selection cycle. Selection of *G. barbadense* strains in periods of high temperature at low elevations resulted in development of Pima strains with greater heat tolerance.

SALT TOLERANCE. Genetic differences to salt tolerance during late growth stages have been observed in cotton strains grown in saline soils. Salt tolerance during germination, early growth, and during late vegetative growth has been observed in a strain of Acala cotton.

Disease Resistance

Many disease problems are associated with the cotton plant. Breeding for host-plant resistance has been an effective method of control of the major disease pathogens. Development of multidisease resistance has received much attention in the breeding of resistant cultivars.

SEEDLING DISEASE COMPLEX. Several soil fungi, including *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani* Kuehn., and *Thielaviopsis basicola* (Berk. & Br.), reduce the potential yield of cotton by causing seed rotting and damping-off of cotton seedlings. Cotton is particularly vulnerable to seedling disease when planted in cold, wet soil. Progress in breeding for resistance to seedling disease may be attained by selecting for rapid germination and seedling vigor in cold wet soils, combined with seedling disease resistance.

FUSARIUM WILT-ROOT KNOT NEMATODE COMPLEX. Fusarium wilt is caused by a soil-inhabiting fungus, *Fusarium oxysporum*, Schlect. f. sp. *vasinfectum* (Atk.) Snyd. and Hans. Fusarium wilt is most severe on light, sandy soils. The disease damages the water-conducting tissues of the plant, causing wilting and premature killing (Fig. 19.9). The disease is associated with injury caused by the root knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood, which provides openings through which the wilt fungus enters the root. Both nematode and wilt resistance are required to give a cultivar maximum protection. The principles of survival and progeny testing were introduced to cotton breeding before 1900 by selection of surviving plants on wilt-infested soils, followed by progeny-row testing. Highly resistant cultivars did not become available until the 1950s. High resistance to root knot nematode is essential for high resistance to fusarium wilt. Resistance to the fusarium wilt-root knot nematode complex is quantitatively inherited.

VERTICILLIUM WILT. The fungus causing verticillium wilt, *Verticillium dahliae* Kleb., may persist in the soil for many years. The disease is widespread throughout the United States Cotton Belt and in cotton-growing areas around the world. The fungus attacks cotton plants at any stage of growth, but symptoms are most noticeable with the onset of fruiting. Affected plants are stunted, shed leaves and young bolls, and have stems with vascular discoloration.

Fig. 19.9.
Comparison of a fusarium wilt-susceptible cultivar (center rows) with a fusarium wilt-resistant cultivar. The cotton was planted on soil infested with the fusarium wilt pathogen.

Sources of tolerance were found in *G. barbadense*. Screening for resistance may be conducted on wilt-infested soils or by artificial inoculation techniques.

BACTERIAL BLIGHT. Bacterial blight (also called blackarm, angular leaf spot, and boll blight) is a bacterial disease caused by *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye. The disease is found almost everywhere that cotton is grown. Symptoms are angular water-soaked leaf spots, elongated black lesions on the stems, blighted spots on the bolls, and failure of bolls to open. The bacterial blight pathogen is spread by hard, driving rains or sprinkler irrigation. The organism is pathologically specialized, and numerous genes conferring race-specific resistance have been identified. Combinations of two or more major genes, combined with minor or modifier genes, are required for a high level of resistance. Genes for resistance have been identified from 11 diploid and two tetraploid species of *Gossypium*.

BOLL ROTS. Boll rots may be caused by several primary pathogens and saprophytic pathogens which enter the boll through cracks, insect injury, or other access points. Boll rots reduce yield, weaken and stain the lint, and infect the seeds. One breeding approach has been to utilize a mutant narrow-leaf type, known as *okra-leaf*, to produce open canopies so that sunlight and wind will dry the bolls rapidly. A mutant bract type, known as *frego bract*, in which the bracts curl outward leaving the flower buds and bolls well exposed, also facilitates rapid boll drying. A mutant strain known as *nectariless* removes extra floral nectaries, which may be points of pathogen invasion.

BREEDING FOR MULTIPLE DISEASE RESISTANCE. Cotton seedlings may be simultaneously evaluated for resistance to several common disease pathogens. The procedure consists of sequential inoculation of cotton seedlings growing in controlled environments with different disease pathogens. A sequential inoculation and selection procedure for evaluating cotton seedlings for resistance to root knot nematodes, bacterial blight, fusarium wilt, and verticillium wilt consists of the following steps (Fig. 19.10):

• Germinate cotton seeds from a genetically mixed population in soil heavily infested with root knot nematodes (*Meloidogyne incognita*).

• Inoculate cotyledons of 10- to 12-day-old seedling plants with races of the bacterial wilt pathogen (*X. campestris* pv. *malvacearum*) by scratching the cotyledon with a bacterial-laden toothpick.

• Inoculate four-week-old nematode-tolerant and bacterial-wilt-resistant plants with a virulent culture of *F. oxysporium*, discarding susceptible plants after 12 to 14 days.

• Inoculate surviving plants from previous disease infections at age of 8 to 10 weeks with a culture of *Verticillium dahliae* and grow resistant plants to maturity.

The breeding populations to be evaluated are generated by crossing among cultivars resistant to the various diseases. During the test periods, temperatures are adjusted to give optimum symptom expression for each disease.

Insect Resistance

Insect pests cause serious losses in cotton each year. Development of tolerance by cotton insects to chemical insecticides, the high cost of insecticidal control, and environmental concerns and legal restrictions on use of chemicals suggest that a greater effort must be devoted to development of insect-resistant cotton cultivars.

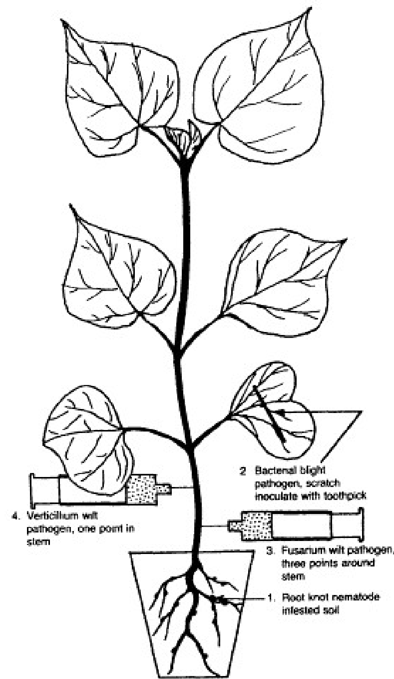


Fig. 19.10.
Steps in sequential inoculation of cotton seedlings in breeding for multiple disease resistance. (1) Germinate seeds in root knot nematode-infested soil. (2) Inoculate seedling with bacterial blight pathogen by scratching the cotyledon with a bacterial-laden toothpick. (3) Inject fusarium wilt pathogen into stem. (4) Inject verticillium wilt pathogen into stem. Discard susceptible plants after each step and inoculate only resistant plants in next step.

BOLL WEEVIL (*ANTHONOMUS GRANDIS*). The boll weevil attacks the young squares, bolls, or terminal bracts of cotton, often causing the squares to shed. Bolls set early in the season while the weevil population is still small may escape injury. This led to development of rapid-fruiting and early maturing cultivars to escape weevil damage. Resistance per se has been difficult to find, but several morphological and biochemical mutants have been identified that alter normal feeding, oviposition, and resting and result in reduced boll weevil populations. Nonpreference for the boll weevil has been observed in male-sterile cottons with cytoplasm derived from *G. harknessii*.

COTTON BOLLWORMS. The cotton bollworm (*Helicoverpa zea)-tobacco* budworm (*Heliothis virescens*) complex and pink bollworm (*Pectinophora gossypiella*) are serious cotton insect

pests in many areas of the world. Characters that suppress insect population development, such as glabrous leaves, absence of nectaries, and high gossypol content in the square, have been used in breeding for resistance. Resistance to the pink bollworm has been reported in some diploid wild species. Intense efforts to genetically engineer resistance to *Lepidoptera* insects by insertion of the *Bt* gene from *Bacillus thuringiensis* are under way (see Fig. 8.13).

OTHER INSECTS. Success has been attained in breeding cotton resistant to leafhoppers (jassids) in Africa, India, and Australia. In all instances, resistant cultivars possessed a heavy pubescence. The nectariless character has been effective in reducing populations of the tarnished plant bug and the cotton fleahopper.

Fiber Quality

Cotton fiber is the major commercial product from cotton. Cottonseed oil and cake are secondary products, yet cottonseed is the second-most important oilseed in the world. The fiber develops in bolls consisting of three to five locks. The cotton fibers are borne on the seeds, each fiber being an outgrowth of a single epidermal cell. Cotton fibers are separated into two groups according to length. The outer and longer layer, called *lint*, contains long fibers separated from the seed in ginning. An inner and shorter layer, called *linters*, or fuzz, contains short fibers that remain attached to the seed after ginning. The lint fibers are used in spinning cotton yam, and the linters or fuzz fibers are used in making rayon and cellulose products.

The cotton fiber cell is a thin-walled tubular structure that elongates until it reaches its maximum length. The tubular fiber cell is thickened by the deposition of cellulose in successive layers on the inner wall, and the hollow core inside, or *lumen*, becomes smaller. *Fiber maturity* refers to the thickness of the fiber wall; mature fibers have thick inner cell walls. The spinning performance of cotton fiber is associated with the *length, strength, and fineness* of the fibers. Cotton types vary in these characteristics. Special instruments are available that accurately measure each of these qualities in samples of cotton fiber.

FIBER LENGTH. Fiber length is important because it contributes to the quality of the yam. Variation in the length of the cotton fibers are found within a cultivar and even within a single boll. Uniformity in staple length improves spinning performance, increases the utility of the cotton, and reduces waste. Improvement in the quality of cotton fiber has been made by breeding cultivars with increased staple length and greater uniformity in fiber length.

FIBER STRENGTH. Fiber strength is important in determining yam strength. Cotton from cultivars that produce weak fibers is difficult to handle in manufacturing processes. The structure of the inner layers of the cotton fibers affects its tensile strength. Cotton types and cultivars differ in fiber strength, and high fiber strength is difficult to obtain without sacrificing yield. Pima cotton has greater fiber strength than Upland cotton; among Upland types, the Acala cultivars have the strongest fibers. The storm-proof cultivars traditionally produce the weakest fibers, but fiber strength has been improved in recently released cultivars.

FIBER FINENESS. Cotton fibers from some cultivars feel soft and silky; fibers from other cultivars feel coarse and harsh. The difference in the way they feel is determined by the fineness or coarseness of the fibers. Fiber fineness is associated with perimeter, or diameter, of the fiber and with the thickness of the fiber wall. Extra-long-staple Pima cultivars produce fibers with small perimeters and fine texture. Storm-proof cultivars produce fibers with large

perimeters and coarse texture. Eastern, Delta, and Acala types have fibers that are intermediate in fineness. Within a cultivar, fiber perimeter is relatively constant, variations in fineness being associated with fiber wall thickness.

COTION QUALITY-TESTING LABORATORIES. The Cotton Division of the United States Department of Agriculture has a laboratory at Clemson, South Carolina, to evaluate fiber quality of cotton cultivars developed by United States Department of Agriculture and State Agricultural Experiment Station research workers. Laboratories to evaluate ginning performance of publicly developed cotton cultivars and breeding lines are operated by the United States Department of Agriculture at Stoneville, Mississippi; Mesilla Park, New Mexico; and Lubbock, Texas. Private fiber-testing laboratories have been developed by cotton merchants and private cotton breeders.

Seed Quality

Stand establishment is affected by the germination and vigor of the seed planted. To be mechanically planted, all fuzz and lint must be removed from the seed, either by flame or acid treatment. Genetic improvement in seedling vigor, cold tolerance, and resistance to seedling disease would permit earlier planting of the cotton cultivar in temperate climates.

Processing quality is affected by the oil content of the cotton seed and presence of undesirable pigments in the oil. While much emphasis has been given to breeding cotton seed free of undesirable pigmentation, only minor attention has been given to selecting cultivars for higher oil content. The cotton plant normally produces pigmented glands in the leaves, stems, and seeds, which contain *gossypol*, a terpenoid compound that causes discoloration in cottonseed oil and in egg yolks when cottonseed meal is fed to poultry, reduces availability of lysine in cottonseed protein, and causes toxicity if cottonseed meal is fed in excess to young swine or poultry. A glandless character controlled by two recessive genes, gl_2 and gl_3, was introduced into commercial cultivars to improve seed quality, but insects have a preference for glandless cotton.

Study Questions

1. How are cultivars of cotton maintained? How does this differ from other crop plants?

2. How many different genomes have been identified in *Gossypium?* Which of these genomes are found in cotton used commercially? What are the uses of the various types of cotton?

3. Describe the cotton flower. Describe how breeders make pollinations in cotton.

Further Reading

Endrizzi, J.E. 1991. The origin of allotetraploid species of *Gossypiurn*. p. 449-69. *In* T. Tsuchya and P.K. Gupta (eds.) Chromosome engineering in plants: genetics, breeding, evolution. Elsevier, Amsterdam.

Hillocks, R.J. 1992. Cotton diseases. CAB International/Univ. Ariz., Tucson, AZ.

Kohel, R.J., and C.F. Lewis (eds.). 1984. Cotton. Agron. Monograph No. 24. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Lee, J.A. 1987. Cotton. p. 126-60. *In* W.R. Fehr (ed.) Principles of cultivar development. Vol. 2. Crop

species. Macmillan Publishing Co., New York, NY.

Lewis, C.F. 1970. Concepts of varietal maintenance in cotton. Cotton Grow. Rev. 47:272-84.

Matthews, G., and J. Tunstall (eds.) 1994. Insect pests of cotton. CAB International and Univ. Arizona Press, Tucson, AZ.

Meredith, W.R. Jr. 1991. Contributions of introductions to cotton improvement. p. 127-46. *In* H.L. Shands and L.E. Wiesner (eds.) Use of plant introductions in cultivar development. Part 1. Crop Sci. Soc. Am. Spec. Publ. No. 17. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Munro, J.M. 1987. Cotton. 2nd ed. John Wiley & Sons, Somerset, NJ.

Murray, E.E., D.L. DeBoer, and E. Firoozabady. 1993. Transgenic cotton. p. 153-68. *In* S-d Kung and R. Wu (eds.) Transgenic plants, Vol. 2. Academic Press, Inc., San Diego, CA.

Percival, A.E., and R.J. Kohel. 1990. Distribution, collection, and evaluation of *Gossypium*. Adv. Agron. 44:225-56.

Shepherd, R.L. 1986. Cotton resistance to the root knot-fusarium wilt complex. II. Relation to root-knot resistance and its implications on breeding for resistance. Crop Sci. 26:233-37.

Shepherd, R.L., and A.J. Kappelman. 1986. Cotton resistance to the root knot-fusarium wilt complex. I. Relation to fusarium wilt-resistance and its implications on breeding for resistance. Crop Sci. 26:228-32.

Smith, C.W. 1992. History and status of host plant resistance in cotton to insects in the United States. Adv. Agron. 48:228-32.

20. Breeding Cross-Pollinated Forage Crops

Breeding and use of improved forage cultivars have progressed rapidly in the last few decades, but this has not always been so. In the United States, breeding of forages did not receive as much attention in the early part of this century as the breeding of cereals. Many forage species are recent domesticates compared to cereals or cotton, so breeding progress has been slow. As late as 1950, seed of many forage species were sold without cultivar identity because improved cultivars were not available.

Why Breeding Cross-Pollinated Forage Species Is Unique

Although procedures in breeding forage crops are based upon the same genetic principles utilized for other crops, forage breeding presents new challenges to be overcome by the breeder. Problems arise from the diversity in pollination of the different species, irregularities in fertilization and seed setting, the perennial nature of most forage species, and differences in the evaluation and maintenance of new strains. For example:

• Forage crops include both grass and legume species.

• Most important forage species are cross-pollinated. The heterozygosity in cross-pollinated species makes it difficult to propagate individual lines from seed and maintain their identity.

• Many forage species have small floral parts, making artificial hybridization tedious and pollination control difficult.

• Self-incompatibility is common in many forage species, limiting the extent to which they may be self-pollinated.

• Some grasses reproduce largely by apomixis (seed setting without union of sperm and

egg), presenting problems in crossing and obtaining gene recombination.

• Many forages are poor seed producers or produce seed of low viability.

• Many forages produce weak seedlings, and stands are not easily established.

• Isolation and clean land on which new strains may be increased without contamination are not always available.

• The initial evaluation of selected plants or lines in the breeding nursery is generally based on the performance of spaced plants, or rows, which may not accurately represent the performance of the strain in a sward as forage species are commonly grown.

• Forage species are often seeded in mixtures with other species, which complicates the evaluation of individual strains.

• Strains may perform differently with different systems of grazing management.

• Most forages are long-lived perennials, and many years are required to evaluate persistence and productiveness of new strains.

• Many forage species are polyploids, which increases their genetic complexity.

• Seed production often occurs in areas other than where cultivar development occurs, making it difficult to simultaneously improve forage and seed yield.

Pollination, Fertilization, and Seed Setting

Pollination and fertilization vary with different species of forage crops, and many are polyploids (Table 20.1). Although there are exceptions, the annual species of grasses and legumes are self-pollinated, and the perennial species are cross-pollinated. Korean and common lespedeza, vetches, and several other annual forage species, many of which have only minor commercial importance, are normally self-pollinated. Slender wheatgrass, a short-lived perennial, is also self-pollinated. Procedures for breeding self-pollinated forage species are essentially similar for breeding self-pollinated cereals as described in Chapter 9.

Bromegrass, timothy, redtop, orchardgrass, bermudagrass, crested wheatgrass, tall fescue, red clover, alfalfa, white clover, birdsfoot trefoil, sweetclover, and many other important perennial forage species are normally cross-pollinated. Species like bluegrass, dallisgrass, and buffelgrass produce seed both sexually and by apomixis. Buffalograss is dioecious, producing staminate and pistillate flowers on different plants (Fig. 20.1). In this chapter we discuss the breeding procedures for this diverse group of forage species.

Flowering and Seed Setting in Forage Grasses

Forage grasses differ in their floral structures, self-fertility, and flowering mechanism.

FLORAL STRUCTURES AND POLLINATION. In many forage grasses, the inflorescence is a pyramidal, branched *panicle as* in oat (Fig. 20.2A); in other forage grasses, flowers are sessile along the axis and form a *spike*, as in wheat (Fig. 20.2B). The spikelet is the unit of the grass inflorescence. Arrangement of the spikelet can differ with the species and may be a distinguishing feature of the species. Two *glumes*, enclosing the florets, are attached at the base of each spikelet on opposite sides of the rachilla. The number of florets within the spikelet normally varies from one to four or five, depending upon the species. A typical grass *fioret* consists of a large outer bract or *lemma*, and a smaller inner bract or *palea*, usually three stamens and a pistil (Fig. 20.3). The pistil bears a one-celled ovary, one ovule, and two styles

Table 20.1.
Mode of pollination or seed setting, chromosome number, and growth habit of some important cultivated species of forage grasses and legumes[a]

Crop	Species	Chromosome number x	Chromosome number 2n	Growth habit
Normally self-pollinated forage grasses				
Brome, annual	*Bromus tectorium*	7	14	Annual
Millet, foxtail	*Setaria italica*	9	18	Annual
Wheatgrass, slender	*Agropyron trachycaulum*	7	28	Short-lived perennial
Normally self-pollinated forage legumes				
Clover, low hop	*Trifolium dubium*	7	14	Winter annual
Lespedeza, common	*Kummerowia striata*	11	22	Annual
Lespedeza, Korean	*Kummerowia stipulacea*	10	20	Annual
Vetch, common	*Vicia sativa*	6	12	Winter annual
Vetch, hairy	*Vicia villosa*	7	14	Winter annual
Normally cross-pollinated forage grasses				
Bermudagrass	*Cynodon dactylon*	9	18,36	Perennial
Bromegrass	*Brornus inermis*	7	56	Perennial
Elephantgrass (napiergrass)	*Pennisetum purpureum*	7	28	Perennial
Fescue, meadow	*Festuca pratensis*	7	14	Perennial
Fescue, tall	*Festuca arundinacea*	7	42	Perennial
Millet, pearl	*Pennisetum americanum*	7	14	Annual
Orchardgrass (cocksfoot)	*Dactylis glomerata*	7	28	Perennial
Pangolagrass	*Digitaria decumbens*	9	27	Perennial
Redtop	*Agrostis alba*	7	28,42	Perennial
Reed canarygrass	*Phalaris arundinacea*	7	14,28	Perennial
Ryegrass, perennial	*Lolium perenne*	7	14	Perennial
Stargrass	*Cynodon nlemfuensis*	9	18,36	Perennial
Sudangrass[b]	*Sorghum bicolor*	10	20	Annual
Timothy	*Phleum pratense*	7	42	Perennial
Wheatgrass, crested	*Agropyron desertorum*	7	28,42	Perennial
Wheatgrass, fairway	*Agropyron cristatum*	7	14	Perennial
Wheatgrass, western	*Agropyron smithu*	7	42,56	Perennial
Normally cross-pollinated forage legumes				
Alfalfa, purple blossom	*Medicago sativa*	8	32	Perennial
Alfalfa, yellow blossom	*Medicago falcata*	8	16,32	Perennial
Alfalfa, variegated	*Medicago media*	8	32	Perennial
Birdsfoot trefoil	*Lotus corniculatus*	6	12,24	Perennial
Clover, alsike	*Trifolium hybridum*	8	16	Biennial
Clover, red	*Trifolium pratense*	7	14	Biennial
Clover, white	*Trifolium repens*	8	32	Perennial
Sweetclover, white blossom	*Melilotus alba*	8	16	Biennial
Sweetclover, yellow blossom	*Melilotus officinalis*	8	16	Biennial
Largely apomictic grasses				
Bahiagrass	*Paspalum notatum*	10	20,40	Perennial
Bluegrass	*Poa pratensis*	7	28,56,70	Perennial
Buffelgrass	*Cenchrus ciliare*		32,34,36,40,54	Perennial
Dallisgrass	*Paspalum dilatatum*	10	40,50	Perennial
Guineagrass	*Panicum maximum*	8	16,32,40,48	Perennial
Kikuyugrass	*Pennisetum clandestinum*	9	36	Perennial
Weeping lovegrass	*Eragrostis curvula*	10	40,50	Perennial
Dioecious grasses				
Buffalograss	*Buchloë dactyloides*		56,60	Perennial

[a] Species names from Terrell, E.E. 1977. A checklist for names of 3,000 vascular plants of economic importance. USDA Agricultural Handbook, No. 505.

[b] Partially cross-pollinated.

Fig. 20.1.
Staminate and pistillate plants of buffalograss (Buchloë dactyloides), a species with dioecious flowering habit. (A) Staminate plant. (B) Pistillate plant.

with feathery stigmas. At the base of the ovary are the lodicules, which swell at the time of flowering and force open the lemma and palea. The anthers are exserted from the flower and shed pollen as they open. Wind is the principal pollinating agent of cross-pollinated species of forage grasses, although occasional florets may be pollinated by insects. Blooming normally begins near the apex of the inflorescence and progresses more or less regularly toward the base. Flowers of many grasses bloom most abundantly during the early morning due to stimulation by light; but some species bloom or have an alternative period of blooming in the afternoon in response to higher temperatures. Anthesis is favored by sunshine and temperatures of 20 to 25ºC or above and is hindered by cool or cloudy weather. Photoperiod and vernalization requirements for flowering in cool-season grasses must be met to obtain floral initiation. These requirements will differ with the species and genotypes within species.

SELF-STERILITY AND SELF-INCOMPATIBILITY. Breeders need to know the extent of self-fertility present. Annual self-pollinated species of forage grasses normally set seed freely after self-fertilization, but perennial cross-pollinated species vary considerably in this respect. Self-fertility may be demonstrated by bagging inflorescences to exclude foreign pollen and

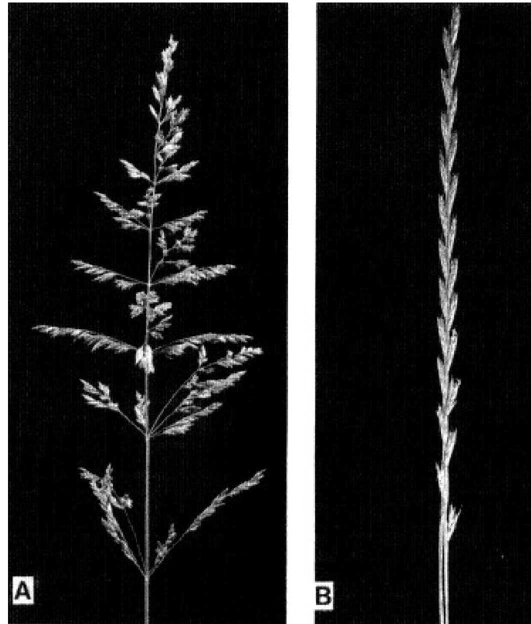


Fig. 20.2.
Types of grass inflorescence. (A) Panicle of bluegrass (Poa pratensis). (B) Spike of ryegrass (Lolium perenne).

comparing the number of seeds set in bagged and in open-pollinated heads on the same plant. Self-fertility varies with individual plants within a species and with levels of ploidy.

A reduction in seed-set from selfing cross-pollinated forage grasses indicates that self-sterility, or self-incompatibility, is present. Yet in all of the self-sterile species, relatively self-fertile plants may be isolated. The latter is important to the breeder, for the amount of self-fertility determines the extent to which inbreeding may be practiced. It is generally assumed that self-sterility in grasses results from a two-loci gametophytic system of self-incompatibility (Chapter 7). The two-loci system has been verified in rye, meadow fescue, and wild species of *Hordeum and Phalaris*. In the "grass system," two multiallelic incompatibility loci, S and Z, interact to produce an incompatible mating if the alleles are identical at both the S and the Z loci. The two-loci system results in a higher percentage of compatible matings than does the one-locus system, which is found in certain legumes, such as red and white clover.

APOMIXIS. Some grass species reproduce by apomixis (see Table 20.1). Apomixis was described in Chapters 2 and 7. In buffelgrass, an apomictic species, it has been shown that the apomictic reproductive process is genetically controlled with progenies segregating for

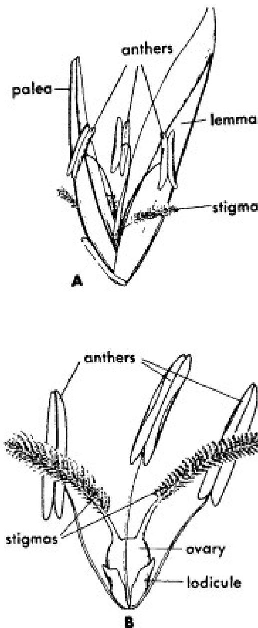


Fig. 20.3.
Grass flower. Typical floret at time of blooming. (A) The lemma and palea have been forced open by the swelling of the lodicules, and the stigma and the anthers have been exposed. (B) Pans of the grass flower with lemma and palea removed.

sexuality and apomixis. With apomixis, genetic segregation is absent, and superior heterozygous plants may be uniformly propagated, as with vegetative propagation or cloning. Once a desirable apomictic genotype is produced, the seed may be increased and the genotype released as a new improved cultivar.

SELFING AND CROSSING PROCEDURES. Self-pollination of plants in normally cross-pollinated forage species is accomplished by enclosing inflorescences in bags or sleeves made of parchment or glassine. The reduction in seed setting obtained with self-fertilization has been described already.

Several techniques may be utilized in making artificial cross-pollinations and hybridization of grasses:

• Conventional hand emasculation and pollination.

• Bagging together inflorescences of the parent clones following emasculation of the seed parent.

• Mutual pollination by bagging together inflorescences of the parent clones without emasculation.

The small size of forage grass spike-lets makes emasculation and pollination more tedious than with cereal grains, although the procedures are similar. When parent inflorescences are bagged together, seed set can generally be improved by shaking the bags during periods of anthesis to disseminate the pollen. This procedure is similar to the *approach method* described for pollination of small grains. Mutual pollination, obtained by bagging together inflorescences of the parent clones without emasculation, depends upon self-incompatibility to prevent the production of selfed seed. Artificial crossing may be facilitated by genetic and cytoplasmic male sterility in species where these fertility-regulating mechanisms are available.

Flowering and Seed Setting in Forage Legumes

As in grasses, the forage legumes vary in floral structures and fertility relationships.

FLORAL STRUCTURES AND POLLINATION. The flower of a typical legume has a standard petal, two wing petals, and two partially united keel petals. There are usually ten stamens. Nine of the stamens have their filaments joined to form a tube enclosing the style; the tenth stamen remains free of the others. The five petals partially join to form a corolla tube enclosing the stamens and the pistil. The corolla tube is longer in red clover than in the other clovers or alfalfa. Nectar is secreted at the base of the corolla tube.

In self-pollinated legumes such as lespedeza, soybean, and various field beans or peas, the pollen is shed directly upon the stigma when the anthers open. In Korean lespedeza two types of flowers are borne on the same plant, *chasmogamous* (showy) and *cleistogamous* (inconspicuous), their ratio being dependent on environmental conditions. In the cleistogamous flowers, the pollen germinates before the anthers open, with some of the pollen tubes entering the stigma through the anther wall.

In *red clover*, the stigma protrudes slightly above the anthers at the time of flowering (Fig. 20.4). The keel petals form a receptacle, enclosing the staminal tube, with a small opening at the tip. When an insect alights on the keel and inserts its proboscis into the corolla tube to obtain nectar, the weight of the insect's body presses down the keel, exposing the anthers and the stigma. Pollen carried by the insect is dusted over the stigma, and fresh pollen is rubbed off onto the insect from the anthers. When the insect leaves the flower, the keel returns to its former position and conceals the anthers. From four to eight insect visits are required to exhaust the pollen supply of a flower.

In *birdsfoot trefoil*, pollen is dispersed by means of a "piston apparatus." The keel petals conceal the anthers and the stigma and form a conical cavity above the anthers, with a small hole in the apex of the cone (Figs. 20.5 and 20.6). The anthers dehisce inside the keel and fill the cone with a mass of sticky pollen. When the insect alights on the flower and depresses the keel from the weight of its body, the anthers are forced up into the cone with a pistonlike movement. The pressure compresses the pollen and forces a ribbon of it through the opening in the apex of the cone, in much the same manner that toothpaste is squeezed from a tube. The sticky pollen covers the underside of the insect. When the pressure on the keel is removed, the organs return to their normal position. Pollen may be pumped from a particular flower as many

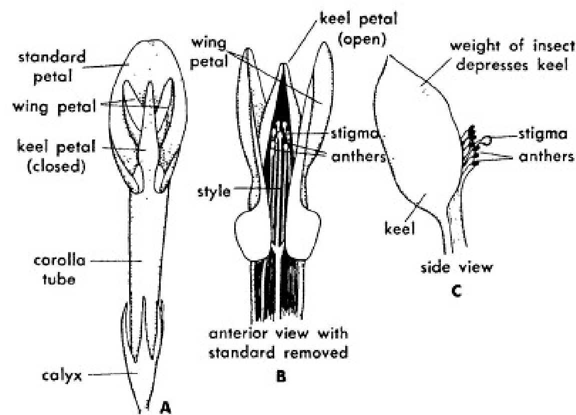


Fig. 20.4.
Flower structure and pollinating mechanism in red clover (Trifolium pratense).

Fig. 20.5.
Flower of birdsfoot trefoil (Lotus corniculatus) showing standard and wing petals and keel with stigma exposed.

as eight times if the keel is not depressed too low.

In *alfalfa*, pollen is dispersed by an explosive action, commonly known as *tripping*. The keel petals, which are held down under tension, conceal the staminal column (Fig. 20.7A). When the keel is pressed down by the weight of the insect (honeybee, the alkali bee, or the alfalfa leaf cutter bee), the stamens and stigma are snapped upward and free of the keel (Fig. 20.7B), with a force similar to that produced by the release of a spring under tension (Fig. 20.8). The insect is struck by the staminal column, which often unseats it, and its underside is covered with a mass of sticky pollen, which is carried to the next flower it visits (Fig. 20.9). There, some of the pollen is rubbed on the stigma, and more pollen is added to the load. Alfalfa flowers are usually tripped by bees, although automatic tripping by wind, rain, or heat may occur occasionally. A flower may be tripped by hand by using a small object such as toothpick or pencil to apply pressure on the keel.

SELF-STERILITY AND SELF-INCOMPATIBILITY. Many normally cross-pollinated forage legumes are highly self-sterile, limiting the production of seed by self-pollination and the development of inbred lines. Highly self-sterile species include red clover, alsike clover, white clover, alfalfa, sweetclover, birdsfoot trefoil, and others. As in forage grasses, it is generally assumed that self-sterility is the result of self-incompatibility mechanisms as described in Chapter 7. One-locus, gametophytic self-incompatibility systems have been positively identified and described in red clover, alsike clover, and white clover. With the gametophytic incompatibility system, the restraint to self-fertility is located in the styler tissue. When the pollen grain contains an incompatibility allele matching an allele in the self tissue, the growth rate of the pollen tube is retarded to such an extent that it rarely reaches the ovule (see Fig. 7.1). Pollen tubes grow at a normal rate if the incompatibility allele in the pollen differs from those in the self tissue and fertilization will occur in a normal manner. Large numbers of incompatibility (*S*) alleles have been identified in white clover, so that cross-pollinations are highly effective. Partial seed-set may be observed sometimes in self-incompatible red clover,

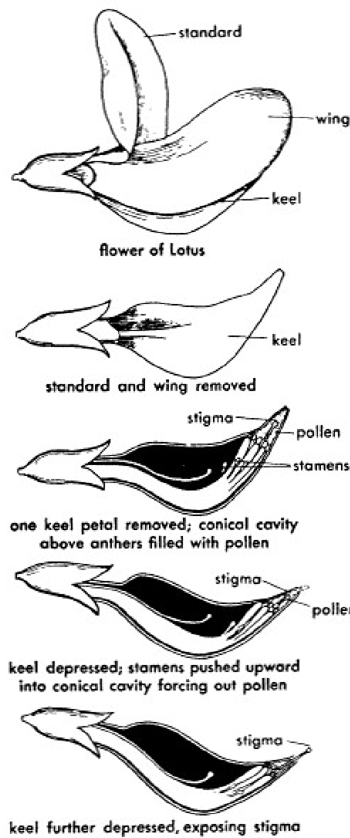


Fig. 20.6.
Flower structure and pollinating mechanism in birdsfoot trefoil.

a condition that is called *pseudo-self-compatibility*. The pseudo-self-compatibility may be increased by exposure of clones to high temperatures around 32ºC. Utilization of pseudo-self-compatibility has been proposed as a means for increasing seed-set in inbred lines of red clover. A noninhibiting or self-fertility allele, if present, permits the pollen tube to grow at the same rate that it would with an unrelated allele. Thus, clover plants may range from complete incompatibility, through various degrees of pseudo-self-compatibility, to high self-fertility.

