Principles of Plant Breeding

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If there's farming, there's life; if there's a farmer, there's India

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Lesson 1	Plant breeding as a Dynamic Science
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Objectives

- Introduction to plant breeding as a dynamic science for crop improvement.
- Important milestones, and scientific discoveries that have happened till date.

Glossary of terms

1. **Variation**: The differences existing among various individuals in a population.
2. **Variability**: The range of variation in a population.
3. **Genotype**: The genetic constitution of an individual. It is determined by the set of genes contained.
4. **Phenotype**: The phenotypic expression of an organism.
5. **Selection**: The preferential contribution of an individual to next generation.
6. **Plant breeder**: A qualified or trained person who knows the principles of genetics as applicable to crop improvement.

1. Introduction

Agriculture is a recent human venture with just 1200 years ago that woman began collecting plants from wild and cultivating them for food, feed and other basic requirements of life. Early humans gathered whatever they could find in the wild that was edible. With change in lifestyle from wandering to sedentary, they brought and planted desirable plant types at their abode. This was the beginning of the era of domestication of plants. This was followed by discrimination (selection) among the available natural biological diversity which gradually but systematically favoured characteristics that increased the utility of the plants to human beings.

With time, domesticated plants become markedly different from their wild progenitors in specific ways that were valuable and advantageous to humans. The settled agriculture led to rapid domestication of a number of cultivated crops that we see today. The early efforts to enhance

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productivity of crops could not essentially be classified under plant breeding as much of the improvements were brought about by cultural management practice such as irrigation and fertilizer in fact very little of research on plants before 1800 could come under plant breeding

Except few studies on botany and evolution of plants. There are reports that attempts to improve rice in China and Tulips and Hyacinths in Netherlands could be thought of an earliest plant breeding activities.

2. Definition

Plant breeding can be defined as an art, a science, and technology of improving the genetic make up of plants in relation to their economic use for the man kind. In technical terms, plant breeding can be defined as the management of genetic variability. In fact, plant breeding uses selection and other methods to utilize the available genetic variability in crop species for improvement in

Economically important traits. Much credit goes to those early men and women who brought wild plants into cultivation and transformed them into domesticated species that shaped our present civilizations. The changes we make to improve crops are often rather modest in comparison to the changes they achieved without modern technology. The principles of Mendelian genetics provided the scientific basis for plant breeding and over a period of time since the rediscovery of Mendelian laws of inheritance in early 20th century, it has evolved as one of the important areas of scientific activity that has contributed in understanding the nature of inheritance of economic traits and the subsequent endeavour to improve these traits in desirable direction. Some of the standard definitions of plant breeding are:

Vavilov (1926): Plant breeding is the evolution directed by will of man

Stebbins (1957): Plant breeding is merely a continuation of natural evolution of crop plant species changing its course in the direction of greater use to mankind

Frankel (1958): Plant breeding is the adjustment of plants to service of man

Smith (1966): Plant breeding is an art and science of improving genetic pattern of plants in relation to their use.

Riley (1978) defined plant breeding as a technology of developing superior crop plants/ varieties for various purpose.

Pohelman and Sleeper (1995): Plant breeding is the art and science of improving the heredity of plants for benefit of humans

Bernardo (2002): Plant breeding is science, art and business of improving plants for human benefit.

Acquaah (2007): Plant breeding is a deliberate attempt to change, with respect to the heredity of the plants, to an advantage.

Plant breeding has been defined in many ways, but, with all definitions converging at its primary objective of improving crop plants in respect of economically important traits. It has been defined as the application of genetics to develop crop varieties more suited to human needs than their ability to just survive in wild. The changes brought about during domestication and subsequent attempt by early man to improve plants got stabilized and were heritable. Such changes are primarily sought with an aim to fulfill the human needs for food, feed, and other requirements. In fact, among all the scientific disciplines of agriculture, the pressure and expectation on the plant breeding to deliver has been enormous. Plant breeding as a science has been evolving as much are the challenges confronting agriculture. Initial focus of almost all breeding operations globally was improvements of productivity of economic traits (Production Breeding). With high yielding varieties replacing old landraces, there was a transition from heterogeneity to uniformity. This resulted in outbreak of disease and pest epidemics forcing plant breeding to shift focus from production to resistance (Resistance Breeding). Breeders were lucky to have resistance governed by oligo-genes, thus were easy to transfer but due to higher selection pressure witnessed breakdown and forced a change of focus from vertical resistance to horizontal resistance. Further, with deterioration of biophysical resource base for agriculture, triggered largely by human activities plants began to confront various abiotic stresses such as drought, submergence, salinity, acidity, etc. Plant

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breeders again had to broaden the spectrum of their operations and currently plant breeders attempt not only to develop improved varieties but varieties that can yield better under adverse climates. In fact, with climatic change looming large, plant breeders have an added responsibility of designing crop cultivars suitably to cope up predicted losses in productivity due to global warming (Resilience). There is also focus on developing crop cultivars that have predetermined levels of expression of traits related to yield, quality and resilience using both conventional and molecular approaches (Breeding by design).

Plant breeding involves both art and science of improving plants by manipulating their genetic patterns in relation to their economic use. The art of plant breeding is the intuition, skill and judgment of his work. The art does not only involve the wisdom of choice of the plants but also the methods and tools available with a plant breeder. Thus as Duvick (1996) stated that, “Breeders universally depend on experiences and art more than on genetics”. The best plant breeders are the best artists. They may or may not be the best geneticists.

The science behind the plant breeding derives bulk of its principle from the science of genetics. Duvick (1996) has appropriately pointed that even though useful and significant contributions have, and will continue to, come from genetics and its allied branches, the practical plant breeding based on plant breeders skill will surely not change much in its structure and impact.

3. Application of Genetics in Plant Breeding

There are generally five different areas of genetics that have been applied to plant breeding:

Qualitative genetics: Where inheritance is controlled by alleles at a single locus, or at very few loci

Population genetics: Which deals with the behaviour (or frequency) of alleles in populations and the conditions under which they remain in equilibrium or change. Thus allowing predictions to be made about the properties and changes expected in populations

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Quantitative genetics: For traits where the variation is determined by alleles at more than a few loci, traits that are said to be controlled by polygenic systems. Quantitative genetics is concerned to describe the variation present in terms of statistical parameters such as progeny means, variances and covariances

Cytogenetics: The study of the behaviour and properties of chromosomes being the structural units which carry the genes that govern expression of all the traits

Molecular genetics: Where studies are carried out at the molecular level. Molecular techniques have been developed to investigate and handle both qualitative and quantitative characters.

Conventional breeding (classical breeding or traditional breeding), is the development of new varieties (cultivars) of plants by using older tools and natural processes, as opposed to the newer, more sophisticated and sometimes radical tools of molecular plant breeding.

Molecular breeding may violate natural biological boundaries in the manipulation of the genetics of plants. They are able to introduce into the new cultivar desirable genes that are alien to its species. In conventional breeding, breeders assemble desirable traits from different but usually closely related plants into the new cultivar using the techniques of crossing (hybridization).

4. Plant Breeder and His Job

Plant breeders are the professionally educated people who use the principles of genetics to change the trait expression in the desirable direction. Plant breeders are mainly concerned with the dissection of the phenotypic variation into various components such as genotype, environment and G x E to be able to manipulate Genotype effectively and efficiently to maximize the genetic gain. Thus a primary requirement for the successful plant breeder is adequate knowledge of genetics and its allied disciplines. Plant breeder is essentially a system biologist who understands the components of G, E, G x E, locate new sources of genetic variation that corrects the weakness in the cultivars and introgresses them

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efficiently; and integrates the relevant disciplines to create a superior variety that is stable across locations and is

acceptable to growers and consumers. Plant breeders essentially use their knowledge of genetics, quantitative genetics, statistics and mathematics to estimate the important genetic parameters such as genetic variation, heritability, gene action and G x E that are critical to designing an efficient breeding programme that is likely going to yield some amount of improvement in trait expression to be of some economic use. However, successful plant breeders need to have adequate knowledge of physiology, pathology, entomology, soil science and experimental designs. The advent of new technologies in late 1980's such as genetic transformation for developing transgenic, genomics for high throughput sequences of whole genomes and marker assisted selection for using DNA sequence polymorphism for precise selection in gene transfer especially from wild relatives, have opened up new vistas for plant breeding and even more challenges for a plant breeder. Today's plant breeders thus need to expand the spectrum of their knowledge to include such cutting edge technologies even though it cannot be expected that a conventional plant breeder can be equally proficient in modern plant breeding techniques.

Identification/creation of useful variation: Genetic variation or variability is indispensable raw material for plant breeding. The common conventional method of creating a nonexistent gene is via mutagenesis (use of mutation agents to induce variation). Variability exists in many plant **gene banks** (repositories for plant germplasm) to which scientists have access. Gene banks may be operated on a small scales by national governments. However, comprehensive operations are undertaken by international entities such as the international research centers. The desired variability may already exist in the breeder's local collection and in remnants from previous breeding projects.

Selection and Evaluation: Selection is simply discriminating among the available or created variability to identify individuals with the desired combination of genes (genotype) or expressed trait(s). With the

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introduction of genetics and statistics into modern plant breeding, breeders have developed standard breeding methods for the species, the genetics of the trait of interest and the type of product desired. There are selection or breeding methods for species based on their modes of reproduction, genetics or whether the product should be uniform or variable. The final selection cycle in breeding results in a small number of genotypes that are potential candidates for advancing as cultivars for release to producers. These genotypes are subjected to rigorous *evaluation* under conditions which must include those under which the cultivars will be commercially grown. Evaluations may be conducted at multiple locations and over multiple years. Included in such evaluations are standard cultivars of known performance (for comparison), which could be replaced should superior performers emerge from the trials.

Certification and Cultivar Release: In countries with more advanced agricultural operations, there exist standardized and approved protocols for releasing new cultivars to growers. There may be national crop certifying agencies that oversee the seed certification process for various crops. The ultimate purpose of seed certification is to ensure that the seed produced by the plant breeder reaches the public (consumer) in its highest quality, original genetic identity and highest genetic purity.

Multiplication and Distribution: Certified seed is multiplied by certified seed growers contracted by independent breeders and seed companies to mass-produce released cultivars for sale to growers. New cultivars are sold to consumers via a variety of outlets. Commercial seed companies have elaborate sales mechanisms.

5. Aims of plant breeding

- Plant breeding seeks to change the trait expression to desirable direction and level depending upon the trait as well as the crop. For traits with economic value usually a higher performance is sought such as yield and its components and post harvest quality. Whereas

for traits such as maturity a lower value is desirable. For traits with end use value such as color, shape, size, quality, diverse benchmarks are used depending upon the consumer preference, industrial utility or other factors.

- Plant breeding as a technology essentially seeks to change expression of traits of economic importance for meeting human needs. The basic raw material for developing improved plant types through directed selection progress is genetic variability. The desired level and direction of trait expression is accomplished through simple selection if enough variability is available for a trait. In case of low genetic variability of traits, hybridization is done to combine desirable levels followed by selection.
- The advent of biotechnology has opened up avenues for creation of requisite variability by cutting across the barriers imposed by sexual compatibility. However all these operations beginning from survey of variability, its creation and utilization are largely dictated by objectives of breeding which determine appropriate breeding methodologies to be undertaken. The goals of plant breeding are evolving as in plant breeding itself.

6. Objectives of plant breeding

1. Higher yield: Since the beginning of plant breeding, even before Mendel's laws of inheritance were recognized, yield has been undauntedly the foremost objective if plant breeding driven by need to feed the ever increasing human population. Since plants are primary producers in the ecosystem and have the capacity to supply basic nutritional ingredient, they are ideal sources of food, feed, and other human requirements. The plants that have been targeted for improvements through plant breeding include the cereals such as wheat, rice, maize, barley, oats, sorghum: pulses such as beans, cowpea, chickpea, lentil, sugar crops such as sugarcane and sugar beet, fruits such as orange, mango, apple, watermelon, walnut, almond, Oil crops such as groundnut, soybean, Rapeseed-Mustard, Olive, sunflower, soybean; fibre crops such as cotton, jute,etc Vegetables such as cabbage, cauliflower, brinjal, tomato, onions etc; Starch crops such as potato, sweet potato; Spices such as chilli, garlic,

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cardamom, saffron etc as well as a large array of medicinal plants dispersed throughout the world and also the floricultural crop there are yet other crop who have specific economic values. In spite of the phenomenal growth in world population, the crop productivity driven by the efforts of strenuous breeding effort at national and international research institutions has been able to keep pace with population growth.

However a major concern that brings in extra yard of pressure plant breeders is that land resources are constantly declined and more land is becoming unfit for agriculture. This coupled with yield platues in many of major crops poses one of the breeders to develop crop cultivars that could meet the human requirements within the limitation of non-expandable land area and deteriorating bio-physical resources base. Presently global population stands in excess of 6 billion and is expected to reach 8 billion in 2030 and 9 billion in 2050. To add to the problems are about 10-35 hectares of land that are annually lost to other uses, with help of this land coming from cropped area. Although it cannot be thought of that all the required impetus to enhancing crop productivity will come from plant breeding efforts but there is no doubt either that a significant contribution has to come from plant breeding

2. Better quality: Different classes of crop plants that serve as human food and animal feed such as cereals, pulses, oil seeds, vegetables, fruits, sugar crop etc have certain unique nutritional characteristics whereby they are quite rich in some nutritional components, very poor in certain essential nutrients or have higher qualities of certain anti-nutritional factors. Thus enhancement of quality as a breeding objective primarily seeks to target such area and aims to manipulate crop compositions to desired levels. Thus enhancement of quality refers to improvement of nutritional profile of crop plant either to improve quality per se, combating nutritional deficiency, remove or decrease the content of anti-nutritional factors, or enhancing market value of foods or crops based on certain parameters.

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The foregoing discussion can be explained with the help of following examples:

Rice is a major food crop being consumed by about half of world population, about 80% Asians. Rice being poor in Vitamin A result in large scale occurrence of vitamin A deficiency in developing nations with about 2.5 million children becoming blind every year and half of them dying within 12 months. A viable strategy to combat such a problem would be to enhance the levels of β-carotene content such as phytoene synthase, phytoene desturase and lycopene cyclase were transferred from various sources such as **Daffodil** (*Narcissus pseudonarcissus*) and ***Erivinia carotovora*** to rice to develop **Golden rice** which has enhanced levels of β-carotene.

Maize is another important cereal, which even though is relatively rich in proteins, but the proteins of maize are poor in essential amino acids such as lysine and Tryptophan. Dr.S.K.Vasal a CIMMYT maize breeder made Tryptophan content of maize by using a recessive mutation known as "**Opaque-2**". (The name has its origin to opaque kernels of grains carrying opaque-2 mutation) already discovered by Mertz in 1920. The newly developed lines had higher lysine and Tryptophan with better grain characteristics (a disadvantage with original mutant) and was

designated as quality protein maize.

Similarly pulses are a very rich sources of protein but contain certain antinutritional factors such as protease Inhibitors, amylase inhibitors, Lectins, Tannins, Saponins, phytates and Oligosaccharides (Raffinose, stachyose). The proteins of pulses is also poor in sulphur containing amino acids such as cysteine and methionine. Pulses like soybean have an undesirable beamy flavor caused by activity of enzyme "**Lipooxygenase**".

Brassicas oilseeds have certain undesirable fatty acids such as erucic acid and glucosinolates. Breeders have identified low erucic acid gene sources

in B. compestris (Torpe), B. napus (liho) and B. juncea (Zem-1 & Zem-2) as well as low glucosinate lines in B. napus (Brownoski) and B. compestris (polish). These have been used to develop low erucic acid (<2%) and low glucosinate (<3µmoles) brassica oilseed varieties called as **double zero lines (00')**. Brassica varieties with low erucic acid, low glucosinate and low fibre content are called as **triple zero lines ("000")**.

In case of wheat quality is usually determined in terms of its suitability for various end products such as breeds, chappatis, biscuits, noodles etc. Hard wheat are usually good suitable for biscuits, pastries, cakes etc. The quality of wheat flour is determined by protein gelatine which consist of gladdens and gluten ins, that give elasticity and extensibility to dough.

In case of cotton, the quality is determined in terms of various fibre parameters such as fibre length, strength, fibre fineness, uniformity ratio, elongation etc. Breeding methods such as selection and hybridization has resulted in a large spectrum of varieties with improved attributes in respect of above traits. India has a distinction of developing just commercial hybrid in cotton developed in 1970 at GAU by Dr. C.T.Patel. It was an intra-hirsutum hybrid developed from a cross between **G-67** & **American Nectriless** and named as H-4(**Shankar-4**). This was followed by inter-specific hybrid **Varalaxmi** developed by Dr.B.Katarki at UAS, Dharwad by crossing **Laxmi** with **SB-289** in 1972.

Crops and the quality traits

Rice : Cooking quality, aroma, kernel characteristics

Wheat : Milling and baking quality

Maize: Protein content and aminoacid profile

Barley: malting quality

Forages: Protein content, Digestibility, low toxins

Pulses: Protein content, low antinutritional factirs

Cotton: Fibre length, strength and fineness

Oilseed : Oil quality, essential fatty acids, Low erucic acid and glucosinolate

Vegetables nutritive value and keeping quality

Fruits: Cololur, size, shape and keeping quality, juice quality

Sugarcane: Sugar recovery

Medicinal plants : Recovery and composition of essential oils

Floricultural crops: Flower colour, shape, size, vase life, fragrance

3. Resistance to biotic stresses : Biotic factors such as diseases, pests, nematodes, rodents cause tremendous losses to crop yields, therefore it is an important component of breeding prograammes to develop crop varieties that have inbuilt ability to ward off the damage caused by biotic factors. The production breeding driven by the urge to increase food grain production resulting in an array of high yielding varieties in almost all crops which lead to the replacement of old and traditional landraces with a few high yielding varieties thereby creating a situation of virtual monoculture as these HYV's were pushed for large scale cultivation. A new problem began to emerge in shape of periodic outbreaks of disease and pests. This came as an added responsibility to plant breeder for they had to add another feature to crop cultivars in addition to higher yield potential.

Resistance, when achieved, is the most reliable and economical way of controlling disease and pest therefore, breeders have incorporated it as one of the most important component of their programs and constitute a vital component of varietal characteristics. Control of plant disease through use of resistant varieties will greatly help in stabilization of yield. In fact, some of the outstandin achievements in plant breeding in recent times have been in the field of resistance breeding. The primary goal of

plant breeding lies in identification of source of resistance and utilizing them in development of new improved varieties. A classical example of this area is development of **Thatcher** crossed to leaf resistant variety "**Hope**" and resulted in development of "**New Thatcher**" wheat. Similarly, B. P. Pal extensively made planned crosses in wheat and developed **NP-809** which was resistant to all the three rusts. Resistance breeding can help in enhancement and stabilizing productivity by removing bottleneck genes which tend to limit the expression of inherent yielding ability of plant under the influence of disease or pest.(Joshi,1963)

. These kinds of efforts by plant breeders have resulted in the development of **multilines** as proposed by **Norman Borlaug**. Gene pyramiding is also being attempted in recent time using molecular markers and great success has been achieved in case of blight disease of rice.

4. Resistance to abiotic stresses: Along with diseases and pest cultivated crops are also exposed to a number of abiotic stresses such as drought, salinity, acidity, submergence in varying degree of intensity, which can potentially limit the yielding ability of crops. These stresses have become even more prominent in recent times due to global climate changes that have resulted in erratic rainfall patterns, degradation of soil and pollution of water. As against disease and pest resistance, the pathways governing the plant resilience to such abiotic stresses are often more

complex involving more than one genetic and physiological mechanism, thus making it difficult to manipulate. Therefore the achievements in thus field are not many even though a number of crop varieties have been developed that possess fair tolerance to drought, flooding, salinity etc. A major problem in breeding varieties for such stresses is the cumbersome and laborious screening procedure. However, such traits, as against yield, can be selected even at cellular level. Thus specific approaches such as mutation and transgenic technology can be of great value.

Breeding for abiotic stress tolerance is even more significant in new of the fact that any initiative to increase the aerable land will largely come from bringing more marginal and stress-proof area under cultivation, where the growing condition are always limiting in more or the other growth

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parameters this is especially true for poor nation who cannot afford the costly amelioration practices such as irrigation, fertilizer application, drainage or soil amendments. The arable constitutes a major 3% of earth's surface and is being constantly determined as a result of soil erosion, salinisation, nutrient mining and acidification. This coupled with increased population will actually decrease the per capita arable land from current level of 0.28 ha to 0.17 ha by 2025. To add to the agony, Global agriculture is about to lose about 10-30% to post harvest loses

especially in poor countries. Thus enhancing productivity in these, seemingly, no so favourable parameters, assumes greater significance

5. Early maturity: Earliness is the most desirable character which has several advantages. It requires less crop management period, less insecticidal sprays, permits new crop rotations and often extends the crop area. It also helps in preventing crop damage by insects and diseases as in case of cotton..

6. Growth habit: Growth habit especially the determinate groth habit is desirable in case of pulses such as common bean, Mung, Pigeon Pea (*Cajanus cajan*), Cotton (*Gossypium sp.*), etc.

7. Dormancy: Vivparity (germination of seeds even before harvesting in the standing crop if there are rains at the time of maturity) is a problem in many crops such as Greengram, Blackgram, Barley and Pea, etc. A period of dormancy has to be introduced in these crops to check loss due to germination. However, some other cases where crops are inherently dormant such as Kala zeera it may be desirable to remove dormancy.

8. Agronomic Characteristics: Agronomic traits include various morphological traits such as plant height, tillering, branching habit,

panicle/ear/pod type, ease of harvesting etc that need to be considered while breeding a new variety.

9. Elimination of Anti-nutritional factors: Various chemical and biochemical factors that limiyt the utility of a crop are called as antinutritional factors. A number of antinutritional factors have been reported in crop plants:

Crop and their antinutritional factors

Pulses: Lectins, raffinose, Stachyose, verbasose

Oilseed brassica: Erucic acid, glucosinolates

Forages: HCN

Lathyrus: Lathyrin

Sorghum: HCN

Cotton: Gossypol

Soybean: Lipoxygenase

Cowpea: Trypsin inhibitors

Beans: Raffinose, stachyose

10. Shattering resistance: Shattering is serious problem in pulse crops like cowpea and green gram therefore is an important objective in such crops.

11. Synchronous Maturity: In crops like Greengram, Cowpea, common bean and Cotton where several pickings are required for crop harvest, it is desirable to have synchronous maturity.

12. Photo and Thermo insensitivity: In order to ensure that crops are introduced in newer/non-conventional areas, it is essential to have light and temperature insensitive varieties. It also helps in fitting crops in new rotations for crop diversification.

13. Wider adaptability: Adaptability refers to ability of a variety to perform uniformly over a wide range of environmental factors. Such an attribute enhances the suitability of a crop variety for large scale cultivation.

14. Biofortification: Recently there has been great emphasis on biofortification of crops in respect of limiting nutritional components such as Fe, Zn, amino acids etc.

15. Pharmacrops: It refers to modification of plants to generate plant derived pharmaceuticals to supply low cost drugs in view of the fact that plants produce a lot of biomass.

16. Multiple use crops: In case of crops which have multiple purpose economic products, it is attempted to improve on account of multiple uses. Example Cotton (cotton fibre, cotton seed oil and oil cake).

17. Plant Architecture for mechanized farming: It involves changing plant architecture such as number and position of leaves, branching pattern of stem, height and position of harvestable organs.

18. Water and nutrient use efficiency: in view of water and fertilizer becoming limiting inputs for agriculture, a major breeding goal is to develop crop varieties having better water and nutrient use efficiency.

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1800 i. Knight used artificial hybridization to develop several new fruit varieties.

ii. Le couteur and Shireff used individual plant selections and progeny test to develop some useful cereal varieties

1840 John Le Couteur: They developed the concept of progeny test individual plant selection in cereals.

1847 “Reid’s Yellow Dent” maize developed

1856 Vilmorin developed the progeny test and used this method successfully in the improvement of sugar beets.

1859 Darwin publiushed the *Origin of Species*

1865 Mendel, G.J (Austria): Discovered principles of inheritance in garden pea.

1866 Mendel proposed laws of inheritance

1873 The work of Patrick Shireff was first published.

1877 Darwin conducted experiments on hybridisation

1879 Beal determined the effect of heterosis in maize

1890 Rimpau (Sweden): First made inheritance cross between bread wheat (Triticum aestivum) and rye (***Secale cereale***), which later on gave birth to triticale.

1899 Hopkins described the ear-to-row selection method of breeding in maize

1900 Nilson-Ehle, his associates developed the individual plant selection method in Sweden.

1900 Mendel’s laws of heredity rediscovered independently by Correns of Germany, de Vries of Holland, and von Tschermak of Austria

1903 i. Johannsen proposed the pureline theory that provided the genetic basis for individual plant selection.

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ii. The science of genetics began with the rediscovery of Gregor Johan Mendel's paper in 1900 by Hugo de veris, Tshermark and Correns which was originally published in 1866.

iii. The modern plant breeding methods have their bases in the genetic and cytogenetic principles.

iv. The discovery of chromosomes as carriers of genes has led to the development of specialized plant breeding methods for chromosome engineering.

v. The totipotency of plant somatic and gametic cells allows regeneration of complete plants from single cells. This, coupled with the development of recombinant DNA technology, has enabled the transfer of desirable genes from any organism into plants.

vi. Crop varieties developed in this manner are already in cultivation in several countries.

1908 Shull and East proposed Overdominance theory of heterosis

1908 Hardy of England and Weinberg of Germany developed the law of equilibrium of populations

1909 Nilson.Ehle proposed multiple factor hypothesis

1909 Hardy of England and Weinberg of Germany developed the law of equilibrium of populations

1910 Bruce, Keeble and Pellew proposed Dominance theory of heterosis

1914 Shull coined the term heterosis

1917 Jones supported dominance theory of heterosis

1917 Jones developed first commercial hybrid maize

1919 Hays, H.K. Garber, R.J Gave initial idea about recurrent selection. They first suggested use of synthetic varieties for commercial cultivation in maize.

1920 East E.M and Jones, D.F, also gave initial idea about recurrent selection.

1921 Sewall Wright described systems of mating.

1921 Mooers established the importance of G x E interaction.

1922 Harlan and Pope gave Backcross method

1925 East and Manglsdorf described gametophytic self-incompatibility

1926 Pioneer Hi-bred Corn Company established as first seed company

1926 Vavilov identified 8 main and 3 subcenters of crop diversity and law of parallel variation.

1927 Karpechenko produced intergeneric hybrid between raddish and cabbage

1928 Stadler used X rays for mutation

1934 Dustin discovered colchicines

1935 Nahaher U proposed origin of tetraploid species of brassica by U triangle

1936 East, E.M: Supported over dominance hypothesis of heterosis proposed by East and Shull in 1908.

1937 Harrington proposed mass pedigree method

1937 Balkeslee and Avery established use of colchicine

1939 Goulden proposed Single seed descent method

1940 Jenkins proposed Recurrent selection

1940 Harlan proposed the Bulk method

1945 Hull coined the term Recurrent selection

1950 Hughes and Babcock: First discovered sporophytic system of self-incompatibility in *Crepis foetida.*

1952 Jenson proposed the use of multilines

1953 Borlaug outlined a method for multiline development in wheat

1958 Thoday gave the concept of disruptive selection

1963 Van der Plank gave the concept of vertical and horizontal resistance

1964 Borlaug, N.E: Developed high yielding semi dwarf varieties of wheat which resulted in green revolution.

1965 Grafius, J.E: First applied Single Seed Descent (SSD) method in oats.

1967 Frankel coined the term “Genetic resources”

1968 Donald gave the concept of ideotype

1970 C T Patel developed first coton hybrid

1970 Borlaug received Nobel Prize for the Green Revolution

1974 Establishment of International Board of Plant Genetic Resources.

1976 Yuan Long Ping et al: Developed world’s first rice hybrid (CMS based) for commercial cultivation in China.

1978 First CMS based hybrid rice in China

1980 Botstein et al demonstrated use of RFLP

1983 First transgenic tobacco in USA

1987 First transgenic cotton by Monsanto in USA

1990 Williams et al established use of RAPD

1991 Release of first pigeonpea hybrid **ICPR-8** in India by ICRISAT

1994 “**FlavrSavr”** tomato developed as first genetically modified food produced for the market

1995 *Bt* corn developed

1996 **Roundup Ready®** soybean introduced

1997 identification of **terminator gene** in USA

2000 Arabidopsis genome sequenced

2002 Draft sequence of rice genome published in *Science*

2004 Roundup Ready® wheat developed

2008 Monsanto. USA: Identificaton of traitor gene, which responds to specific brand of fertilizers and insecticides.

2008 Papaya genome sequenced

2009 Firs MAS derived wheat variety Patwin released

2009 First transgenic papaya X17-2 with virus resistance released

2010 Soybean genome sequenced

2011 Charpentier and Doudna isolated components of CRISPR-Cas9 system

2012 Watermelon genome sequenced

2012 Muskmelon genome sequenced

2012 Tomato genome sequenced

2013 Peach genome sequenced

2013 Sweet orange genome sequenced

2013 Beet root genome sequenced

2014 Rapeseed (*Brassica napus*) genome sequenced

2015 First non-browning apple transgenic Arctic-Golden Delicious released

2016 First delayed ripening pineapple transgenic PinkGlow released

2017 Chenopodium genome sequenced

2018 Non-browning apple transgenic Arctic-Fuji released

2018 First genome edited Soybean variety Calyxt with modified oil composition developed

2019 Brinjal genome sequenced

2020 *Eruca sativa* (Rocket salad) genome sequenced

2020 Jojoba genome sequenced

2020 Loquat genome sequenced

2021 Prof Rattan Lal receives World Food Prize

2021 st gene edited GABA rich tomato released

8. History of plant breeding in India

Timeline Development

1871 The Government of India created the Department of Agriculture

1892 First scientist appointed in Departmennt of Agriculture

1905 The Imperial Agricultural Research Institute was establish in Pusa, Bihar

1921 Central Cotton Committee established

1934 The buildings of the institute damaged in earthquake

1936 Shifted to New Delhi

1946 Name was changed Indian Agricultural Research Institute

1901-05 Agricultural Colleges were established at Kanpur, Pune, Sabour, Llyalpur, Coimbatore

1929 Imperial council of Agricultural Research was established

1946 Name was change to Indian Council Agricultural Research

1921 Indian Central Cotton Committee was established – Notable researches on breeding and cultivation of cotton.

1954 First multiple race resistant wheat variety NP 809 developed by B P Pal

1956 Project for intensification of regional research on cotton, oilseeds and millets (PIRRCOM) was initiated to intensify research on these crops – located at 17 different centres through out the country

1957 All India Coordinated maize improvement project was started with objective of exploiting heterosis

1960 First Agricultural University established at Pantnagar, Nainital, U.P.

1961 The first hybrid maize varieties released by the project

1965 Worlds first pearl millet hybrid released

1965 First bajra hybrid HB-1 released

1966 16 AICRP's established.

1966 Dr N G P Rao developed first sorghum hybrid CSH-1 (Swarna)

1970 Dr C T Patel developed worlds first cotton hybrid H-4 at Gujarat Agricultural University

1980 First sunflower hybrid BSH-1 released

1991 First pigeonpea hybrid ICPH-8 developed by ICRISAT Hyderabad

2001 PPVFR Act passed in India

2002 First Bt Cotton hybrid grown officially

2002 National Seed Policy unveiled in India

2003 First derived basmati Pusa 1121 developed by ICAR

2014 First drought tolerant rice variety DRR Dhan 42 released in India

TYPES OF PRODUCTS/VARIETIES

Plant breeding products are genetically different forms ranging from homozygous to heterozygous as well as populations called as cultivar or variety. Various types of cultivars developed are:

Purelines: Purelines are derived from single self fertilized plant by successive selfing. They are genetically and phenotypiclly uniform but vulnerable owing to narrow genetic base. They are relevant to self pollinated crops are cannot be reconstituted

Hybrids: Hybrids are first generation of a cross between genetically diverse plants and are based on exploitation of heterosis. Hybrids are heterozygous but can be homogenous of heterogenous depending upon the type of hybrid. Hybrids are also narrow based as they have only two

parents. They are relevant to both cross and self pollinated crops are can be reconstituted

Populations: Populations are a group of sexually reproducing, freely interbreeding individuals that share a common gene pool. They are highly heterozygous and heterogenous are based on explioitation of additive effects. They are usually developed in cross pollinated crops and are having wider adaptability owing to broader genetic base. They are relevant to cross pollinated crops are cannot be reconstituted except in case of synthetics.

Multilines: They are mixtures of nearly isogenic lines containing different genes of resistance against different races. They are relevant to self pollinated crops are can be reconstituted

Varietal blends: They are similar to multilines with the difference that the component lines are not isogenic lines but different purelines or varieties. They are relevant to self pollinated crops are can be reconstituted

Inbred lines: They are derived by successive selfing of a single cross pollinated plant. They are highly homozygous and homogenous. They are relevant to cross pollinated crops are cannot be reconstituted

Broad lines: They are similar to inbred lines with the difference that sib mating is done instead of selfing to achieve homozygosity. They are relevant to cross pollinated crops are cannot be reconstituted

Clones: Clonal cultivars are genetically uniform but tend to be highly heterozygous. Uniformity of plant types is maintained through vegetative rather than sexual reproduction. Cultivars are vegetatively propagated by

asexual reproduction (clones) including cuttings, tubers, bulbs, rhizomes and grafts(e.g. potato, peaches, apples, chrysanthemums). A cultivar can also be classified as a clone if it is propagated through obligate apomixis (e.g. buffelgrass).

Apomictic Cultivars: *Apomixis* is the phenomenon of producing seed without the fertilisation and is as such genetically identical to the mother plant. They are as good as clonally propagated varieties and can be used to fix the heterosis.

Table 1.1. Features of different crop cultivars/products

Variety type	Genetic constitution	Nature
Pureline	Homozygous	Homogenous
Hybrid	Heterozygous	Homogenous
Synthetic	Heterozygous	Heterogenous
Composite	Heterozygous	Homogenous
Multilines	Homozygous	Homogenous
Varietal blends	Homozygous	Homogenous
Inbred line	Homozygous	Homogenous
Broad line	Homozygous	Homogenous
Clone	Heterozygous	Heterogenous
Apomictic variety	Heterozygous	Homogenous
Landrace (SP crops)	Homozygous	Heterogenous
Landrace (CP crops)	Heterozygous	Heterogenous

Course Name	Principles of Plant Breeding
Lesson 2	Genetic Basis of Plant Breeding
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

- Understand the genetic principles of plant breeding
- Understand the nature of variation and its exploitation by selection and hybridization

Glossary of terms

Transposition: - Transposition refers to the ability of genes to move between chromosomes.

Transformation: - Transformation refers to transforming a host plant by introduction of a foreign gene from an unrelated sources or over-expression/suppression of a native gene through recombinant DNA technology.

Epigenetic variation: - These are the epigenetic variations (variations without change in genome structure or organization) that arise during tissue culture. eg. Somaclonal variation

Additive genetic variance: Gene effects associated with the average effects of individual genes. It measures the breeding value of genotypes and is always fixable through selection.

Dominance variance: Gene effects associated with intra-allelic interactions of genes at segregating loci and measures breeding behaviour of alleles in heterozygotes. It is of practical application in heterosis breeding.

Relative dominance: It is the ratio of dominance variance to additive genetic variance.

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Heritability: It measures the relative importance of genetic components of variance to total variance.

Origin of Genetic Variation

Genetic variation is the variation attributable to the action and interaction of genes governing various traits. There are many genetic mechanisms that cause such variations to arise:

Recombination: This is perhaps the most significant source of genetic variation in sexually propagation crop species. It arises as a result of crossing over that occurs in the pachytene stage of prophase of meiosis-I, Wherein exchange of chromosome parts between homologous chromosomes occurs. Such a reciprocal exchange of chromosome segments, result in independent assortment of genes. The number of possible combination of genes in F2 is where "n" is the number of genes. If a trait is governed by 10 genes, the number of possible gene combinations in F_2 would be 4^{10}=1048576. Genetic recombination also creates variability upon hybridization of divergent parents.

Mutation: - Mutations are a vital source of genetic variation that are heritable. A striking example of a useful mutation that has been exploited by plant breeders is opaque- 2 mutation in maize that leads to higher lysine and tryptophan content. Mutation may occur either due to damages to DNA or replication errors or may result from chromosomal aberrations such as addition, deletion and translocation. Mutation may arise spontaneously under natural processes or may be induced artificially using physical or chemical mutagens.

