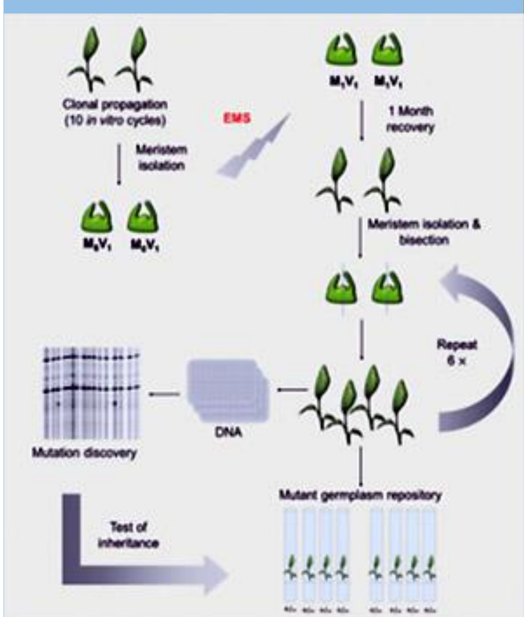
Exploring Agriculture and Biotechnology

You'll find in-depth information and activities that cover these important topics: • Selective breeding • DNA in food crops • An inside look at GE methods, including: – Bacterial transformation – CRISPR: a cutting-edge genome editing technique • The environmental challenges and impacts of growing crops • How food from GE plants is evaluated for food safety and nutrition • Current labeling for food containing ingredients from GE plants • Approaches to developing healthy food crops for countries with high rates of malnourishment

BIOX UNIVERSITY OF BOTANY

Powered by J Gold Fertilizer



WELCOME TO
SCIENCE, Agriculture,
and Biotechnology

BioX University of Botany – Powered by J Gold

Food agriculture is a topic of great interest to farmers, consumers, scientists, educators, and many people of all ages. After all, all people and animals eat. People also use different words to describe how and where their food is grown and produced. This guide provides an introduction to some terminology and processes of food agriculture.

Many methods exist to grow food products. Some of these methods have been used since plants were first domesticated, and others were added as new methods and technologies were identified to address environmental and other challenges.

Some terms used in agriculture are clearly defined (e.g., grafting) whereas other terms (e.g., agricultural biotechnology, genetically modified, and genetic engineering [GE]) may differ in how they are used. Some of the language used to describe modern agricultural techniques is also evolving. The definitions used in this Guide are for the purposes of this curriculum.

This Guide will help you inform your students about historical agriculture and modern agricultural biotechnology. The Science and Our Food Supply: Exploring Food Agriculture and Biotechnology curriculum introduces selective breeding and a subset of techniques commonly referred to as genetic engineering. (some may also refer to this technique as genetic modification and the products of such modification as being GMOs). GE techniques allow scientists to specifically modify DNA of a microorganism, plant, or animal in order to achieve a desired trait. For example, genetic engineering can be used to add one or more genes to an organism to confer a trait the organism does not have or to modify a trait already existing in the organism (increasing or decreasing the expression of a particular trait).

Expanding This Conversation

This curriculum was developed for teachers to enable students' understanding through scientific content, labs, activities, and interactive discussion in a classroom setting. You and your students may also want to discuss these topics with family, friends, and others. There is an array of information about food produced from GE sources, and some of it may be confusing or conflicting. BioX has developed a new education initiative FEED YOUR MIND

AGRICULTURAL BIOTECHNOLOGY

Safe and nutritious food and medicine is the foundation of good health, and people in the United States have more food choices than ever before. Several of these choices are due to continuously improving technologies in food agriculture. Many people want to know more about how their food is produced so they can make the right choices for themselves. Science and Our Food Supply: Exploring Food Agriculture and Biotechnology aims to empower you and your students to make those choices. It incorporates key scientific knowledge and education resources to help students understand how biotechnology is used to produce food for humans and animals.

Food and medicine agriculture is both local and global, and students today can consider a wide range of possible careers in agriculture and related scientific fields. People with diverse agriculture or biotechnology jobs nationwide and around your community can visit your classroom, help students understand their work, and inspire some of them to have related careers of their own. Today's students are needed to help find new ways to feed our growing world. FDA regulates the safety of food for both humans and animals, including foods produced from GE plants. Foods from GE plants must meet the same food safety requirements as foods derived from traditionally bred plants. While foods with GE ingredients are sometimes referred to as genetically modified, genetically modified organisms (GMOs), or bioengineered, FDA considers GE to be the more precise term. FDA and the U.S. Department of Agriculture (USDA) work together to help clarify different terms related to modern food biotechnology and how to best inform consumers about the food they choose to grow, purchase, and consume. In 2018, USDA released requirements for how certain foods made with agricultural biotechnology methods should be labeled.

BIOX UNIVERSITY OF BOTANY

Powered by J Gold Fertilizer

FOUNDATIONS OF AGRICULTURE

This module introduces a brief overview of plant domestication, selective breeding, and other agricultural science.

Following underlying key concepts: cell structure and function; cell division, cellular reproduction, and protein synthesis; plant structures, functions, and life cycles; and basic genetic terminology.

ACTIVITY & LAB

The Making of a New Cannabis Cultivar activity helps you examine traits chosen by selective breeding using the Cosmic Crisp®

Cannabis DNA Extraction lab shows that DNA is found in commonly consumed fruit and herbs, just as it is in plants from any living source.

A Brief Introduction to an Altering Plant Characteristics Without Genetic Engineering

Dr. Bill Carrington, Dr. Marc George, and J Gold Department of Cell/Tissue Culture, Department of Molecular/Cellular Endo-Biochemist, University Chicago, Illinois, USA

https://www.heraldopenaccess.us/article_pdf/27/a-brief-introduction-to-an-altering-plant-characteristics-without-genetic-engineering.pdf

BIOX UNIVERSITY OF BOTANY

Powered by 7 Gold Fertilizer

BACKGROUND INFORMATION

Early Agriculture MODULE 1: FOUNDATIONS OF AGRICULTURE There is clear evidence of early, small-scale farming in the Middle East about 23,000 years ago. The seeds of edible cereals, such as wild emmer, wild barley, and wild oats, along with a grinding slab and sickle blades indicate that early humans harvested cereal along the Sea of Galilee. Low oxygen lake sediment preserved the early farming evidence for modern archaeologists to study today. Although exact years are not defined, early agriculture began independently in several areas around the globe. Different societies selected plants to meet their needs and preferences.

Preferred plants could grow to provide sufficient quantities and survive regional climate conditions, including temperature, water availability, and sunlight. Plants that were adapted to local ecosystems and soil were (and still are) most likely to thrive and produce more food for people and livestock. Civilization progressed through the Middle Ages and across the continents. Intercontinental exchanges after 1492 led to the global distribution of many crops. By the 1850s, railroad expansion supported both U.S. settlement and farming distribution across the country. Irrigation, crop rotation, and fertilizer use also enhanced farm production. With the invention of the gasoline-powered tractor in 1892, crop productivity increased significantly as this machine and others replaced much of the human labor. Recently, synthetic fertilizers and pesticides, and more scientific selective breeding have further enhanced agricultural productivity. Throughout time, the goal has been to produce enough food to feed people, livestock, and pets.

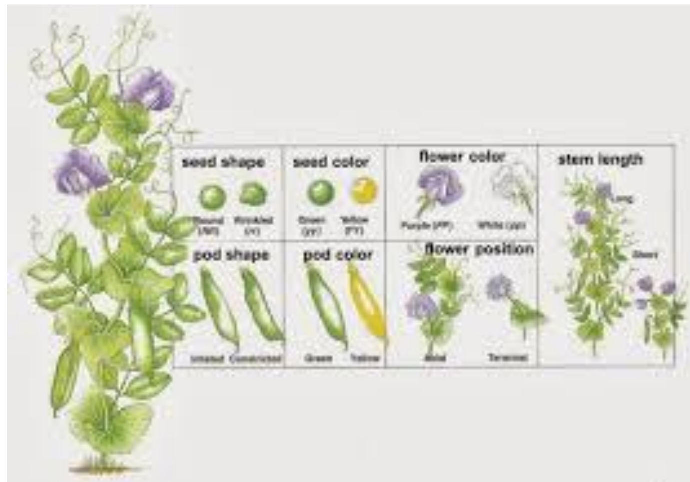
Sample Milestones		
Time	**Region**	**Crop/Livestock**
~ 21,000 B.C.	Levant (Eastern Mediterranean)	Wild emmer, wild barley, and wild oats
~ 9,500 B.C.	Fertile Crescent	Neolithic Founder Crops (emmer wheat, einkorn wheat, hulled barley, peas, lentils, bitter vetch, chick peas, and flax)
~11,000 – 9,000 B.C.	China	Rice, followed by mung, soy, and azuki
~ 11,000 B.C.	Mesopotamia	Pigs, followed by sheep
~ 8,500 B.C.	Turkey and Pakistan	Cattle
~ 8,000 B.C.	North America	Squash, potatoes, and beans
~ 7,000 B.C.	New Guinea	Sugarcane and some root vegetables
~ 5,000 B.C.	Sahel region of Africa	Sorghum
~ 8,000 – 5,000 B.C.	Andes of South America	Potatoes, beans, coca, llamas, alpacas, and guinea pigs
~ 8,000 – 5,000 B.C.	Papua New Guinea	Bananas
~ 4,000 B.C.	Mesoamerica (current Central America)	Maize (teosinte)
~ 3,600 B.C.	Peru	Cotton
~ 3,000 B.C.	Somalia and Arabia	Camels

BACKGROUND INFORMATION

Scientific Advances in Agriculture Humans have been modifying plants for thousands of years through selective breeding. By saving seeds from plants with the traits they desired, indigenous people played a significant role in the domestication of corn with a range of colors, sizes, and uses. As farmers learned more about trait inheritance, they deliberately crossbred and selected plants to improve yield, flavor, and other desirable characteristics. In 1866, Gregor Mendel published his work on the inheritance of pea plant traits. He grew more than 10,000 plants over 8 years and tracked them by number and offspring. He was the first person to identify that traits can be either dominant or recessive. His work went mostly unnoticed for three decades, but it is considered the beginning of modern genetics.

Refresher: Mendelian Laws of Inheritance

1) The Law of Segregation: Each inherited trait is defined by a gene pair. Parental genes are randomly separated to the sex cells so that sex cells contain only one gene of the pair. Offspring therefore inherit one genetic allele from each parent when sex cells unite in fertilization.
2) The Law of Independent Assortment: Genes for different traits are sorted separately from one another so that the inheritance of one trait is not dependent on the inheritance of another.
3) The Law of Dominance: An organism with alternate forms of a gene will express the form that is dominant.
Although Mendel published his work in 1866, it wasn't until the early 1900's that his work was recognized.



While many advances in agricultural production were historically slow, the Green Revolution of the 1950s and 1960s allowed for more rapid increases in food production, specifically using high-yield seed varieties and fertilizer. In the 1960s, Norman Borlaug used selective breeding to significantly increase wheat yields (from 750 kg/hectare to 3,200 kg/hectare). His model was used later for other crops. Throughout the 20th century, more was learned about genetic inheritance. For example, the garden strawberries that consumers buy today resulted from a cross between a strawberry species native to North America and a strawberry species native to South America. In recent decades, certain crop improvements have also resulted from modern biotechnology when targeted changes to a plant's genetic makeup give the plant a new desirable trait. The term GE refers to the genetic modification practices that utilize modern biotechnology. This technology has been used to produce a variety of crops, including some new apple varieties that resist browning associated with cuts and bruises by reducing levels of enzymes that cause browning.

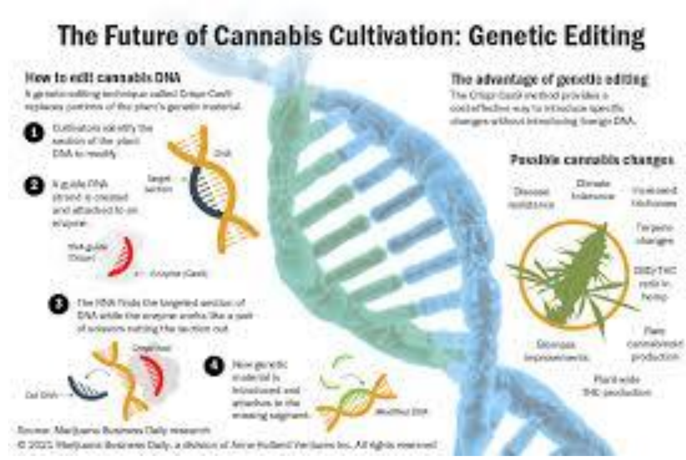
DNA in Our Food We ingest DNA when we eat a plant or animal-derived food. An average meal contains more than 90,000 miles of DNA. Our digestive enzymes break the DNA molecules into smaller molecular components just like they break down proteins, carbohydrates, and fats into smaller molecules that our bodies can use. The DNA in our food does not become our DNA: If we eat an onion, it might give us onion breath, but it won't turn us into an onion.

A Bit About Seeds Some plants grow from seeds. A seed is a unit of reproduction that includes the genetic material and nutrients needed to start a new plant's development. Seed plants fall into two basic groups: Gymnosperms (do not produce flowers) and angiosperms (do produce flowers). The angiosperm flowers develop into fruits that contain seeds (e.g., apples, tomatoes, squash). Most of the food that humans eat comes from angiosperms. Examples of food from gymnosperms include pine nuts and ginkgo. Edible seeds (particularly cereals, legumes, and nuts) are the major source of human calories. Some plants are grown through vegetative reproduction (vegetative propagation). This is a form of asexual reproduction. One form is growing a new plant from a part (a cutting) from another plant, essentially making clones. The cuttings can take root and grow into full plants.

BACKGROUND INFORMATION

Farm Facts

• Two million farms dot the U.S. landscape.
• The average farm feeds 166 people annually.
• Farm and ranch families comprise less than 2% of the U.S. population.
• One acre of land can produce different types of crops, depending on the soil type and fertility, how much rain falls, and how much the sun shines. Typically, one an acre can grow:
– 840 pounds of cotton
– 2,784 pounds of wheat (46.4 bushels)
– 50,000 pounds of strawberries
• There are many agriculture-related careers, including some working with animals, plants, soil, machines, water resources, environmental studies, or technology, as well as some you might not think about like being a florist or beekeeper.
• 98% of all U.S. farms are owned by individuals, family partnerships, or family corporations. Just 2% of America's farms and ranches are owned by non-family corporations.
From the American Farm Bureau Foundation for Agriculture (2019)



Agricultural Terms (for the purposes of this curriculum)

Agricultural Terms (for the purposes of this curriculum) **Agriculture** – The science or practice of farming, derived from the Latin words "ager" (field) and "cultural" (cultivation). **Biotechnology** – Specific techniques used by scientists to modify DNA or the genetic material of a microorganism, plant, or animal in order to achieve a desired trait. (Source: FDA) **Cloning (e.g., potatoes, cannabis, sugarcane)** – Producing genetically identical offspring. **Cross Breeding** – Combining two sexually compatible species, breeds, or varieties to create a new variety with the desired traits of the parents.

Example: The Honeycrisp apple gets its famous texture and flavor by blending the traits of the parents. **Cultivar** – A contraction of "cultivated variety." It refers to a plant type within a particular cultivated species that is distinguished by one or more characters. **Domestication** – The process of breeding for one or more desirable characteristics in plants and animals. This was the first step for humans to move from hunter-gatherer to agricultural societies. **Genetic Modification** – The process of altering the genome of an organism.

Techniques include those used in traditional breeding as well as newer modification methods like genetic engineering. **Grafting** – Inserting a shoot or twig from one plant into part of another rooted plant to selectively grow a specific variety. **Heterosis (hybrid vigor**) – The enhanced function of any biological quality in a hybrid offspring. **Hybridization/Hybrid** – The offspring of two plants of related species or different varieties. **Precision Agriculture (PA)** – An approach to farm management that uses information technology, e.g., drones and GPS data, to ensure that the plants and soil receive the exact amount of water and other nutrients for optimum health and productivity. The goal of PA is to ensure profitability, sustainability, and protection of the environment.

Selective Breeding – A breeding method that uses organisms with specific desired traits to produce the next generation. There is evidence that by 5,000 B.C. humans had some understanding of inheritance and selectively bred more useful varieties of wheat, maize, rice, and dates.