In alfalfa, the self-sterility system differs from the system described for red clover. Incompatibility in alfalfa may result from either failure of self-pollen tubes to penetrate the style, failure of self-pollen tubes to enter the ovule and complete fertilization following normal growth in the style, or abortion and failure of embryos to develop into seeds after fertilization. The genetic basis for these self-sterility reactions in alfalfa has not been clarified. Individual clones of alfalfa differ in the extent to which they will set seed following inbreeding. Some clones are relatively self-fertile, while others are highly self-sterile. Comparisons of self-fertility may be made from the average number of seed set per. tripped flower. The self-fertility of alfalfa clones may be increased with exposure to higher temperatures.

MALE STERILITY. *Genetic male sterility* has been reported in red clover, alfalfa, and perennial ryegrass. In all cases, the male-sterile character is controlled by recessive alleles. However, the male-sterile character has not generally been used to develop cultivars of these crops, but instead, used by the breeder to assist in making cross-pollinations.

A cytoplasmic male sterility system (**cms**) was identified in alfalfa by examining large numbers of plants in the field for pollen production. To identify male-sterile plants, a few flowers from each plant are tripped onto a piece of dark glass or plastic. Small amounts of pollen can then be distinguished against the dark background. Plants without visible pollen are

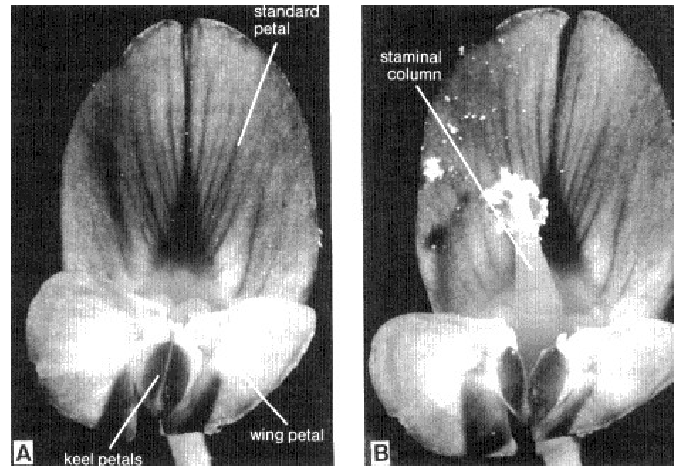


Fig. 20.7.
Flower of alfalfa (Medicago sativa). (A) Before tripping. (B) The same flower after tripping. The staminal column (pistil and stamens) have been released from the keel petals.

examined again on successive days as additional flowers develop. Plants totally devoid of pollen are assumed to be male-sterile and are tested by making pollinations from male-fertile plants. Failure of the progeny plants to produce pollen would provide substantial evidence of **cms**. Although a gene that restores fertility to the plants with sterile cytoplasm has been identified, fertility restoration is unnecessary in alfalfa grown for forage. Attempts were made to utilize the **cms**-restorer gene system in alfalfa for production of commercial hybrid alfalfa seed, but the system was uneconomical due to poor seed-set.

SELFING AND CROSSING PROCEDURES.

Various techniques are utilized in selfing and crossing forage legumes. Self-pollination is accomplished by bagging the flowers to exclude foreign pollen and by tripping or otherwise manipulating the bagged flowers by hand. Alfalfa flowers are tripped by applying pressure to the

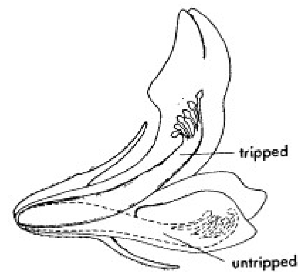


Fig. 20.8.
Flower structure and pollinating mechanism in alfalfa. Position of staminal column (pistil and stamens), tripped and untripped.

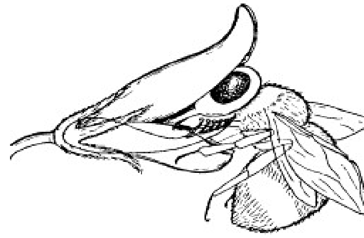


Fig. 20.9.
Mechanism by which pollen is deposited on a bee when an alfalfa flower is tripped.

keel with a toothpick or pencil or by rolling the racemes between the fingers. Self-pollination can also be obtained by caging individual clones with bees.

Legume flowers are emasculated by removing the corolla, staminal tube, and anthers with small forceps, leaving the pistil intact. Anthers and pollen are sometimes removed from flowers with suction, washed off with a jet of water, or killed by immersing the flower in an alcohol solution or hot water. In crops that have a high degree of self-sterility, such as red clover, it may be unnecessary to emasculate, especially if the pollen parent has a dominant marker gene so that plants originating by self-pollination may be identified. Plants should be checked for presence of self-fertility genes before eliminating the emasculation procedure entirely.

Crossing techniques with forage legumes generally fall into two procedures in which the pollen is transferred to the stigma by hand or by insects. A camel's hair brush or small piece of cardboard may be used with hand pollinations. Cross-pollination by bees or other insects is accomplished by growing the two parents in a cage in which honeybees, bumblebees, or leaf-cutter bees are present (see Fig. 12.15). The bees may be cleansed of pollen before being placed in a cage by washing them with water. The pollen grains will absorb water, causing them to burst.

Vegetative Propagation

A clone is a group of plants propagated asexually from a single plant (genotype). Most forage crops lend themselves to asexual propagation by:

• stolons (a runner or creeping stem above ground that produces roots) (Fig. 20.10A),

• rhizomes (underground stems that develop roots)(Fig. 20.10B),

• dividing the crowns,

• stem cuttings (Fig. 20.11), or

• tissue culture techniques (Chapter 8).

Grass plants that spread by stolons or rhizomes are easily divided to form clones. Buffalograss, redtop, creeping bentgrass, and bermudagrass spread by stolons. Bluegrass, bromegrass, western wheatgrass, and sideoats grama spread by rhizomes. Bunch-type grass plants, such as tall fescue, may be divided by cutting through the crown with a sharp scalpel or knife. Legumes, such as alfalfa, the clovers, lespedeza, and birdsfoot trefoil, and some grasses, such as napiergrass and reed canarygrass, are readily propagated by stem cuttings (Fig. 20.11). The cuttings are rooted in moist sand or in slowly moving water at temperatures of 18 to 25°C. Rooting may be stimulated by treatment with growth hormones, although such treatments may not be necessary.

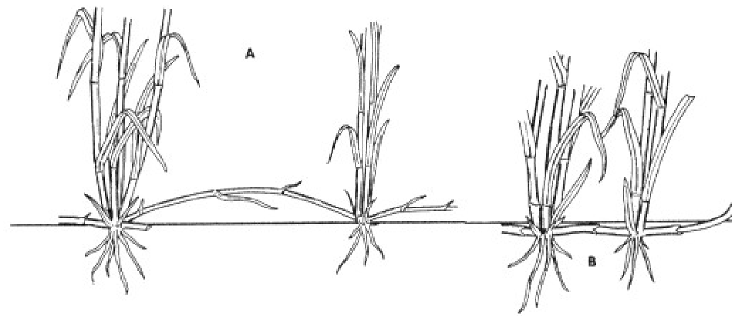


Fig. 20.10.
Vegetative propagation in grasses. (A) Stolons. (B) Rhizomes.

Vegetative propagation is used by the forage breeder to:

• establish clones from superior plants,

• maintain original genotypes used in synthetic cultivars,

• propagate strains or cultivars that are poor seed producers, or

• propagate genotypes that have unusual chromosome numbers and could not be propagated by seed due to sterility.

Progeny testing is eliminated with vegetatively propagated materials. Extensive acreages of 'Coastal' and other cultivars of bermudagrass, which are sterile or produce seed sparingly, have been established in the southern United States by vegetative sprigs. 'Zoysia,' a grass used in lawns in temperate climates, is propagated by vegetative sprigs (Fig. 20.12).

Tissue culture techniques provide an additional procedure for clonal propagation. However, it would be impractical for species that may be vegetatively propagated by procedures described above. Meristem tip cultures may be utilized to obtain virus-free propagating stocks in species such as red clover.

Procedures for Breeding Cross-Pollinated Forages Species

In developing breeding procedures for a particular species, the breeding behavior of the species must be understood and breeding techniques utilized that will efficiently exploit the genetic variability available. Although forage crops represent a large group of species with complex breeding systems, some generalizations that provide a background for the development of the breeding procedures in cross-pollinated species may be made:

• Individual plants of cross-pollinated forage crops, like open-pollinated corn, are highly heterozygous. Each plant in an open-pollinated population is a different genotype.

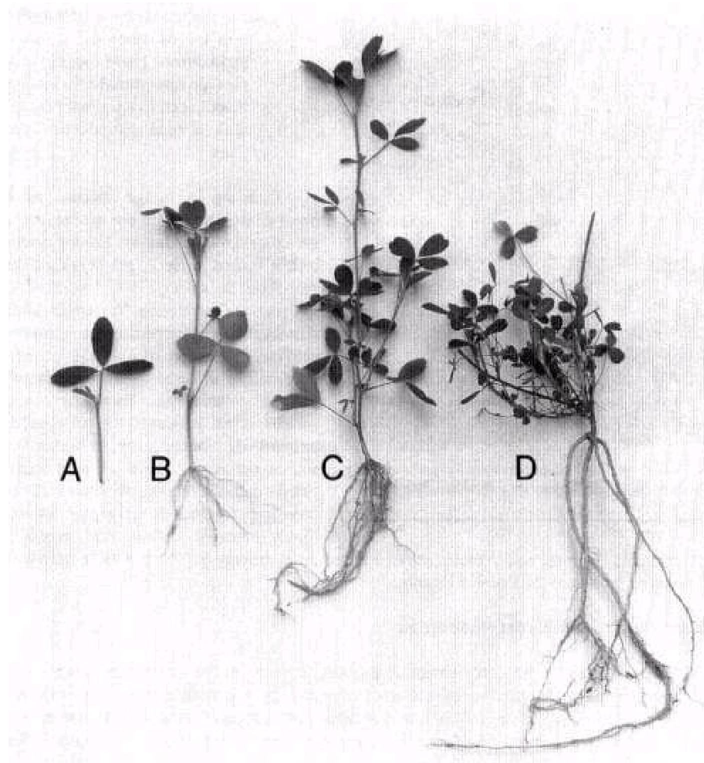


Fig. 20.11.
Vegetative propagation of alfalfa by stem cuttings. (A) Stem cutting before rooting. (B) Development after 6 weeks. (C) Development after 2 months. (D) Development after 5 months.

• Inbreeding leads to depression of vigor and loss of fertility, although species and genotypes within the species vary considerably in these respects.

• Plants or genotypes of most species of forage grasses and legumes may be propagated vegetatively as clones.

• Clones, like inbreds of corn, differ in ability to combine with other clones and produce F_1 progenies with superior performance.

• Recurrent selection may be utilized to increase frequency of favorable genes in a random mating population of the forage species, if genetic variability for the character is present in the population and screening techniques are available to identify superior genotypes.

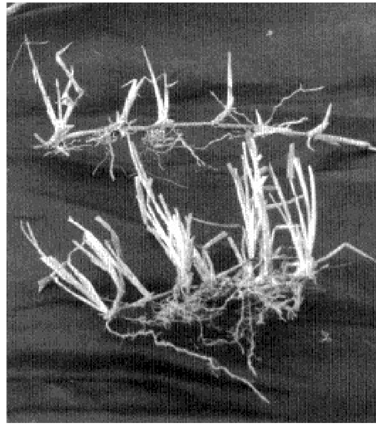


Fig. 20.12.
Propagation of zoysiagrass. Vegetative sprigs are used to propagate this species and certain other grasses.

• Self-incompatibility or apomixis may be hindrances to the utilization of conventional breeding procedures. These systems also provide opportunities to employ breeding techniques not possible with normal reproductive systems.

Breeding in forage species, as in other crops, starts with the *assembly of a germplasm source nursery*. Having assembled a source nursery, *the breeder must search out the most efficient procedure for utilizing the germplasm resources* available in the development of an improved cultivar. The choice of a breeding procedure will be affected by different factors such as whether the particular forage species is an improved or a relatively unimproved forage crop, whether the source nursery is clonal or seed-propagated, and the major objectives of the breeding program. A forage species with highly improved cultivars, such as alfalfa, will normally require using more advanced breeding procedures man with a relatively unimproved crop such as a native legume.

Assembly of Germplasm Resources

The initial step in the development of a breeding program for any forage species is to assemble germplasm that may be utilized as a source nursery. Germplasm may be acquired and propagated as *seed lots*, either bulked seed lots from a population of plants or selfed seed from individual plants, or as *living plant materials* that can be vegetatively propagated as clones. The latter is preferred, as the breeder is then working with specific genotypes and is unhampered with pollination control problems. The principal sources from which the seed or plant materials originate are:

• local ecotypes,

• introductions from the area where the species is indigenous,

• improved cultivars,

• hybrid or polycross progenies, and

• improved populations from recurrent selection programs.

Maintenance of the source nursery may be by clones (Fig. 20.13) or by seed. With long-lived perennials, superior clones will generally persist for years, although they may need to be renewed occasionally. The seed collections may be maintained by bulked seed or selfed seed, provided selfing does not reduce vigor and fertility too drastically. In biennials, or short-lived

Fig. 20.13.
Source nursery of birdsfoot trefoil clones. Note differences in flowering, area covered, and survival. Superior clones from the source nursery are utilized in development of synthetic cultivars.

perennials, such as red clover, reproduction by seed is generally necessary. Maintenance by bulked seed requires special attention due to possible contamination from foreign pollen, or to genetic shifts in the population if the environment where the seed is produced differs from that where the accession originated.

Domestication of New Species

Some species of grasses and legumes are utilized for forage or for revegetation in their native habitats even though improved cultivars are not available. Domestication and cultivation of these species would be enhanced by improved cultivars. A source of seed would also be available for reseeding as desired. Examples of cultivars from recently domesticated species include 'Sourdough' bluejoint reed grass [*Calamagrostis canadensis* (Michx.) Beauv.] and 'Florida' carpon desmodium [*Desmodium heterocarpon* (L.) DC.].

Mass Selection

The simplest breeding procedure for improvement of cross-pollinated forage species is mass selection, which, as described in Chapters 9 and 10, involves harvesting and bulking seed without progeny evaluation. Mass selection from an established pasture or meadow permits exploitation of the benefits from natural selection and the utilization of local ecotypes.

Domestication of new species usually involves a mass selection procedure. Mass selection may be accomplished by (1) harvesting en masse from a local ecotype, (2) harvesting en masse after rouging inferior or off-type plants, (3) harvesting and bulking seed from superior plants from a natural ecotype, or (4) successive cycles of mass plant selection. Mass selection of superior plants, as in (3) and (4), is based on visual evaluation and is effective if superior genotypes can be identified from the phenotypes.

Single-Plant Selection

Single plants of cross-pollinated forage species may be propagated from selfed or open-pollinated seed or may be propagated as clones. Development of cultivars from single plants in seed-propagated cross-pollinated species is considered a hazardous breeding procedure because the genetic base of the new cultivar will be too narrow. Propagating from selfed seed would lead to rapid inbreeding and would generally be undesirable. However, successful cultivars have been developed starting with an open-pollinated plant and propagating from sib-pollinations in succeeding generations.

Vigorous clones from natural populations or F_1 hybrid plants may serve as the source of new cultivars in species that can be propagated by vegetative sprigs. Cultivars of forage crops may be developed and seed propagated from single plants that reproduce apomictically. Apomictic cultivars of Kentucky bluegrass, buffalo grass, and love grass have been developed by this procedure.

Synthetic Cultivars

The synthetic cultivar method of breeding forage crops originated at the University College of Wales, located at Aberystwyth. It was originally described by T.J. Jenkin in 1931 and was called *strain building*. Jenkin's conception of a strain varied from a single plant, to multiple plants, or even to an entire population. According to Jenkin,

> The multiple-plant strain has the initial advantage over the single-plant strain in that there is less danger of loss of vigor from inbreeding and, theoretically at least, the greater the number of plants used the less danger from this source. On the other hand, the greater the number of plants used, the greater the danger of lack of uniformity in the progeny, and of divergence from the original type in subsequent generations.

Development of synthetic cultivars has become the breeding method most frequently used in forage crops.

The synthetic forage cultivar is constituted by one to three generations of random mating of a limited number of genotypes (clones) selected on the basis of high *general combining ability*. General combining ability is defined as the *average performance of a strain in a series of crosses. The* theory of the synthetic is to exploit hybrid vigor from combining superior clones, while avoiding loss of vigor from close inbreeding by restricting the number of generations of seed increase. The procedure for producing a synthetic utilizing a polycross test nursery was described in Chapter 10 and illustrated in Fig. 10.9. The steps in the development of the synthetic involve:

- Visual evaluation of clones in a source nursery.

- Establishment of a clonal line nursery for additional evaluation of phenotypically

superior clones.

• Evaluation of the combining ability of the superior clones.

• Synthesizing the cultivar by intercrossing a limited number of clones with superior combining ability.

• Advancing the synthetic cultivar by two or three generations through random mating to stabilize the population and to increase the quantity of seed required to meet commercial needs.

A further feature of the synthetic is that the clones are maintained so that the synthetic can be reconstituted.

The *source nursery* serves as a potential source of clones for use in a synthetic cultivar (Fig. 20.13). Superior clones are identified by visual evaluation for vigor, disease and insect resistance, persistence, and other desired features according to the particular species. A *clonal line nursery* is vegetatively established by dividing clones from the source nursery and planting in rows of eight to ten spaced plants. The clonal lines may be clipped to measure yield potential and recovery, subjected to disease or insect pests, and evaluated for forage quality. Replication of the clonal lines in the nursery permits measurement of genetic and environmental variances.

The combining ability of superior clones from the clonal line nursery is evaluated by *polycross, single cross*, or *diallel* yield trials. The *polycross nursery* (see Fig. 10.9) is established in isolation by clonal propagation of 40 to 50 selected clones, spaced in a manner to permit random interpollination. Open-pollinated seed is harvested and bulked by clones. With legume species the clones may be isolated in cages and interpollinated by bees. Seeds are harvested separately from each clone in the polycross nursery and planted in a yield trial. Single- or diallel-cross procedures to obtain seed for growing yield trials are more laborious than the polycross and are seldom used for that reason.

In recent years it has become more common to evaluate clones by an ***S_1* *yield nursery*** rather than by the polycross procedure. With **S_1** yield procedure (Fig. 20.14), selfed or open-pollinated seed is harvested directly from the clonal line nursery. The **S_1** procedure eliminates making the polycross, hence it is more rapid and less expensive but does not evaluate combining ability of the clones. From the polycross or **S_1** performance, clones are selected for making the synthetic.

The synthetic cultivar is constituted by random interpollination among the selected clones (**Syn 1** generation), followed by one to three generations (**Syn 1** to **Syn 3**) of open-pollination in isolation to produce the quantity of seed required for commercial distribution. The number of clones entering into the synthetic varies widely—commonly 5 to 12 will be used. As few as two have been used in alfalfa and tall fescue. The two-clone populations of alfalfa were unsuccessful because the pollinating bees tended to favor specific clones, leading to seed produced by self- or sib-pollination rather than by cross-pollination. The tendency is to use a larger number of clones than was used earlier. In some commercial cultivars of alfalfa registered with the Crop Science Society of America, several hundred clones are reportedly used. When such a large number of clones are used, they will seldom be maintained for reconstitution of the synthetic. Another procedure used by some commercial breeders is to produce enough **Syn 1** seed that it may be placed in cold storage and used for the life of the cultivar.

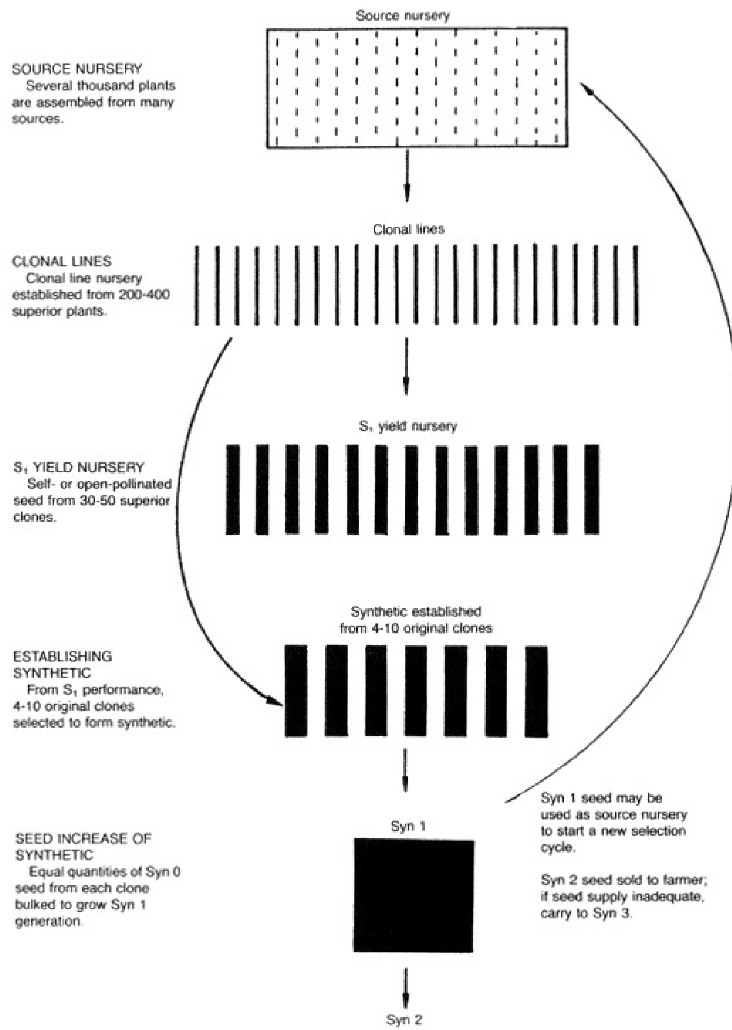


Fig. 20.14.
Procedure for developing a synthetic cultivar on the basis of S_1 yield performance. (See Fig. 10.9 for procedure for developing a synthetic cultivar on the basis of polycross progeny performance.)

Composite Cultivars

A composite cultivar in a forage crop may be developed by blending seed of two or more strains or by interpollinating clones in isolation. The composite cultivar is maintained by open-pollination after it is initially constituted and is not reconstituted as originally produced. In this regard, the composite cultivar differs from the synthetic cultivar in which the original component clones (or inbred lines) are maintained for reconstitution of the cultivar. In forming the composite cultivar, equal quantities of seed from each component strain, or clone, would normally be blended to form the initial breeder seed stock. Cultivars developed as composites are sometimes mistakenly referred to as synthetics.

Hybridization

Intraspecific and interspecific hybridization may be utilized to combine genes for favorable characteristics from two or more parent cultivars as in self-pollinated crops. The selection procedures following intraspecific crossing is different from those used with self-pollinated crops and will vary with different breeders. A general procedure is to select and intercross $\mathbf{F_2}$ or $\mathbf{F_3}$ plants with genes for desired characters, repeating the process several times until plants are obtained that breed true for the characters being combined. This procedure was utilized in breeding cultivars of sudangrass and red clover.

The *backcross* may be utilized to add genes for particular characteristics to established cultivars or breeding lines. The procedure for the backcross was described in Chapter 9. Because individual plants in cross-pollinated cultivars differ in genotype, crosses need to be made to many plants in the recurrent parent at each backcross generation to recover a representative sample of the cultivar population.

Interspecific crosses offer opportunities for obtaining gene recombinations not possible with intraspecific hybridization and for extending the range of genetic variability beyond that of a single species. The manipulation of breeding materials by interspecific hybridization requires an intimate knowledge about the cytology and chromosome relationship among the species with which the breeder is working.

Utilization of Hybrid Vigor

Hybrid vigor may be utilized in forage crop breeding by (1) vegetative propagation of superior $\mathbf{F_1}$ hybrids, (2) producing $\mathbf{F_1}$ hybrid seed utilizing **cms**, (3) producing $\mathbf{F_1}$ hybrid seed by crossing self-incompatible, cross-compatible clones, and (4) utilizing first-generation chance hybrids.

VEGETATIVE PROPAGATION OF $\mathbf{F_1}$ HYBRID PLANTS. Forage cultivars may be produced by vegetative propagation of superior $\mathbf{F_1}$ hybrids. 'Coastal' bermudagrass is the classic example due to its extensive use as an improved cultivar in the southern part of the United States. To obtain widespread distribution of Coastal, it was necessary to develop procedures for producing, certifying, distributing, and planting vegetative sprigs of the new cultivar.

$\mathbf{F_1}$ HYBRID CULTIVAR UTILIZING CMS. $\mathbf{F_1}$ hybrids of sudangrass and pearl millet have been produced utilizing **cms** and grown as forage crops. Fertility-restoring genes are unnecessary in these crops because they are used as forage. Because sudangrass grows to extreme heights when permitted to mature, recessive dwarfing genes from sorghum are transferred by backcrossing.

F_1 HYBRID FROM CROSSING SELF-INCOMPATIBLE, CROSS-COMPATIBLE CLONES. Many species of grasses and legumes have gametophytic self-incompatibility where genotypes may vary from virtually complete incompatibility to almost complete self-fertility. Production of $\mathbf{F_1}$ hybrid seed by crossing pairs of self-incompatible genotypes has been utilized in breeding bahiagrass and some other species. The procedure used is to cross two clones that are highly self-incompatible, yet cross-compatible, and that express heterosis in the hybrid progeny.

FIRST-GENERATION CHANCE HYBRID. First-generation chance hybrids may be produced by mixing seed of several inbred lines in equal proportions to plant the seed production fields. The seed harvested will be a mixture of hybrid seed and selfed seed. This procedure was used to produce 'Gahi 1' pearl millet. Seed produced in this manner cannot be marketed as hybrid seed in the United States because United States federal seed regulations specify that seed labeled "hybrid" must contain not less than 95% hybrid seeds.

Breeding Apomictic Species

Buffelgrass (*Cenchrus ciliaris* L.) is an example of a species that reproduces apomictically. A single sexually reproducing plant of buffelgrass was discovered whose progeny segregated into either completely sexual or apomictic types. Subsequently it was learned that the plant was heterozygous for method of reproduction. This sexual plant, propagated clonally, provides a tool for producing hybrid plants, which then reproduce by apomixis, thus permanently fixing the $\mathbf{F_1}$ hybrid vigor. Two procedures may be utilized: (1) selection of apomictic plants from a selfed progeny of a heterozygous sexual clone, or (2) crossing heterozygous sexual clones as the seed parent with an apomictic clone as pollinator and selecting apomictic segregates exhibiting hybrid vigor. 'Higgins' buffelgrass was the first improved apomictic cultivar released of this species followed by others. These cultivars are completely uniform because they reproduce apomictically.

Breeding Polyploid Cultivars

Autoploid forms of a crop, as a consequence of their larger cell size, often produce larger plants than the related diploids from which they originated. Fertility is usually reduced in the autoploids, and they do not produce seed as freely as diploids. It is generally believed that autoploidy would be a more productive method for breeding forage and root crops, where the increase in plant size could be utilized, rather than a method for breeding grain crops, where yields are dependent upon high seed production. Because many species of forage crops are already polyploids and do not tolerate further increases in chromosome number, attention in breeding autoploids is generally given to diploid species, and preferably to those with low chromosome numbers. Red clover is an example of a forage crop where artificially induced autoploid cultivars have been successful, particularly in Europe.

The production of alloploids offers opportunities for combining the desirable characteristics of two related species. Most success is attained in combining diploid species with low chromosome numbers. At the Welsh Plant Breeding Station in the United Kingdom, forage cultivars have been developed successfully by producing allotetraploids of Italian ryegrass (*Lolium multiflorum*) × perennial ryegrass (*L. perenne*), Italian ryegrass × meadow fescue (*Festuca pratensis*), and perennial ryegrass × meadow fescue. The three diploid parent species utilized in the crosses have the chromosome number $2n = 2x = 14$. The new allotetraploids ($2n = 4x = 28$) are essentially new species. The tetraploid cultivars produced are propagated by seed.

Application of Biotechnology in Forage Breeding

The use of *transgenic technology* offers the opportunity for breeders to introduce DNA into forage species that is not available in cross-compatible forage germplasm sources. Tall fescue, orchardgrass, alfalfa, white clover, and birdsfoot trefoil have had DNA transformed into them. At the present time, no *transgenic cultivars* of forages have been released. In the future, attempts will likely be made to develop transgenic cultivars that have resistance to pests, herbicides, and the introduction of DNA for forage quality attributes such as higher protein, higher digestibility, and reduced lignin synthesis, among others. Today, most forage crops, particularly the forage grasses, do not have reliable plant regeneration systems which are necessary to develop transgenic cultivars. Because there are many different forage species, the use of biotechnology techniques in cultivar development will likely be only for economically important species such as alfalfa and a few others. Transgenic cultivars will be developed as more crop species, including forage crops, become mapped at the molecular level. Several forage crops, including alfalfa and tall fescue, are being mapped at the molecular level using *restriction fragment length polymorphisms* (*RFLPs*). Employment of biotechnology techniques for development of transgenic forage cultivars will require continued generation of improved forage germplasms by conventional breeding procedures.

Breeding Objectives

Objectives in breeding forage crops vary with the species, the region of production, and the utilization of the crop, whether for hay, pasture, or for other purposes. Because there are many forage crops, it is impossible to enumerate a group of objectives that will apply with equal importance to all species. Ultimately, it is necessary to study each species individually, to identify the conditions that are limiting production and quality in the area where the breeder is working, and to determine whether heritable improvements may be made.

Yield

Foremost consideration needs to be given to improving forage yield, but improving seed yield, except for those species that are vegetatively propagated, is also important. High forage and high seed yield are not always compatible traits. Strains selected for high forage yield frequently are poor seed producers, or strains selected for high seed yield are poor forage producers. As a result, it may be necessary to compromise between high forage yields and satisfactory seed yields in determining which strain to increase.

FORAGE YIELD. Good forage production of acceptable quality is an essential characteristic in an improved cultivar. Forage yield is measured by the quantity produced, but forage quality and animal performance must not be overlooked. Failure to make desired gains in forage yield may be offset by improving forage quality. The factors affecting quality and animal performance are considered later in this chapter.

The type of plant that will produce a satisfactory yield of forage will depend upon the particular species and whether it is to be utilized for pasture or for hay. Low-growing leafy plants capable of persisting under heavy grazing may be selected as a basis for pasture-type cultivars, while vigorous tall-growing plants that set seed freely may be more suitable for hay-

type cultivars. Many grasses and legumes are grown in mixtures, and comparative yields of strains under competitive conditions with other species may differ from comparative yields in pure stands.

Current experiences in forage breeding indicate that major increases in yield potential have resulted following (1) initial selection within unimproved populations, (2) several cycles of recurrent selection for specific characteristics such as yield, winter hardiness, or pest resistance, (3) utilization of hybrid vigor or polyploidy, or (4) reduction of yield losses through increased tolerance to production hazards, such as cold, drought, disease pathogens, nematodes, or insects. After these gains have been attained, breeding for forage yield per se has generally resulted in more modest gains than have been attained in breeding for higher grain yields in cereals.

SEED YIELD. Breeding for high seed production involves selection for various characteristics, according to the species with which one is working, such as early ripening to escape drought, heat, or frost; adaptation to day length and temperature in the area where seed of the cultivar is to be produced; nonshattering; and synchronization of flowering within the population. A high degree of self-fertility may not be desirable in cross-pollinated species. Attempts to select alfalfa strains with greater self-tripping to facilitate seed production were unsuccessful because they led to self-fertilization and inbreeding, which reduced seed yields in advanced generations.

Some species of legumes and grasses are poor seed producers in humid climates like the eastern half of the United States, owing to excessive rainfall and high humidity during the pollinating period or high incidence of disease. Commercial seed of adapted species often is produced elsewhere where environmental conditions for seed production are more favorable, such as the western United States and Canada. However, seed of breeding lines must still be produced by breeders in the areas where they are working.

Greater Seedling Vigor

A common reason for failure to obtain satisfactory stands of a new seeding of a forage grass or legume is the inability of the seedling plant to become established quickly so that it may survive unfavorable environmental conditions, such as heat, drought, cold, and insects, or compete with weeds or other crop species with which it may be associated. Development of strains with greater seedling vigor would increase the ability of the seedling plant to cope with these adverse growth conditions. This characteristic is particularly desirable if a species is moved out of an area of optimum environment into marginal production areas. Seedling vigor is directly related to seed size and weight and may be increased by selecting for larger and heavier seeds with more endosperm reserves.

Persistence of Stands

Longevity of stands is essential in perennial forage species, where the maintenance of a dense stand is desirable. Persistence in perennial species may be reduced by disease, insects, drought, high temperature, cold, unfavorable soil conditions, or excessive defoliation from cutting or grazing. Breeding for resistance to these pests or adverse environmental conditions will result in the development of more persistent cultivars.

DISEASES THAT REDUCE STANDS. Disease is an important cause for the failure of stands of forage crops to persist. Bacterial wilt may reduce the stands of alfalfa so that cultivars

susceptible to the wilt pathogen become unproductive after the second or third year, whereas resistant cultivars will remain productive for a period of many years (Fig. 20.15). Diseases that reduce stands and are amenable to control by breeding include bacterial wilt of alfalfa, crown rot of clovers, southern anthracnose on red clover, nematodes, and many others.