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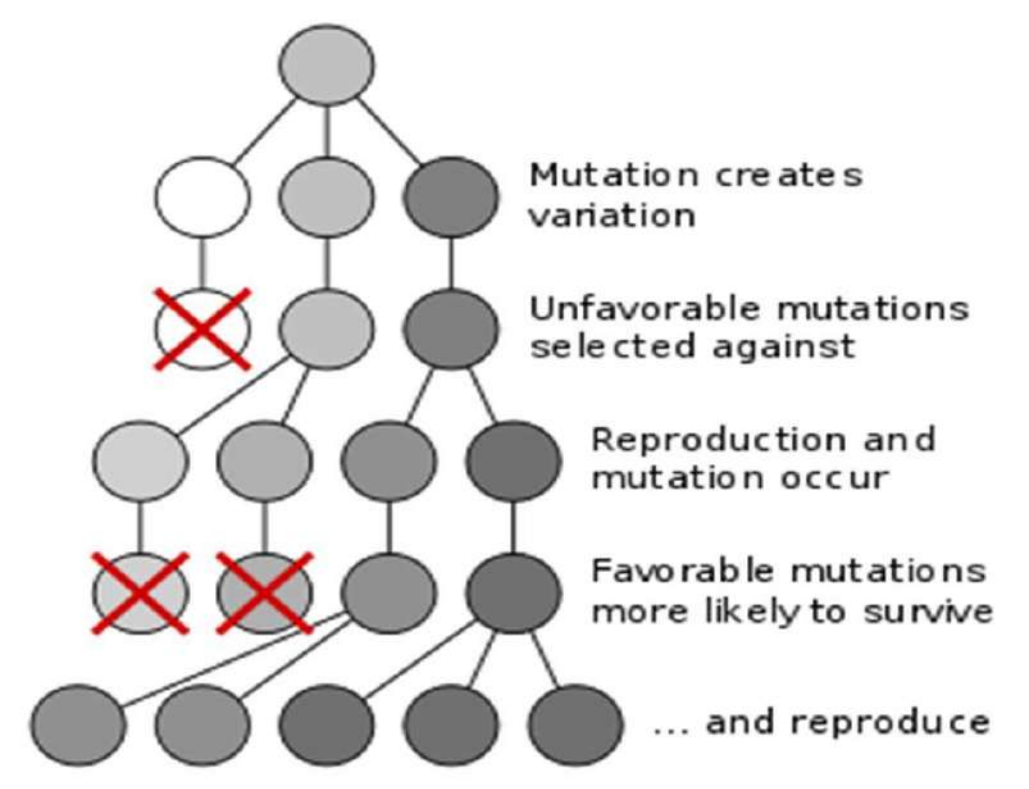

Fig 1: Role of mutations in creating genetic variation (Source:www.ck12.org/)

Transposition: - Transposition refers to the ability of genes to move between chromosomes. It is regarded as illegitimate recombination as the relocation usually occurs between non-homologous chromosomes, and cause genetic rearrangement that can lead to altered trait expression.

Transformation: - Transformation refers to transforming a host plant by introduction of a foreign gene from an unrelated sources or over-expression/suppression of a native gene through recombinant DNA technology. This is an unconventional source of creating genetic variability as it cuts across the sexual barriers. It is generally used where no variability exists for a trait in the cultivated/wild germplasm.

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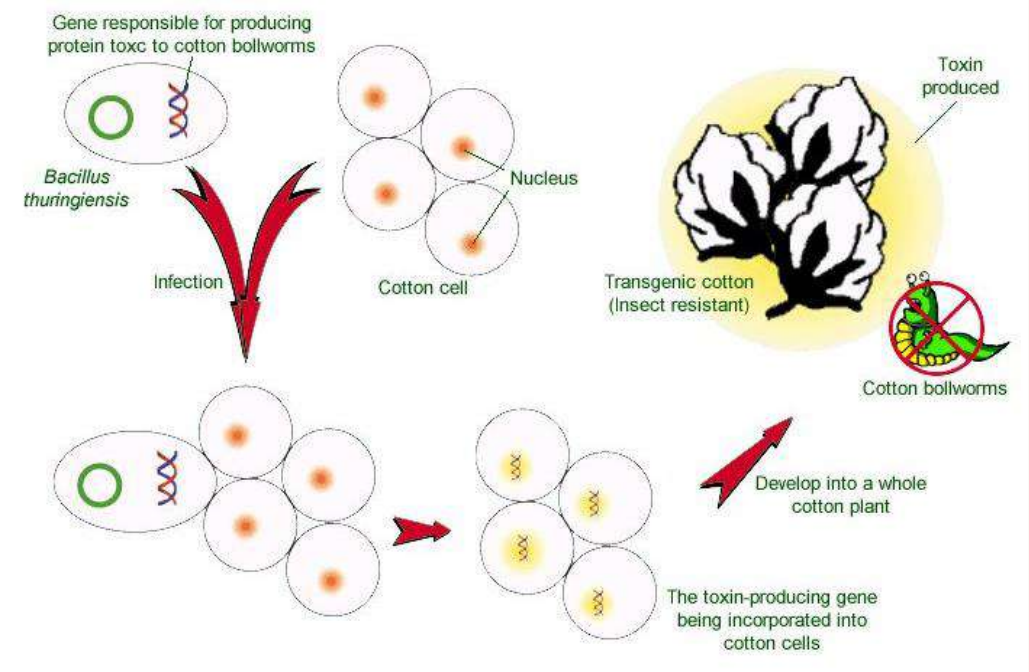

Fig 1: Development of Bt cotton by transformation (Source: www.edutik.hk)

Epigenetic variation: - These are the epigenetic variations (variations without change in genome structure or organization) that arise during tissue culture. They are much desirable than mutations because of higher frequency and non-lethality of soma clonal variants. However, selections can only be made for certain traits that could be identified at cellular level and yield arguably cannot be a candidate in this case.

Ploidy modifications: New variability may arise naturally through modifications in chromosome number as a result of hybridization (between unidentical genotypes), or abnormalities in the nuclear division processes.

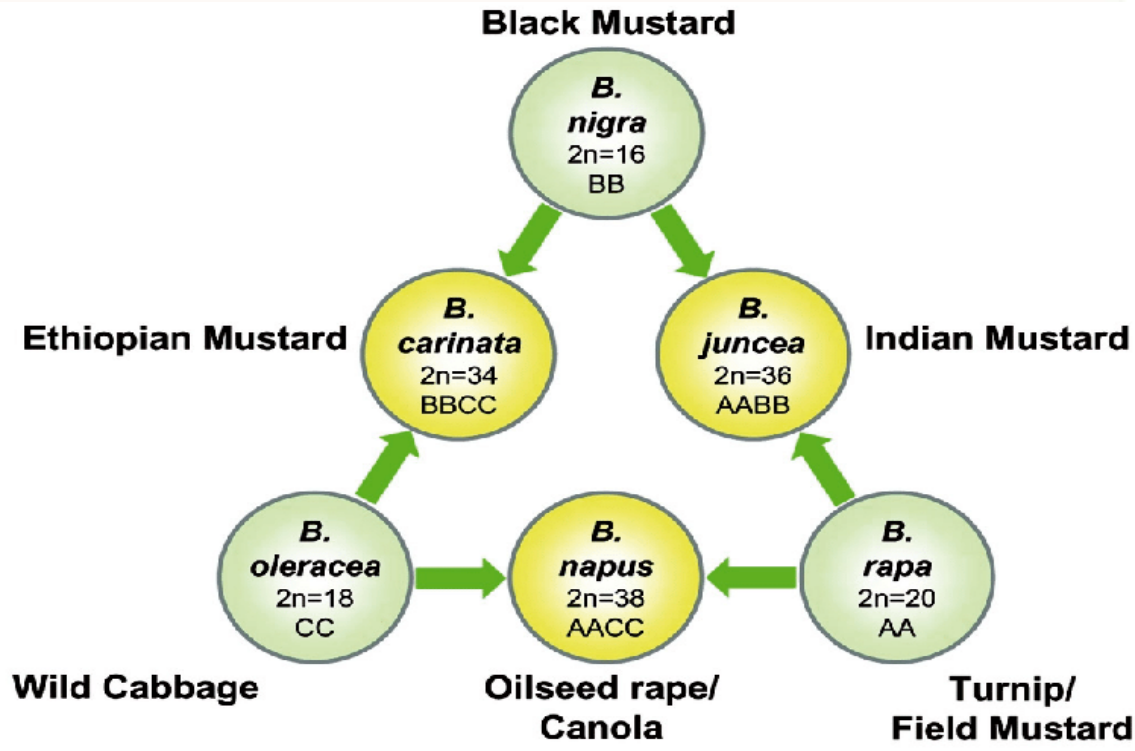

Fig 2: Evolution of various Brassica species by ploidy modification (U triangle) (Source: Nagahari U)

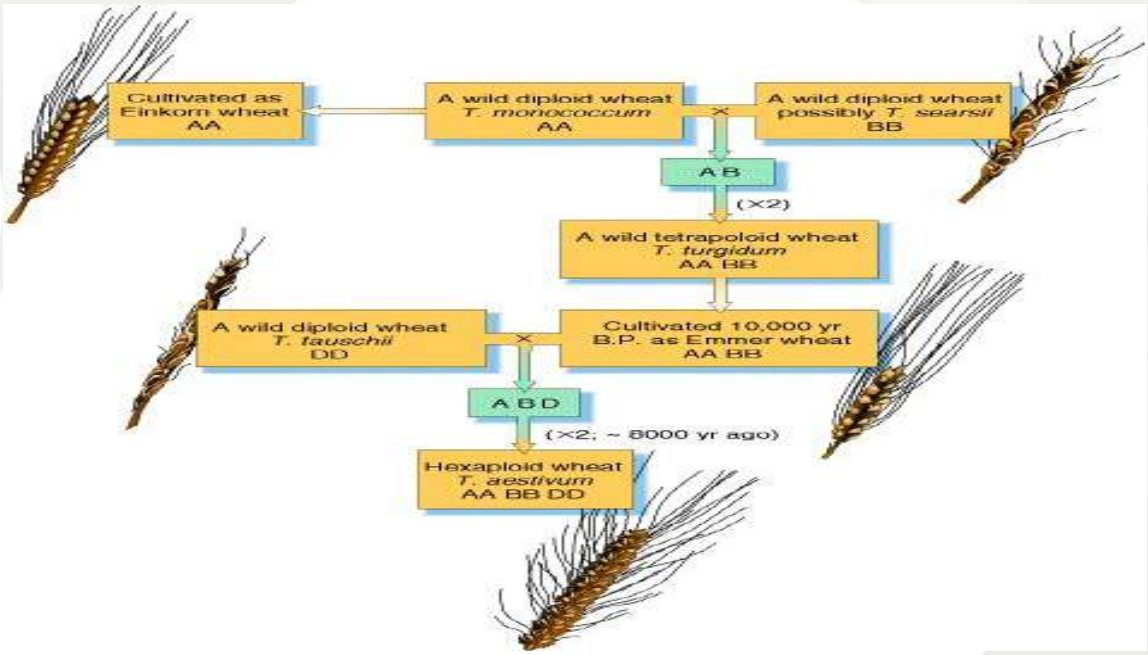

Fig 3: Evolution of Hexaploid wheat by ploidy modification (Source: www. https://www.cerealsdb.uk.net)

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Table 2.1. Qualitative v/s Quantitative Genetics

Particulars	Qualitative genetics	Quantitative genetics
Number of genes	Very few	Very large
Effect of genes	Large	Small cumulative
Environmental Vulnerability	Small	Substantial
Trait expression	Type of expression	Degree of expression
Scale of variability	Discrete	Continuous
Mating pattern	Individuals	Populations
Statistical analysis	Counts and ratios	Quantitative genetics parameters

GENETIC PRINCIPLES & PLANT BREEDING

Number of Genes Controlling a Quantitative Trait: In plant breeding, quantitative traits are distinguishable by measuring (**metric traits**) of the plants (instead of counting). Quantitative traits are controlled by polygenes (multiple genes) and are also called *polygenic traits.* The genes have effects that are too small to be individually distinguished. Polygenic inheritance exhibits a phenotypic pattern that is continuous (non-discrete) because segregation occurs at a large number of loci. As a consequence, quantitative traits are more susceptible, than qualitative traits, to modification by the variation in environmental factors to which plants in the population are subjected. It is challenging to measure the role of the environment on trait expression because it is difficult to measure the environmental effect on plant basis. Another aspect of polygenic trait inheritance is that it

is advantageous to breeders if desirable polygenes occur in tight linkages (linkage blocks are transferred together)

Gene Action: The effects of quantitative genes do not fall into discrete categories; it is more useful to describe quantitative traits by their gene action rather than by the number of genes by which they are encoded. The genetics of a quantitative trait centers on the study of its variation. It is in terms of variation that the primary genetic questions are formulated. Further, the researcher is interested in partitioning variance into its components that are attributed to different causes or sources. The genetic properties of a population are determined by the relative magnitudes of the components of variance. In addition, by knowing the components of variance, one may estimate the relative importance of the various determinants of phenotype. Total variance of a quantitative trait may be mathematically expressed as follows:

$V_P = V_G + V_E + V_{GxE} + V_{G \times G} + V_e$, where

V_P = total phenotypic variance of the segregating population;

V_G = genetic variance;

V_E = *environmental variance*;

V_{GxE} = variance associated with the genetic and environmental interaction

$V_{G \times G}$= variance associated with the gene-gene interaction, and

V_e= variance associated with error

The four types of gene action are additive, dominance, overdominance, and epistatic. Additive gene action occurs when each additional gene enhances the expression of the trait by equal increments. In a breeding program, selection is most effective for additive variance; it can be fixed by breeding (i.e. a cultivar that is homozygous can be developed).

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Dominance gene action pertains to allelic relationship at the same locus. Dominance gene effects are deviations from additivity that make the heterozygote resemble one parent more than the other. When dominance is complete, the heterozygote is equal to the homozygote in effects making it impossible for the breeder to distinguish between the two phenotypes. Because both kinds of phenotypes will be selected in a breeding program, fixing superior genes will be less effective where dominance gene action prevails.

Overdominance gene action exists when each allele at a locus produces a separate effect on the phenotype, and their combined effect exceeds the independent effect of the alleles (i.e., *aa* = 1, *AA* = 1, *Aa* = 2). Plant breeders can fix overdominance effects only in the first generation (i.e. F1 hybrid cultivars) through apomixis (asexual reproduction via seed), or through chromosome doubling of the product of a wide cross). Epistasis is the non-allelic interaction of genes. Epistatic gene action, when it occurs in qualitative traits, manifests as the masking of one gene expression by another. In quantitative traits, epistasis is described simply as non-allelic gene interactions which can result in an effect where none existed (e.g., *Aabb* = 0, *aaBB* = 0, but *AaBb* = 4).

Quantitative genetics has made significant contribution to genetic improvement of crop plants by providing the basic framework for science-based plant breeding. However, beyond the generalization that phenotype is the manifestation of genotype expressed in environment, little is known about the architecture of quantitative traits in terms of the number of loci conditioning a trait, the number of alleles segregating per locus, the allelic frequencies, effect of allelic substitutions, the linkage relationship among loci, the non-allelic interactions and expression and regulation of genes. Thus, characterization of genetic components using appropriate mating

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designs & field experimentation is imperative, as it helps in estimation and partitioning of various components, which eventually determine the applicability and success of breeding procedures. The following components of genetic variance are of practical consideration in crop improvement programmes.

Additive genetic variance associated with the average effects of individual genes. It measures the breeding value of genotypes and is always fixable through selection.

Dominance variance associated with intra-allelic interactions of genes at segregating loci and measures breeding behaviour of alleles in heterozygotes. It is of practical application in heterosis breeding.

Epistatic variances associated with inter allelic (non-allelic) interaction of genes at two or more segregating loci. Epistasis involving additive effects is fixable and as such exploited in intra- population improvement. Other epistatic effects are used in hybrid breeding.

Relative dominance, which is the ratio of dominance variance to additive genetic variance, decides the appropriate breeding strategy to be employed for seeking improvement in a quantitative trait. The values less or more than unity indicate over-dominance, unit values indicate complete dominance while as values approaching zero indicate absence of dominance.

Heritability that measures the relative importance of genetic components of variance to total variance (broad sense) or relative proportion of fixable component (VA) in total genetic variation (narrow sense). Heritability estimates in cultivated plants are categorized as low (5-10%), medium (10-30%) and high (30-60%). Heritability estimates are usually low for medium for yield

components, morphological traits and quality traits but are usually high for maturity traits as well as chemical composition characters.

Genotype X environment interaction, a component which causes bias in estimation of additive and dominance components. Thus, relative importance of these components from single environment experiment is of little practical value

Heritability and Plant Breeding: Plant breeders practice selection in the field on the basis of phenotype (the product of the interaction of genotype and the environment). If a trait is significantly influenced by the environment (e.g. a quantitative trait), a less desirable genotype may be accidentally selected to advance the breeding program. The expected breeding progress or gain will not be realized. *Heritability* is the concept of the reliability of the phenotypic value of a plant as a guide to the breeding value (additive genotype) of a metrical trait. It does not measure genetic control, but rather how this control can vary (broad sense, $H = VG/VP$ or narrow sense, $h2 = VA/VP$). It is also the property of the trait, the population, and the environment. Quantitative traits tend to have lower heritability versus qualitative traits, and hence selection for the former is challenging in breeding. A high heritability (especially narrow sense heritability) is desired in breeding for rapid progress.

The Breeders' Equation: After the plant breeder has determined that a trait can be improved through breeding, genetic variability is assembled or generated to initiate the breeding program. As the program continues, rounds of selection are implemented with the goal that the trait of interest would be progressively improved with each round. The difference between the mean phenotypic value (value of an individual judged by the mean value of its progeny; it is the value that is transferred from an individual to its progeny) of the offspring of the selected parents and the whole of the parental

generation before selection is called the *response to selection (R)* or the *genetic gain* or *genetic advance* (change of population mean between generations following selection. The response to selection depends on three factors :

The total (phenotypic) variation in the population in which selection will be conducted;

Heritability of trait; and,
Selection pressure i,e. proportion of population that is selected for the next generation) to be imposed by the plant breeder.

The mean phenotypic value of the individuals selected as parents for the next generation expressed as a deviation from the population mean is called *selection differential (S).* Response to selection is a function of heritability as follows:

$$\mathbf{R = h^2. S}$$

Qualitative traits are easier to select (breed) than quantitative traits (low heritability). When heritability is high, a small number of top performers may be selected (high selection pressure). The reverse is true when heritability is low. Further, when heritability is unity (VA = VP; no environmental variance), progress with selection should be perfect. On the other hand if heritability is zero, (R = 0); in theory, the breeder can predict the response to selection in one generation (heritability estimate is valid for one generation). The response to selection in one generation may be mathematically expressed following:

$$X_0 - X_p = i \times h^2 \times \sigma P - = R \text{ (or } G\ i \times h^2 \times \sigma P),$$

where X_0 = mean phenotype of the offspring of selected parents,

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X_p = mean phenotype of the whole parental generation,

R = the advance realised in one generation of selection,

h^2 = heritability,

P = phenotypic standard deviation of the parent population,

i = intensity of selection,

G = genetic gain or genetic advance

Managing Variability

The variability in plant populations can be managed as follows:

If variation is adequate: we go for selection

If variation is inadequate or dispersed: we go for hybridization

If variation is absent: we go for mutation

If variation is present in sexually isolated populations: we go for transgenics

Genetic basis of selection

1. Selection is effective only if heritable variation is present.
2. Selection does not act on the gene itself but acts on genotype through phenotype and thus ultimately changes frequency of genes and genotypes.
3. Selection itself does not create new genes or genotypes but influences their relative frequency by changing their contribution to the progeny.

GENETIC BASIS OF HYBRIDISATION IN SELF POLLINATED CROPS

The basic genetic considerations in hybridization in self-pollinated crops are:

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1. Gene recombination in segregating generations: taking gene as a unit of inheritance, there are three factors that affect gene recombination through hybridization:

A. *Number of genes differentiating the parents:* This determines the number of different genotypes derived from the cross.

B. Number of alleles at each locus: Which further complicates the situation if a trait is multiallelic.

C. Linkage: Linkage, when present will favour linked loci thereby promoting frequency of parental types at the expense of recombinants. However, it does not influence the rate of attainment of homozygosity, but does affect the proportion of homozygous individuals by reducing value of **"n"** in the formula **{(2m-1)/2m}n**. moreover, different homozygotes will not be in equal proportion, those involving linked loci will be more frequent. It acts as a conservative force that tends to retain existing gene combinations. It is highly desirable if the desirable genes are linked but delays breeding progress if the desirable and undesirable genes are linked. Natural selection favours certain gene combinations especially in case of housekeeping genes that are essentially required to be together.

2. Progress of attaining homozygosity: F_2 is the most variable population as all the genes are segregating simultaneously, but the latter generations witness rapid attainment of homozygosity and by F5, with just five genes, almost 85% population will be homozygous. Our aim as plant breeders is to be able to pick the best homozygote, an endeavour, that is greatly influenced by number of genes, linkage relationships and population size to be handled in F_2.

3. Nature of successful gene combinations: Populations under selfing after crossing tend to comprise of homozygous individuals. The job of

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plant breeder is to be able to pick the best homozygote that comprises paired combinations of identical genes at different loci that interact well with each other. These cooperating genes by virtue of paired nature should be able to replicate the structure in next generation.

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Course Name	Principles of Plant Breeding
Lesson 3	Limitations, Major Achievements, Goal Setting for Future
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Major limitations of plant breeding
2. Achievements in Plant Breeding
3. Goal setting and future perspectives in plant breeding

Glossary of terms

Green revolution: A dramatic increases of rice and wheat yields in many countries, in particular of Southeast Asia and Mexico by identification of dwarf and semi- dwarf genes, allowed the development of new cultivars

Genetic base: the level of genetic diversity in a population

Genetic bottleneck: Lack of variability or presence of undesirable correlations in populations that hinders crop improvement

Yield plateau: The level after which it is seemingly hard to enhance yield levels

Genetic bottleneck: Phenomenon where certain crop species have inherently low genetic variability for economically important traits.

MAJOR ACTIVITIES IN PLANT BREEDING

Identification/creation of useful variation: Genetic variation or variability is indispensable raw material for plant breeding. The common conventional method of creating a non-existent gene is via mutagenesis (use of mutation agents to induce variation). Variability exists in many plant **gene banks** (repositories for plant germplasm) to which scientists have access. Gene banks may be operated on small scales by national governments. However, comprehensive operations are undertaken by international entities such as the international research centres. The desired variability may already exist in the breeder's local collection and in remnants from previous breeding projects.

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Selection and Evaluation: Selection is simply discriminating among the available or created variability to identify individuals with the desired combination of genes (genotype) or expressed trait(s). With the introduction of genetics and statistics into modern plant breeding, breeders have developed standard breeding methods for the species, the genetics of the trait of interest and the type of product desired. There are selection or breeding methods for species based on their modes of reproduction, genetics or whether the product should be uniform or variable. The final selection cycle in breeding results in a small number of genotypes that are potential candidates for advancing as cultivars for release to producers. These genotypes are subjected to rigorous *evaluation* under conditions which must include those under which the cultivars will be commercially grown. Evaluations may be conducted at multiple locations and over multiple years. Included in such evaluations are standard cultivars of known performance (for comparison), which could be replaced should superior performers emerge from the trials.

Certification and Cultivar Release: In countries with more advanced agricultural operations, there exist standardized and approved protocols for releasing new cultivars to growers. There may be national crop certifying agencies that oversee the seed certification process for various crops. The ultimate purpose of seed certification is to ensure that the seed produced by the plant breeder reaches the public (consumer) in its highest quality, original genetic identity and highest genetic purity.

Multiplication and Distribution: Certified seed is multiplied by certified seed growers contracted by independent breeders and seed companies to mass-produce released cultivars for sale to growers. New cultivars are sold to consumers via a variety of outlets. Commercial seed companies have elaborate sales mechanisms.

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IMPACT OF PLANT BREEDING:

Genetics based plant breeding is one of the significant impacts in science that has driven spectacular success especially during last 5-6 decades. Green revolution in wheat and rice, single cross hybrids in maize, high profitable seed industry and improvement of non-yield parameters such as quality of produce have delivered their impacts and now agriculture is preparing for evergreen revolution that again will derive major thrust from plant breeding. The challenge now is to add ecological dimension to crop productivity improvement. In spite of unprecedented increase in population, the period between 1960 - 2000 has witnessed remarkable surge in productivity levels of most crops and much of transformation of agriculture has undoubtedly been driven by crop genetic improvement, even though it would be a mistake to discredit other factors such as better crop, management practices, Mechanization, effective extension as well as better educated farmers which are responsible to technological interventions. However, many questions have been posed; First how much impact has been generated by plant breeding driven crop improvement and second, whether the impact is uniform across crops and growing environments. In fact, many of the observers believe that the so-called Green Revolution did not fully justify the above questions. They argue that field increases were realized in wheat and rice only were limited to Asia, Latin America and were irrigate environments. The impact of crop genetic improvement is considered from three points of consideration i.e. development and release of high yielding varieties, adoption of high yielding varieties and impact of high yielding varieties on yield enhancement. Up to 2000, more than 8000 high yielding varieties have been released under 40 breeding programs across 100 countries.

MAJOR ACHIEVEMENTS OF PLANT BREEDING

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Plant breeding efforts have led to significant gains in yield per unit area of major crop plants over the past 50 years. It has been established that the linear increase in production and productivity has been equally contributed by plant breeding (high yielding varieties) and better management (fertilizer, irrigation). Rates of gain attributable to genetic improvements have averaged 1% per year, have generally been linear, and show no sign of slackening. Improvements in tolerance to environmental stress, in grain-to-straw ratios, and in standability, as well as maintenance of required levels of resistance to disease, insect and nematode pests, have been the major genetic causes of higher achieved yields and will continue to be the foundation for further gains in productivity and stability. Broadened genetic diversity is also an increasingly important goal to promote stability and increase productivity potentials. Proportionately large research inputs are now needed to maintain desired rates of improvement, compared to earlier years. It seems likely that contributions from biotechnology will become increasingly important in years to come if improvement rates are to be maintained. Average world yield of cereals has almost tripled in the last five decades, increasing from 1.35 t/ha in 1961 to 3.51 t/ha in 2009. This has allowed raising the production of cereals from about 900 t in 1961 to 2800 t in 2016, with an expansion of only 9% of the cultivated area. Increases in the production of cereals have outpaced the growth of population, so that in 2009 the cereal production per capita was of 365 kg/person/year, compared to 285 kg/person/year in 1961. The unparalleled yield improvements in the last century have resulted from improved cultivation techniques and new varieties, and although different estimates exist depending of the crop and region, the effect attributable to genetic gain has been very important, in some cases, like maize in the US, reaching up to 75%.

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Many examples exist of the success of scientific plant breeding before the onset of genomics, but one of the most frequently reported is the case of yield improvements of maize in the US. Maize is a monoecious, and consequently outcrossing, crop for which 59 landraces have been identified in its centre of origin (Mexico). Since 1930 maize yields in the US have increased by more than five- fold and the trend seems to be continuing with no evidence of a plateau effect in the short term. These impressive yield increases have been preceded by the adoption of breeding innovations, which, sequentially, have been selection within open-pollinated varieties, double and three-way hybrids, simple F1 hybrids, and GMO F1 hybrids (mostly resistant to insects and herbicides). Another outstanding example of the achievements of plant breeding, this time in autogamous crops, comes from the so-called **Green Revolution**, which resulted in dramatic increases of rice and wheat yields in many countries, in particular of Southeast Asia and Mexico. The identification of dwarf and semi- dwarf genes, allowed the development of new cultivars that could be subjected to higher rates of fertilizer without lodging, resulting in dramatic yield increases. More recently, development of hybrids heterotic for yield, particularly in rice, has resulted in further significant yield increases.

The achievements in increasing the productivity of crops have resulted from the combination of the newly developed cultivars in combination with improved crop management techniques and practices, many times following a co-evolutionary process, so that new varieties are obtained for adaptation to new management practices or growing conditions, and vice versa, in a clear example of exploitation of genotype x environment interaction. At present, the combination of conventional breeding techniques with new biotechnological tools and approaches is clearly allowing further developments, so that breeders are witnessing the onset of a new biotechnology-based revolution of plant breeding. In this respect, genetically modified (GM) crops have allowed significant increases in

the yield and quality of many crops, and although GM crops are subjected to a tight legal and social scrutiny, in particular in some countries, they have shown that in many cases allow higher and stable yields that contribute to more production with less land. New genomic tools and approaches, which have been developed thanks to the revolution in molecular biology and advances in DNA sequencing technologies are in most cases widely accepted, even for developing varieties for organic farming, and are leading to a genomics-based plant breeding.

IMPORTANT CONTRIBUTIONS TO PLANT BREEDING IN INDIA

Name	**Crop**	**Achievements**
Dharm Pal Singh	Rapeseed and Mustard	
C. T. Patel	Cotton Breeder	World's first interspecific cotton hybrid
V. Santhanum	Cotton Breeder	Upland and Egyptian Cotton
D.S Athwal	Wheat and Pearl millet Breeder	Father of wheat revolution
Ram Dhan Singh	Wheat Breeder	Development of C-591
K. Ramaiah	Rice Breeder	Indica-japonica hybridisation programme
N.G.P. Rao	Sorghum Breeder	NRCS Hyderabad CSH hybrids

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M. S. Swaminathan	Wheat and Rice	Father of Green Revolution in India
B.P. Pal	Wheat Breeder	NP series varieties including NP-809
T.S. Venkataraman	Sugarcane Breeder	Sugarcane breeding institute, Coimbatore
R S Paroda	Plant Genetic resources	Plant genetic resource conservation in India
E A Sidiq	Rice breeder	High yielding rice varieties

UNDESIRABLE CONSEQUENCES / LIMITATIONS OF PLANT BREEDING

Over a period of time, plant breeding has emerged as a useful technology to develop high yielding varieties for ensuring food and nutritional security. However, there are certain obvious undesirable effects and limitations that have limited the progress of plant breeding. Achievements in any technology come at a certain cost that is justified by the economic impact of its products. Also, there are certain inherent Lacunae with any technology or the Lacunae that evolve when the technology is put to practice. Plant breeding essentially, is a technology that uses principles of genetics to direct evolution of crops in desired direction. However, there are certain limitations that put a ceiling on the level of improvement that could be effected through plant breeding.

1. Reduction in genetic diversity: Plant breeding has replaced heterogeneity with uniformity by large scale cultivation of a few high

yielding varieties thereby decline the diversity that was present in traditional cultivars and farmer's landraces. This is all the more important in view of the fact that the success of plant breeding depends on the level of variability that needs to be maintained for consistent gains through selection process.

2. Narrow genetic base: The varieties developed have been bred using a very few parents thereby rendering them narrow based that makes them vulnerable to climatic vagaries as well as less adaptable. The genetic diversity in domesticated species is invariably low as compared to their wild counterparts.

3. Lack of perfect plant types: A perfect plant type for most of the crops is yet to be designed in practice. Such a plant is supposed to possess optimum expression of all traits that eventually determine yield levels in a given environment. Though, ideotype breeding in rice through use of NPT lines has given encouraging results in form of super rice varieties/hybrids but same level of accomplishment is yet to be achieved in most other crops.

3. Inherent genetic bottleneck / Low genetic variability: Certain crop species have inherently low genetic variability for economically important traits. It slows down progress in breeding even if a variety of conventional breeding methodologies have been used. This is especially true in case of vegetatively propagated, clonal crops where gene flow is precluded.

4. Negative correlations: In certain crops there are undesirable negative correlations between traits which have slowed down the breeding progress. In pulses, yield and protein content are negatively correlated and thereby breeding for high yielding varieties has been less rewarding in view of the concomitant compromise on protein content which is the important trait of pulses.

5. Undesirable associations: Sometimes, plant breeding leads to undesirable combinations. The examples of man-made crops having undesirable combination of characters are *Raphanobrassica* and Pomato. Pomato, which is a cross between Potato and tomato was bred with the objective of having roots like potato and shoot like tomato but breeders eventually ended up having the reverse.

6. Increased susceptibility to minor diseases and pests: Uniformity of crop varieties with low genetic diversity has enhanced the vulnerability of crops to diseases and pests. Continuous culture of a few varieties across large areas and over large number of years helps build pest and disease inoculum thereby enhancing crop susceptibility.

8. G × E Interactions: Unpredictable G **×** E interaction coupled with faulty experimental designs mars the efficiency of selection in segregation generation due to blurring effect of environment and biased estimates of genetic parameters.

CHALLENGES FOR PLANT BREEDERS

1. The world population is projected to hit 9 billion by 2050, an increase of 2 billion from today's global population. The population growth has outstripped the growth in food production. Securing the world's food supply is one of the greatest challenges of plant breeders. The agricultural sector faces major a challenge as the global food reserves have already fallen to the lowest level in thirty years. Hunger and poverty around the globe must be addressed while safeguarding the life-support systems provided by the world's natural environment. The matter of concern, however, is that the rate of increase of food crop production has declined to a third that of 25 years ago.

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2. Worldwide, 40-50% of crops are still lost each year to competition with weeds, diseases and pests – a figure comparable with farming in Europe 500 years ago. Together, crop losses and agrochemical costs amount to hundreds of billions of dollars each year.

3. Regions like Africa have not harnessed the benefits of Green Revolution and include some of the poorest nations hit hard by hunger and malnutrition. Over 180 million people in sub-Saharan Africa live below the poverty line a number likely to exceed 300 million by 2020. In fact, Africa imports 25% of its grain needs. To add to the problem, Africa's crop production per unit area is the lowest in the world with average maize yield is just over a third the global average and rice yield, one quarter while as sweet potato, half of global average.

4. An estimated 800 million people survive on a limited diet of a few staples that do not provide adequate macronutrients, and micronutrient deficiencies are even more common.

5. Climate change is looming large, that will worsen the already grim scenario of life support systems as well as biophysical resource base for agriculture. Drought, heat, flood, changed disease and pest scenario are some of the implications of climate change that will need to be addressed.

FUTURE PROSPECTS

Plant breeding as a technology has evolved since the scientific foundations were provided by Mendels Laws of inheritance and the subsequent validation of principles of genetics. It has provided strong impetus to the development of crop varieties having enhanced productivity, improved quality and resilience to diseases and pests. However, in view of burgeoning human and animal population, the

challenge of feeding the ever-increasing number of humans and provide feed and fodder to the livestock is never ending. In view of deteriorating biophysical
resource base and the looming challenges of climate change, plant breeders are faced with the challenge of constantly improve the yielding ability of varieties as well as enhance their resilience to diseases, pests and abiotic stresses that are looming large in view of changed climate and its implications. In India food production has enhanced from a mere 50 million tons in 1950-60 to about 300 million tons in 2019-20, an increase, that has to a large extent been propelled by plant breeding. Even in the current scenario and the future challenges of ensuring food and nutritional security, plant breeding will be in the forefront of technologies that will be used to overcome the challenges.

There are certain issues such as yield plaguing, loss of biodiversity, emerging biotic and abiotic stresses and declining public investment in agricultural research. The role of plant breeding needs reorientation in a way that the varieties for meeting the future demands have to be bred with the inbuilt ability to perform optimally under varied stresses. Further in view of renewed focus on nutritional security, biofortification has assumed an important dimension in breeding programmes. Breeding programmes under the aegis of Harvest Plus, a Generation challenge programme of CGIAR, have focused on main crops such as rice, maize, wheat, common bean, cassava, sweet potato and pearl millet for biofortification with iron, zinc, vitamin A using conventional breeding and MAS in view of diverse regulatory mechanisms regarding genetically modified crop varieties. Plant breeding has also emerged as a multidisciplinary subject embracing research output from diverse areas of study like biotechnology, biochemistry, physiology, pathology, entomology, molecular biology etc to enhance the precision of understanding and application. Plant breeding will continue to produce improved cultivars. Biotechnology will be used increasingly, albeit at slower pace than expected. Plant breeding of the future will seamlessly

integrate conventional and molecular breeding. Emerging challenges to plant breeding in respect of being able to meet the expectations of stakeholders and justify the public investment in research will need an integration of classical and modern breeding methodologies to enhance productivity with less land, water and inputs. Plant breeding will also have to be specialized as well as appropriately integrated in terms of new approaches. Organic farming is gaining momentum world over and breeding needs to be directed to develop varieties that are suitable under organic low input systems. Similarly Participatory Plant Breeding is emerging as a viable solution to enhance adoption rates of varieties through farmer driven selection process where farmers are actively involved in varietal development as well as selection process.