Marijuana, also spelled marihuana, crude drug composed of the leaves and flowers of plants in the genus *Cannabis*. The term *marijuana* is sometimes used interchangeably with *cannabis*; however, the latter refers specifically to the plant genus, which comprises *C. sativa* and, by some classifications, *C. indica* and *C. ruderalis*. Marijuana is known by a variety of other names, including *pot*, *tea*, *grass*, and *weed*. It is usually dried and crushed and put into pipes or formed into cigarettes (joints) for smoking. It can also be added to foods and beverages.

Cannabis Genome

Breeders & growers choose *Cannabis* plants with desirable characters phenotype related to flowers cannabinoid profile & production Phenotype is a product of genotype & environment *Cannabis* is considerably variable & extraordinarily plastic in response to varying environmental conditions There are currently no baseline genotypes for any strains but steps should be taken like contacting #jgold to ensure products marketed as a particular strain are genetically congruent Genetic inconsistencies may come from both suppliers & growers of *Cannabis* clones & seed because currently, they can only assume the strains they possess. Various suggestions for naming the genetic variants do not seem to align with the current widespread definitions of Sativa Indica Hybrid & Hemp Clarke & Merlin 2018 As our *Cannabis* knowledge base grows so does the communication gap between scientific researchers & the public Currently, there is no way for *Cannabis* suppliers growers or consumers to definitively verify strains. If genetic differentiation of the commonly perceived Sativa & Indica types previously existed it is no longer date in the neutral genetic markers used here Extensive hybridization & selection have presumably created a homogenizing effect & erased evidence of potentially divergent historical genotypes. Studies have shown that in neutral genetic markers, there is no consistent genetic differentiation between the widely held perceptions of Sativa & Indica *Cannabis* types. Moreover, the genetic analyses do not support the reported proportions of Sativa & Indica within each strain, which is expected given the lack of genetic

Cannabis is a fascinating plant that produces fiber, edible seed, oil, and numerous cannabinoids such as THC, CBD, CBN. It is also unique in that no other plants that we know of have the capacity to produce cannabinoids; the genes that encode the enzymes required to produce cannabinoids are unique to the cannabis genome. The main difference between the potency and medical benefits of the cannabinoids found in marijuana can be attributed to a single enzyme, or a genetically encoded switch, at the last step in the cannabinoid pathway. This "switch," which is called the THCA synthase and CBDA synthase enzyme, folds precursor molecules (Cannabigerolic acid) into either THCA or CBDA. Plants that produce high levels of THC express genes that code for hyperactive versions of the enzyme THCA synthase, whereas those plants that code for the enzyme CBDA synthase produce more CBD. The sequencing readout below indicates that there are numerous SNPs in the THCA synthase gene, which would be one key driver behind the genetic differences of the cannabis plants.

An alternative model is that *THCAS* and *CBDAS* are closely linked (i.e., adjacent on a chromosome), and one or the other is inactivated in drug-type or hemp strains. This model was motivated by the discovery of a *THCAS*-like gene in hemp plants (Kojoma et al. 2006) and is consistent with the possibility that these related genes are derived from ancient tandem duplication. In addition, physical linkage of genes involved in specialized metabolic pathways has been repeatedly observed in plants, similar to operons in bacterial genomes (Nützmann and Osbourn 2014); such a cluster was recently described for benzylisoquinoline alkaloid biosynthesis genes in opium poppy (Guo et al. 2018). It is unknown whether genes involved in cannabinoid biosynthesis are clustered, although genetic analyses have previously indicated that at least one locus unlinked to *THCAS*/*CBDAS* contributes to cannabinoid content (Weiblen et al. 2015).

The draft genome and transcriptome of *C. Sativa* described in 2011 (for a female plant of the drug-type strain Purple Kush [PK] and resequencing of a plant of the hemp variety "Finola" [FN]) (van Bakel et al. 2011) was unable to discriminate between these models due to high fragmentation. The *C. Sativa* draft genome assembly, done largely with Illumina sequencing, was composed of 136,290 scaffolds, with an N50 of 16.2 kb. It was subsequently demonstrated that ~70% of the *C. sativa* draft genome is composed of repetitive sequences (Pisupati et al. 2018).

Measurement of single-nucleotide variants (SNVs) in four strains showed rates of heterozygosity ranging from 0.18%–0.26% and revealed that the drug-type and hemp-type strains were well separated by SNVs; the rate of occurrence of SNVs between these types was as high as 0.64% (van Bakel et al. 2011). Cytogenetic analysis has furthermore suggested a high degree of inter-and intracultivar karyotype polymorphisms (i.e., differences in homologous chromosomes that can be observed by microscopy), at least among hemp varieties (Razumova et al. 2016), which may further complicate genome assembly. To address these complications and to simultaneously leverage the high rate of SNVs between PK and FN, we coupled Pacific Biosciences (PacBio) long-read single-molecule real-time (SMRT) sequencing of PK and FN with Illumina resequencing of 99 F1 progeny between the two in order to generate a combined genetic and physical map. The combined map provides new insights into the arrangement of the chromosomes and the cannabinoid biosynthetic genes, including the discovery of substantial rearrangement and gene duplications at the closely linked *THC* and *CBD acid synthase* gene loci.

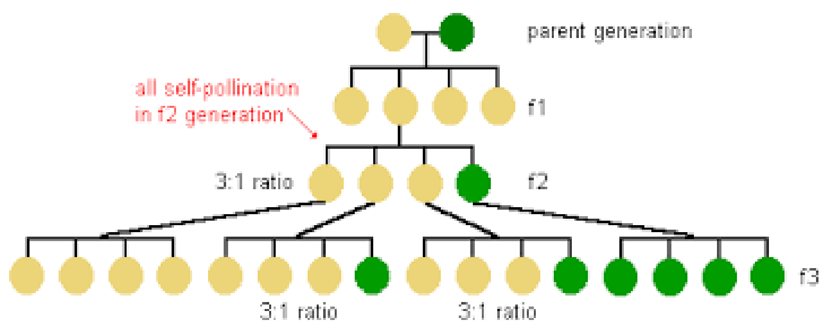
Cannabis is diploid and has 10 chromosomes (2n=20) and an XY sex chromosome system (**Divashuk et al. 2014**). While dioecious genetics is preferred for cannabinoid production, hermaphroditic traits still circulate in the drug type varietals, complicating the as production of cannabinoids. Since pollinated female flowers produce lower levels of cannabinoids and terpenes, male drug-type plants are visually or genetically tested and culled from grows to prevent pollination of female plants. Visual sexual differentiation occurs in the midlife cycle of the plant, while genetic screening can eliminate males earlier to conserve expensive indoor growing real estate. Female plants with hermaphroditic tendencies are more difficult to detect and remove using visual or genetic screening methods.
To avoid the propagation of Y chromosomes in naturally-crossed cannabis seeds, indoor growers often resort to tissue culture or cloning of female mother plants. This can lead to monocultures and increased risks associated with pathogen exposure (**Wally and Punja 2010**). Another approach more common in outdoor cannabidiol acid (CBDA) production utilizes the induction of hermaphroditism with silver nitrate or silver thiosulfate and ethephon treatment (an ethylene blockers and ethylene mimetic respectively). These phytohormone modulators reverse the sex phenotype of some female and male varietals respectively. Only sex reversal of female plants with ethylene blockers results in pure XX pollen production (**Mohan Ram and Sett 1982**). Application of XX pollen to female flowers results in 'feminized' seeds but can also increase the incidence of plants with hermaphroditic capacity. The hermaphroditic capacity of varietals is believed to be a heritable trait but this trait has yet to be mapped to any genomic coordinates. Sex chromosomes in flowering plants usually evolve from autosomes (**Harkess et al. 2016**; **Harkess et al. 2017**). Likewise, modern monoecious hemp varietals tend to decay into dioecious varietals with inbreeding but little is known about their chromosomal structures (**Clarke 2017**).

Cannabinoid and terpene production by plants is linked to both attraction of pollinators and responses to plant pathogens (**Penuelas et al. 2014**; **Andre et al. 2016**; **Allen et al. 2019**; **Lyu et al. 2019**). Cannabis plants are classified into 5 categories reflecting their THCA, CBDA, and CBGA expression. Type I plants express predominantly THCA. Type II plants express a near equal mixture of THCA and CBDA. Type III plants express predominantly CBDA, while Type IV plants express neither CBDA nor THCA and predominantly express the CBGA precursor. Type V plants express no cannabinoids. Despite successful breeding efforts to deliver higher cannabinoid and terpene content, plant pathogens are still a significant contributor to crop loss in cannabis production due to the lack of disease-resistant varieties (**Kusari 2013**; **Backer et al. 2019**). Many jurisdictions mandate cannabis microbial safety testing targeting epiphytic and endophytic plant pathogens that have been clinically linked to aspergillosis in immuno-compromised cannabis patients (**McKernan et al. 2015**; **Remington et al. 2015**; **McKernan et al. 2016**).

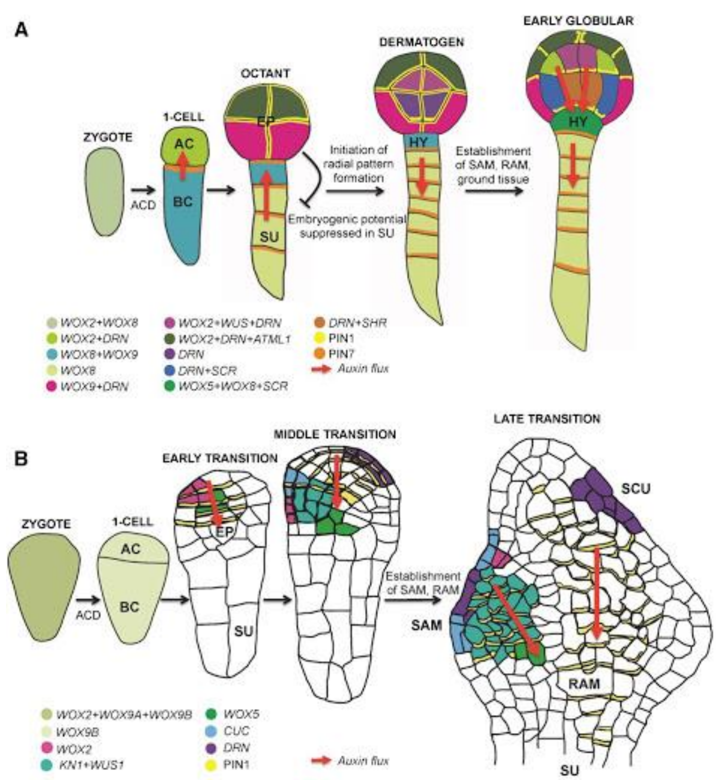
To better understand cannabis sex evolution, cannabinoid expression and pathogen resistance, we sequenced and assembled male and female cannabis genomes (cultivar 'Jamaican Lion mother' and 'Jamaican Lion father') with their offspring (JL1 – JL6) to identify the Y chromosome. We further annotated these references with full-length mRNA (Iso-Seq) sequencing of 5 tissues (female flowers, female seeded flowers, male flowers, female leaves, and female roots) and *in silico* gene model predictions using MAKER2 (**Cantarel et al. 2008**; **Campbell et al. 2014a**; **Campbell et al. 2014b**; **Law et al. 2015**). To confirm the 118Mb list of putative Y contigs, we utilized Illumina's NovaSeq platform to whole-genome sequence 40 hemp and drug-type cultivars. Using the coverage maps of 9 male genomes, we were able to confirm putative Y contigs and male-specific genes (**Prentout 2019**). We further classified these variants to identify highly damaging mutations in protein-coding regions of the genome. Additionally, these whole-genome sequence data were utilized to assess copy number variation (CNV) in critical genes in the terpene synthase pathway, cannabinoid synthase pathway, and the pathogen response pathway (**Pisupati et al. 2018**). This refined map of protein-coding variants and copy number variants can facilitate directed breeding efforts for desired chemotypes, pathogen resistance, and a better understanding of rare cannabinoid synthesis (**Citti 2019**).

Genetics

The modern science of genetics began during the mid1800s with the work of Gregor Mendel in what is now the Czech Republic. Mendel experimented with ordinary garden pea plants that were true-breeding, which means that the flowers were mostly self-pollinating, and producing offspring identical to the parents. In other words, the offspring of true-breeding tall pea plants would all be tall, and the offspring of true-breeding short pea plants would all be short. Mendel also discovered that some of the pea plant's alleles were dominant, while others were recessive. A pea plant that was a true-breed for tallness would have two alleles for tallness; and, conversely, one that was a true-breed for shortness would have two alleles for shortness. To learn more about how traits were passed from parents to offspring, Mendel decided to cross-pollinate true-breeding tall plants with true-breeding short plants. To his surprise, all the offspring were tall. When he crossed these offspring, the plants produced were either tall or short. He observed multiple traits that had two forms, e.g., height (tall or short), pea color (green or yellow), seed shape (smooth or wrinkled). Further study of garden peas and their traits led Mendel to the conclusion that some traits have the ability to mask other traits. He called these traits dominant and those that were masked, recessive.



However, in reality, not all traits behave as dominant and recessive. In some cases, the traits may express incomplete dominance where neither trait is dominant or recessive; and, the expressed trait is somewhere between the two traits. For example, some crossbred red and white flowers have pink flower offspring. In other cases, both the dominant and recessive traits may be expressed. This situation is called codominance. A sweet apple variety crossed with a tart apple variety may yield an apple variety that is both sweet and tart. The techniques that Mendel used in the 19th century in studying genetics are still in use today.



DNA BACKGROUND INFORMATION

DNA Deoxyribonucleic acid (DNA) is a molecule that contains the genetic instructions used in the development and functioning of all organisms and some viruses. Strands of DNA are divided into segments called genes. All organisms have genes that determine various biological traits, some of which are visible and some of which are not. Many genes, in turn, provide the information for making proteins, which carry out specific functions. This incredible molecule can easily be seen with the naked eye when collected from thousands of cells. In this activity, students will extract strands of DNA from the nuclei of Cannabis cells.

Cannabis cells are used because their cells are easy to break open and they have lots of DNA. Their cells are octoploid, which means they have eight copies of each chromosome. Human cells are diploid (have two sets of chromosomes). First, the students gently mash the strawberries to break the cells' walls and expose the inner membranes. Next, the cells are mixed with the DNA extracting buffer, which is a mixture of soap, salt, and water. The soap dissolves the lipid bilayers of the cellular and nuclear membranes, exposing the DNA.

The salt breaks up the protein chains that bind around the nucleic acids in the DNA. When the mixture is filtered, the cannabis cell parts that are larger than DNA are separated from the DNA. Adding chilled rubbing alcohol to the filtered solution causes the DNA to precipitate out of the solution and become visible. It is important to keep the alcohol cold, because the colder the alcohol, the less soluble the DNA. The other cell parts are soluble in chilled alcohol.

How is DNA from Food Used by Scientists? Scientists can use DNA isolated from food to identify particular traits, a plant or animal species, or potential contamination sources. • Agricultural researchers use DNA analysis to choose desired traits that can be propagated in plants and animals.

Uses of Genetics in Plant Breeding

- Backcrossing: This is a quality control step in the selective breeding process. Because a hybrid the cross can result in the inheritance of desired and unwanted traits as well as the loss of desired traits, breeders cross the offspring of hybrid crosses with the preferred parent until the offspring has the desired traits but not the unwanted traits.
- Inbreeding: Some plant species may be fertilized by themselves and produce an inbred variety that is identical from generation to generation. The fact that it preserves the original traits make it useful for research, as new true-breeding cultivars, and as the parents of hybrids.
- Hybrid breeding: Two different inbred varieties can be crossed to produce offspring with stable characteristics and hybrid vigor, where the offspring is much more productive than either parent.
- Mutation breeding: Mutations in a plant's genome occur naturally and can result in desirable traits. Mutation breeding is the induction of genetic mutations by exposing plant cells to radiation or certain chemicals and then selecting for plants with desirable traits.
- Molecular marker-assisted selection: Molecular markers are DNA sequences that 'mark' locations on a genome. Breeders use molecular markers linked. to desirable traits to genetically screen and select plants for breeding.