CLIMATIC CONDITIONS THAT REDUCE STANDS. Resistance to cold is an important characteristic, which determines the limits for the production of many species. Increased frost tolerance would enable bermudagrass to be grown farther north in the United States and to remain green longer into the fall and winter. Production areas for orchardgrass, birdsfoot trefoil, and many other species could be increased if hardier cultivars were developed. Advances have been made in the development of hardier cultivars of alfalfa. The early introduction of the 'Grimm' cultivar from Germany and 'Ladak' from the high plains of India made it possible to grow alfalfa successfully into the northern plains states and in Canada where previously cultivars had not survived. These introductions are used today as parent materials in the breeding of hardy cultivars. Types of alfalfa with branching roots, known as creeping rooted alfalfas, have been selected to develop strains resistant to winter injury resulting from heaving and increased tolerance to grazing animals.

In regions of low rainfall, resistance to drought is important for the survival of many grasses and legumes. Drought resistance is generally associated with earlier maturity, reduced leaf area, and slow recovery after grazing. Drought-resistant species frequently tend to stop growth during midsummer periods of high temperature and dry soil but have the ability to renew growth when supplied again with soil moisture. Selection for succulent, quick-recovery types within these species should be avoided if the objective is to obtain maximum drought resistance. Early maturity may enable a forage cultivar to escape drought or midsummer heat or enable the plant to mature seed before frost.

OTHER FACTORS. Improved strains must be sufficiently aggressive to compete with weeds, or with other forage species in a mixture, if the strains are to survive. This characteristic is important because forage strains are frequently seeded in mixtures. Some grasses may be too aggressive, reducing the stand of the legumes with which they are associated and lowering the nutritive value of the herbage. In such cases it may be desirable to breed for less aggressiveness.

Fig. 20.15.
Comparative injury by bacterial wilt pathogens and stem nematodes to alfalfa strains growing in Nevada. Strains A, C, D, and F are resistant to the bacterial wilt pathogen and stem nematodes. Strain B is susceptible to the bacterial wilt pathogen and the stem nema-tode. Strain E is resistant to the bacterial wilt pathogen and susceptible to stem nematodes.

Some legumes, such as alfalfa, do not persist well under close grazing. Creeping-rooted types, with horizontal rootstocks 10 to 30 cm underground from which shoots arise, are more persistent under grazing, in addition to being more resistant to cold and drought.

In tall fescue, the presence of an endophytic fungus [*Acremonium coenophialum* Morgan-Jones Gams, previously *Epichloe typhina* (Pers.) Tul.] is related to stand persistence, particularly in stress environments consisting of poor soil fertility, low moisture, and high temperatures.

Resistance to Diseases

Some of the major benefits from forage breeding have come about by developing cultivars with resistance to disease-infecting agents. Breeding for resistance to the bacterial wilt pathogen was a major step in improving longevity of stands and forage yield in alfalfa. Bacterial wilt was the most destructive disease of alfalfa in the United States. Alfalfa plants with bacterial wilt disease are stunted, leaves wilt during hot weather, and stands are rapidly reduced. Early breeding research in alfalfa centered mostly on development of wilt-pathogen-resistant cultivars. Today, breeding in alfalfa has broadened to combine resistance to several disease and insect pests in *multiple-resistance breeding* programs.

Forage diseases may be incited by different types of pathogens, which may attack the plant in various ways. Several root- and crown-root disease pathogens commonly infest many grass and legume species. Root- and crown-rot diseases are characterized by loss of stand, increased winter injury, unthrifty appearance, and reduced yields. Root- and crown-rot injury may be caused by a complex of several pathogens, making breeding for resistance difficult. Selection for resistance may be done in the field where several pathogens may be present or in the greenhouse with infection by specific pathogens. Resistance to root- and crown-rot is usually complex in inheritance.

Leaf-spot and stem diseases are of special interest to the forage breeder because they affect forage yield and quality. The foliar diseases reduce photosynthetic activity, cause partial defoliation, and reduce yield. Because protein is higher in leaves than in stems, defoliation results in steminess and decline in protein content. Yellowing of leaves from leaf spot damage will reduce the carotene content. Diseased tissue in alfalfa may contain an increase in content of the estrogen coumestrol. Resistance to leaf-spot pathogens is often simply inherited in contrast to the complex inheritance of resistance to root- or crown-rot pathogens. Some leaf-spot pathogens, such as rust, may be highly race-specialized, complicating the breeding problem.

Bacterial diseases are common to alfalfa, lespedeza, and other forages. With bacterial wilt disease, the vascular system of the infected plant becomes clogged, interfering with water movement and causing the plant to wilt. The bacteria often enter through wounds caused by mechanical or insect injury. Significant progress made in breeding for bacterial wilt resistance in alfalfa has already been cited. Inheritance of resistance to the bacterial wilt pathogen in alfalfa has proven to be complex.

Virus diseases are widespread in both legumes and grasses. Often, the presence of these diseases may go unnoticed. In legumes, the virus may cause yellowing, stunting, deformation of leaves, reduction in stands, lower nutritive value, and reduced forage yields. In grasses the virus may cause discoloration, stunting, and reduction in tillering and seed setting. Visible symptoms are not always apparent. Tall fescue and other grasses are common alternate host plants to barley yellow dwarf virus (BYDV) and other viruses in the United States. Many virus diseases infect a wide range of species. More than 40 alternative host species have been

identified for BYDV. Breeding for resistance to viruses in forages has received only minor attention thus far but may be expected to increase as the losses from virus injury become better documented.

Nematodes are found associated with roots and stems of many forage species. The root-knot nematode causes branching and deformation of roots, stunting, and reduced stands. In alfalfa, the stem nematode feeds and reproduces mainly on basal shoots just above the crown. Crowns become swollen, and stands are reduced. Lesions caused by nematode injury provide avenues for bacterial or fungal root-rotting organisms to enter. The cultivars 'African,' 'Moapa,' and 'India' have been used as sources of resistance to root-knot nematode and 'Lahontan' and 'Trek' as sources of resistance to stem nematode (Fig. 20.15).

Resistance to Insects

Forage yield and quality may be reduced by insect injury. Loss in yield may result from insects feeding on the plant or from stunting following injection of toxins during insect feeding. Insects may serve as vectors for disease pathogens or viruses, or injury from insect feeding may provide the avenue for entrance of disease pathogens. Insects feeding on the floral organs may severely curtail seed production. Breeding for insect resistance has been conducted with several forage species, but the major effort has been with alfalfa.

Breeding for Multiple Pest Resistance in Alfalfa

The success in breeding for bacterial-wilt resistant cultivars in alfalfa led to breeding for resistance to other diseases and to insect pests (Table 20.2). Incorporation of genes for resistance to two or more of these pests into germplasm populations and new cultivars is being accomplished through *multiple pest resistance breeding* programs.

Table 20.2.
Some common disease and insect pests of alfalfa

Disease/Insect	Species name
Alfalfa Diseases and Causal Pathogen	
Bacterial leafspot	*Xanthomonas alfalfae* (Riker, Jones, and Davis) Dows.
Bacterial wilt	*Clavibacter michiganense* McCulloch
Anthracnose	*Colletotrichum trifolii* Bain
Common leafspot	*Pseudopeziza medicaginis* (Lib.) Sacc.
Downy mildew	*Peronospora trifoliorum* de Bary
Fusarium root rot	*Fusarium* spp.
Phytophthora root rot	*Phytophthora megasperma* Drechs.
Pythium root rot	*Pythium* spp.
Rhizoctonia crown and root rot	*Rhizoctonia solani* Kuehn.
Verticillium wilt	*Verticillium albo-atrum* Reinke & Berthier
Sclerotinia crown and stem rot	*Sclerotinia trifoliorum* Eriks.
Alfalfa mosaic virus	AMV
Stem nematode	*Ditylenchus dipsaci* (Kühn) Filipjev
Alfalfa Insect Pests	
Spotted alfalfa aphid	*Therioaphis maculata* Buckton
Pea aphid	*Acyrthosiphon pisum* Harris
Blue alfalfa aphid	*Acyrthosiphon kondoi* Shinji
Potato leafhopper	*Empoasca fabae* Harris
Alfalfa weevil	*Hypera postica* Gyllenhal

The general procedure in alfalfa breeding has been to first develop germplasm populations with a high level of resistance to a specific disease or insect pest by recurrent phenotypic selection. Resistant clones from different populations are intercrossed, followed by several generations of random mating and phenotypic recurrent selection for multiple resistance. Selection for multiple resistance within the random mating population may be accomplished in several ways. For example, if resistance to three pests is being combined:

• Screen population for pest A, keeping only plants with an acceptable level of resistance, then screen those plants for resistance to pest B, and so on. Plants with resistance to all three pests are intermated to start the next cycle. This procedure is called *independent culling*.

• Screen intensively for pest A and establish a high level of resistance through one or more cycles of selection, then repeat for pest B and for pest C. This procedure is *tandem selection*.

• Develop a *selection index* in which a value is assigned for resistance to a particular pest. The plants are rated for the level of resistance for each pest according to the assigned values and the sum of the ratings will be the score for a particular plant. Plants with the highest scores are kept to start the next selection cycle. This procedure necessitates making personal judgments on the value to assign for each trait.

All of the methods are dependent upon having a reliable field or greenhouse screening procedure for evaluating resistance for each of the traits.

Forage Quality

Forages are utilized as feed for livestock, chiefly as pasture or hay. Both quantity and quality of the forage consumed contribute to the animal response. Progress in breeding for quantity or yield of forage may be supplemented by improving the quality of the forage. Cultivars for improved quality may be developed for (1) greater nutritional value, (2) increased intake and digestibility, and (3) lower content of toxic substances. Near infrared reflectance spectroscopy is used by forage breeders to select for improved forage quality (see discussion in Chapter 12).

NUTRITIVE VALUE. The nutritive value of forage is determined by its protein, fiber, mineral, and vitamin content. Chemical analyses for crude protein, crude fiber, mineral content, or vitamins are measures of forage quality. By themselves, they are an inadequate measure of quality because they do not tell how completely the animal can utilize the forage. Because leaves are higher in protein, minerals, and carotene and lower in fiber than stems, breeding for increased leafiness or a higher level of xanthophyll and carotene in the leaf is a direct way of increasing nutritive value. Leaves damaged by disease or insect injury are lower in protein and vitamins than healthy leaves. Losses in nutritive value of this nature may be reduced by breeding for resistance to pests. Leaf percentage was increased in pearl millet by introduction of single dwarfing genes that reduced height and the stem:leaf ratio. Nutritive value is also affected by maturity with protein decreasing and lignification increasing in older tissues. Breeding for improved mineral contents has been emphasized in several forage grass species.

INTAKE AND DIGESTIBILITY. Production of meat and milk by grazing animals is related to *intake* and *digestibility* of the forage. Intake refers to the amount of forage consumed and

digestibility to how well the forage ingested by the animal is utilized. Intake and digestibility are somewhat related as intake is generally highest with forage that has high digestibility. Intake of leafy strains is generally higher than with strains having a low ratio of leaves to stem. The degree of harshness or hairiness of the leaf may affect intake. Forage plants being grazed are more readily consumed if they are making a rapid growth, high in carbohydrates and fiber and do not contain too much water. Intake may be reduced by the presence of toxic substances, or by flavones or off-flavored compounds produced by plant disease. Intake of tall fescue infected with the fungal endophyte is reduced during periods of high temperatures, a condition referred to as "rescue toxicosis."

Digestibility differs with particular plant tissues and the maturity of the tissues. Lignified portions of forage tissue, such as cell wall or stem structural tissue, are poorly digested, while cell contents may be completely digested. Digestibility is higher in leaf tissue than in stem tissue and declines as the tissues mature due to increased lignification. Breeding for increased leaf mess and rapid recovery after grazing are ways to improve digestibility. Increased leafiness may not be compatible with maximum forage yield because high-yielding genotypes generally will have a high proportion of stem tissue. The quality will be affected by the relative digestibilities of the leaf and stem tissues in the species and genotype under study and by the stage of maturity. Breeding for disease resistance may increase digestibility by reducing the proportion of disease-infected leaf or stem tissue in the forage.

Intake and digestibility may be assessed by grazing or feeding trials. Feeding trials are expensive to conduct and often are impractical because seed quantity of experimental strains is insufficient in early stages of selection to grow the amount of forage needed for a feeding trial. Today, most plant breeders rely upon near infrared reflectance spectroscopy to obtain estimates of in vitro digestibility. The near infrared reflectance spectroscopy instrumentation must be calibrated with good chemical procedures, such as digesting forage samples in rumen fluid or fungal-produced enzymes.

TOXIC SUBSTANCES. Some forage species may produce toxic substances that reduce the palatability of the forage or have harmful effects on animals consuming the forage. Sudangrass under certain environmental conditions may produce cyanogenetic glycosides, which cause hydrocyanic (HCN) poisoning in livestock. Cultivars of sudangrass have been developed with low genetic potential for glycosidal formation of HCN, thereby reducing the danger of pasturing sudangrass.

Sweetclover contains O-coumaric and coumaric acid glycosides, which are variously included under the term *coumarin*. Coumarin may cause a bleeding disease in animals feeding on sweetclover. Breeding for low coumarin content has been a major objective in breeding sweetclover. Two genes for low coumarin were transferred to white sweetclover (*Melilotus alba*) by interspecific hybridization from *M. dentate* using embryo culture and grafting techniques, leading to the development of improved cultivars.

The presence of the endophyte in tall rescue leads to the synthesis of an alkaloid, ergovaline. Ergovaline is at toxic levels during the warmer periods of the grazing season and leads to an abnormality in ruminants called fescue toxicosis. Cattle exhibiting fescue toxicosis have rough hair coats, elevated body temperature, poor milk production, poor conception rates, poor weight gains, depressed serum prolactin levels, and generally have an unthrifty appearance.

Tannins are present in lespedezas, birdsfoot trefoil, crown vetch, hop clover, and some other forage species. Strains of serecia lespedeza selected for low tannin were higher in in vitro dry matter digestibility than strains selected for high tannin.

Seed Increase of New Cultivars

After new cultivars of forage crops are developed, seed must be produced in quantities sufficient to be readily available to farmers at a reasonable price. Failure to produce needed supplies of seed has limited the use of some new forage cultivars. Problems related to rapid seed increase of forage crop cultivars differ from those related to rapid seed increase of grain crop cultivars. In the east, south, and midwest United States, seed production of many forage species is often uncertain because weather conditions are unfavorable. On farms in these areas seed production is frequently a sideline, is harvested only if the forage is not needed for hay or pasture, and the seed crop and price are good. In the United States, grass and legume seed are most efficiently produced in specialized seed-growing areas, such as irrigated areas in the western United States (Fig. 20.16). When seed stocks of eastern or midwestern forage cultivars are increased in western seed-producing areas, care must be taken to prevent genetic changes before the seed is returned to its area of adaptation.

Fig. 20.16.
Seed increase field of Ladino white clover in Oregon. Many forage species will produce high yields of superior quality seed in the irrigated areas of the western United Statesand Canada. Seed yields and quality are often unsatisfactory for forage species grown in the humid climate of the eastern United States.

Study Questions

1. Why has forage breeding lagged behind the breeding of other crop plants?

2. Why is it often necessary to have animal performance trials as an integral component of the forage breeding program?

3. Why is it difficult to simultaneously improve forage and seed yield?

4. What is forage quality and what are the quality characteristics that plant breeders want to improve in forage crops?

5. How does forage breeding differ from breeding of cereal crops?

Further Reading

Asay, K.H. 1991. Contributions of introduced germplasm in the development of grass cultivars. p. 115-25. *In* H.L. Shands and L.E. Wiesner (eds.) Use of plant introductions in cultivar development I. Crop Sci. Soc. Am. Publ. No. 17. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Burton, G.W. 1983. Improving the efficiency of forage-crop breeding. p. 138-40. *In* J.A. Smith and V.W. Hays (ed.) Proc. 14th Int. Grassland Congress, Lexington, Kentucky, Westview Press, Boulder, CO.

Gorz, H.J., F.A. Haskins, and K.P. Vogel. 1983. Cyanogenesis in dhurrin-containing forage grasses. p. 152-55. *In* J.A. Smith and V.W. Hays (ed.) Proc. 14th Int. Grassland Congr., Lexington, Kentucky, Westview Press, Boulder, CO.

Hanna, W.W. 1993. Improving forage quality by breeding. p. 671-75. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Hanna, W.W., and E.C. Bashaw. 1987. Apomixis: Its identification and use in plant breeding. Crop Sci. 27:1136-39.

Hanson, A.A. 1972. Breeding of grasses. p. 36-52. *In* V.B. Younger and C.M. McKell (eds.) The biology and utilization of grasses. Academic Press, New York.

Hanson, A.A., D.K. Barnes, and R.R. Hill, Jr. (eds.). 1988. Alfalfa and alfalfa improvement. Agronomy Monograph No. 29. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Hovin, A.W. 1980. Cool-season grasses. p. 285-98 *In* W.R. Fehr and H.H. Hadley (eds.) Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Jenkin, T.J. 1931. The method and technique of selection, breeding and strain-building in grasses. *In* The breeding of herbage plants: Technique adopted at the Welsh Plant Breeding Station, Bull. 3. Imperial Bureau of Plant Genetics: Herbage Plants, Aberystwyth, Wales.

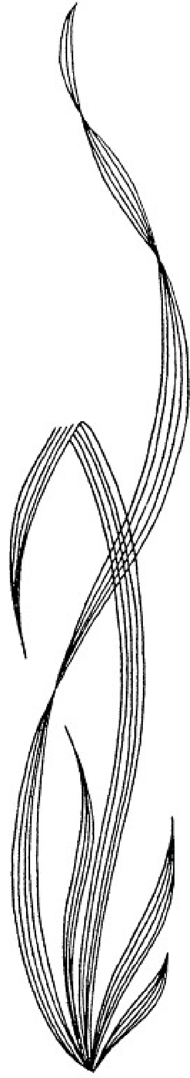
Norris, K.H. 1989. NIRS instrumentation. p. 12-17. *In* G.C. Marten, J.S. Shenk, and F.E. Barton II (eds.) Near infrared reflectance spectroscopy (NIRS): Analysis of forage quality, USDA-ARS Handbook No. 643.

Pedersen J.F., and D.A. Sleper. 1993. Genetic manipulation of tall rescue. Agric. Ecosys. Environ. 44:187-93.

Schmidt, S.P., and T.G. Osborn. 1993. Effects of endophyte-infected tall fescue on animal performance. Agric. Ecosys. Environ. 44:233-62.

Taylor, N.L. (ed.). 1985. Clover science and technology. Agronomy Monograph No. 25. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Tysdal, H.M., T.A. Kiesselbach, and H.L. Westover. 1947. Alfalfa breeding. Nebr. Res. Bul. 124.



IX
APPLICATIONS:
FIELD CROPS THAT ARE VEGETATIVELY PROPAGATED

21. Breeding Potato

Potato (*Solanum tuberosum*) is one of the most productive and widely grown food crops in the world and produces approximately twice as many calories per hectare as rice or wheat. It is adapted to a wide range of climates and can be found in both tropical and temperate environments and at elevations from sea level to 4000m. Western and Central Europe, the former USSR, and China are major production areas. It is cultivated both in large tracts and in home gardens. The potato is highly nutritious because it contains significant concentrations of vitamin C and the amino acids essential for good human nutrition. Because the potato is an important food crop worldwide, an International Potato Center (CIP) was established in Lima, Peru, near the region in which potato was domesticated, in 1971 to improve potato cultivation in developing nations.

The potato is unique and different from other crops discussed in this textbook because edible food materials are stored in underground parts, called tubers (Fig. 21.1). The potato is also unique because it is propagated asexually or vegetatively through these tubers. Vegetative propagation via tubers has certain disadvantages such as (1) transmission of disease, (2) expense of transporting bulky and heavy tubers as compared to seeds, and (3) sprouting of tubers before the planting season. Vital diseases are commonly distributed through the tubers so production of disease-free seed becomes a major concern for large-scale commercial growers and distributors of tubers for seed. Sprouting of tubers before the planting season is a problem for small farmers and growers who save tubers for planting the next season but do not have cold storage facilities. However, a distinct advantage of vegetative propagation is that once a superior plant is identified by the breeder, it can be released as an improved cultivar and maintained in its original genetic state. The only other vegetatively propagated crop discussed in detail in this textbook is sugarcane (Chapter 22).

An interesting aspect in the breeding of potato is its autotetraploid origin, which makes it difficult to study the inheritance of certain traits. Traits inherited in a *tetrasomic* manner from autotetraploids are much more complex than traits inherited in a *disomic* manner from diploids. For a discussion on disomic versus tetrasomic inheritance, please see Chapter 5. However, as we will see, the autotetraploid nature can be exploited to maximize heterosis for production of superior yielding potato cultivars.

One of potato's greatest attributes is the diverse source of germplasm that can be utilized by the breeder. Almost all of the related wild relatives can be easily crossed to *Solanum tuberosum* to incorporate resistance to abiotic and biotic stresses and to improve upon

heterozygosity. Virtually no other crop has such an array of wild germplasm that can be used so readily in cultivac development. The genetic potential for further advancements in yield ace due to these unique characteristics of the potato.

Classification of Potato

The potato belongs in the genus *Solanum*, in the family *Solanaceae*, or the nightshade family. This family also includes many other important commercial plants such as tomato, tobacco, eggplant, various species of chili peppers, petunia, as well as poisonous nightshades. The genus *Solanum* contains approximately 2000 species, including over 150 tuber-bearing species which form a polyploid series from diploids (2*x*) to hexaploids (6*x*) with 75% of them being diploid. Most of the species of *Solanum* are herbs or small thorny shrubs.

The cultivated potato belongs to the species *Solanum tuberosum* and is considered to be an *autotetraploid* with a genomic formula of $2n = 4x = 48$. This is the only species of the tuber-bearing *Solanums* that has been cultivated outside its native area. *Solanum tuberosum* is generally believed to have originated in the Andes region from central Peru to central Bolivia. From there, the potato reached Europe, and, after prolonged selection for tuber yield and earlier maturity under longer day lengths than prevailed in its native home, many alterations in plant and leaf characteristics and photoperiodic response have taken place. Types with similar plant and leaf characteristics and photoperiodic response ace found growing also in the long day conditions of Chile. This led early Russian investigators to suggest that the European potato came from Chile, but this view has generally been refuted. It is now considered more likely that Chile may be a secondary center of origin of *Solanum tuberosum*, where evolutionary changes occurred similar to those which later took place under selection in Europe. Further evidence for the Andean region of Peru and Bolivia as the original home of the cultivated potato is the fact that the native cultivated diploid species, *S. stenotomum*, believed to be the first domesticated potato species and a progenitor of the tetraploid *tuberosum* species, is also found growing in the Andean area. Although most of the native species of potato are located in South America, it has been

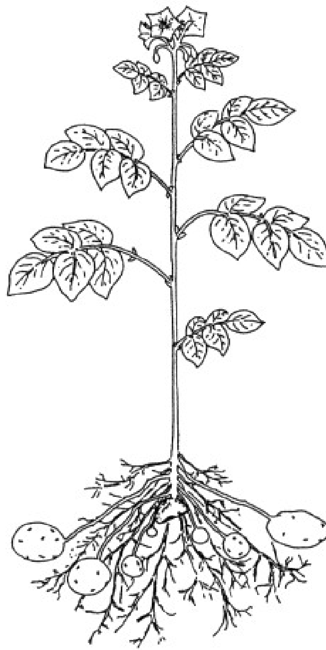


Fig. 21.1.
Plant of potato showing reproduction by flowers and tubers. In the potato plant the food materials are stored in underground parts called tubers.

postulated that the tuber-bearing species may have originated in the Mexican area and migrated southward into the Andes in very early times, where they hybridized with native Andean species. Species of wild potato are also found in Mexico, southwestern United States, and Guatemala and other countries in Central America. This area, along with the Central Andean region of Peru-Bolivia, is considered to be the primary center of origin for the potato.

Botany of Potato

The potato flower is 3 to 4 cm in diameter and contains five sepals and petals, and a two-celled ovary with a single style and bilobed stigma. The corolla varies in size with the cultivar. The color of the corolla varies from purplish to nearly white. The petals are united and tubular. The stamens are attached to the corolla tube and bear erect anthers which form a close column or cone around the style (Fig. 21.2A and B). Anthers are bright yellow except for those produced on male-sterile plants, which have light yellow or yellow-green anthers. In some clones the buds abscise, and mature flowers are never formed. Pollen production in some commercial cultivars is very poor; many cultivars produce practically no pollen at all. Seeds are produced in a berry, often called the *seed ball* or *apple* (Fig. 21.2C).

Flowers in the cultivated potato open mostly in early morning, although a few may continue to open throughout the day. Self-pollination in nature is the rule. Cross-pollination is most often accomplished by bumblebees, which are the main carriers of pollen. Wind-pollination plays a minor role in nature. Germination of the pollen is completed after 30 minutes, and the ovary is fertilized within 12 hours. Obstacles to seed production in the potato include:

- failure to flower,
- dropping of buds and flowers either before or after fertilization,
- low pollen production and failure to produce viable pollen,
- male sterility, and
- self-incompatibility.

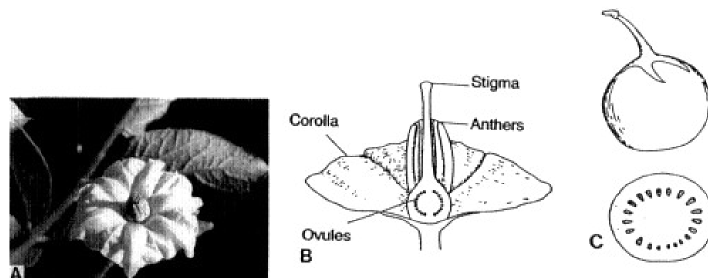


Fig. 21.2.
(A) Flower of potato. Note how anthers form a close column or cone around the style.
(B) Longitudinal section of a potato flower showing corolla, anthers, style and stigma protruding above the cone of anthers, and ovules. (C) Berry or "seedball."

Asexual reproduction in the potato is by tubers. The sprouts arise by germination of buds in the eye of the tuber (Fig. 21.3). The tuber varies in the length of the period of dormancy according to the cultivar. In the United States, most cultivars have a relatively short dormancy period, whereas in many other countries selection is practiced for longer dormancy so that tubers can be shipped to other nations. Vegetative propagation is also possible by rooting stem cuttings.

Flowering in Potato

The potato requires long day lengths (around 16 hours), abundant rainfall, and cool temperatures to flower. Under most normal growing conditions, the day lengths in the early pan of the season will favor flowering over tuber production, which requires short days (around 12 hours). High heat at the time of flowering may lead to floral abscission while still in the bud stage, giving the appearance that the cultivars don't flower well. While many older cultivars, in particular 'Russet Burbank,' flower sparsely and are often male-sterile, the newer cultivars usually flower abundantly and many are male-fertile. Today, flowering and seed set are not serious handicaps for the breeder. Various techniques are often used to induce flowering such as periodic removal of tubers, girdling or constriction of the stem, and grafting of young potato shoots onto tomato or other compatible *Solanaceous* plants. Among the cultivars currently used for breeding, selecting for increased flowering and seed set does not cause reduced tuber yield. Flowering and tuber yield are uncorrelated as are berry or seed set and tuber yield.

Fig. 21.3.
Asexual reproduction occurs by tubers in potato. The sprout has germinated from the bud in the eye of the tuber.

Sterility and Incompatibility

Reduced seed set in flowers of the cultivated potato may result from *male sterility* or *incompatibility. The* problem is complex, and many nuclear and cytoplasmic genetic systems are responsible. In certain cases, F_1 progeny, which are both male- and female-fertile, results when reciprocal crosses are made. In other crosses, male sterility occurs in the F_2 progeny when the cross is made in only one particular direction. Male-sterile progeny may have deformed flowers with indehiscent anthers or shrivelled microspores which do not separate. Failure to produce pollen, or production of poor-quality pollen, is another common cause of sterility in *S. tuberosum*. The failure to produce pollen may be an inherent characteristic with sterility dominant to fertility. Presence of a tetrasomic gene, which is lethal when present in a homozygous condition, or partly lethal when present in the heterozygous condition, has also been reported.

ENDOSPERM BALANCE NUMBER HYPOTHESIS. Successful development of embryos and seeds requires proper endosperm development. In Chapter 2 we learned that the endosperm (3*n*) is formed as a result of fertilization of the *polar nuclei* or *central nucleus* (2*n*) by a male

nucleus ($1n$) (see Fig. 2.6). The $2n$ embryo results from the fertilization of an egg ($1n$) by a male nucleus ($1n$). The *endosperm balance number* (EBN) *hypothesis* states that normal endosperm development occurs when the ratio of maternal to paternal EBN contribution to their progeny is 2 to 1. Any deviation from this ratio (2EBN maternal: 1EBN paternal) will result in no seed set. The following rules determine what the parental EBN contributions to their progeny will be:

- Gametes have one half the parental EBN.
- Because the female contributes two polar nuclei to the endosperm, the maternal EBN contribution is two.
- The paternal EBN contribution is one.
- For unreduced ($2n$) gametes, both parental contributions are doubled.

In a $2x$(2EBN) × $2x$(2EBN) cross between genotypes which produce only reduced ($1n$) gametes, the maternal contribution would be two EBN while the paternal contribution would be one EBN. Therefore, this cross should result in successful seed set according to the EBN hypothesis because of the 240-1 ratio of maternal to paternal EBN contributions. The EBN is assigned to a genotype based on the results of crosses with standard genotypes of known EBNs. The EBN is the phenotypic expression resulting from the combined interaction of a small number of genes (5 to 7) affecting endosperm success or failure.

This ratio is an important consideration in breeding potato because crosses between parents of differing ploidy levels are possible and unreduced gametes from both male and female parents occur (Fig. 21.4). when illustrating the EBN concept, it is important to include the EBN in parentheses following the ploidy levels in a cross. For example, in $4x$(4EBN) × $2x$(2EBN) and $2x$(2EBN) × $4x$(4EBN) crosses where all parents produce reduced gametes, a *triploid block* is present and no triploid progeny result because of the EBN hypothesis. However, if in a $4x$(4EBN) × $2x$(2EBN) cross the $4x$ female produces a reduced (n=$2x$=24) egg and the male produces an unreduced ($2n$=$2x$=24) gamete, the 2-to-1 ratio of maternal to paternal EBN contribution is satisfied and viable $4x$ seeds result. This result has important practical ramifications because most commercial potato cultivars in the United States are $4x$(EBN) and most wild potato species are $2x$(2EBN).

INCOMPATIBILITY. Incompatibility is present in some species of the genus *Solanum*. It has been shown in several diploid species that the gametophytic system of incompatibility is genetically

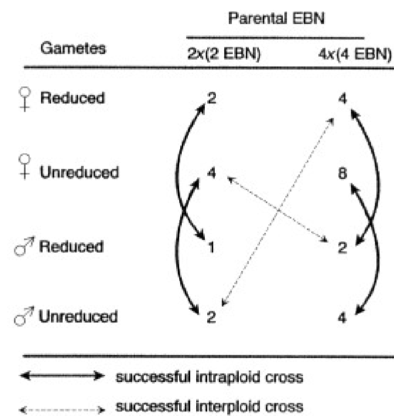


Fig. 21.4.
Normal endosperm development and seed set occur when the endosperm balance number (EBN) is in a ratio of 2EBN maternal: 1EBN paternal. Intra- and interploid crosses that are likely to produce viable endosperm and seeds are indicated.

controlled by a series of alleles, S_1, S_2, S_3, and etc.(see Fig. 7.1). In certain cases, the incompatibility system is modified by a factor, *R*, which when present in the style prevents fertilization from a pollen tube carrying *S* alleles.

Crossing Techniques

Flower buds that are mature are selected for emasculation just prior to crossing. It is particularly important to emasculate just prior to crossing if pollinations are done in the field as the wind can break off the stigmas before pollination occurs if they are emasculated too far ahead of pollination. Mature buds are plump, with the petals ready to separate. The remaining buds and opened flowers in the bunch are removed to facilitate emasculation of the selected buds and to prevent contamination of the emasculated flowers by the open flowers. There is a limit to the number of flowers from an inflorescence that will set fruit/seed, so removing the extra flowers increases the chances that the pollination will be successful. The petals of the selected flowers are gently pushed apart along the sutures and the five stamens removed with fine-pointed forceps without breaking the style. The emasculated flowers are then bagged. Inserting a branch with one or two leaves into the bag helps in maintaining a humid climate inside the bag. In fully self-sterile parents, emasculation is unnecessary.

Pollination can be done at any time of the day so long as the temperature is not too high. Open flowers are collected from the plant to be used as a male. The flowers are laid out to dry overnight. The following morning the pollen is collected from them by shaking into gelatin capsules such as those used in the pharmaceutical industry (other small tubes can also be used). For large quantities of flowers, the pollen is shaken out by placing the flowers in the top section of a sieve, and the sieve is then shaken at high speed. The pollen falls through and is collected in the bottom chamber of the sieve and transferred to the smaller capsules or tubes for storage. For smaller quantities of flowers, a modified toothbrush or doorbell buzzer is used to vibrate the pollen free. The flowers are inverted over glassine paper and the vibrating portion of the toothbrush or buzzer is touched to the anthers. The pollen falls onto the slick paper and is then transferred into the capsules or tubes. Pollen can be stored desiccated in the refrigerator for 1 to 2 weeks and in the freezer for 6 months to a year. To make the pollination, the stigma is dipped in the pollen in the capsule or tube, and then the pollination tag is attached and the bag is placed over the flower and left on until the fruit is harvested. Setting of seed may be observed in about 7 to 10 days. Average seed set per berry varies with the cultivar, but levels of 50 to 200 seed per fruit may be obtained.