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Course Name	Principles of Plant Breeding
Lesson 4	Sexual Reproduction (Cross and Self-Pollination)
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the process of development of male and female gametes in flowering plants.
2. Implications of sexual reproduction in plant breeding.

Glossary of terms

Amphimixis: Fusion of male and female gametes resulting in formation of a zygote,

Hermaphrodite: A flower containing both stamens and pistil is a perfect or hermaphrodite flower.

Staminate: A flower containing only stamens (male organs).

Pistillate: A flower containing only pistil (female organs).

Monoecious: A flower in which staminate and pistillate flowers occur on the same plant.

Dioecious: A flower in which staminate and pistillate flowers occur on different plants.

Sporogenesis: Production of microspores and megaspores.

Gametogenesis: The production of male and female gametes in the microspores and the megaspores, respectively, is known as gametogenesis.

Triple fusion: Fusion of diploid polar nuclei with one of the sperm cells to form the triploid endosperm.

Syngamy: fertilization of female gamete by male gamete.

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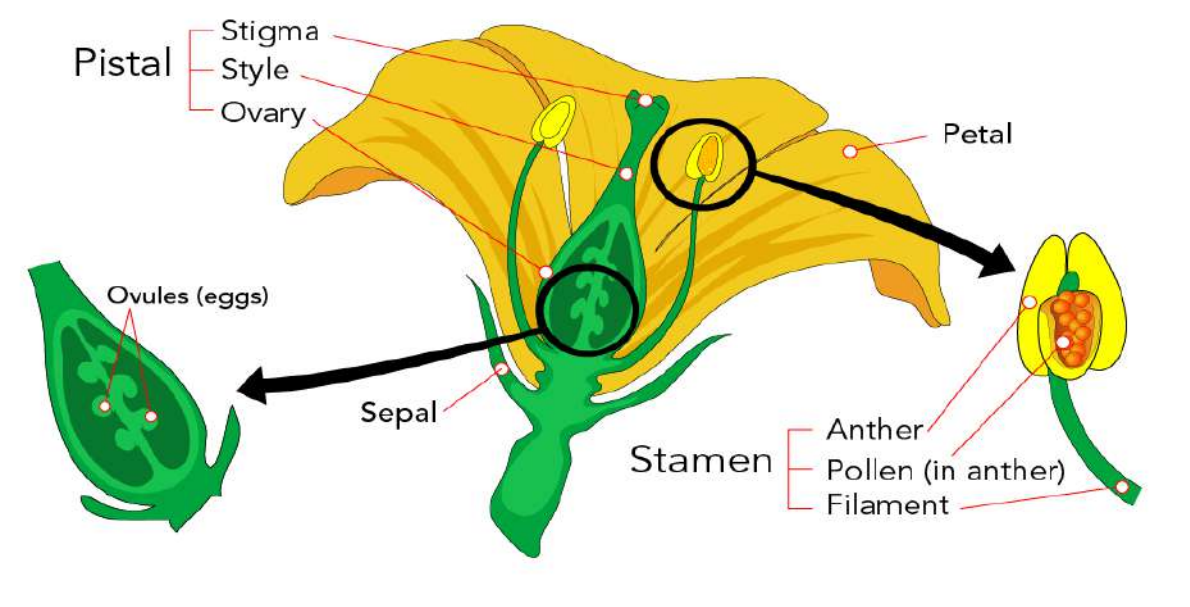
Double fertilization: The process of fertilization of two embryo sac cells by two male gametes is called as double fertilization.

Sexual reproduction or amphimixis involves fusion of male and female gametes resulting in formation of a zygote, which develops in to an embryo. In crop plants, male and female gametes are produced in specialized structures known as flowers. A flower usually consists of sepals, petals (or their modifications), stamens and/or pistil.



A flower containing both stamens and pistil is a perfect or hermaphrodite flower. If it contains stamens, but not pistil, it is known as staminate, while a pistillate flower contains pistil, but not stamens. Staminate and pistillate flowers occur on the same plant in a monoecious species, such as maize, cucurbits, colocasia, castor (*Ricinus communis*), coconut, etc. But in dioecious species, staminate and pistillate flowers occur on different plants, e.g., papaya, date palm (*Phoenix dactylifera*), pistachio (*Pistacia vera*), spinach, pointed gourd etc. In crop plants, meiotic division of specific cells in stamen and pistil yields microspores and

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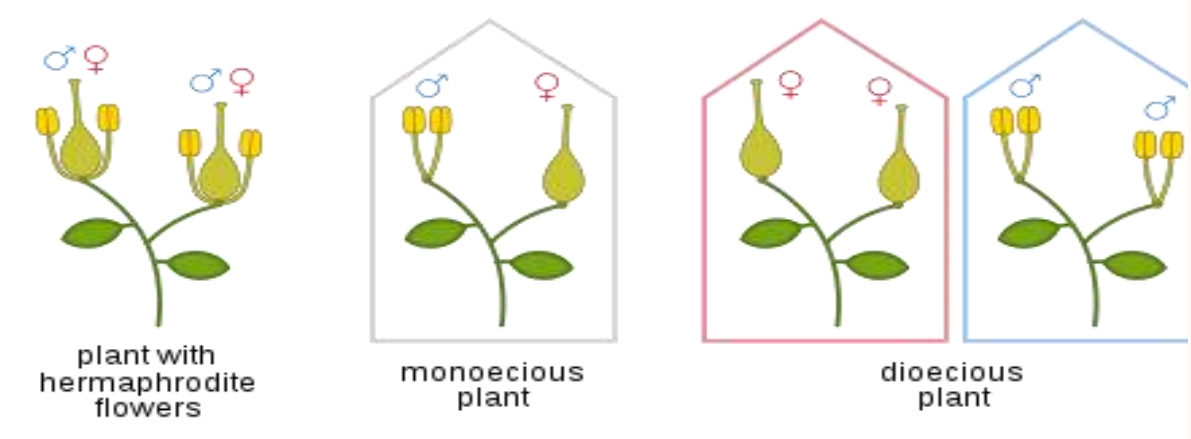
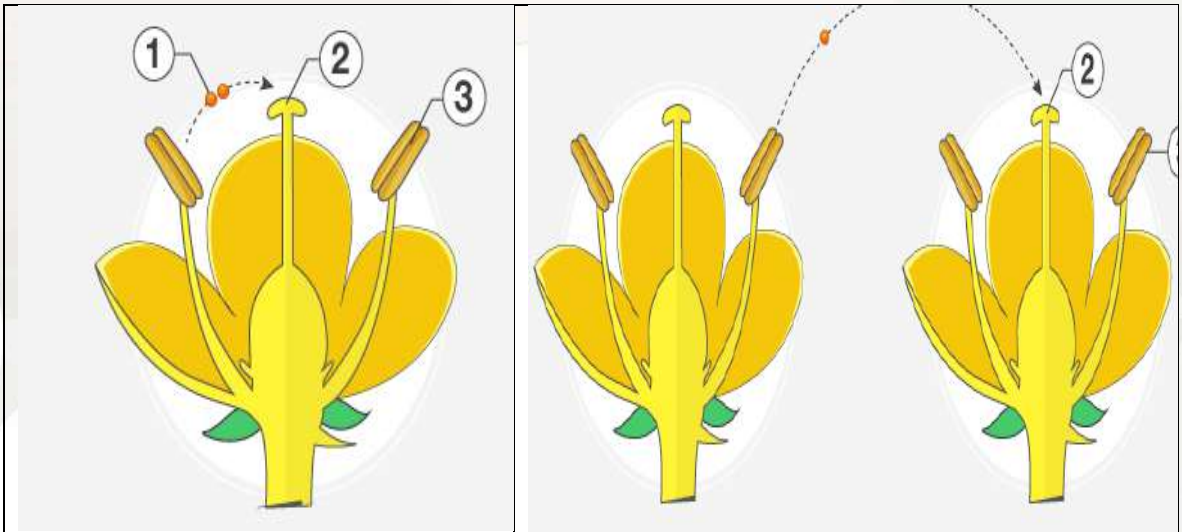
megaspores, respectively. This is followed by mitotic division of the spore nuclei to produce gametes; the male and female gametes are produced in microspores and megaspores, respectively.





Self Pollination	Cross Pollination

Basic features of sexual reproduction

- It is the production of offspring by the fusion of egg and sperm, which are the sex cells or gametes.

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- Upon fertilization, the male and female gametes unite to form a zygote, which develops into a mature organism.

- It results in the combination of genetic material from two parents.

Sporogenesis

Sporogenesis refers to the production of microspores and megaspores. Male gametes i.e., microspores are produced in anthers (microsporogenesis), while female gametes i.e., megaspores are produced in ovules (megasporogenesis). Each anther has four pollen sacs, which contain numerous pollen mother cells (PMCs) which undergoes meiosis to produce four haploid cells or microspores. This process is known as microsporogenesis. The microspores mature into pollen grains mainly by a thickening of their walls. Megasporogenesis occurs in ovules, which are present inside the ovary. A single cell in each ovule differentiates into a megaspore mother cell. The megaspore mother cell undergoes meiosis to produce four haploid megaspores. Three of the megaspores degenerate leaving one functional megaspore per ovule.

Gametogenesis: The production of male and female gametes in the microspores and the megaspores, respectively, is known as gametogenesis and includes microgametogenesis and megagametogenesis. The development occurs late in plant development within defined organs, the anther for the male which contains the haploid pollen and the ovule for the female which contains the embryo sac and is the progenitor of the seed. The initiation process occurs in a similar manner for both male and female gametes with the differentiation of archesporial cells and initiation of meiosis.

Microgametogenesis: Male gamete formation is initiated by periclinal divisions (parallel to the anther wall) in several adjacent hypodermal cells

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within the anther primordium, which develop into archespores, typically with four situated at the four corners of each anther primordium. The archesporial cells then divide mitotically to form the inner primary sporogenous cells, which later go to form pollen mother cells (PMCs) and primary parietal cells, The primary parietal cells go through further mitotic divisions to form the anther wall comprising of the endothecium, middle cell layer and tapetum. The tapetum surrounds the sporogenous cells and serves to nourish the developing microspores during the process of PMC meiosis and microspore maturation. Callose (β1,3 glucan) forms around the PMCs and later between the meiotic products. After meiosis, the callose is broken down by the release of a β1,3 glucanase by the tapetum. The microspores then go through a process of wall deposition, coordinated by the tapetum but also in part by the microspores/immature pollen grains. This is followed by two mitotic divisions to produce functional male gametes. These can occur during the late stages of pollen development in species that produce tricellular pollen, e.g., *Arabidopsis*.

Alternatively, the first mitotic division (pollen mitosis I; PMI) may occur in the pollen grain so that, when released, the mature pollen is bicellular, e.g., tobacco, and the further mitotic division occurs during pollen tube development. The two cells formed have very distinct fates; the larger vegetative cell accumulates a dense cytoplasm containing lipids, proteins and carbohydrates. This does not divide further, but provides storage compounds for the active period of growth associated with the production of a pollen tube. Double fertilization is characteristically seen in plants, with one sperm cell fusing with the egg cell to form an embryo, whilst the other fuses with the diploid central cell to form the endosperm. The endosperm is a triploid tissue that serves to nourish the developing embryo prior to the commencement of functional photosynthesis by the developing seedling.

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Megagametogenesis: Female gamete formation occurs within the ovule. Different types of female gametophyte development are reported in plants but more than 70% of flowering plants exhibit the *Polygonium* type pattern resulting in an eight nucleate embryo sac. Each ovule comprises of a nucellus that encloses the embryo sac, which is encased within two integuments that are the progenitors of the seed coat. The nucellus contains the spore-bearing tissue (**mega-spore mother cell** (MMC)). The MMC goes through meiosis to form **four haploid megaspores.** However, three of these degenerates via programmed cell death, usually leaving only one functional megaspore at the chalaza end. This then goes through three successive mitotic divisions to form the eight-nucleate embryo sac. Two of these nuclei (**polar nuclei**) then fuse to form the central cell, which is diploid and is later fertilized by one of the sperm cells to form the **triploid endosperm** (**Triple fusion**). Three of the cells form the egg apparatus comprising two synergids and an egg cell which is fertilized by one of the male gametes to form the embryo (**Syngamy**). The process of fertilization of two embryo sac cells by two male gametes is called as double fertilization. A typical embryo sac therefore comprises of seven cells with eight nuclei. The nucellus is usually encapsulated by two integuments, except for a small cleft at the base, termed the micropyle, through which the pollen tube grows to allow double fertilization by the two sperm cells.

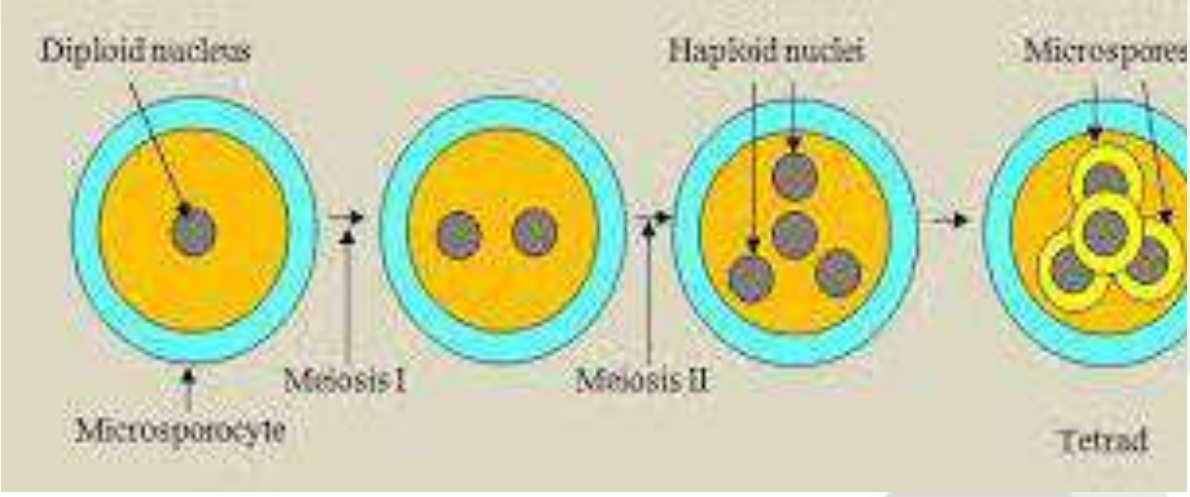

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Fig 21.1: Microsporogenesis (Source: Google Images)

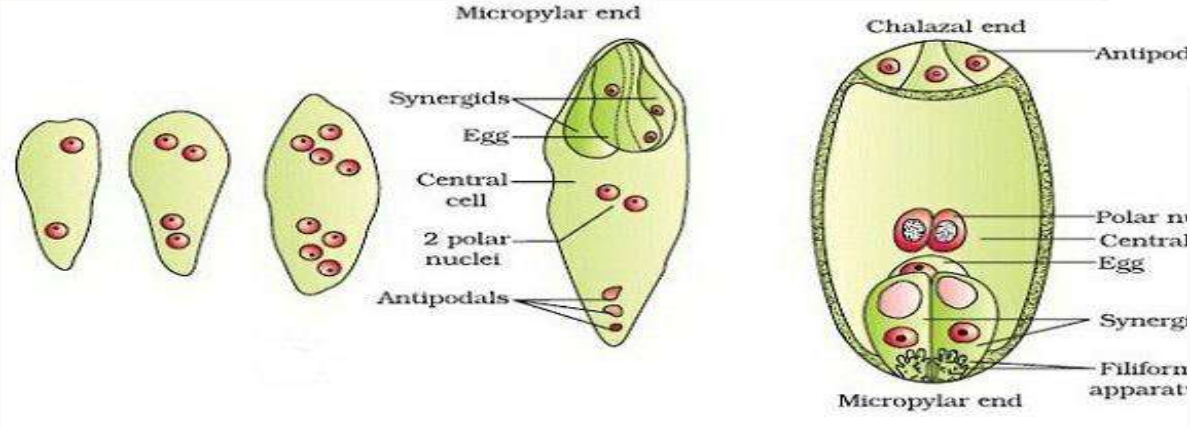

Fig 21.2: Megagametogenesis (Source: Google Images)

Implications of Sexual Reproduction

Sexual reproduction makes it possible to combine genes from two parents into a single hybrid plant. Recombination of these genes produces a large number of genotypes. This is an essential step in creating variation through hybridization. Almost the entire plant breeding is based on sexual reproduction. Even in asexually reproducing species, sexual reproduction, if it occurs, is used to advantage, e.g., in sugarcane, potato, sweet potato etc.

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Course Name	Principles of Plant Breeding
Lesson 5	Asexual Reproduction
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the various asexual reproduction mechanisms in crop plants.
2. Implications of asexual reproduction in plant breeding.

Glossary of terms

Asexual reproduction: A mode of reproduction that does not involve fusion of male and female gametes.

Vegetative reproduction: The regeneration of plants from the vegetative parts of the plant.

Apomixis: The regeneration of plants from seeds/embryos that develop without fertilization.

Bulbils: Bulbils are modified flowers that develop into plants directly without formation of seeds. These are vegetative bodies; their development does not involve fertilization and seed formation. The lower flowers in the inflorescence of garlic naturally develop into bulbils.

Adventive Embryony or Sporophytic budding: Embryo develops directly from vegetative cells of the ovule, such as nucellus, integument, or chalaza without development of embryo sac.

Apospory: Embryo develops from cells other than embryo sac cells i.e., cells of integuments or nucleus. e.g.Citrus and Mango, *Hieraceum, Malus, Crepis, Ranunculus,* etc.

Diplospory: Embryo sac is produced from the megaspore, which may be haploid or, more generally, diploid. Generally, the meiosis is so modified that the megaspore remains diploid. It is of two types

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Parthenogenesis: The embryo develops from embryo sac without pollination.

Apogamy: In apogamy, synergids or antipodal cells develop into an embryo. Like parthenogenesis, apogamy may be haploid or diploid depending upon the haploid or diploid state of the embryo sac.

ASEXUAL REPRODUCTION

Asexual reproduction is a mode of reproduction that does not involve fusion of male and female gametes. The regeneration of plants takes place from the vegetative parts of the plant (vegetative reproduction) or may arise from embryos that develop without fertilization (apomixis). Asexual reproduction yields clones, offspring completely identical to the parent. Although a few animals can regenerate in this fashion, in general plants are better candidates for cloning. Tissues of many plants retain totipotency, the ability to regenerate the entire plant structure. In fact, many plants reproduce asexually under natural conditions. Basic features of asexual reproduction are:

- It involves only one organism i.e. different sexes are not involved.
- The cell divisions during this type of reproduction are either mitotic or amitotic.
- New individuals produced are genetically identical to the single parent.
- It is a fast mode of multiplication.

1. Vegetative Reproduction

In nature, a new plant develops from a portion of the plant body. This may occur through modified underground and sub-aerial stems, and through bulbils.

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Underground Stems: The underground modifications of stem generally serve as storage organs and contain many buds which may develop into shoots and produce plants after rooting. The underground stems include tubers (Potato, Kala zeera), Bulb (onion, garlic), Rhizome (ginger, turmeric) and Corm (saffron, gladiolus).

Sub-aerial Stems: These modifications include runner, stolon, sucker etc. Subaerial stems are used for the propagation of mint, date palm etc.

Bulbils: Bulbils are modified flowers that develop into plants directly without formation of seeds. These are vegetative bodies; their development does not involve fertilization and seed formation. The lower flowers in the inflorescence of garlic naturally develop into bulbils.

Implications of Vegetative Reproduction

Vegetatively reproducing species offer unique possibilities in breeding. A desirable plant may be used as a variety directly regardless of whether it is homozygous or heterozygous. Further, mutant buds, branches or seedlings, if desirable, can be multiplied and directly used as varieties. There are certain characteristics of clonal propagation that have breeding implications.

- Clonal species with viable seed and high pollen fertility can be improved by hybridization.

- Unlike hybridization of sexual species, which often requires additional steps to fix the genetic variability in a genotype for release as a cultivar (except for hybrid cultivars), clonal cultivars can be released immediately following a cross, provided a desirable genotype combination has been achieved.

- When improving species whose economic parts are vegetative products, it is not important for the hybrid to be fertile.

- Because of the capacity to multiply from vegetative material (through methods such as cuttings or micropropagation), the breeder only needs to obtain a single desirable plant to be used as stock.

- Heterosis, if it occurs, is fixed in the hybrid product. That is, unlike hybrid cultivars in seed-producing species, there is no need to reconstitute the hybrid. Once bred, heterozygosity is maintained indefinitely.

- It is more difficult to obtain large quantities of planting material from clones in the short term.

- Plant species that are vegetatively parthenocarpic (e.g., banana) cannot be improved by hybrid methodology.

- Species such as mango and citrus produce polyembryonic seedlings. This reproductive irregularity complicates breeding because clones of the parent are mixed with hybrid progeny.

- Clonal crops are perennial out breeders and intolerant to inbreeding. They are highly heterozygous.

2. Apomixis

In apomixis, seeds are formed but the embryos develop without fertilization resulting in formation of the plants obtained from such seeds, identical in genotype to the parent plant from which they are derived. Apomixis is of two types based on whether sexual reproduction may occur or not. It is designated as obligate if sexual reproduction does not occur and crops are exclusively apomictic. However, if sexual

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reproduction occurs, it is termed ad facultative. Apomixis occurs in about 400 plant species but none of the important commercial crops reproduces by apomixes, which however is possible to induce by genetic engineering. In either case, the apomixes is classified as follows, based on the source of cells from which the embryo develops.

Adventive Embryony or Sporophytic budding: Embryo develops directly from vegetative cells of the ovule, such as nucellus, integument, or chalaza without development of embryo sac. It occurs in mango, citrus, orchids etc.

Apospory: Embryo develops from cells other than embryo sac cells i.e., cells of integuments or nucleus.E.g.Citrus and Mango, *Hieraceum, Malus, Crepis, Ranunculus,* etc.

Diplospory: Embryo sac is produced from the megaspore, which may be haploid or, more generally, diploid. Generally, the meiosis is so modified that the megaspore remains diploid. Diplospory leads to parthenogenesis or apogamy.

Parthenogenesis: The embryo develops from embryo sac without pollination. It is of two types

- **Gonial parthenogenesis:** Embryos develop from egg cell,
- **Somatic parthenogenesis:** Embryos develop from any cell of the embryo sac other than the egg cell.

Apogamy

In apogamy, synergids or antipodal cells develop into an embryo. Like parthenogenesis, apogamy may be haploid or diploid depending upon the haploid or diploid state of the embryo sac. Diploid apogamy occurs in *Antennaria, Alchemilla, Allium* and many other plant species.

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Implications of Apomixis

- It produces seed progeny which are exactly the same as the mother plant. Thus, apomixis helps in the preservation of good characters over generations for crop plants.
- It helps in the production of hybrid seeds with a combination of desirable characters. It also prevents the loss of specific characters from a hybrid.
- It helps in the cost-effective and time-efficient production of seeds.
- Apomictic species are highly heterozygous and inbreeding leads to severe inbreeding depression.
- Apomixis helps in fixation f heterosis

Artificial Methods of Asexual Reproduction

Various methods of asexual reproduction are frequently employed to give rise to new plants. They include grafting, cutting, layering, and micropropagation.

Grafting: Grafting has long been used to produce novel varieties of roses, citrus species, and other plants. In grafting, two plant species are used: part of the stem of the desirable plant is grafted onto a rooted plant called the stock. The part that is grafted or attached is called the scion. Both are cut at an oblique angle (any angle other than a right angle), placed in close contact with each other, and are then held together. Matching up these two surfaces as closely as possible is extremely important because these will be holding the plant together. The vascular systems of the two plants grow and fuse, forming a graft. After a period of time, the scion starts producing shoots, eventually bearing flowers and fruits. Grafting is widely used in viticulture (grape growing) and the citrus industry. Scions capable of producing a particular fruit variety are grafted onto root stock with specific resistance to disease.

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Cutting: Plants such as coleus and money plant are propagated through stem cuttings where a portion of the stem containing nodes and internodes is placed in moist soil and allowed to root. In some species, stems can start producing a root even when placed only in water. For example, leaves of the African violet will root if kept undisturbed in water for several weeks.

Layering: Layering is a method in which a stem attached to the plant is bent and covered with soil. Young stems that can be bent easily without any injury are the preferred plant for this method. Jasmine and bougainvillea (paper flower) can be propagated this way. In some plants, a modified form of layering known as air layering is employed. A portion of the bark or outermost covering of the stem is removed and covered with moss, which is then taped. Some gardeners also apply rooting hormone. After some time, roots will appear; this portion of the plant can be removed and transplanted into a separate pot.

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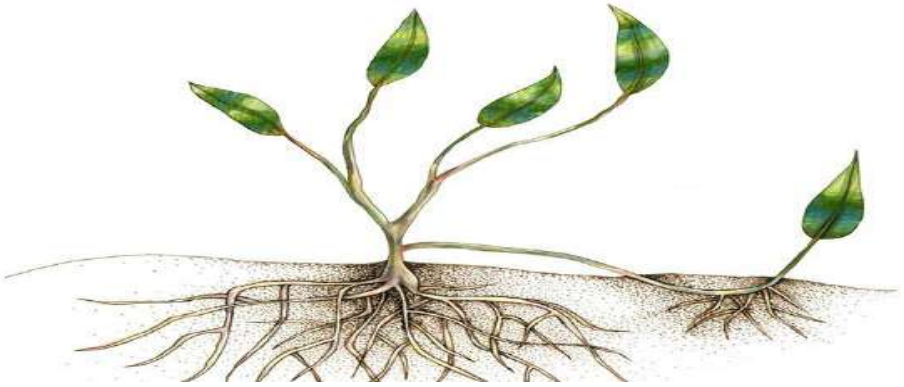
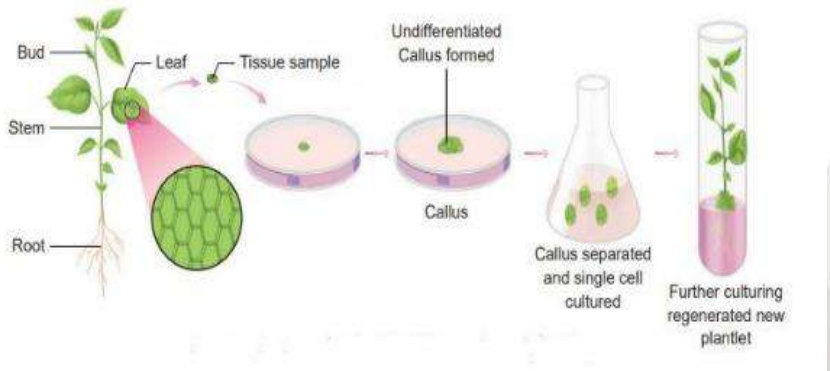


Micropropagation: Micropropagation or plant tissue culture is a method of propagating a large number of plants from a single plant in a short time under laboratory conditions. This method allows propagation of rare, endangered species that may be otherwise difficult to grow under natural conditions, are economically important, or are in demand as disease-free plants.

Plant tissue culture requires certain plant parts called as Explants such as a stem, leaf, embryo, anther, or seed. The explants are thoroughly sterilized using a combination of chemical treatments standardized for that species. Under sterile conditions, the explant is put in a plant tissue culture medium that contains all the minerals, vitamins, and hormones required by the plant and are varied in composition to promote rooting and shoot formation following callus (an undifferentiated mass) formation. These plants thus produced can be separated; they are first grown under greenhouse conditions (Hardening) before they are moved to field conditions.



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Course Name	Principles of Plant Breeding
Lesson 6	Pollination Control Mechanism
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand various mechanisms that promote self and cross pollination.
2. Comparative analysis of various systems of self incompatibility and male sterility systems.

Glossary of terms

Bisexuality*:* Presence of male and female organs in the same flower is known as bisexuality. The presence of bisexual flowers is a must for self pollination. All the self- pollinated plants have hermaphrodite flowers.

Cleistogamy: In this case, flowers do not open at all. This ensures complete self pollination since foreign pollen cannot reach the stigma of a closed flower. Cleistogamy occurs in rice, wheat, oats, barley and in a number of other grasses.

Chasmogamy*:* In some species, the flowers open, but only after pollination has taken place. This occurs in many cereals, such as, wheat, barley, rice and oats. Since the flower does open, some cross-pollination may occur.

Homogamy*:* Maturation of anthers and stigma of a flower at the same time is called homogamy. As a rule, homogamy is essential for self-pollination.

Dicliny: Dicliny or unisexuality is a condition in which the flowers are either male or female.

Dichogamy: Stamens and pistils of hermaphrodite flowers may mature at different times facilitating cross -pollination.

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Heterostyly: When styles and filaments in a flower are of different lengths, it is called heterostyly. It promotes cross pollination, such as linseed.

Herkogamy: Hinderance to self-pollination due to some physical barriers such as presence of hyaline membrane around the anther is known as herkogamy. Such membrane does not allow the dehiscence of pollen and prevents self-pollination such as in alfalfa.

Self-Incompatibility: It refers to a condition wherein an otherwise normal pollen is not able to effect fertilization of its own female gamete.

Male sterility: It refers to a situation in which the pollen grains produced are sterile due diverse mechanisms that interfere with the development and maturation of pollen.

Heteromorphic system: When self incompatibility is associated with differences in floral morphology, it is known as heteromorphic system. In this system self incompatibility results due to differences in the length of style and stamen.

Distyly: It refers to two types of styles (short and long) and stamens (low and high).

Tristyly: When style has three different positions, it is referred to as tristyly.

Homomorphic self incompatibility: In case where flowers of incompatible types do not exhibit floral differences, the incompatibility reaction is determined by genetically defined mechanisms in which the gene products of different S genes are involved either gametophytically or sporophytically.

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Gametophytic self incompatibility: Mechanism of self incompatibility governed by genetic constitution of gametophyte.

Sporophytic self incompatibility: Mechanism of self incompatibility governed by genetic constitution of plant bearing the gametophyte

Bud–Pollination: Pollination of immature buds with mature pollens has been successful in production of large quantity of self seed both in gametophytic and sporophytic systems in *Brassica*, *Nicotiana* and *Petunia*.

***In vitro*-fertilization:** Placing of pollen grains in direct contact with ovules has been reported to result in breakdown of self incompatibility in many crop species.

Mentor Pollen: Mentor effects can be defined as the capacity, in mixed pollinations, of foreign compatible pollen to induce the growth of the incompatible pollen grains present in the mixture. Mentor pollen has been found to overcome self – incompatibility in Cola, and Lotus.

Genetic male sterility: In this case the genetic factors governing male sterility are present in nucleus.

Cytoplasmic male sterility: In this case the genetic factors governing male sterility are present in cytoplasm (mitochondria).

Cytoplasmic-genetic male sterility: in this case the genetic factors governing male sterility are present in cytoplasm (mitochondria) as well as nucleus.

Environmental-Genetic Male Sterility: This male sterility system is controlled by nuclear gene expression, which is influenced by environmental factors such as temperature, daylength, or both.

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Classification of plants based on pollination

- I. Natural or Normally self pollinated crops: These crops show high degree of self pollination(>95%) and cross pollination is less than five per cent e.g., Barley, Ragi, Wheat, Beans, tomato, pea, Grams, Groundnut, Sesamum and Tobacco.

- II. Naturally or normally cross pollinated crops: In these crops, cross pollination occurs predominantly (>95%) with a very little (i.e., 5 %) self pollination.E.g.,

- a. Field Crops i.e., Alfalfa, Castor, Beans, hemp, Maize, Rye, Castor, Mustard, Sugarcane and Sunflower.

- b.Horticulture Crops.E.g., Almond, Apples, Banana, Cherries, Chestnut, Citrus, Datepalm, Grapes, Fig, Papaya, Mango, etc.)

- c. Vegetables.E.g., All Cruciferous plants.

- III. Often cross- pollinated crop: These crops are normally self-pollinated. However, cross pollination in these crops usually exceeds five per cent due to various agencies e.g., okra, brinjal, chilli, Sorghum, Cotton, Safflower, etc.

Mechanisms promoting self-pollination

Self-pollinated species, as a rule, must have hermaphrodite flowers. But in most of these species, self -pollination is not complete and cross -pollination may occur up to 5%. The degree of cross-pollination in self-pollinated species is affected by several factors, e.g., variety environmental conditions like temperature and humidity, and location. Various mechanisms that promote self-pollination are generally more

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efficient than those promoting cross -pollination. These mechanisms are listed below.

1. Bisexuality: Presence of male and female organs in the same flower is known as bisexuality. The presence of bisexual flowers is a must for self-pollination. All the self-pollinated plants have hermaphrodite flowers.

2. Cleistogamy. In this case, flowers do not open at all. This ensures complete self-pollination since foreign pollen cannot reach the stigma of a closed flower. Cleistogamy occurs in rice, wheat, oats, barley, pea and in a number of other grasses.

3. Chasmogamy: In some species, the flowers open, but only after pollination has taken place. This occurs in many cereals, such as, tomato, wheat, barley, rice and oats. Since the flower does open, some cross-pollination may occur.

4. Floral structure: In crops like tomato and brinjal, the stigmas are closely surrounded by anthers. Pollination generally occurs after the flowers open. But the position of anthers in relation to stigmas ensures self-pollination. Similarly in some species, flowers open but the stamens and the sigma are hidden by other floral organs. In several legumes, e.g., pea, mung, urd, soybean and gram, the stamens and the stigma are enclosed by the two petals forming a keel.

5. Non-receptive stigmas: In a few species, stigmas become receptive and elongate through staminal columns. This ensures predominant self -pollination.

6. Homogamy: Maturation of anthers and stigma of a flower at the same time is called homogamy. As a rule, homogamy is essential for self-pollination.

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Mechanisms promoting cross pollination

Nature promotes variation through free exchange of genes that create new gene combinations. Consequently, nature has created more mechanisms that promote cross pollination as compared to self pollination. Several mechanisms that facilitate cross pollination are described briefly.

1. Unisexuality or Dicliny: Dicliny or unisexuality is a condition in which the flowers are either male or female.

 Monoecy. Male and female flowers occur in the same plant, either in the same inflorescence, *e.g.,* Cucurbits, Castor, mango and coconut, or in separate inflorescences, chestnut, strawberries, rubber, grapes and cassava.

 Dioecy. The male and female flowers are present on different plants, *i.e.,* the plants in such species are either male or female, *e.g.,* papaya, date, hemp, asparagus, and spinach. In general, the sex is governed by a single gene, e.g., asparagus and papaya. In some cases, there are hermaphrodite plants in addition to males and females, and a number of intermediate forms may also occur.

2. Dichogamy: Stamens and pistils of hermaphrodite flowers may mature at different times facilitating cross -pollination.

 Protogyny. In crop species like bajra and cole crops, pistils mature before stamens.

 Protandry. In crops like maize, onion and sugar beet, stamens mature before pistils.

3. Non-receptive flowers: In Lucerne or alfalfa, stigmas are covered with a waxy film. The stigma does not become receptive until this waxy film is

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broken. The waxy membrane is broken by the visit of honey bees which also effect cross-pollination.

4. Heterostyly: When styles and filaments in a flower are of different lengths, it is called heterostyly. It promotes cross pollination, such as linseed, brinjal.

5. Herkogamy: Hinderance to self-pollination due to some physical barriers such as presence of hyaline membrane around the anther is known as herkogamy. Such membrane does not allow the dehiscence of pollen and prevents self-pollination such as in alfalfa.

6. Self-Incompatibility: It refers to a condition wherein an otherwise normal pollen is not able to effect fertilization of its own female gamete.

7. Male sterility: It refers to a situation in which the pollen grains produced are sterile due diverse mechanisms that interfere with the development and maturation of pollen.

SELF INCOMPATIBILITY

The term self incompatibility was originally coined by Stout in 1917. Koelreuter, in the middle of 18th century, first reported self incompatibility in ***Verbascum phoeniceum*** plants. Self incompatibility refers to the inability of a functional or viable pollen grain to effect fertilization of its own female gamete upon its landing on the stigma. It is one of the natural mechanisms that promotes crossing over by regulating the acceptance or rejection of pollen grains. It has been reported in about 300 species belonging to 70 families of flowering plants. SI prevents inbreeding by making selfing impossible and preventing mating between close relatives that have inherited the same incompatibility type. SI is present in 60% of all flowering plants. Two genetic mechanisms of SI have been recognized: GSI and SSI. In GSI the incompatibility type of the pollen is controlled by its own haploid

genotype, whereas in SSI, the pollen incompatibility type is controlled by the diploid (sporophyte) genotype of the parental anther in which it was produced.

The well-known heteromorphic SI systems of distyly and tristyly are under sporophytic genetic control whereas 'homomorphic' SSI, where no such differences in floral morphology distinguish incompatibility.