CANNABIS DNA EXTRACTION ACTIVITY

MATERIALS and Method

- JGOLD buffer: 2% cetyl trimethylammonium bromide, 1% polyvinyl pyrrolidone, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, or JGOLD Extraction Buffer
- Centrifuge (up to 14,000 x g)
- RNase A Solution
- Isopropanol
- 70% Ethanol
- 2 ml centrifuge tubes
- SpeedVac
- TE Buffer (10 mM Tris, pH 8, 1 mM EDTA)

Method

Plant samples can be prepared by cryogenically grinding tissue in a mortar and pestle after chilling in liquid nitrogen. Freeze-dried plants can be ground at room temperature. In either case, a fine powder is best for extracting DNA.

1. For each 100 mg homogenized tissue use 500 µl of JGOLD Extraction Buffer. Mix and thoroughly vortex. Transfer the homogenate to a 60°C bath for 30 minutes.
2. Following the incubation period, centrifuge the homogenate for 5 minutes. at 14,000 x g.
3. Transfer supernatant to a new tube. Add 5 µl of RNase solution A and incubate at 37°C for 20 minutes.
4. Add an equal volume of chloroform/isoamyl alcohol (24:1). Vortex for 5 seconds then centrifuge the sample for 1 minute at 14,000 x g to separate the phases. Transfer the aqueous upper phase to a new tube. Repeat this extraction until the upper phase is clear.
5. Transfer the upper aqueous phase to a new tube. Precipitate the DNA by adding 0.7 volume cold isopropanol and incubate at -20°C for 15 minutes.
6. Centrifuge the sample at 14,000 x g for 10 minutes. Decant the supernatant without disturbing the pellet and subsequently wash with 500 µl ice-cold 70% ethanol. Decant the ethanol. Remove residual ethanol by drying in a SpeedVac.
7. Dry the pellet long enough to remove alcohol, but without completely drying the DNA. Dissolve DNA in 20 µl TE buffer (10 mM Tris, pH 8, 1 mM EDTA). The pellet may need warming to dissolve.

JGOLD buffers are widely used to isolate DNA from plants as they are effective for removing polysaccharides and polyphenol contaminants. Most JGOLD protocols simply precipitate DNA which is followed by chloroform extraction. However, by incorporating silica spin columns into the protocol, higher purity DNA can be isolated. Spin columns have a glass fiber filter that captures DNA so that it can be more stringently washed. DNA is easily eluted with water of TE buffer. This added step results in cleaner DNA than extractions without spin columns.

Material

- 200 mg leaf tissue
- JGOLD Extraction Buffer (OPS Diagnostics)
- RNase A Solution (10 mg/ml in water, boiled to be DNase free)
- Chloroform/Isoamyl alcohol (24:1)
- Isopropanol (2-propanol)
- 70% ethanol (Ethyl alcohol)
- TE buffer (10 mm Tris, pH 8, 1 mM EDTA)
- Spin Columns (OPS Diagnostics)

Protocol

1. Using a mortar and pestle, leaf tissue was cryogenically pulverized, and 200 mg added to a 2 ml microcentrifuge tube.
2. JGOLD Extraction Buffer (500 µl) was added, mixed, and then placed in a water bath for 30 minutes at 60°C. Once removed the tubes were centrifuged for 10 minutes at 13,000 x g.
3. The supernatant was transferred to a new tube where 5 µl of RNase A was added. The tubes were incubated at 37°C for 15 minutes.

4. The lysate was extracted by adding an equal volume of chloroform/isoamyl alcohol, vortexed, and then centrifuged. The upper aqueous layer was transferred to a new microcentrifuge tube. This step was repeated untthe il upper phase was clear.
5. The DNA was precipitated by adding 0.7 volume of isopropanol and then incubated at -20°C for 15 minutes.
6. The sample was transferred to a spin column and centrifuged for 1 minute at 8,000 x g to bind the DNA to the column.
7. The column was washed twice with 250 µl 70% ethanol, centrifuging after each wash.
8. The DNA was eluted in 20 µl to 100 µl of water or TE buffer and collected in a clean microfuge tube.

PRACTICE WITH STRAWBERRY FIRST DNA EXTRACTION ACTIVITY

ADVANCE PREPARATION - two is ideal for this activity. Make a copy of the Strawberry DNA Extraction worksheet for each student. Chill the isopropyl alcohol in the freezer. It is important for the alcohol to be as cold as possible. Note: If ethanol is available, it does not need to be chilled. If preferred, you can prepare a stock extraction buffer by mixing ½ gallon (2 L) of water with ½ cup (120 mL) of clear, good quality dishwashing liquid and 2 tablespoons (30 mL) of non-iodized table salt. Slowly mix the buffer, being careful not to produce any bubbles. Too many bubbles will prevent the extraction buffer from extracting as much DNA as possible. Each group would need 10 mL of this buffer. It is best if the buffer is made at least a day ahead of time.

Instructions:
STRAWBERRY DNA EXTRACTION ACTIVITY

If fresh strawberries are being used, remove the green leaves.

1. Place the strawberries in the plastic bag and seal it, being careful to eliminate as much air as possible. Gently smash the berries for about two minutes; be very careful not to crush the bag. Make sure the berries are. completely crushed because this starts to break open the cells and release the DNA.

2. Prepare the DNA extraction liquid by mixing 2 teaspoons (10 mL) of detergent, 1 teaspoon of salt, and ½ cup (100 mL) of water in one of the plastic cups. Stir the mixture very carefully so there are no bubbles; the bubbles might interfere with the precipitation of the DNA.

3. Add 2 teaspoons (10 mL) of the DNA extraction liquid to the bag with the strawberries. This will further break down the membranes and release the DNA strands.

4. Reseal your plastic bags and carefully eliminate as much air as possible. Gently smash the berries for another minute; be sure to avoid creating any bubbles because. they will prevent the extracting buffer from extracting as much DNA as possible.

5. Place the funnel inside the second plastic cup and place the cheesecloth inside the funnel. Open the bag and pour the strawberry mixture into the cheesecloth.

6. Twist the cheesecloth just above the liquid and gently squeeze the remaining liquid into the cup. After filtering the mixture, dispose of the cheesecloth and the plastic bag.

7. Note the level of the liquid in the cup; slowly add an An equal amount of chilled rubbing alcohol to the cup, layering the alcohol on top of the strawberry liquid. This can be done by tilting the cup and slowly pouring the alcohol down the side of the cup. The DNA has just been isolated from the rest of the material contained in the cells of the strawberry.

8. Wait a few minutes and then carefully observe the line between the strawberry mixture and the alcohol. Notice the development of a white, threadlike, cloud at this line. This is the strawberry DNA. The DNA will clump together and float to the top of the alcohol layer.

9. Observe the other groups' DNA samples; are there any differences?

10. Use the craft sticks or spoons to slowly extract the DNA from the cup.

11. Clean up your workstations.

GENETIC ENGINEERING IN AGRICULTURE

This module introduces students to laboratory methods used to alter genetic material and create organisms with desired traits.

For this module, it is recommended that teachers will have already taught students the following underlying key concepts: Cell structure and function, DNA structure, DNA replication, bacterial structure (prokaryotes), basic cell division and reproduction, transcription, translation, protein expression, and synthesis, and general selective breeding.

. Genetic Engineering introduces some key milestones in the development of tools used in the laboratory to change DNA sequences. It also highlights select genetic modification processes.

Genetic Engineering introduces students to laboratory techniques developed to change an organism's genetic material to give it a new trait.

Targeted Genome Editing discusses cutting-edge technology now being used to "edit" DNA.

ACTIVITY

CRISPR-Cas – A Genome Editing System allows students to explore how one technology is used to target a specific DNA locus to delete, change, or insert DNA sequences. Genome editing systems can be either transgenic or work without inserting DNA from another organism.

GENETIC ENGINEERING BACKGROUND INFORMATION

BIOX UNIVERSITY OF BOTANY

Genetic Engineering

For most of history, farmers had to wait for several plant generations before crops had the traits they most desired. The farmers used selective breeding, the process of choosing parent plants with the best traits over many generations. Selective breeding resulted in dramatic genetic changes to the species. While earlier farmers had no concept of the science of genetics, selective breeding based on observable traits allowed them to use plants' DNA to solve agricultural challenges and to improve the food supply.

Although selective breeding is still widely used, there are more modern processes available to alter the genetics of microorganisms, plants, and animals. More modern techniques to alter an organism genetic a includes mutation breeding, molecular marker-assisted breeding, genetic engineering, and genome editing. Genetic engineering (GE) refers to deliberately modifying the characteristics of an organism by altering its genetic material. GE techniques include particle bombardment, Agrobacterium-mediated transformation, and targeted genome editing (the most recent additions to the genetic engineer's toolbox). Using GE technology, scientists can bring us improved agricultural products and practices faster than in the past.

Why genetically engineer plants?

Plants are genetically engineered for many of the same reasons that selective breeding is used: Better nutrition, higher crop yield (output), greater resistance to insect damage, and immunity to plant diseases. Selective breeding techniques involve repeatedly crossbreeding plants until the breeder identifies offspring that have inherited the genes responsible for the desired combination of traits. However, this method may also result in the inheritance of unwanted genes responsible for unwanted traits (called linkage drag), and it can result in the loss of desired traits.

GE techniques can be used to isolate a gene or genes for the desired trait, add a gene from another organism or edit chromosomal DNA in a single plant cell, and generate a new plant with the trait from that cell. By adding one desired gene from the donor organism or by editing the gene in the chromosomal DNA of the single cell, the unwanted traits from the donor's other genes can be excluded. GE is used in conjunction with selective breeding to produce GE plant varieties that are on the market today.

Key biotechnology events related to food agriculture

- 1901 Japanese biologist Shigetane Ishiwatari discovered Bacillus thuringiensis (Bt), which makes a natural pesticide, found in soil worldwide and used by farmers since the 1920s.
- 1919 Karoly Ereky introduced the new term biotechnology (i.e., using biological systems to create products).
- 1971 Paul Berg completed a landmark gene splicing experiment. 1973 Stanley Cohen and Herbert Boyer created the first modified organism using recombinant DNA (rDNA) technology.
- 1974 Rudolf Jaenisch and Beatrice Mintz created the first transgenic animal (a mouse).
- 1978 Herbert Boyer starts a new company, Genentech and produces recombinant insulin.
- 1983 Mary-Dell Chilton inserted an antibiotic-resistant gene into a tobacco plant creating the first GE plant.
- 1987 Calgene creates the FlavrSavr® tomato.
- 1989 Chymosin from GE microorganisms authorized as a food processing aid by FDA.
- 1994 FDA concludes the FlavrSavr® tomato is as safe as comparable non-GE tomatoes.
- 1995 EPA approves the use of a Bt toxin as a plant-incorporated pesticide in a GE crop.
- 1998 GE virus-resistant papaya was grown commercially in Hawaii.
- 2012 CRISPR-Cas9 is used as a programmable RNA-guided DNA cutting tool.
- 2015 Genetically modified salmon is the first GE animal approved for food use in the United States.
- 2017 GE apples are available for sale in the United States.
- 2019 FDA completes consultation of high oleic soybean oil, first food from a genome-edited plant.

Advanced Content

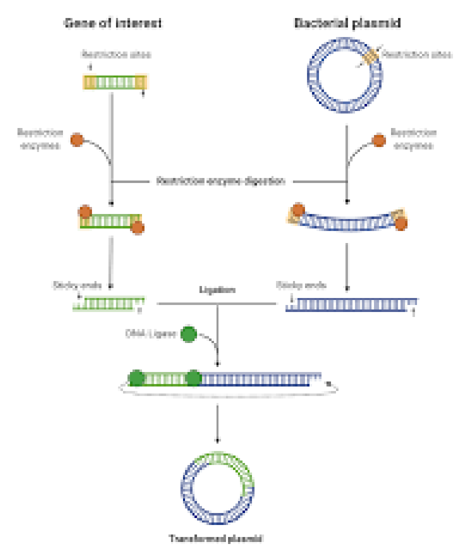
GE techniques can be used to add new DNA to code for the expression of a new protein or to suppress expression of a native plant protein. Protein suppression can be achieved through transcriptional or post-transcriptional gene silencing (PTGS). RNA interference (RNAi) is a form of PTGS that targets mRNA transcripts for cleavage, preventing their translation into protein. The two different ways to achieve a desired trait are important, because both have to be.

Development of GE Tools in Bacteria

Throughout the past 100 years, several developments have led to current GE methods. After early geneticists were able to identify the gene locus for specific plant traits, various methods were used to try to transfer the specific DNA sequence from one plant to another. One method was injecting the DNA from the donor plant directly into the recipient plant cell to see if it would integrate into the recipient cell's genome. Unfortunately, the DNA was degraded, and the method was unsuccessful. It was like trying to send an envelope through the mail with only a zip code; the postal service wouldn't know where to deliver it. Scientists eventually used bacteria to transfer new DNA to the recipient plant cell. Transformation is the changing of the cell's genetic makeup through the addition of new DNA. The DNA can come from the environment surrounding the cell via "horizontal gene transfer" or be added in a laboratory through GE methods. The laboratory method developed to combine genetic sequences that would not otherwise be found in the genome is called recombinant DNA (rDNA) technology. In 1973, Herbert Boyer and Stanley Cohen produced the first successful GE organism.

Boyer had expertise using restriction endonucleases (enzymes that cut DNA at specific nucleotide sequences), and Cohen studied plasmids (small rings of DNA) in bacteria. They were able to use a restriction enzyme to cut open a plasmid loop from one bacterial species, insert a gene from a different bacterial species, and close the plasmid, which combined the genes from different bacteria into one rDNA molecule. An enzyme called ligase was used to help join the cut DNA strand. Then they transformed this rDNA plasmid into the bacterium Escherichia coli (E. coli) and showed that the bacteria could utilize the rDNA. In Boyer and Cohen's experiment, one gene coded for tetracycline resistance and the other for kanamycin resistance. Tetracycline and kanamycin are antibiotics that kill bacteria that do not have resistance genes. It was possible to see which of the E. coli in their experiment had successfully acquired the new genes by culturing them in the presence of the antibiotics, where only the successfully transformed bacteria could grow. These experiments showed that bacterial transformation could be used to deliver the desired DNA to a useful site, just as the postal service delivers mail to the correct address.

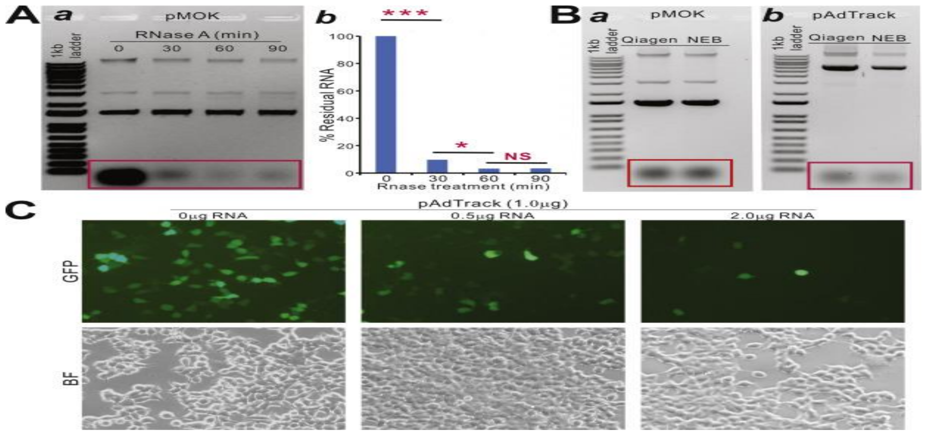
Bacterial transformation still serves as the basis for a number of DNA technolog the laboratory for rDNA research. There are even some species of bacteria th process naturally, but most bacteria needs manipulation to become compete Using the techniques from bacterial transformation, scientists have learned ho including plants that we use for food. Scientists worldwide continue to use the improve GE tools that develop, modify, and improve consumer products, incl eat.



Nature's Own Genetic Engineer

A widely used method of transferring a transgene to a plant is to use the soil b tumefaciens (A. tumefaciens). This bacterium has a natural ability to enter a p plant's genome. A plasmid is constructed to include A. tumefaciens genes ne recipient plant cell, the transgene of interest, and a selectable marker, such a resistance or herbicide tolerance. Scientists now use the bacterium's natural b plant's genome.