Genetics of Potato

The tetraploid nature of potato can be exploited by the breeder to improve desirable characteristics. It is well known that asexually propagated species such as potato have evolved taking advantage of *nonadditive or epistatic gene action*. Therefore, the potato breeder must be knowledgeable on the use of breeding procedures that can accommodate nonadditive gene action. Because of the potato's autotetraploid nature, *intralocus interactions (heterozygosity*) and *interlocus interactions (epistasis*) are important when selecting breeding procedures to improve certain traits. It is assumed that increased heterozygosity leads to increased *heterosis*. Heterosis in potato occurs when the progeny outperform the best parent or the parents' mean. The level of heterozygosity is influenced by how different the four alleles are within a locus. The more

diverse the alleles are within a locus, the higher the heterozygosity and the greater the number of increased interlocus or epistatic interactions.

To see how increased heterozygosity can lead to more epistatic interactions, it is necessary to identify the allelic conditions possible in an autotetraploid. Five tetrasomic conditions are possible at an individual locus in an autotetraploid:

- $a_1a_1a_1a_1$, a monoallelic locus where all alleles are identical,
- $a_1a_1a_1a_2$, an unbalanced diallelic locus where two different alleles are present in unequal frequency,
- $a_1a_1a_2a_2$, a balanced diallelic locus where two different alleles occur with equal frequency,
- $a_1a_1a_2a_3$, a triallelic locus where three different alleles are present, and
- $a_1a_2a_3a_4$, a tetraallelic locus where four different alleles are present.

It is hypothesized that the tetraallelic condition provides the maximum heterosis because more interlocus interactions are possible for this tetrasomic condition than for the other configurations (Table 21.1). For example, in the tetraallelic condition, six first order interactions are possible:

- a_1a_2, a_1a_3, a_1a_4, a_2a_3, a_2a_4, and a_3a_4;

four second order interactions are possible:

- $a_1a_2a_3$, $a_1a_2a_4$, $a_1a_3a_4$, and $a_2a_3a_4$; and

one third order interaction is possible:

- $a_1a_2a_3a_4$.

Table 21.1.
The number of first, second, and third order interactions possible and their sums for the five different tetrasomic conditions in an autotetraploid

	No. of allelic interactions				
Tetrosomic condition	First order	Second order	Third order	Total	Portion of haploids (2*x*) conserving one first order interaction
$a_1a_2a_3a_4$	6	4	1	11	All
$a_1a_1a_2a_3$	3	1	0	4	5/6
$a_1a_1a_2a_2$	1	0	0	1	2/3
$a_1a_1a_1a_2$	1	0	0	1	1/2
$a_1a_1a_1a_1$	0	0	0	0	None

Note: If haploids are made from plants having these same possible tetrasomic loci, the portion of haploids conserving one first order interaction is given when an unreduced (2*n*) gamete is produced assuming double reduction is lacking.

This is a total of 11 different interactions possible for the tetraallelic condition. This is in contrast to the monoallelic condition, which has no interactions. The highest level of heterosis

will occur as the frequency of tetraallelic loci increase. The greatest number of interlocus or epistatic interactions will also occur as the frequency of tetraallelic loci increase. In breeding for improved tuber yield in potato, intra- and interlocus interactions have been shown to be important. Procedures that maximize the frequency of tetraallelic loci should be considered in breeding potato for increased yields.

Breeding Methods for Potato

Potato is unique in the fact that improvements can be made using an array of breeding procedures. Potato can be improved by conventional breeding procedures, such as recurrent selection through the use of cytogenetics and through the use of new biotechnology procedures. Biotechnology advancements allow for development of *monoploids* (1*x*) from diploids, such as *S. phureja*, and protoplast fusion. Knowledge of cytogenetics facilitates the use of *dihaploids (2x)* from 4*x S. tuberosum* and meiotic mutants that give rise to unreduced gametes (Chapter 5). The fact that *androgenic dihaploids* are possible from 4*x S. tuberosum* is a distinct advantage because breeders can study and incorporate single-gene traits at the diploid level. This advantage is due to the fact that disomic genetic ratios are easier to interpret and work with than tetrasomic genetic ratios. The majority of potato haploids ($2n$=$2x$=24) used in germplasm enhancement are derived from $4x \times 2x$ crosses in which the 2*x* male parent is a particular accession of *S. phureja* that is capable of inducing high haploid frequencies. Anther culture has not been as successful a means of deriving haploids or monoploids because many of the resulting derivatives turned out to have originated from anther walls, tapetum, or 2*n* pollen grains and thus were not the expected product. With this vast array of breeding procedures available, the plant breeder has a tremendous amount of flexibility in improving potato.

Hybridization

CONVENTIONAL HYBRIDIZATION BREEDING. The hybridization procedure is the principal method of improving the potato. The procedure starts with the selection of desirable parents. Selection of the parental materials is important because it determines the potential for success of the hybridization breeding procedure. Crosses are made between commercial cultivars or with plants developed from population improvement procedures.

Because potato is a vegetatively propagated crop, commercial cultivars utilized as parents are heterozygous and segregation of characters will be found in the F_1 generation following hybridization. Clonal selection is practiced in the F_1 generation and rarely is an F_2 generation grown. Tubers obtained from each selected F_1 plant can be grown in F_1 rows for evaluation and to increase the amount of seed tubers. Each row represents the clonal increase from a single F_1 plant. During the next season the clones can be grown and evaluated in longer rows and replicated if sufficient seed tubers are available. Selected clones, after seed tubers have been increased in sufficient quantity, can be tested at multiple locations for evaluation of genotype × environment interactions, screening for disease and insect resistance, or measuring comparative yield potential.

UNILATERAL AND BILATERAL SEXUAL POLYPLOIDIZATION. Breeding procedures that can increase the frequency of tetraallelic loci include *unilateral sexual polyploidization* and

bilateral sexual polyploidization, discussed in Chapter 5. Unilateral and bilateral sexual polyploidization when coupled with *first division and second division restitution mechanisms* (also discussed in Chapter 5) will lead to an increased frequency of tetraallelic loci and hence an increased number of intra- and interlocus interactions. Because many diploid *Solanums* readily hybridize among themselves and with the tetraploid *S. tuberosum*, and the fact that dihaploids ($2x$) are easily obtained from *S. tuberosum*, uni- and bilateral sexual polyploidization are powerful plant-breeding procedures to use in improving yield in potato (Fig 21.5).

Unreduced gametes are produced from dihaploids and naturally occurring diploids. The significance of unreduced gametes from naturally occurring diploids or from dihaploids is that desirable epistatic interactions are passed from parent to progeny intact. Normal meiosis transfers only additive gene action, but unreduced gametes in potato transfer non-additive gene action. As indicated in Chapter 5, meiotic mutants have been discovered that lead to production of unreduced gametes in sufficient frequency to be useful in a potato breeding program.

A high frequency of haploids obtained from a highly heterozygous S. *tuberosum* plant will have first order interactions conserved (Table 21.1). For example, all haploids are expected to conserve first order interactions if the initial constitution of the locus is tetraallelic. This is true if there is no *double reduction*. Double reduction results from a crossover between the centromere and the allele in question, and sister alleles end up in the same gamete producing homozygous gametes such as a_1a_1, a_2a_2, a_3a_3, and a_4a_4 Because these gametes are homozygous, no first order interactions are conserved.

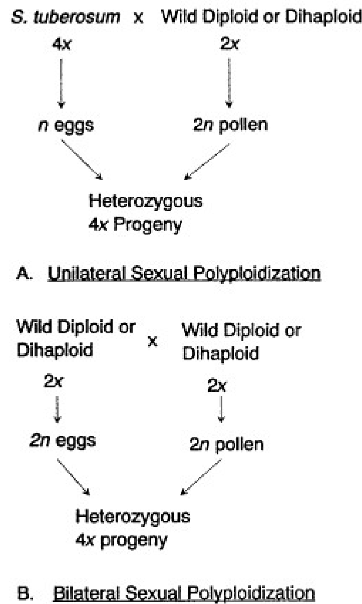


Fig. 21.5.
Procedures for introducing diploid germplasm into the cultivated potato is illustrated. Highly heterozygous progeny can be obtained from unilateral (A) and bilateral (B) sexual polyploidization. An example of unilateral sexual polyploidization involves a cross between a S. tuberosum female with a wild diploid or dihaploid male. Reduced gametes (n=2x=24) are formed by the 4x female and unreduced gametes (2n=2x=24) are produced by the 2x male leading to highly heterozygous 4x progeny. The 2x parent could also be a hybrid between a haploid and a wild species because this allows the breeder to select for traits at the 2x level and take advantage of disomic ratios prior to crossing to the tetraploid. Bilateral sexual polyploidization involves unreduced (2n = 2x = 24) eggs and pollen from both 2x parents which results in highly heterozygous 4x progeny.

In Figure 21.5, it is shown how unilateral and bilateral sexual polyploidization can be used to improve the cultivated potato. Both of these procedures are used to transfer unadapted germplasm into the $4x$ germplasm pool. Again, as with any breeding procedure, care must be

taken to select the best parents possible. With unilateral and bilateral sexual polyploidization, it is possible to incorporate simply inherited traits at the diploid level, either with the wild diploids or with the dihaploids. After the desirable gene combinations are obtained at the diploid level, they can easily be transferred to the 4x level through unreduced gametes.

In addition, monoploids can be synthesized from diploids or dihaploids. They can occur by *parthenogenesis* or by *androgenesis* (anther culture from 2*x* plants). Monoploids lend themselves for use in mutation breeding because all of the loci are in the *hemizygous* condition and desirable recessive genes are visible. Screening at the monoploid level amounts to selecting at the gametophytic level. Once the desired monoploid is developed the chromosome number can be increased to the diploid level and incorporated into the breeding scheme as suggested in Fig. 21.5.

The International Potato Center uses haploids and unreduced gametes to improve pest resistance in potato. The strategy used is to obtain 2*x* plants from as many diverse backgrounds as possible to improve upon the chance of having different alleles at a given locus. Pest resistance is identified at the diploid level and transferred to the 4*x* gene pool. Breeders have successfully transferred root-knot nematode resistance from the wild diploid *S. sparsipilum* to the 4*x* level by first division 2*n* pollen. Other pest resistance transferred from wild diploids to the cultivated potato via 2*n* gametes include the potato tuber moth, resistance to cyst nematode, bacterial wilt, early and late blight, and several other diseases.

Protoplast fusion can also be used by the breeder to improve potato. In Fig 21.6 assume that a_1, a_2, a_3, and a_4 are desirable alleles controlling specific traits located in four different wild diploid potato species. The goal is to obtain a single 4*x* plant with all four desirable alleles. This can be accomplished by crossing the diploids and selecting within the two diploid F_1 progeny for the desirable phenotype. Once the two desirable diploids are found, they can be advanced to the 4*x* level utilizing protoplast fusion.

Breeding Objectives of Potato

The breeding objective must be clearly defined if the breeder is to make meaningful progress. Selection of parents will depend upon the objectives in mind. Furthermore, the breeder of potato as with other crops is not concerned with only one objective, but many. Careful selection of parents before selection cannot be over emphasized when improvement of multiple traits is the goal.

Tuber Yield

The primary objective in breeding potato is increased tuber production. Tuber yield is influenced by the number

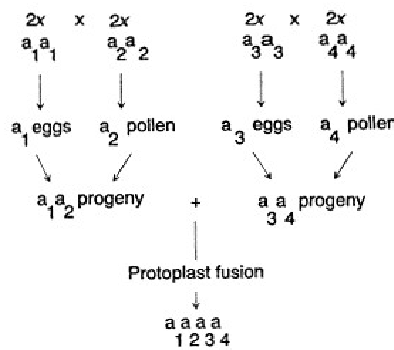


Fig. 21.6.
Desirable genes from unrelated diploids can be combined in a cultivated 4x potato plant through a combination of conventional crossing and through protoplast fusion. Unrelated diploids with different desirable alleles are mated to form two single-crosses, each with different alleles. Protoplast fusion allows the final progeny to include the four desirable recessive alleles in one plant.

of tubers produced per plant and weight of the individual tuber. The number of tubers produced per plant ranges from 3 to 10 with each underground stem producing approximately 3 tubers. The number of tubers is highly correlated with the number of stems produced while a negative relationship exists between number of stems per plant and the number of tubers per stem.

In selecting for improved tuber yields, the response to photoperiod needs to be considered. The relationship of the photoperiod to the potato is complex. In general, vegetative growth is favored by long days and moderate temperatures, stolon growth is favored by long warm days, while tuber yield is favored by long days to stimulate vegetative growth followed by short days to activate tuberization. The amount of foliage present has a strong influence on tuber yield up to a leaf area index (LAI) of three, after which the correlation between tuber yield and leaf area decreases. Tuber shape is also affected by day length, with largest tubers produced in long-day conditions, but the smoothest and most uniform tubers are produced in short-day conditions. Cultivars and species of potatoes differ in their photoperiod response. The reaction to photoperiod is inherited, and a large number of genes are involved.

Maturity

Most of the past breeding efforts concentrated on developing cultivars for temperate environments. Therefore, yields in warmer climates have been low. Yields in warmer climates can be increased using proper management techniques. Maturity is an important consideration when the breeder must develop cultivars for regional adaptability. Earliness is of value in areas where the favorable growing periods are short. Further, early maturing cultivars are also needed where two or more crops of potato are grown during the year. Early maturing cultivars are more economical in use of irrigation waters. Early cultivars tend to escape insect infestations such as aphids when grown in the fall in many locations. An early crop may escape frost injury or disease infestation. Early maturing strains usually flower more sparsely than late strains.

Heat, Frost, and Drought Resistance

Resistance to heat, frost, and drought are desirable to prevent losses when the potato crop is grown under these adverse climatic conditions.

TOLERANCE TO HIGH TEMPERATURE AND HEAT. The development of a potato plant may be divided into three periods. These are germination, growth, and tuberization. Germination and growth are favored by warm temperatures, while tuberization is favored by cool temperatures, preferably below 18ºC. Normally, there is a reduction in size of tubers with temperatures above 18 to 20ºC during the tuberization period. Practically no tuberization takes place with temperatures above 29ºC. In most warmer climates where potato is produced, tuberization would be improved if cultivars tolerating higher temperatures during tuber formation could be developed.

Breeding materials may be screened for heat tolerance by testing for foliage resistance to high temperature and tuberization during high temperatures. For example, if plants are kept in a controlled environment at 50ºC for 8 hours during the night for a period of 14 days, susceptible plants will deteriorate significantly within 3 days. Clones are tested for tuberization by growing in a greenhouse at 30 to 38ºC during the tuberization period and then comparing the amount of tuber formation.

FROST RESISTANCE. In certain areas the fall-grown potato crop may be injured by frost before tuberization is completed. Cultivars that can tolerate frost would be desirable for such areas. Screening for frost resistance is conducted in controlled environment chambers at freezing temperatures and in the field in areas where frost occurs. A detached leaf technique may be used for quick and large-scale screening for frost resistance. With this technique, leaves of the potato are exposed to temperatures of around -5°C for 8 hours. Leaves from susceptible plants lose their turgidity and become discolored due to osmosis from the chloroplasts, effects which can be visually observed immediately.

DROUGHT RESISTANCE. Drought resistance is desirable when potato is grown in areas where there are no irrigation facilities or where irrigation facilities are inadequate. Screening for drought resistance can be done either in the field or in pots under simulated drought conditions.

Breeding for Disease Resistance

The potato suffers from a number of diseases incited by viruses, fungi, and bacteria. The complete array of diseases will not be discussed here; rather, a few will be pointed out to illustrate the complexity of breeding for disease resistance in potato.

The potato breeder recognizes two types of resistance:

• *general* or *field resistance, and*

• *race-specific resistance*.

Field resistance is *polygenic* and controlled by many minor genes, each with small individual effects. Race-specific resistance is the resistance of a genotype to a particular physiologic race of the pathogen. Resistance to a specific race of the patbogen is usually inherited as a single dominant gene. It is more difficult to accumulate genes for field resistance into a cultivar than to transfer single genes for resistance to specific races. Field resistance is more lasting than race-specific resistance because the protection offered through race-specific resistance may be breached by a single gene mutation in the pathogen. The changed pathogen may then infect the previously resistant cultivar. Field resistance slows down the rate at which disease increases in the field, and race-specific resistance reduces the amount of inoculum which starts the infection. (See discussion in Chapter 12.)

LATE BLIGHT. Late blight caused by *Phytophthora infestans* is the most important fungal parasite of potato. It causes decay of the foliage and tubers. Late blight may be spread by planting infected tubers or by wind-blown spores in the field. Prolonged wet and warm weather favors infection. Many races of the pathogen have been found, and breeding is complicated because foliage resistance and tuber resistance may differ in the same plant.

Resistance to late blight is found in several wild species of *Solanum*. In Europe several major genes, designated R_1, R_2, R_3, R_4, and etc., have been identified in differential cultivars and are used to identify the specific physiologic races of the blight pathogen.

Screening of seedlings for resistance to late blight may be done both in the laboratory and in the field. In the laboratory small filter paper disks dipped in spore suspensions of the late blight organism are placed on detached leaflets, and the leaflets are incubated in moist chambers until sporulation develops on the leaf. Detached leaves may also be treated with hormones to induce rooting. The rooted leaves will continue to grow in soil for a sufficient length of time to be used in tests for resistance. Both procedures permit the plant to continue

growing in the field while the test for blight resistance is being made. In the field, screening can be done in areas where the disease is known to occur by planting the materials to be tested along with known susceptible and resistant cultivars. Screening of cultivars in the field has its limitations as evaluation for resistance is made only to the specialized races present. Because field resistance can't be observed if the strain carries at the same time genes for hypersensitivity to races present in the field, arrangements need to be made to evaluate the materials at other locations where other large and virulent groups of physiologic races of the late blight organism are present.

VIRUS DISEASES. Several viruses infect the potato crop. These are virus X, virus A, virus Y, leaf roll, virus S, virus C, and spindle tuber, as well as several soil-borne viruses. Virus C is closely related to virus Y. Virus X, virus S, and spindle tuber are transmitted by contact. Virus Y and leaf roll are transmitted by aphids.

Virus X is the most widely distributed virus of potato and is present throughout south and southeast Asia as well as Europe and America. There are many strains of the virus and three types of resistance have been reported. These are:

- resistance to infection,
- hypersensitivity, and
- extreme resistance or immunity.

The latter two are used mostly by the breeder; however, the immunity type of resistance is generally preferred. Hypersensitivity is controlled by a single dominant gene, *Nx*, inherited in a tetrasomic fashion. Another gene, *Nb*, has been described for field immunity to the strain B of the X virus. Other genes for resistance to virus X include *Ne, Nr, Rx,* RX_{acl}, and Rx_{adg}.

Screening for virus X can be done in several stages. Initially the seedlings are grown in flats or boxes and sprayed with a suspension of virus X grown on tobacco leaves. Susceptible seedlings are rejected and the remainder are planted in pots. The potted plants are then inoculated by introducing the virus mechanically by abrasion and checked for resistance. The next step is to graft an infected scion of tobacco or *Datura* onto a stalk of potato observed to be resistant in previous tests. After two days the test for virus in the potato is made, either serologically or by inoculation of an indicator plant like *Gomphrena globosa*, with sap from the grafted potato stalk. The latter test is required to identify latent virus in the potato, symptoms of which can't be identified except by serological tests or by the graft test.

As indicated, many other viruses attack potato. The discussion on breeding for resistance to virus X has implications for breeding for resistance to other viruses.

CHARCOAL ROT. Charcoal rot is caused by *Macrophomina phaseoli*. Infection occurs when potatoes are grown at high temperatures. The disease develops in the stored tuber. Clones of the diploid *S. chacoense* along with other sources have been found to be resistant. The S. *chacoense* clones are also of interest because they have the ability to tuberize under high temperatures. Inoculation for charcoal rot may be done by inserting toothpicks containing the organism into tubers. The tubers are incubated at temperatures of 32 to 34ºC, and the extent of development of the disease in the tuber is observed in comparison with that in susceptible check cultivars.

Breeding for Insect Resistance

Important insect pests of potato include nematodes, aphids, and certain beetles. Attempts at breeding resistant cultivars have been primarily focused against nematodes and aphids.

The root knot nematode, *Meloidogyne incognita*, and the cyst nematodes, *Globodera pallida* and *Globodera rostochiensis*, are important nematodes on potato. Other nematodes exist as well. To screen for resistance in the laboratory, potato tubers are sprouted in small pots with sterilized soil into which several hundred larvae are released one week after planting. Pots are kept at a temperature near 25ºC, and the roots are scored for nematode damage after 75 days. In the field, screening may be done by growing plants in areas known to be infested with nematodes along with susceptible cultivars as checks.

Aphids are a serious pest problem on potatoes. The resistance of potato cultivars to aphids appears to be associated with the pubescence of the leaves. Aphid resistance is desirable because it would help give protection to virus diseases transmitted by aphids.

The Colorado potato beetle, *Leptinotarsa decemlineata*, is a common pest of potato. The larva and adult Colorado potato beetles damage plants by feeding on their leaves. It is not uncommon to lose 50% of the total yield to this pest. Other beetles are also problems on potatoes.

Breeding for Improved Quality

Potatoes are either consumed directly or they are processed. The amount of potatoes used for processing is increasing. Processed potatoes have various uses, including being used in the fast-food industry, made into snacks, used as a starch source, and for alcohol production, among other uses. High quality is an important breeding objective because it has direct relationships to consumer acceptance and higher premiums in the marketplace.

Some of the desirable features of high tuber quality include good keeping quality, medium size, good grading, good shape, proper color, no cracks, flatness of the eyes, and proper skin texture, among others. For most purposes the optimum tuber size for best grading should be between 150 and 200 g and should be round-oval in shape. If tubers are used for french fries, long oval ones are preferred and high levels of reducing sugars are not desirable because they lead to discoloration of fries and chips. If the tubers are going to be fed to livestock or used in the production of alcohol, a high starch content is needed. It is important that tubers have good keeping quality and do not degenerate in storage, either in viability in the case of seed or in nutritive value in the case of potatoes to be used for food. Cultivars differ markedly in ability to be stored. Thick-skinned potatoes have better keeping qualities than thin-skinned. Keeping quality is associated with nonsprouting and resistance to storage diseases.

Cultivars differ in their cooking qualities, some requiring prolonged cooking, while others cook easily. Freedom from after-cooking darkening is also desirable. White tubers are preferred to red ones, and they sell at higher prices in most markets. Little attention has been given to breeding tubers with better nutritive value, especially tubers rich in protein content and vitamins. More breeding work needs to be done on improving nutrient content, particularly for developing countries, as lack of protein is a serious problem. Cultivars with shallow eyes are preferred by consumers as there is less loss in preparation of the tuber for cooking, but deep eyes in seed potatoes afford protection for the growing tip.

True Potato Seed (TPS)

Breeders have long sought to increase potatoes by seed. The production of potato from true potato seed has several advantages compared to tubers, including:

• production of virus-free stocks as viruses are generally not transmitted by seed,

• reduce storage problems because refrigeration of true potato seed is not necessary,

• lower shipping costs for true potato seed,

• easier shipping of true potato seed because 100 g true potato seed will seed a hectare while 2000 kg of seed tubers are needed to seed the same area,

• comsumption of all tubers produced as none need to be saved for next year's seed crop.

The objective of true potato seed is to have completely homogeneous progeny. This can best be accomplished by the use of $4x$ families from $4x \times 2x$ crosses where the $2x$ parent produces $2n$ gametes. It is important that both parents be adapted to the area where the homogeneous progeny are going to be grown. Studies have shown that higher seedling vigor and tuber yields resulted from this approach compared to progeny produced from $4x \times 4x$ crosses or progeny obtained from open pollinated seed.

Study Questions

1. What is the balanced endosperm number hypothesis and why is it important in potato breeding?

2. What makes potato breeding unique as compared to many other crops?

3. What breeding methods are available to the potato breeder to develop high yielding heterotic cultivars?

Further Reading

Grun, P. 1990. The evolution of cultivated potatoes. Econ. Bot. 44(suppl.3):39-55.

Hancock, J.F. 1992. Starchy staples and sugars, p. 239-57. *In* Plant evolution and the origin of crop species. Prentice Hall, Englewood Cliffs, NJ.

Hawkes, J.G. 1990. The potato: evolution, biodiversity and genetic resources. Smithsonian Institution Press, Washington, D.C.

McKinlay, R.G., A.M. Spaull, and R.W. Straub. 1992. Pests of solanaceous crops, p. 263-26. *In* R.G. McKinlay (ed.) Vegetable crop pests. CRC Press, Inc., Boca Raton, FL.

Mendoza, H.A., and R.L. Sawyer. 1985. The breeding program at the International Potato Center (CIP). p. 117-37. *In* G.E. Russell (ed.) Progress in plant breeding 1. Butterworth & Co. Ltd., London, U.K.

Peloquin, S.J., and R. Ortiz. 1992. Techniques for introgressing unadapted germplasm to breeding populations, p. 485-512. *In* H.T. Stalker and J.P. Murphy (eds.) Plant breeding in the 1990s. Redwood Press Ltd., Melksham, U.K.

Plaisted, R.L. 1980. Potato. p. 483-94. *In* W.R. Fehr and H.H. Hadley (ed.) Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Agron., and Soil Sci. Soc. Am., Madison, WI.

Ross, H. 1986. Potato breeding--problems and perspectives. *In* W. Horn and G. Röbbelen (eds.) Advances in plant breeding. J. Plant Breeding (suppl. 13). p. 132. Verlag Paul Parey, Berlin.

Rowe, R.C. (ed.). 1993. Potato health management. APS Press, Am. Phytopath. Soc., St. Paul, MN.

Rich, A.E. 1983. Potato diseases. Academic Press, New York, NY.

22. Breeding Sugarcane

The breeding of sugarcane (*Saccharum* spp.) differs from that of other major field crops. A *cultivar* in cultivated sugarcane refers to a specific genotype, or *clone*, that is propagated vegetatively through *seedcanes* or *setts*. *The* cultivated sugarcane clone is a complex, heterozygous polyploid that originated by chance segregation in a hybrid population. Most commercial clones contain germplasm introgressed from three or more different *Saccharum* species. The agronomic performance of the clone can be evaluated through field trials, but its genetic contribution as a parent in a breeding program is difficult to evaluate due to its heterozygous genotype. Sugarcane is widely grown in the tropical and subtropical areas of the world. In tonnage of sugarcane produced, Brazil and India lead all countries. In the United States, sugarcane is grown in Florida, Hawaii, Louisiana, and Texas.

Species of Sugarcane

Sugarcane is classified in the genus *Saccharum*, tribe Andropogoneae, family Gramineae. Within the genus *Saccharum* there are three species of cultivated sugarcane (*S. officinarum* L., *S. barberi* Jesw., and *S. sinense* Roxb.) and two species of wild sugarcane (*S. robustum* Brandes and Jeswiet ex Grassl, and *S. spontaneum* L.) (Fig. 22.1). Sugarcane clones presently in cultivation are complex hybrids among these species and cannot be classified as belonging to any particular species. Another species of *Saccharum, S. edule* Hassk., has an edible inflorescence used for food but has little or no sugar and can scarcely be regarded as sugarcane.

SACCHARUM OFFICINARUM. This species includes the tropical, "noble" canes indigenous to the New Guinea region of the South Pacific. Canes of *S. officinarum* are found only in cultivation in native gardens and are no longer found growing wild. They are characterized by thick stems, soft rinds, high cane yield or tonnage, low fiber, and high content and purity of sucrose. Originally, they were grown as chewing canes and for centuries were the only cultivated canes in the tropical regions of the South Pacific. The term "noble" was applied to

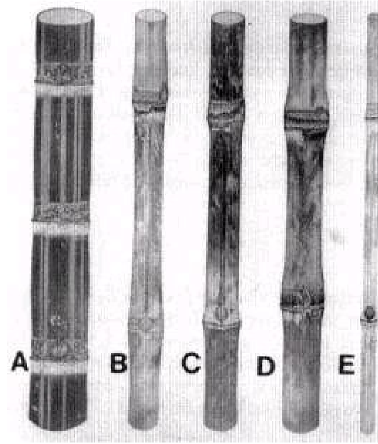


Fig. 22.1.
Canes of the cultivated and wild species of sugarcane.
(A) S. officinarum (noble cane), (B) S. barberi (north Indian cane), (C) S. sinense (Chinese cane), (D) S. robustum (wild cane), and (E) S. spontaneum (wild cane).

the tall, handsome, large-barrelled, colorful canes of this species by early Dutch researchers in Java. The canes of S. *officinarum* do not withstand well the rigors of drought, such as occurs in the subtropical climate of north India, or occasional frost, as occurs in the temperate climate of southern United States. Particular clones are resistant to rust, smut, Fiji disease, or downy mildew, but outbreaks of red rot and mosaic at one time virtually eliminated the species from large-scale commercial cultivation.

SACCHARUM BARBERI AND S. SINENSE. *Saccharum barberi*, the north India sugarcane, and *S. sinense*, the Chinese sugarcane, are indigenous to north India, Bangladesh, and the Burma-China region. The species are reported to have originated by hybridization between *S. officinarum* and *S. spontaneum*. The canes are characterized by thin stems, great vigor, early maturity, and wide adaptability. They can withstand light frost and drought, features which adapt them for cultivation in north India. They are poor in cane yield but intermediate to good in sucrose content, resistant to red rot and sereh disease, susceptible to smut, and vary according to the clone from susceptible to immune for mosaic disease. Clones of these species contribute genes for vigor, hardiness, and disease resistance in sugarcane crosses.

SACCHARUM ROBUSTUM. *Saccharum robustum* is a wild species that originated in New Guinea. The species has great vigor and wide adaptability. The canes are tall with medium thickness, high in fiber, low in sugar content, susceptible to mosaic, but some clones are resistant to eye-spot disease. *S. robustum* has not been utilized extensively in the breeding of commercial cultivars.

SACCHARUM SPONTANEUM. The clones of the wild species, *S. spontaneum*, form a complex group with great diversity and much natural hardiness. The distribution of this species is widespread, being found in India, China, Taiwan, the Philippines, the South Pacific Islands, and Africa. In general, the clones are perennial, rhizomatous, high tillering with slender stalks, high in fiber, and low in sucrose. Most clones flower abundantly with good seed set. Particular clones of *S. spontaneum are* resistant to rust, smut, sereh, mosaic, red rot, or pythium root rot. Clones of *S. spontaneum are* widely used in sugarcane breeding to contribute genes for vigor, hardiness, tillering capacity, and disease resistance.

Related Genera

The genus *Saccharum* and four related genera, *Erianthus, Miscanthus, Narenga*, and *Sclerostachya*, constitute what is known as the *Saccharum complex*. Intergeneric crosses have been successfully accomplished between sugarcane and genera within this group. These related genera possess a reservoir of genes for agronomic characters and disease resistance that have not been used in the breeding of commercial clones of sugarcane due to meiotic abnormalities in the hybrid progenies. Several backcrosses to sugarcane will undoubtedly be needed to transfer genes for desirable characteristics from these genera to sugarcane and recover usable commercial clones.

Origin of Sugarcane

Cultivated sugarcane had two geographic centers of origin, New Guinea and the northern India-Burma-China region. The large-barrelled, tropical species, *S. officinarum*, probably originated from the wild species, *S. robustum*, in the New Guinea region. As *S. officinarum* migrated outward from its center of origin, it became modified through natural hybridization with related genera. The north India-Burma-China sugarcanes, *S. sinense* and *S. barberi*, based on appearance, probably have one and maybe two unidentified species involved in their origin.

Botany of Sugarcane

Sugarcane flowers sparsely except in tropical climates. The flowering response differs with the genotype of the clone, the temperature, the photoperiod, the soil moisture, and the nutrition of the clone. Flowering is favored by warm nights and high humidity and inhibited by cool weather and high altitudes. Flowering is undesirable in commercial canes as it leads to rapid maturity and reduces total sugar yield. *In sugarcane breeding, flowering and production of fertile pollen and true seeds are requirements for obtaining genetic recombination*.

The sugarcane inflorescence consists of an open, branched panicle, known as an arrow, that bears thousands of flowers (Fig. 22.2). The flowers are borne in paired spikelets, one sessile and one pedicellate (Fig. 22.3). The flowers open in early morning, usually between 5 and 6 a.m. Flowering starts at the top of the arrow and proceeds downward, requiring 7 to 14 days for completion. Most flow-

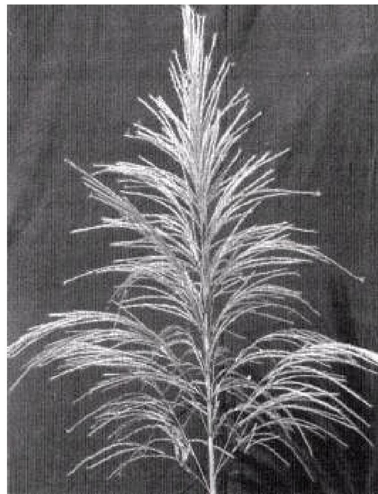


Fig. 22.2.
Flowering arrow of sugarcane plant. The sugarcane arrow may bear thousands of flowers.

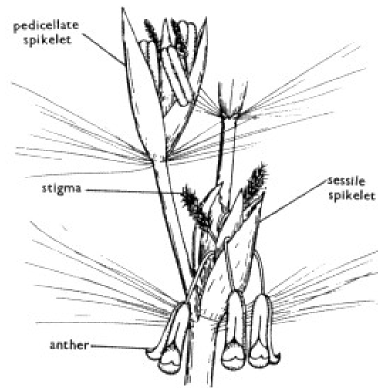


Fig. 22.3.
Part of inflorescence of sugarcane plant showing sessile and pedicellate spikelets. The sessile spikelet flowers before the pedicellate spikelet.

ers are perfect; others are imperfect with either the pistil or, more frequently, the stamens aborting. Sugarcane clones vary widely in fertility and seed production; from high pollen production to male-sterility, from complete self-fertility to self-sterility, and even self-incompatibility. The seeds produced are extremely small in size averaging 200 to 300 seeds per gram. The seeds are often poorly developed and inviable. The seeds and accompanying floral structures, including the long silken hairs at the base of the spikelets, are referred to as fuzz (Fig. 22.4). The fuzz breaks off easily and may be carried away by the wind. In breeding experiments care must be taken to prevent loss of the seed in this manner. This is usually accomplished by bagging the tassel about 15 to 20 days after a cross has been made. As seeds mature and fall, they are caught in the bag and are harvested about 30 days later.