Main features of self incompatibility

- Self incompatibility is an important outbreeding mechanism which prevents autogamy and promotes allogamy.
- Self incompatible species do not produce seed on self pollination but lead to normal seed set on cross pollination.
- It maintains high degree of heterozygosity in a species due to outbreeding and reduces homozygosity due to elimination of inbreeding or selfing.
- Self incompatibility results due to morphological, genetic, physiological and biochemical causes. It is not under simple genetic control.
  - Self incompatibility reaction can operate at any stage between pollination and fertilization.
  - Self incompatibility has been reported in about 70 families of angiosperms including several crop species.

Table : Classification of self incompatibility

Basis of classification	Types of self incompatibility	Brief Description
Flower Morphology	(a) Heteromorphic	SI is associated with differences in flower morphology.

	(i) Distyly (ii) Tristyly	Styles and stamens are of two types, *i.e.,* short and long. Styles and stamens have three positions, *i.e.,* short, medium and long.
	(b) Homomorphic i. Sporophytic ii. Gametophytic	The flowers do not differ in Morphology. SI is governed by genotype of pollen producing plant. SI is governed by the genetic constitution of gametes.
Number of Alleles Involved	(a) Monoallelic (b) Diallelic (c) Polyallelic	SI is controlled by a single gene. SI is governed by two genes. SI is governed by several genes.
Site of Expression	(a) Stigmatic (b) Stylar (c) Ovarian	SI genes express on the stigma. SI genes express in the style. SI genes express in the ovary
Pollen Cytology	(a) Binucleate (b) Trinucleate	The pollen grains have two nuclei. The pollen grains have three nuclei.

Heteromorphic system

When self incompatibility is associated with differences in floral morphology, it is known as heteromorphic system. In this system self incompatibility results due to differences in the length of style and stamen. This system is again of two types, *viz.,* (a) distyly, and (b) tristyly.

Distyly: It refers to two types of styles (short and long) and stamens (low and high). This system operates in the family *Primulaceae*. In primula,

there are two types of flowers: *viz.,* (1) thrum type which has short style and high anthers, and (2) pin type with long style and low anthers. The crosses are compatible only between the style and stamens of matching length. In other words, crosses are compatible between pin X thrum or thrum X pin but not between pin X pin and thrum X thrum flowers.

Tristyly: When style has three different positions, it is referred to as tristyly. In tristyly anthers and style have three positions in the flowers, viz., short, medium and long. Tristyly is common in the family Lythraceae. Tristyly is found in *Lythrum salicaria*. The position of style are genetically controlled by two genes (Ss and mm.) The S gives rise to short style, s and M to medium style and s and m to long style. Short style may have Ssmm, SsMm or SsMM genotypes; medium style has ssMm or ssMM genotypes; and long style has ssmm genotype.

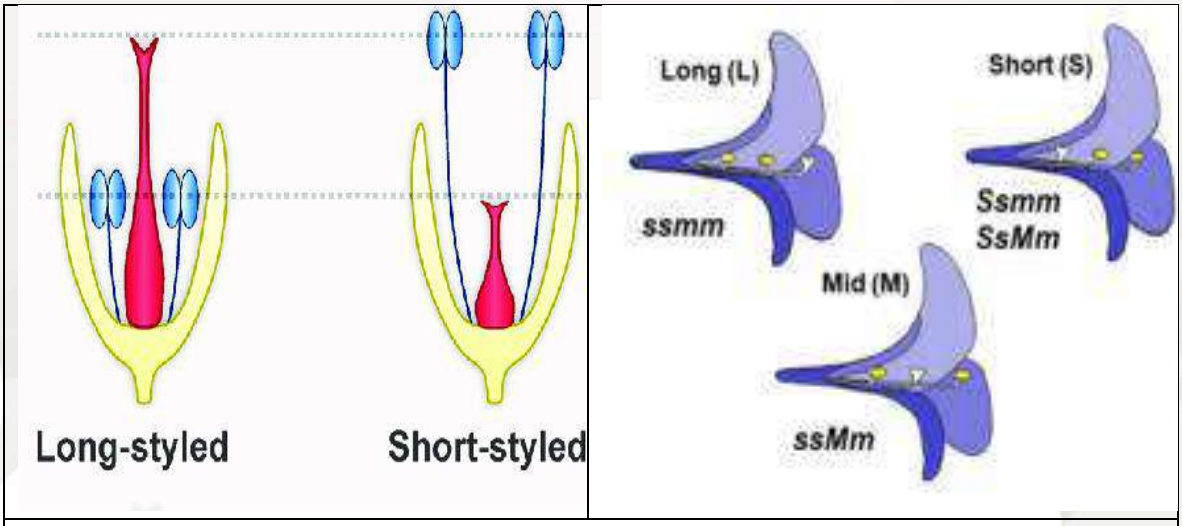

Fig: Distyly and Tristyly

Homomorphic self incompatibility

In case where flowers of incompatible types do not exhibit floral differences, the incompatibility reaction is determined by genetically defined mechanisms in which the gene products of different S genes are involved either gametophytically or sporophytically.

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Gametophytic self incompatibility: Gametophytic self incompatibility the most widespread of the SI systems reported in *Solanaceae, Rosaceae, Scrophulariaceae, Leguminoseae, Onagraceae, Campanulaceae, Papaveraceae and Poaceae*. Two different single-locus gametophytic SI systems have been studied. The first of these is the S-RNase system originally found and extensively characterized in members of the *Solanaceae* and subsequently also reported in the *Rosaceae* and *Scrophulariaceae*. The second is found in the *Papaveraceae,* namely, *Papaver rhoeas* L.

In the Solanaceae and other families that possess an SRNase- based SI system, an incompatible pollen grain on the stigma germinates and begins to grow through the transmitting tract of the style. However, the growth of this incompatible pollen tube is arrested when it has reached about one-third of the way through the style. Analysis of stylar proteins from *Nicotiana alata* resulted in the identification of a 32 kDa S-glycoprotein that exhibited genetic linkage to the *S*-locus.

Sporophytic self incompatibility: Sporophytic self incompatibility has been identified in six families other than the *Brassicaceae*: the *Asteraceae*, *Convolvulaceae, Betulaceae, Caryophyllaceae, Sterculiaceae and Polemoniacea*e. Among these families SSI has been investigated at a molecular level only in the *Convolvulaceae* and the *Asteraceae.*

The genetics of SSI was first elucidated in two species from the Asteraceae: *Crepis foetida* and *Parthenium argentium*. Later, SSI was described in species from the *Brassicacea*e. As with GSI, SSI is controlled by a single polymorphic locus, *S*. The fundamental genetic difference between SSI and GSI is that in SSI, the incompatibility phenotype of the pollen is determined by the diploid parental genome. Hence, pollen grains carry the products of two SI (*S*) alleles, instead of one. Diploid sporophytic expression of SI allows dominance interactions to occur

between *S*-alleles and such interactions can occur independently for pollen and stigma. This often results in very complex patterns of compatibility and incompatibility, with reciprocal differences in incompatibility between individuals being a regular feature associated with differing dominance effects in pollen and stigma.

Dominance interactions can also give rise to individuals homozygous for recessive *S*-alleles: an event that is impossible under GSI. With Sporophytic self- incompatibility, the total number of *S* alleles needed to sustain a functional SI system is two, with the one dominant allele exerting its effect on both pollen and stigma. This is the situation with heteromorphic SI, where incompatibility and floral morphology are controlled by just two sporophytically expressed *S*-alleles, *S* and *s* with complete dominance. In the multiallelic 'homomorphic' Sporophytic self incompatibility systems of the *Brassicaceae* and *Asteraceae*, between 30 and 40 *S* alleles are typically found within natural populations

Main features of GSI system

1. Self incompatibility in majority of species is governed by a single gene S which has large number of multiple alleles. However, in rye self incompatibility reaction is governed by two loci.

2. In this system alleles have individual action in the style without interaction.

3. Pollen grains are unable to germinate or function on a pistil having similar alleles as that of pollen. The pollen tube growth is usually inhibited in the style or ovary.

4. This system gives rise to three types of pollinations, *viz.,* (1) fully incompatible (S1 S2 x S1 S2) in which both allele are common in the pollen and ovule, (2) half the pollen is compatible (S1 S2 x S1 S3) in which

one allele is different, and (3) fully fertile (S1 S2 x S3 S4) when both alleles differ in pollen and ovule.

5. Gametophytic system permits recovery of male parent only in the partially fertile crosses which are obtained when one allele differs in the cross, *viz.,* S1 S2 x S1 S3. This cross would give rise to S1 S3 and S2 S3 progeny.

6. Plant species belonging to gametophytic self-incompatibility system have binucleate pollen.

7. All gametophytic systems (except in Gramineae) operate with wet stigma surfaces and there is not direct interaction between one pollen grain and one surface cell because germination takes place in a common fluid medium.

8. The biochemical substance which is associated with the incompatibility response of the pollen develops very late, *i.e.,* during pollen formation in gametophytic system.

Main features of SSI system are

- In this system also self incompatibility is controlled by a single gene S which has multiple alleles.
- The alleles may show dominance, individual action or interaction in either pollen or style as per the allelic combinations involved.
- This system exhibits inhibition of pollen germination or pollen tube growth on the stigma of same flower.
- The sporophytic system contains a form of dominance in which S1is dominant over all other alleles, S2 is dominant over all except S1 and

so on (S1 > S2 > S3 > S4). In this system, crosses between different genotypes are either fully fertile or completely sterile.

- Pollen grains from both heterozygous and homozygous plants react in a similar fashion due to dominance effect of male parent.
- The system permits recovery of parental genotypes in some crosses.
- This system of self incompatibility generally have tri nucleate pollen and operate with a dry stigma.
- The biochemical substance which is associated with the incompatibility develops very early in sporophytic system that is before pollen development.

Measures to overcome self incompatibility

There are several ways and means to overcome self incompatibility in crop plants.

Bud–Pollination: Pollination of immature buds with mature pollens has been successful in production of large quantity of self seed both in gametophytic and sporophytic systems in *Brassica, Nicotiana* and *Petunia*. This method is now used for the development of inbred lines for hybrids seed production in *Brassica* species. The bud pollination should be done 2 – 4 day before opening. Generally in young buds the stigmas lack adhesivity which can be achieved if the stigma is smeared with the exudates from an open flower.

Delayed Pollination: Delayed Pollination also leads to self fertilization in some self incompatible species. Pollination of aged pistil several days after maturity with normal incompatible pollen resulted in some degree of self fertilization in *Brassica* and *Linum*. However, such results could not

be achieved with other plant species. This indicated that Brassica and *Linum* aging leads to break down of self incompatibility.

Late season pollination: Self pollination at the end of flowering season also leads to seed set in some species like *Nicotiana, Petunia* and *Abutilon*. This may be due to loss of capacity to produce active incompatibility substances at the end of flowering season.

Irradiation: Irradiation of style with X-rays immediately before selfing resulted in breakdown of self incompatibility in *Petunia*. Gamma irradiation significantly increased seed setting upon selfing in *Lycopersicon peruvianum* and *Nicotiana alata*. However, these effects were observed in one generation and were lost in the next generation. Mutation plays an important role in overcoming self incompatibility. If S1 is mutated to S' It will be compatible with both S1 and S2.

High Temperature: Treatment of style at temperature ranging from 30℃ to 60℃ leads to breakdown of self incompatibility in many plant species like, *Malus*, *Pyrus, Prunus, Trifolium, Lycopersicon* and rye having gametophytic single locus system of self incompatibility. Heat treatment inactivates the substance or enzyme which causes self incompatibility.

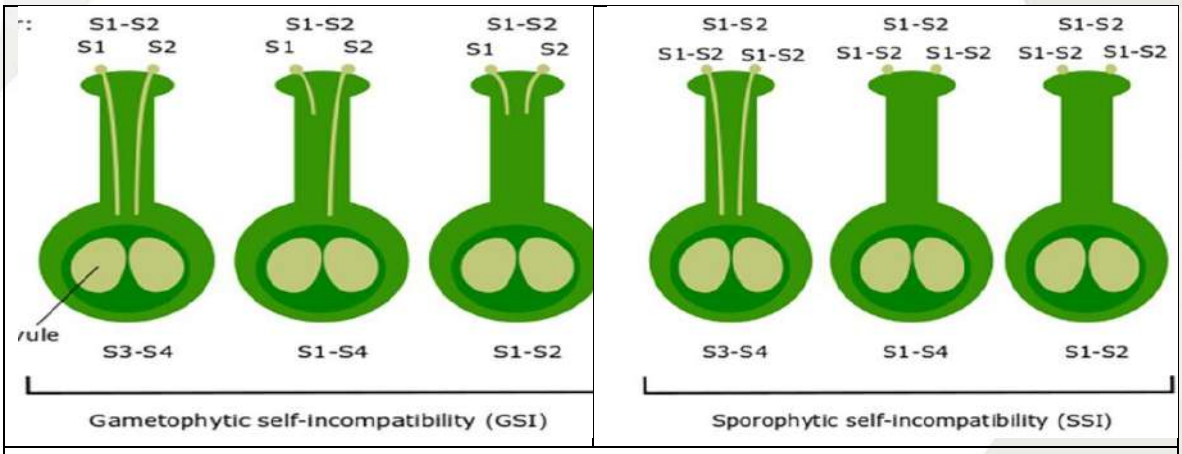

Fig: Gametophytic and Sporophytic systems of incompatibility (a) Gametophytic and (b) Sporophytic

Mutilation of style: Removal of stigma in species having stigmatic incompatibility results in certain amount of seed production in some crops. This method was found to be successful in *Brassica oleracea*. In this species, self incompatibility could be broken by mutilating the stigma with steel wire brush during pollination.

***In vitro*-fertilization:** Placing of pollen grains in direct contact with ovules has been reported to result in breakdown of self incompatibility in many crop species. This indicates that restriction of interference from the stigma, the style or the ovary leads to breakdown of self incompatibility. Self seeds were obtained in *Petunia axillaris* by in vitro fertilization.

Mentor Pollen: Mentor effects can be defined as the capacity, in mixed pollinations, of foreign compatible pollen to induce the growth of the incompatible pollen grains present in the mixture. Mentor pollen has been found to overcome self – incompatibility in Cola, and Lotus.

Hormones and Inhibitors: Kinetin and morophocitin have been found to retard the abscission of petioles in *Catharanthus* and it is therefore, probable these substances or related compounds could also be used to produce seed upon selfing in self-incompatible species.

Surgical Techniques: The application of pollen to the cut surface of the style after the removal of stigma (*Brassica*) or the introduction of pollen into this ovarian cavity (*Petunia*) can result in successful self-pollination in plant.

Electric Aided Pollination: It has been shown that self-incompatibility barrier could be partially broken down in *Brassica oleracea* by applying a direct electric potential difference of 100 V between pollen and stigma during pollination. The effect measured as the number of seeds per self-

pollinated flower which was comparable to method used in Brussels sprouts by removal of the stigma.

UTILIZATION OF SELF INCOMPATIBILITY IN PLANT BREEDING

Self incompatibility is of great significance to plant breeders. It is an important pollination control device which prevents autogamy and promotes allogamy. In plant breeding it is useful in two main ways: *viz.,* (1) in the production of hybrids, and (2) combining desirable genes from different sources.

1. Production of Hybrids: Self incompatibility provides a way for hybrid seed production without emasculation and without resorting to genetic or cytoplasmic male sterility. Self incompatibility has been utilized for production of commercial hybrids in *Brassica* (cabbage and Brussels sprouts) and sunflower, two self incompatible lines (strain) are planted in the alternate row for hybrid seed production. The harvest from both the lines would be hybrid seed. In Japan, self incompatibility is used for commercial seed production in some cruciferous crops.

2. Combining Desirable Genes: Self incompatibility system permits combining of desirable genes in a single genotype from two or more different sources through natural cross pollination which is not possible in self compatible species. Moreover, knowledge of self incompatibility specially in fruit crops, helps fruit growers to increase the yield of fruit by providing suitable pollinators.

LIMITATIONS

1. It is very difficult to produce homozygous inbred lines in a self-incompatible species. Bud pollination has to be made to maintain the parental lines.

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2. Self-incompatibility is affected by environmental factors such as temperature and humidity. Incompatibility is reduced or broken down at high temperature and humidity. Hybrid seed produced under unfavourable conditions using self- incompatibility may contain high proportion of sib crossed seeds which is not desirable. High temperature and humidity are favourable for maintenance of parental lines.
3. Sometimes bees visit only one parental line in the seed production plot resulting in sib mating. For example in Brussels sprouts, when two distinct morphological parental lines are used in hybrid seed production programme, bees have preference in visiting only one of these lines. This also poses problems in the used of self incompatibility in hybrid seed production programmes.

MALE STERILITY

Male sterility refers to the absence of functional pollen in an otherwise hermaphrodite flower. It is usually caused by mutation especially in genes governing energy systems of the tapetal cells of developing pollen that causes premature abortion and non-functionality of pollen grain. Tapetum, the innermost cell layer surrounding pollen grain, is a physiologically active site and nourishes the developing gametes. However, it degenerates later on and is not found as an organized tissue in mature anthers. The tapetum and other surrounding cell layers suffer early degeneration in sterile pollen as compared to fertile ones. In 1763, Kölreuter first observed anther abortion within species and species' hybrids and is thought to arise spontaneously via mutations in nuclear and/or cytoplasmic genes. Male sterility is more prevalent than female sterility due to following reasons:

- Male sporophyte and gametophyte are less protected from environment than ovule and embryo sac.

- It is easy to detect male sterility, because a large number of pollens for study are available.
- Easy to assay male sterility: staining technique (caramine, lactophenol or iodine); female sterility (fst) requires crossing.
- Male sterility has propagation potential in nature (can still set seed) and is important for crop breeding, female sterility does not.
- **Male Sterility is usually manifested in following forms;**
- Absence or malformation of male organs (stamens) in bisexual plants or no male flowers in dioecious plants.
- Failure to develop normal microsporogenous tissue- anther.
- Abnormal microsporogenesis--deformed or inviable pollen.
- Abnormal pollen maturation; inability to germinate on compatible stigmata.
- Non- dehiscent anthers but viable pollen- sporophytic control.
- Barriers other than incompatibility preventing pollen from reaching ovule.

Classification of Male Sterility

A. On the basis of phenotype:

***Structural male sterility*: A**nomalies in male sex organs (or missing all together).

Sporogenous male sterility: **S**tamens form, but pollen absent or rare due to microsporogenous cell abortion before, during, or after meiosis.

Functional male sterility: Viable pollen form, but barrier prevents fertilization such as failure of anther dehiscence and exine formation or inability of pollen to migrate to stigma.

B. On the basis of genotype:

Genetic male sterility**:** In this case the genetic factors governing male sterility are present in nucleus. It has arised due to spontaneous mutations in nuclear genes which in most cases are recessive mutations while as in some cases inheritance has been reported to be polygenic. It has been reported in about 175 species. Usually, **MsMs** or **Msms** plants are fertile while as **msms** are male sterile. A cross between a male sterile and male fertile plant yields all male fertile plants if the fertile plant is homozygous where as if it is heterozygous then fertile and sterile plants are recovered in the ratio of 1:1. There are usually no reciprocal differences in this case. Genetic male sterility has been exploited commercially only in a few countries. The genetic constitutions of various lines and their progeny upon crossing are shown as below:

A line= msms: Male sterile (sterile line)
B line =Msms: Male fertile (maintainer line)
R line = MSMS: Male fertile (restorer line)

Restoration step:

msms × MsMs = Msms (all fertile)
(male sterile) (male fertile)
Mantainance step:
msms × Msms = Msms : msms (1:1 fertile and sterile plants)
(male sterile) (male fertile)

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***Cytoplasmic male sterility*:** In this case the genetic factors governing male sterility are present in cytoplasm (mitochondria) and arise as a result of mutation in the *atp6* gene causing tapetal degeneration. It is less common than genetic male sterility, is maternally inherited and follows non-mendelian inheritance with reciprocal differences. The normal and sterile cytoplasms are designated as N and S respectively. A cross between a sterile and normal plant always yields sterile plants and as such it does not produce seed. Therefore, this kind of system is not used in seed crops but can be useful in ornamentals and vegetables where seed is not the economical product. In fact, the male sterile plants tend to produce larger flowers and bloom longer.

S × N = S (all sterile)

***Cytoplasmic-genetic male sterility*:** In this case the genetic factors governing male sterility are present in cytoplasm (mitochondria) as well as nucleus. In fact, this system is regarded as a result of nuclear-mitochondrial genomic conflict, with the dominant genes in nucleus tend to restore fertility. However, these restorer genes are different from the genes governing genetic male sterility and are designated as *Rf* genes by overcoming the deleterious effect of mitochondrial mutations. This system involves

1. Cytoplasmically determined MS plants known as A line in the genetic constitution.

2. Fertile counter parts of A line known as maintainer line or B line in the genetic constitution.

3. Restorer plants used to restorer the fertility in commercial seed plots known as R lines in the genetic constitution.

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Requirements for CGMS system

A-line

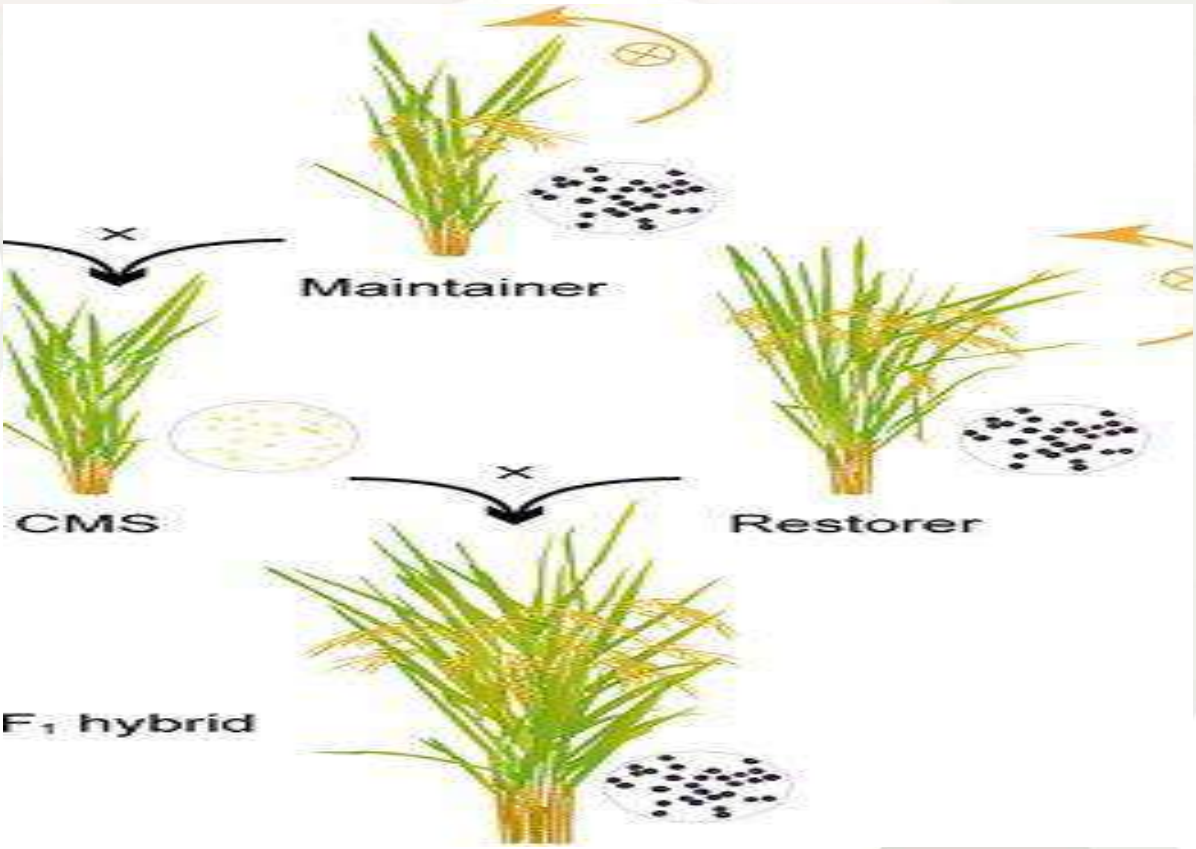
- Stable Sterility
- Well developed floral traits for out-crossing
- Easily, wide-spectrum, & strongly to be restored

B-line

- Well developed floral traits with large pollen load
- Good combining ability

R-line

- Strong restore ability
- Good combining ability
- Taller than A-line
- Large pollen load, normal flowering traits and timing



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Limitations in using Male Sterile Systems:

1. Existence and maintenance of A, B & R Lines is laborious and difficult.
2. If exotic lines are not suitable to our conditions, the native/adaptive lines have to be converted into MS lines.
3. Adequate cross pollination should be there between A and R lines for good seed set.
4. Synchronization of flowering should be there between A & R lines.
5. Sterility should be stable over the environments.
6. Fertility restoration should be complete otherwise the F_1 seed will be sterile.
7. Isolation is needed for maintenance of parental lines and for producing hybrid seed.

Environmental-Genetic Male Sterility: This male sterility system is controlled by nuclear gene expression, which is influenced by environmental factors such as temperature, daylength, or both. This male sterility system was first observed in pepper by Martin and Crawford in 1951 and subsequently in different crops (Table 1). However, this system has been exploited commercially only in rice because of the pioneering work of Chinese scientists. Male sterility expression in EGMS lines is governed by a single nuclear recessive gene or pair of nuclear recessive genes that are sensitive to environmental conditions such as photoperiod, temperature, or a combination of both. Under natural conditions, there is a constant interaction of photoperiod and temperature and it is therefore difficult to separate the effects of photoperiod and temperature on fertility. Depending on the environmental factor(s) influencing expression of the sterility-inducing gene(s), EGMS is classified in the following categories:

TGMS: temperature-sensitive genic male sterility: TGMS lines are sensitive to the temperature for the expression of male sterility or

fertility. For example, most TGMS lines remain male sterile at high temperature (day temperature >30 ºC/night >24 ºC) and they revert back to partial fertility at a lower temperature (day <24 ºC/>16 ºC night),

rTGMS: reverse temperature-sensitive genic male sterility: Reverse TGMS (rTGMS) lines are sensitive to low temperature (<24 ºC day/>16 ºC night) for the expression of male sterility, whereas, at a higher temperature (>30 ºC day/24 ºC night), they become male fertile, which is just the reverse of the TGMS system.

PGMS: photoperiod-sensitive genic male sterility: PGMS lines are sensitive to the duration of daylength for the expression of sterility or fertility. For example, most PGMS lines remain male sterile under long-day (>13.75 h) conditions and revert back to fertility under short-day (<13 h) conditions.

rPGMS: reverse photoperiod-sensitive genic male sterility: PGMS lines that express sterility under short daylength and fertility under long daylength are known as reverse PGMS (rPGMS). This category is yet to be found.

PTGMS: photothermosensitive genic male sterility: PTGMS lines are sensitive to both photoperiod and temperature. Temperature is the key factor since PTGMS lines become completely male sterile or fertile beyond a particular temperature range, that is, >30 ºC or <24 ºC, without any influence of photoperiod. But, within this temperature range (24–32 ºC), photoperiod influences the PTGMS lines, that is, longer photoperiod hours will enhance male sterility at lower temperatures.

Characteristic features of ideal EGMS lines

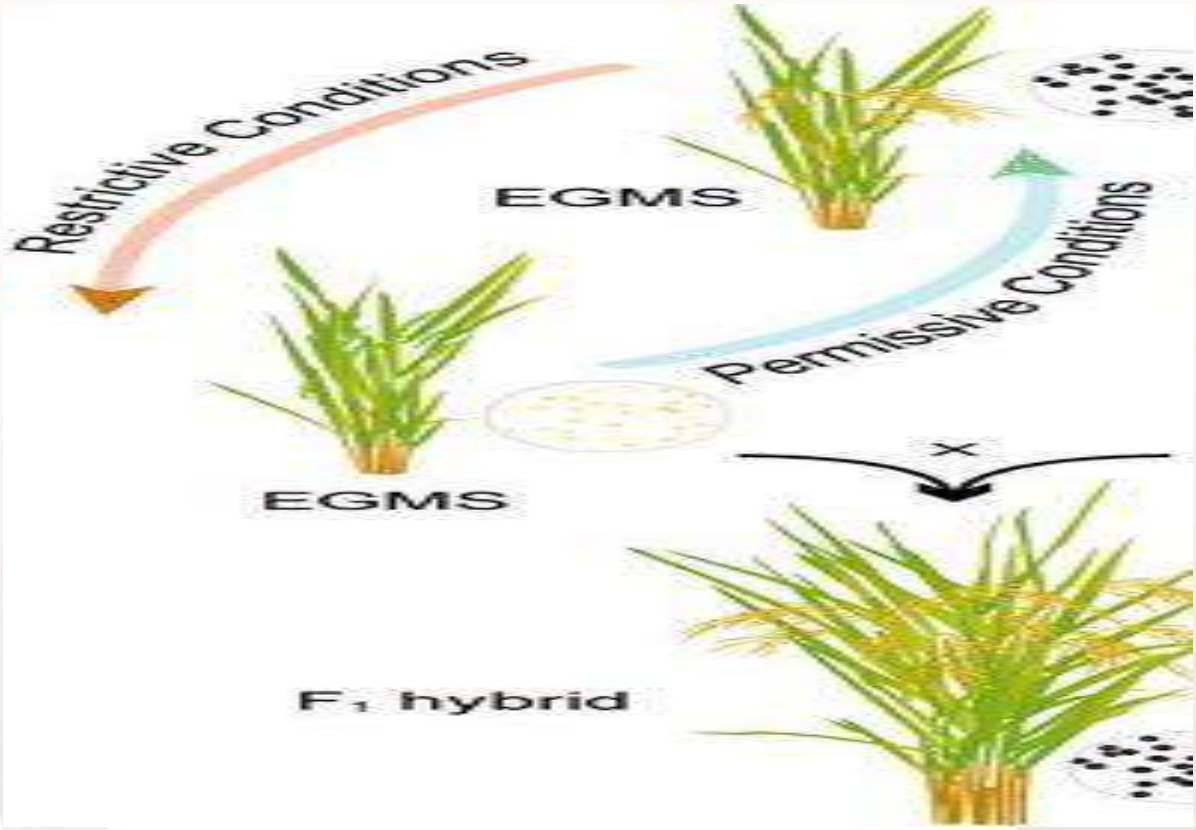
- The proportion of male sterile plants in a population of more than 1,000 plants during the critical sterility period should be 100%.
- The pollen sterility of each male sterile plant should be more than 99.5%.
- EGMS lines should have clearly defined sterility- fertility alteration regimes.
- The male sterile phase should last for more than 4 consecutive weeks.
- Seed setting during the fertile phase should be more than 30%.
- The critical temperature or photoperiod for inducing sterility should be as low as possible for more stability of the EGMS lines, for example, <27 ºC (maximum) or <13 h photoperiod.

Advantages of the EGMS system

- There is no need for a maintainer line for seed multiplication, thus making seed production simpler and more cost-effective.
- Any fertile line can be used as a pollen parent (PP); therefore, the frequency of heterotic hybrids is higher among two-line hybrids than among three-line hybrids, thereby increasing hybrid breeding efficiency.
- Negative effects of sterility-inducing cytoplasm are not encountered.
- The EGMS trait is governed by major genes, thus enabling their easy transfer to any genetic background and thus increasing diversity

among the female (EGMS) parents, which helps in reducing potential genetic vulnerability among the hybrids.

- Since there is no need for restorer genes in the male parents of two-line hybrids, this system is ideal for developing indica/ japonica hybrids because most japonica lines do not possess restorer genes.



Disadvantages of the EGMS system

Since the sterility trait is conditioned by environmental factors, any sudden change such as temperature fluctuation because of a thunderstorm, typhoon, etc., will influence the sterility of EGMS lines.

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Course Name	Principles of Plant Breeding
Lesson 7	Selection
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University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
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Objectives

1. Understand the genetic basis of selection
2. Understand various types and elements of selection and utility in plant breeding

Glossary of terms

Natural selection: Natural selection acts through variation in genotypic fitness that includes traits like adaptability, survival and viability.

Balanced polymorphism: When both the forces are operative, the increase in gene and genotypic frequency by one force is compensated by other and soon the population reaches an equilibrium state called as balanced polymorphism**.**

Artificial selection: Artificial selection is done by plant breeders to change the mean of population in a desired direction for a trait under selection.

Response to selection: Change in mean performance of selected population to base population

Selection intensity: Percentage of plants selected to be advanced to next generation from a population.

Selection differential: Difference between the mean of the population and mean of the selected individuals.

Heritability: The ratio of genetic variance to the total variance i.e. phenotypic variance is known as heritability.

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Genetic Advance: Genetic advance is the difference between the mean of the selected plants in the original population and the mean of the progeny raised from the selected plants in the next generation.

Progeny testing

Evaluation of the worth of plants on the basis of performance of their progenies is known as progeny test.

SELECTION

The fundamental steps of any breeding programme are:

1. Utilizing the available genetic variability through selection, if it is adequate,
2. Creation of useful variation, if there is no variability or the available variability isinadequate, and
3. Exploiting the newly created variation through selection.

Selection refers to preferential contribution of genotypes to next generation and is accomplished by isolation of desirable plant types from the population and elimination of undesirable ones in line with the objectives and trait. There are two agencies involved in carrying out selection : one is Nature itself (**Natural selection**) and the other is man (**Artificial selection**). Selection under both forces has been constantly changing the population structure that has directed evolution of new species as well as governed the survival and shape of plant species.

Natural selection: Natural selection acts through variation in genotypic fitness that includes traits like adaptability, survival and viability. Fitness in broader term can be defined as the number of progeny that survive up to maturity and produce atleast one individual. Suppose in a population, Aa produces 100% viable individuals, AA only 80% and aa only 60%, there is a disadvantage of 20% and 40% respectively for AA and aa. This disadvantage is called as **selective disadvantage** or **selection coefficient**.

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The situation is also referred to as **heterozygous advantage**. The changes under natural selection are brought about by two forces namely selection and mating design. When both the forces are operative, the increase in gene and genotypic frequency by one force is compensated by other and soon the population reaches an equilibrium state called as **balanced polymorphism.**

Artificial selection: Artificial selection is done by plant breeders to change the mean of population in a desired direction for a trait under selection. The **Response to selection** which is defined as change in mean performance of selected population to base population depends upon the population selected, heritability and influence of environment. Like natural selection, here also the change in gene and genotypic frequency is brought about by selection and mating design.

GENETIC BASIS OF SELECTION

Selection is the process by which certain individuals are allowed to contribute more towards progeny at the expense of others. Admittedly it must be the guiding factor which resulted in the evolution of new species some of which were domesticated and laid the foundation of present day agriculture. Selection was the predominant component of Mother Nature's repertoire of tools that governed the survival and shape of plant species over centuries. Even the early farmers and some biologists used it successfully to improve plants without knowing the basis of its action.

1. Selection is effective only if heritable variation is present.
2. Selection does not act on the gene itself but acts on genotype through phenotype and thus ultimately changes frequency of genes and genotypes.
3. Selection itself does not create new genes or genotypes but influences the irrelative frequency by changing their contribution to the progeny.

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BASIC PRINCIPLES OF SELECTION

Notwithstanding the highly complex genetic situation imposed by linkage and espistasis, the basic principles of selection are outlined as follows :

1. Selection does not create any new variation: It only utilizes the variation already present in a population.

2. **Selection operates on existing variability**: The main function of the selection exercise is to discriminate between individuals. This is possible only when sufficient variation is present in the material subjected to selection pressure. Thus, selection acts on the existing variation it cannot create new variation.

3. **Selection acts only through heritable differences**: Only the selected individuals are permitted to contribute to the next generation / progenies. Therefore, should there be greater influence of non-heritable agencies on the individuals selected; the parent-progeny correlation will be greatly vitiated. Hence, the variation among individuals to be selected must be genetic in nature, since it is the genetic variation that tends to close the gap between phenotype and genotype. Environmental variability cannot be of any use under selection.

4. Selection favours some individuals in reproduction at the expense of others: As a consequence of its past evolutionary history and breeding structure, a population or a crop consists of highly genetically variable individuals with regards to such diverse phenomena as differential viability, differential maturity, differences in mating tendencies, fecundity, and duration of reproductive capacity. Hence some individuals tend to become superior to others for some or other traits desirable under domestication. These superior individuals are retained for reproduction while others discarded under selection.

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5. Selection changes gene and genotypic frequencies: There is a gradual change in gene and genotypic frequencies in a population under selection for a metric trait. The frequencies can be further modified if different mating designs are employed among selected individuals.