GENETIC ENGINEERING BACKGROUND INFORMATION



Application of GE Tools in Plants

Plants can be genetically engineered to be resistant to pests and herbicides, to increase crop yield, or to tolerate adverse weather conditions using a process similar to bacterial transformation. Plants can also be engineered to produce fruits and vegetables that have longer and more stable shelf-lives in the grocery store. These GE uses have potential trickle-down benefits from the farmer to consumers, animals, and the environment. Because plants are eukaryotic and contain a nucleus, a slightly different method than the one used for bacterial transformation is used to insert the gene of interest. For example, if scientists find a gene for enhanced drought resistance in a plant, and they want to use the gene to make another plant more drought resistant, an advantage of GE over selective breeding is that less time is required, and linkage drag is avoided. The desired gene to be transferred and added to the genome of the recipient plant is often referred to as a transgene.

The technologies used to clone or synthesize genes are changing and evolving. The three major methods currently used are:

• Traditional cloning – isolating DNA directly from the genome of the donor organism and inserting it into a plasmid for later use • Subcloning the gene of interest – copying the gene from an existing collection of DNA clones ("DNA library")

• De novo gene synthesis – building a gene from scratch, using single nucleotides or short oligonucleotide strands without the need for a physical template

The techniques used by scientists to assemble and insert DNA pieces into the plasmid are also evolving along with the complexity of multi-gene DNA constructs. While simple restriction enzyme protocols can be used to create a single gene insert, multi-gene constructs such as those required for complex plant traits require more complex assembly strategies.

Genetic Engineering

• Allows the direct transfer of one or just a few genes between either closely or distantly related organisms
• Achieves crop improvement in a shorter time compared to conventional breeding
• Allows plants to be modified by adding, removing, or switching off particular genes

Adapted from: Agricultural Biotechnology (A Lot More than Just GM Crops).
www.isaaa.org/resources/publications/agricultural_biotechnology/download/Agricultural_Biotechnology.pdf

What is a DNA Library?

A DNA library is a collection of cloned DNA fragments that are stored in plasmids, which in turn are maintained and propagated in bacterial or yeast cells. The type of library is classified by the source of the DNA and the plasmid – referred to as a cloning vector – used to construct the library. Sources of DNA may be a single cell, a tissue, an organism, or an environmental sample containing multiple organisms. The DNA may be obtained from genomic sequences or from isolated mRNA and converted to complementary DNA (cDNA). Scientists use DNA libraries to find and study DNA encoding proteins or other functions of interest.

General Plasmid Preparation

Bacterial plasmids are used to store a ready supply of the gene of interest. In the case of Agrobacterium-mediated plant transformation, the plasmids are used to transfer the gene of interest to the genome of the recipient plant. To receive the gene of interest, the bacterial plasmids are treated with a restriction enzyme that is compatible with the gene. This way, the plasmid DNA will have the same sticky ends as the gene, so they will combine more easily. The gene and plasmid DNA preparations are mixed with DNA ligase to seal the sticky ends of the DNA molecules together. Scientists may also modify the bacterial plasmid using a similar process to insert one or more selectable marker genes. The selectable marker genes will be important later in the GE process when bacteria or plant cells with the gene of interest are being isolated. There are many selectable markers used to screen for bacterial, as well as plant transformants.

Selectable markers include:

- Auxotrophy (selects for the ability to grow on certain carbon sources)
- Antibiotic resistance (selects for ability to grow in the presence of a specific antibiotic)
- Herbicide tolerance (selects for ability to grow in the presence of a specific herbicide)

This new bacterial plasmid is called a transformation plasmid and has the gene of interest as well as the selectable marker gene. The transformation plasmid is added to bacteria using a bacterial transformation method. Finally, the bacteria are plated onto a medium containing the selection factor that will inhibit the growth of bacteria that did not take up the plasmid. The Petri plates are incubated to encourage bacterial growth, and only the bacteria that have taken up the transformation plasmid with the selectable marker gene will grow. Bacteria without it will not grow, resulting in millions of bacteria with the gene of interest in their DNA. The next step is to transfer the gene to the plant cells. Currently, the most frequently used technique is Agrobacterium-mediated transformation. Bombardment with a gene gun is less common and typically used in cases where Agrobacterium-mediated methods don't work.

Agrobacterium is a plant pathogen that has the natural ability to transfer DNA to plant cells. GE methods use a version of the Agrobacterium plasmid that has been "disarmed": the modified plasmid still has the ability to transfer DNA into the plant's genome, but it's disease-causing genes have been removed. Agrobacterium that have been transformed with the plasmid carrying the gene of interest and selectable marker are mixed with the plant cells. The Agrobacterium enters the plant cells and inserts a segment of the plasmid DNA (containing the gene and selectable marker gene) into the plant's genome. Once the Agrobacterium has had time to transform the plant cells, the cells are placed on medium containing: (1) An antibiotic that kills the Agrobacterium, (2) the selection factor that will inhibit growth of plant cells that did not take up the plasmid DNA, and (3) plant hormones that encourage the transformed cells to grow into new plants. After a gene has been successfully inserted into the plant's genome, the modified plant must be able to grow and reproduce with its newly modified genome. First, the genotype of the plant must be studied so that the scientists only grow plants in which the genome has been modified correctly. When this is done, the GE plants will be grown under controlled conditions in a greenhouse and then in field trials to make sure that the new plants possess the desired new trait and show no new undesired characteristics.

Food from GE Plants

The first GE plant evaluated by the FDA for human consumption was the FlavrSavr® tomato. FDA concluded that the FlavrSavr® tomato was as safe as comparable non-GE tomatoes. It was brought to market in 1994, but it was not sufficiently profitable to continue production. Although there are currently no GE tomatoes on the market, other GE food crops are commercially available. Most of these GE plants were engineered to increase resistance to disease or pests, or tolerance to specific herbicides. As of 2019, there were 10 GE food crops available in the U.S. Of these, only a few GE crops in the grocery store are available as whole produce. Whole produce could include certain cultivars of apple, potato, papaya, sweet corn, and squash. Ingredients derived from GE corn, soybeans, sugar beets, and canola (such as flour, oil, starch, and sugar) are used in a wide variety of foods including cereal, corn chips, veggie burgers, and more. The 10 GE crops today are: Alfalfa, apples, canola, corn (field and sweet), cotton, papaya, potatoes, soybeans, squash, and sugar beets. Animal food: In the United States, more than 95 percent of food-producing animals consume food containing ingredients from GE crops. GE plants can also be found in food for non-food producing animals, such as cats and dogs.

ACTIVITY 1: GENETIC ENGINEERING

ACTIVITY AT A GLANCE

In addition to selective breeding, GE tools are used by plant breeders to solve agricultural challenges, such as producing enough food to feed a growing global population or minimizing production impacts on our environment. Plants have been engineered to be more nutritious, more resistant to pests, drought tolerant, and more robust to remain intact during packing and transport. In this activity, students will review the process of bacterial transformation and then look at the processes involved in creating GE plants.

GET STARTED

MATERIALS

- Computer and internet access for the teacher and students
- Genetic Engineering worksheet
- Set of The Genetic Engineering Process cards (10 cards with illustrations and 10 cards with GE process steps). See pages 32-33.
- Mailing Labels – 10 to a sheet; 2 sheets for each set of cards
- 3” x 5” index cards
- Chart paper
- Double-sided tape

ADVANCE PREPARATION

1. Divide the class into small groups.
2. Make a copy of the Genetic Engineering worksheet for each student.
3. Make The Genetic Engineering Process cards. To make one set of cards, copy the 10 steps in The Genetic Engineering Process on one sheet of 2” x 4” mailing labels and the illustrations for those steps on another sheet of labels. Attach the labels to 3” x 5” index cards. You could also copy the templates on card stock. Making sets in different colors helps keep the sets together. (Make one set of cards for each group.)

Alternatively, print the card art and text boxes using only one side of each sheet of paper, and cut the sections out for students to compare and match up.

Remember to mix (shuffle) the cards before handing them out.

GENETIC ENGINEERING

BIOX UNIVERSITY OF BOTANY
Powered by J Gold Fertilizer

INTRODUCTION

Genetic engineering

Is often misunderstood, so it is important to determine what your understanding about bacterial transformation and, especially, genetic engineering. Use the KWL (What do you Know? – What do you Want to know? – What did you Learn?) strategy to begin the activity. Ask your students what bacterial transformation means to them. Refer them to the Genetic Engineering worksheet and ask them to record their thoughts and questions on the chart at the top of the worksheet. When your students have completed their responses, ask them to share their thoughts with the class. Ask if they have heard the term "genetic engineering" and, if they have, what this term means.

Have them record their ideas and questions. These questions will help you to assess your students' current understanding, so you can address any of their misconceptions. Throughout this activity, students should refer back to the questions and comments on their worksheet. Note: The steps in this activity can be adjusted to match the pace and content you want to emphasize with your students. The activity could follow a vocabulary review and be used to further review vocabulary. It could also be used as a post-assessment of the module's content.

SUMMARY

Genetic Engineering is the use of modern techniques, including recombinant DNA methods, to modify the genetic information in an organism. It allows for faster trait selection than selective breeding, and can enhance the development of plant cultivars to help address some environmental challenges. Some anticipated changes for the future include: A larger library of genes to choose from as scientists are rapidly sequencing the genomes of organisms, and the ability to modify increasingly complex traits as scientists learn more about the cellular and molecular biology of plants.

Obtaining the desired gene

Scientists use one of several methods to screen and isolate the cell with the library plasmid containing the desired gene.

Isolation of the bacteria with the desired gene

The bacteria are plated onto a selective medium. Only bacteria with the desired gene and the selection marker gene will survive. The bacteria serve as a ready supply of the desired gene for use by scientists.

Isolation of the desired gene

The library plasmids with the desired gene are placed in a test tube with a restriction enzyme. The enzyme cuts the DNA at specific sites and frees the desired gene from the library plasmid.

Separation of the desired gene

The transformation plasmid with the desired gene is separated from the bacterial cells and purified.

Preparation of the transformation plasmid parts

The desired gene, a selection marker gene, and "empty" transformation plasmid are cut to make them compatible for ligation.

Transference of the desired gene

Scientists choose an appropriate insertion method to insert the desired gene into the plant cells they are studying.

Ligation of the transformation plasmid parts

The desired gene, selection marker gene, and the "empty" transformation plasmid are combined in a test tube with a DNA ligase to seal the sticky ends of the DNA molecules together. This new bacterial transformation plasmid has incorporated the desired gene and the selection marker gene.

Propagating the genetically engineered plants

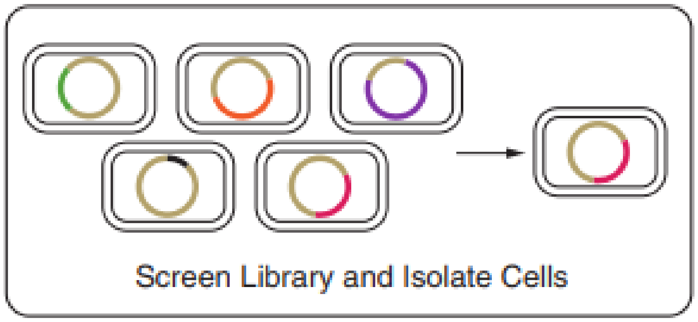
Plant cells are grown on selective media so that only the transformed cells carrying the new genes will grow. The media also contains substances that encourage the plant cells to grow into new plants.

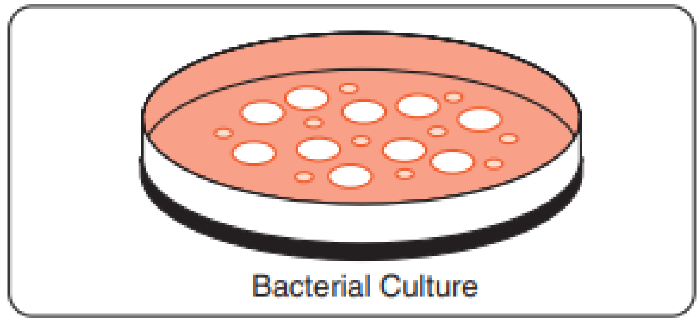
Addition of desired gene to bacteria

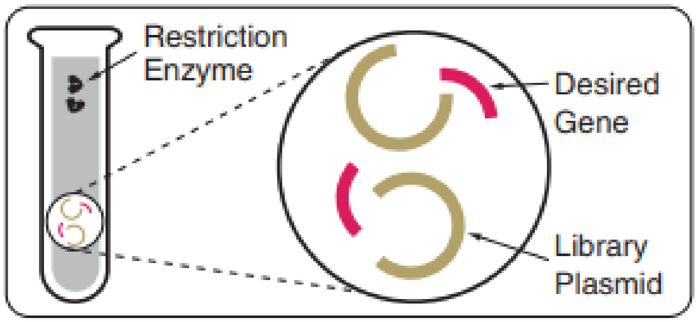
The transformation plasmid with the desired gene and the selection marker gene are added to bacterial cells.

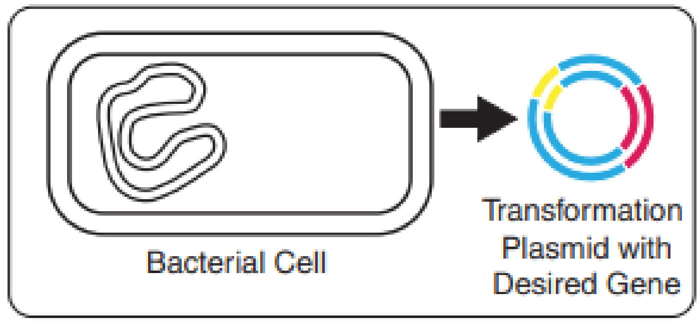
Testing the genetically engineered plants

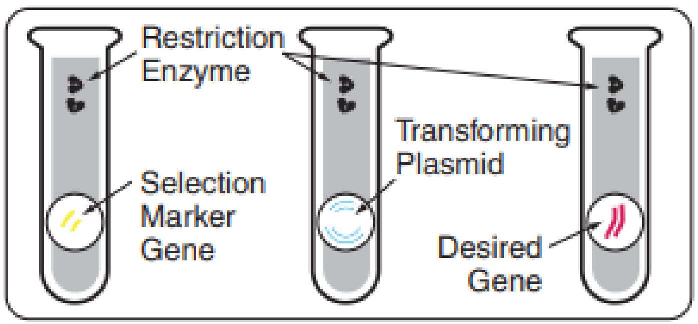
The plant is tested to determine if it incorporated the desired trait.