Commercial sugarcane clones are propagated vegetatively by stem cuttings. The stem sections are about one meter in length and contain a lateral bud at each node that germinates to form shoots and roots (see Fig. 2.10). The shoot develops into a primary stem from which secondary stems or tillers arise. The leaves may be loose on the stem and break away easily, in which case they are said to be *free trashing*. Leaf sheaths that adhere tightly are undesirable as they hold water, permitting root primordia to develop aerial roots. Breeding clones are propagated by single-bud cuttings (Fig. 22.5). Several single-bud cuttings may be made from each selected clone. Stem nodal sections about 5 to 8 cm in length are prepared; each stem section contains a node and a bud or eye at the node. Upon germination of the bud, shoots and roots develop from the nodal bud. Thousands of the nodal buds can be germinated in relatively small areas of greenhouse benches (Fig. 22.5).

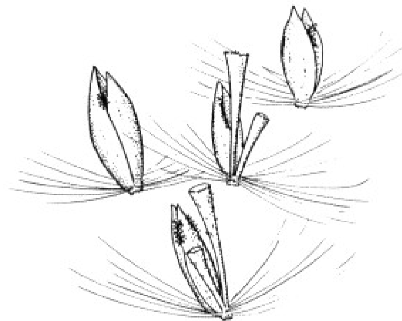


Fig. 22.4.
Fuzz or tree seeds of sugarcane. The stalk of the pedicellate spikelet and a rachis segment remains attached to the sessile spikelet. The pedicellate spikelet breaks free, and the long, silky hairs at the base permit the wind to carry the seed for long distances.

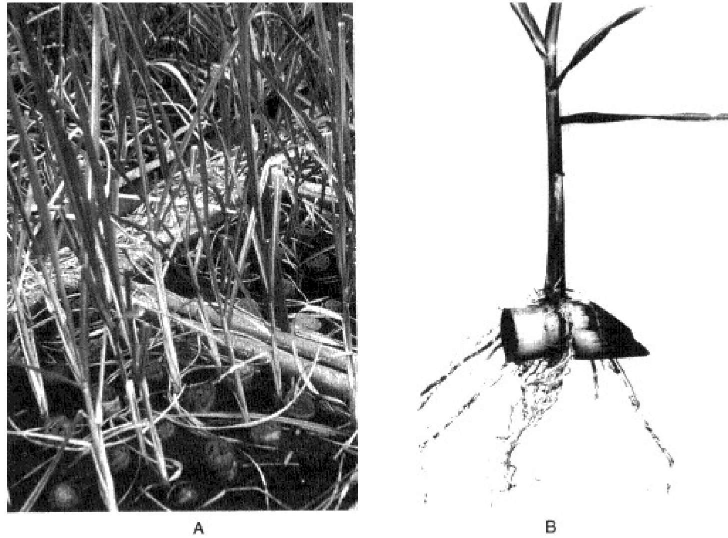


Fig. 22.5.
Sugarcane is propagated asexually through stem cuttings. (A) Propagation of sugarcane breeding clones from stem nodal sections growing on a greenhouse bench. (B) Each cutting contains an eye or bud that germinates and forms roots and shoots.

Selfing and Crossing Techniques

Sugarcane is normally cross-pollinated. If self-pollination is required in the breeding program, the arrow is covered, usually with a paper bag as in corn, or isolated from arrows of other clones. Cross-pollinations may be made either by bringing arrows from the male and female clones together in isolation and permitting natural cross-pollination or by dusting pollen from the male clone over a flowering arrow of the female clone. Generally, emasculations are not made due to the large number and small size of flowers on the arrow and to the 10- to 14-day period it takes for the entire sugarcane arrow to complete flowering. In temperate climates, parent clones are potted, placed on rail carts, and moved into a *crossing house* (Fig. 22.6). Within the crossing house, temperature and humidity are controlled for optimum pollen production and stigma receptivity, and photoperiod is controlled to promote synchronization of flowering.

Male and female clones for specific crosses are placed together in isolated cubicles. Because the cubicle in the crossing house is free of wind currents that would disseminate the pollen, the arrow of the male clone is placed above the arrow of the female clone so that pollen falls directly on the female flowers (Fig. 22.7). Tapping the male plants lightly each day during

Fig. 22.6.
Sugarcane clones on rail carts preparatory to being moved into a tall crossing house. The air-layers are visible as black bulges on the lower stems just above the cans.

the pollination period helps to scatter the pollen over the female inflorescence. Natural self-sterility of the female parent is depended on to prevent self-pollination. The modem crossing house has sufficient height to accommodate tall canes growing in a container, with tie bars to which the plants are fastened to hold them erect.

Crossing is accomplished also by dusting pollen from the male plant over the flowers on an arrow of the female plant, a process called *pollen-loading*. Arrows collected from male plants in early morning and exposed to light at normal temperature will shed pollen. The pollen is collected on a clean paper and dusted over isolated female parent arrows daily during the flowering period. Sugarcane pollen normally remains viable only for a few hours, but viability will be retained for several weeks if the pollen is dried and stored at a temperature of –20ºC, or for periods up to one year if stored at –80ºC. The seed or fuzz ripens about three weeks following pollination. Sugarcane seeds retain their viability only for a short period of time under normal storage temperatures, but the life of the seed is extended if dried and stored at temperatures of 0 to 5 ºC.

Maintenance of Breeding Stocks

Clones to be used as parents in sugarcane crosses are normally selected while still growing in the field. Prior to flowering, the parent clones are potted and transported to the crossing area or crossing house. Preservation of the flowering stalks of parent clones is prolonged by rooting while the stalk is still attached to the mother plant, a process called *airlayering* or *marcotting*. An alternative procedure for preservation of flowering stalks and maintaining freshness is to keep detached stem sections alive in a weak *sulfurous acid* solution.

AIRLAYERING OR MARCOTTING. About three weeks before flowering, a polyethylene strip containing a mixture of moist potting soil, or sphagnum moss, is wrapped around a bud of the sugarcane stalk about two nodes above the ground level. If kept moist, roots will develop from the airlayered bud (Fig. 22.8). The airlayered stalk is severed below the rooted node,

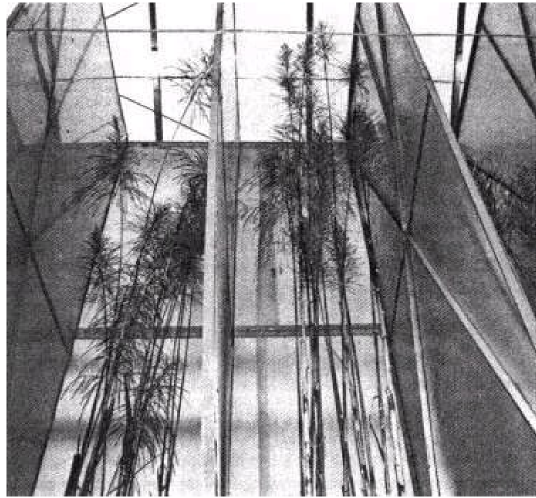


Fig. 22.7.
Sugarcane parent clones isolated in cubicles in the crossing house. Because there is no air circulation within the crossing cubicles, the male clone is suspended above the female clone so that the pollen as it matures will fall directly over the female flowers.

potted, and transferred to the crossing house. Airlayering keeps the tassels fresher and increases seed production compared to nonairlayered stalks.

SYNCHRONIZATION OF FLOWERING Arrows severed from the base of the cane and transported to a central crossing area or crossing house may be kept alive in a fresh condition for several weeks by immersing the cut end in a weak sulphur dioxide solution containing 150 ppm SO_2, 75 ppm H_3PO_4, and 37.5 ppm of each H_2SO_4 and HNO_4. The solution is recharged daily and changed twice weekly. The arrows from hybrid clones and most clones from *S. officinarum* and *S. robustum* continue to flower in a normal manner and remain alive for three to four weeks permitting the seeds to mature. Survival rate for clones of *S. spontaneum* is generally lower than for other species.

Synchronization of Flowering

Synchronization of flowering in parent clones is necessary to make cross-pollinations. Flower initiation in sugarcane is sensitive to photoperiod and temperature. It is often possible to induce flowering in nonflowering clones or to hasten flowering in late flowering clones by reducing the length of the daylight period. Flowering may be delayed and vegetative growth continued in early flowering clones by increasing the daylight period. Increasing the temperature also hastens flowering, and flowering is delayed and pollen fertility reduced by lowering temperature. In temperate climates, crossing is done in crossing houses where both photoperiod and temperature are controlled. The flowering response of clones to photoperiod and temperature may vary with the clone.

Fig. 22.8.
Airlayered or marcotted stalks of sugarcane. A polyethylene strip containing a soil mixture, or sphagnum moss, is wrapped around the node of the sugarcane stalk and kept moist. The stalk will root within a three-week period and may then be severed from its natural root system. Note the root development within the clear plastic marcot.

Cytogenetics and Genetics

The *Saccharum* species are extremely complex allopolyploids, with small chromosomes that are difficult to study, and high chromosome numbers that vary with the particular clone examined. The irregular number is due to presence of aneuploids and to meiotic irregularities that result in gains or losses of single chromosomes. The basic chromosome numbers in *Saccharum* are 6, 8, and 10. *S. officinarum* is an octoploid with a basic chromosome number of 10 and 2n number of 80. The wild species, *S. robustum*, has a basic chromosome number of 10, with 2n numbers of 60 and 80 being most common. Clones of *S. barberi* have been divided into four groups based on chromosome number. The wild species, *S. spontaneum*, contains one polyploid group with a basic chromosome number of 8, and 2n numbers of 40, 48, 56, 64, 72, 80, 96, 104, 112, and 120; and a second polyploid group with a basic chromosome number of 10 and 2n numbers of 40, 50, 60, 70, 80, 100, and 120. The number of chromosomes in commercial clones generally varies between 2n= 100 and 2n-130.

Interspecific crosses can usually be made among clones of the five species within the genus *Saccharum*, although peculiar chromosome numbers are observed in the progenies of certain crosses. Due to abnormalities in fertilization and embryo formation, the somatic chromosome number is transmitted to the progeny instead of the gametic number of the pistillate parent, when *S. officinarum* is used as the maternal parent in crosses with S. *spontaneum, S. barberi*, or *S. sinense*. This phenomenon does not occur when *S. officinarum* is used as the pollen parent as illustrated here:

Cross	Chromosomes in F_1
S. officinarum × *S. spontaneum*	*2n 4- n*
S. officinarum × *S. barberi*	*2n + n*
S. officinarum × *S. sinense*	*2n + n*
S. officinarum × *S. robustum*	*n + n*
S. spontaneum × *S. officinarum*	*n + n*
S. barberi × *S. officinarum*	*n 4- n*

S. sinense × *S. officinarum*	$n + n$
S. robustum × *S. officinarum*	$n + n$

Exceptions will be found among particular clones within each species. Various explanations have been proposed to explain the phenomenon, but none are universally accepted.

In the breeding of sugarcane, it has been a general practice to cross the different species with the noble cane, *S. officinarum*, to combine the high sugar yield of the *officinarum* clones with hardiness and disease resistance of the other species, a procedure called *nobilization*. Usually, two to three backcrosses to the noble parent are necessary to recover satisfactory sucrose content.

Simple Mendelian genetic studies are virtually impossible in sugarcane owing to the high polyploid number and irregular transmission of individual chromosomes, to meiotic irregularities arising with cross-fertilization, and to sterility problems that make crossing and selfing difficult. Each clone is a different heterozygous genotype which is not reproducible through floral reproductive processes. Greater attention is given to quantitative genetic inheritance in sugarcane than to the inheritance of qualitative characters. Inbreeding, where possible, leads to the rapid loss of vigor. Inbreeding is restricted in particular clones due to presence of self-sterility or self-incompatibility. Quantitative inheritance studies suggest that additive genetic variance is important for many agronomic characters and disease resistance and nonadditive variance is important for cane and sugar yield.

Biotechnology

Plant cell and tissue culture techniques may be utilized to supplement conventional breeding programs in sugarcane. Micropropagation has been proposed as a means for obtaining pathogen-free seed canes for planting or long-term storage, to facilitate shipping and movement of breeding canes through quarantine restrictions, and to provide a source of genetic variants in a breeding program. The latter is a questionable use, considering the genetic complexity in present clones, and, in a clonal propagated crop, the importance of striving for stability in maintenance of clones rather than increasing diversity. Research on genetic transformation was slow to develop due to problems associated with utilization of the *Agrobacterium* technique in monocots. However, utilization of the particle gun technique for insertion of the DNA has now permitted transformation in the sugarcane to be accomplished.

Methods of Breeding

The methods of breeding sugarcane include:

• *germplasm collection*, which plays an important role in supplying sources of breeding materials,

• *clonal selection* from populations of wild clones to isolate genotypes with desirable genes that can be transferred to commercial clones, and

• *hybridization among commercial clones* to obtain genetic recombinations.

Hybridization is the breeding procedure by which new cultivars are developed in sugarcane.

Germplasm Collections

Germplasm collections containing cultivated clones with a wide range of diversity and representative clones from the different *Saccharum* species are normally maintained by sugarcane breeding stations for use in their breeding programs. The germplasm collections of sugarcane differ from germplasm collections of wheat, or maize, as they are maintained as living clones rather than by seeds (Fig. 22.9). The largest germplasm collection is maintained at the Indian Sugarcane Breeding Institute, Coimbatore, India, with about 3500 clones. The United States Department of Agriculture maintains about 3000 clones at the Sugarcane Field Station, Canal Point, Florida, and about 2000 clones at the National Clonal Germplasm Depository, Miami, Florida. About 3000 clones are maintained at the Copersucar Technology Center, Piracicabu, Brazil. The germplasm collections contain both commercial clones and clones from wild species and related genera. The commercial clones contain useful genes for sugar yield and purity. The clones from wild sugarcane species and related genera constitute a depository for genes contributing to hardiness, drought and cold resistance, salt tolerance, and disease and insect resistance.

Clonal Selection

Clonal selection refers to the isolation of a specific genotype that is maintained by vegetative propagation. Sugarcane breeding began by selection of clones from wild populations. Clonal selection is currently utilized to isolate desirable genotypes from a genetically mixed population, such as a wild population, or a segregating population obtained by hybridization, or from populations originating through recurrent selection.

Fig. 22.9.
Collection of clones of the wild sugarcane species, S. spontaneum, maintained at the Sugarcane Research Institute, Coimbatore, India. S. spontaneum is used as a parent in breeding for hardiness and disease resistance.

Hybridization

Hybridization between clones, followed by selection within the hybrid population, is the procedure by which sugarcane cultivars are currently developed. Because the sugarcane plant is heterozygous, segregation will occur and selections are made in the F_1 generation. Crosses are made freely, with several thousand F_1 seedlings grown from a single cross. If a particular cross is found to have seedlings with desirable characteristics in the progeny, it may be repeated. If a clone is found to contribute desirable characteristics to a series of progenies (exhibits good general combining ability), it may be used as a parent in a large number of crosses.

Several types of crosses are made in the breeding of sugarcane, which are referred to as:

BIPARENTAL CROSSES. Biparental crosses are crosses between two specific clones. This is the most common type of cross. Arrows of two parent clones are brought together in isolation to permit open-pollination. If the female parent is self-sterile, hybrid seeds will be harvested. If the female parent is self-fertile, both selfed and crossed seeds will be harvested. An alternative procedure is to isolate a self-sterile parent as female and pollinate by hand.

AREA CROSSES. Crosses in which several male-sterile clones are brought together in isolation and pollinated by a single male parent. Unless the female parent clones are self-sterile, they will cross among themselves.

MELTING-POT CROSSES OR POLYCROSSES. Arrows of selected clones are brought together in isolation, permitting natural cross-pollination to occur. Seeds from melting-pots are harvested and kept separate by clones, in which case only the maternal parent is known. If the clones are selected for a common outstanding character, such as yield, sugar content, or frost resistance, the harvested seeds may be used as the first selection cycle in a recurrent selection procedure.

Selection Procedures Following Hybridization

A selection procedure in which seedlings are planted in field plots as single plants is illustrated in Fig. 22.10. In the first season, sugarcane seeds are germinated in greenhouse flats or seed beds soon after the seed is harvested (Fig. 22.11). Several thousand F_1 seedlings may be grown from each cross, with a total of 100,000 seedlings being grown each season in a normal breeding program. The seedlings are transplanted to a field nursery when 6 to 12 weeks old. An alternative planting method is to plant the seedlings in bunches of 3 to 15 seedlings. The bunch planting system permits growing a larger number of seedling plants on available land area and reduces labor costs of transplanting compared to transplanting single seedlings. Evaluation of individual seedling plants growing in a bunch is more difficult than when seedlings are grown singly. Preliminary screening occurs in the second and third seasons, with about 10% of the plants grown from each crop being selected. Plants are selected in early stages on the basis of vigor, stalk size, erectness, freedom of disease, and sugar yield and quality. Yield trials are conducted from the fourth through the eighth season, in replicated plots after which a superior clone, if identified, is increased and released as a cultivar for commercial production.

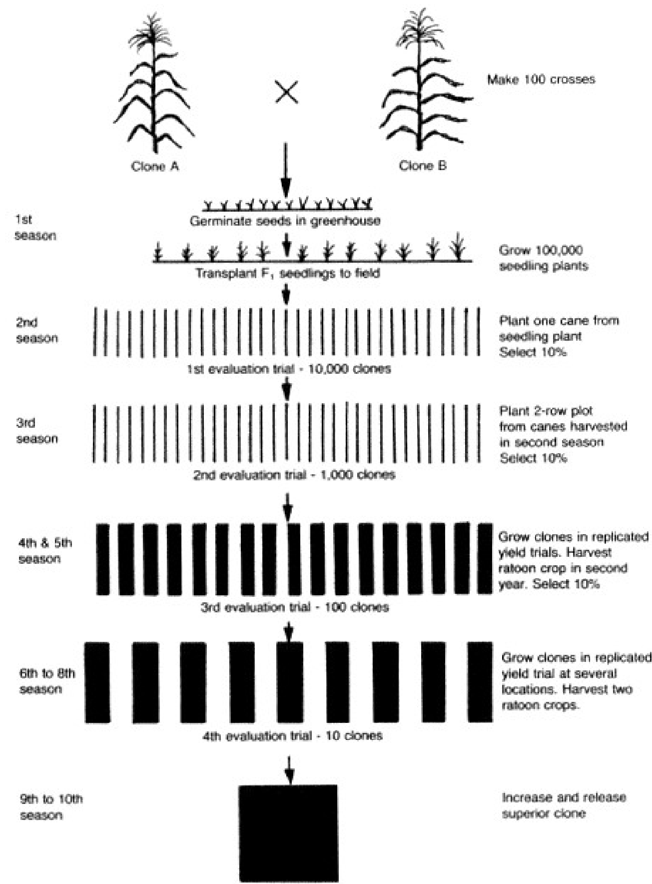


Fig. 22.10.
A typical selection procedure following a biparental cross in sugarcane. Seeds are germinated and seedling plants grown in the first season, followed by preliminary evaluation trials in the 2nd and 3rd seasons and yield trials in the 4th to 8th seasons.

Cultivars

Descriptions of new sugarcane cultivars are published and the published descriptions used as the basis for future identification of the clone. In the United States, new cultivars are registered by the Crop Science Society of America, and the descriptions are published in *Crop Science*. It has become the practice in sugarcane breeding to name new sugarcane cultivars by letters that identify the sugarcane breeding station where the clone was selected, followed by a number to identify the specific clone. Letters identifying clones from a representative group of sugarcane breeding stations throughout the world are as follows:

Symbol	Breeding Station
B	Central Sugar Cane Breeding Station, Barbados, British West Indies
CO	Sugarcane Breeding Institute, Coimbatore, India
CP	USDA Sugarcane Field Station, Canal Point, Florida
H	Hawaiian Sugar Planters' Association, Honolulu, Hawaii
L	Louisiana State University, Baton Rouge, Louisiana
M	Mauritius Sugar Industry Research Institute, Reduit, Mauritius
A	South African Sugar Association, Natal, South Africa
POJ	Java Sugar Experiment Station, Pasuruan, Java
T	Texas A&M Research and Extension Center, Weslaco, Texas
Q	Bureau of Sugar Experiment Stations, Brisbane, Queensland, Australia

A sugarcane cultivar tends to decline in yield after being grown for a few years in a particular area, making it necessary to replace the cultivar with a new clone in order to maintain high yield. While the exact cause of the deterioration in yield is not always determined with certainty, the yield decline is often attributed to changes in disease patterns.

Breeding Objectives

Yield, sugar content, and disease resistance have received major attention as breeding objectives in sugarcane breeding. Other objectives that are important in particular breeding programs are maturity; resistance to lodging; resistance to environmental stress from frost, drought, flooding, high temperature, or salinity; disease resistance; insect resistance; and ability to produce ratoon crops.

Potential for Cane and Sugar Yield

The sugarcane plant stores large quantities of juice containing sucrose in the stalk. Thus, tonnage of canes per unit of land area is one criterion of sugar yield. Because thick and tall stalks can store more juice than thin and short stems, primary selection in tropical countries is for vigor of growth and for tall, large-barrelled canes with high tillering capacity. In subtropical areas with short growing seasons, as in Louisiana, selection is made for shorter stalks and high tillering. Juiciness of the stem, and sugar content and recovery, are also important in yield of sugar per unit of land area. More progress has been made in increasing total sugar yield by breeding for increased tonnage than has been made by breeding for

Fig. 22.11.
Sugar seedlings growing in flats at the Sugarcane Breeding Institute, Coimbatore, India.

increased sucrose content. Yield of cane harvested is also influenced by cultivar response to fertilization, resistance to climatic adversities, and resistance to disease and insect pests.

Maturity

Maturity in sugarcane breeding refers to the stage of plant growth when maximum sugar accumulation has been achieved. The sugarcane plant is mature when sugar accumulation is uniform from the bottom to the top of the stalk. Sugarcane cultivars may be classified as early, intermediate, or late. Cultivars that mature early are desired for milling in the beginning of the season, with progressively later maturing cultivars being milled as the season progresses.

Lodging Resistance

Sugarcane plants grown with high fertility and optimum moisture need strong stalks so they will stand without lodging. Lodged canes fail to develop full normal growth, provide favorable environments for the development of disease, and deteriorate in sugar content and quality. Resistance to lodging from strong winds and rainstorms require a thick, stout stalk, a healthy and well developed root system, and freedom from disease or insect injury that weakens the stalk. While tall plants are necessary and desirable for maintaining high yield, they are more susceptible to damage by strong wind and rain. A balance between excessive height and reduced yield from shorter plants may need to be achieved.

Resistance to Adverse Environments: Frost, Drought, Waterlogging, Salinity

Resistance to cold and occasional frost is required for sugarcane cultivars in north India and southern United States. Tolerance to frost and drought is found in canes of *S. barberi* and *S. sinense*, the indigenous sugarcanes of north India, and in canes of the wild species *S. spontaneum*. Combining genes for frost and drought hardiness of these species with genes for high sugar yield from *S. officinarum* has been a major objective in the breeding of sugarcane

for marginal environments. Certain clones of *S. spontaneum* are able to withstand waterlogged conditions for long periods. Resistance to waterlogging is characterized by the production of a large matrix of fibrous roots extending from the base of the stem to the surface of the water. Breeding for tolerance to salinity generally involves selection for tolerance to higher levels of sodium ions.

Disease Resistance

The breeding of sugarcane has been closely related to outbreaks of serious diseases in the crop. The sereh disease forced the abandonment of susceptible clones of the noble sugarcanes in Java. Mosaic virus combined with red rot almost forced abandonment of the sugarcane industry in Louisiana. The diseases were later controlled by breeding resistant clones. Due to the diverse genetic background in sugarcane, resistance appears to be available to most any disease provided the breeder can devise efficient and effective screening techniques. Decline in yields of new cultivars after a few seasons of cultivation is usually believed to result from changes in pathogen populations.

Breeding for resistance to sugarcane disease pathogens is generally based on increasing tolerance controlled by quantitative inheritance rather than utilization of a single gene-specific type of resistance. Breeding procedures to increase disease resistance levels involve:

• the selection of parent clones with a high degree of resistance,

• crossing resistant clones to generate transgressive segregates with improved resistance, and

• screening seedling populations to identify the superior segregates.

Screening procedures are required that will identify resistant parent clones and Fl seedling plants.

S. spontaneum has been used widely in crosses as a source of disease resistance. *S. officinarum* and *S. robustum* have also served as sources of disease resistant genes. Genes for resistance to rust, caused by *Puccinia melanocephala* H. and P. Sydow, and for resistance to sugarcane smut, caused by *Ustilago scitaminea*, have been found in clones of *S. officinarum* and *S. spontaneum*; genes for resistance to red rot, caused by *Physalospora tucumanesis* Speg., and to sugarcane mosaic, a virus disease, have been found in *S. spontaneum*; genes for tolerance to eye-spot disease, caused by *Helminthosporium sacchari* (B. de haan) Butl., were found in clones of *S. robustum*. Genes for resistance are transferred from wild species to cultivated clones by the nobilization procedure.

Insect Resistance

The most destructive insect pests attacking sugarcane are the sugarcane stem or stalkborers. *Diatraea saccharalis* F. is a common sugarcane borer in the United States, but other species are prevalent in other countries. Resistance to the sugarcane borer has been identified in clones of *S. spontaneum*. Resistance may result from unattractiveness of the leaf for egg deposition, inability of young borers to become established, high fiber which hinders feeding of borers, or tolerance and ability to produce good yields in spite of borer attack.

Production of Ratoon Crops

Production of a ratoon crop refers to the production of a second crop after the first crop of sugarcane has been harvested. Growing a ratoon crop saves the cost of replanting a new crop. Clones vary in their ability to survive and produce a profitable ratoon crop. The number of crops harvested from a single planting may vary from one to as many as six to eight in tropical countries.

Quality

Factors considered in quality of sugarcane include millability, yield of juice, sugar content of juice, and quality of juice. Millability refers to characteristics of the cane that make it possible to recover the sucrose from the stalk by normal methods of extraction. Characteristics desirable for good millability are moderate hardness of rind, good length of fibre, long internodes, and low fibre-sucrose ratio. Yield of juice and sucrose content of the juice are important as they determine the sugar yield. The most important factor in quality of the juice is the percent sucrose. Other factors of importance are total solids, brix (the percent of solids in the juice), and the nonsugar fraction of the juice. Breeding for sugar quality has been facilitated by development of hand-held instruments for measuring brix which can be taken to the field so that measurements may be taken on individual canes. Little progress has been made in increasing sucrose content in new hybrid canes over the content of the best of the older clones of *S. officinarum*.

Study Questions

1. Where is the center of origin for sugarcane? Identify the various species of sugarcane and comment on their usefulness in breeding new cultivars.

2. Describe the breeding methods used in sugarcane improvement. How does sugarcane breeding differ from the breeding of other crop plants?

3. How are interspecific crosses used in the breeding of sugarcane?

Further Reading

Dunckelman, P.H., and B.L. Legendre. 1982. Guide to sugarcane breeding in the temperate zone. United States Dept. Agri., Agri. Res. Ser., ARM-S-22. U.S. Gov. Print. Office, Washington, D.C.

Heinz, D.J. 1991. Sugarcane cytogenetics, p. 279-93. *In* T.Tsuchiya and P.K. Gupta (eds.) Chromosome engineering in plants: genetics, breeding, evolution. Elsevier Science Publishers, Amsterdam. Netherlands.

Heinz, D.J. (ed.) 1987. Sugarcane improvement through breeding. Elsevier Science Publishers, Amsterdam, Netherlands.

Herbert, L.P., and M.T. Henderson. 1959. Breeding behavior of certain agronomic characters in progenies of sugarcane crosses. U.S. Dep. Agri. and La. Agr. Exp. Sm. Tech. Bul. 1194.

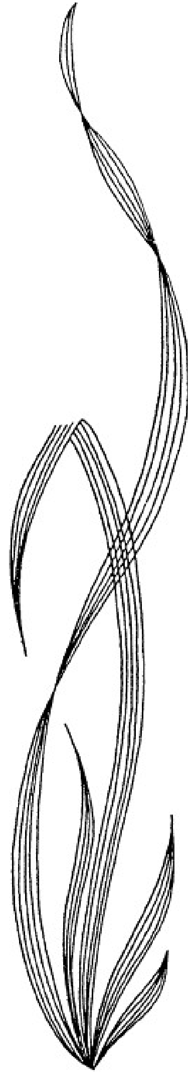
James, N.I. 1980. Sugarcane. p. 617-29. *In* W.R. Fehr and H.H. Hadley (eds.) Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Miller, J.D., and P.Y.P. Tai. 1992. Use of plant introductions in sugarcane cultivar development, p. 137-49. *In* H.L. Shands and L.E. Wiesner (eds.) Use of plant introductions in cultivar development. Part 2. Spec. Publ. No. 20, Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am.,

Madison, WI.

Stevenson, G.C. 1965. Genetics and breeding of sugar cane. Longmans, London, England.

Walker, D.I.T. 1987. Trends in sugarcane breeding, p. 3-26. *In* A.J. Abbott and R.K. Atkin. Improving vegetatively propagated crops. Academic Press, London.



X
MAINTENANCE AND SEED PRODUCTION OF IMPROVED CULTIVARS

23. Cultivar Increase, Maintenance, and Seed Production

To facilitate the systematic increase and rapid distribution of new improved cultivars, fairly extensive and well-defined seed production practices are used (Fig. 23.1). In the development of these practices, two assumptions have generally been made:

• the development of the cultivar is the primary function of the breeder; and

• increase and distribution can be handled most expeditiously by seed producers, who are experienced in the art of growing, conditioning, and marketing of pure seed.

Much of the detail in distribution of seed of cultivars developed through publicly supported breeding programs has centered around the steps by which the breeder turns over seed of a new cultivar to the seed grower, and the procedures by which pure seed of the new cultivars are increased, distributed, and certified. Seed of new cultivars developed through privately supported breeding programs is normally increased by the seed company conducting the breeding research program and is distributed through the originator's usual marketing channels. It is with these developments that we are concerned in this chapter.

Public and Private Plant Breeding and Seed Distribution

Plant breeding in the United States and Canada is conducted by the public and private sectors. The publicly supported plant breeding projects in the United States are conducted by tax-supported agencies such as the United States Department of Agriculture, the state agricultural experiment stations, and the agricultural colleges. In Canada they are conducted by the Canada Department of Agriculture, the provincial governments, and the agricultural colleges.

Fig. 23.1.
Seed production field of intermediate wheatgrass near Clarkston, Utah. Grass and legume seed production fields are usually planted in rows to conserve seed of a new cultivar and to facilitate weed control.

Improved cultivars developed by tax-supported institutions, such as state or provincial agricultural experiment stations or colleges of agriculture, are considered public property. However, this does not preclude releasing a cultivar on an exclusive basis. In many instances today, tax-supported institutions will give exclusive marketing rights to a private company for the purpose of obtaining royalties to support plant breeding research and to improve upon the distribution of the cultivar. Nevertheless, it is in the public interest that new cultivars developed by these public agencies be increased rapidly and distributed in an orderly fashion. To this end, seed improvement associations have been organized in most states in the United States. Several of the New England states have consolidated their seed improvement associations. In Canada, this function is handled by the Canadian Seed Growers' Association. Through these organizations, procedures have been developed for the increase, distribution, certification, and maintenance of improved cultivars originating at public institutions.

Improved cultivars developed by the private seed industry are the property of the originator. Seed sales from cultivars allow the private seed industry to recover investment in its breeding programs. The extent to which plant breeding is done by public or by private agencies varies with the economic importance of the crop, the resources of the industry, and the potential for sales of the improved cultivars. In crops with large recurrent sales of seed, such as hybrid corn, hybrid sorghum, sugar beets, cotton, or alfalfa, private breeders have developed breeding programs to a much greater extent than with crops in which the recurrent seed sales are small. Formerly, private industry in the United States participated very little in the breeding of self-pollinated crops, such as wheat or soybean, or in the breeding of forage crops. This was due to the ease with which cultivars of self-pollinated crops may be reproduced. A farmer, or another seed producer who purchased seed of the new cultivar, using

wheat as an example, could harvest and sell seed of the new wheat cultivar without compensation to the breeder.

The situation regarding the breeding of self-pollinated crops changed in the United States with passage in 1970 of the Plant Variety Protection Act. This act gives the originators exclusive right to control the sales of seed of sexually reproduced cultivars. This has spurred private seed companies to invest in breeding of small grains, soybean, and forages, crops that had previously been given little attention by the private breeder.

In Western Europe, breeding of small grains and forages has been conducted by private breeders to a larger extent than in the United States or Canada. In the European countries, the exclusive rights to marketing of cultivars by seed producers who develop them are strongly protected. However, in these countries the testing of potential cultivars, approval for naming new cultivars, and procedures for maintaining purity are supervised closely by governmental institutions. In many of these countries cultivars may be marketed as seed only after testing and approval by designated government agencies. A similar situation exists in Canada, where only government-licensed cultivars may be produced and sold as seed. The licensing applies to publicly developed cultivars and to privately developed cultivars.

Before a cultivar is distributed from a state experiment station in the United States, it is normally tested thoroughly in the state where it originated and from which it is being distributed. Through regional cooperation the tests may be conducted over an area of several states. The results of these tests are available to assist the breeder in making final decisions regarding release and recommendations about the area of adaptation of the new cultivar. Generally, less information is available to guide the grower regarding the acceptance of a new cultivar developed by a private breeder. For this information the grower must rely largely upon the integrity of the company and upon published information from local yield trials that include the privately developed cultivars. In many states, corn and sorghum hybrids and other crops developed by private companies are planted in yield trials conducted at appropriate locations by the state agricultural experiment station and the data published for use by growers.

Private seed companies which conduct breeding programs generally have established outlets for marketing the seed of their new cultivars. Many of the larger companies, such as those that produce hybrid corn or sorghum, or seed cotton, have sales staffs in the field. The technical operations by which pure seed stocks are increased and maintained are similar, whether carried out by a public institution through a seed improvement association or by a commercial seed company by its seed production staff.