Basic requirements for selection to operate are:

1. Adequate variation must be present in the population.
2. The variation should be heritable.
3. There should be no undesirable linkages and gene interactions

ELEMENTS OF SELECTION

Selection intensity: Percentage of plants selected, to be advanced to next generation, from a population.

Selection intensity (I): It is the amount of selection applied expressed as the proportion of the population favored (selected). The selection intensity is inversely proportional to the percentage proportion selected (PS), as reflected in Table 1.

Table1. : Relationship between selection intensity and proportion of population selected

Selection intensity (I)	**2.64**	**2.42**	**2.06**	**1.76**	**1.40**	**1.16**	**0.97**	**0.80**	**0.34**	**0**
% selected	**1**	**2**	**5**	**10**	**20**	**30**	**40**	**50**	**80**	**100**

Thus, larger the size of I, more stringent is the selection pressure (hence low fraction is selected) and vice versa. Then, no selection means all the members of a population are allowed to reproduce (I=0, PS=100%), and zero selection means the whole population is rejected (PS=0). However, in real selection experiments, as the desired alleles become preponderant after each cycle of selection, I is also changed. Selection

intensity (I) is of the greatest consequence is bringing about changes in the gene frequency under selection. However, since the latter does not mean undue loss of desirable alleles, or undue load of population size, the choice of an arbitrary value of I may be hazardous in a plant breeding programme. The small size of I (i.e. low selection pressure) may cause a large population size to be handled in the next generation, which will unnecessarily be taxing on time and resources. On the other hand, a large size of I (high selection pressure) might cause allelic erosion due to genetic drift (i.e. changes in gene frequencies due to sampling error or small sample size under selection in a finite population not due to genetic causes). The limit of selection intensity is set by two factors :

(i) population size, and (ii) inbreeding.

Selection differential: Difference between the mean of the population and mean of the selected individuals. Expressed in terms of standard deviation and is designated as 'S'.

Selection differential (S): S is the average superiority of selected individuals over the mean of population of their origin. It is considered in the same parental generation before selection is made. An arbitrary culling level, k (i.e. I) is fixed for a trait and individuals beyond that level are selected. The average of all such selected individuals can be designated by X. then the mean of selected individuals(X) exceeds the parental population mean by the measure of S. That is S=X-μ. Therefore, wider the phenotypic variability (i.e. phenotypic variance, and phenotypic standard deviation, that measures variability), greater is the possibility of S being large.

Heritability: In crop improvement, only the genetic component of variation is important since only this component is transmitted to the next generation. The ratio of genetic variance to the total variance i.e. phenotypic variance is known as heritability.

$H = Vg / Vp$

$Vp = Vg+Ve$ Where Vp = phenotypic variance

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Vg = genotypic variance

Ve = error variance of environmental variance

Heritability estimated from the above formula is known as the broad sense heritability. This is valid when homozygous lines are studied. But when segregating generations are studied genotypic variance consists of (a) additive variance (b) dominance variance (c) and variance due to epistasis. Dominance variance is important when we are dealing with hybrids i.e. F_1 generations. In self pollinated crops we release varieties only after making them homozygous lines. Hence additive variance is more important in such cases. The proportion of additive genetic variance to the total variance is known as narrow sense heritability. If heritability is very high for any character it can be improved. Improvement of characters with low heritability is very difficult.

Genetic Advance: Genetic advance is the difference between the mean of the selected plants in the original population and the mean of the progeny raised from the selected plants in the next generation. It can be predicted by the following formula.

Genetic advance (GA) = P x H x K

K = selection intensity 2.06 when 5% of the population is selected

P = phenotypic standard deviation of the character in the population

H = heritability in broad sense

SELECTION FOR MORE THAN ONE TRAIT

A successful cultivar is invariably selected due to desirability in a wide array of characters. Therefore selecting individuals with optimal levels of trait expression from a population usually involves simultaneous evaluation for more than a single character. When more than a single character is to be considered in a selection scheme a plant breeder can

make selection by either ***tandem selection, independent culling*** of a number of characters or by using some defined ***selection index***.

Tandem selection (or sequential single trait selection): In this mode of selection, the breeder focuses on one trait at a time (sequential improvement). One trait is selected for several generations. Once the desired level of improvement is achieved, another trait is focused on for the next period. It is effective when genetic correlation does not exist between the traits of interest or when the relative importance of each trait is not constant.

Independent cull selection: To examine this consider a simple case where there are only two traits to be included in the selection decision. If independent culling is used then the breeder will choose **target values** for each of the two characters independently. In order for a genotype to be selected, then the phenotype must exceed (or be less than, depending on the trait of interest) the target values of both of the characters simultaneously. Therefore each of the genotypes from the initial base population will fall into one of four possible categories. Which, for example if we are selecting for greater expression of both characters, will be:

- Greater than the target value set for both trait 1 and trait 2
- Greater than the target value set for trait 1 but less than the target value set for trait 2
- Less than the target value set for trait 1 but greater than the target value set for trait 2
- Less than the target value set for both trait 1 and trait 2

With this form of selection, only the genotypes which fall into category 1 (i.e. greater than both target values for each trait) would be retained, while all other categories would be discarded.

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Index selection: Index selection involves creating an equation which includes values recorded for both traits. Selection indices can be either additive or multiplicative. For example, an additive selection index for the i^{th} genotype with only two traits would be represented by:

$Ii = (w1 \times xi1) + (w2 \times xi2)$, where

Ii is the index value,

*w*1 and *w*2 are the **weights** for each variate and

*x*1 and *x*2 are the actual recorded values for each character of the i^{th} genotype.

Obviously if **n** characters were included in the index value then the index equation would be represented by:

$Ii = (w1 \times xi1) + (w2 \times xi2) + \cdots + (wn \times xin)$

The difficulty in all selection index schemes is how to determine the ***index weights*** (i.e. the *wi* s). The difference in results between index selection and independent culling are primarily related to the association between the two (or more) traits and the differences in their relative weightage. If there is good association between the traits (i.e. high expression in one trait is related to high expression in the other, and *vice versa*) and both are nearly equally valued, then there may be little difference between the genotypes selected by either method. If, however, there is poor association between trait (i.e. high expression in one trait is not related to a similar high expression in the other trait) or one character is of vital importance, then there could be a large difference in the genotypes that would be selected by index selection over independent culling.

Progeny testing

Evaluation of the worth of plants on the basis of performance of their progenies is known as **progeny test**. This was developed by Louis de Vilmorin and so it is also known as the **Vilmorin Isolation principle**.

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Vilmorin worked on sugar beet plants. The progeny test serves two valuable function;

1. Determines the breeding behaviour of a plant i.e. whether it is homozygous or heterozygous.
2. Whether the character for which the plant was selected is heritable i.e. is due to genotype or not. Selections have to be based or phenotype and so it is necessary to know the genotype of the selected plant.

ERRORS IN SELECTION

Whenever selection is applied, there is a chance that an error will occur. Errors in selection occur because the true genotype value is masked by environmental effects or because of administrative or clerical error.

a. Rejected in the first year and selected in the second year
b. Selected in the both years
c. Selected in the first year but rejected in the second year
d. Rejected in both years

Type I error:
Where genotypes have been rejected in the first year and selected in the second. If the proportion of genotypes selected in year 1 is *p1, then the Type* I error is calculated by *c/(c+b).*

Type II error:
Where genotypes are selected in the first year but rejected in the second year. If the proportion of genotypes selected in year 2 is *p2, then the*Type II error is given by *a/(a + b).*

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Course Name	Principles of Plant Breeding
Lesson 8	Hybridization
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University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the genetic basis of hybridization
2. Comparative analysis of various situations of hybridization based on parental contribution

Glossary of terms

Hybridization: Hybridization refers to crossing of two genetically unrelated plants or lines of dissimilar genotype

Intervarietal Hybridization: The parents involved in hybridization belong to the same species; they may be two strains, varieties or races of the same species. It is also known as intraspecific hybridization.

Biparental Cross: In a simple cross, two parents are crossed to produce the F_1. The F_1 is selfed to produce F2 or is used in a backcross programme.

Transgressive segregants: Plants in F_2 or advanced segregating generations that are superior to either parent.

Bottleneck: An inherent weakness of a genotype that can be corrected by crossing to an appropriate trait source

HYBRIDIZATION

Selection procedures such as mass and pure line selection are used when the variability present in base population is adequate enough. When the variability is not adequate in a population for selection to be effective, there is a need to create variability. A simple and effective way of creating variability for the traits for which improvement is sought is to cross two parents having desirable combinations of traits and then selecting among the segregating generations for desirable combinations of traits. Hybridization refers to crossing of two genetically unrelated plants or lines of dissimilar genotype. It is accomplished by planned crossing of

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parents identified on the basis of objectives. The term cross is often used to denote the products of hybridization, i.e. the F_1 as well as the segregating generations. In hybridization, which can clearly be differentiated from hybrids, the finished products are usually retrieved from segregating generations whereas the hybrids are the first generation of a cross between two genetically unrelated plants such as inbreds, varieties or populations.

GENETIC BASIS OF HYBRIDISATION IN SELF POLLINATED CROPS

The basic genetic considerations in hybridization in self-pollinated crops are:

1. Gene recombination in segregating generations: taking gene as a unit of inheritance, there are three factors that affect gene recombination through hybridization:

A. *Number of genes differentiating the parents:* This determines the number of different genotypes derived from the cross. The different hybrid combinations that have to be handled in F2 and the implications on the smallest population size are derived as follows:

Number of allelic pairs	**Gametes possible in F_1**	**Genotypes possible in F_2**	**Smallest perfect population size in F_2**	**Kinds of phenotypes in F_2**	
				Dominance	**No dominance**
1	2	3	4	2	3
2	4	9	16	4	9
3	8	27	64	8	27
10	1024	59049	10874576	1024	59049

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21	20971452	10460353203	10460353203	-	-
n	**2^n**	**3^n**	**4^n**	**2^n**	**3^n**

The above formula complicates the situation for plant breeder in terms of the population he has to handle in F2. With 21 different loci in parents, in case of rice, the space required to handle 4 trillion plants will be millions of acres in a pedigree, bulk or SSD method. However, with backcross, the situation is much less complex, with possible genotype number reduced to 20 million. Therefore, in case of parents, divergent for large number of alleles, it is better to go for
backcross, in a situation if most of the desired traits are concentrated in one parent.

B. Number of alleles at each locus: Which further complicates the situation if a trait is multiallelic.

Number of heterozygous loci	Number of alleles	**Genotypes possible in F_2**
1	2	3
2	4	9
n	2^n	3^n

C. Linkage: Linkage, when present will favour linked loci thereby promoting frequency of parental types at the expense of recombinants. However, it does not influence the rate of attainment of homozygosity, but does affect the proportion of homozygous individuals by reducing value of **"n"** in the formula **{(2m-1)/2m}n**. moreover, different homozygotes will not be in equal proportion, those involving linked loci will be more frequent. It acts as a conservative force that tends to retain existing gene combinations. It is highly desirable if the desirable genes

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are linked but delays breeding progress if the desirable and undesirable genes are linked. Natural selection favours certain gene combinations especially in case of housekeeping genes that are essentially required to be together.

2. Progress of attaining homozygosity:

- F_2 is the most variable population as all the genes are segregating simultaneously, but the latter generations witness rapid attainment of homozygosity
- By F_5, with just five genes, almost 85% population will be homozygous.
- Our aim as plant breeders is to be able to pick the best homozygote, an endeavour, that is greatly influenced by number of genes, linkage relationships and population size to be handled in F_2.

3. Nature of successful gene combinations:

- The job of plant breeder is to be able to pick the best homozygote that comprises paired combinations of genes at different loci that interact well with each other.
- The successful gene combinations depend upon number of traits for which parents are differing
- The chances of isolating better trait combinations are increased with a greater number of recombination cycles under selfing following hybridization.

OBJECTIVES OF HYBRIDIZATION

1. Creation of useful genetic variation: The chief objective of hybridization is to create genetic variation. When two genotypically different plants are crossed, the genes from both the parents are brought

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together in F_1. Segregation and recombination produce many new gene combinations in F_2 and the later generations, i.e. the segregating generations. The degree of variation produced in the segregating generations would, therefore, depend on the number of heterozygous genes in the F_1. This sill, in turn, depends upon the number of the genes for which the two parents differ. If the two parents are closely related, they are likely to differ for a few genes only. But if they are not related, or are distantly related, they may differ for several, even a few hundred, genes. However, it is not likely that the two parents will ever differ for all their genes.

2. Combination of desirable traits: The hybridization aims at combination of desirable characters into a single variety by crossing different parents. These characters may be governed by oligogenes or polygenes. The intensity of the character in the new variety is either comparable to or, more generally, lower that in the parent variety from which it was transferred. In this approach, increase in the yield of a variety is obtained by correcting the weaknesses in the yield contributing traits, e.g., tiller number, grains per spike, test weight is that for disease resistance. The backcross method of breeding was designed for combination breeding, and often pedigree method also fulfils the same purpose. In combination breeding, the genetic divergence between parents is not the major consideration. What is important is that one of the parents must have in a sufficient intensity the character(s) under transfer, while the other parent is generally a popular variety.

3. Derivation of transgressive segregants: Transgressive segregants are those plants recovered from segregating generations that outperform the parents for traits under consideration. Transgressive segregation is the production of plants in an F2 or advanced generations generation that are superior to both the parents for one or more characters. Such plants are produced by an accumulation of plus or favorable genes from both the parents as a must combine well with each other, and should

preferably be genetically diverse, i.e., quite different. This way, each parent is expected to contribute different plus genes which when brought together by recombination give rise transgressive segregant. As a result, the intensity of character in the transgressive segregant, i.e., the new variety, is greater than that in either of the parents. The pedigree method of breeding and its modifications, particularly the population approach, are designed for the production of transgressive segregants.

4. Removal of bottlenecks: In the methods such as backcross the major emphasis is on transfer of such traits from adapted or semi-adapted sources of variation for traits that eliminate certain bottlenecks in otherwise adapted varieties. Therefore, in backcross breeding the selection focuses on retrieval of trait of interest while seeking to retain the maximum genetic background of the original variety.

CONSIDERATIONS IN HYBRIDISATION

1. The breeding objective: The objective is very important in a planned hybridization programme. The objective determines the choice of parents as well as choice of method to be employed.

2. The Choice of parents: Parents need to be carefully chosen on the basis of positive and negative traits as well as distribution of traits. Two parent hybridization should not be a rule as the perfect situation of equal distribution of traits is rarely existent in natural populations. In case the traits are distributed among a large number of parents, multiple crosses should be attempted to create adequate desirable variability.

3. Ability to retrieve desirable genotypes in segregating generations: Our ability to retrieve desirable segregants in segregating populations depends upon the population size and stage of selection. Selection in early generations should focus on identification of homozygotes and cull out heterozygotes which can be deceptive especially in case of

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overdominance. Small population size invariably hinders isolation of genotypes with all desirable alleles due to limitations posed by minimum population size. Mild selection should be employed at early generations and selection at F_6 will be ideal as the probability of identification is enhanced, ambiguity due to heterozygotes are removed and parent-offspring relationship is good.

TYPES OF HYBRIDIZATION

The plants or lines involved in hybridization may belong to the same variety, different varieties of the same species, different species of the same genus or species from different genera. Based on the taxonomic relationship of the two parents, hybridization may be classified into two broad groups namely intervarietal and distant hybridization.

A. Intervarietal Hybridization: The parents involved in hybridization belong to the same species; they may be two strains, varieties or races of the same species. It is also known as intraspecific hybridization. In crop improvement programmes, intervarietal hybridization is the most commonly used. In fact, it is so common that it may often appear to be the only form of hybridization used in crop improvement. an example would be crossing of two varieties of wheat, rice or some other crop. The intervarietal crosses may be simple or complex depending upon the number of parents involved.

Simple biparental Cross: In a simple cross, two parents are crossed to produce the F_1. The F_1 is selfed to produce F_2 or is used in a backcross programme.

Complex Cross: in a complex cross, more than two parents are crossed to produce the hybrid, which is then used to produce F_2 or is used in a backcross. Such a cross is also known as convergent cross because this crossing programme aims at converging, i.e., bringing together, genes

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from several parents into a single hybrid. A few examples of convergent cross are described as:

Three Parents (A, B, C)
A X B
F_1 (A X B) X C
Complex hybrid (A X B) X C

Four Parents (A, B, C, D)
A X B C X D
F_1 (A X B) X (C X D)
Complex hybrid (A X B) X (C X D)

Eight Parents (A, B, C, D, E, F, G, H)
A X B C X D E X F G X H
F_1 (A X B) X (C X D) (E X F) X (G X H)
[(A X B) X (C X D)] X [(E X F) X (G X H)]
Complex hybrid [(A X B) X (C X D)] X [(E X F) X (G X H)]

B. Distant Hybridization: Distant hybridization includes crosses between different species of the same genus or of different genera. When two species of the same genus are crossed, it is known as interspecific hybridization; but when they belong to two different genera; it is termed as intergeneric hybridization. Generally, the objective of such crosses is to transfer one or few simply inherited characters like disease resistance to a crop species. Sometimes, interspecific
hybridization may be used for developing a new variety, e.g., Clinton oat variety was developed from a cross between *Avena sativa* x *A. byzantina* (both hexaploid oat species), and CO 31 rice variety was developed from the cross *Oryza sativa* var. indica x *O. perennis*. Almost al the present-day sugarcane varieties have been developed from complex crosses between *Saccharum officinarum* (noble canes), *S. barberi* (Indian canes) and other *Saccharum* species, e.g., *S. spontaneum* (Kans.). The improvement in

fiber length of Indian Cotton (*Gossypium arboreum)* has been brought about by crossing it with American cultivated Cotton; many improved varieties have resulted from such crosses. Intergeneric hybridization may also be used to develop a new crop species, e.g., **Triticale** from a cross between *Triticum* sp. and *Secale cereale* (rye). Wild species often provide genes which are not present in the cultivated species. For example, many of the genes for rust resistance in wheat are derived from related wild species. Distant hybridization is likely to become increasingly important in the correction of specific defects of crop species. In many cases, wild species may contribute valuable 'yield genes' as well to the cultivated species.

CONSIDERATIONS IN PARENTAL COMBINATIONS & HANDLING OF SEGREGATING GENERATIONS

The choice of parents in a hybridization programme is determined by the distribution of desired traits among the prospective parents. In case of **adapted x adapted** cross where two parents contribute equally in terms of trait (**50:50 situation**), a normal pedigree/bulk/Single seed descent method will be effective. In case the contribution of parents towards trait improvement is skewed (**75:25 situation**), it is better to go for one backcross of F1 to the better parent in order to retrieve 75% from the better parent and then follow normal pedigree method. In case the trait contribution is provided by more than two parents, complex crosses involving three, four, six or eight parents should be employed in a convergent crossing scheme where trait contributions are proportionately derived from the parents. In case of an extremely skewed situations (**99:1 situation**), which is usually found in case of **adapted x unadapted** crosses, backcross involving repeated crossing of F1 to adapted parent is adopted to reconstitute the adapted parent (**background selection**) as well as derive the trait of interest from unadapted parent (**foreground selection**).

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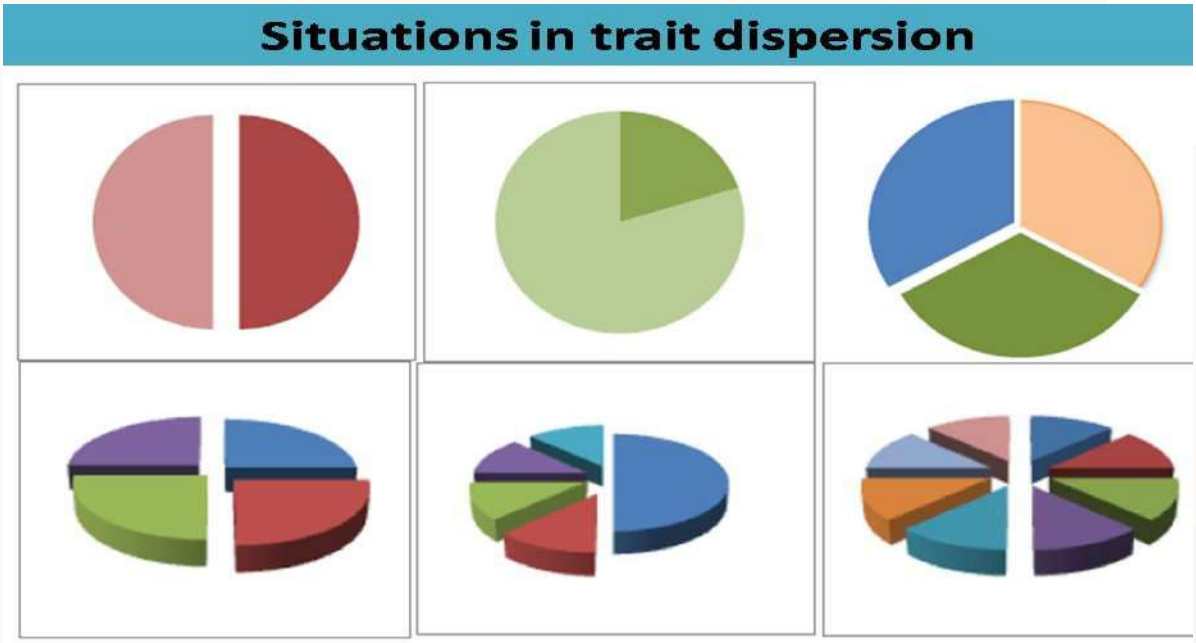
Fig 8.1: Different situations of parental combinations



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Course Name	Principles of Plant Breeding
Lesson 9	Mass Selection
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the procedure of Mass selection
2. Know the applications, merits and demerits of mass selection

Glossary of terms

Bulking: mixing of seeds of selected lines

Land race: A variety that is cultivated and maintained by farmers over a long period of time

Broad based population: A population which has a broad genetic base

MASS SELECTION

It is the earliest method of selection. Man has always practiced mass selection consciously or unconciously from the time of domestication. In its most basic form mass selection consists of selecting individuals on the basis of phenotypic superiority and mixing the seeds for using as base material for next cycle of selection. The process is repeated upto seven generations to achieve fair amount of homozygosity as well as nullify the environmental influences. In recent
times, mass selection has been modified in several ways to enhance the efficiency of selection.

Requirements of Mass selection

1. Genetically broad-based population
2. Simply inherited traits
3. High heritability of the traits under selection
4. Low G x E interaction
5. No negative associations between traits

Procedure for evolving variety by mass selection

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First year: A large number of phenotypically superior with more or less uniform expression of desirable characters are selected. The number may vary from few hundred to few thousand. The seeds from the selected plants are composited to raise the next generation.

Second year : Composited plant progenies should be planted in a preliminary field trial along with standard checks as well as the original variety to assess the gain from selection. Phenotypic characteristics of the variety are critically examined and evaluated.

Third to sixth year : The variety is evaluated in coordinated yield trials at several locations. It is evaluated in an initial evaluation (IET) trial for one year. If found superior it is promoted to main yield trials for 2 or 3 years.

Seventh year : If the variety is proved superior in main yield trials it is multiplied and released after giving a suitable name.

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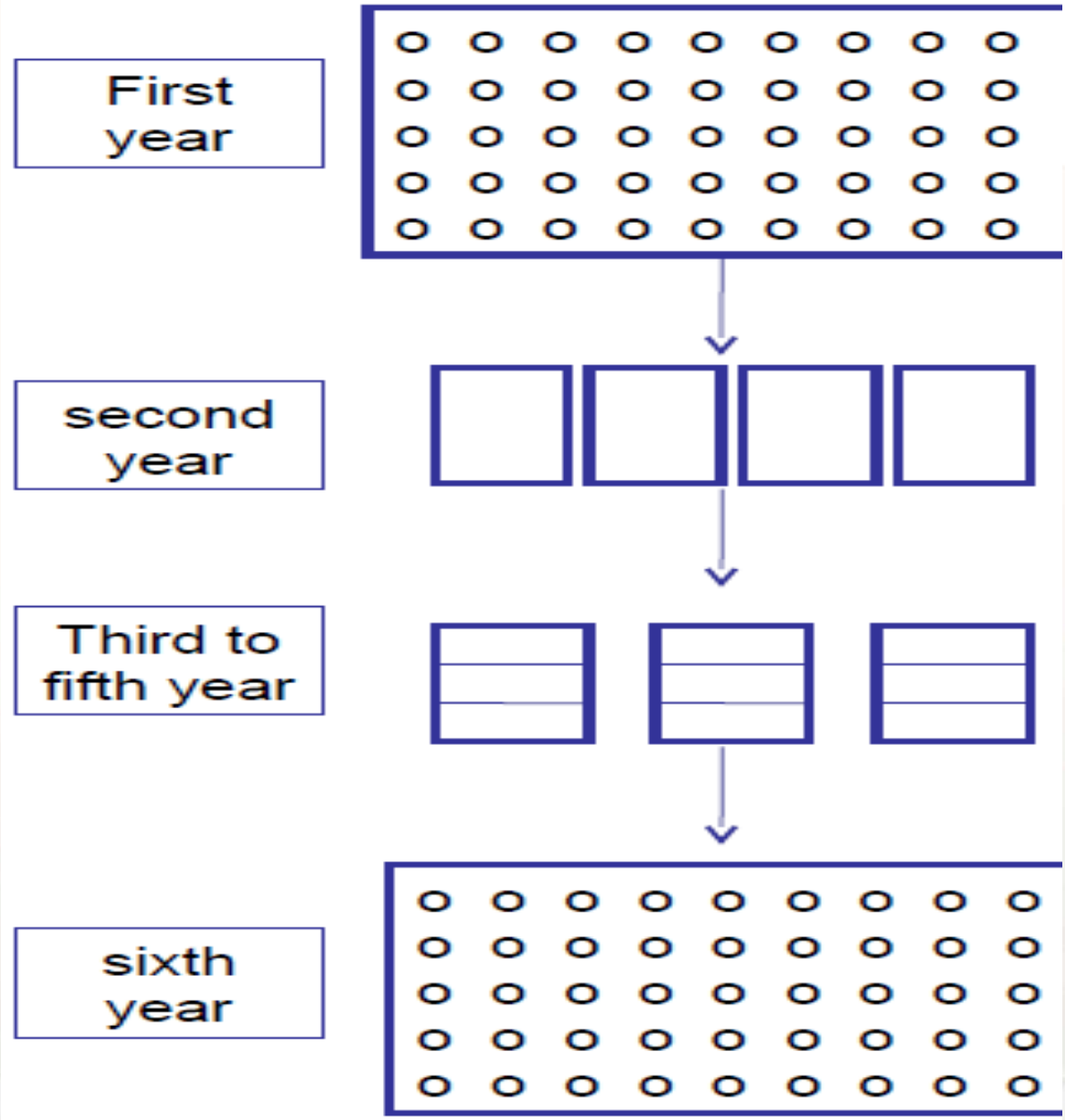

Fig. 9.1. Procedure of mass selection

Applications

1. Purification of released varieties
2. Maintenance of released varieties
3. Development of new varieties from a variable population

Merits of Mass selection:

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1. It is the simplest method and can be practiced both in self- and cross-pollinated crops
2. The varieties developed through mass selection are more widely adapted as they are fairly heterogenous.
3. It retains considerable variability and hence further improvement is possible in future by selection
4. Helps in preservation of land races
5. Useful for purification of pure line varieties
6. Improvement of characters governed by few genes with high heritability is possible.
7. Less time consuming and less expensive.

Demerits of mass selection

1. Since the selected progenies are bulked, the varieties are not uniform
2. Since no progeny test is done, the genotype of the selected plant is not known, whether it is homozygous or heterozygous. This is especially important in case of overdominance, where heterozygotes can be deceptive and upon segregation, will necessitate further selection.
3. Since selection is based on phenotype it is not sure that whether the performance is because of genetic potential or environmental effect.
4. Characters which are governed by large number of genes with low heritability cannot be improved efficiently.
5. It cannot create any new genotype but utilizes existing genetic variability.

Mass selection with Progeny test

Mass selection is usually carried out in a broad-based variable population without any progeny test. However, Allard (1960) has advocated that progeny test should be used in mass selection. This will help in removing poor plants that were selected die to selection errors as well as remove the bias caused by heterozygosity and G x E interaction. The efficiency of

progeny test in mass selection in overcoming selection errors can be explained by following table.

Selected plant				Rejected plant			
Plant in a progeny row	**P**	**G**	**E**	**Plant**	**P**	**G**	**E**
1	500	450	+50	1	100	150	50
2	350	450	-100	2	250	150	-100
3	400	450	-50	3	200	150	-50
4	490	450	+40	4	110	150	40
5	420	450	-30	5	180	150	-30
6	510	450	+60	6	90	150	60
MEAN	**445**	**450**	**-5**	MEAN	**155**	**150**	**-5**

The above table clearly shows how the progeny row evaluation of selected plants helps in minimizing G x E interaction. This is achieved by harvesting the best plants separately and to grow and compare their progenies. The poorer progenies are destroyed and the seeds of the remainder are harvested. Thus, the selection is not based on the phenotype of the selected plants but also on the performance of their progeny. Progeny selection is usually more effective than usual phenotypic selection especially for quantitative characters of low heritability. However, progeny testing requires an extra generation; hence gain per cycle of selection must be double that of simple phenotypic selection to achieve the same rate of gain per unit time.

Varieties developed by Mass selection in Horticultural crops

Bittergourd: Pusa Mousami, Priyanka, Konkan Tara, Arka Harit and Pusa Vishesh.

Muskmelon: Arka Jeet, Arka Rajhans and MH1

Cauliflower: Pusa Ageti, Pusa Drum Head, Golden Acre, Pusa Mukta, KGMR-1

Raddish: ArkaNishant

Shallot: Arka Kalyan, Arka Niketan

Garlic: Agrifound White, Yamuna Safed, Yamuna Safed 2 and Yamuna Safed 3

Amaranth; CO1, CO2, CO3, CO4

Coriander: Arka Isha

Capsicum: Arka Basant, Arka Gaurav, Arka Mohini

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Course Name	Principles of Plant Breeding
Lesson 10	Pureline Selection Method
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the procedure of pureline selection
2. Know the applications, merits and demerits of pureline selection

Glossary of terms

Pureline: Line derived by continuous selfing of a single self pollinated plant
Vulnerability: Increased susceptibility of purelines to diseases and pests
Progeny selection: A method in which plants are selected from a base population based on the performance of progeny of selected plants
Pureline theory: A theory that states that variation within purelines is not heritable but environmental
Dead end: A situation where further improvement in a line is not possible due to its extremely narrow genetic base as in purelines

JOHANNSEN'S PURELINE THEORY

Johannsen (1903) made his studies with beans, selecting this plant because it belongs to the self -pollinated group and contains characters that are easy to measure. Johannesen bought 16,000 seeds of "Princess" variety in 1901 and took 150 seeds of different sizes out of which 100 represented the average weight of whole lot and 25 represented smallest and 25 largest beans. The offsprings of largest and smallest beans deviated from the mean in the same direction as expected by Galton's law of regression.
He defined pure line as progeny of a single homozygous plant produced through self-fertilization. So, in 1901 crop season, he identified 19 plants, each of which originated from single carefully measured bean in original seed lot. In this way, 19 pure lines with difference for seed weight, were established. The seed of each of 19 plants was divided into different 'sublines' or weight classes of 10 cg size. The offsprings from each sub-

line were raised in 1902 crop season and seed weight of offsprings of each weight class or subline was again averaged.

Johannesen hypothesized that differences within pure lines are due to environment and would thus not be utilized by selection. So, the seed of 60 cg and 70 cg from 1902 crop has same genetic constitution and difference of 10 cg is due to environment as a result of which the progeny from both of these is very similar i.e., a difference of only 1.70 cg. So, irrespective of the size of difference in parental seed even for six generations, the progeny mean did not deviate from the characteristic mean of 64 cg of the line. In addition, he demonstrated the differences in the kind of variability present between and within purelines through parent-offspring correlations. Johannesen's experiments clearly demonstrated that:

1. Continuous inbreeding in self-pollinated crops leads to homozygosity. A mixture of pure lines includes genetic variation and thus responds to selection in the desired direction.
2. The progeny of different individuals of the pure line tends to congregate around the line mean because differences among individuals of pure line are only due to environment and not genetic factors.
3. Once a pure line has been identified further selection within the line will not lead to more improvement because of lack of hereditary variation within the pure lines due to exactly same genotype of all individuals in a pure line.
4. The observed performance (Phenotype of an individual) is the joint contribution of genotype and environment which makes a difference of phenotype from genotype. The progeny test is critical tool to differentiate between genetic and environmental variation.

GENETIC BASIS OF PURE LINE

Self-pollination increases homozygosity with a corresponding decrease in heterozygosity. The effect of homozygosity and heterozygosity may be

illustrated by taking an individual heterozygous (Aa) for a single gene. Suppose an individual heterozygous for a single gene (Aa) and the successive generations derived from it are subjected to self-pollination. Every generation of self -pollination will reduce the frequency of heterozygote Aa to 50 per cent of that in the previous generation. There is a corresponding increase in the frequency of the two homozygotes AA and aa. As a result, after 10 generations of selfing, virtually all the plants in the population would be homozygous, *i.e.,* AA and aa. On the other hand, the frequency of heterozygote Aa would be only 0.097 per cent, which is negligible. It is assumed here that the three genotypes AA, Aa and aa have equal survival. If there is unequal survival, it may increase or decrease the rate at which homozygosity is achieved. If Aa is favoured, the rate of increase in homozygosity
would be lower than expected. But if Aa is selected against, homozygosity would increase at a faster rate than expected.

No. of generations of selfing	**Frequency of different genotypes**			**Frequency of homo- and heterozygotes**	
	AA	**Aa**	**Aa**	**Homozygotes**	**Heterozygotes**
1	0	100	0	0	100
2	25	50	25	50	50
3	37.5	25	37.5	75	25
4	43.75	12.5	43.75	87.5	12.5
5	46.875	6.25	46.875	93.73	6.25
6	48.437	3.125	48.437	96.87	3.125
7	49.218	1.562	49.218	98.436	1.562
8	49.608	0.781	49.608	99.216	0.781
9	49.803	0.39	49.803	99.606	0.39
N	**½** $[(2^m - 1) / 2^m]^n$	**1-** $[(2^m - 1) / 2^m]^n$	**½** $[(2^m - 1) / 2^m]^n$	$[(2^m - 1) / 2^m]^n$	**1-** $[(2^m - 1) / 2^m]^n$

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m = No. of generations of self-pollination
n = No. of genes segregating

Origin of variation in pure lines

Pure lines show genetic variation after some time because of the following reasons.

1. **Mechanical Mixture**: During cultivation, harvesting threshing and storage, other genotypes may get mixed up.

2. **Natural hybridization**: Through pure lines are produced in self pollinated crops, some amount of natural cross pollination occurs in them also can be avoided by isolation and rouging.

3. **Mutation**: occur spontaneously in nature at random

Characteristics of purelines

1. All the plants within a pureline have the same genotype
2. The variation within a pureline is environmental and non-heritable
3. Purelines are stable

General procedure for evolving a variety by pureline selection

Pureline selection has been the most commonly used method of improvement of self pollinated crops. Almost all the present-day varieties of self-pollinated crops are purelines. Pureline selection has several applications in improvement of self-pollinated crops. It is used to improve:

1. Local varieties
2. Old pureline varieties and
3. Introduced varieties

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The pureline selection has three steps.

1. Selection of individual plants from a local variety or some other mixed population.
2. Visual evaluation of individual plant progenies and
3. Yield trials

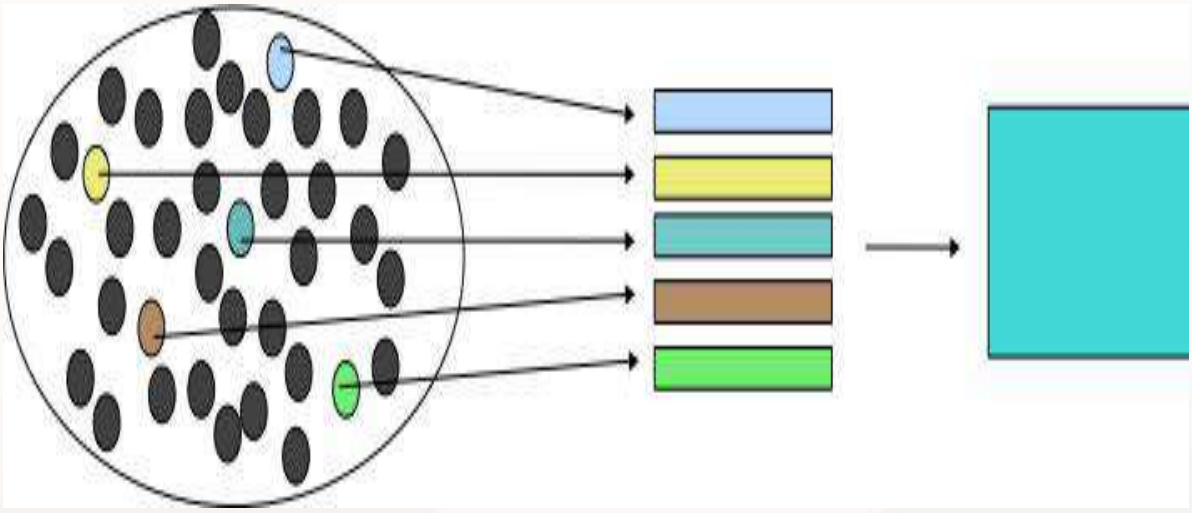

Fig.10.1. General outline of pureline method

PROCEDURE

1. Selection

First year: A large number of plants (200-3000) which are superior to the rest are selected from a local variety or mixed population and harvested separately (in some cases individual heads or stems may be selected). The number of plants to be selected depends upon the breeder's discretion but should be as large as possible in view of the available time, land, funds, labour etc. It is advisable to select for easily observable characters such as flowering, maturity, disease resistance, plant height etc.