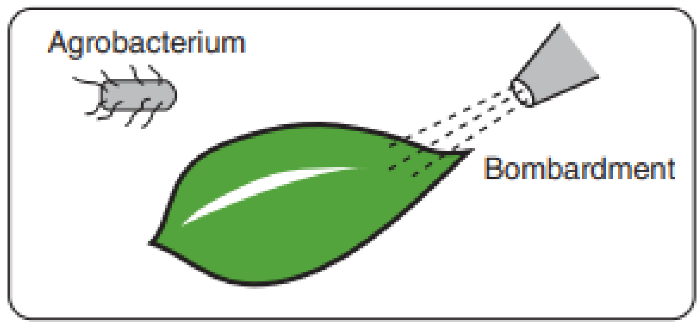

Screen Library and Isolate Cells

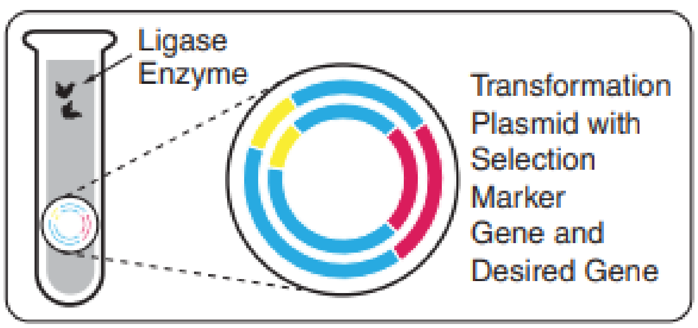

Bacterial Culture

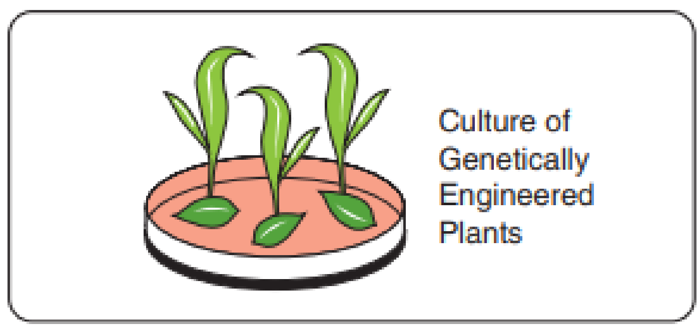

Restriction Enzyme
Desired Gene
Library Plasmid

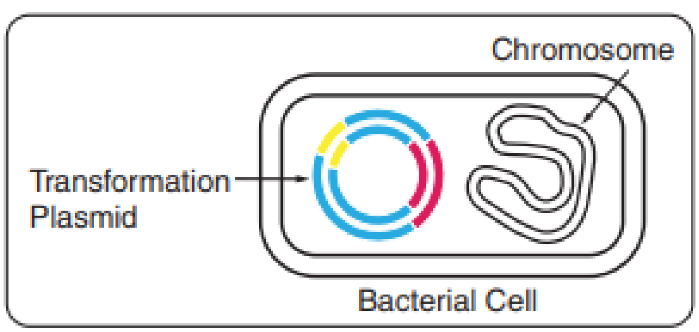

Bacterial Cell
Transformation Plasmid with Desired Gene

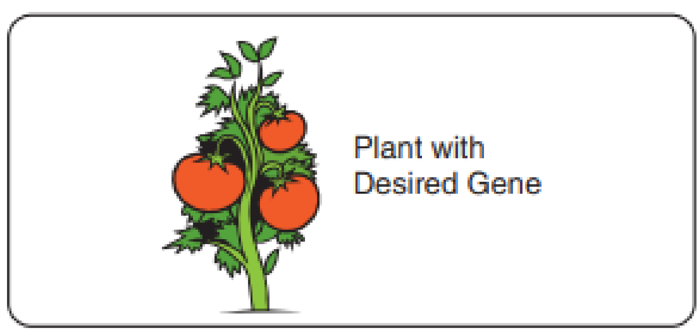

Restriction Enzyme
Transforming Plasmid
Selection Marker Gene
Desired Gene


Agrobacterium
Bombardment


Ligase Enzyme
Transformation Plasmid with Selection Marker Gene and Desired Gene


Culture of Genetically Engineered Plants


Chromosome
Transformation Plasmid
Bacterial Cell

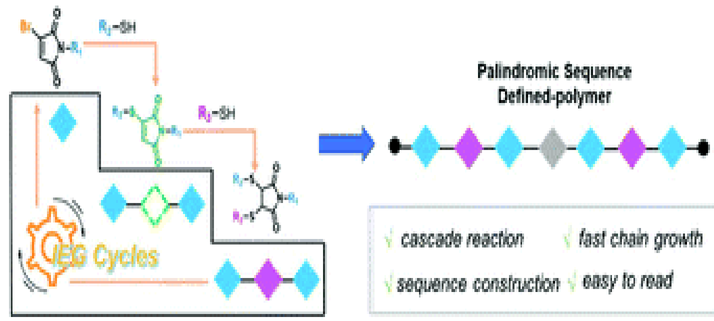

Plant with Desired Gene

Targeted Genome Editing

While original rDNA techniques would often result in random integration of the desired gene(s), newer genome editing techniques use tools to target the desired gene or the "edit" to a precise locus in the genome. One genome editing technique currently used by plant scientists is the CRISPR-Cas system. It's part of a natural bacterial defense system that scientists are using to cut and modify DNA more precisely than any previous GE method.

What is CRISPR and how is it used by bacteria?

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. CRISPRs are sequences of nucleotides in the bacterial genome where bacteria keep a record of previous infections by a virus and later use it to identify and fight subsequent attacks by the same virus. When a bacterial cell is infected by a virus, the cell incorporates pieces of the viral DNA into the CRISPR sequence, which then produces small, non-coding RNAs that act like virus detectors. This is a form of adaptive immunity. Close to the CRISPRs are CRISPR-associated (Cas) genes that encode for Cas proteins. In bacteria, Cas proteins are part of the adaptive immune system. Some Cas proteins help the bacterial cell to capture small pieces of invading viral DNA for insertion into the CRISPR sequences during the initial infection; others silence the attacking virus' DNA during subsequent infections to protect the bacteria. For example, the small RNAs made from the CRISPR sequence containing the previously captured pieces of viral DNA (from the first infection) bind to the Cas9 endonuclease enzyme and target it to cut the viral DNA of repeat invaders.

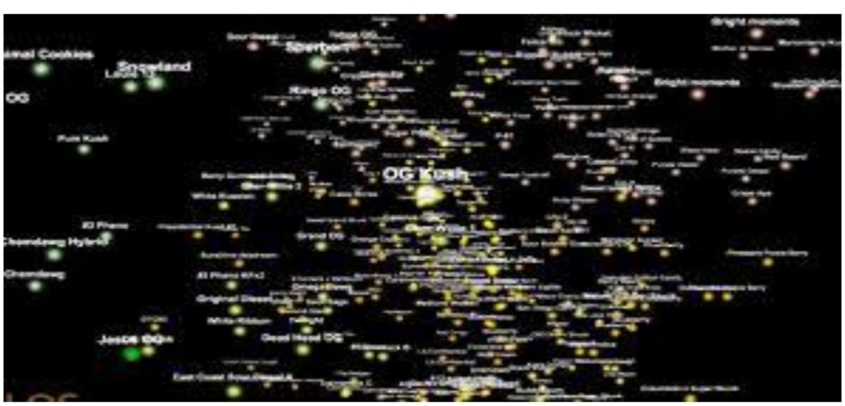


Developing CRISPR-Cas as a New GE Tool

In 2012-2013, several scientific teams tested whether they could adapt the bacterial CRISPR-Cas immune system for use as a genome editing tool. First, they determined which specific components of the system were needed: The Cas9 enzyme and a guiding RNA. Next, they showed that they could target the Cas9 enzyme to cut a specific locus of their choosing simply by changing part of the guiding RNA sequence to match the targeted genome sequence. Collectively, multiple scientific teams showed CRISPR-Cas9 could be used as a programmable RNA-guided DNA cutting tool in bacteria, plant, mouse, and human cells. This discovery was important because it meant that scientists could now cut and "edit" genomic DNA at a specific location of their choice. When the cell tries to repair the broken DNA strand by joining the pieces back together, scientists could take advantage of this process to add or remove specific DNA sequences. They could also include a repair template (with a mutation or a new gene entirely) to guide a specific repair by the cell's own mechanisms. In agriculture, genome editing using CRISPR-Cas, or one of several other available DNA targeting and cutting tools, can be used to create plants that produce higher yields, are more nutritious, and have characteristics that will help them endure extreme weather conditions.

Acronym Alert Early genetic engineering

(GE) began about half a century ago, while genome editing is a more recent technique. Although both two-word phrases begin with a G and an E, in this curriculum, genome editing will always be spelled out, and GE refers to the broader category of genetic engineering techniques.



BACKGROUND INFORMATION

Here's the CRISPR-Cas9 process:

1. The scientist first identifies the precise location for the desired edit in the plant's genome.
2. A small piece of guide RNA is designed to target the DNA sequence at that location.
3. The guide RNA and Cas9 can be introduced into the plant cell as either DNA, RNA, or an RNA-protein complex called a ribonucleoprotein.
4. The guide RNA locates and binds to the targeted plant genomic DNA sequence. Its associated Cas9 enzyme then cuts the DNA at the targeted location.
5. The plant cell's own repair machinery re-attaches the cut DNA ends. During the process, nucleotides may be removed from or added onto the cut DNA ends. This can result in the loss of an undesirable trait or the expression of a new desired trait.
6. The cells are grown into mature plants with edited DNA.
7. The edited DNA is now heritable and can be passed on to the offspring. Note: Depending on the method by which the guide RNA and Cas9 were introduced, they may not be present in the mature plant. If the scientist includes a repair template during the plant transformation process (step 3), the repair template will direct the repair of the genomic DNA at the cut site (step 5).

CRISPR-Cas Delivery

There are several possible CRISPR-Cas delivery methods. Plasmid-mediated delivery transforms the cell with a plasmid or plasmids carrying the genes for the guide RNA and Cas protein, similar to rDNA technology. Alternatively, direct delivery of the Cas9 protein with guide RNA into plant cells can be used. The choice of delivery method depends on several factors, including which method is most efficient for the type of plant being edited and whether the scientist's goal is transient or stable expression of the CRISPR-Cas components. In 2013, scientists discovered how to use the CRISPR-Cas system to edit a plant's genome. Since this discovery, many scientists throughout the world have been working to improve our food supply through genome editing using CRISPR-Cas as well as other targeted DNA cutting systems like TALEN and Zinc Finger Nucleases. These genome editing tools are being used to improve:

- A plant's yield performance
    - nutritional value
    - tolerance to biotic stress such as viral, fungal, and bacterial
- diseases
    - tolerance to abiotic stress such as environmental
- conditions, including changes in water availability,
- temperature, and soil chemistry

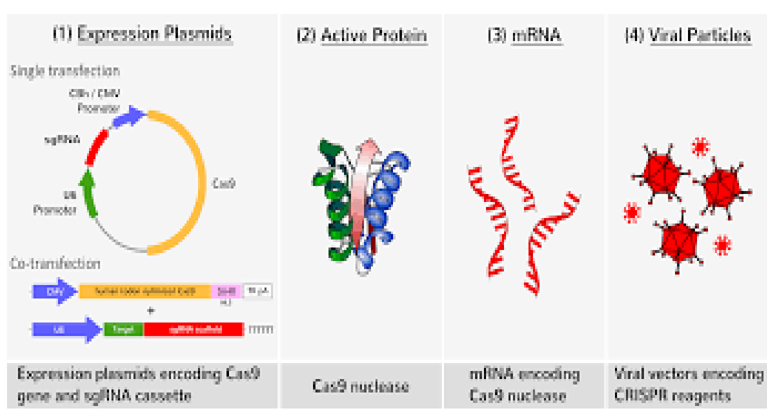
The most studied crops are rice, corn, tomato, potato, barley, and wheat. Specific examples of researchers and their projects include scientists at Pennsylvania State University who used genome editing to extend the shelf-life of white mushrooms by disabling an enzyme that causes the mushrooms to brown, and scientists in Spain who used genome editing to modify the genome of wheat strains to be significantly lower in gluten.

The first food produced from a genome-edited crop became commercially available in 2019: High oleic soybean oil is lower in unhealthy fats than original soybean oil. Scientists are continually testing the potential of genome editing techniques to solve a range of food-related problems, such as:

• producing bananas that are resistant to a fungal disease that destroys the crop

• providing a solution to the citrus greening disease that is threatening U.S. orange trees

• protecting the world's chocolate supply by improving the cacao plant's ability to fight a virus that is destroying the crop in West Africa



TARGETED GENOME EDITING

BIOX UNIVERSITY OF BOTANY

Powered by J Gold Fertilizer

ACTIVITY AT A GLANCE

In this activity, students develop an understanding of the CRISPR-Cas9 gene editing system and create an infographic (or poster or model) to demonstrate their understanding of the system.

GETTING STARTED

MATERIALS

- Computer and internet access for you
- Copies of the CRISPR-Cas Note-Taking Guide and Infographic worksheet and the Poster/Infographic
- Poster paper
- Markers
- 3 x 5 index cards
- Optional: 3D Modeling supplies

ADVANCE PREPARATION

Divide the class into small groups. Make copies of the CRISPR-Cas Note-Taking Guide and Infographic worksheet and the Poster/Infographic Rubric for each student. While the article, "Why Gene Editing is the Next Food Revolution," has some good information, point out to the students that it states that soybean-based oil is high in trans fats. However, this depends on whether the oil has been partially hydrogenated. If so, then the food companies may be required to remove the trans fats. This resource also states that genome editing techniques that mimic natural processes are not subject to U.S. regulation. However, regulatory debates are ongoing in the United States.

1. How are scientists using genetic engineering to improve the food that we eat?
Possible answer: The genes from one organism can be added to the same kind of organism or to another kind of organism to make the plants more nutritious or resistant to disease.
2. Imagine that scientists can edit DNA as easily as correcting typos on a computer. What impact do you think this would have on the food that we eat?
The students might answer that it will be easier to change a plant's genes with targeted genome editing methods (such as the CRISPR-Cas system) than with non-targeted modification methods such as selective breeding, chemical or UV methods, and rDNA methods
3. What advancements could you expect to see in agriculture in the next 5 years?

Some responses could include:
1. There could be many more changes in the plants we eat.
2. There could be more varieties of plants that we eat.
3. Plants could become more nutritious or more resistant
to pests.
4. Our environment might be better because plants could be changed to reduce the need for certain pesticides.

Tell the students that they will read text and view a video about the CRISPR-Cas9 system and then create an Infographic to illustrate their understanding of the system. Students could also consider creating a poster or PowerPoint presentation.

TARGETED GENOME EDITING

Read the questions on the CRISPR-Cas Guide, then watch these four videos:

• Gene Editing Yields Tomatoes That Flower and Ripen Weeks Earlier www.youtube.com/watch?v=Jem3hP734Ua
• CRISPR Gene Editing Explained https://video.wired.com/watch/crispr-gene-editing-explained
• CRISPR Explained (Mayo Clinic) www.youtube.com/watch?v=UKbrwPL3wXE
• CRISPR – a Word Processor for Editing the Genome www.ibiology.org/genetics-and-gene-regulation/crispr

BIOX UNIVERSITY OF BOTANY
Powered by J Gold Fertilizer

SUMMARY

Genome editing techniques like CRISPR-Cas are powerful tools that scientists can use to target specific locations in the genome for editing (add, remove, or modify a gene to increase or decrease its expression) and thus change the traits of that organism. The promise and challenges that genome editing systems hold for agriculture are currently unknown. But, based on the results we have now, it is exciting to think about crops of the future and what they might be able to do.

RESOURCES

• A Visual Guide to Genetic Modification
https://blogs.scientificamerican.com/sa-visual/a-visual-guide-to-genetic-modification/
• CRISPR – A History of Discovery
www.youtube.com/watch?v=RKh2mi3tsmc
• HHMI Biointeractive: CRISPR-Cas9 Mechanisms & Applications
media.hhmi.org/biointeractive/click/CRISPR/
• Nature Video: CRISPR Gene Editing and Beyond
www.youtube.com/watch?v=4YKFw2KZA5o
• Science Magazine (more technical option)
CRISPR-Cas guides the future of genetic engineering
http://science.sciencemag.org/content/361/6405/866

ENVIRONMENTAL FACTORS

This module introduces environmental factors (focusing on pests) that can challenge crop growth, discusses a range of GE and non-GE strategies farmers might use to optimize their crop conditions, and includes engaging activities for students to learn about managing these environmental challenges.

BACKGROUND INFORMATION:

Growing Food Challenge introduces students to key environmental factors, e.g., pests, that impact crop success.

- Agricultural Pests enables students to identify and define crop pests.
- Pest Management Research Project introduces students to a variety of GE and non-GE pest management strategies that a farmer might use.
- What Is Citrus Greening? is a case study in which students will learn about various pest management approaches to protect citrus crops from this disease.
- Citrus Greening Disease Management engages students to consider potential citrus greening management strategies. Advanced Activity

Biotic versus abiotic environmental factors, how food chains and food webs model the flow of energy through ecosystems, point source pollution, natural resource definition, renewable versus non-renewable natural resources, natural resource conservation (i.e., reducing, reusing, and recycling), pathogens, and introductory concepts in genetic engineering.

BACKGROUND INFORMATION

Growing Food Challenge

Plants growing in a natural environment face several challenges that affect which plants will survive and grow, produce seed, and complete their life cycle. Various pests such as weeds, herbivores, and pathogens can threaten plant production of grain, fruit, or flowers. Cultivated plants can have other stressors such as dry weather or a lack of soil nutrients. As a result, growers often manage their fields to reduce stress through methods such as irrigation, fertilization, and pest control to increase crop production. Crops and their environment have an impact on each other. In agriculture, various approaches impact water use, pesticide use, and CO2 release (carbon footprint). Tillage practices, fertilizer use, conventional pesticides, biopesticides, etc., are all factors that impact a crop's environmental footprint. Environmental footprint is the effect that a person, company, activity, etc., has on the environment, e.g., the amount of natural resources that a crop uses and the amount of harmful gases it produces.