In general, education of the public, seed regulatory laws, and competition have forced seed producers to set high standards in the sale of seed of new and established crop cultivars. Use of certified seed, whenever available, is one assurance of obtaining seed accurately labeled for purity and quality. It is not an assurance of obtaining an adapted cultivar, unless the cultivar has been tested and has been found to be suitable for production in the area where the buyer expects to plant the seed.

Classes of Certified Seed

The procedures for certification of seed developed as a means of assuring that the seed has a high standard of purity and quality. Four classes of seed are recognized by seed certification agencies:

• *Breeder seed* is seed or vegetative propagating material directly produced or controlled by the originating plant breeder or institution. Breeder seed provides the source for the increase of foundation seed.

• *Foundation seed* is the direct increase from breeder seed. The genetic identity and purity of the cultivar is maintained in foundation seed. Production is carefully supervised or approved by representatives of an agricultural experiment station or other authorized agency. Foundation seed is the source of all certified seed classes, either directly, or through registered seed. In many countries, foundation seed is called *basic seed*.

• *Registered seed* is the first-generation increase of breeder or foundation seed. Registered seed maintains satisfactory genetic identity and purity of the cultivar for the production of certified seed. Registered seed is used as the source of certified seed.

• *Certified seed* is the first-generation increase of breeder, foundation, or registered seed. Certified seed must be handled so as to maintain sufficient genetic identity and purity of the cultivar that it will be approved and certified by the certifying agency.

Foundation, registered, or certified seed is identified by a distinctive tag on each bag of seed (Fig. 23.2). A white tag is used for breeder and foundation seed, a purple tag for registered seed, and a blue tag for certified seed.

A green tag is used in those instances where the cultivar is not certified but the originator wishes to have the cultivar produced under the same standards of seed quality as used for certified seed. The green tag program is not part of the national seed certification effort. It is often issued for *branded* cultivars. A branded cultivar is one in which the exact name of the cultivar is not stated. For example, more than one company may wish to sell the same cultivar but use their own brand name for marketing purposes.

Each state seed-certifying agency established the procedure by which each class of seed may be produced and the standards of purity for each class of each crop within their state. However, the standards may not fall below the minimum standards approved by the Association of Official Seed Certifying Agencies. Each state seed-certifying agency publishes the standards applicable in its state and assumes responsibility for inspecting, sampling, and testing seed lots and certifying those that meet certification standards.

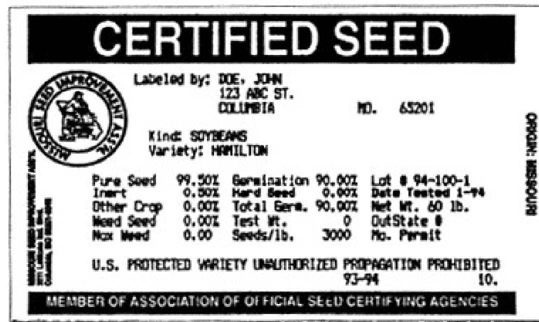


Fig. 23.2.
Using certified seed assures good seed quality.

How a New Cultivar Reaches the Farmer

A typical procedure by which a new cultivar of a field crop developed by a state agricultural experiment station reaches the farmer (Fig. 23.3) may be described as follows:

(1) A cultivar is ready for release and distribution when it has been proven to be distinctly superior to and different from existing commercial cultivars in at least one or more characteristics, and at least satisfactory in all other important respects.

(2) The decision for release is made by the breeder in consultation with appointed boards of review, according to procedures established by the state agricultural experiment station for release of a new cultivar. The plant breeder makes a limited increase of breeder seed of the new cultivar, the amount varying from a few to a few hundred kilograms. The breeder seed is then generally turned over to the agency responsible for making the foundation seed increase.

(3) Foundation seed is increased from breeder seed. The organization making the foundation seed increase varies in different states. In most states, a foundation seed program has been developed within the agricultural experiment station. In some states, foundation seed is produced by private foundation seed stocks organizations closely associated with the agricultural experiment station.

(4) At least one year before distribution by the originating station, each state experiment station in the region of adaptation of the new cultivar is normally informed of plans to release the cultivar, and seed is supplied to them in quantities to permit field plot testing at one or more locations.

(5) The cultivar is named at the originating station, in consultation with representatives of other state agricultural experiment stations within the region, and the United States Department of Agriculture in the case of cooperative state-federal breeding programs.

(6) Prior to distribution, either breeder or foundation seed is shared within the region with other state agricultural experiment stations, which may wish to make a simultaneous increase and distribution of the new cultivar. An adequate quantity of foundation seed should be available to supply immediate needs for the production of registered and certified seed before release or distribution is made.

(7) Foundation seed is the source of registered seed. Distribution of foundation seed, plus a public announcement, constitutes a formal release by the originating station. Initial distribution of foundation seed is usually made only to experienced growers to conserve the seed supply of a new cultivar, but later distribution may be made to growers of registered or certified seed without restriction insofar as the supply of seed permits.

(8) Registered seed is the source of certified seed, although certified seed may

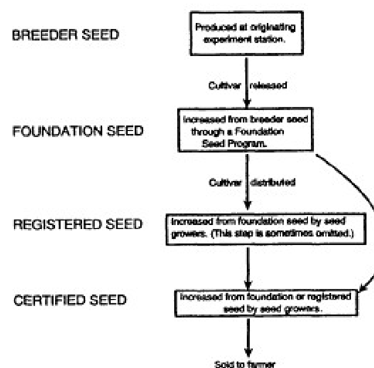


Fig. 23.3.
Procedure by which a new cultivar reaches the farmer.

be produced directly from foundation seed when the registered class is omitted. Certified seed is sold to farmers without restriction.

(9) New, improved crop cultivars may be registered by the Crop Science Society of America. A description of each cultivar registered by the society is published in *Crop Science* and becomes the official description of the cultivar.

How a Cultivar Is Certified

Exact certification procedures vary from state to state and with different crops. In general, certification involves the following steps:

(1) The grower must plant foundation or registered seed of an approved cultivar.

(2) The seed must be planted on clean ground. The field should not have been planted in the previous year to another cultivar of the same crop, or to other crops that might volunteer and affect the purity of the crop being certified, such as rye preceding wheat. Noxious weeds are removed before harvest, and borders are clipped where necessary to maintain seed purity.

(3) In cross-pollinated crops, isolation of the seed-producing field is required, either by planting a specified distance from all other fields of the same crop or by planting a specified number of pollinator rows around the border of the field to reduce the opportunity for cross-pollination with other cultivars planted in neighboring fields.

(4) Off-type plants and mixtures are rogued by the grower before harvest, or before flowering in the case of a cross-pollinated crop.

(5) Field inspections are made by representatives of the seed-certifying agency to check on the purity of the cultivar, freedom from other crop plants, freedom from noxious weeds, amount of disease that might affect certification, and general conformity by the grower to the seed certification rules. Inspections are made at the time that purity and diseases can best be observed.

(6) Seed inspections are made by representatives of the seed improvement association as necessary to observe and supervise the harvesting, conditioning, bagging, and other processing operations. Representative samples are drawn by the inspector from each lot of seed after it has been conditioned, bagged, and prepared for sale. The seed samples are tested for impurities, germination, and other factors affecting seed quality according to the particular crop. Only seed meeting or exceeding minimum standards of the association in all respects is accepted for certification.

(7) Official tags supplied by the seed-certifying agency are sealed on the bags of seed approved for certification. The tag is labeled to show that the seed meets the specific standards for the crop and the state and federal seed laws (Fig. 23.2).

The production, conditioning, and marketing of certified seed is exclusively the responsibility of the grower. The responsibility of the seed improvement association is to verify that the grower follows the regulations outlined by the association and that the seed meets the prescribed standards for certification of that particular crop.

Agencies Concerned with Seed Certification in the United States

In the United States, various public and private agencies are concerned with the testing, increase, release, and distribution of new cultivars of field crops and the maintenance of pure seed stocks of the cultivar after the initial distribution has been completed. Although the details of organization may differ from one state to another, the overall pattern of these organizations is quite similar.

State Agricultural Experiment Stations and the USDA

New crop cultivars are developed by state agricultural experiment stations and the United States Department of Agriculture. If the breeding effort is cooperative between the agricultural experiment station and the United States Department of Agriculture, the state agricultural experiment stations are usually responsible for release of the new cultivars within the boundaries of their state. The participation of the state agricultural experiment stations in the final increase and distribution of the new cultivars varies somewhat from state to state according to the specific relationships of the experiment station with the seed-certifying agency and other seed increase organizations operating within the state. Joint releases by two or more states are commonly made.

Seed-Certifying Agencies

Seed certification programs are conducted in nearly every state and in Canada. The Canadian Seed Growers' Association, organized in 1900, and the Canadian Department of Agriculture certify seed on a national basis in Canada, but in the United States seed certification is a responsibility of the individual states. The purpose of seed certification by the official seed-certifying agencies is to maintain and make available to the public sources of high-quality seeds and propagating materials of cultivars grown and distributed to ensure genetic identity. Certified seed is high in purity and of good seeding value.

Cultivars eligible for certification have resulted from systematic plant breeding procedures and have distinctive and identifiable characteristics. Without a planned method for maintaining genetic purity, there is always danger of losing cultivar identity.

Cultivar purity is the first consideration in seed certification. Other factors, such as weeds, diseases, viability, mechanical purity, and grading, are also important but the extent to which they are considered in the certification process varies from state to state. One of the most effective methods of preventing the wider distribution of weeds is to plant weed-free seed. Adverse effects of plant diseases can be reduced by planting clean seed from disease-free fields. Properly cleaned and graded seed is easier to plant and gives more uniform stands.

Approximately four million acres of seed crops are certified annually in the United States and Canada as indicated by the application for all certified classes received by seed certification agencies (Table 23.1).

AOSCA

The Association of Official Seed Certifying Agencies (AOSCA), formerly the International Crop Improvement Association, was organized in 1919. Its membership includes the seed-

Table 23.1.
Seed certification applications of some major crops received by seed certification agencies in the United States and Canada in 1992*

Crop	Foundation seed acres	Registered seed acres	Certified seed acres
Wheat	35,490	224,715	969,280
Oat	4,527	26,076	85,559
Barley	13,167	64,544	242,695
Rice	914	20,393	97,787
Soybean	49,924	131,398	560,054
Cotton	48,041	49,035	146,174
Grasses	6,072	9,954	324,157
Alfalfa	2,486	399	152,110
Clover	5,567	370	22,619

*Adapted from AOSCA Production Publication No. 46.

certifying agencies in the various states and Canada. Its major function is coordination of the certification programs of the member seed-certifying agencies. This has been accomplished by the establishment of minimum certification standards. In the United States, these minimum standards have been incorporated into the Federal Seed Act. Procedures have also been established for interagency certification of seeds. For example, a potential certified lot of grass seed grown in one state may be shipped to a seed-processing plant in another state for final conditioning, bagging, tagging, and sealing. By mutual agreement the seed-certifying agencies of the two states may combine efforts, each making the field or processing plant inspections necessary in their respective state to complete final certification on the seed.

OECD

The Organization for Economic Cooperation and Development (OECD), established in 1961, which includes as members most countries of Western Europe, has developed schemes for cultivar certification of herbage and oil, cereal, sugar and fodder beet, maize, and tree, and several other seeds moving in international trade. The objective is to encourage the use of seed of high quality in the participating countries and to facilitate movement of quality seed among the countries. In addition to the OECD countries, the United States, Canada, Japan, New Zealand, Australia, and several others participate in the plan. From July 1, 1991, to June 30, 1992, 110,979,837 kilograms of seed were produced and certified in the United States under the OECD Seed Schemes. While most countries of Western Europe have developed improved forage cultivars, due to unfavorable weather conditions they often experience difficulty in producing seeds. Under the OECD program, forage seeds may be produced and certified in a country other than the country in which the cultivar was developed. This enables seed producers to arrange for seed increase of their cultivars in another country where seed production conditions are more favorable. The seed is certified by the local seed certification organization in the country where the seed is produced, and the seed is then returned to the country of origin for distribution. This scheme has permitted European seed producers to arrange for the production and certification of their forage seeds in the favorable seed production areas of the western United States and Canada.

In the OECD scheme, terminology and tag colors differ from the system in the United

States. Breeder seed is designated prebasic; foundation or registered seed is designated basic seed; certified seed is designated first-generation certified seed, and the progeny of certified seed as second- or successive-generation certified seed.

United States Federal and State Seed Laws

The United States Federal Seed Act requires seeds in interstate commerce and certain imported seeds to be correctly labeled with respect to origin, cultivar name, purity, germination, and other quality factors according to the specific crop species. The United States Federal Seed Act is enforced by the United States Department of Agriculture, Agricultural Marketing Service. In addition to the Federal Seed Act, the various states have seed laws to regulate labeling of seeds moving in intrastate commerce. The seed legislation is designed to provide what is frequently referred to as "truth in labeling," with respect to seeds, by requiring the seller to inform the buyer accurately about the seed.

Agricultural Extension Service

The Agricultural Extension Service, through its extension agronomists and agents, offers useful service by encouraging the general use of pure seed of improved cultivars throughout the state. It assists in the education of seed growers and the seed trade regarding certification procedures and disseminates information regarding new cultivars and performance trials of currently available cultivars.

Intellectual Property Rights

The protection of plant breeders' rights can be accomplished through the *Plant Variety Protection Act* (PVPA), through the use of *plant patents*, or through *trade secret protection*. It is important for the breeder to have protection of intellectual property (crop cultivars) so that monetary support in the form of royalties or fees collected from sales of cultivars can be used to support further research and cultivar development.

Plant Variety Protection Act

The Plant Variety Protection Office of the United States Department of Agriculture administers the Plant Variety Protection Act in the United States. The purpose of the Plant Variety Protection Act is to encourage the development of novel cultivars of sexually reproduced plants and to make them available to the public, providing protection to those who breed, develop, or discover them. Novel cultivars are described as having the following characteristics:

• *Distinctive*, which means a cultivar differs by one or more identifiable morphological, physiological, or other characteristics.

• *Uniformity*, which means a cultivar must demonstrate that the variations in the cultivar are describable, predictable, and commercially acceptable.

• *Stability*, which means that the cultivar must remain essentially unchanged with regard to essential and distinctive characteristics with a reasonable degree of reliability commensurate with that of cultivars of the same type.

Superiority over existing cultivars is not a requirement for being novel. Originators of novel cultivars of sexually reproducible crop plants may apply to the Plant Variety Protection Office for "protection" of the cultivar. If granted, the originator then possesses exclusive rights to the production and sale of seed of the protected cultivar. The originator may elect to specify that the cultivar may be sold by cultivar name only as a class of certified seed and may designate the number of generations from breeder seed to certified seed. Protection for sale of seed does not prohibit grain of the cultivar from being sold for purposes other than seed. Both publicly and privately developed cultivars are eligible for protection under the act. Protection of intellectual property through the Plant Variety Protection Act is more popular than through patents. Protection through the Plant Variety Protection Act is good for 18 years.

The Plant Patent Act of 1930 provided for the protection of asexually propagating plants. It was not until 1985 that patents could be obtained on sexually reproducing plants. There are certain requirements that must be met before the United States Government will issue a patent on a plant or crop cultivar. The three most important requirements are:

• *Novelty*, which means that the person who is the first inventor must show that it is original in some manner.

• *Utility*, which means something that is capable of being used beneficially for the purpose for which it was developed.

• *Non-obviousness*, which means that the crop cultivar or plant is something that goes beyond what people who have ordinary skill in the art would know.

Non-obviousness is the most difficult of the three to comprehend. The question needs to be asked would it be obvious to the inventor (plant breeder) guided by other previous patents and considering any other information that might ordinarily be available in that particular field (plant breeding), such as for example, publications? If it is obvious, the criteria are not upheld and a patent would not be issued. Once the patent is obtained, protection is good for 17 years.

Many seed companies rely upon trade secret protection rather than protection through patents or The Plant Variety Protection Act. For example, companies have used trade secrets to protect inbred seed lines used in the production of hybrid cultivars. In certain instances, the trade secret might give more protection because the information is kept secret. One of the disadvantages of protecting crop cultivars through trade secrets is that the information does not become public as is true for plant variety protection and plant patents.

Practical Problems in Seed Production

Seed production problems are peculiar to each specific crop. Solutions to these problems have generally been found from long-term experience.

Small Grains, Rice, and Soybean

The small grains, except for rye, and soybean are self-pollinated, and extensive isolation is not required to maintain cultivar purity. Normally a strip a few meters in width, which may be mowed, uncropped, or planted to some other crop, is all that is needed to separate two cultivars. With cross-pollinated rye, isolation of 200 to 300 m from fields planted with other cultivars of rye is required to prevent mixing.

The combine, if used to harvest more than one cultivar of a crop, can be a major source of a cultivar mixture because it is difficult to clean thoroughly. Custom seed cleaners are also sources of cultivar mixtures, unless carefully cleaned between cultivars of the same crop. Presence of red rice is undesirable in the production of seed rice. Red rice has a distinctive red pigmentation running through the kernel. It may be detected by hulling, pearling, scarifying, or scratching the kernel by some mechanical means.

Breeder seed in small grains and soybean is normally produced from plant-to-row, head-to-row, or head-to-hill plantings. The procedure is to select several hundred or more plants or particles and plant each to a row or hill. Only rows or hills that conform to the cultivar type are harvested. The seed is bulked to start a pure seed increase. If one of these procedures is used to purify a cultivar, several hundred rows should be grown. Otherwise, if one off-type row is overlooked and included in the seed, the actual percentage of the off-type in the cultivar may be increased. Cultivar purity may be maintained also by planting small seed plots each year and roguing the off-type plants. Through this procedure, the percentage of mixture will be decreased each year.

Hybrid Corn

In normal production of hybrid seed corn, three classes of seed are commonly produced, namely, inbred, single-cross, and three-way-cross.

(1) Inbred seed requires the most care in production. Basic seed lots of inbreds are maintained by hand pollinations, but larger lots may be increased by open-pollination in isolated fields. Careful roguing is required to remove any off-type plants that may have originated from stray pollen.

(2) Single-cross and three-way-cross seed may be produced in limited amounts by hand pollinations, but large quantities needed for commercial use are produced by open-pollinations, of the inbreds involved in isolation. Single-cross and three-way-cross seed now constitute the major portion of the hybrid seed production. A common planting pattern is one pollen row to four seed parent rows (Fig. 17.8), or a ratio of 1:2:1:4 pollinator to seed-producing rows may be planted (Fig. 23.4). The pollen-producing rows are frequently destroyed after pollination is completed to avoid mixture with the seed parent during harvest. Off-type plants or plants of doubtful origin in parent rows are removed. The roguing may be done any time before harvest in the ear parent, but it must be done before pollen is shed in the pollen parent.

Harvesting of seed may be done by mechanical pickers or picker-shellers. Seed is dried at temperatures of 40 to 46°C. Temperatures need to be controlled carefully because higher temperatures may reduce germination. After drying and shelling, the seed is cleaned, sized, treated, and bagged. Seed is separated into two shapes, flat and round, and sized according to width, thickness, and length of the kernel. With each particular kernel size, information on the corn planter plate designed for use with seed of those dimensions is provided to the purchaser. Using the proper plate makes for greater accuracy in dropping seeds and obtaining uniform stands. Seed is normally treated with a combination fungicide and insecticide and bagged either by seed count or by weight.

Hybrid Sorghum

The production of hybrid sorghum seed is similar to the production of hybrid corn seed. Seed of the **A-, B-**, and **R-lines** must be increased and maintained, and seed of the **A-line** ×

Fig. 23.4.
Commercial seed-production field of single-cross hybrid corn planted in a 1:2:1:4 ratio of male pollinator to female seed-parent (detasseled) rows. Four of the six seed-parent rows are adjacent to a pollinator row, and none is more than two rows from a pollinator row. Compare with Figure 17.8.

R-line cross produced to provide the hybrid seed. The pollinators, **B-** and **R-lines**, are produced by growing in isolation. Usually 200 m is required for isolation, but larger distances may be needed to obtain isolation from sudangrass pollen. Breeder seed of these parent lines may be maintained by bagging heads to ensure self-pollination. The seed parent, or **A-line**, is maintained by pollination from the **B-line** in isolation. The **A-line** × **R-line** cross, which produces the hybrid seed, is usually planted in ratios of 12 seed-producing to 4 pollinator rows (Fig. 18.11). Rogues or off-type plants need to be removed before pollen shedding occurs. Also, plants shedding pollen in the seed-producing rows should be removed. As with hybrid corn seed, growout tests to evaluate hybrid seed purity and the effectiveness of the restorer genes are normally conducted during winter months in a suitable climate. Seed harvesting and processing procedures are similar to those for hybrid corn. Harvesting at a moisture content above 17% should be avoided to prevent cracking or injury to the seed, and seed harvested above 13% moisture needs to be dried.

Cotton

Various procedures are used by the seed cotton grower to maintain cultivar purity. Some system of progeny-row testing is most common. In addition to the plant-to-row plots used in the progeny-row tests, a series of first, second, and third year, or more, multiplication plots are used to increase seed from approved progeny-rows.

Pure seed cotton production necessitates some control over the ginning process so that seed

cotton can be ginned without mixing cultivars. Care must be taken to clean completely pickers, vehicles in which the cotton is hauled, and warehouse floors where the seed cotton may be delivered.

Cotton seed is delinted by mechanical means or, more commonly, by treatment with acid (Fig. 23.5). Delinting the cotton seed facilitates seed treatment and planting of the seed.

Forage Crops

Rapid expansion in forage seed production has followed advances in cultural practices and mechanization of production and harvesting operations. As a result, seed production has become a specialized operation. A few of the seed production practices followed by growers of forage seed are listed:

(1) *Planting seed production fields in rows* gives satisfactory seed yields and makes possible the planting of large acreages with a minimum amount of foundation or registered seed (Figs. 23.1 and 23.6).

(2) *Planting rate* should be the lowest possible amount of pure live seed that will assure an even stand within the row.

(3) *Irrigation* may be used to establish stands and to increase production of established fields.

(4) *Fertilization*, especially the application of nitrogen to grass seed crops, will usually increase seed yields.

(5) *Cultivation and weed control are* essential to good seed production. Seeding in rows will facilitate weed control.

(6) *Insect control* is essential to prevent loss caused by damage to the plant from insects.

(7) *An adequate supply of pollinating insects* is necessary to obtain good seed yields of forage legumes, such as clover or alfalfa. Honeybees can be used to supplement the natural pollinators. From five to ten hives per hectare are generally considered sufficient. Leaf cutter bees and alkali bees are useful pollinators also.

(8) *Harvesting and threshing* is most frequently accomplished by direct combining, or

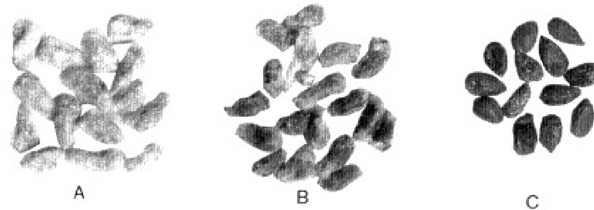


Fig. 23.5.
Seed of cotton. (A) Natural. (B) Mechanically delinted. (C) Acid delinted.

Fig. 23.6.
An alfalfa seed field being grown in California. This planting is only 5 months old and will produce seed the same year that it is planted. The harvested seed may be returned to the eastern United States for marketing.

combining from the windrow, but various types of harvesting machines have been developed and are used with different forages. In some crops the field may be vacuumed. The material picked up by the vacuum machine is then threshed.

(9) *Conditioning* of forage seeds is done with specially designed seed cleaning and processing equipment. Modem seed-conditioning plants are planned for efficient operation and highly automated (Fig. 23.7).

Vegetatively Propagated Forages

Cultivars of some forages such as bermudagrass, bahiagrass, and zoysiagrass are propagated by vegetative means because they produce little or no seed (see Fig. 20.12). The

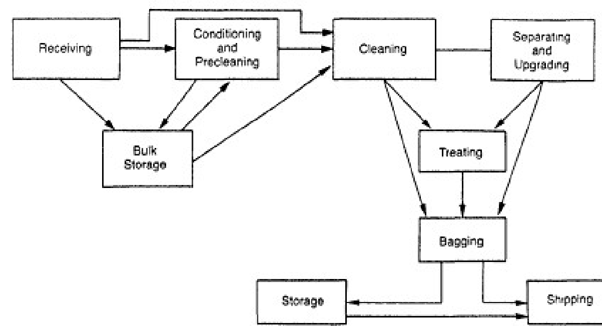


Fig. 23.7.
Flow chart for seed conditioning. Modem seed-conditioning plants are highly automated and have flexibility for handling seed, depending upon the kind and condition of the seed on arrival and its final destination.

original distribution of coastal bermudagrass was made by means of vegetative sprigs. Certification procedures and minimum certification standards have been established for vegetatively propagated grasses by the Association of Official Seed Certifying Agencies. This makes it possible to buy certified stocks of vegetatively propagated forage grasses.

Study Questions

1. Why is it necessary to have a seed-certification program?

2. What is the Association of Official Seed Certifying Agencies and what role does this organization play in the release of improved cultivars in the United States?

3. What are the various classes of seed produced in the United States and what are their functions?

Further Reading

Ackigoz, E., and R.P. Knowles. 1983. Long-term storage of grass seeds. Can. J. Plant Sci. 63:669-74.

Baenziger, P.S., R.A. Kleese, and R.F. Barnes (ed.). 1993. Intellectual property rights: Protection of plant materials. Crop Sci. Soc. Am. Spec. Publ. 21. Crop Sci. Soc. Am., Am. Soc. Agron., and Soil Sci. Soc. Am., Madison, WI.

Caldwell, B.E., and J.A. Schillinger (ed.). 1989. Intellectual property rights associated with plants. Am. Soc. Agron. Spec. Publ. 52. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Canadian Seed Growers' Association. 1988. Regulations and procedures for pedigreed crop production. Circ. 6-88. Ottawa. 65pp.

Crop Science Society of America. 1968. Registration of field-crop cultivars, parental lines, and elite germplasm by the Crop Science Society of America. Crop Sci. 8:261-62.

Duvick, D.N. 1993. Possible effects of intellectual property rights on erosion and conservation of plant genetic resources in centers of crop diversity. p. 505-9. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop

Sci. Soc. Am., Inc., Madison, WI.

Hebblethwaite, P.D. (ed.). 1980. Seed production. Butterworths, London.

Orzolek, M.D., and D.R. Daum. 1984. Effect of planting equipment and techniques on seed germination and emergence: A review. J. Seed. Tech. 9:99-113.

Parsons, F.G. 1985. The early history of seed certification, 1900-1970. p. 3-7. *In* M.B. McDonald, Jr., and W.D. Pardee (ed.) The role of seed certification in the seed industry. Crop Sci. Soc. Am. Spec. Publ. 10. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Siebeck, W.E., D.L. Plucknett, and K. Wright-Platais. 1993. Privatization of research through intellectual property protection and its potential effects on research at the international centers. p. 499-504. *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson (eds.) International crop science I. Crop Sci. Soc. Am., Inc., Madison, WI.

Stefferud, A. (ed.). 1961. Seeds: importance, life processes, production, processing, certification, and marketing. U.S. Dep. Agric. Yearb. Agric. 1961.

Wright, H. 1980. Commercial hybrid seed production. p. 161-76. *In* W.R. Fehr and H.H. Hadley (eds.) Hybridization of crop plants. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Wych, R.D. 1988. Production of hybrid seed corn. p. 565-607. *In* G.F. Sprague and J.W. Dudley (eds.) Corn and corn improvement. 3rd ed. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI.

Youngberg, H.W., and R.J. Buker. 1985. Grass and legume seed production. p. 72-79. *In* M.E. Heath, R.F. Barnes, and D.S. Metcalfe (eds.) Forages, the science of grassland agriculture. 4th ed. Iowa State Univ. Press, Ames, IA.

GLOSSARY

A

A-Line: the male-sterile parent line in a cross to produce hybrid seed. The A-line is the seed-producing line, commonly used with reference to production of hybrid sorghum, hybrid wheat, etc.

Acclimatization: adjustment of a species or a population to a changed environment over a number of generations.

Additive gene effects: gene action in which the effects on a genetic trait are enhanced by each additional gene, either an allele at the same locus, or genes at different loci.

Alien chromosome substitution: exchange of single chromosomes or pairs of chromosomes from other species or genera.

Allele: an alternative gene. Alleles are located on corresponding loci of homologous chromosomes.

Alloploid (allopolyploid): an organism with more than two sets of chromosomes in the somatic cells, the additional sets having been derived from different species.

Amphiploid (amphidiploid): an alloploid possessing the total chromosome complement of the parent species.

Aneuploid: an individual with other than an exact multiple of the haploid chromosome complement.

Anisoploid: mixture of diploid, triploid, and tetraploid plants obtained with seed harvested from a mixture of diploid and tetraploid plants.

Anther: the pollen-bearing portion of the stamen.

Anther culture: the culturing of anthers in vitro for the purpose of generating haploid plantlets.

Anthesis: the process of dehiscence of the anthers; the period of pollen distribution.

Antibiosis: antagonistic association in which one organism has an injurious effect on normal growth and development of another.

Apomixis: an asexual reproduction process that substitutes for sexual reproduction in certain flowering plants, leading to the formation of seeds.

Asexual reproduction: reproductive process that does not involve the union of gametes.

Autoploid (autopolyploid): an organism with more than two sets of chromosomes in the somatic cells, the sets derived from the same species.

B

B-Line: the fertile counterpart, or maintainer of the **A-line**. The **B-line** does not have fertility restorer genes and is used as the pollen parent to maintain the A-line. Commonly used with reference to production of hybrid sorghum, hybrid wheat, etc.

Backcross: (1) in breeding, a cross of a hybrid with one of its parents or with a genetically equivalent organism; (2) in genetics, a cross of a hybrid with a homozygous recessive. *See also* Testcross.

BC_1, BC_2, etc.: symbols used to designate the first backcross generation, the second backcross generation, etc.

Bilateral sexual polyploidization: formation of a polyploid plant where unreduced gametes are produced by both parents.

Biological yield: the total yield of plant material.

Biotype: (1) a population in which all individuals have an identical genotype; (2) a physiologic race.

Bolting: production of seed stalks the first season in a biennial crop, as in sugar beets.

Breeder seed: seed (or vegetative propagating material) increased by the originating, or sponsoring, plant breeder or institution, used as the source for the increase of foundation seed.

Bulk-population selection: selection procedure in self-pollinated crops. Segregating populations are propagated as bulks until segregation is virtually ceased, at which time selection is initiated.

C

Callus: a mass of undifferentiated cells, originating from an anther, microspore, plant tissue explant, or other cellular source, when cultured in vitro.

Centromere: that area of the chromosome where spindle fibers are attached.

Certified seed: the progeny of breeder, foundation, or registered seed, produced and so handled as to closely maintain genetic purity or identity of a cultivar. Certified seed is approved and certified by an official seed certification agency.

Character: the expression of a gene as revealed in the phenotype.

Chiasma (plural chiasmata): visible association of nonsister chromatids to permit genetic exchange between paired chromosomes leading to genetic recombination.

Chromatid: one of two threadlike structures formed in the duplication of a chromosome to form daughter chromosomes.

Chromosome: a structural unit in the nucleus, which carries the genes in a linear constant order; it preserves its individuality from one cell generation to the next and is typically constant in number in any species.

Chromosome engineering: changes in chromosome quantity or arrangement for the purpose of altering the phenotype of the plant.

Cleistogamy: pollination and fertilization in an unopened flower bud.

Clone: a group of plants originating by vegetative propagation from a single plant.

Codominance: gene products occur independently and are expressed phenotypically. For example, in an allelic series, if the heterozygote A_1A_2 produces two phenotypes, one specific for A_1 and one for A_2, and not intermediate to A_1A_1 *and* A_2A_2, the alleles are said to express codominant behavior.

Coefficient of variation: the standard deviation expressed as a percentage of the mean.

Colcemid: a synthetic equivalent of colchicine.

Colchicine: an alkaloid extracted from seeds or corms of *Colchicine autumnale*, which induces polyploidy by arresting spindle formation during mitosis.

Combining ability, general: the average or overall performance of a genetic strain in a series of crosses.

Combining ability, specific: the performance of specific combinations of genetic strains in crosses in relation to the average performance of all combinations.

Composite: a mixture of genotypes from several sources, maintained by normal pollination.

Correlation: a mutual relationship between two things such that an increase or decrease of one is generally associated with an increase or decrease of the other. Linear correlation is measured by the Correlation Coefficient, which may range in value from -1 to +1.

Cross-fertilization: *see* Fertilization.

Crossing over: an interchange of segments between the chromatids of two homologous chromosomes at meiosis.

Crossover value: the percentage of crossing over in a hybrid population; a term used mostly in determining linkage percentage, particularly in chromosome mapping.

Cross-pollination: *see* Pollination.

Cryopreservation: *see* Freeze preservation.

Cultivar: synonymous with variety; the international equivalent of variety.

Cytology: the science dealing with the structure, function, and life history of the cell.

Cytoplasm: the protoplasm of a cell excluding the nucleus.

Cytoplasmic: pertaining to or centered in the cytoplasm.

Cytoplasmic inheritance: inheritance dependent upon hereditary units in the cytoplasm.

D

Dehiscence: splitting open of a fruiting structure or anther.

Detassel: removal of the immature tassel as practiced in the production of hybrid seed corn.

Determinate: descriptive of an inflorescence in which the terminal flower opens first, thus arresting the prolongation of the floral axis.

Dichogamous flowers: flowers whose male and female sex organs are mature at different times, which promotes cross-pollination.

Digestibility: with reference to forage, characteristics of forage that affect its capability of being digested by an animal.

Dihybrid: the result of a cross between parents that differ by two specified genes.

Dioecious: having staminate and pistillate flowers on different plants of the same species.

Dioecy: state of being dioecious.

Diploid: having two sets (genomes) of chromosomes; chromosome number of 2*n*, as in a zygote. Somatic or body tissue is normally diploid, in contrast to haploid germ cells.

Disomic inheritance: simple Mendelian genetic ratios that are characteristic of diploids with bivalent chromosome pairing behavior.