II. Evaluation :

Second year: Progenies of individual plants selected in 1st year are grown separately with proper spacing (plant to row or head to row). The

progenies are evaluated by taking elaborate date on visual characters such as plant height, duration, grain type, ear characters besides yield. The number of progenies should be reduced as much as possible. Disease epiphytotic may be created to test the progenies for disease resistance, poor, weak, diseased, insect attacked and segregating progenies are rejected. The superior progenies are harvested separately. If necessary the process may be repeated for one or more years.

III. Yield trials :

Third year: The selected progenies, now called as cultures are grown in replicated trial for critical evaluation of yield etc. The best local variety is used as a check and should be grown at regular intervals, after every 15 or 20 cultures for comparison. This is known as preliminary yield trial. Superior cultures based on observable characters and yield is selected. The number is drastically reduced.

Fourth & Fifth years: The superior cultures are tested against the local checks in yield trials. Observations are recorded on many characters like diseases resistance, days to flower, days to maturity, and height of the plant ear characters, test weight and yield. The data is subjected to statistical analysis to identify really superior cultures. If necessary the trials may be extended for one more year or season. Inferior culture are rejected and a few (4-5) promising cultures are
selected.

Sixth, Seventh and Eighth years: The promising cultures selected are evaluated at several locations along with strains or cultures of other breeders and local checks. One or two promising cultures are selected.

Ninth year: The best progeny identified earlier is multiplied, named and released as a variety for official release of any variety (approval from the variety releasing committee of the state or central is necessary).

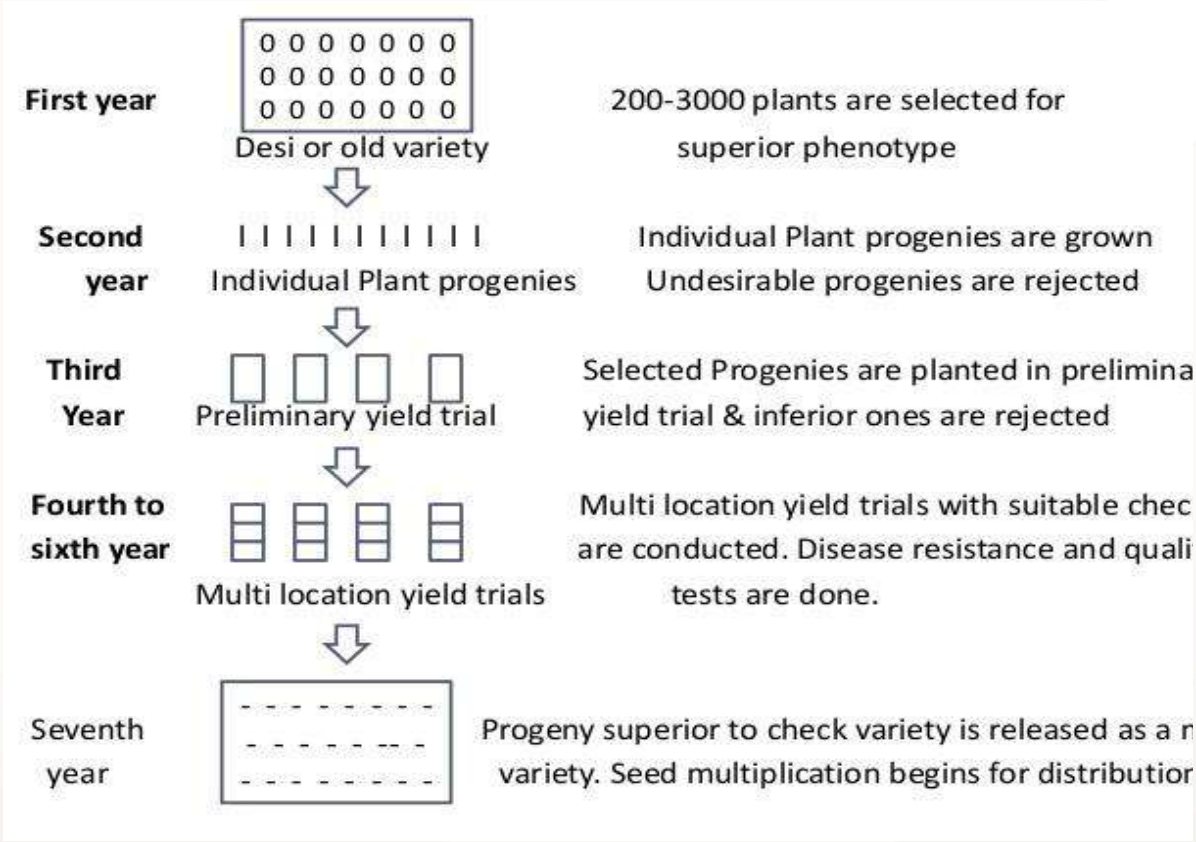

Fig. 9.3: Schematic representation of pureline method

Advantage of pureline selection

1. The purelines are extremely uniform since all the plants in the variety will have the same genotype.
2. Attractive and liked by the farmers and consumers.
3. Purelines are stable and long test for many years.
4. Due to its extreme uniformity the variety can be easily identified in seed certification programmes.

Disadvantages of pureline selection

1. New genotypes are not created by pureline selection
2. Improvement is limited to the isolation of the best genotype present in population. No more improvement is possible after isolation of the best available genotype in the population.
3. Selection of purelines require great skill and familiarity with the

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crop.

4. Difficult to detect small differences that exist between cultures
5. The breeder has to devote more time
6. Pure lines have limited adaptability hence can be recommended for cultivation in limited area only.

Differences between Mass and Pureline selections

Particulars	**Mass selection**	**Pureline selection**
Nature	More of art	More of science
Crops	Used both in self and cross pollinated crops	Practiced in self-pollinated crops only
Number of plants Selected	Large number of plants are selected	Comparatively less number of plants are selected
Handling selected plants	The produce of the selected plants is mixed and sown as such in next year	Produce of individual plants is kept separate and progeny rows are raised next year
Pollination control	No control of pollination	Pollination is controlled
Nature of product	Variety developed is heterozygous and not uniform	Variety is homozygous, homogeneous and uniform
Stability of variety	Due to heterozygosity the variety deteriorates quickly	Due to homozygosity the variety lasts long
Adaptability	Wider adaptability due to heterozygosity	Narrow adaptability due to homozygosity
Scope for further improvement	Selection within a variety is effective	Selection within a pureline variety is not effective

Vulnerability to diseases	Less vulnerability to diseases	Greater vulnerability to Diseases
Testing of progeny	No progeny test	Progeny as well as individual plants tested
Repetition of process	To be repeated each 2-3 years for purification	No need for repetition

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Course Name	Principles of Plant Breeding
Lesson 11	Pedigree Selection Method
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the procedure of pedigree selection
2. Know the applications, merits and demerits of pedigree selection

Glossary of terms

Pedigree: Details of all parents, grandparents, etc., of a particular genotype are known back to natural population

Progeny bulks: Bulk seed of all the selected lines/families from F_2 onwards

Correspondence: The relation between performance of parents and progenies

Early generation selection: Selection in F_2 or F_3 generation in a pedigree breeding programme

Trait dispersion: The amount of distribution of desirable traits in parent in a pedigree breeding programme

Pedigree Method

In the pedigree method, individual plants are selected from F_2 and subsequent generations, and their progenies are tested. During the entire operation a record of all parent off-spring relationships is kept. This is known as pedigree record. Individual plant selection is continued till the progenies show no segregation. At this stage the selection is done among the progenies, multilocation tests are conducted and the selected genotypes released as varieties. The pedigree may be defined as a description of the ancestors of an individual and it generally goes back to some distant ancestors. It is useful to know the relationship of two individuals and useful for selection of parents and prediction of outcome of the cross. The method was first proposed by Vilmorin in France in the

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1830's while as Institute at Svalof used pedigree type breeding in the 1880's and later on H. H. Lowe used it in in 1927.

Procedure of pedigree method

1st year: A cross is made between the parents possessing desirable characters.

2nd year: All the F_1 seed is space planted for optimal expression so that each F_1 plant produces enough seeds. Raise as many F_1 plants as possible to produce large number of F_2 seeds. Harvest in bulk.

3rd year: Grow 2000-10000 plants of F_2 (based on crop and its spacing) giving wide spacing for full expression of the characters in F_2 generation plants. Grow parents also for comparison. Depending upon the facilities and objectives of the programme about 100-500 superior plants are selected. The selection in F_2 can be done for simply inherited characters like head type disease resistance etc. and selection for characters governed by many genes like yield will should be reserved for later generations. The selected plants are harvested separately and given serial numbers and description entered in pedigree registers.

4th year: Progeny rows of F_3 i.e. seeds of selected F_2 plants in one row are space planted along with parents and checks. From superior progeny rows, individual plants with desirable characters are selected (about 50-100 families and about 5 plants in each family are harvested separately). Diseased, lodging and other undesirable progenies are discarded.

5th year: F_4 plants raised again as head to row. Desirable plants are selected from desirable rows and harvested separately.

6th year: F_5 plants raised in 3 row plots i.e. seeds of each selected plant sown in 3 rows. By this time many families might have become fairly homozygous. For comparison, check variety is grown for every 3 or 5

block. Progenies are evaluated for yield and the inferior ones are rejected. The number should be reduced to 25- 50. Superior plants from superior progenies are selected. Plants from each progeny are bulked.

7th year: F_6 individual plant progenies are grown in multi-row plots and evaluated. Inferior progenies are rejected and superior progenies are selected. Plants of each progeny are harvested in bulk. Diseased and inferior plants from the progenies are removed.

8th year: F_7 preliminary yield trial with 3 or more replications are conducted to identify superior lines. The progenies are evaluated for many characters including yield. Standard commercial varieties must be included as checks. Two to five outstanding lines are selected and advanced to coordinated yield trials.

9th, 10th & 11th year: Selected lines are tested in several localities for 2 or 3 years for adaptation tests. Lines are evaluated for all characters mainly yield and disease resistance. A line that is superior to commercial variety in yield and other characters is selected

11th and 12th year: Selected superior lines is named, multiplied and released as a new variety. Number of year can be reduced if generations are advanced during off seasons either in green house or under irrigated conditions. Several modifications for the above described pedigree method are followed by breeders depending upon the crop, time and availability of funds and facilities like labour, land etc.

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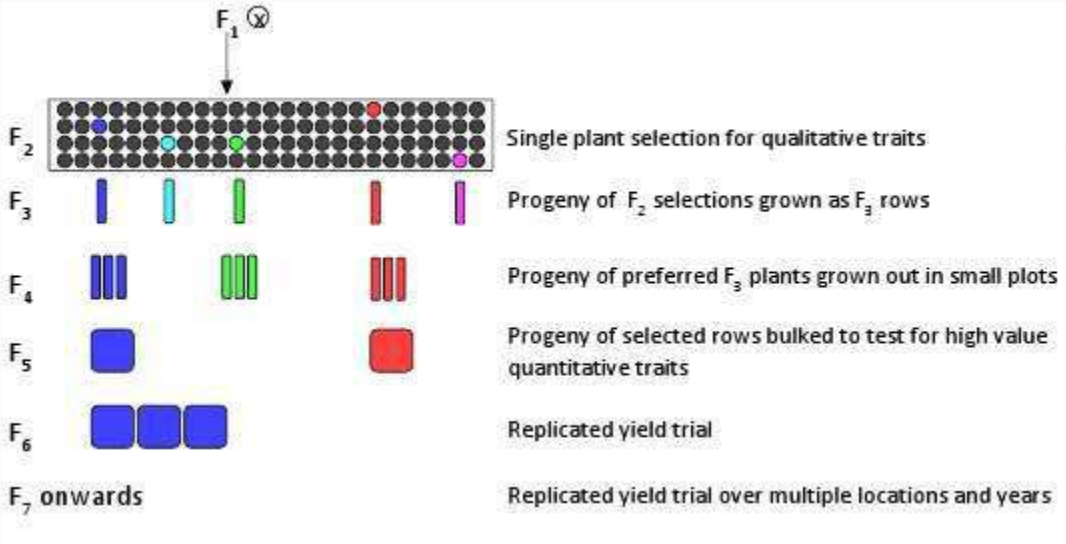

Fig. 10.3.General scheme of pedigree method

Early generation tests:

The objective of this test is to find out superior crosses and superior progenies in early generations i.e. in F_2 and F_3. We need not advance all the crosses and all selected progenies in each cross up to F_8. Much labour, time and cost would be saved by this early generation testing. A more reliable information about the potential crosses and progenies may be obtained by conducting replicated tests (preferably in more location) and evaluating them for yield and other characters in F_2 or F_3 itself. A desirable cross or progeny should have high mean yield, high genetic variance and high expected genetic advance under selection. Other crosses and progenies are rejected in the beginning i.e. F_2 and F_3 generations itself.

F_2 progeny testing: Another modification for pedigree method. In F_2 make as many single plants selections as possible. From F_3 to F_6 advance the progenies in bulk making selections of the progenies as a whole and discarding the inferior progenies. Thus, each of the progeny is derived from the single plant selected in F_2 generation. In F_6 make single plant selections in each of the progeny. Compare the yields of the single plants with progenies from which they are selected. Select superior single plant

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progenies and advance to preliminary yield trials, multi-location tests etc. There are two advantages

1. No. of crosses can be handled simultaneously
2. Natural selection operates from F_3 to F_6 since they are advanced in bulk.

Early generation selection v/s advanced generation selection

Selection in the early generation stages differs from later selection because:

- Many thousands of lines are to be screened
- Each plant in F_2 is unique and so experimental designs with large numbers of plots and high replication are not possible
- Selection is often carried out on highly heterozygous populations where dominance effects can be large and can mask the true genotype being selected.
- In case of overdominance, the selected plants can be highly deceptive.

Efficiency of early generation selection

The efficiency of early generation selection has been examined in a number of different crops. When breeding an autogamous species, for example pea or beans, selection will be influenced by the highly heterozygous nature of the breeding lines in the early generations. Segregation effects can be avoided by advancing towards homozygosity prior to selection but has not been common in the past because of time restraints or cost factors. Visual assessment of yield and
yield components have been examined and visual evaluation of yield from single rows or small plots has proved unreliable in predicting actual yield in subsequent generations. The highest yielding progeny bulks, derived from F_2 and F_3 single plants, do not necessarily produce the highest yielding segregants. Visual selection for yield on individual plants results in only a random reduction in population size with little or no

effect in increasing yield. Even when the actual yield of an early generation of a cereal pedigree bulk breeding scheme (say F_2 or F_3*)* was measured and it was found to be significantly correlated with yield. In later generations (say F_5 or F_6*)* the association found between segregating populations was usually so poor that it was questionable whether selection at the early stages (along with the expense that this would incur) would be justified.

Selection for yield *per se* in the early segregating generations of other inbreeding species has also been shown to produce an effect which is no better than random. Examples from past research include chickpea, cotton, soybean and rice. In addition selection for yield components such as seed size in chickpea and grains per ear in spring barley was shown to be slightly more effective in the early generations than selection for yield itself. The large numbers and small plots used in the early generations dictates that selection is only carried out for characters which are highly heritable. Often these only include single gene traits. As might be expected, selection for qualitative disease resistance in the early generations has been found to be more effective than selection for quantitatively inherited resistance or other polygenic characters. The efficiency of selection in a pedigree bulk breeding scheme has been related to the heterozygosity of the bulks under selection. As homozygosity increases, selection becomes more effective. Homozygosity can be accelerated by single seed descent. However, care is needed to ensure that in single seed descent there is not a non-random loss of genetic material. Homozygosity can also be accelerated by various doubled haploid techniques. Again however, care must be taken in the use of these procedures as there is evidence of non-random success and a strong genotypic response to *in vitro* regeneration.

Application of Pedigree Method:

1) Selection of desirable plants from the segregating population in self- pollinated crops.

2) This method is commonly used to correct some specific weaknesses of an established variety (Combination breeding).
3) It is also used in the selection of new superior recombinant type's i.e Transgressive breeding.
4) This method is suitable for improving specific characteristics such as disease resistant, plant height, maturity etc.

Advantages of the pedigree method

- It provides maximum opportunity for the breeder to use his skill and judgement in selection of plants. Inferior genotypes can be discarded early in the process, allowing the use of higher volumes for early generations in the programme, and retaining only the good ones for the later, expensive, replicated experiments stage.
- It is well suited for the improvement of characters which can be easily identified and are simply inherited.
- Different locations and environments can be used in each growing cycle, allowing selection for different traits not expressed everywhere. Populations can be replicated in hot spots, i.e. environments where the desired selection traits are expressed at a maximum, to increase the probability of selecting for specific traits.
- The breeder can manage the amount of genetic variance they want to keep within and between families, as well as the number of families.
- Different qualitative and quantitative traits can be selected at the same time in different generations, including traits being expressed at plant as well as grain level
- Transgressive segregation for yield and other quantitative characters may be recovered.

Disadvantages of the pedigree method

- Cannot be utilized in environments where genetic variability for the characters of interest is not expressed. If one cannot use off-season nurseries, there will be an associated increase in the length of time

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for cultivar development compared with other methods of breeding.

- Valuable genotypes may be lost in early generations, if sufficient skill and knowledge are lacking in the breeder, at the time of selection.
- No opportunity for natural selection
- Difficult to handle large number of crosses
- Maintenance of records, selections, growing progeny rows etc. are time consuming and laborious. An experienced person must do the selection (at least we flatter ourselves in thinking so).
- Requires more land and labour than other methods of inbreeding.

Expected additive genetic variability among and within lines during inbreeding process without any selection

Generation of lines	**Additive Genetic Variability**	
	Among Lines	Within Lines
F2:3	**1**	**1/2**
F3:4	**1.5**	**1/4**
F4:5	**1.75**	**1/8**
F5:6	**1.875**	**1/16**

Table 10.2: Genotypic frequency of populations under selfing in pedigree method

Generation	**AA**	**Aa**	**aa**	**Homozygotes (%)**
F1	0	1	0	0
F2	0.25	0.50	0.25	50
F3	0.375	0.25	0.375	75
F4	0.437	0.125	0.437	87.50
F5	0.4688	0.0625	0.4688	93.75
F6	0.4844	0.0312	0.4844	96.88
F7	0.4922	0.0156	0.4922	98.44
F8	0.4961	0.0078	0.4961	99.22

F_n	$2^{m-1}/2^{m+1}$	$1/2^m$	$2^{m-1}/2^{m+1}$	$2^{m-1}/2^m$

Varieties Released by Pedigree Method

Rice: Krishna, Ratna, Sabarmati, Padma, Jaya, Bala, Kaveri, etc.

Wheat: HD 2281, HD 2285, HD 2380, ND 2402, Janak, Pratap, Raj 2535 DWR 39, WH 331, WL 616, etc.

Cotton: LH 900, LH 1556, F 846, F 1054, F 1378, HS 6, H 1098, RST 9, Vikas, Khandwa 3, Sharda, Abhadita, Sahana, MCU 9, MCU 11, LRA 5166, Anjali, Surabhi, Suvin etc.

Pigeon pea: T21, Prabhat

Green gram: T2, T44, T51. Sheela, etc.

Chickpea: T1, T2, T3, T5, Radhey, etc.

Pea: Pant Matar 2, Jawahar Matar 1, Jawahar Matar 4, Him Palam Matar-1, Palam Triloki etc.

Tomato: Pusa Ruby (Sioux × Improved Meeruti), Arka Ahuti

Chilli : Andhra Jyothi (G5), Pusa Jawala, Punjab Lal, Jawahar 218, K2, PKM.1, Pusa Sadabahar, Arka Suphal, Him Palam Mirch-1, Him Palam Mirch-2

Capsicum: Spartan Garnet, Spartan Emerald, Sonnette

Ridge Gourd: Swarna Uphar, Arka Sumeet, Arka Sujat

Water Melon: Durgapura Meetha, Durgapura Lal, Arka Manik

Musk Melon: Pusa Sharbati, Punjab Sunehri

Round Melon: Arka Tinda

Brinjal: Arka Unnati, Arka Keshav

Palak: Arka Anupama

Cauliflower: Arka Vimal

Guava: Arka Amulya

Papaya: Surya
Mango: Arka Puneet, Arka Neelkiran, Arka Anmol

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Course Name	Principles of Plant Breeding
Lesson 12	Backcross Method
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the operational procedure of backcross method
2. Application, merits and demerits of Backcross method

Glossary of terms

Backcrossing: A breeding method used to move one or only a few desirable genes from an agronomically poor crop line to an elite line. This is done by crossing a donor parent to an elite line, and crossing offspring with the 'desired gene(s)' back to the elite parent.

Recurrent parent: . The well adapted, high yielding variety, lacking one or two characters and hence receives these genes from other variety is called as **recurrent parent**

Non-recurrent parent: The variety which donates one or two useful genes is called as Donor parent or Non-recurrent parent.

Isogenic lines : Isogenic lines are identical in their genotype, except for one gene. Such lines are useful in studying the effects of individual genes on yield and other characteristics

Simultaneous Transfer : A backcross scheme in which genes for the different characteristics transferred simultaneously in the same backcross programme.

Stepwise Transfer : A backcross scheme in which the recurrent parent is improved sequentially for many genes or characters.

Congruent Backcrossing: Congruent backcrossing involves crossing of backcrosses are alternated with both the parents instead of one in conventional backcrossing. This is used to overcome interspecific barriers.

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Marker assisted backcross: A backcross scheme where selection for complex traits is done using molecular markers.

BACK CROSS

Pedigree and bulk methods are usually useful in case of 50:50 situation (adapted x adapted crosses), where both parents contribute to the trait almost equally. However, in many cases, such as disease resistance, male sterility etc, the variation is not present in the cultivated germplasm but is available in wild/unadapted germplasm. In such a situation (99:1), it is desired to get the trait of interest from unadapted source and reconstitute the otherwise adapted variety. The method used is called as back cross as it requires continuous backcrossing of F1 to the adapted parent to achieve the objectives of reconstitution of adapted variety as well as flushing out the unadapted parent genome baring the trait of interest.

Breeders of early 20th century engaged in the development of disease resistant varieties observed that pureline selections with genes for resistance from intra-or interspecific hybridization were inferior to the generally acceptance superior parent in yield or quality characteristic. To overcome this problem, Harlan and Pope (1922) suggested the back cross method by which an undesirable allele at a particular locus is replaced by the desirable allele in otherwise elite variety.

Back cross procedure conserves all good characteristics of a popular adapted variety and incorporates a desirable character from another variety. Back cross refers to a cross between a hybrid (F 1 or a segregating generation) and one of its parents is known as backcross. The well adapted, high yielding variety, lacking one or two characters and hence receives these genes from other variety is called as **recurrent parent** and

The variety which donates one or two useful genes is calleda as **Donor parent or Non-recurrent parent**.

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Requirements of a back cross programme

1. Existence of a good recurrent parent variety which requires improvement is some qualitatively inherited character or a quantitative character with high heritability.
2. A suitable donor parent must be available possessing the character or characters to be transferred in a highly in tense form.
3. High expressivity of the character under transfer through several back crosses in the genetic back ground of the recurrent parent.
4. The character to be transferred must have high heritability-preferably determined by one or few genes.
5. Simple testing technique for detecting the presence of the character under transfer.
6. Recovery of the recurrent genotype in a reasonable number of back cross generations.

Applications of back cross method

Backcross method is applicable to both S.P. & C.P. crops.

1. Inter varietal transfer of simply inherited characters : characters governed by one or two major genes – Eg. disease resistance, used color. Linkage drag : Failure of transfer of simply inherited characters like disease resistance by B.C. method due to a tight linkage between the gene being transferred and some other undesirable gene.
2. Inter varietal transfer of Quantitative characters : Quantitative characters with high heritability can be transferred. Eg. Early ness, Pl. height seed size, seed shape.
3. Inter specific transfer of simply inherited characters : Mostly disease resistance from related species into a cultivated species. Eg. 1. Leaf and stem rust resistance from *Triticum timopheevii T. monococcum, Aegilops*

*speltoides and rye (*S. cereale *) to T. aestivum* Black arm resistance from several *Gossypium* species to *G. hirsutum*

4. Transfer of cytoplasm : Back Cross methodin used to transfer cytoplasm from one variety or species to another. This is especial desirable in cases of Cytoplasmic or Cytoplasmic-genetic male sterility. E. Transfer of *T. timopheevii* cytoplasm to T. aesticem

5. Transressive segregation : Back cross method may be modified to obtain transgressive segregants. It may be modified is one of the following two ways.

- The F1 may be Back crossed only 1 or at most 2 times to the recurrent parent leaving much heterozygosity for transgressive segregants to appear.

- To or more recurrent parents may be used in the back cross programme to accumulate genes from them in to the back cross progeny. Such a modification of the back cross would produce a new variety that would not be exactly like any one of the recurrent parents.

6. Production of Isogenic lines : Isogenic lines are identical in their genotype, except for one gene. Such lines are useful in studying the effects of individual genes on yield and other characteristics. Isogenic lines and easily produced using the back cross method.

7. Germplasm conversion : Conversion of photosensitive germplasm lines (using as recurrent parent) to photo insensitive line (using a photo insensitive line as a donar or non-recurrent parent.

Situations of trait transfer under backcrossing

A. Transfer of a Dominant Gene: Let us suppose that a high yielding and widely adapted variety A is susceptible to stem rust. Another variety B is resistant to stem rust, and that resistance to stem rust is dominant to

susceptibility. A generalized scheme of the backcross programme for the transfer of rust resistance from variety B to variety A is given below.

Hybridization : Variety A is crossed to variety B. Generally, variety A should be used as the female parent. This would facilitate the identification of selfed plants, if any.

F1 Generation : F1 plants are backcrossed to variety A. Since all the F1 plants will be heterozygous for rust resistance, selection for rust resistance is not necessary.

First Backcross Generation (BC1) : half of the plants would be resistant and the remaining half would be susceptible to stem rust. Rust resistant plants are selected and backcrossed to variety A. BC1 plants resistant to rust may be selected for their resemblance to variety A as well.

BC2-BC5 Generations: In each backcross generation, segregation would occur for rust resistance. Rust resistant plants are selected and backcrossed to the recurrent parent A. Selection for the plant type of variety A may be practiced, particularly in BC2 and BC3.

BC6- Generation : On an average, the plants will have 98.4 per cent genes from variety A. Rust resistant plants are selected and selfed; their seeds are harvested separately.

BC6 F2 Generation : Individual plant progenies are grown. Progenies homozygous for rust resistance and similar to the plant type of variety A are harvested in bulk. Several similar progenies are mixed to constitute the new variety.

Yield Tests : The new variety is tested in a replicated yield trial along with the variety A as a check. Plant type, date of flowering, date of maturity, quality etc. are critically evaluated. Ordinarily, the new variety would be identical to the variety A in performance. Detailed yield tests are,

therefore, generally not required and the variety may directly be released for cultivation.

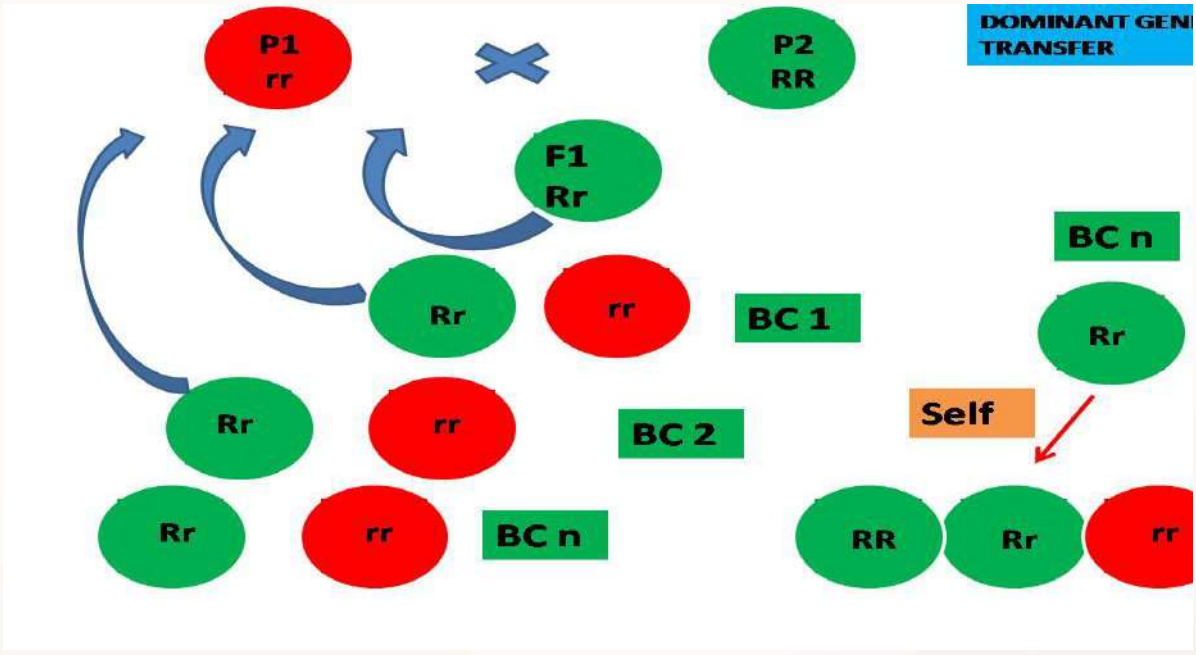

Fig 12.1. Schematic transfer of a dominant gene

B. Transfer of a Recessive Gene

When rust resistance is due to a recessive gene, all the backcrosses cannot be made one after the other. After the first backcross, and after every two backcrosses, F2 must be grown to identify rust resistant plants. The F1 and the backcross progenies are not inoculated with rust because they would be susceptible to rust. Only the F2 is tested for rust resistance. A generalized scheme for the transfer of a recessive gene for rust resistance is given below.

Hybridization : The recurrent parent is crossed with the rust resistant donor parent. The recurrent parent is generally used as the female parent.

F1 Generation : F1 plants are backcrossed to the recurrent parent.

BC1 Generation : Since rust resistance is recessive, all the plants will be rust susceptible. Therefore, there is no test for rust resistance. All the plants are self pollinated.

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BC1 F2 Generation : Plants are inoculated with rust spores. Rust resistant plants are selected and backcrossed with the recurrent parent. Selection is done for the plant type and other characteristics of the variety A.

BC2 Generation : There is no rust resistance test. Plants are selected for their resemblance to the recurrent parent A, and backcrossed with the recurrent parent.

BC3 Generation : There is no disease test. The plants are self-pollinated to raise F2. selection is usually done for the plant type of variety A.

BC3F2 Generation : Plants are inoculated with stem rust. Rust resistant plants resembling variety A are selected and backcrossed to variety A. Selection for plant type of A is generally effective.

BC4 Generation : There is no rust resistance test. Plants are back-crossed to variety A.

BC5 Generation : There is no rust test. Plants are self -pollinated to raise F2 generation.

BC5F2 Generation : Plants are subjected to rust epidemic. A rigid selection is done for rust resistance and for the characteristics of variety A. Selfed seeds from the selected plants are harvested separately.

BC5F3 Generation : individual plant progenies are grown and subjected to rust epiphytotic. A rigid selection is done for resistance to stem rust and for the characteristics of variety A. Seeds from several similar rust resistant homogeneous progenies are mixed to constitute the new variety.

Yield Tests : It is the same as in the case of transfer of a dominant gene.

C. Transfer of quantitative trait into a Recurrent Parent from same source In a situation where a trait under transfer is quantitative but all the genetic factors are present from same donor parent, it is recommended that each backcross should be followed bt at least two generations of recombinations to help recover desirable gene combinations containing all or most of the factors related to the trait.

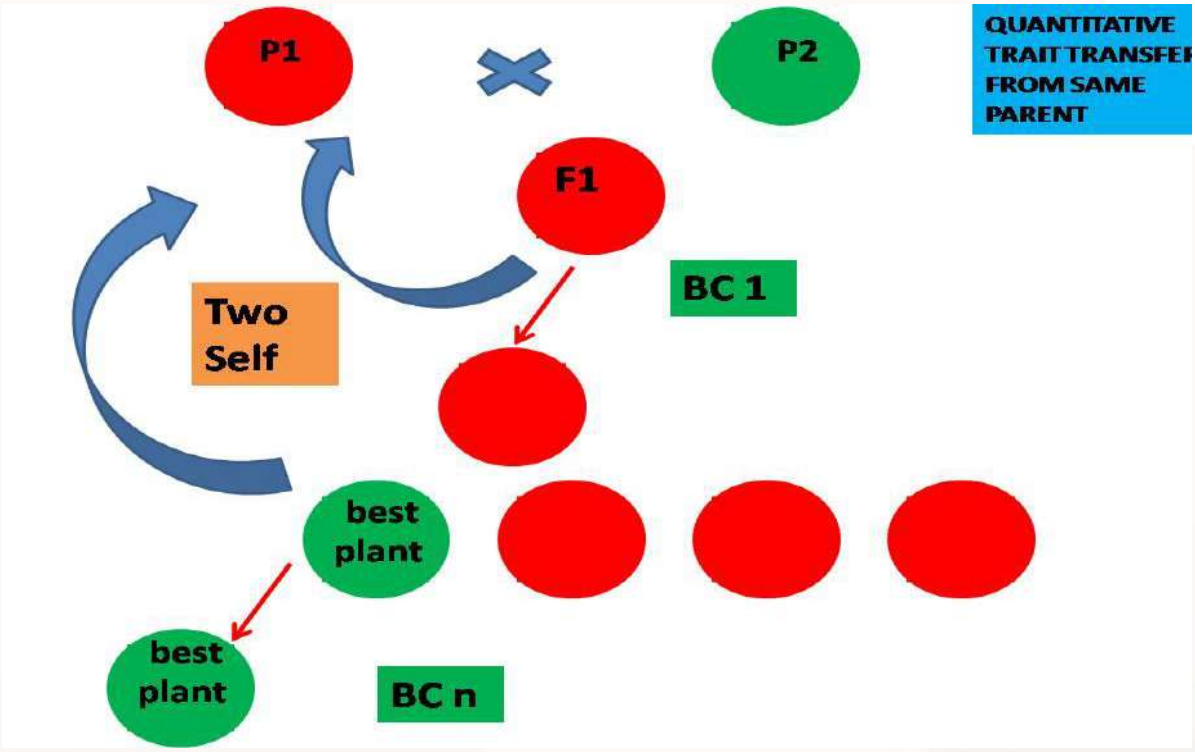


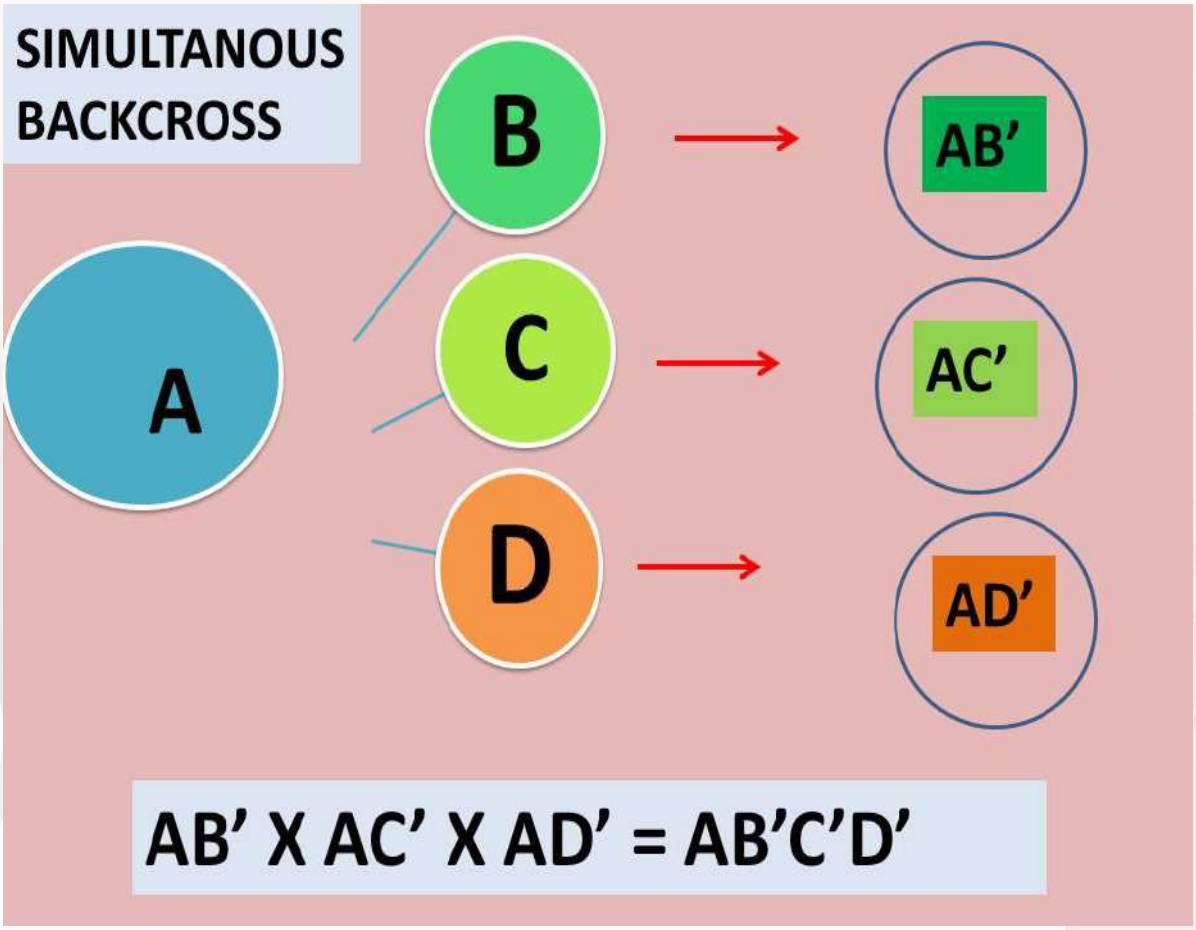
Fig 12.3. Transfer of quantitative trait

D. Transfer of quantitative trait into a Recurrent Parent from different sources: In a situation where a trait under transfer is quantitative but the genetic factors are present from diverse donor parents, it is recommended that simultaneous backcrosses be done to diverse parental sources and at the end when all the genes are recovered in the similar genetic background, convergent crossing be done to develop a genotype containing all the factors for the trait.