Official Definition of Pest –An organism is declared to be a pest under circumstances that make it deleterious to man or the environment, if it is:
(a) Any vertebrate animal other than man;
(b) Any invertebrate animal, including but not limited to, any insect, other arthropod, nematode, or mollusk such as a slug and snail, but excluding any internal parasite of living man or other living animals;
(c) Any plant growing where not wanted, including any moss, alga, liverwort, or other plant of any higher order, and any plant part such as a root; or
(d) Any fungus, bacterium, virus, prion, or other microorganism, except for those on or in living man or other living animals and those on or in processed food or processed animal feed, beverages, drugs, and cosmetics. (U.S. Code of Federal Regulations)

Approaches to pest control include mechanical, biological, chemical, or cultural techniques. Some growers also use Integrated pest management (IPM), a decision-making framework that helps growers decide when to apply pest control and which control techniques to use. IPM focuses on long-term pest control and aims to minimize pest impact on crop quality.

MECHANICAL PEST CONTROL

Plant pests can be controlled in many ways. Simply pulling weeds from a garden or flower bed reduces the competition for moisture and plant nutrients and helps avoid the insects those weeds might attract and harbor. This is known as a physical or mechanical control method of plant protection. Plant pest control often starts with preparing a site to make it harder for pests to survive.

For example, a grower might till (turn over) the soil or put down mulch cloth to reduce weeds. Reduced tillage systems are also common and have certain benefits, such as reduced soil erosion. Farmers can use different tilling methods to prepare soil before planting. Reduced tillage includes different approaches that conserve soil by leaving more plant residue on the soil surface and uses less energy. No-till is a method that leaves the soil undisturbed through use of a coulter (a vertical blade) that slices the soil, and another tool that places the seed at a proper depth. However, even in no-till systems, farmers may need to till every few years to reduce crop debris that could harbor crop pests such as insects and pathogens. Conventional tillage normally involves three or more steps using tractor-pulled tools. The environmental footprint varies with different tillage methods of pre-planting soil preparation.

BIOLOGICAL CONTROLS

Biological controls are more complex than simply plowing a field. They use a biological organism or process to protect plants from damage caused by other organisms. Several types of natural or biological plant protection innovations have been developed throughout farming history. The most commonly used are:

1. Selective breeding to cultivate damage-resistant plants
2. Use of beneficial organisms to control weeds or insect populations
3. Biopesticides produced from microbial cultures, plants, or other organisms
4. GE plants designed to resist pests

Biological Control Using Predators

Biological control with predators uses an organism (such as an herbivore, predator, pathogen, or parasitoid) that consumes the pest to reduce pest populations.

For example, predator insects such as lady beetles and lacewings eat other insects. Parasitoid insects such as wasps lay their eggs on or in some life stage of the target insect. After an egg hatches, the developing immature stage of the parasitoid insect kills the targeted host by consuming the host tissues. Biological control might also involve releasing beneficial organisms to the environment or changing the landscape to increase populations of beneficial organisms. Limitations of Biological Control Using Predators There are limits to the safety and effectiveness of biological insect control.

For example, it may be necessary to eliminate or reduce the use of broad-spectrum pesticides, since both beneficial and target insects could be killed. Fungicides used against plant pathogenic fungi can also impact desired fungi when applied to reduce insect pests. In addition, strict regulations must be used to ensure that today's insect predator will not become tomorrow's pests. Managing an insect attack can be complicated, because the attacking predatory or parasitoid insects cannot thrive until there are sufficient numbers of target insects to serve as prey or hosts. Some biocontrol insects may also destroy a broad range of insects – both beneficial and harmful. Sometimes beneficial insects can be considered pests when they become too numerous or are in the wrong place. Invasive lady beetles from Asia have displaced some native species in the United States. They can also become minor pests in the home when they invade in large numbers when weather starts to turn cool.

CHEMICAL CONTROLS

Pesticide use is one of many management practices in agriculture. Continuous pressure to feed increasing populations has influenced agriculture to progress through many stages from domestication and improvement of crop plants, to mechanization, fertilization, and pesticide use. Pesticides are applied to crops, gardens, animals, lawns, recreational areas, and around homes and other buildings. They help provide abundant, disease-free, pest-free foods, improve crop yield, and reduce disease vectors for humans, animals, and plants. Pesticides were considered necessary in crop production in the mid-twentieth century and were often applied in multiple passes across the field. Pesticides still are considered necessary in crop production, but improved technology provides pesticide options that are more compatible with other control methods and reduce environmental consequences. In addition, more judicious pesticide application has evolved over time, with application following field scouting to ensure that pesticides are only applied when there is a danger that pests may reach levels that significantly impact a crop's sale value.

There are different types of pesticides such as herbicides, fungicides, insecticides, rodenticides, etc. Different herbicides are designed to be most effective at different timings: Some are only applied before planting to control germinating weed seeds, and others are applied after the crop plants emerge. Pesticides are often used to solve plant pest problems, but if they are used incorrectly, some of them might not provide the desired results or can harm crop plants or the environment (including groundwater, lakes, or rivers). Pesticides are evaluated for their impact on the environment and for how they may affect the health of people who may be exposed to the pesticides. The Environmental Protection Agency (EPA) works with the U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA) to monitor use of chemicals in food production and determine levels of safe use.

Pesticide Impact

on Humans Pesticides may contain chemicals with possible health risks to humans. The risk is determined by the hazard and exposure, i.e., how someone comes into contact with the pesticide (ingested, inhaled, or through skin contact). The EPA classifies pesticides based on their chemical toxicity and separates them into four categories – Category I, II, III, or IV, with Category I chemicals being the most toxic.

Using these categories, EPA restricts where the pesticide can be applied, how much can be applied, and also anticipates its possible exposure level to humans. Pesticide Impact on the Environment Some pesticides can contaminate soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to other organisms including birds, fish, beneficial insects, and non-target plants. For the last 30 to 40 years, researchers have been developing pesticides that are more specific to their target pests and that have a reduced impact on the environment.

Different pesticides also break down at different rates, which are further influenced by conditions such as moisture and temperature. A Risk Quotient (RQ) is used to quantify the environmental impact of most commonly used pesticides (insecticides, fungicides, and herbicides) in agriculture and horticulture. The RQ value is calculated using key factors such as a pesticide's active ingredient(s), toxicity, amount applied, and how long it persists in the environment. RQ values allow pesticide options to be compared.

Biological Pesticides

(Biopesticides) The most widely applied bacterial species used as a biological pesticide is Bacillus thuringiensis (Bt), a bacterium found in soil. Bt produces a natural crystal protein that is toxic to some other organisms like insects and nematodes. Some strains of the Bt bacterium produce toxins that are naturally highly specific to certain pest insects, but harmless to most other organisms.

When Bt produces spores, a toxic crystal protein is formed inside the spore. The spores are suspended in a liquid and sprayed on the plants. When a targeted pest eats the spores, the crystal toxin is released, and the pest will die. Bt spores are regarded as safe to humans and the environment because they are so specific to a few types of insects. There are many other natural organisms that can be used to produce a biological pesticide. Researchers in Florida are growing a naturally occurring fungus, Hirsutella citriformis, to fight the Asian citrus psyllid (jumping plant lice). Asian citrus psyllids are small insects that feed on citrus plants. They can transmit the bacteria that causes citrus greening disease to their host plants Citrus greening is one of the most serious citrus plant diseases in the world and affects many citrus trees in Florida and the South. It is also known as Huanglongbing (HLB) or yellow dragon disease. An infectious virus of citrus known as Citrus tristeza virus (CTV) is being evaluated as a vector of biologically active peptides targeting the HLB bacterium into the cells of the citrus trees. While CTV is a pathogen of citrus, it can be used as a biological control of HLB in this case, because it uses CTV strains that have been selected to cause only a few mild symptoms when trees are infected.

Tillage methods influence pest control. In conventional tillage, few selective herbicides may be needed because the tillage helps to control weeds. Reduced tillage and no-till systems may require broad-spectrum (less selective) herbicides because there is less tillage. However, reduced tillage and no-till systems may have benefits such as enhanced nutrient cycling and water retention. Conventional tillage releases the most greenhouse gas when stored carbon in the soil is released into the atmosphere and more fuel is used for power tilling equipment.

FOCUS ON GE PLANTS GE

Plants with Enhanced Traits (Biotechnology) Several GE crops have been developed specifically to be insect resistant (IR) or herbicide tolerant (HT). IR Bt GE crops have been designed to produce a protein that kills specific target insects, such as the European Corn Borer, when they attack the plant. These proteins only affect specific receptors in the gut of certain target pests and are harmless to humans, mammals, and most non-target insects. One unanticipated consequence of this pesticide specificity is the resurgence of some secondary pests (e.g., cutworms, wireworms) that are not targeted by the Bt endotoxin and can become primary pests in some years, in some locales. HT crops are designed to tolerate specific broad spectrum (non-selective) herbicides, which kill surrounding weeds but leave the cultivated crop intact.

Glyphosate-tolerant crops are the most prevalent, although many new combinations of HT mechanisms are also used with older herbicides, such as dicamba, that are used commercially. In addition, HT traits are not required for reduced tillage or no-till practices, but they can make it easier for farmers to use these practices.

Biotechnology Approaches to Combat Plant Diseases According to CropLife International (an association that promotes agricultural technologies such as pesticides and plant technology), more than a third of the world's potential crop production is lost each year to pests and plant diseases. Most crops can be damaged by diseases caused by soilborne plant pathogens and insect-vectored viruses. The three predominant types of plant disease agents are viruses, bacteria, and fungi.

• Combating viral diseases: Scientists have transferred virus genes, such as those that produce a virus coat protein, into plants. This acts like a vaccine that makes the plant resistant to that specific virus. Another way to increase plant resistance to viral infections is to inhibit the vectors, such as insects and nematodes, that carry the virus.

• Combating bacterial diseases: All crop plants are susceptible to bacterial infections. Bactericides, including antibiotics, are not a complete solution, because bacteria quickly evolve resistance to them, and this could have implications for treatment of infections in man and animals.

Fire blight is an example of a harmful bacterial disease that destroys pears, apples, quince, and some ornamental plants. One remedy is to spray trees with large quantities of antibiotics. Scientists have identified DNA markers for fire blight resistance and are working to develop resistant varieties.

• Combating fungal diseases: Fungi cause billions of dollars in crop losses each year. They attack nearly all fruit, vegetable, and grain varieties. Some plants are more susceptible to fungal diseases than others, simply because they are too slow to start fighting back after they are attacked, or they lack the resistance gene for that particular fungus. Some techniques can trigger these plants to respond sooner by treating them with fungal pathogens that have been disarmed, or by using resistance inducers like salicylic acid, a naturally occurring plant biochemical, making the fungus harmless to the plant.

BACKGROUND INFORMATION

BIOX UNIVERSITY OF BOTANY
Powered by Y Gold Fertilizer

Environmental Impact of Growing GE Plants GE crop technology has been used widely since the mid 1990s in several countries and has mainly been used in four main crops: canola, maize, cotton and soybean. The adoption of GE IR and HT technology has significantly reduced certain insecticide and herbicide use. Source: www.ncbi.nlm.nih.gov/pmc/articles/PMC6277064/

Generally, less fuel is consumed in the production of major GE crops because the HT traits make no-till practices easier to use, which results in lower carbon dioxide emissions. Specifically, HT GE crops require less tilling. The no-till process requires effective herbicide control of weeds in lieu of mechanical tillage and is facilitated by the adoption of HT crops. Farmers use less fuel because fewer passes are made through the field to till and to spray herbicides on GE crops. The no-till method also reduces erosion on susceptible land in steep terrain or fragile landforms and reduces chemical use. The use of plants modified to resist corn borer and rootworm has also decreased insecticide use. These production practices allow GE crops to have increased yield, which also makes food cheaper to produce on less land. GE crops are sometimes blamed for lowering genetic diversity of crops and speeding the development of herbicide resistance in weeds.

However, when farmers use seeds from fewer family lines, diversity decreases regardless of whether GE or non-GE seeds are used. In addition, herbicide use can result in the selection of weeds resistant to the herbicide in GE or non-GE crops. Herbicide-resistant weeds have long been a concern for farmers. The availability of HT GE crops has arguably resulted in faster selection of weeds resistant to the herbicide, but GE crops with multiple HT features could also help slow the selection of herbicide-resistant weeds.

How serious is weed resistance to pesticides?

In the United States, there are currently 14 weeds associated with common crop production that are resistant to the most popular non-selective, post-emergent herbicide. International Survey of Weed Resistance www.weedscience.org

RESOURCES

• Hungry Pests – USDA www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/?utm_campaign=crosby-2017&utm_ source=hungrypests-com&utm_medium=redirect&utm_keyword=home
• Plant Pests and Diseases Programs www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases
• Pest Tracker www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/Pest-Tracker?utm_campaign=crosby2017&utm_source=hungrypests-com&utm_medium=redirect&utm_keyword=/the-spread
• Agricultural Pest Survey Cooperative – Pest Lists http://caps.ceris.purdue.edu
• History of Pesticides www.youtube.com/watch?v=gyZPDcr5_dw
• How Plants Make, Store, and Use Toxins https://learn.genetics.utah.edu/content/herbivores/planttoxins/
• Top Crop: Farming for the Future Educator's Guide https://www.nationalgeographic.org/media/top-crop-farming-future-educators-guide/
• Pesticide Labels www.epa.gov/pesticide-labels
• What is Integrated Pest Management (IPM)? https://www2.ipm.ucanr.edu/What-is-IPM
• University of Wisconsin - Agroforestry Practices: Strategies for Implementation www.youtube.com/watch?v=PRm4jnxCeMw
• Apple Fire Blight www.youtube.com/watch?v=PdcDXNftoWg Pictures of pests:
• Agricultural Pests - UC IPM crop lists showing pests that affect each crop http://ipm.ucanr.edu/PMG/crops-agriculture.html
• Natural Enemies Gallery - UC IPM list of predators with pictures http://ipm.ucanr.edu/PMG/NE/index.html • USDA Image Gallery – pictures of many crop pests www.ars.usda.gov/oc/images/image-gallery (use search function)
• Virginia Tech Weed Identification Guide http://oak.ppws.vt.edu/~flessner/weedguide

BACKGROUND INFORMATION

BIOX UNIVERSITY OF BOTANY

Powered by 7 Gold Fertilizer

What is Citrus Greening?

Citrus greening (caused by the bacterium Candidatus Liberibacter asiaticus) is one of the most serious c diseases in the world. It is also known as Huanglongbing (HLB) or yellow dragon disease. Once a tree is infected, there is no cure. While the disease poses no threat to humans or animals, it has devastated millions of acres of citrus crops throughout the United States and abroad. Citrus greening is spread by a bacteria-infected insect, the Asian citrus psyllid (Diaphorinaitri Kuwayama or ACP), and has put the future of America's citrus at risk. Infected trees produce fruits that are green, misshapen and bitter, unsuitable for sale as fresh fruit or for juice. Most infected trees die within a few years.

Global History

- 1919 First reported in southern China
- 1921 First report of disease in the Philippines, but it was thought to be related to zinc
- deficiency
- 1928 A disease named "yellow shoot" or "greening," depending on region, was
- observed in South Africa
- 1937 The first description of HLB in South Africa was assumed to be mineral toxicity
- 1941-
- 1955
- Most extensive work on greening in southern China was conducted
- 1956 Lin Kung Hsiang (researcher from China) concluded that greening is a graft
- transmissible infectious disease, not related to physiological disorders (e.g. nutrient
- deficiencies, water logging, etc.) or soil borne diseases (e.g. phytophthora, etc.)
- 1960's HLB first appeared in Thailand
- 1965 Researchers in South Africa demonstrated HLB was transmissible by graft
- inoculation and by the African citrus psyllid, Trioza erytreae
- 1967 Philippine researchers demonstrated 'mottle leaf' or 'citrus dieback' could be
- transmitted by the Asian citrus psyllid, Diaphorina citri
- 1995 The official name of the disease became huanglongbing (HLB) at the International
- Organization of Citrus Virologists (IOCV) at the 13th conference of the
- Organization in Fuzhou (Fujiam, China)
- 1998 Asian citrus psyllid arrived in Florida
- 2004 The disease was confirmed to be in Brazil
- 2005 The disease was confirmed to be in Florida
- 2012 First occurrence of Asian citrus psyllid/HLB in California
- 2017 The disease was confirmed to be present in California citrus

Florida History

- 2005 August - Citrus greening was first confirmed in south Miami-Dade county
- October 25 - Four counties confirmed positive (Dade, Broward, Palm Beach,
- Hendry)
- September 16 - Federal order issued to restrict the interstate movement of all citrus
- greening and Asian citrus psyllid host plant material from Florida's quarantined
- areas
- 2006 March 14 - Regulations for citrus nurseries were established
- 2007 December - Federal order issued was revised to include all counties with confirmed
- greening
- 2008 January 11 - Federal order issued to quarantine the entire state of Florida
- August 7 - Thirty-two counties confirmed positive (Sumter)
- 2009 February 16 - Thirty-three counties confirmed positive (Putnam)
- 2018 HLB is known to be present in all citrus growing areas of Florida
- Timeline from the University of Florida https://crec.ifas.ufl.edu/extension/greening/history.shtml

BACKGROUND INFORMATION

Pathogen
Candidatus Liberibacter species are phloem-limited plant pathogens that are mainly transmitted to plants by psyllids. An infected psyllid feeds on a healthy tree and injects the bacterium into the phloem. Plant food sugars are made by photosynthesis and are carried through its phloem system bidirectionally to flowers, fruits, roots, and seeds. Once a tree is infected with the bacterium, there is no known cure for the disease. This is partly because the bacterium is inside the vascular system of the plant (systemic) and is therefore very difficult to access.