Dominant: (1) a gene that expresses itself in a hybrid to the exclusion of its contrasting (recessive) allele; (2) a character that is expressed in a hybrid phenotype to the exclusion of the contrasting (recessive) character.

Dominant gene effects: gene action with deviations from the additive such that the heterozygote is more like one parent than the other.

Double-cross: (1) a cross between two single-crosses; (2) the $\mathbf{F}_1$ progeny of a cross between two single-crosses.

Doubled haploid: a diploid plant produced by doubling the chromosome content of a haploid plant. A doubled haploid will be homozygous at all loci.

Duplicate genes: two or more pairs of genes that produce identical effects, whether alone or together.

E

Egg: the female gamete or germ cell.

Emasculate: to remove the anthers from a bud or flower before pollen is shed. Emasculation is a normal preliminary step in crossing to prevent self-pollination.

Embryo: the rudimentary plant in a seed. The embryo arises from the zygote.

Embryo culture: the culturing of an immature embryo on a sterile nutrient medium.

Embryo sac: typically, an eight-nucleate female gametophyte. The embryo sac arises from the megaspore by successive mitotic divisions.

EMS: ethyl methane sulfonate, a chemical mutagen.

Endosperm: triploid tissue that arises from the triple fusion of a sperm nuclei with the polar nuclei of the embryo sac. In seeds of certain species, the endosperm persists as a storage

tissue and is used in the growth of the embryo and by the seedling during germination.

Endosperm balance number hypothesis (EBN): normal endosperm and seed development occurs if the endosperm tissue results from fusion of maternal and paternal nuclei in a ratio of 2 maternal: 1 paternal.

Epiphytotic: sudden and usually widespread development of a destructive disease in plants.

Epistasis: interaction between nonallelic genes in which a gene exerts a dominant effect over a gene at another locus.

Euploidy: variations in chromosome numbers that are multiples of complete sets basic to a species.

Evolutionary breeding: breeding procedure in which the cultivar is developed from an unselected progeny of a cross, or multiple crosses, that have undergone evolutionary changes.

F

F_1, F_2, etc.: symbols used to designate the first generation, the second generation, etc., after a cross.

Fatuoid: a mutant commonly occurring in cultivated oat and which resembles wild oat (*Avena fatua*).

Fertility-restoring genes: nuclear genes that act to restore fertility in plants with male-sterile cytoplasm.

Fertilization: union of an egg and a sperm (gametes) to form a zygote. Self-fertilization is the union of an egg with a sperm from the same flower or from another flower on the same plant, or within a clone. Cross-fertilization is the union of an egg with a sperm from a plant of a different clone.

First division restitution: failure of anaphase I where all chromosomes go to one pole, producing a gamete with unreduced chromosome numbers.

Floret: a small flower from an inflorescence, as in a grass panicle or a composite head.

Foundation seed: seed stocks increased from breeder seed, and so handled as to closely maintain the genetic identity and purity of a cultivar. Foundation seed is the source of certified seed, either directly or through registered seed.

Freeze preservation: conditioning and preservation of plant cells, tissues, or organs at extremely low temperatures, usually in liquid nitrogen.

Frego-bract: a mutant bract type in cotton in which the bracts curl outward, exposing flower buds and bolls.

G

Gene: the unit of inheritance, located on the chromosome; by interaction with other genes, the cytoplasm, and the environment, it affects or controls the development of a character; a segment of DNA that determines an amine acid sequence in a polypeptide. The basic unit of heredity.

Gene frequency: the frequency of a specific allele at a locus.

Gene interaction: modification of gene action by a nonallelic gene.

General resistance: nonspecific host plant resistance.

Genetic advance: the expected gain in the mean of a population for a particular quantitative character by one generation of selection of a specified percent of the highest-ranking plants.

Genetic engineering: in plants, the transfer of DNA from a donor plant species to a recipient species by means of a bacterial plasmid, virus, or other vector.

Genetics: the science dealing with heredity.

Genome: a set of chromosomes, such as contained within a gamete; corresponds to the haploid number of chromosomes within a diploid species.

Genotype: (1) the material basis of heredity; (2) the potential hereditary genetic stocks within a species, taken collectively.

Genotypic ratio: the proportions of the different genotypes in a particular progeny.

Germplasm: (1) the material basis of heredity; (2) the potential hereditary genetic stocks within a species, taken collectively.

Germplasm collection: a collection of genotypes of particular species, from different sources and geographic locations, used as source materials in plant breeding. *See also* World collection.

Glume: the outer husks or bracts of each spikelet in grasses.

Gossypol: a polyphenolic compound present in darkly pigmented glands that occur throughout the cotton plant.

H

Haploid: having a single set (genome) of chromosomes in a cell or an individual; the reduced number (n), as in a gamete.

Harvest index: the proportion of the biological yield that is grain.

Heaving: lifting effect of the soil due to alternate freezing and thawing. Heaving may result in the lifting up of plants and may tear them loose from the soil, or shear off roots.

Hemizygous: where a plant carries only one dose of an allele. In a haploid formed from a diploid, all loci are represented by a single allele.

Heredity: the transmission of genetic characters from parents to progeny; the genetic characters transmitted to an individual by its parents.

Heritability: capability of being inherited; that portion of the observed variance in a progeny that is inherited.

Heritability, broad-sense: heritability estimated from the total genetic variance.

Heritability, narrow-sense: heritability estimated from the additive portion of the genetic variance.

Heterosis (hybrid vigor): (1) the increased vigor, growth, size, yield, or function of a hybrid progeny over the parents that results from crossing genetically unlike organisms; (2) the increase in vigor or growth of a hybrid progeny in relation to the average of the parents, referred to as the mid-parent value.

Heterozygous: having unlike alleles at corresponding loci of homologous chromosomes. An organism may be heterozygous for one or several genes. *See also* Homozygous.

Hexaploid: having six sets (genomes) of chromosomes; chromosome number of $6x$.

Homoeologous: having hemology, as with homologous or partially homologous chromosomes originating from different genomes.

Homologue: a homologous chromosome.

Homologous chromosomes: chromosomes that synapse or pair at the first division in meiosis. Each member of a pair has a corresponding sequence of gene loci and is derived from a different parent.

Homozygous: having like genes at corresponding loci on homologous chromosomes. An organism may be homozygous for one, several, or all genes. *See also* Heterozygous.

Horizontal resistance: *see* General resistance.

Hybrid: (1) the first generation offspring of a cross between two individuals differing in one or more genes; (2) the progeny of a cross between species of the same genus or of different genera.

Hybridization: (1) the crossing of individuals of unlike genetic constitution; (2) a method of breeding new cultivars that utilizes crossing to obtain genetic recombination.

Hybridize: to produce hybrids by crossing individuals with different genotypes.

Hybrid vigor: *see* Heterosis.

I

Immune: free from attack by a given pathogen; not subject to the disease.

Inbred line: (1) a pure line usually originating by self-pollination and selection; (2) the product of inbreeding.

Inbreeding: breeding closely related organisms; in plants, usually by self-pollination. (Also, by sib-pollination, backcrossing.)

Inbreeding coefficient: the probability that two alleles at a particular locus are identical by descent.

Incompatibility: failure to obtain fertilization and seed formation after self-pollination, usually due to failure of the pollen tube to penetrate the stigma or to reduced growth of the pollen tube in the stylar tissue.

Independent assortment: the chance distribution of two or more pairs of segregating genes to the gametes.

Indeterminate: descriptive of an inflorescence in which the terminal flower is last to open. The flowers arise from axillary buds, and the floral axis may be indefinitely prolonged by a terminal bud.

Inflorescence: (1) a flower cluster; (2) the arrangement and mode of development of the flowers on a floral axis.

Inherit: receive from one's predecessors. In organisms, chromosomes and genes are transmitted from one generation to the next.

Inoculate: to place inoculum where it will produce an infectious disease.

Inoculum: spores, bacteria, or fragments of mycelium of pathogens that can infect plants or soil.

Introduction: *see* Plant introduction.

Irradiation breeding: *see* Mutation breeding.

Isolines: lines that are genetically similar except for one gene.

L

Landrace: early cultivated forms of a crop species, evolved from a wild population.

Lemma: the lower of the two bracts enclosing each floret in the grass spikelet.

Line: a group of individuals from a common ancestry. A more narrowly defined group than a strain or cultivar.

Linkage: the relationship between two or more genes that tend to be inherited together because they are located in the same chromosome. This results in parental combinations occurring more frequently than recombinations in the progeny.

Linkage group: a group of genes arranged in a linear order on a chromosome.

Linkage map: a diagram of a chromosome showing the relative position of the genes.

Lint: long fibers of cotton separated from the seed in ginning.

Linters: short fibers of cotton that generally remain attached to the seed in ginning. Also called Fuzz.

Locus: the position of a particular gene on a chromosome (plural, Loci).

Lodging: the bending or breaking-over of a plant before harvest.

Lodicule: one of two scalelike structures at the base of the ovary in a grass flower.

M

M_1, M_2, etc.: symbols used to designate the first generation, second generation, etc., following exposure to mutagenic agents (ionizing radiations, chemical mutagens, etc.). *See also* **R_1, R_2**, etc.

Male sterility: a condition in which pollen is absent or nonfunctional in flowering plants.

Male sterility, cytoplasmic: male sterility resulting from specific cytoplasmic-genic interactions. Sterile cytoplasm is denoted by symbols S or *cms*, in contrast to normal

cytoplasm N.

Male sterility, genetic: male sterility resulting from action of specific genes.

Mass selection: a system of breeding in which seed from individuals selected on the basis of phenotype is composited and used to grow the next generation.

Megaspore: one of the four haploid spores originating from the meiotic divisions of the diploid megaspore mother cell in the ovary and which gives rise to the megagametophyte.

Megaspore mother cell: diploid cell in ovary that gives rise, through meiosis, to four haploid megaspores.

Meiosis: two successive nuclear divisions, in the course of which the diploid chromosome number is reduced to the haploid.

Meristem tip culture: the in vitro culturing of plant tissue from the meristem tip region for the purpose of regenerating pathogen-free plants.

Microspore: one of the four haploid spores originating from the meiotic division of the microspore mother cell in the anther and which gives rise to the pollen grain.

Microspore mother cell: diploid cell in the anther which gives rise, through meiosis, to four haploid microspores.

Mitosis: a process of nuclear division in which the chromosomes are duplicated longitudinally, forming two daughter nuclei each having a chromosome complement equal to that of the original nucleus.

Modified single-cross: the progeny of the cross between a single-cross, derived from two related inbred lines, and an unrelated inbred line.

Monoecious: having staminate and pistillate flowers on the same plant.

Monoecy: state of being monoecious.

Monogerm: a sugar beet seed with a single germ, in contrast to a multigerm seed.

Monohybrid: the result of a cross between parents that differ by one specified gene.

Monoploid: haploid plant produced from a diploid species; contains basic (x) chromosome number for that species. *See also* Polyhaploid.

Monosome: a chromosome that has no homologue present; a haploid chromosome in an otherwise normal diploid individual.

Multiline cultivar: a composite of isolines.

Multiple alleles: a series of alleles, or alternative forms, of a gene. A normal heterozygous diploid plant would bear only two genes of an allelic series. Multiple alleles arise by repeated mutations of a gene, each mutant giving different effects.

Multiple genes: two or more genes at different loci, which produce complementary or cumulative effects on a single quantitative genetic trait. *See also* Polygenes.

Mutant: an organism that has acquired a heritable variation as a result of mutation.

Mutation: a sudden variation in the hereditary material of a cell. Mutations may be gene mutations or chromosomal changes. A gene mutation is a change in a gene from one allelic form to another. Chromosomal changes include deletions, duplications, inversions, and interchanges.

Mutation breeding: the use of mutagenic agents to increase the frequency of mutant plants useful in the breeding of improved cultivars.

Mutation, macro-: a mutation with an effect of sufficient magnitude that it can be recognized with certainty in a single plant.

Mutation, micro-: a mutation with a small effect that can be detected only by measurement of a group of plants.

N

Nectariless: devoid of leaf and extrafloral nectaries in cotton.

Non-disjuction: irregular distribution of sister chromatids or homologous chromosomes at meiosis leading to gametes without the expected chromosome complement.

Nonpreference: plant resistance to insects through suppression of feeding or oviposition.

Nonrecurrent parent: parent not involved in a backcross. Also donor parent. *See also* Recurrent parent.

Nullisomie: an otherwise normal diploid plant that lacks a specific chromosome pair.

O

Oeimene: a volatile terpene produced in alfalfa flowers, which gives them aroma.

Okra-leaf: a mutant narrow-leaf type in cotton.

Open-pollination: natural cross-pollination.

Outcross: cross-pollination, usually by natural means, with a plant differing in genetic constitution.

Ovary: the enlarged basal portion of the pistil, in which the seeds are borne.

Overdominance: the combined effect on a genetic trait of two alleles such that the heterozygote is more extreme than either homozygote. (The effect of AA^1 is greater than either AA or A^1A^1.)

Ovule: the structure that bears the female gamete and becomes the seed after fertilization.

P

Palatability: with reference to forage, characteristics of forage that affect animal intake.

Palea: the upper of the two bracts enclosing each floret in the grass spikelet.

Particle: an open and branched inflorescence with pediceled flowers.

Parthenocarpy: the production of fruits without fertilization and, normally, without seeds.

Parthenogenesis: the development of an individual from a gamete without fertilization.

Partial dominance: lack of complete dominance; the production of a hybrid intermediate between the parental types.

Pathogen: an organism capable of inciting a disease.

Pathogenicity: the ability of an organism to incite a disease.

Pedigree selection: selection procedure in a segregating population in which progenies of selected F_2 plants are reselected in succeeding generations until genetic purity is reached.

Pentaploid: having five sets (genomes) of chromosomes; chromosome number of $5x$.

Perfect flower: flower possessing both stamens and pistils.

Phenotype: (1) physical or external appearance of an organism as contrasted with its genetic constitution (genotype); (2) a group of organisms with similar physical or external makeup.

Phenotype ratio: the proportions of the different phenotypes in a particular progeny.

Phenotype value: the numerical description of a phenotype with respect to a particular quantitative character measured in metric units.

Physiologie race: a group of pathogens of the same species, which are similar in physiological and pathological characteristics, especially in ability to parasitize cultivars of a particular host. *See also* Biotype.

Pistil: the seed-bearing organ in the flower, composed of the ovary, the style, and the stigma.

Pistillate flower: a flower bearing pistils but no stamens.

Plant introduction: (1) transport of a collection of seeds, plants, or vegetative propagating materials from one ecological area into another; (2) collection of seeds, plants, or vegetative propagating materials that have been transported from one area into another.

Plasmid: a circular, extrachromosomal body within a bacterial cell. Plasmids may be used as vectors for carrying a DNA sector in gene-splicing experiments.

Polar nuclei: two centrally located nuclei in the embryo sac that unite with the second sperm in a triple fusion. In certain seeds, the product of this triple fusion develops into the

endosperm.

Pollen grain: the male gametophyte, originating from a microspore.

Pollen mother cell: *see* Microspore mother cell.

Pollen tube: a tube developing from the germinating pollen grain. The sperm cells pass through the pollen tube to reach the ovule.

Pollination: transfer of pollen from the anther to a stigma. Self-pollination is the transfer of pollen from an anther to the stigma of the same flower or another flower on the same plant, or within a clone. Cross-pollination is the transfer of pollen from an anther on one plant to a stigma in a flower on a different clone.

Polycross: an isolated group of plants or clones arranged in some fashion to facilitate random interpollination.

Polycross progeny: progeny from a selection, line, or clone outcrossed to other selections growing in the same isolated polycross nursery.

Polygenes: multiple genes.

Polyhaploid: haploid plant produced from a polyploid plant; contains a multiple of the basic (x) chromosome number for that species.

Polyploid: an organism with more than two sets (genomes) of chromosomes in its body cells.

Progeny selection: selection based on progeny performance.

Progeny test: a progeny, or groups of progenies, grown for the purpose of evaluating the genotype of the parent.

Protandry: a dichogamous flower whose male sex organs are active before the female sex organs.

Protogyny: a dichogamous flower whose female sex organs are active before the male sex organs.

Protoplast: a plant cell devoid of a cell wall.

Pseudo-self-compatibility: partial seed setting following self-pollinationin an otherwise self-incompatible plant.

Pure line: a strain in which all members have descended by self-fertilization from a single homozygous individual. A pure line is genetically pure (homozygous).

Purity: with reference to sugar beets, the ratio of sucrose to total solids dissolved in sugar beet juice.

Q

Quantitative character: a character that is influenced by a group of genes at different loci, which are cumulative in their effect.

R

R_1, R_2, etc.: symbols used to designate the first generation, second generation, etc. following exposure to ionizing radiations. *See also* M_1, M_2, etc.

R-Line: the pollen parent line, containing fertility-restoring genes, crossed with the A-line in the production of hybrid seed.

Random amplified polymorphic DNA (RAPDs): DNA fragments that are amplified using the polymerase chain reaction. RAPD fragments are used as genetic markers in genome mapping, chromosome tagging, phylogenetic studies, and cultivar identification.

Recessive: the condition of a gene such that it does not express itself in the presence of the contrasting (dominant) allele.

Reciprocal crosses: two crosses between two plants or strains in which the male parent of one cross is the female parent of the second cross, for example, **A** × **B** and **B** × **A**.

Recombination: formation of new gene combinations as a result of cross-fertilization between individuals differing in genotype.

Recurrent parent: parent to which hybrid material is crossed in a backcross. *See also*

Nonrecurrent parent.

Recurrent selection: a breeding system designed to increase the frequency of favorable genes of a quantitatively inherited characteristic by repeated cycles of selection.

Reduction division: a nuclear division in which the chromosomes are reduced from the diploid to the haploid number. *See also* Meiosis.

Registered seed: the progeny of breeder or foundation seed and so handled as closely to maintain the genetic identity and purity of a cultivar. Registered seed is the source of certified seed. Registered seed must be approved and certified by an official seed certification agency.

Resistant: characteristic of a host plant such that it is capable of suppressing or retarding the development of a pathogen or other injurious factor.

Restitution nucleus: chromosome numbers are not reduced in number during meiosis.

Restriction fragment length polymorphism (RFLP): different fragment lengths of restriction endonuclease digested DNA detected by a defined probe between individuals. RFLPs are used as genetic markers in genome mapping, chromosome tagging, phylogenetic studies, and cultivar identification.

Rhizome: an underground stem, usually horizontal and often elongated, distinguished from a root by the presence of nodes and internodes and sometimes scalelike leaves and buds at the nodes.

Roguing: removing off-type plants from a population of plants or a cultivar.

S

S_0: symbol used to designate the original selfed plant.

S_1, S_2, etc.: symbols for designating first selfed generation (progeny of So plant), second selfed generation (progeny of S_1 plant), etc.

Second division restitution: abnormal anaphase II where homologous chromosomes do not go to opposite poles, leading to gametes with unreduced chromosome numbers.

Seed: a mature ovule with its normal coverings. A seed consists of the seed coat, embryo, and, in certain plants, an endosperm.

Segregation: the separation of homologous chromosomes (and genes) from different parents at meiosis.

Selection: (1) any process, natural or artificial, that permits an increase in the proportion of certain genotypes or groups of genotypes in succeeding generations; (2) a plant, line, or strain that originated by a selection process.

Selection intensity: the percentage of the population selected.

Self-fertile: capable of fertilization and setting seed after self-pollination.

Self-sterile: failure to complete fertilization and set seed after self-pollination.

Semigamy: abnormal fertilization in which reduced or unreduced male and female gametes participate in embryo formation but they do not fuse, leading to chimeric sectoring in the offspring.

Sexual reproduction: reproduction involving germ cells and union of gametes.

Shattering: the fortuitous loss of seed from a plant before harvest.

Single-cross: (1) a cross between two inbred lines, or pure-line cultivars (in self-pollinated species); (2) the progeny of a cross between two inbred lines or pure-line cultivars.

Single-seed-descent: selection procedure in which F_2 plants and their progenies are advanced by single seeds until genetic purity is virtually attained.

Somaclonal variation: genetic variation occurring in *in vitro* culture of plant tissue.

Somatic: referring to diploid body cells, normally with one set of chromosomes coming from the male parent and one set from the female parent.

Span length, 2.5%: the length that 2.5% of the cotton fibers in the sample will equal or exceed.

Span length, 50%: the length that 50% of the cotton fibers in the sample will equal or exceed.

Species: in classification, a subdivision of a genus. A group of closely related individuals descended from the same stock.

Specific resistance: host plant resistance to specific biotypes of a pathogen; interaction of host plant gene-conditioning resistant reaction with a pathogen gene for pathogenicity-conditioning avirulence.

Sperm: a male gamete.

Spike: an inflorescence with a more or less elongated axis, along which the flowers are sessile or nearly so.

Spikelet: a unit of the inflorescence in the grasses, composed of the glumes, the rachilla, and the florets.

Square: an unopened flower bud in cotton with its accompanying bracts.

Stamen: the pollen-bearing organ in the flower.

Staminate flower: a flower bearing stamens but no pistil.

Standard deviation: a statistic that measures variability of individual measurements in a population; the square root of the variance.

Steckling: small sugar beet stored over winter and planted for the production of seed. Stecklings are grown from unthinned seed.

Sterility: failure to complete fertilization and obtain seed as a result of defective pollen or ovules, or other aberrations.

Stigma: the portion of the pistil that receives the pollen.

Stolon: a trailing stem, capable of forming roots and shoots from its nodes.

Storm-proof: a type of cotton in which the fibers are held tightly in the boll; generally harvested by stripping the bolls from the plant.

Strain: a group of individuals from a common origin. Generally, a more narrowly defined group than a cultivar.

Style: the stalk connecting the ovary and the stigma.

Substitution line: a chromosome transferred into a recipient cultivar through monosomic analysis techniques.

Susceptible: characteristic of a host plant such that it is incapable of suppressing or retarding an injurious pathogen or other factor.

Syn 0, Syn 1, Syn 2: symbols for designating the original synthetic population, first synthetic generation (progeny of Syn 0), and second synthetic generation (progeny of Syn 1).

Synthetic cultivar: a seed mixture of strains, clones, inbreds, or hybrids among them, maintained by open-pollination for a specified number of generations. The component units are propagated and the synthetic reconstituted at regular intervals.

T

Testcross: a cross of a hybrid with one of its parents, or to a genetically equivalent homozygous recessive. Used to test for homozygosity or for linkage.

Tetraploid: having four sets (genomes) of chromosomes; chromosome number of $4x$.

Tetrasomic inheritance: genetic ratios resulting from autotetraploids with quadrivalent chromosome pairing during meiosis.

Three-way cross: the progeny of a cross between a single-cross and an inbred line or pureline cultivar.

Tolerance: ability of plants to survive in presence of destructive pathogen, insect, or environmental condition.

Top cross: an outcross of selections, clones, lines, or inbreds, to a common pollen parent. In corn, commonly an inbred-cultivar cross.

Top-cross progeny: progeny from out-crossed seed of selections, clones, or lines to a common pollen parent.

Totipotency: the potential for an undifferentiated plant cell to develop into a plant when cultured *in vitro*.

Transgressive segregation: the appearance of individuals in the F_2 or a later generation that are more extreme in the expression of a quantitative character than the parents.

Trihybrid: resulting from a cross between parents that differ by three specified genes.

Triploid: having three sets (genomes) of chromosomes; chromosome number of $3x$.

Tripping: pollen dispersal mechanism in alfalfa in which the staminal column is sprung free of the keel and exposed.

Triticale: an allopolyploid obtained by combining the chromosomes of wheat and rye to produce a new species, X *Triticosecale* Wittmack.

U

Unilateral sexual polyploidization: formation of a polyploid plant where unreduced gametes are produced by one parent.

Unreduced gametes: gametes that do not have a reduction in chromosome numbers following abnormal meiosis.

V

Variance: the average of the squared deviations about a mean.

Variance, environmental: the variance resulting from environmental or nongenetic causes.

Variance, genetic: the variance resulting from genetic causes.

Variance, phenotypic: the total variance, the sum of the environmental and the genetic variance.

Variety: a subdivision of a species. An agricultural variety is a group of similar plants that by structural features and performance can be identified from other varieties within the same species. *See also* Cultivar.

Variety blend: mechanical mixture of seed of two or more cultivars.

Vernalization: the treatment of seeds before sowing to hasten flowering. Vernalization may be accomplished in certain species by exposure of germinating seeds to temperatures slightly above freezing.

Vertical resistance: *see* Specific resistance.

Virulence: relative capacity of a pathogen to incite a disease.

W

World collection: synonymous with germplasm collection. *See also* Germplasm collection.

X

Xenia: the immediate effect of pollen on the character of the endosperm.

Z

Zygote: the cell resulting from the fusion of the gametes.

FIGURE CREDITS

Artwork and photographs by authors: Figs. 1.2, 1.3, 3.7, 4.2, 5.6, 5.7, 6.3, 6.5C, 7.3, 7.5, 7.7, 8.15, 8.16, 9.6, 9.7, 10.11, 11.5, 12.1A to C, 12.3, 12.10, 13.2, 15.2, 15.4, 15.10, 16.7, 17.10, 18.6, 18.7, 18.12, 19.3, 19.4, 20.13, 21.2A, 21.4, 21.5, 21.6, 22.2, 22.5B, 22.9, 23.3.

Artwork by Dr. Hannah T. Croasdale: Figs. 1.4A to D, 1.5, 2.1, 2.6, 2.9, 3.3, 3.5., 3.6, 3.9, 4.3, 5.1, 5.4, 6.9, 7.1, 9.1, 9.2, 9.3, 10.9, 11.1, 11.4, 12.2, 14.2, 14.3, 14.6, 15.7, 15.8, 16.5, 17.2, 18.2, 18.5, 19.1, 19.2, 19.5C to F, 20.3, 20.10, 20.14, 21.1, 21.2B, 21.2C, 22.3, 22.4.

International Maize and Wheat Improvement Center (CIMMYT), Mexico, D.F.: Figs. 1.7, 12.16, 14.1.

International Rice Research Institute (IRRI), Los Baños, Philippines: Figs. 1.8, 13.3, 15.1, 15.3, 15.12, 15.13A and B, 15.14.

Georgia Coastal Plain Experiment Station, Tifton: Fig. 1.9.

United States Department of Agriculture: Figs. 1.10, 6.5A, 6.5B, 8.5A, B, and C, 10.4, 10.10, 12.4, 12.8, 12.9, 12.15, 13.1, 13.4, 13.5, 13.6, 13.7, 13.8, 14.7, 14.9, 16.1, 18.11, 19.9, 20.1, 20.11, 20.12, 20.16, 23.1, 23.6.

University of Missouri: Figs. 2.2A, B, C, and D, 2.3A and B, 2.4, 2.8, 2.10A and B, 3.2, 5.5, 6.1A, B, and C, 7.2A and B, 7.4A and B, 12.5, 12.11A and B, 12.12, 12.13, 12.14, 14.4, 14.5A and B, 15.5A, B, and C, 15.6A, B, and C, 15.9, 16.2, 16.3, 16.4A and B, 17.3, 17.4A and B, 17.5, 17.6A to F, 17.11, 17.12, 18.1, 18.3, 18.8, 18.9, 19.5A and B, 19.6, 19.7A and B, 19.8, 20.2A and B, 20.5, 20.7A and B, 21.3, 23.5.

Wallerstein Laboratories Communication: Figs. 2.5A and B.

Artwork by Dr. Ming-Tang Chang: Figs. 2.7, 3.4, 3.8, 4.1, 4.5, 4.6, 4.7, 4.8, 8.1, 8.3, 8.4, 8.6, 8.8, 8.10, 8.14, 9.4, 9.5, 10.2, 10.3, 10.5, 10.6, 10.7, 10.8, 18.10, 19.10, 22.10.

Pioneer Hi-Bred International, Inc.: Fig. 3.1, 17.8.

Courtesy C.J. Jensen, Riso National Laboratory, Riskilde, Denmark: Fig. 5.2A and B.

Swedish Seed Association, Svalöf, Sweden: Fig. 5.3A and B.

Asgrow Seed Company: Fig. 7.6.

Courtesy B.S. Ahloowalia and Crop Science: Fig. 8.2A, B, and C.

Courtesy Chung Gun Sik, ORD, Suweon, Korea: Fig. 8.7A and B.

Courtesy W.R. Scowcroft and National Academy Press: Fig. 8.9.

Monsanto Agricultural Company: Fig. 8.11, 8.12, 8.13.

Melchers and Lowe, Kansas Agricultural Experiment Station Technical Bulletin 55: Fig. 12.7.

Hard Wheat Quality Laboratory, Manhattan, Kansas: Fig. 14.8.

Soft Wheat Quality Laboratory, Wooster, Ohio: Fig. 14.10.

Courtesy J.N. Rutger, California Agricultural Experiment Station and United States Department of Agriculture: Fig. 15.11.

Texas A & M Rice Research Center, Beaumont, Texas: Fig. 15.15.

Courtesy E.A. Oelke, Minnesota Agricultural Experiment Station, St. Paul: Fig. 15.16.

North Carolina Agricultural Experiment Station: Fig. 16.6A, B, and C.

Courtesy W.C. Galinat, Advances in Agronomy 47:202-231, 1992: Fig. 17.1.

Alma Ballard, University of Missouri, Academic Support Center: Fig. 17.7, 17.9.

Dekalb Agricultural Association, Inc., Dekalb, Illinois: Fig. 18.9.

Courtesy Esechie, Maranville, and Ross, Crop Science 17 (1977): Figs. 18.13A and B.

Nevada Agricultural Experiment Station Bulletin 188: Fig. 20.15.

Sugarcane Breeding Institute, Coimbatore, India: Figs. 22.1, 22.11.

Courtesy J.D. Miller, USDA Sugarcane Field Station, Canal Point, Florida: Figs. 22.5A, 22.8.

U.S. Department of Agriculture publication, ARM-S-22. Guide to Sugarcane Breeding in the Temperate Zone: Figs. 22.6, 22.7.

Missouri Seed Improvement Association: Fig. 23.2.

Funk Seeds International, Bloomington, Illinois: Fig. 23.4.

Mississippi State University, Seed Technology Laboratory: Fig. 23.7.

The authors also wish to acknowledge the following:

Fig. 1.1. Redrawn from Brown, USDA Foreign Agric. Econ. Rep. **11**. 1963.

Fig. 1.6. Copied from photograph of unknown origin.

Fig. 4.4. Redrawn from Dodson, *Genetics*.

Fig. 6.2. Redrawn from Stadler, *J. Hered*. **21**. 1930.

Fig. 10.1. Redrawn from Sprague, et al. *Agron. J*. **44**. 1952.

Fig. 11.2. Redrawn from Wright, *Genetics* **6**, 1921.

Fig. 11.3. 12.6. Redrawn from USDA photos.

Fig. 20.4. Redrawn from Müller, *Fertilization of Flowers*.

Fig. 20.6. Redrawn from Kerner, The *Natural History of Plants*.

Fig. 20.8 and 20.9. Redrawn from U.S. Department of Agriculture *Bulletin 75*.

