Genetic Approaches to Chickpea (*Cicer arietinum* L.) Varietal Development

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Introduction

Chickpea is the world's second most important food legume, rich in protein (18%-24%), fiber, vitamin B, and minerals. India is the largest producer and consumer of chickpeas, with 14.81 million hectares under cultivation, 18.09 million metric tons of production, and a productivity of 1,221 kg/ha (FAOSTAT, 2022). India accounts for 72% of global chickpea production, with Madhya Pradesh contributing 45-50% of the country's total output. The crop is predominantly grown as rainfed across most parts of the country.

Despite India being the leading producer of chickpeas globally, it continues to import chickpea seeds from other countries, highlighting the need for further productivity enhancement. Understanding gene action, which refers to the mode of gene expression within a genetic population, is crucial for selecting suitable parents for hybridization programs and for choosing appropriate breeding strategies aimed at improving various quantitative traits.

Varietal Improvement in Chickpea

The varietal improvement will be possible through many different approaches.

1. Conventional breeding methods: Introduction, selection, hybridization, mutations *etc*.
2. Pre-Breeding methods: Wide hybridization, back crossing, introgression with special techniques.
3. Molecular Breeding: Marker assisted selection by using molecular markers and QTLs.
4. Omics Technology: Genomics, proteomics, transcriptomics, metabolomics *etc*.
5. Transgenics: Recombinant DNA technology, gene editing techniques *etc*.

Conventional breeding methods:

Various procedures that are used for genetic improvement of crop plants are referred to as plant breeding methods or plant breeding procedures. In chickpea, mainly three methods are employed for improvement of crop.

1. Selection: a. mass selection, b. pure line selection
2. Hybridization: a. bulk method, b. single seed descent method, c. pedigree method.
3. Mutational breeding

Selection permits the reproduction only in those plants that have the desirable characteristics, the plant has been selected.

Mass Selection: It refers to selection of superior plant on the basis of phenotype from a mixed population, their seeds are bulked and used to raise the next generation. This is a good method for improvement of old cultivars & land races. This is also used for the purification of improved cultivars. These varieties are stable in their performance than pure line varieties. *Ex*.: JG 74 and CSG 8962.

Pure line selection

A large number of plants are selected from a homozygous population of self-pollinated crop & are harvested individually, are evaluated and best progeny released as a new variety. Pure line variety is obtained from a single homozygous plant of a self-pollinated crop. These are more uniform but poor adaptable due to narrow genetic base. *Ex*.: DCP 92-3, Chaffa, Dahod yellow, BDN 9-3, JG 315, KWR 108, GNG 146.

Hybridization

This involves emasculation and pollination techniques. Select small buds that are ready to open in 2 days. Open the flower keel petals with forceps and remove anthers, then close the flower. Pollinate with fresh and fully opened flower in the next day morning under bright sunshine hours.

The knowledge on flower structure is important to perform hybridization programme. The flower of chickpea is solitary, axillary & polypetalous. The outer most petal is large called standard petal. Two lateral petals are lanceolate and curved, called wing petals(alae). Two anterior and partly fused innermost petals are called keel petals or carina. Stamens are ten in number, diadelphous (9 anthers are fused + 1 anther is free) condition and ovary is superior. Majority of buds commence opening between 8 am to 11am. After fertilization, pod formation starts in 5-6 days. Fruits are containing 1-3 seeds.

Pedigree method: Pedigree refers to record of the ancestry of an individual selected plant. It is a method of genetic improvement of self-pollinated species in which superior genotypes are selected from segregating generations and proper record of ancestry of selected plants are maintained in each generation. It is used when the both parents have good agronomic traits or well adapted. This method is used for the improvement of polygenic traits. *Ex.:* T2, T1, T3, T5, Radhey *etc.*

Pre-Breeding: The gene pool in the genus *Cicer* according to their crossability with *Cicer arietinum* (from easiest to hardest, GP1, GP2, and GP3, respectively), the pod set percentage is ranged from 10-35% in wide hybridization in chickpea crop. The wild species are the major sources of majority pest and disease resistance, abiotic stress tolerance genes and also for special nutritive characters and also have higer genetic diversity.