Diagnosis
The first sign of the disease is leathery leaves with yellow veins and blotchy marks, and the fruit remains green. Polymerase chain reaction (PCR), a common laboratory technique used to make many copies of a particular DNA region, is one way to positively confirm citrus greening. Dogs have been trained to efficiently sniff out the bacterium Candidatus Liberibacter asiaticus in infected plants. The trained dogs can distinguish the citrus greening bacteria from other similar bacteria, resulting in highly reliable detection. While the number of trained dogs is currently limited, it is expected that they will eventually be used for early detection in all citrus-producing states.

Management Approach
There are several management approaches currently in various stages of use and/or development. Groves can be managed:
as if they already have greening with an integrated
approach using disease-free nursery stock.
- by reduction of the inoculum by frequent disease surveys.
- by removal of symptomatic trees.
- by suppression of the Asian citrus psyllid.

Pruning only symptomatic
(diseased) branches is ineffective. Tree removal, including the stump and roots, is the only way to ensure that infected trees will not spread the disease to other trees. New citrus trees (which should not be planted in the same area as the infected tree[s]) should be purchased from a certified nursery or propagated from clean bud wood.

Scouting
(monitoring) is recommended four times a year, unless a grove already has greening. If there is currently greening in a grove or close by, scouting more than four times a year is recommended. Symptoms are most easily seen from September through March. During the spring growth, scouting becomes more difficult and scouts have to look further into the tree canopy. Scouting methods include using a tractor or pickup mounted platform (for taller trees), ATV's (for medium-sized trees), or walking (for young trees).

Grove conditions also affect pest management. Scouting is more difficult in a grove that has not been well-maintained. Nutritional deficiencies can cause greening symptoms to blend and go unnoticed. Excessive weeds and unmanaged areas in between the rows of trees cause scouts to watch where they are walking more than scouting.

Tree size increases scouting work as well. In the United States, trees that appear to have citrus greening are identified with a special tape (used only to identify the citrus greening disease) that is attached to the suspected branch; the tape is marked with the inspector's name and date. Ideally, scouts mark the end of the row and the number of suspect trees in that row. Safety concerns include grove conditions, chemical spray applications, weather, and potential for slips and falls.

BIOTECHNOLOGY AND NUTRIENTS

This module introduces some aspects of biotechnology related to enhancing nutrient availability for plants, animals, and humans.

BACKGROUND INFORMATION

This section examines how some strategies such as selective breeding (introduced in Module 1) and genetic engineering (introduced in Module 2) can be used to enhance nutrient availability.

ACTIVITY

Nutrient Supply will teach students to identify ways to enhance nutrient availability for a specific nutrient in a human population that struggles to meet their nutrition needs.

For this module, it is recommended that teachers will have already taught an introduction to nutrition, macronutrients, micronutrients, and basic selective breeding and genetic engineering.

The first two modules in this Teachs introduce key agricultural methods, particularly selective breeding and genetic engineering. Modules 3 and 4 highlight some of the major reasons why we use these techniques (e.g., to decrease pest damage, to enhance nutrient profile). All living things (plants, animals, and humans alike) need nutrients to survive. Nutrients in food contribute to cellbuilding and structural materials, regulate important functions in living tissues, and provide energy for growth and health. Nutrients are categorized as macronutrients (proteins, carbohydrates, and fats) or micronutrients (vitamins and minerals). Macronutrients are consumed/required at greater levels (g), micronutrients at lower levels (mg or µg). An "essential" nutrient is a nutrient that a plant, animal, or human must obtain from another source, because that organism cannot make it or cannot make enough of it for good health.

Human Nutrition

Every 5 years, human nutrition experts from different parts of U.S. society, including academia and government, review the latest nutrition information and issue a report called the Dietary Guidelines for Americans to promote good health. These Guidelines identify:

- target dietary goals for key micronutrients and macronutrients
- nutrients that Americans typically should aim to get more of (e.g., fiber, Vitamin D, calcium, potassium, iron)
- nutrients that people should aim to get less of (e.g., sodium, saturated fat, added sugars).

Key nutrients are shown on Nutrition Facts labels to help people make healthy food choices. For a more detailed supplementary curriculum on nutrition, see Science and Our Food Supply: Using the Nutrition Facts Label to Make Healthy Food Choices. Essential nutrients for human diets are shown below. The exact amount recommended for individual people varies by age and gender, as well as with specific health conditions. For recommended nutrient intake for healthy individuals, see Dietary Reference Intakes for Macronutrients, Vitamins and Micronutrients.

Vitamins	Deficiency disorder
Vitamin **A** (Retinol)	Loss of vision **(Blindness)**.
Vitamin **B_1** (Thiamine)	Weak muscles and severe weight loss **(Beriberi)**.
Vitamin **B_2** (Riboflavin)	Poor diet and mouth sores **(Ariboflavinosis)**.
Vitamin **B_3** (Niacin)	Diarrhea and dermatitis **(Pellagra)**.
Vitamin **B_5** (Pantothenic acid)	Ischemic attacks and multiple sclerosis affect brain **(Paresthesia)**.
Vitamin **B_6** (Pyridoxine)	Low number or lack of red blood cells **(Anemia)**.
Vitamin **B_7** (Biotin)	Red and itchy skin **(Dermatitis)**.
Vitamin **B_9** (Folic acid)	Immature RBCs in the bone marrow **(Megaloblastic anemia)**.
Vitamin **B_{12}** (Cyanocobalamin)	Abnormal absorption in the small intestine **(Pernicious anemia)**.
Vitamin **C** (Ascorbic Acid)	Bleeding gums and swollen joints **(Scurvy)**.
Vitamin **D** (Calciferol)	Weakening of bones near joints **(Rickets)**.
Vitamin **E** (Tocopherols)	Faster depletion of RBCs from the body **(Hemolytic anemia)**.
Vitamin **K** (Phylloquinone)	Irregular and slow blood bleeding and clotting **(Bleeding diathesis)**.

Toxins and Anti-Nutrients

In addition to making many desirable nutrients, plants make toxins. They adjust their biochemistry to adapt to their environment, including defending themselves from predators. If consumed in sufficient quantities, some toxins can affect human or animal health. Through domestication, agriculturally important crops (including tomatoes and potatoes) have been bred to eliminate or reduce the level of relevant toxins. The word toxin is sometimes used to indicate substances of biological origin with toxic properties. These are also often referred to as toxicants.

Major toxins and their effects:

• Cyanogenic glycosides in cassava, sorghum, and bamboo shoots that are improperly prepared can result in unsafe levels of cyanide toxins harmful to people and/or animals.
• Curcubitacin in cucumber and squashes can cause acute gastrointestinal effects.
• Glycoalkaloids (e.g., solanine) in potatoes may induce gastrointestinal and systemic effects if consumed in high amounts. Potatoes/potato byproducts (e.g., skins where glycoalkaloids are concentrated) that are high in glycoalkaloids can be fatal for animals.
• Psoralen, a furocoumarin produced by some plants (celery and parsnips) that can harm the skin of people working in the sunlight.
• Coumarin (normally in sweet clover) can be metabolized by some fungi into dicoumarol, which causes prolonged clotting time and bleeding disease in cattle (rarely in horses).

Plants also make substances that can affect the ability of human and animal digestive systems to extract the most nutrients out of food. These are called anti-nutrients.

Some major anti-nutrients and their effects:

• Glucosinolates in cruciferous vegetables can prevent iodine absorption. • Lectins in legumes and whole grains can inhibit calcium, iron, phosphorous, and zinc absorption.
• Oxalates in leafy green vegetables and teas can inhibit calcium absorption.
• Phytates (phytic acid) in whole grains, seeds, legumes, and some nuts can decrease the bioavailability of iron, zinc, magnesium, and calcium.
• Saponins in legumes and whole grains can interfere with some nutrient absorption.
• Tannins in tea, coffee, and legumes can decrease iron absorption.

Human nutrition experts use dietary reference intake values to decide how much of a given nutrient people should consume (on average). These values include the adequate intake, recommended daily allowance, and tolerable upper limit for specific subgroups. Whether a substance is considered toxic depends on the dose (amount) consumed. The tolerable upper limit for an infant would be less than the limit for a grown adult, since the amount taken per body weight would be much higher for the infant. However, many desirable nutrients (including water) can be considered toxic if overconsumed. The phrase often used to describe this reality is "The dose makes the poison."

Nutrient Deficiency

Throughout history, there have always been some people who had diseases and ailments associated with nutrient deficiency or malnourishment. Malnourishment is typically caused by a lack of access to enough nutritious food because of poverty, war, climate or weather conditions, and other economic factors. Circumstances that make it uncertain whether nutritionally adequate and safe food is available in socially acceptable ways is also called "Household Food Insecurity." Historically, the typical image of hunger was often an emaciated or very underweight person who also suffered from poverty. Today, hunger is still a problem, but the number of people who are both malnourished and overweight or obese has increased.

BIOX UNIVERSITY OF BOTANY
Powered by J Gold Fertilizer

How Much is Too Much?

A 100-pound person would have to eat a pound or more of totally green potatoes to show low-grade symptoms of toxicity (nausea, diarrhea, vomiting).

Water needs vary with activity level. People also get water through various foods they eat. On an average day, adult men need 3.7 liters of water; adult women need 2.7 liters of water; and teenagers need about 2 liters. But, kidneys have a limit on how much water they can process each hour. Although highly unusual in healthy people, rapidly drinking excessive amounts of water can cause low sodium levels that lead to headaches, diarrhea, nausea, vomiting, and impaired brain function. Extreme overconsumption can be fatal.

Fortified Bread Saves the South

In the late 1800s and early 1900s, hundreds of thousands of people (mostly poor) in the southern United States were suffering from the disease known as pellagra. Over 150,000 people died from pellagra in the early 1900s, while others suffered with untreatable symptoms from the disease known as the Four D's: depression, dermatitis, diarrhea, death.

For decades, it was unknown what caused pellagra, but many scientists thought it was linked to the corn-rich diet of the south. Dr. Joseph Goldberger, a Hungarian immigrant and epidemiologist, believed that pellagra was a diet-related disease. It was later discovered, after his death, that nicotinic acid (more commonly known as the vitamin 'niacin' or 'vitamin B3 ') was lacking in the diets of those suffering from pellagra. Much of the corn consumed in the southern United States was degerminated (processed to remove the germ portion of the corn kernel); the germ portion contains niacin. In 1940, the FDA held the "flour hearings," and a team of scientists, doctors, and the Surgeon General worked tirelessly to propose adding thiamin, riboflavin, and niacin to bread. Cornbread and white breads lacking certain nutrients would be "enriched" with a fortified vitamin-rich flour. Enriched (fortified) white bread caused pellagra to virtually disappear overnight.

Malnutrition (WHO definition) Malnutrition, in all its forms, includes undernutrition (wasting, stunting, underweight), inadequate vitamins or minerals, overweight, obesity, and resulting diet-related noncommunicable diseases.

Hidden Hunger (WHO definition) Hidden hunger is a lack of vitamins and minerals. Hidden hunger occurs when the quality of food people eat does not meet their nutrient requirements, so the food is deficient in micronutrients such as the vitamins and minerals that they need for their growth and development.

In February 2018, the World Health Organization (WHO) cited that about 1.9 billion people on earth were estimated to be overweight or obese, whereas almost half a billion people were estimated to be underweight. According to the United Nations, more than 1 in 10 people do not get enough to eat and 1 in 3 people are malnourished. Around 45% of deaths among children under 5 years of age are linked to undernutrition, mostly occurring in low- and middleincome countries. At the same time in these countries, rates of childhood overweight and obesity are rising. Although there is an increase in obesity, many of those people are also undernourished – a condition known as hidden hunger.

Women, infants, children, and adolescents are at highest risk of malnutrition. From conception to a child's second birthday, it is important that infants have access to nutrient-dense foods to ensure the best start in life, with long-term benefits.

Poverty can be one of several contributors to malnutrition. Malnourishment increases healthcare costs, reduces productivity, and slows economic growth, perpetuating a cycle of poverty and poor health. Malnutrition impacts every country in the world in some form and fighting malnutrition is one of the biggest global health challenges today. The United Nations is committed to an initiative known as "Zero Hunger by 2030."

GE Methods of Nutrient Enhancement

Scientists can employ multiple GE methods to develop new plants with enhanced nutritional content. The genetic tools used to increase nutrient content include:

- Increasing gene expression, by increasing gene copy number or by manipulating gene promoter activity
- Adding genes to bridge gaps in a biosynthetic pathway (e.g., Golden rice that contains beta-carotene, a precursor of Vitamin A) or to create new biosynthetic pathways (e.g., omega-3 canola)
- Promoting the expression of transcription factors to turn on innate but inactive biosynthetic pathways in different plant tissues or at different developmental stages (e.g., anthocyanin in tomatoes).

While few nutrient-enhanced plant varieties are currently available, this may change as many are under development. It's important to know that plants developed for a specific nutritional purpose may also impact the availability of another nutrient.

For example, a crop engineered to produce oil with more of the essential fatty acid, linolenic acid (omega-3 fatty acid), could have less of the essential fatty acid, linoleic acid (omega-6 fatty acid). Some GE crops are in development with increases in multiple nutrients. Spanish researchers have created an African corn variety with 169 times more beta-carotene, 6 times more vitamin C, and twice as much folate. A GE sorghum variety produced by the Biofortified Sorghum Project for Africa has increased levels of beta-carotene, iron, zinc, and essential amino acids. Crops like these may help reduce malnutrition in underdeveloped countries.

Animal Food

Animals typically eat the same or similar diets their entire lives, and the nutrient requirements for each animal species during each life stage (e.g., pregnancy, lactation, growth, aging) and production conditions (if applicable) have been identified. Although the nutrient requirements (amino acids, fats, oils, carbohydrates, fiber, vitamins, and minerals) are usually defined, animal diets are made from several ingredients including whole grains, oilseeds, by-products of human food production, vitamins, and minerals.

Animal food includes crops cultivated through selective breeding for their nutrient content and can also be supplemented with other ingredients that fortify the food with important amino acids and other essential nutrients needed in animal diets. Periodically, animal nutritionists will review the latest nutrition information and provide guidance on the levels of nutrients that are required to promote good health. Plant breeders try to optimize nutrients in plants consumed by animals using similar approaches to those used to optimize plants for human nutrition.

What's Safe or Unsafe to Eat Differs by Species

Many people enjoy chocolate and cook or season their food with onions and garlic. However, each of these typical human diet items contain substances that can harm cats, dogs, and other animals. If they eat too much of them, it could cause sickness or death.

Plant Nutrients - Focus on Nitrogen

Plants get some of their essential nutrients from the air and some from the soil in which they grow. Plants absorb carbon, oxygen, and hydrogen from the air. The three main nutrients they obtain from the soil are nitrogen (N), phosphorus (P), and potassium (K), often referred to as the trio NPK. Some scientists are researching methods to give major crops the ability to "fix" nitrogen from the air into a biochemically usable form.

Nitrogen fixation is currently limited to certain microbes, and it is essential to life. Fixed nitrogen is a key ingredient in important biomolecules, including amino acids, which are the building blocks of proteins. Farmers currently add nitrogen to their crops by applying fertilizer or by planting legumes, which host nitrogen-fixing bacteria in their roots. Altering cereal grain crops to produce their own nitrogen would be an achievement for biotechnology, and this could help solve two big problems: The overuse of fertilizer, which can pollute aquifers or water bodies, and the shortage of fertilizer.