E. Transfer of many traits into a Recurrent Parent

Simultaneous Transfer : Genes for the different characteristics may be transferred simultaneously in the same backcross programme. The characters to be transferred are bought together into the hybrid by successively crossing each of the non-recurrent parents to the recurrent

parent or the hybrid thus produced. But in such a case, a larger backcross population would be needed than in the case of transfer of a single character. Further, the breeding programme may be delayed because the conditions necessary for the selection of all the characters may not occur every year. Sometimes, the two genes under transfer may be linked. In such a case, the transfer becomes very easy, and selection for only one gene may be
necessary.



Stepwise Transfer : The recurrent parent is first improved for one character. The improved recurrent parent is then used as the recurrent parent in a backcross programme for the transfer of the second character. If additional characters are to be transferred, they are transferred one at a time in a stepwise fashion. This approach takes much longer time for the transfer of two or more characters.

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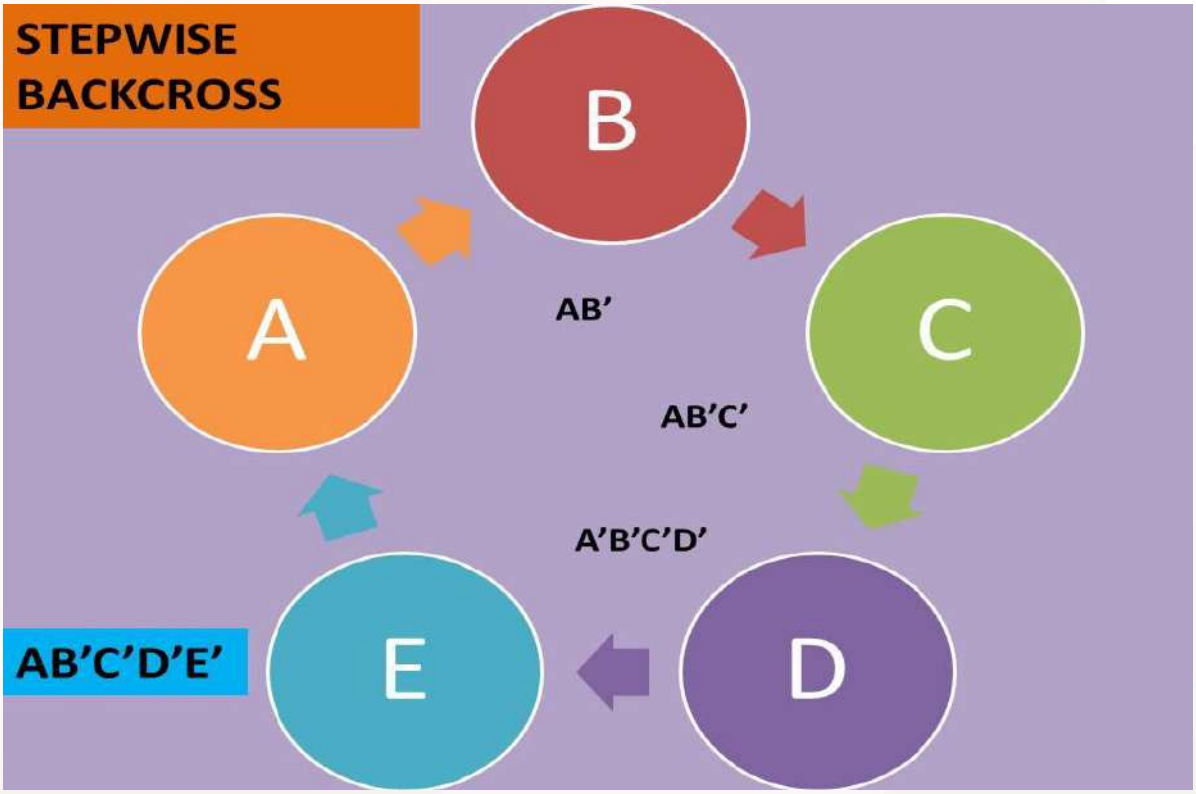

Simultaneous But Separate Transfers : Each character is transferred to the same recurrent parent in simultaneous but separate backcross programmes. The resulting improved versions from the different programmes are then crossed together. Homozygous lines for the characters being transferred are then selected from the segregating generations using the pedigree method. This approach appears to be the most suitable of the three strategies.

Merits

1. The genotype of new variety is nearly identical with that of the recurrent parent, except for the genes transferred. Thus the outcome of a backcross programme is known beforehand and it can be reproduced any time in the future.
2. It is not necessary to test the variety developed by the backcross method in extensive yield tests because the performance of the recurrent parent is already known. This may save upto 5 years' time and a considerable expense.

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3. The backcross programme is not dependent upon the environment, except for that needed for the selection of the character under transfer. Therefore, off – season nurseries and green-houses can be used to grow 2-3 generations each year. This would drastically reduce the time required for developing the new variety.
4. Much smaller populations are needed in the backcross method than in the case of pedigree method.
5. Defects, such as susceptibility to disease, of a well-adapted variety can be removed without affecting its performance and adaptability. Such a variety is often preferred by the farmers and the industries to an entirely new variety because they know the recurrent variety well.
6. This is the only method for inter-specific gene tra nsfers, and for the transfer of cytoplasm.
7. It may be modified so that transgressive segregation may occur for quantitative characters.

Demerits

1. The new variety generally cannot be superior to the recurrent parent, except for the character that is transferred.
2. Undesirable genes closely linked with the gene being transferred may also be transmitted to the new variety.
3. Hybridization has to be done for each backcross. This often difficult, time taking and costly.
4. By the time the backcross programme improves it, the recurrent parent may have been replaced by other varieties superior in yielding ability and other characteristics.

Table.12.1.. Genotypic frequency of populations under backcrossing

Generation	A1A1	A1A2	P1	P2
F1	0	1	0.5	0.5
BC1	0.5000	0.5000	0.75	0.25
BC2	0.7500	0.2500	0.875	0.125
BC3	0.8750	0.1250	0.9375	0.0625
BC4	0.9375	0.0625	0.96875	0.03125
BC5	0.9688	0.0313	0.984375	0.01562
BC6	0.9844	0.0156	0.9921875	0.00781
BC7	0.9922	0.0078		
Fn	2^t-1/2t	$1/2^t$	2^{t+1}-$1/2^{t+1}$	$1/2^{t+1}$

Table.12.2. Comparison between pedigree and backcross methods

PEDIGREE	**BACKCROSS**
F1 and the subsequent generations are allowed to self-pollinate	F1 and the subsequent generations are backcrossed to the recurrent parent
The new variety developed by this method is different from the parents in agronomic and other characteristics	The new variety is identical with the recurrent parent, except for the character under transfer
The new variety has to be extensively tested before release	Usually extensive testing is not necessary before release
The method aims at improving the yielding ability and other characteristics of the variety	The method aims at improving specific defects of a well adapted, popular variety
It is useful in improving both qualitative and quantitative characters	It is useful for the transfer of both quantitative and qualitative characters

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	provided they have high heritability
It is not suitable for genes transfer From related species and for producing substitution of addition lines	It is the only useful method for gene transfers from related species and for producing addition and substitution lines
Hybridization is limited to the production of the F1 generations	Hybridization with the recurrent parent is necessary for producing every backcross generation
The F1 and the subsequent generations are much larger than those in the backcross method	The backcross generations are small and usually consist of 20-100 plants in each generation
The procedure is the same for both dominant and recessive genes	The procedures for the transfer of dominant and recessive genes are different

Congruent Backcrossing

Congruent backcrossing involves crossing of backcrosses are alternated with both the parents instead of one in conventional backcrossing. This is used to overcome interspecific barriers. The scheme of congruent backcross and genetic constitution of progenies is as follows:

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Cross	Hybrid type	Genetic constitution		
		A	:	B
A × B	F_1	50		50
F1 × A	BC_1	75		25
BC1 × B	CBC_2	37.5		62
CBC2 × A	CBC_3	68.8		31
CBC3 × B	CBC_4	34.4		65
CBC4 × A	CBC_5	67.2		32

Course Name	Principles of Plant Breeding
Lesson 13	Bulk Method
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand operational procedure of Bulk method
2. Know the merits and demerits of Bulk method

Glossary of terms

Bulk method: This method relies on natural selection from F_2-F_6 generations and to harness superior natural selection as well as homozygosity of individual plants, by doing single plant selections in F6. Also known as mass method or evolutionary method 'or' Population method of breeding.

Single-seed-descent method: A modification of the bulk in which a single seed/pod from each of the plants in segregating generations is bulked to raise the next generation.

Mass pedigree method: A modified pedigree method in which population are exposed to the natural conditions of stress and selection is done only in the year when such stress conditions occur.

Bulking: Mixing of seeds of all the selected plants in segregating generations

Natural selection: Selection exerted by natural forces that screen out plants based on fitness in a bulk breeding programme

The bulk method was first proposed by Nilsson Ehle in 1908 at Svalof. This method is also known as **mass method or evolutionary method** 'or' **Population method** of breeding. This method relies on natural selection from F_2-F_6 generations and to harness superior natural selection as well as homozygosity of individual plants, by doing single plant selections in F_6.

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- Isolation of Homozygus lines
- Waiting for the opportunity for selection
- Opportunity for natural selection.
- F_2 and subsequent generations are harvested in mass as bulk to raise the next generation.
- At the end of the bulking period (after attaining homozygosity) individual plants are selected and evaluated similar manner as pedigree method of breeding.

Role of natural selection under bulking

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- Natural selection is mostly effective in increasing adaptive capacity of genotypes.
- Considerable variability is retained in final product despite population is substantially homozygous.
- However, genotypes with higher competitive ability may not necessarily be high yielders

Procedure for bulk method

The exact procedure for the bulk method would vary depending upon the objective of breeder. The following procedure is described for the isolation of homozygous lines. The breeder may introduce various modifications in the scheme to suit his needs.

Hybridization: Parents are selected according to the objective of the breeding programme. A simple or a complex cross is then made depending upon the number of parents involved.

F_1 Generation: F_1 is space-planted and harvested in bulk. The number of F_1 plants should be as large as possible; usually more than 20 plants should be grown.

F_2-F_6 Generations: F_2 to F_6 generations are planted at commercial seed rates and spacings. These generations are harvested in bulk. During this period, environmental factors, disease and pest outbreaks would change the frequencies of different genotypes in the population. Artificial selection is generally not done. The population size should be as large as possible, preferably 30,000-50,000 plants in each generation.

F_7 Generations: About 30-50 thousand plants are space-planted. 1000 to 5000 plants with superior phenotypes are selected and their seeds harvested separately. Selection is based on the phenotype of plants, grain characteristics, disease reaction, etc.

F_8 Generation: Individual plant progenies are grown in single or multi-row plots. Most of the progenies would be reasonably homozygous and are harvested in bulk. Weak and inferior progenies are rejected on the basis of visual evaluation. Only 100-300 plant progenies with desirable characteristics are saved. Some progenies which show segregation are generally rejected unless they are of great promise. In promising progenies, individual plants may be selected; preliminary yield trial will be delayed for one year in such cases.

F_9 Generation: Preliminary yield trial is conducted by using standard commercial varieties as checks. The progenies which are superior than the checks are advanced. Quality test may be conducted to further reject undesirable progenies. The progenies are evaluated for height, lodging resistance, maturity date, disease resistance and other important characteristics of the crop species.

F_{10}-F_{13} Generations: Replicated yield trials are conducted over several locations using standard commercial varieties as checks. The lines are evaluated for important characteristics in addition to yield, disease resistance and quality. If a line is superior to the standard varieties in yield trials, it would be released as a new variety.

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F_{14} Generation: Seed of the released variety is increased for distribution to the cultivators.

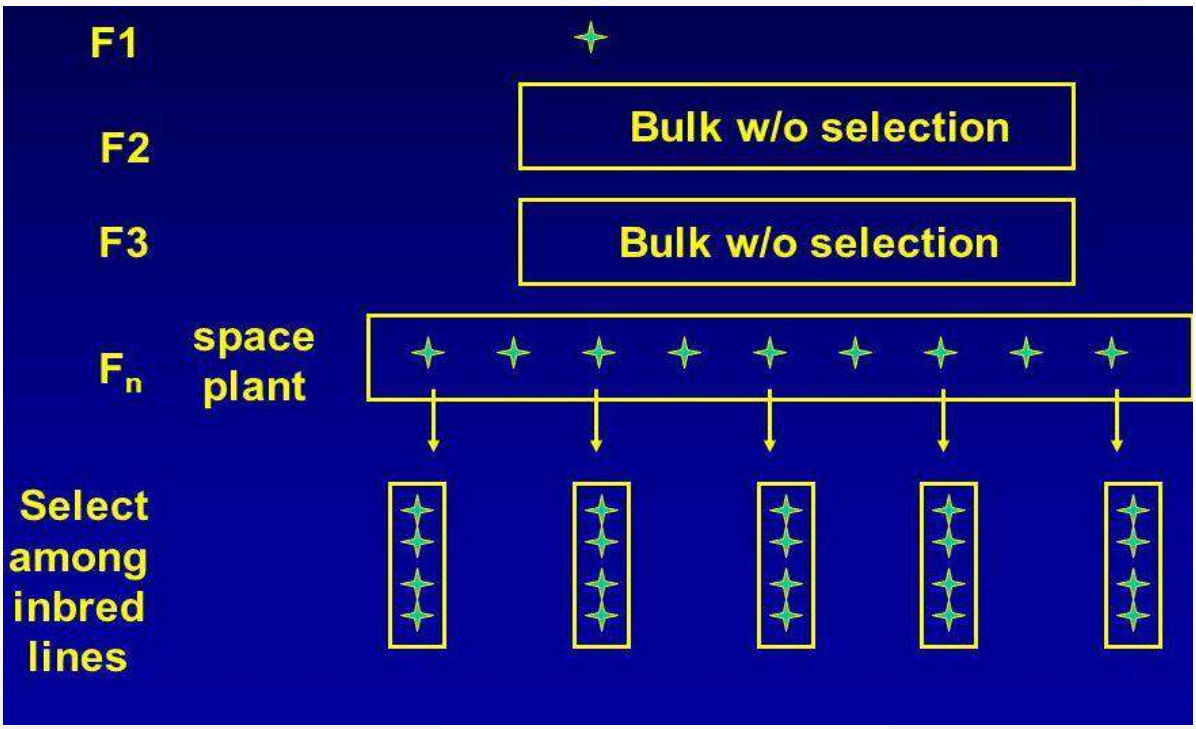

Fig. 13.1. General scheme of bulk method

Merits of bulk method

1. The bulk method is simple, convenient and less expensive.
2. Since, each F_2 plant is equally represented till F_6, no chance of elimination of good genotypes in early generations.
3. Artificial or natural disease epiphytic, winter killing high temperature etc. eliminates undesirable types and increases the frequency of desirable type. Thus, isolation of desirable types becomes easier.
4. Progenies select from long term bulks are superior to the selection from F_2 or short-term bulk.
5. Since, little work and attention is needed in F_2 and subsequent generation more no. of crosses can be handled.
6. No pedigree records are kept which help to save time.

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7. Since large population is grown, transgressive segregants are more likely to appear and increase due to natural selection. Hence, there is a greater chance to isolate good segregants than pedigree method.

Demerits of bulk method

1. The major disadvantage of bulk method is that it takes a much longer time to develop a new variety. Natural selection is invariably slow and gains under natural selections may not be optimum.
2. In short-term bulks, natural selection has little effect on the genetic composition of populations. But short -term bulks are useful for the isolation of homozygous lines and for specific objectives as in Harlan's mass -pedigree method.
3. It provides little opportunity for the breeder to exercise his skill or judgement in selection. But in the modified bulk method, the breeder has ample opportunity for practicing selection in the early segregating generations.
4. A large number of progenies have to be selected at the end of the bulking period.
5. Information on the inheritance of characters cannot be obtained which is often available from the pedigree method.
6. In some cases, at least, natural selection may act against the agronomically desirable types.
7. Natural selection selects for fitness and competitive ability which may not necessarily translate into higher yielding ability.
8. Invariably the natural and artificial selection go in opposite direction.

Table.13.1. Comparison between bulk and pedigree method.

PEDIGREE METHOD	BULK METHOD
Most widely used Breeding method	Used only to a limited extent

Individual plants are selected in F_2 and subsequent generations and individual plant progenies are grown	F_2 and subsequent generations are grown in bulk
Artificial selection; artificial disease epidemics etc. are an integral part of the method	Mainly natural selection. In certain cases, artificial selection may be essential
Natural selection does not play any role	Natural selection determines the composition of the pop n at the end of the bulking period
Pedigree Records have to be maintained which is often time consuming and laborious	No pedigree records are maintained
Generally, it takes 12-13 years to release a new variety	Generally, it takes 12-13 years to release a new variety
Requires close attention of breeder from F_2 onwards	It is quite simple and does not require much attention
Planting (spacing) the segregating generations are space planted to permits effective individual plant selection	The bulk populations are generally planted at commercial planting rate
Population size is small in comparison to bulk	The population size is large

SINGLE-SEED-DESCENT METHOD

A modification of the bulk method is the single-seed-descent method, proposed by **Goulden**, which is becoming increasingly popular. In this method, a single seed/pod from each of the one to two thousand F_2 plants is bulked to raise the F_3 generation. Similarly, in F_3 and the subsequent generations one random seed/pod is selected from every

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plant present in the population and planted in bulk to raise the next generation.

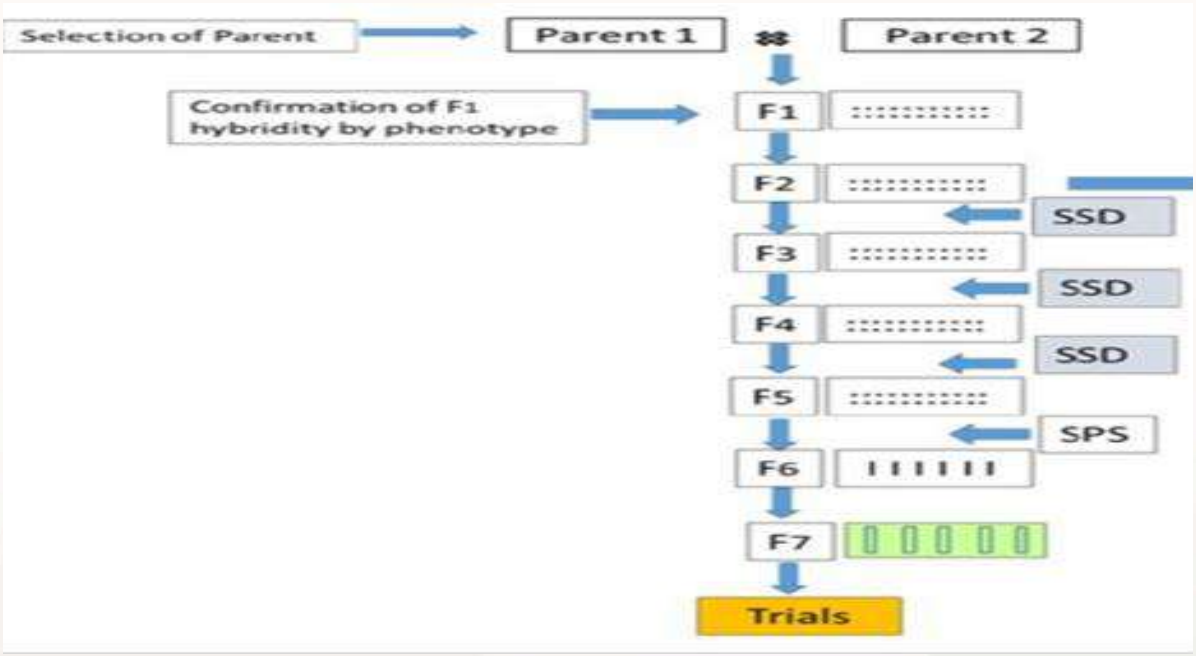
This procedure is followed till F_5 or F_6 when the plants would have become nearly homozygous. In F_5 or F_6, a large number (1 to 5 hundred) of individual plants are selected and individual plant progenies are grown in the next generation. Selection is done mainly among the progenies, and the number of progenies is sufficiently reduced to permit replicated trial in the next generation. Individual plants may be selected only from outstanding families not showing segregation. Thus, preliminary yield trials and quality tests begin in F_7 or F_8 and coordinated yield trials in F_8 or F_9.

The objective of single-seed-descent method is to rapidly advance the generations of crosses; at the end of the scheme, a random sample of homozygous or near homozygous genotypes/lines is obtained. F_2 and the subsequent generations are grown at very high plant densities as vigour of individual plants is not important. In each year, 2-3 generations may be raised using off-season nurseries and greenhouse facilities.

The important features of this scheme are :

(1) Lack of selection, natural or artificial, till F_5 or F_6 till the population is reasonably homozygous, and
(2) Raising of F_3 and later generations from a bulk of one seed from each F_2 and the subsequent generation plant in order to ensure that each F_2 plant is represented in the population. As a result of the speed and economy, the single seed- descent scheme is becoming increasingly popular with the breeders.
(3) Advances the generation with the maximum possible speed in a conventional breeding method;
(4) It requires very little space, effort and labour
(5) It makes the best use of greenhouse and off -season nursery facilities

(6) It ensures that the plants retained in the end population are random sample from the F_2 population.
(7) It does not permit any form of selection (which is implied in the scheme) during the segregating generations.



Advantages of the single-seed descent procedures

- They are easy to manage and speed up the inbreeding process as no special laboratories or techniques are needed in comparison with other methods, e.g. double haploid production.
- Procedures are well suited for environments where otherwise only one generation per year can be grown in the field.
- This method can be less expensive than field selection where the costs of land and land management are high.

Disadvantages

- The size of the population should be adjusted for germination losses.

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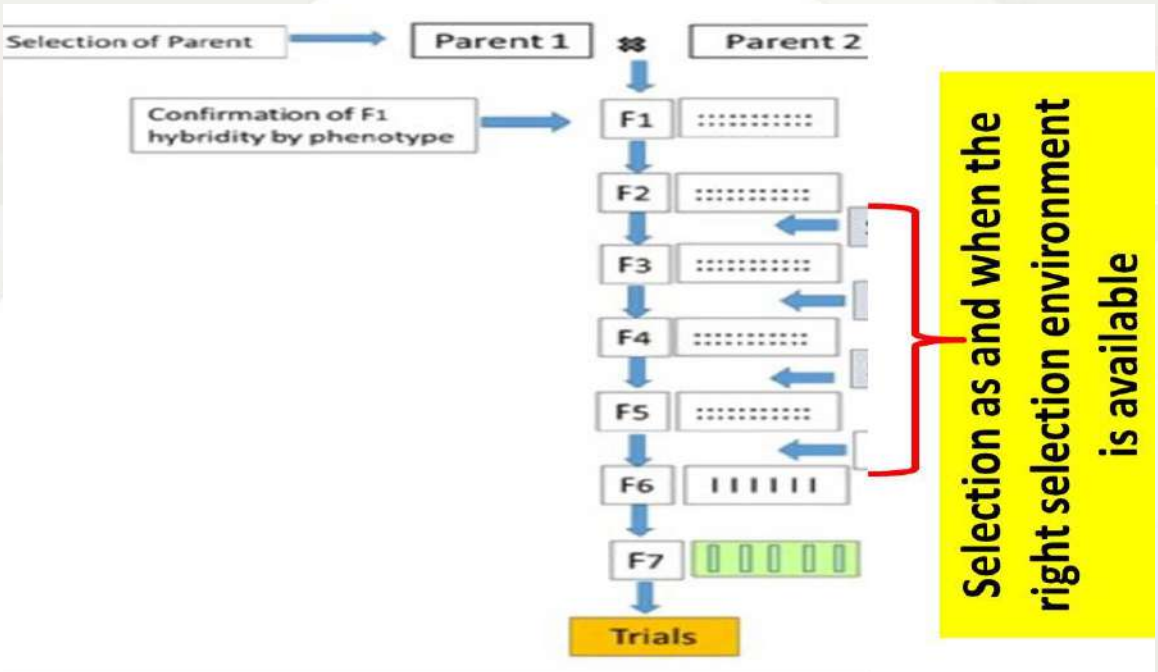
- All the F_2 plants may not be present in the line evaluation due to germination losses, decreasing the genetic variance.
- The amount of seed available at the end of the inbreeding process is reduced, needing additional growing cycles just to multiply seed, thus delaying the total process.

MASS PEDIGREE METHOD :

This is another modified pedigree method. Crosses are made and further generations grown in bulk or as mass until suitable season occurs for making desirable selections against drought, insect and diseases etc. The population will be exposed to the natural conditions of vagaries. From the remaining population individual plants are selected and harvested progenies are evaluated for yield and other characters in preliminary yield trials and further generations are preceded as in pedigree method till release of variety. The advantages of both bulk and pedigree methods can be obtained and large number of crosses can be handled at a time. The disadvantage is that it takes a bit longer time.



VARIETIES DEVELOPED BY BULK METHOD

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Carrot: Pusa Kesar

Pea: Jawahar Mattar-1

Brinjal : PLR-2

French Beans: Cometa, Majestoso, Pitanga, Requinte

Course Name	Principles of Plant Breeding
Lesson 14	Hybrid Breeding
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand various steps in hybrid breeding in cross pollinated crops
2. Understand various methods of development and evaluation of inbred lines

Glossary of terms

Hybrid: It is the "First generation of a cross between two genetically unrelated parents", such as pure lines, varieties, populations, inbreds etc.

Inbred lines: Lines developed by continuous selfing of a single heterozygous cross pollinated plant

Single Cross hybrid: A cross involving two inbred lines where one line is essentially sister line of other

Three way cross: A cross involving three inbred lines

Double cross: A cross involving four inbred lines

Sister lines: Lines that are more that 50% genetically similar

Tester: A line used to test GCA or SCA of an inbred line

HYBRID BREEDING

Hybrid by definition is the "**First generation of a cross between two genetically unrelated parents**", such as pure lines, varieties, populations, inbreds etc. Hybrid breeding is undoubtedly the most important practical achievement of the science of plant breeding. Hybrid breeding exploits the phenomenon of heterosis that arises upon crossing of genetically diverse parents. The early plant breeders recognized the benefits of

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mating diverse individuals as increased luxuriance, superior expression of economic traits and enhanced adaptability (Herbert, 1837; Gartner, 1849). Darwin (1876) in his book, “The effects of cross and self fertilization in vegetable kingdom” concluded that inbreeding is generally deleterious and cross fertilization is beneficial. He was first to conduct experiments comprising selfed and crossed progenies in maize.

Beal (1880) conducted similar experiments in maize and outlined a procedure for hybridization to enhance yield. Hybrid breeding has largely been driven by the successful application in maize. In fact the spectacular increases in U.S. maize yields that witnessed dramatic upward shift from1860 to 2000 when breeders progressively shifted from open-pollinated varieties through double cross hybrids in 1920’s to single cross hybrids in 1960’. The greater yield potential of single cross hybrids could be harnessed by availability of highly productive inbred lines that were purged of their genetic bottlenecks and could thus overcome the limitation posed by seed yield potential of lines.

East and Hayes (1912) were first to propose hybrid breeding on an alternative crop improvement strategy in allogamous crops. However, due to non-availability of good inbred lines that limits seed production potential of lines, Jones (1918) advocated use of double cross hybrids. First double cross hybrid in U.S.A. was **Burr Leaming Dent** in 1922 in maize. Thus began the successful joining of hybrid culture which gave great impetus to development of superior inbred lines and by 1965 when double cross hybrids began to show yield plateauing, single cross hybrids were already making inroads. Thus was also accompanied by evolution of breeding methodologies that lead to successful exploitation of hybrid superiority. Fischer (1949) outlined that hybrid breeding is essentially a three step procedure:

1. Foundation individuals chosen to start the process.
2. Constant inbreeding to near homozygosity.

3. Crossing of chosen lines.

Fisher also outlined that selection ideally has to operate at all three steps but since it is not effective at first and second stage, great focus should be on the third stage. In stage-I, the selection is usually not always fruitful a selection on basis of phenotype does not necessarily mean that lines developed from selected individuals will eventually result in productive hybrids. Selection is not either effective under inbreeding due to rapid fixation of genotypes as heterozygosity is reduced by 50% through each generation of inbreeding. For the sake of simplicity, hybrid breeding in allogamous crops can be broadly outlined into following steps:

1. Development and improvement of Inbred lines
2. Testing of Inbred lines
3. Combination of Inbred lines into heterotic hybrids.

Types of Hybrids:-

Once the lines have been identified on the basis of agronomic feature, vigor and combining ability, the lines are combined into heterotic hybrids. The male and female parents are chosen based on the desired features and planted in appropriate male: female ratios ranging from 1:2, 1:4 to 1:6 depending upon the pollen shedding ability of male parent. Depending upon the nature and number of parental lines, following types of hybrids are produced: -

- Single Cross hybrid= $I_1 \times I_2$
- Modified single cross hybrid= $(I_1 \times I_1') \times I_2$
- Three-way cross hybrid= $(I_1 \times I_2) \times I_3$
- Modified Three-way cross hybrid= $(I_1 \times I_2) \times (I_3 \times I_3')$
- Double cross hybrid= $(I_1 \times I_2) \times (I_3 \times I_4)$
- Triple Cross Hybrid= $(I_1 \times I_2) \times (I_3 \times I_4) \times (I_5 \times I_6)$
- Top Cross Hybrid= $I_1 \times V$
- Double Top Cross Hybrid= $(I_1 \times I_2) \times V$

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- Varietal Cross Hybrid= $V_1 \times V_2$
- Population Cross Hybrid= $P_1 \times P_2$

The number of all possible crosses among n number of inbred lines in different schemes is as follows:

- All possible pairwise single crosses excluding reciprocal crosses = n(n-1)/2
- Total number of three way crosses = n(n-1) (n-2) / 2
- Total number of double cross hybrids = n(n-1) (n-2) (n-3) / 8

Among all types of hybrids listed above single cross hybrid is most productive. It is heterozygous but homogenous while rest all are heterozygous as well as heterogeneous.

Advantages of Hybrids

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1. They give maximum performance for economic traits under optimal management.
2. They have stability of performance in stress conditions.
3. Reduced time for cultivar development.
4. It provides for joint improvement of traits.
5. Hybrids are highly uniform, thus are suitable for mechanization as well as industrial requirements.
6. Hybrids are reproducible
7. They have propriety control of parents.

Disadvantages of Hybrids

1. Hybrid seed is invariably costlier than corresponding OPV's.
2. Seed replacement rate in hybrids is 100% i.e. seed has to be purchased every year. However, in India where most of the seed replacement is vertical through farmers own saved seeds popularization of hybrids is difficult.

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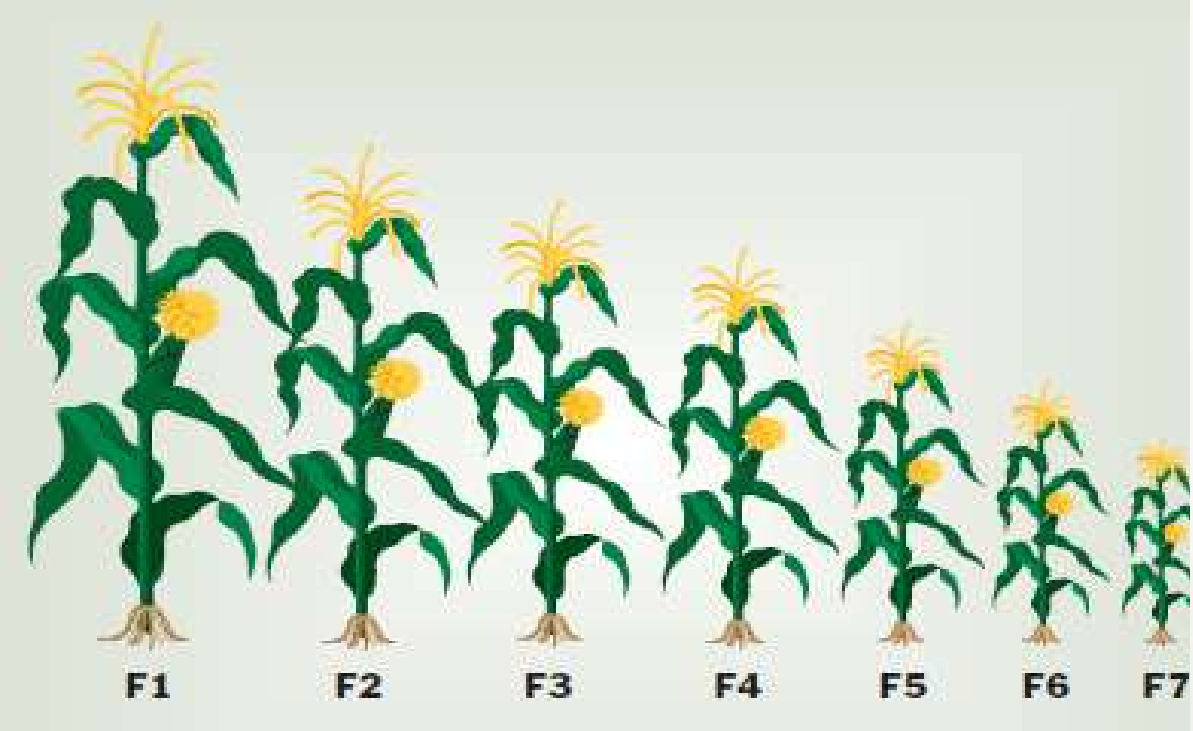
3. Hybrids are not feasible in the areas where no seed industry set up is present to support hybrid seed production.
4. Hybrids are not feasible for marginal, low input agricultural systems.
5. Hybrids usually have a narrow genetic base and are thus fragile in terms of their ability to adapt to changing situations.