Table 1. Sources of disease resistance traits identified in *Cicer* species

Disease/pests	Resistant wild species
Diseases	
Ascochyta blight	*C. judaicum, C. pinnatifidum, C. cuneatum*
Fusarium wilt	*C. bijugam, C. reticulatum, C. echinospermum*
Botrytis grey mould	*C. judaicum, C. pinnatifidum, C. bijugam*
Rust	*C. reticulatum, C. echinospermum*
Phytophthora root rot	*C. pinnatifidum, C. reticulatum, C. bijugam*
Stem rot	*C. pinnatifidum, C. judaicum, C. Yamashitae*
Pests	
Helicoverpa pod borer	*C. bijugam, C. reticulatum, C. echinospermum, C. microphyllum*
Pulse beetle	*C. cuneatum, C. judaicum, C. judaicum*
Leaf miner	*C. judaicum, C. bijugam, C. reticulatum*
Root lesion nematode	*C. reticulatum, C. echinospermum*
Bruchids	*C. reticulatum*
Cyst nematode	*C. reticulatum, C. bijugam, C. pinnatifidum*
Root knot nematode	*C. bijugam, C. judaicum*

Table 2. Varieties developed from wide hybridization

S. No.	Wild *sps.*	Varieties developed	Character Improved
1	*C. reticulatum, C. bijugum, C. judaicum, C. pinnatifidum*	FLIP87-82C, ILC8262, ILC8617	Cold tolerance
2	*C. reticulatum*	L550	Introgression of yielding gene (6-17% YA)
3	*C. reticulatum X Pusa 256 x Pusa 362*	Pusa 1103	High Yield
4	*C. reticulatum x C. judaicum*	IPC 71	Pre-breeding line

Mutational breeding: The genetic improvement of various traits through the use of different types of mutagens (chemical/induced) called as mutational breeding. It is clearly planned and effective for screening of large populations**.** ***Ex.:*** Mutant G 130, BG 1003, WCG 2, WCG 3, WCG 10, Sadhbhavana, BG 408, BG 413, BG 417, Kiran (RSG-2), Ajay, Atul, Girnar, BGM 547 *etc.*

Genomics in chickpea

Genomics/MAS/GAB includes the development of molecular markers for a character to analyse genetic diversity, mapping, and identification of regions (QTLs) associated with desirable traits. Use of genomics tools in chickpea has allowed the development of new cultivars through MABC, Genomic Selection, MARS *etc.*

Ethiopia became the first country to release a climate resilient chickpea variety, Geletu using MABC (ICC 4958 x JG 11). Pusa chickpea 10216 is the first molecular breeding variety with enhanced drought tolerance released in India (ICC 4958 x Pusa 372). Super Annigiri 1 also developed by the University of Agricultural Collaboration with from ICRISAT. It is resistant to fusarium wilt disease introgressed through MABC to Annigiri variety. It has been identified for release in A.P., Karnataka, Maharashtra and Gujarat *etc.*

Transcriptomics in Chickpea:

Two biological techniques are used to study the transcriptome, namely DNA microarrays, a hybridization technique and RNA-*seq*, a sequence-based approach. This is under progress in developing the improved varieties of chickpea.

- DNA micro arrays: As an alternative to genome mapping, microarrays have recently been applied in crop species to identify and assess the function of putative genes thought to be involved in plant abiotic stress and defense responses. Micro arrays provide a high through put means to study the gene expressions of thousands of transcripts simultaneously.
- RNA-*seq* is the preferred method and has been the dominant transcriptomics technique since 2010.

Proteomics & Metabolomics:

Study of entire set of proteins produced or modified by the plant. It denotes the large-scale experimental analysis of proteins and, *esp.* protein purification and quantification by mass spectrometry. It is more complicated than genomics and transcriptomics because the plant's genome is more or less constant, but proteins are differed from cell to cell and from time to time. Biological processes are more complex related to abiotic stress tolerance, which cannot be understandable by using transcriptomics and proteomics. Hence, metabolomics was emerged, which means study of cell metabolites. This focus on high through put characterization of small molecule metabolites in biological pathways. Metabolites are the substrates, intermediate and products of metabolism. Metabolites bridges the gap between genotype and phenotype.

Transgenic Chickpea:

Generally, increase the level of expression of different proteins/ osmolytes are responsible for conferring tolerance/ resistance against abiotic/biotic stresses. Hence, it is necessary to transfer useful genes to desired plants using genetic modification methods, gene editing like ZFNs, TALENs, CRISPR Cas9 and RNA Silencing *etc.* In chickpea, production of GM varieties is under progress.

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