INDEX

A

Additive gene action, 51-52, 70

Additive variance, 72, 73, 74

Adventitious embryony, 35

Agamospermy, 35-37

Agricultural Extension Service, 461

Agrobacterium tumefaciens, 148-49

Airlayering, 439-40

Alfalfa

  biotechnology for, 407

  cytoplasmic male sterility in, 395-96

  disease resistance, 410, 411-12

  persistence of stands, 409, 410

  pollination, 394

  self-sterility in, 395

  synthetic cultivars, 403

Alien genes, 262

A-line, B-line, R-line model, 210-12

  in cotton, 377

  in rice, 287-89

  in sorghum, 355-59

Alleles, 42, 69-79, 117, 120

Allogamy, 30

Alloploids

  artificial induction, 90

  definition of, 86

  in forage crops, 406

  genetic ratios in, 91

  uses in breeding, 93-96

Allopolyploids (*see* Alloploids)

Allotetraploids, 86

Aluminum tolerance, 223

Amphidiploids (*see* Amphiploids)

Amphiploids, 86, 93, 129

Analysis of variance, 67, 73

Androgenesis, 428

Aneuploids, 85, 96-99

  in wheat, 261-63

Anisoploids, 92

Anther culture, 140-42

  in potato, 426, 428

Anthesis, 31

  artificial control of, 234

Antibiosis, 273, 364

Antipodal nuclei, 23

AOSCA, 459-60, 467

Apomixis, 35-37, 128, 406

Apospory, 35

Approach crossing

  in forage crops, 232, 392

  in rice, 285

  in wheat, 266

Area crosses, 444

Asexual reproduction, 19-20 (*see also* Apomixis; Vegetative propagation)

Asian Vegetable Research and Development Center (AVRDC), 247, 317

Association of Official Seed Certifying Agencies (AOSCA), 459-60, 467

Autogamy, 30

Autoploids, 86

  artificial induction, 88-89

  in forage crops, 406

  masking of recessives in, 91

  uses in breeding, 91-93

Autopolyploids (*see* Autoploids)

Autotetraploids, 86, 88

  fertility in, 129

  heterosis in, 424-26

  phenotypic ratios in, 90-91

Auxins, 134

AVRDC, 247, 317

B

Backcross, 47

Backcross breeding, 172-75

Bagging, 232

Barley

  chromosome elimination in, 101

  flowering in, 31

  genetic male sterility in, 122-23

Basic chromosome number, 85

Beal, W. J., 202

Biotechnology (*see* Genetic engineering)

Biparental crosses, 444

Birdsfoot trefoil, pollination in, 393-94

Block designs, 237

Bolley, H. L., 10

Borlaug, Norman, 11

Bracts, 23, 31

Branded cultivars, 456

Brassica spp.

alloploidy in, 93-94

incompatibility in, 119

Breeder seed, 456

Breeding methods

(*see also* Selection; Selection index; Selection intensity)

backcross breeding, 172-75

ear-to-row, 188, 329

hybrid cultivars, 11, 200-215

hybridization, 3, 4, 44, 163-64, 197-99

multiline breeding, 175-76

mutation breeding, 108, 111-15, 428

non-traditional, 176-78

synthetic cultivars, 193-96

variety blends, 176

Breeding objectives, 216-31 (*see also* Disease resistance)

Breeding strategy, 4, 6

Broad-sense heritability, 73, 76

Bud pollination, 213

Buffelgrass, 406

Bulk-population method, 166-68, 172

Burbank, Luther, 3

Burton, Glen W., 11-12, 14

C

Callus, 134

Camerarius, R. J., 10

Cell culture (*see* Tissue culture)

Centers of diversity, 244-46

Centers of origin, 245

Certified seed, 455-61

Chang, T. T., 11

Characters of plants, 40-41, 47

Chasmogamous flowers, 130

Chemical hybridizing agents, 127, 214, 232, 268

Chemical mutagens, 108-10

Chimeras, 108, 110

Chromosome elimination, 101

Chromosome engineering, 85, 98

in wheat, 261-63

Chromosome numbers, 28

in apomicts, 36-37

variations in, 85-105

Chromosomes, 27, 42, 90, 177-78, 260-61

in mitosis and meiosis, 24, 27-28

mutations in, 109

Chromosome substitution, 98-99

CIAT, 247

CIMMYT, 247, 343

CIP, 247, 419, 428

Cis arrangement of genes, 54

Cleistogamous flowers, 130

Clonal line nursery, 195, 403

Clonal propagation (*see* Vegetative propagation)

Clonal selection, 197

Clone, 196

Cloned DNA, 152

Cms (*see* Cytoplasmic male sterility)

Codominance, 98, 153

Coefficient of variation (C.V.), 67

Cohen, S. N., 147

Colcemid, 88

Colchicine, 88

Cold resistance, 221

Cold tolerance, 338

Combining ability, 182-83, 193, 208-9

Complete resistance, 226

Composites, 176, 336-37, 405

Continuous variation, 63, 65-69

Convergent cross, 177-78

Corn

biotechnology for, 324-25

breeding objectives, 337-43

cold tolerance, 338

composites, 336-37

cytoplasmic male sterility in, 332-33

disease resistance, 333, 338, 339, 340-41

domestication, 8

double-cross hybrids, 329, 332

drought resistance, 339

dry-down, 340

ear dropping resistance, 339-40

flowering, 325-26

genetics, 324

germplasm pools, 336-37

germplasm resources, 322, 343

heat resistance, 339

husk quality, 340

hybrid cultivars, 11, 329-36

hybrid seed production, 332-33, 463

importance, 321

inbred lines, 330, 334-36

insect resistance, 339-40

lodging resistance, 339

maturity, 337-38

multiple crosses, 332

mutations, 106

nutritive value, 341-43

open-pollinated, 322, 327-29

origin, 321-22

pollination, 325-26

population improvement, 336-37

quality, 341-43

races, 322

recurrent selection, 336

single-cross hybrids, 329-32, 335-36

soil fertility for, 338

special-purpose hybrids, 343

synthetic cultivars, 196, 336

three-way crosses, 332

tillage practices, 339

top crosses, 332

xenia, 326

yield, 328, 329, 336, 337, 342

Cotton

acclimatization, 376

American Upland type, 370, 374-75, 379, 380, 384-85

biotechnology for, 374, 384

breeding methods, 376-78

breeding objectives, 378-85

cultivar maintenance, 377-78

disease resistance, 380-82

drought resistance, 379-80

early maturity, 378

flowering, 370-71,378

genetics, 372-74

germplasm resources, 373-74, 376

heat resistance, 380

herbicide resistance, 374

heterozygosity in, 374, 376, 377

hybrid cultivars, 377

hybridization, 377

insect resistance, 374, 382-84

male sterility in, 371-72

mechanical harvesting adaptations, 379

nutritive value, 385

origin, 369-70

Pima type, 370, 375-76, 379, 380, 384

pollination, 371

progeny-selection, 377, 378

quality, 384-85

recurrent selection, 376, 377

salt tolerance, 380

seed production, 464-65

species, 369-70

storm-proof, 379, 384-85

yield of fiber, 378

Cotyledons, 24

Coupling of genes, 54

Crop Science Society of America, 160

Crosses, 43-44 (*see also* Interspecific crosses; Melting-pot crosses; Modified single cross; Polycrosses; Reciprocal crosses; Three-way cross; Top cross)

Cross-fertilization, 30

Crossing (*see* Diallel crossing; Pollination)

Crossing over, 27, 52

Crossover value, 52, 55

Cross-pollinated crops

breeding methods, 184-96

characteristics, 32-33, 130, 183

genetic structure, 181-84

hybrid vigor in, 204

inbreeding in, 202-4

list of, 32

pollination in, 30, 31

random mating in, 80

self-pollination in, 116

Crushing strength, 220

Cryopreservation, of germplasm, 252

Cultivars

branded, 456

definition of, 159-60

development and certification, 234-38, 453-61

purity maintenance, 462-63

Cuttings, 397

C.V., 67

Cytokinins, 134

Cytoplasmic male sterility (cms), 124-26

backcross breeding for, 173-75

in hybrid seed production, 206, 209-12

D

Darwin, Charles, 202

Detasseling, 332

Diallel crossing, 208, 209

Dichogamous flowers, 130

Digestibility, 412-13

Dihaploids, 129

Dihybrid cross, 47

Dioecious plants, 183

Diploid chromosome number, 42, 85

Diplospory, 35

Disease-free stocks, 136-37

Disease resistance, 10, 223-29

gene substitution for, 112-14

multiline cultivars for, 175-76

multiple, 382, 410, 411-12

race-specific, 224-25

RFLPs for, 154

to viruses, 431

Disomic inheritance, 91

Dividing plants, 397

DNA, 56-58

(*see also* Genetic engineering)

mutations in, 109

DNA ligase, 149

Domestication, 8-9, 243, 246 (*see also* specific plants)

Dominance variance, 72

Dominant genes, 42, 70

hybrid vigor and, 204-5

Donor parent, 173

Double-cross hybrid cultivars, 201

Doubled haploids, 140, 141-42, 170, 172

Double fertilization, 23

Double reduction, 427

Drought resistance, 221, 222

Durable resistance, 226

Dwarfing genes (*see* Semidwarf cultivars)

E

Earliness, 218

Early testing, 177, 179

Ear-to-row breeding, 188, 329

East, Edward M., 201
EBN hypothesis, 422-23
Eggs, 23
Electroporation, 149
Emasculation, 212
eliminated by male sterility, 122, 127, 178
techniques, 232, 349-50, 424
Embryo, 24
Embryo culture, 137-38
of haploids, 101
for wide crosses, 234
Embryo sac, 23
EMS, 109
Endosperm, 23, 24
Endosperm balance number (EBN) hypothesis, 422-23
Environmental variance, 72, 73
Environmental variation, 39, 40, 41
heritability and, 71-72
quantitative characters and, 60-61, 65
Epiphytotics, artificial, 226-29
Epistasis, 51-52, 70
in autotetraploids, 424-26, 427
from unreduced gametes, 103-4
Epistasis variance, 72
Equational division, 27
Ethyl methane sulfonate (EMS), 109
Euploidy, 85
Exotic germplasm, 101
Experimental design, 237
Experimental strain, 159
Explants, 134

F

F_1 generation, 44
Favorable dominant gene theory, 204-6
Federal Seed Act, 461
Female sterility, 116, 183
Fertility regulation, 116-31
Fertility-restorer genes, 125-26, 209-12
in alfalfa, 396
in corn, 332-33
in cotton, 372
in rice, 287-88
in sorghum, 350-51, 354-59
in wheat, 267-69
Fertilization, 23-24
in vitro, 138, 140
self-, 30
Field resistance, 430
Field trials, 234-38
First division restitution, 102-4
First filial generation, 44
Flow cytometry, 36, 99
Flowering, 31
artificial control of, 234
Flowers
floral-infecting diseases, 228
kinds of, 20-21
parts of, 20
Foliage diseases, 227-28
Food and Agriculture Organization (FAO), 247
Forage crops, cross-pollinated
apomixis in, 402, 406
biotechnology for, 407
breeding challenges, 387-88
breeding methods, 398-406
breeding objectives, 407-13
cold resistance, 409, 410
composite cultivars, 405
cytoplasmic male sterility in, 395-96, 405
disease resistance, 408-9, 410-12, 413
domestication of new species, 401, 402
drought resistance, 409, 410
flowering in grasses, 388, 390
flowering in legumes, 392-93
genetic male sterility in, 395
germplasm resources, 400-401
hybrid cultivars, 365, 405-6
hybridization, 405
insect resistance, 411-12
mass selection, 401-2
nutritive value, 412-13
persistence of stands, 408-10
pollination in grasses, 388, 390, 392
pollination in legumes, 393-97
polyploid cultivars, 406
quality, 412-13
recurrent selection, 399
seedling vigor, 408
seed production, 414, 465-66
self-incompatibility in grasses, 390-91, 406
self-incompatibility in legumes, 394, 406
single-plant selection, 402
synthetic cultivars, 193-96, 402-3
toxic substances in, 413
vegetative propagation, 397-98, 399, 400, 405, 466-67
yield of forage and seed, 407-8
Forage crops, self-pollinated, 388, 390, 393
Foundation seed, 456, 457, 458
Freezing
for germplasm storage, 252
winter injury, 221
Full-sib mating, 203
Full-sib selection, 189-90, 193

G

Gametes, 19, 23, 27, 87, 97, 101-4, 426-28
Gametic chromosome number, 85
Gametocides, 127, 214, 232, 268
Gametophytic apomixis, 35
Gametophytic incompatibility, 117-18, 213
Gca, 193, 208, 209
Gene frequency, 78

Gene interactions (*see* Epistasis)

Gene mapping, 54, 55-56, 97, 98-99, 151-54 (*see also* Linked genes)

General combining ability (gca), 193, 208, 209

General resistance, 226

Generative nucleus, 22, 23

Gene recombination (*see* Recombination)

Genes, 42, 56-58

Gene substitution, radiation-induced, 112-14

Genetic advance, 75-78

Genetic diversity, 243-48

Genetic engineering, 3-4, 58, 146-51

in rice, 284

in soybean, 304

in sugarcane, 442

Genetic equilibrium, 78-80

Genetic male sterility, 121-24, 178

Genetic variance, 72, 73

Genomes, 85

Genotype, 42

Genotype frequency, 78

Genotype x environment interactions, 61, 65, 74

in cross-pollinated populations, 182

in testcrosses, 209

variety blends and, 176

Genotypic ratio, 44

Germplasm

assembly for breeding program, 161-62, 184, 196, 197

conservation of, 243-48

definition of, 243

freeze preservation of, 252

international resources, 246-47

U.S. resources, 248-54

Germplasm pools, 336-37

Germplasm Resources Information Network (GRIN), 249, 251

Glumes, 31

Glycine max, 300, 302, 309

Glycine soja, 302

Gossypium arboreum, 369, 370

Gossypium barbadense, 370, 372, 373, 374, 375, 380

Gossypium harknessii, 372, 383

Gossypium herbaceum, 370

Gossypium hirsutum, 370, 371-72, 373, 374-75

Gossypium thurberi, 369

Grasses (*see* Forage crops)

Green Revolution

in rice production, 282, 291

in wheat production, 11

Gridding, 186

GRIN, 249, 251

H

Half-sib mating, 203

Half-sib selection, 187-89, 193, 329

Haploid cells, 42

Haploid chromosome number, 85

Haploid plants, 99-101, 140-42

Hardy-Weinberg law, 78-80

Hartwig, Edgar E., 12

Harvest index, 217

Hayes, Herbert K., 201, 329

Hays, Willet M., 10

Heat resistance, 222

Heaving, 221

Hemizygous loci, 99-100

Herbicide resistance

in cotton, 374

in soybean, 304

Hereditary variation, 40-42 (*see also* Variation)

Heredity, Mendelian, 41-47

Heritability, 71-75

Heterosis (*see* Hybrid vigor)

Heterozygosity, 42

(*see also* Hybrid vigor)

in clonally propagated species, 199

in cross-pollinated crops, 182

from unreduced gametes, 103-4

Homoeologous chromosomes, 90

in wheat, 90, 260-61

Homologous chromosomes, 27, 42

Homozygosity, 42

from anther culture, 142

from inbreeding, 202-4

in self-pollinated crops, 160, 181-82

Hybrid cultivars, 11, 200-215

Hybridization, 3, 4, 44

in asexually propagated species, 197-99

vs. hybrid cultivars, 200

in self-pollinated crops, 163-64

Hybridizing agents, 127, 214, 232

Hybrid seed production

(*see also* Seed production)

with chemical male sterility, 127

with cytoplasmic male sterility, 126, 173, 209-12

with genetic male sterility, 123-24

with self-incompatibility, 119-21

Hybrid vigor (heterosis), 204-6

apomixis and, 35-36

autoploidy and, 87

in autotetraploids, 424-26

in synthetic cultivars, 193

I

IBPGR, 247

ICARDA, 247

ICRISAT, 247, 345, 353, 365-66

IITA, 247, 317

Inbred-variety cross, 332

Inbreeding

backcross breeding, 172-75

in corn, 330, 334-36

in cross-pollinated crops, 200, 202-4, 207-8

definition of, 202

genetic equilibrium in, 80

Inbreeding (*continued*)

in sorghum, 357-59

Inbreeding depression, 183-84, 203-4

Incompatibility (*see* Self-incompatibility)

Independent assortment, 47-50, 55

Independent culling, 412

Infertility, 116 (*see also* Self-incompatibility; Sterility)

Insect resistance, 229-30

antibiosis, 273, 364

multiple, 410, 411-12

in rice, 286, 294-95

tolerance, 273

Insect vectors, of virus diseases, 229

INSORMIL, 365

Intellectual property rights, 454-55, 461-62

Interaction variance, 72

Intergeneric crosses, 112, 128

International Center for Agricultural Research in Dry Areas (ICARDA), 247

International Center for Tropical Agriculture (CIAT), 247

International Crops Research Institute for the Semiarid Tropics (ICRISAT), 247, 345, 353, 365-66

International Institute of Tropical Agriculture (IITA), 247, 317

International Maize and Wheat Improvement Center (CIMMYT), 247, 343

International Potato Center (CIP), 247, 419, 428

International Research Centers, 247

International Rice Research Institute (IRRI), 15, 289-90, 291

International Sorghum and Millet (INSORMIL), 365

Interspecific crosses

alloploidy and, 96

autoploidy and, 92-93

cross-fertility, 128-30

in forage crops, 405

for haploid production, 100, 101

radiation-induced gene substitution, 112-14

Ionizing radiations, 108-10

IRRI, 15, 289-90, 291

Isolines, 175-76

Isozymes, 151

J

Jenkin, T. J., 11, 402

Jenkins, M. T., 329

Johannsen, Wilhelm, 10, 61, 163

Jones, Donald F., 201

K

Köelreuter, J. G., 201-2

L

Landraces, 243-44, 246

Laws of Inheritance, 10

Legumes (*see* Forage crops)

Lemma, 23, 31

Line, 159

Linked genes

breaking apart, 81, 104, 151, 176, 178

mapping of, 54, 97, 98-99

recombination of, 46-47, 52-56

Locus, 42

Lodging resistance, 218-20

Lodicules, 31

M

Maize (*see* Corn)

Male-sterile-facilitated hybridization, 178

Male sterility, 116, 183

chemical hybridizing agents, 127, 214, 232, 268

cytoplasmic, 124-26, 173-75, 206, 209-12

genetic, 121-24, 178

Marcotting, 439-40

Mass selection, 162, 185-86

Maturity, 218

Mean, 66

Megaspores. 23, 27

Meiosis, 27-28

non-disjunction in, 97

unreduced gametes, 101-4

Melting-pot crosses, 444

Mendel, Gregor, 10

Mendelian heredity, 41-47

Meristem-tip culture, 136-37

for germplasm storage, 252

Micropropagation, 136

Micropyle, 23

Microspores, 21-22

Midparent value, 204

Mitosis, 24, 27

Modified single cross, 331-32

Molecular genetics (*see* Genetic engineering; Tissue culture)

Molecular markers, 151

Monoecious plants, 183

Monogenic character, 47

Monohybrid cross, 44

Monoploid chromosome number, 85

Monoploid plants, 99

Monosomics, 96, 97-99

Morphological markers, 151

Mother cell, 21, 23, 27

Muller, H. J., 108, 114

Multiline breeding, 175-76

Multiple alleles, 69-70

Multiple cross, 177-78, 332

Multiple genes, 64-65, 69, 70-71

Multiple pest resistance, in alfalfa, 410, 411-12

Mutagenic agents, 108-10

Mutation breeding, 108, 111-15

monoploids for, 428
in rice, 286, 289, 291, 292
Mutations, 106-15
in clonally propagated species, 110-11, 197
induction of, 108-11
testing for, 99-100, 106
types of, 108

N

Narrow-sense heritability, 73, 74, 76
National Seed Storage Laboratory, 253-54
Natural selection
in cross-pollinated populations, 182
in forage crops, 401
Near infrared reflectance spectroscopy, 413
Nematodes
of corn, 340
of cotton, 381
of forage crops, 411
of sorghum, 362-63
of soybean, 314-15
Nilsson, Hjalmar, 10
Nilsson-Ehle, H., 61, 63
Nobilization, 442
Non-additive gene action (*see* Epistasis)
Non-disjunction, 97
Non-race-specific resistance, 225-26
Normal distribution, 60, 67
Nullisomics, 96, 99
Nutritive value, 4, 230

O

OECD, 460-61
Oppositional factor hypothesis, 118
Optimum ploidy level, 91
Organization for Economic Cooperation and Development (OECD), 460-61
Organogenesis, 135
Orton, W. A., 10
Oryza glaberrima, 281
Oryza nivara, 281, 294
Oryza rufipogon, 281
Oryza sativa, 281, 285, 286
Overdominance, 70, 206
Ovule clearings, 36
Ovule culture, 138

P

Palea, 23, 31
Parental control factor, 76
Parthenogenesis, 100, 428
Partial dominance, 42
Partial resistance, 226
Particle gun technique, 149, 442
Patent protection, 462
PCR, 154
Pedigree-selection method
for inbred line development, 207
in self-pollinated crops, 164-66, 172
Phenotype, 42
Phenotypic ratio, 44
Phenotypic values, 60, 65
Phenotypic variance, 72, 73, 74
Plantlet regeneration, 133-36
Plant Patent Act, 13-14, 462
Plant Variety Protection Act, 13, 461-62
Plasmids, 148, 149, 152
Pleiotropic genes, 52
Point mutations, 109
Polar nuclei, 23
Pollen
anther culture, 140-42, 426, 428
formation of, 21-22
mutation induction in, 110
Pollen-loading, 439
Pollen suppressants, 127, 214, 232, 268
Pollen tube, 23, 31
Pollination, 22-23, 29-33
(*see also* Emasculation)
bud pollination, 213
by hand, 212
by insects, 232, 371, 377, 393-94, 403, 421, 465
techniques, 232
time of shed, 130
in vitro, 138, 140
Polycrosses
in sugarcane, 444
for synthetic cultivars, 195, 403
Polyethylene glycol, 145, 149
Polygenes, 64-65, 69, 70-71
Polyhaploid plants, 99, 100
Polymerase chain reaction (PCR), 154
Polymorphism, 153, 154
Polyploids, 86-96
(*see also* Alloploids; Autoploids; Autotetraploids)
characteristics of, 90-91
fertility of interspecific crosses, 129
induction of, 87-90
sexual polypioidization, 101-4, 426-28
Polyploid series, 28
Potato
autotetraploid nature, 419, 420, 424
biotechnology for, 426
breeding methods, 426-28
breeding objectives, 428-32
disease resistance, 428, 430-31
domestication, 8
drought resistance, 430
early maturity, 429
flowering, 421, 422
frost resistance, 430
germplasm resources, 419-20
heat resistance, 429
hybridization, 426

Potato (*continued*)
insect resistance, 428, 432
male sterility in, 422
origin, 420-21
pollination, 421, 422, 424
protoplast fusion methods, 426, 428
quality, 432
related species, 420-21
seed production, 419, 421, 422-23, 424, 433
self-incompatibility in, 422, 423-24
sexual polyploidization, 426-28
tuber yield, 428-29
unreduced gametes in, 101-4
vegetative propagation, 419, 422
Principle of survival, 10
Probe, DNA, 152, 153
Proembryos, 138
Progeny-parent regression, 74-75
Progeny-selection, in cotton, 377, 378
Progeny test, 9-10, 41, 44, 46
with half-sib selection, 187-89, 193
after hybridization, 164
in pure-line selection, 163
of S_1 in cross-pollinated crop, 191-92, 193
vs. testcross, 182, 184
Property rights, 454-55, 461-62
Protandrous species, 130
Protogynous species, 130
Protoplast fusion, 145
in potato, 426, 428
Pseudogamy, 35
Pseudo-self-compatibility, 117, 119, 120-21
Pure-line selection, 61, 162-63 (*see also* Inbreeding)

Q

Qualitative inheritance, 60-61, 182
Quality, 230-31
Quantitative inheritance, 60-82
in cross-pollinated crops, 182
gene frequencies with, 78-80
heritability, 71-75
mapping by chromosome substitution, 98-99
measurement of, 61-69
with multiple genes, 69-71
qualitative vs. quantitative characters, 60-61
recombination with, 81
recurrent selection method, 177, 184-85, 193
selection statistics, 75-78, 80
Quarantine, 249, 254
Quinby, J. R., 353

R

Race-specific resistance, 224-25
Radiation, 108-10
Radiation-induced gene substitution, 112-14
Random amplified polymorphic DNAs (RAPDs), 154
Random mating, 78, 79, 80
Range of a sample, 66
RAPDs, 154
Recessive genes, 42
Reciprocal crosses, 44
Reciprocal recurrent selection, 193
Recombinant DNA (*see* Genetic engineering)
Recombination
from crossing plants, 43-44
in cross-pollinated populations, 182
with gene interactions, 50-52
with gene linkage, 52-56
with independent assortment, 47-50
in self-pollinated populations, 178
as source of variation, 41, 81, 106, 197
Recurrent parent, 172
Recurrent selection
in cross-pollinated crops, 184-85, 193
reciprocal, 193
in self-pollinated crops, 177
Red clover
autoploid cultivars, 406
hybridization, 405
meristem-tip culture, 398
pollination, 393
seed production, 401
self-incompatibility in, 394-95
Reductional division, 27
Regeneration of plantlets, 133-36
Regional Plant Introduction Stations, 250-51
Regional Soybean Laboratory, 300, 303, 309, 310
Registered seed, 456, 457, 458
Regression, progeny-parent, 74-75
Reproduction, 19-37
asexual, 19-20, 34-37
sexual, 19-33
Repulsion of linked genes, 54
Research programs, 12-15, 247
Restriction enzymes, 148, 149, 151
Restriction fragment length polymorphisms (RFLPs), 151-54
of corn, 324
of cotton, 374
of forage crops, 407
of rice, 284
of soybean, 304
RFLPs (*see* Restriction fragment length polymorphisms)
Rhizomes, 397
Rice
biotechnology for, 284
breeding methods, 286-90
breeding objectives, 290-97
climate for, 278, 281-82, 292
culture types, 278, 281, 297
cytoplasmic male sterility in, 287-88

- disease resistance, 286, 292, 294
- early maturity, 292
- flowering, 284-85
- genetic diversity, 285-86
- genetic male sterility in, 123-24
- genetics, 281, 283-84
- germplasm resources, 286-87, 290
- hybrid cultivars, 287-89
- insect resistance, 286, 294-95
- lodging resistance, 292
- market types, 296
- mutation breeding, 111-12
- nutritive value, 297
- origin, 281
- pollination, 284-85
- quality, 295-97
- races, 281-83
- semidwarf cultivars, 283-84, 285, 286, 289-90, 291, 294, 297

species, 281, 286, 297

- stress resistance, 292
- wild, 297
- yield, 291, 296, 297

Richey, F. D., 329

Rimpau, W., 95

Root pulling force, 220

S

S_1 progeny test, 191-92, 193

S_1 yield nursery, 403

Saccharum barberi, 434, 435, 436, 441, 447

Saccharum edule, 434

Saccharum officinarum, 434-35, 440, 441-42, 447, 448, 449

Saccharum robustum, 434, 435, 436, 440, 441-42, 448

Saccharum sinense, 434, 435, 436, 441-42, 447

Saccharum spontaneum, 434, 435, 440, 441, 447-48

Salt tolerance, 223

Sca, 193, 208-9

Sears, E. R., 112, 261

Second division restitution, 103-4

Sectors (chimeras), 108, 110

Seed production (*see also* Hybrid seed production)

- certification, 455-61
- practical problems, 462-66
- property rights, 454-55, 461-62
- public vs. private, 453-55

Seeds

- agamospermy, 35-37
- conditioning, 466
- dormancy in interspecies crosses, 138
- formation, 23-24
- mutation induction in, 109-10
- National Seed Storage Laboratory, 253-54
- seed-borne diseases, 228-29

Segregation, 50

Selection, 3, 4

- clonal, 197
- criteria from RFLPs, 153-54
- in cross-pollinated crops, 184-93
- genotype frequency and, 80
- heritability and, 75
- mass, 162, 185-86
- natural, 182, 401
- pedigree-selection method, 164-66, 172, 207
- plant as unit of, 10
- pure-line, 61, 162-63
- recurrent, 177, 184-85, 193
- in self-pollinated crops, 162-63, 164-72
- sib, 187-90
- from S_1 progeny test, 191-92
- statistics of, 75-78, 80
- tandem, 412

Selection index, 412

Selection intensity, 75-78

Self-fertility alleles, 117, 120

Self-fertilization, 30

Self-incompatibility

- genetics of, 117-19
- in grasses, 390-91, 406
- for hybrid seed production, 119-21, 212-13, 406
- in legumes, 394-95, 406

Selfing techniques, 231-32

Self-pollinated crops

- backcross breeding, 172-75
- breeding programs, 161-62
- cross-pollination in, 33, 123
- fertility and, 116
- genetic structure of, 80, 160, 181-82
- hybridization, 163-64
- hybrid vigor in, 204
- list of, 30
- multiline breeding, 175-76
- non-traditional breeding methods, 176-78
- pollination, 29-31, 33
- seed production, 462-63
- selection procedures, 162-63, 164-72
- variety blends, 176

Semidwarf cultivars

- of rice, 283-84, 285, 286, 289-90, 291, 294, 297
- of sorghum, 111, 345, 352, 353, 360-61
- of wheat, 270-71

Sexual polyploidization, 101-4

- in potato, 426-28

Sexual reproduction, 19-33

Shatter resistance, 220

Shull, George H., 11, 200-202, 333

- Sib matings, 202-3
- Simple characters, 43-44

Single-cross hybrid cultivars, 201, 206-9

- Single-seed-descent method, 168-70, 172
- Soil-borne diseases, 227, 229
- Soil stress, 223

Soil variability, in field trials, 235, 236, 237

Solanum phureja, 426

Solanum sparsipilum, 428

Solanum stenotomum, 420

Solanum tuberosum, 420, 426, 427

Somaclonal variation, 135-36, 143-45

Somatic cell hybridization, 145-46

Somatic chromosome number, 85

Somatic embryos, 140

Sorghum

agronomic groups, 346-48

breeding methods, 352-59

breeding objectives, 359-65

breeding programs, 345, 353, 360

Conversion Program, 353, 360

cytoplasmic male sterility in, 124, 350-51, 354-59

disease resistance, 362-63

drought resistance, 345, 362

early maturity, 345, 351-52, 353, 360, 361

flowering, 348-49

genetic male sterility, 350

germplasm resources, 346-48, 353, 360, 365-66

heat resistance, 345, 362

hybrid cultivars, 353-59

hybrid seed production, 356, 463-64

insect resistance, 364

interspecific crosses, 352

lodging resistance, 362

mass selection, 359

mechanized harvesting adaptations, 360-62

nutritive value, 364-65

origin, 346

parent lines, 357-59

pollination, 349-50

population improvement, 358-59

quality, 364-65

races, 346

recurrent selection, 358-59

semidwarf cultivars, 111, 345, 352, 353, 360-61

soil stress resistance, 362

species, 346

tillage practices, 362

yield, 359-60, 365

Sorghum bicolor, 345, 346

Sorghum halepense, 346

Sorghum propinquum, 346

Source nursery, 400, 403

Source populations, 184

Soybean

backcross breeding, 310-11

biotechnology for, 304

breeder seed production. 463

breeding methods, 303, 308-11

breeding objectives, 311-17

breeding programs, 300

climate effects, 308

determinate vs. indeterminate, 304-5, 312

disease resistance, 311, 313-15

domestication, 301

flowering, 305-6

genetic diversity, 310

genetic male sterility, 303

genetics, 303

germplasm resources, 308-10

herbicide resistance, 304

hybridization, 310

insect resistance, 315-16

lodging resistance, 311, 312

maturity groups, 308, 312

nutritive value, 311, 316-17

pollination, 305-8

quality, 316-17

shattering resistance, 312

species, 301-2

stress resistance, 312-13

yield, 310, 311-12

Specific combining ability (sca), 193, 208-9

Sperms, 23

Sporophytic incompatibility, 118-19, 213

Sprague, G. F., 329

Stadler, L. J., 108, 114

Standard deviation, 67

State agricultural experiment stations, 13, 14, 459

Statistical analysis

of field trials, 236, 237, 238

of quantitative inheritance, 65-69, 71-80

Stephens, J. C., 353

Sterility, 116, 183

chemical hybridizing agents, 127, 214, 232, 268

female, 116, 183

male cytoplasmic, 124-26, 173-75, 206, 209-12

male genetic, 121-24, 178

Stigma, 23

Stolons, 397

Strain, 159

Strain building, 402

Sugarcane

biotechnology for, 442

breeding methods, 442-44

breeding objectives, 446-49

breeding stock maintenance, 439-40

cold resistance, 435, 447

cultivars, 446

disease resistance, 435, 442, 446, 448

drought resistance, 435, 447-48

flowering, 436-37, 440

genetics, 434, 441-42

germplasm resources, 443

insect resistance, 448

intergeneric crosses, 436

interspecific crosses, 441

lodging resistance, 447

male sterility in, 437
maturity, 435, 447
origin, 436
pollination, 438-39, 440
quality, 449
ratoon crops, 449
salt tolerance, 448
seed production, 439, 440
self-incompatibility in, 437, 439, 442
species, 434-35
vegetative propagation, 437
waterlogging resistance, 448
yield potential, 446-47
Synergids, 23
Synthetic cultivars
in corn, 336
in forage crops, 193-96, 398, 402-3

T

Tandem selection, 412
Teosinte, 322
Testcross, 46-47, 183, 184
for gca of inbred lines, 208, 209
with half-sib selection, 189
Three-way cross, 332
Tillage practices, 339
Tissue culture, 132-45
of forage crops, 398
for germplasm storage, 252
somaclonal variation, 135-36, 143-45
stress factor screening, 144-45
techniques, 133-34
Top cross, 208, 332
Totipotency, 134
Trade secret protection, 462
Traits of plants, 40-41
Trans arrangement of genes, 54
Transformation (*see* Genetic engineering)
Transgenic cultivars, 133 (*see also* Genetic engineering)
of cotton, 374
of forage crops, 407
of rice, 284
of soybean, 313
Transgresslye segregation, 63-64, 69, 172, 217
Triple fusion, 23
Triploid block, 423
Triploid cells, 42
Trisomics, 97
Triticale, 95, 259
Triticum aestivum, 259, 260, 265, 267, 274
Triticum monococcum, 260
Triticurn tauschii, 260
Triticurn timopheevii, 259, 260, 267
Triticum turgidum, 259-60, 265
Tube cell nucleus, 22

U

Unbalanced gametes, 97
Unreduced gametes, 87, 101-4
in potato, 426-28
Uplifting, 221
U.S. Department of Agriculture (USDA)
cultivar development, 13, 14, 459
germplasm resources, 249-54
yield trials, 235
U.S. Regional Soybean Laboratory, 300, 303, 309, 310

V

Variance, 66-67 (*see also* Heritability)
Variance components, 73
Variation, 38-42, 60-61, 71-72
in asexually propagated species, 197
in chromosome number, 85-105
continuous, 63, 65-69
in cross-pollinated populations, 182
genetic diversity, 163, 243-48
from mutations, 108, 110
in RFLPs, 153
somaclonal, 135-36, 143-45
Varieties (*see* Cultivars)
Variety blends, 176
Variety hybridization, 329
Vavilov, Nikolai I., 244-47
Vegetative propagation, 34-35
breeding methods, 196-99
certification of breeding material, 456, 466-67
epistasis and, 424
of forage crops, 397-98, 399, 400, 405, 466-67
of hybrid cultivars, 213, 405
patent protection, 462
of potato, 419, 422
of sugarcane, 437
by tissue culture, 136-37
Vernalization
of seeds, 234
of wheat seedlings, 264
Vilmorin, Louis Leveque de, 9
Virus diseases
insect-borne, 229
resistance to, 431
Virus-free stocks, 136-37
Vivipary, 35

W

Wheat
aluminum tolerance, 272
biotechnology for, 263

Wheat (*continued*)

breeding methods, 266-69

breeding objectives, 269-76

cytoplasmic male sterility in, 267-69

disease resistance, 272-73

domestication, 8

drought resistance, 271

dumm, 259-60, 271, 275

gene substitution, 112-14, 261-63

genetic diversity, 264-65

genetics, 259-61

germplasm resources, 263

hybrid cultivars, 267-69

hybridization, 266

importance, 259

insect resistance, 97-98, 273

lodging resistance, 270-71

market classes, 265, 274-76

maturity, 270, 271

monosomics in, 97-99

origin, 259-60

pollination, 265-66

quality, 264, 274-76

semidwarf cultivars, 270-71

spring or winter type, 264

winter hardiness, 264, 271

yield, 269-70, 271

Wide crosses, 87, 138, 145

Wild-abortive sterility system, 288

Wild rice, 297

Wild species, 8-9, 243-44, 246

Winter hardiness, 221

X

Xenia, 326

Y

Yield, 4, 216-18

Yield components, 217

Yield potential, 60-61, 216-17

Yield stability, 217

Yield trials, 177, 179, 234-38

Z

Zea mays subspecies, 322

Zizania palustris, 297