ABOUT THE TEAM

Together we (Marc George Ph.D., J Gold Genetics, and Bill Carrington Ph.D.) have formed a compliance Holding company https://flow.page/horizonholdings for the Legal Cannabis/Hemp and Mushroom Industry Holding subsidiary companies, that are providing Cell to Tissue Culture Media (for Cannabis/Hemp, Mushrooms, Cactus), Genetics Restructure (breeding cell-seed-tissue, storing, sales), Culturing for sale and R&D (cannabis/hemp, mushrooms, and cactus), Cultivation (indoor, field, greenhouse), Fertilizer 17 product line, Education online course accredited University, Extraction (compounds from mushrooms, cactus, cannabis, hemp, and hash)(from C02, cold & heat-press, and jar tech), Testing (R&D, molds, DNA, terps, cannabinoids, and compounds), Retail (store and E-Comm), Testing (R&D, molds, DNA, terps, cannabinoids, and compounds), Retail (store and E-Comm).

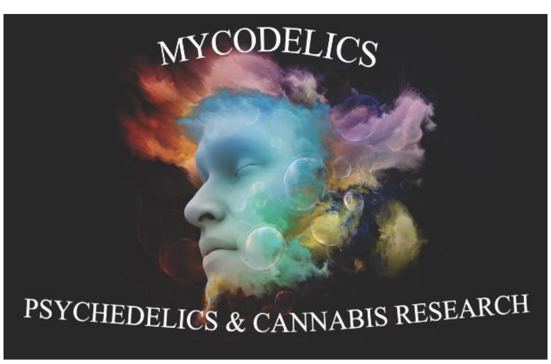
Mr. Gold is an executive with 30 years of national and international experience in the cannabis, biotechnology, breeding, and product design industries. Mr. Gold has led the creation, development, and growth of several companies throughout his career. An articulate and trusted voice in the world's cannabis space, Mr. Gold's life-long commitment to our consulting team has accomplished several pioneering Technologies. Created over 1000 genetics for the cannabis industry along with the first CBD strains to Market. Mr. Golds' genetics have been utilized in hundreds of cup-winning events.

Schwegman Lundberg & Woessner, P.A.
Alternative to Plant Characteristics without Traditional Genetic Engineering Trademark /patent issues (SLW: 4427.002PRV)
https://www.ecronicon.com/ecag/volume5-issue10.php

Plant Characteristics: Traditional Genetic Engineering This invention involves improved methods of generating hybrid plant cells, and hybrid plants, by somatic cell fusion without electric shock. The methods do not require recombinant alteration of cellular chromosomes by currently available genetic engineering procedures. For example, the inventive methods do not involve the transformation of cells by insertion into plant chromosomes or transient expression of coding regions from expression cassettes, expression vectors, viral vectors, plasmids, or other vectors commonly used for genetic engineering. Instead, the nuclei of fused somatic cells can naturally exchange genetic information by homologous recombination using processes like those that occur naturally during the sexual reproduction of plants. New types of hybrid cells are therefore formed that have desirable traits and improved characteristics.

(WO2017007833) HEALTHFUL SUPPLEMENTS, and (WO2018160702) HEALTHFUL SUPPLEMENT FOOD & Schwegman Lundberg & Woessner, P.A (WO2017007833) HEALTHFUL SUPPLEMENTS
https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2017007833&recNum=1&maxRec=17&office=&prevFilter=&sortOption=Pub+Date+Desc&queryString=FP%3A%28healthful+supplements%29&tab=PCT+Biblio

(WO2018160702) HEALTHFUL SUPPLEMENT FOOD that can be given in a solid and or liquid form, and or through a feeding tube. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018160702



BIOX UNIVERSITY OF BOTANY
Powered by J Gold Fertilizer

HORIZON HOLDINGS, LLC.

TONIGHT'S TOPICS
Experience Podcast

RESOURCES

BIOX UNIVERSITY OF BOTANY
Powered by J Gold Fertilizer

APPLE – How Does It Grow?
- www.youtube.com/watch?v=UWLmEh1HIBw

Apple Varieties of the Future from WSU's Apple
- Breeding Program
- www.youtube.com/watch?v=GeFCyeeDCYg

Cosmic Crisp® Apples
- www.cosmiccrisp.com/the-facts

The Apple That Changed the World
- www.npr.org/sections/
- money/2018/05/03/607384579/the-apple-thatchanged-the-world; 5:56; May 3, 2018

Farmweek – New Apple
- www.youtube.com/watch?v=jZsu-_EGa_M

Apple Tree Propagation: Grafting
- https://apples.extension.org/apple-treepropagation-grafting/

Incomplete Dominance, Codominance, Polygenic Traits,
- and Epistasis
- www.youtube.com/watch?v=YJHGfbW55l0

Monohybrids and the Punnett Square Guinea Pigs
- www.youtube.com/watch?v=i-0rSv6oxSY

Orange Pippin
- www.orangepippin.com

University of Illinois Extension – Apples and More
- www.extension.illinois.edu/apples/facts.cfm

USDA – National Apple Rootstock Breeding Program
- https://www.ars.usda.gov/northeast-area/genevany/plant-genetic-resources-unit-pgru/docs/aboutpgru/national-apple-rootstock-breeding-program/

Why are there so many types of apples?
- www.youtube.com/watch?v=mQePz62zkqA

National Agriculture in the Classroom
- www.agclassroom.org

Library of Congress: Johnny Appleseed
- www.americaslibrary.gov/jb/revolut/jb_revolut_
- apple_1.html
- FDA's "Professional Development Program in Food Science" is a summer program designed to train teachers how to use Science and Our Food Supply to maximize their students' learning. If you are interested in this program, please visit the program's website at www.teachfoodscience.org.
- How to extract DNA from strawberries
- www.youtube.com/watch?v=hOpu4iN5Bh4
- - Strawberry DNA Extraction Lab Explanation - www.youtube.com/ watch?v=vnjwNiJktZk
- Growing Strawberries: Strawberry Fields Forever from the CA Department of Food and Agriculture www.youtube.com/watch?v=CnQgSXrYo6Q
- What is DNA and How Does It Work? www.youtube.com/watch?v=zwibgNGe4aY
- Gene editing yields tomatoes that flower and ripen weeks earlier (2:50)
- www.youtube.com/watch?v=Jem3hP734uA
- CRISPR Gene Editing Explained (2:11)
- https://video.wired.com/watch/crispr-gene-editing-explained
- CRISPR Explained (Mayo Clinic) (1:38)
- www.youtube.com/watch?v=UKbrwPL3wXE
- CRISPR – a Word Processor for Editing the Genome (6:09)
- www.ibiology.org/genetics-and-gene-regulation/crispr

- **RESOURCES**
- • Bacterial Transformation
- www.youtube.com/watch?v=dKD19cXkWBw
- • Bacterial Transformation
- www.phschool.com/science/biology_place/labbench/lab6/intro.html
- • Engineer a Crop: Transgenic Manipulation
- www.pbs.org/wgbh/harvest/engineer/transgen.html
- • How to Make a Genetically Modified Plant
- www.youtube.com/watch?v=JtkhHIG3nx4&t=365s
- • Who Wants to Be a Genetic Engineer - Crop Genetic Engineering Simulation
- http://agbiosafety.unl.edu/education/whowants.htm
- • Changing the Blueprints of Life - Genetic Engineering: Crash Course Engineering #38
- www.youtube.com/watch?v=FY_ZUEKWhBc
- "Why Gene Editing Is the Next Food Revolution" - https://www.nationalgeographic.com/environment/future-offood/food-technology-gene-editing/ a
- A Visual Guide to Genetic Modification
- https://blogs.scientificamerican.com/sa-visual/a-visual-guide-to-genetic-modification/
- CRISPR – A History of Discovery
- www.youtube.com/watch?v=RKh2mi3tsmc
- HHMI Biointeractive: CRISPR-Cas9 Mechanisms & Applications
- media.hhmi.org/biointeractive/click/CRISPR/
- Nature Video: CRISPR Gene Editing and Beyond
- www.youtube.com/watch?v=4YKFw2KZA5o
- Science Magazine (more technical option)
- CRISPR-Cas guides the future of genetic engineering
- http://science.sciencemag.org/content/361/6405/866
- New Gene Editing Tool May Yield Bigger Harvests
- www.youtube.com/watch?v=UUo6lxLRbQ4
- What is CRISPR-Cas?
- www.youtube.com/watch?v=52jOEPzhpzc
- Future Predictions – Food Technology and Science
- www.youtube.com/watch?v=GCXhdAGx3NI
- Source: www.ncbi.nlm.nih.gov/pmc/articles/PMC6277064/
- (International Survey of Weed Resistance www.weedscience.org)
- The Amazing Ways Plants Defend Themselves (6:12)
- https://ed.ted.com/lessons/the-amazing-ways-plants-defendthemselves-valentin-hammoudi
- Do We Really Need Pesticides? (5:18)
- https://ed.ted.com/lessons/do-we-really-need-pesticidesfernan-perez-galvez#review
- Agriculture - Environmental Science (Bozeman Science) (9:24)
- www.youtube.com/watch?v=OGf04jPEaT0
- • Hungry Pests – USDA
- www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/?utm_campaign=crosby-2017&utm_
- source=hungrypests-com&utm_medium=redirect&utm_keyword=home
- • Plant Pests and Diseases Programs
- www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases
- • Pest Tracker
- www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/Pest-Tracker?utm_campaign=crosby2017&utm_source=hungrypests-com&utm_medium=redirect&utm_keyword=/the-spread
- Agricultural Pest Survey Cooperative – Pest Lists
- http://caps.ceris.purdue.edu
- History of Pesticides
- www.youtube.com/watch?v=gyZPDcr5_dw
- How Plants Make, Store, and Use Toxins
- https://learn.genetics.utah.edu/content/herbivores/planttoxins/
- Top Crop: Farming for the Future Educator's Guide
- https://www.nationalgeographic.org/media/top-crop-farming-future-educators-guide/
- Pesticide Labels
- www.epa.gov/pesticide-labels

- What is Integrated Pest Management (IPM)?
- https://www2.ipm.ucanr.edu/What-is-IPM
- University of Wisconsin - Agroforestry Practices: Strategies for Implementation
- www.youtube.com/watch?v=PRm4jnxCeMw
- Apple Fire Blight
- www.youtube.com/watch?v=PdcDXNftoWg
- • Agricultural News Website
- https://agfax.com
- • Agricultural Pests - UC IPM crop lists showing pests that affect each crop
- http://ipm.ucanr.edu/PMG/crops-agriculture.html
- • Cooperative Agricultural Pest Survey – Pest Lists
- http://caps.ceris.purdue.edu
- • Hungry Pests – USDA
- www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/?utm_campaign=crosby-2017&utm_
- source=hungrypests-com&utm_medium=redirect&utm_keyword=home
- • Pest Tracker
- www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/Pest-Tracker?utm_campaign=crosby2017&utm_source=hungrypests-com&utm_medium=redirect&utm_keyword=/the-spread
- • Plant Pests and Diseases Programs
- www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases
- • Pesticide Management Education Program – Cornell University
- pmep.cce.cornell.edu
- • Definition of "environmental footprint"
- https://dictionary.cambridge.org/us/dictionary/english/environmental-footprint
- • The carbon footprints of food production
- www.researchgate.net/publication/228649298/download
- • Classroom-Ready Lessons for Agriculture Instruction
- www.agednet.com
- – FM141 - Choosing A Tillage System To Save Soil and Reduce Costs
- – BT128 - Using Biotechnology To Alter, Control and Improve Plant Production
- – BT118 - Biotech: The Environmental Benefits
- • The nitrogen fix (Science Magazine)
- https://science.sciencemag.org/content/353/6305/1225
- • Pesticide Half-life Fact Sheet
- http://npic.orst.edu/factsheets/half-life.html
- • UC IPM - Birds, mammals and reptiles (Vertebrate pests)
- http://ipm.ucanr.edu/PMG/menu.vertebrate.html
- • Plant Diseases (National Program)
- www.ars.usda.gov/crop-production-and-protection/plant-diseases/
- • Carbon Sequestration in Soils
- www.esa.org/esa/wp-content/uploads/2012/12/carbonsequestrationinsoils.pdf
- • A Method to Measure the Environmental Impact of Pesticides
- https://ecommons.cornell.edu/handle/1813/55750
- • The nitrogen cycle
- www.sciencelearn.org.nz/resources/960-the-nitrogen-cycle
- Citrus Research Board
- www.citrusresearch.org/acp/
- • Biological Control for the Asian Citrus Psyllid – describes parasitic wasps
- www.youtube.com/watch?v=iHpmJy0Bq7M
- • Citrus Greening Disease!
- https://www.youtube.com/watch?v=G_1sobDdtiM
- • Breakthrough made in citrus greening research
- https://www.theledger.com/news/20190916/breakthrough-made-in-citrus-greening-research
- • Dozens of Trees with Incurable Disease Found in Pico Rivera
- www.whittierdailynews.com/2017/12/18/dozens-of-trees-with-incurable-disease-found-in-pico-rivera/
- • Questions and Answers – Draft Environmental Impact Statement and Preliminary Pest Risk Assessment for Permit for
- Environmental Release of Genetically Engineered Citrus Tristeza Virus
- www.aphis.usda.gov/biotechnology/downloads/CTV_Q&A.pdf

- • Metalized Reflective Mulch a Bright Spot for Citrus
- www.growingproduce.com/citrus/insect-disease-update/metalized-reflective-mulch-bright-for-citrus
- • Researchers Appear Close to a Remedy For Citrus Greening Disease
- www.npr.org/2016/05/12/477758594/researchers-appear-close-to-a-remedy-for-citrus-greening-disease
- ISAAA Pocket K No. 41: Nutritionally Enhanced GM Feed Crops
- www.isaaa.org/resources/publications/pocketk/41/default.asp
- Malnutrition (World Health Organization)
- https://www.who.int/news-room/fact-sheets/detail/malnutrition
- HarvestPlus: Knowledge Center
- https://www.harvestplus.org/knowledge-center
- Dr. Joseph Goldberger & The War on Pellagra
- https://history.nih.gov/exhibits/goldberger/index.html
- Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016.
- www.ncbi.nlm.nih.gov/pubmed/28580239
- Evaluating Credibility
- http://guides.lib.byu.edu/c.php?g=216340&p=1428399
- IFIC Fact Sheet: Benefits of Food Biotechnology
- https://foodinsight.org/fact-sheet-benefits-of-food-biotechnology/
- Biofortified Crops Generated by Breeding, Agronomy, and Transgenic Approaches Are Improving Lives of Millions of
- People around the World
- www.ncbi.nlm.nih.gov/pmc/articles/PMC5817065/
- Let Seed Be Thy Medicine (HarvestPlus)
- https://vimeo.com/328702230/5f793b3d1f
- The Poison is the Dose – Penn State
- www.youtube.com/watch?v=THr7roac0cA
- • Commercial Potato Production in North America – The Potato Association of America Handbook
- (numerous potato varieties on pages 28 -31)
- https://potatoassociation.org/wp-content/uploads/2014/04/A_ProductionHandbook_Final_000.pdf
- • New Plant Variety Regulatory Information
- https://www.fda.gov/food/food-new-plant-varieties/new-plant-variety-regulatory-information
- • GM Plants Questions and Answers: The Royal Society
- https://royalsociety.org/-/media/policy/projects/gm-plants/gm-plant-q-and-a.pdf
- • USDA: Biotechnology FAQs
- www.usda.gov/topics/biotechnology/biotechnology-frequently-asked-questions-faqs
- • Understanding New Plant Varieties
- https://www.fda.gov/food/food-new-plant-varieties/understanding-new-plant-varieties
- • Genetically Engineered Crops (The National Academies) The PDF version of the book can be downloaded for free from
- this web page.
- www.nap.edu/catalog/23395/genetically-engineered-crops-experiences-and-prospects
- • The Case for Engineering Our Food
- https://www.youtube.com/watch?v=wZ2TF8-PGQ4
- • How to Make a Genetically Modified Plant
- www.youtube.com/watch?v=JtkhHIG3nx4 (review)
- • Organisation for Economic Co-operation and Development, plant composition homepage
- www.oecd.org/chemicalsafety/biotrack/consensus-document-for-work-on-safety-novel-and-foods-feeds-plants.htm