Development and Improvement of Inbred lines

An inbred line can be defined as a nearly homozygous line developed through repeated cycles of selfing in a cross-pollinated crop. Stringfield (1974) gave the idea of "Broad line" in maize to overcome the problem of inbreeding depression. The broad lines are developed through sib-mating rather than selfing. A major advantage of a broad line is that it retains sufficient genetic variability in the population and does not lead to rapid fixation. Moreover, since sib-mating is a mild form of inbreeding, the inbreeding depression is consequently lower. However, a major disadvantage of broad line is that advance to homozygosity is greatly delayed because one generation of selfing is equal to three generations of full-sib mating and five generations of half-sib mating. Inbred lines can be derived from any material, such as open pollinated varieties, synthetics, composites, landraces, germplasm complexes, pools or any heterozygous population that has substantial genetic variability. Various procedures for development of inbred lines are:

Standard Method:- Standard method is based on repeated inbreeding of selected plants in the base population , with selection being exercised at each step of inbreeding. The selfed progenies of selected plants are grown in an ear-to-row fashion and selection is exercised both between and within rows for vigor, disease and pest resistance, gene characteristics and ear characteristics. Selection during selfing neither delays attainment of homozygosity nor has any influence on the combining ability of lines. During early cycles of inbreeding, there may be

drastic inbreeding depression with a tendency of stabilization in subsequent cycles. The selfing is usually carried up to 6-7 generations till the lines reach near homozygosity.



Single Hill Method:- Jones and Singleton (1934) proposed a modification of standard method in which only a single hill containing three plants is used instead of a row containing several plants, in each cycle of inbreeding. The method comes with the advantages of reducing the experimental area for selection of a large number of lines. Thus between line selection is greatly enhanced but within line selection is highly ineffective especially for the traits like lodging and disease resistance due to small number of plants per progeny.

Cryptic Method:- The method was proposed by Hallauer (1967) for simultaneous development of inbred lines as well as single cross hybrids. The method requires prolific plants in which one ear can be selfed and other one crossed, and is based on assessment of full-sib families. Thus plants selected in base population are crossed (x) as well as selfed. Based on the performance of x crosses, the best crosses are selected and

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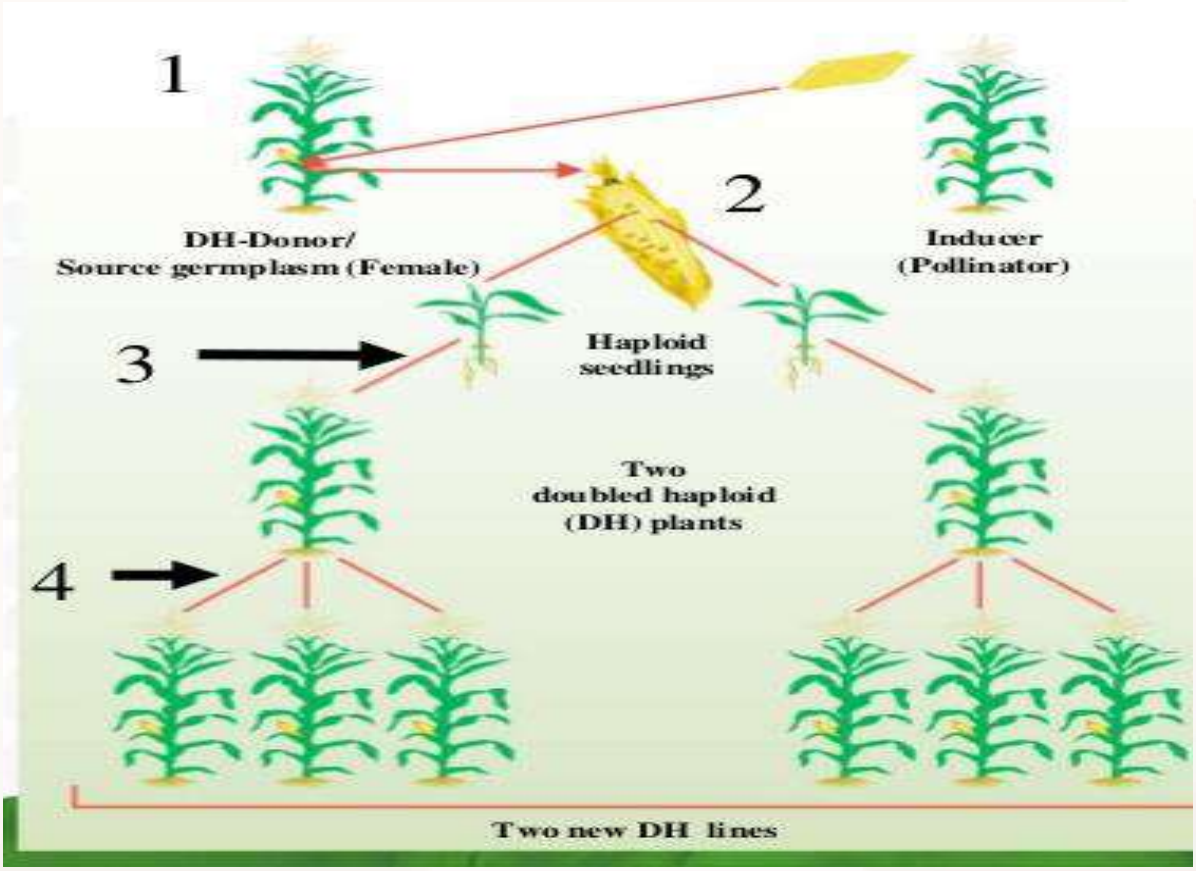
corresponding progenies are planted in pairs to produce x crosses as well as corresponding selfs to produce progenies. The process is continued to x level where desired level of homozygousity is achieved. Such a method can yield good inbred lines as well as superior single cross hybrids. The lines developed can not only be crossed intense but also with lines developed from other breeding programmes in hybrid development.

Homozygous Diploids:- This is the fastest method of deriving homozygous inbred lines and was proposed by Chase (1952). He reported that haploids arise spontaneously in Maize population with a frequency of 1:1000 plants and selfed progenies can be obtained among such monoploids with a frequency of 1:10. A major problem is low frequency and detection of such haploids. A Scutellum marker has been developed to detect such markers in a population. Sarkar and Coe (1978) have an inducer stock (Coe stock6) that enhances the frequency of such haploids up to
5%. Recently, Kernicke (1969) have developed a mutant stock (ig) that also results in higher frequency (3%) of haploids. Anther culture is also being used for rapid development of homozygous diploids especially in China. Wu (1986) has reported development of an elite homozygous line by double haploidy through anther culture that has been used in hybrid development. Similarly, Anderson (1986) also developed an inbred line tol4erant to herbicide by cell culture selection using immature embryo as explants.



Maintenance of Inbred Lines:-

Once the lines have been established they are maintained by sib mating or intermittent selfing and sib-mating (Khekra and Dhillon, 1993) unless selection is exercised to maintain the identity of line under successive generations of maintenance through selfing or sibbing. The changes have been reported to be random or directional towards reduced vigor. The changes may be largely due to mutation or developmental variations.

Evaluation and Testing of inbred lines

This is one of the most critical aspects of hybrid breeding in which the worth of a line is assessed for its suitability as a parent in hybrid combination. Every year thousand of Inbred lines are developed in National and International hybrid breeding programme of allogamous crops but only a handful of them actually go in hybrid development. In order to keep the number of lines, that enter testing phase, to a manageable level, visual selection during the process of inbreeding is of

paramount importance. In fact, Bauman (1981) reported that intensive selection during S1-S4 helps to cull out about 92% of lines. The two important aspects of inbred line evaluation is the time or stage of evaluation.

Testing Procedure:- Screening of lines on the basis of per se performance has mostly been misleading and numerous reports have depicted poor correlation between per se performance of a line and its performance in hybrid combinations (Halauer and Miranda, 1988). Thus an appropriate test of the worth of a line can be done by putting it in the same i.e. hybrid combinations with other lines or a tester to judge its combining ability, which by definition is the ability of a line to produce productive cross combinations with vigor and seed production are no less important especially in view of greater focus on single cross hybrids. A line with poor vigour and seed yielding ability has no worth in commercial hybrid breeding programmes even if it has high positive values for combining ability. In maize, the male and female parent should have following

Male Parent:-

- Lax Tassel with few secondary branches
- Long duration of pollen shedding
- Taller than female parent
- Attractive grain color
- Strong plant
- Resistant to stresses

Female Parent:-

- Productive
- Strong Plant
- Long Cobs with complete exertion
- Low Cob placement
- Shorter Anthesis-Silking interval
- Stay green
- Resistant to stresses

- Erect leaves
- Strong Root system
- Nutrient responsive

Nature of Tester:-

The nature of tester assumes great significance in view of a critical role in classifying of lines on the basis of merit of top-cross test evaluation. Theoretically, for a top-cross, the tester should be broad based (usually an OPV) to provide an estimate of general combining ability of lines. This opinion has been supported by Green (1948) who proposed use of synthetic testers and Matzinger (1953) who suggested use of a tester produced by crossing of eight most commonly used Inbreds. Contrary to this opinion, Hull (1945) proposed use of narrow based tester usually a homozygous recessive line. The opinion was supported by Rawlings and Thompson (1962) and Alison and Curnow (1966). Their premise was that use of narrow based tester would give a better estimate of specific rather than general combining ability which is important for single cross hybrid development.

Rawlings and Thompson have stated two important features of a good tester namely

(i) ability to discriminate effectively between the lines and
(ii) Classify the material correctly.

Hallauer (1975) stated that in addition to above a good tester must be simple and easy to use. However, the nature of a tester is largely dependent on breeding objective. For evaluation of large unselected lines, the ideal tester must be broad based (OPV) to discriminate the lines on the basis of GCA..

Time of Testing

In earlier breeding programmes aimed at hybrid development the usual practice was to test the lines for GCA once they would attain near

homozygosity (S6-S7 stage). Such a practice would allow carrying forward of lines that are likely to be rejected in testing. In view of increasing number of inbred lines being developed, it was felt that a preliminary screening of lines should be done early in the inbreeding process to cull out poor combining lines, so that new lines could be pushed in the inbreeding cycles. Jenkins (1935) suggested a procedure called as “early testing” wherein lines are tested early in the inbreeding process to economize time and resources. His idea was based on the premise that the lines attain their characteristic feature very early in the inbreeding process and remain stable thereafter as the inbreeding progresses. He reported that testing lines simultaneously along with inbreeding could make out differences in the combining ability of lines. However, Richey (1927) argued against the effectiveness of early testing. His argument was based on the following points:-

a. Early testing provide for testing of lines at any levels of inbreeding.
b. Performance of a selfed progeny is not a good indicator of its combining ability before it is purged of recessive alleles of major effect through further inbreeding.

Many other objections have been raised against early testing. One is that the combining ability expression may change with the level of homozygosity, a line has achieved. This is in line with the argument of Richey (1927), wherein it could be concluded that poor performance of a line in top cross test at S1-S2 may not be due to poor combining ability but due to higher genetic load due to recessive alleles. Another objection has been that merely on the basis of early testing; such lines are to be carried forward, which are agronomically so poor that they are likely to be eliminated during subsequent generations of inbreeding. Also the effectiveness of early testing is greatly reduced by the low heritability of important traits like grain yield.

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Retention of a large number of lines during early testing decreases the risk of losing lines that would be genetically superior at homozygosity but at the same time requires large scale testing of lines, at a later generation. In contrast, if small number of lines is retained, there is always some risk as the performance in test crosses may vary between partially inbred lines and homozygous lines. In case of low heritability selfing up to 2-3 generations should be done to increase the likelihood of retaining lines that will perform well at homozygosity. However, in all cases the number of lines retained is larger at earlier selfing generations. An optimum combination of generation of testing and proportion of lines selected (selection intensity) depends upon the population size, expected cost of retesting lines and the risk of losing genetically superior lines.

PREDICTION OF THE PERFORMANCE OF DOUBLE CROSS HYBRIDS

In a double cross hybrid, four inbred parents are involved. Theoretically, the potential of the double cross will be the function of the inbreeding value of these four parental inbreds. Therefore, based on the procedure of testing of the breeding value of inbreds, the performance of a double cross hybrid can be predicted through any of the four methods indicated by Jenkins (1934). Starting with the simplest procedure these methods are:

a) Mean performance of all possible six single crosses with inbred lines A, B, C and D.

Predicted performance of double cross ABCD = (AB +BC + CD + AD + BD + AC)/ N

b) Mean of the four non-parental single crosses involved in (A×B) × (C × D) double cross, viz., (AXC), (AXD), (BXC) and (BXD) (total 4 non-parental single crosses per double cross).

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Predicted performance of double cross ABCD = (AC +AD + BC + CD + BD) / 4

c) Average yield performance of all possible crosses of a set of four inbred lines A, B, C and D with a series of four other lines E, F, G and H.

Predicted performance of double cross ABCD = (AE +AF + AG + AH + BE + BF +….DH) /N

d) Average progeny-performance of four top crosses of inbred lines A,B, C and with a variety.

Predicted performance of double cross ABCD = (A × variety) +(B × variety) + (C × variety) + (D × variety) / 4

Important Hybrids of Horticultural Crops

Crop	Hybrid	Crop	Hybrid
Tomato	Pusa hybrid 1, Pusa hybrid 2, BSS-48, Himsohna, Arka Samrat	Mango	Pusa Arunima, Sindhu, Ratna, Pusa Lalima, Pusa Prathiba, Arka Aruna, Arka Puneet
Brinjal	Arka Anand, Arka Navneet , Pusa Anmol, Shamli	Papaya	Arka Surya, Arka Prabhat
Chilli	Arka Suphal, Arka Meghana, Arka Sweta, Arka Harita, Rani	Passion fruit	Kaveri

Capsicum	Pusa Deepti (Kt – 1), Solan Hybrid – 2, Solan Hybrid - 1:	Pomegranate	Ruby
Bitter gourd	Pusa hybrid-1, Pusa hybrid-2, Urvasi	Watermelon	Arka Aishwarya, Arka Akash, Arka Manik, Arka Jyoti
Ridge gourd	Arka Vikram	Musk melon	Pusa Rasraj, Punjab Hans
Pumpkin	Pusa Alankar	Onion	Arka Kirtiman, Arka Lalima

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Course Name	Principles of Plant Breeding
Lesson 15	General & Specific Breeding Techniques
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
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Objectives

1. Understand various specific breeding methods employed in self and cross pollinated crops
2. Understand the potential role of some non-conventional methods in plant breeding

Glossary of terms

Tissue culture: Development of whole plants from various tissues of a plant using nutrient media

Explant: The part of plant/tissue use in tissue culture is called explant

Hardening: Preparatory phase of taking tissue culture derived plants to filed conditions

Transgenics: Plants containing genes from unrelated source transferred through non-conventional methods

Transformation: Process of transferring a foreign gene in unrelated species

Genetic marker: DNA sequence with a known physical location on a chromosome.

Recurrent selection: Selection generation after generation with intermating of selects

Multilines: They are mixtures of nearly isogenic lines containing different genes of resistance against different races.

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Inter Population Improvement:- Inter population improvement is the set of breeding procedures aimed at cyclical improvement of cross-bred progenies.

Intra population Improvement:- Intra population improvement refers to any method that seeks to improve a population per se by exploiting the genetic variability present in the population

Population refers to a group of freely intermating individuals sharing a common gene pool.

A. TISSUE CULTURE

Tissue culture is the in vitro aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions often to produce the clones of plants. The resultant clones are true-to type of the selected genotype. The controlled conditions provide the culture an environment conducive for their growth and multiplication. These conditions include proper supply of nutrients, pH medium, adequate temperature and proper gaseous and liquid environment. Plant tissue culture technology is being widely used for large scale plant multiplication.

Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Endangered, threatened and rare species have successfully been grown and conserved by micropropagation because of high coefficient of multiplication and small demands on number of initial plants and space. In addition, plant tissue culture is considered to be the most efficient technology for crop improvement by the production of somaclonal and gametoclonal variants. The micropropagation technology has a vast potential to produce plants of superior quality, isolation of useful variants in well-

adapted high yielding genotypes with better disease resistance and stress tolerance capacities.

History of plant tissue culture

The science of plant tissue culture takes its roots from the discovery of cell followed by propounding of cell theory. In 1838, Schleiden and Schwann proposed that cell is the basic structural unit of all living organisms. They visualized that cell is capable of autonomy and therefore it should be possible for each cell if given an environment to regenerate into whole plant. Based on this premise, in 1902, a German physiologist, Gottlieb Haberlandt for the first time attempted to culture isolated single palisade cells from leaves in knop's salt solution enriched with sucrose. The cells remained alive for up to one month, increased in size, accumulated starch but failed to divide. Though he was unsuccessful but laid down the foundation of tissue culture technology for which he is regarded as the father of plant tissue culture. Some of the landmark milestones in tissue culture are summarized follows:

1902 - Haberlandt proposed concept of in vitro cell culture
1904 - Hannig cultured embryos from several cruciferous species
1922 - Kolte and Robbins successfully cultured root and stem tips respectively
1926 - Went discovered first plant growth hormone –Indole acetic acid
1934 - White introduced vitamin B as growth supplement in tissue culture media for tomato root tip
1939 - Gautheret, White and Nobecourt established endless proliferation of callus cultures
1941 - Overbeek was first to add coconut milk for cell division in Datura
1946 - Ball raised whole plants of Lupinus by shoot tip culture
1954 - Muir was first to break callus tissues into single cells
1955 - Skoog and Miller discovered kinetin as cell division hormone

1957 - Skoog and Miller gave concept of hormonal control (auxin: cytokinin) of organ formation 1959 - Reinert and Steward regenerated embryos from callus clumps and cell suspension of carrot (Daucus carota)
1960 - Cocking was first to isolate protoplast by enzymatic degradation of cell wall
1960 - Bergmann filtered cell suspension and isolated single cells by plating
1960 - Kanta and Maheshwari developed test tube fertilization technique
1962 - Murashige and Skoog developed MS medium with higher salt concentration
1964 - Guha and Maheshwari produced first haploid plants from pollen grains of Datura (Anther culture)
1966 - Steward demonstrated totipotency by regenerating carrot plants from single cells of tomato
1970 - Power et al. successfully achieved protoplast fusion
1971 - Takebe et al. regenerated first plants from protoplasts
1972 - Carlson produced first interspecific hybrid of Nicotiana tabacum by protoplast fusion
1974 – Reinhard introduced biotransformation in plant tissue cultures
1977 - Chilton et al. successfully integrated Ti plasmid DNA from Agrobacterium tumefaciens in plants –
1978- Melchers et al. carried out somatic hybridization of tomato and potato resulting in pomato 1981- Larkin and Scowcroft introduced the term somaclonal variation
1983 - Pelletier et al. conducted intergeneric cytoplasmic hybridization in Radish and Grape - 1984 - Horsh et al. developed transgenic tobacco by transformation with Agrobacterium
1987 - Klien et al. developed biolistic gene transfer method for plant transformation
2005 - Rice genome sequenced under International Rice Genome Sequencing Project

Tissue culture in agriculture

Plant tissue culture has a great impact on both agriculture and industry, through providing plants needed to meet the ever increasing world demand. It has made significant contributions to the advancement of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture

- Germplasm conservation
- Embryo culture
- Genetic transformation
- Protoplast fusion
- Haploid production
- Micropropagation

Techniques of plant tissue culture

Micropropagation: Micropropagation starts with the selection of plant tissues (explant) from a healthy, vigorous mother plant. Any part of the plant (leaf, apical meristem, bud and root) can be used as explant. The whole process can be summarized into the following steps:

Preparation of donor plant Any plant tissue can be introduced in vitro. To enhance the probability of success, the mother plant should be ex vitro cultivated under optimal conditions to minimize contamination in the in vitro culture

Initiation stage In this stage an explant is surface sterilized and transferred into nutrient medium. Generally, the combined application of bactericide and fungicide products is suggested. The selection of products depends on the type of explant to be introduced. The surface sterilization of explant in chemical solutions is an important step to remove contaminants with minimal damage to plant cells.

Multiplication stage The aim of this phase is to increase the number of propagules. The number of propagules is multiplied by repeated subcultures until the desired (or planned) number of plants is attained.

Rooting stage The rooting stage may occur simultaneously in the same culture media used for multiplication of the explants. However, in some cases it is necessary to change media, including nutritional modification and growth regulator composition to induce rooting and the development of strong root growth.

Acclimatization Stage At this stage, the in vitro plants are weaned and hardened. Hardening is done gradually from high to low humidity and from low light intensity to high light intensity. The plants are then transferred to an appropriate substrate (sand, peat, compost etc.) and gradually hardened under greenhouse.

TRANSGENICS IN AGRICULTURE

Transgenic plants are plants that have had their genomes modified through genetic engineering techniques either by the addition of a foreign gene or removal of a certain detrimental gene. A foreign gene inserted into a plant can be of a different species or even kingdom. The first transgenic plant was developed through the insertion of nptII bacterial antibiotic resistance gene into tobacco. Since then, with the rapid development in plant molecular biology and genetic engineering technology, a wide variety of transgenic plants with important agronomic traits such as pest resistance and drought tolerance have been developed, ranging from dicots to monocots that are amenable to genetic modifications. The main purpose in the production of transgenic plants is to produce crops, which have ideal traits, quality, and high yield.

Application of transgenic plants

- Improving yield
- Improved nutritional quality
- Resistance to biotic or abiotic stresses
- Transgenic plants as bioreactors for recombinant proteins

Transformation techniques

Plant transformation refers to the process of altering the genetic constituents in a plant of interest by introducing DNA segments into the plant genome to achieve desired gene expression. Numerous types of plant transformation techniques have now been made accessible to the public. These plant transformation techniques can be categorized under two groups: indirect or direct gene transfer. Indirect gene transfer (also known as vector-mediated gene transfer) involves the introduction of exogenous DNA into the plant genome via biological vectors, whereas direct gene transfer methods involve the introduction of exogenous DNA directly into plant genome through physical or chemical reactions.

Agrobacterium-mediated gene transfer: Agrobacterium-mediated transformation is the most common technique used in plant transformation as it is efficient and effective in a wide range of plants. Agrobacteria are indigenous to the soil ecosystem. They are pathogenic Gram-negative bacteria that cause crown gall or hairy root disease in plants. The genetic information for tumor growth is encoded on a tumor inducing plasmid (Ti plasmid) or hairy root-inducing plasmid (Ri plasmid) in the genome of these bacteria. There are generally two types of Agrobacterium species that are commonly used in plant transformation; Agrobacterium tumefaciens and Agrobacterium rhizogenes. A. tumefaciens contains the Ti plasmid which causes crown gall disease, whereas A. rhizogenes contains the Ri plasmid that causes hairy root disease. The discovery of these two species provides efficient vector

systems for the development of transgenic plants when the detrimental genes in Agrobacteria are removed.

Direct gene transfers: Direct gene transfer, as the name suggests, involves the direct introduction of exogenous DNA (naked DNA) into the plant nucleus. In order to introduce foreign DNA into the plant cell, the outer membrane of the cell is first disrupted, permeating it for foreign DNA to enter. Most of the methods under direct gene transfer are simple and effective. However, gene expression in these transgenic plants can be transiently or stably transformed. Direct gene transfer can be categorized into two main groups:

1. Physical gene transfer
2. Chemical gene transfer.

Physical gene transfer disrupts the cell wall and cell membrane via mechanical means. Among these methods, particle bombardment biolistic is the most common one used in plant transformation. The DNA coated with gold or tungsten particles are shot into the target plant cell under high pressure using a “Gene Gun”. The fast-moving particles allow for the penetration of coated DNA through the thick plant cell wall, directing the foreign DNA into its nucleus. The coated DNA will then separate from the metal particles and integrate itself into the chromosomes within the nucleus of the plant cell. This method had been found to be effective in transforming both dicots and monocots which compensates for the less successful Agrobacterium-mediated transformation process. Furthermore, it is also less toxic and applicable to almost all plant cells. The major setbacks of this method, however, lie in the availability of special instruments as well as the delivery efficiency of DNA fragments to the plant nucleus instead of other organelles.

Other physical gene transfer methods include electroporation that uses electrical impulses to facilitate the transfer of foreign DNA into the plant

cells. Plant cells are first incubated in a buffer solution containing foreign DNA, followed by the application of electrical impulses into the buffer, resulting in the formation of temporary transient pores on the cell membrane of the plant to allow the foreign DNA to enter. This method is relatively easy and time saving but is only applicable to protoplasts (cell without cell wall). Hence, this method is not commonly practiced in plant transformation.

Chemical gene transfer approaches involves the use of chemical to disrupt cell membrane enabling the entry of foreign DNA. This particular method is not preferable in plant transformation as it is only effective when applied to protoplasts. One of the most prominent chemicals used in this approach is polyethylene glycol (PEG) that is used for destabilizing the cell membrane in the presence of a divalent cation, thus increasing the permeability of the cell membrane, allowing for the uptake of foreign DNA. The exact mechanism for chemical gene transfer is not fully understood, but it was postulated that PEG increases the osmotic pressure and causes contraction in the protoplast; this facilitates endocytosis of the divalent cation/DNA complex. Besides those, liposome is another chemical method that is used in the transformation of plant's protoplast cells. Liposomes act as vehicles to encapsulate and deliver foreign genetic materials into the protoplast. The lipophilic attribute of liposomes provide easy access into the protoplast in transforming the cell.

C. MOLECULAR MARKERS

A molecular markers (~ genetic marker or DNA marker) is a DNA sequence with a known physical location on a chromosome. Genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromo- some or

a gene. Genetic markers used in genetics and plant breeding can be classified into two categories: classical markers and DNA markers (Xu, 2010). Classical markers include morphological markers, cytological markers and biochemical markers. DNA markers have developed into many systems based on different polymorphism-detecting techniques or methods (south- ern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing) (Collard et al., 2005), such as RFLP, AFLP, RAPD, SSR, SNP, etc.

Classical markers

Morphological markers: Comprise of visible traits, such as leaf shape, flower color, pubescence color, pod color, seed color, seed shape, hilum color, awn type and length, fruit shape, rind (exocarp) color and stripe, flesh color, stem length, etc. They are less abundant and are significantly influenced by environment.

Cytological markers: In cytology, the structural features of chromosomes can be shown by chromosome karyotype and bands. The banding patterns, displayed in color, width, order and position, reveal the difference in distributions of euchromatin and heterochromatin. For instance, Q bands are produced by quinacrine hydrochloride, G bands are produced by Giemsa stain, and R bands are the reversed G bands.

Biochemical/protein markers: Protein markers may also be categorized into molecular markers though the latter are more referred to DNA markers. Isozymes are alternative forms or structural variants of an enzyme that have different molecular weights and electrophoretic mobility but have the same catalytic activity or function. Isozymes reflect the products of different alleles rather than different genes because the difference in electrophoretic mobility is caused by point mutation as a result of amino acid substitution.

Molecular (DNA) markers

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Molecular (DNA) markers are defined as a fragment of DNA revealing mutations/variations, which can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool. Such fragments are associated with a certain location within the genome and may be detected by means of certain molecular technology. Simply speaking, DNA marker is a small region of DNA sequence showing polymorphism (base deletion, insertion and substitution) between different individuals. They are of two types namely:

(i) Genetic or linked markers- those markers which are tightly linked to trait of interest and

(ii) (ii) Direct or Genic markers that are part of gene itself.

Types of Molecular markers

RFLP (Restriction fragment length polymorphism): RFLP markers are the first generation markers and one of the important tools for plant genome mapping. They are based on Southern-Bolttin and caused by mutation events (deletion and insertion) at restriction sites or between adjacent restriction sites in the genome. Gain or loss of restriction sites resulting from base pair changes and insertions or deletions at restriction sites within the restriction fragments may cause differences in size of restriction fragments.

RAPD (Random amplified polymorphic DNA): RAPD is a PCR-based marker system. In this system, the total genomic DNA of an individual is amplified by PCR using a single, short (~ ten nt or b) and random primer. The primer which binds to many different loci is used to amplify random sequences from a complex DNA template that is complementary to it.

AFLP (Amplified fragment length polymorphism): AFLPs are PCR-based markers, simply RFLPs visualized by selective PCR amplification of DNA restriction fragments. Technically, AFLP is based on the selective PCR amplification of restriction fragments from a total double-digest of

genomic DNA under high stringency conditions, i.e., the combination of polymorphism at restriction sites and hybridization of arbitrary primers. Because of this AFLP is also called selective restriction fragment amplification (SRFA)

SSR (Simple sequence repeats): SSRs, also called microsatellites, short tandem repeats (STRs) or sequence-tagged microsatellite sites (STMS), are PCR-based markers. They are randomly tandem repeats of short nucleotide motifs (2-6 bp/nucleotides long). Di-, tri- and tetra-nucleotide repeats, e.g. (GT)n, (AAT)n and (GATA)n, are widely distributed throughout the genomes of plants and animals. The copy number of these repeats varies among individuals and is a source of polymorphism in plants.

SNP (Single nucleotide polymorphism): An SNP is a single nucleotide base difference between two DNA sequences or individuals. SNPs can be categorized according to nucleotide substitutions either as transitions (C/T or G/A) or transversions (C/G, A/T, C/A or T/G). In practice, single base variants in cDNA (mRNA) are considered to be SNPs as are single base insertions and deletions (indels) in the genome.

Table: Comparative analysis of various marker systems (Source: Jiang, 2013)

Parameter	RFLP	RAPD	AFLP	SSR	SNP
Number of loci	<1000	<1000	<1000	1000-10000	<100000
Polymorphism	Moderate	High	High	High	High
Amount of DNA	Large	Medium	Small	Small	Small
Quality of DNA	High	Average	High/ Average	Average	Average
Cloning/ sequencing	Yes	No	No	Yes	Yes

requirement					
Marker type	Southern blotting	PCR	Both	PCR	PCR
Inheritance	Codominant	Dominant	Dominant	Codominant	Codominant
Radioactive detection	Yes	No	Yes/No	No	No
Reliability	High	Low	High	High	High
Type of polymorphism	Single base changes	Single base changes	Single base changes	Changes in repeat length	Single base changes
Technical complexity	Moderate	Low	Moderate	Low	Low
Cost per analysis	Low	Low	Low	Moderate	Low
Ease of use	Not easy	Easy	Moderate	Easy	Easy
Ease of automation	Low	Moderate	Moderate to high	High	High
Major use	Genetics	Diversity	Genetics & diversity	All purposes	All purposes

Considerations for use of molecular markers in plant breeding

DNA quantity and quality. Some marker techniques require large amounts and high quality of DNA, which may sometimes be difficult to obtain in practice, and this adds to the cost of the procedures.

Reliability. Markers should be tightly linked to target loci, preferably less than 5 cM genetic distance. The use of flanking or genic markers will greatly increase the reliability of the markers to predict phenotype

Technical ease. The level of simplicity and the time required for the technique are critical considerations. High-throughput simple and quick methods are highly desirable.

Level of polymorphism. Ideally, the marker should be highly polymorphic in breeding material (i.e. it should discriminate between different genotypes), especially in core breeding material.

Cost of analysis. The marker assay must be cost-effective in order for MAS to be feasible.

Type of inheritance: Co-dominant markers are more desirable as heterozygotes can be distinguished from homozygotes)

Effect on other traits: No detrimental effect on phenotype

Advantages of MAS over conventional phenotypic selection

a. *It may be simpler than phenotypic screening, which can save time, resources and effort.* Classical examples of traits that are difficult and laborious to measure are cereal cyst nematode and root lesion nematode resistance in wheat. Other examples are quality traits which generally require expensive screening procedures.

b. *Selection can be carried out at the seedling stage.* This may be useful for many traits, but especially for traits that are expressed at later developmental stages. Therefore, undesirable plant genotypes can be quickly eliminated.

c. *Single plants can be selected.* With MAS, individual plants can be selected based on their genotype. For most traits, homozygous and heterozygous plants cannot be distinguished by conventional phenotypic screening.

Application of MAS in plant breeding

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1. **Tracing favorable allele(s)** (dominant or recessive) across generations; in order to accumulate favorable alleles, –
2. **Identifying the suitable individuals** among segregating progenies, based on the allelic composition of a part or of the entire genome and
3. **Cultivar identity/assessment of 'purity':** Markers can be used to confirm the true identity of individual plants. The maintenance of high levels of genetic purity is essential in cereal hybrid production in order to exploit heterosis.
4. **Assessment of genetic diversity and parental selection:** Breeding programmes depend on a high level of genetic diversity for achieving progress from selection. Broadening the genetic base of core breeding material requires the identification of diverse strains for hybridization with elite cultivars.
5. **Study of heterosis:** For hybrid crop production, especially in maize and sorghum, DNA markers have been used to define heterotic groups that can be used to exploit heterosis (hybrid vigour).
6. **Identification of genomic regions under selection:** The identification of shifts in allele frequencies within the genome can be important information for breeders since it alerts them to monitor specific alleles or haplotypes and can be used to design appropriate breeding strategies.
7. **Marker-assisted pyramiding**: Pyramiding is the process of combining several genes together into a single genotype. Pyramiding may be possible through conventional breeding but it is usually not easy to identify the plants containing more than one gene. Using conventional phenotypic selection, individual plants must be evaluated for all traits tested.
8. **Early generation marker-assisted selection**: Although markers can be used at any stage during a typical plant breeding programme, MAS is a great advantage in early generations because plants with undesirable gene combinations can be eliminated. This allows

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breeders to focus attention on a lesser number of high-priority lines in subsequent generations.

SYNTHETICS and COMPOSITE

The major shortcomings of hybrid cultivars as discussed coupled with low seed yield and poor vegetative growth of inbred lines has lead to the concept of developing synthetic and composite varieties especially in developing nations where hybrids do not enjoy such dominance as in case of developed countries. Synthetics and Composites are advanced generations of cross fertilized seed mixture of a few selected parents. Bernardo (2002) defined synthetics as random mating populations formed by intermating a group of inbred lines which have been tested for combining
ability while as, composites are a random mating population formed by intermating a group of populations. Hayes and Garber (1919) were first to suggest commercial use of synthetics as an alternative to hybrid cultivars possibly due to ease of development and maintenance and enlarged working life of a synthetic variety.

Basic steps involved in development of synthetic variety are:-

1. Isolation of one generation selfed lines (S_1)

2. Testing of lines in a top cross to assess their GCA.

3. Intercrossing the superior lines to produce the Syn1 generation.

4. Advancing Syn1 generation under random mating for seed increase to produce Syn2 generation.

Wright (1921) was first to give theoretical basis of predicting expected performance of synthetics. The general consideration is that Syn1 will have highest performance and as in case of F_2 of a single cross hybrid there will be depreciation in performance of Syn2. Wright's formula for performance of Syn2 developed from a group of inbred lines is:

$$F_1 (Syn_2) = F_1(Syn_1) – (Syn_1 – P) / N$$

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Where,

F_2= Expected performance of Syn2

F_1= Mean performance of Syn1

P= average performance of Inbred lines

N= number of inbred lines

Operations in producing a synthetic variety

By definition, a synthetic variety consists of all possible crosses among a number of lines that combine well with each other. The lines that make up a synthetic variety may be inbred lines, clones, open-pollinated varieties, short-term inbred lines or other populations tested for GCA or for combining ability with each other. The operations involved in the production of synthetic varieties are briefly described below.

Evaluation of Lines for GCA

GCA of the lines to be used as the parents of synthetic varieties is generally estimated by topcross or polycross test. The lines are evaluated for GCA because synthetic varieties exploit that portion of heterosis, which is produced by GCA. Polycross refers to the progeny of a line produced by pollination with random sample pollen from a number of selected lines. Polycross test is the most commonly used test in forage crops. Polycross progeny are generally produced by open pollination in isolation among the selected lines. The lines that have high GCA are selected as parents of a synthetic variety.

Production of A Synthetic variety

A synthetic variety may be produced in one of the following two ways.

1. Equal amounts of seeds from the parental lines are mixed and planted in isolation. Open-pollination is allowed and is expected to produce crosses in all combinations. The seed from this population is harvested in bulk; the population raised from this seed is the Syn1 generation.

2. All possible crosses among the selected lines are made in isolation. Equal amounts of seed from each cross are composited to produce the synthetic variety. The population derived from this composited seed is known as the syn1 generation.

Multiplication of Synthetic Varieties

After a synthetic variety has been synthesized, it is generally multiplied in isolation for one or more generations before its distribution for cultivation. This is done to produce commercial quantities of seed, and is a common practice in most of the crops, e.g., grasses, clovers, maize etc. But in some crops, e.g., sugar beets, the synthetic varieties are distributed without seed increase, i.e., in the Syn1 generation.

The open-pollinated progeny from the Syn1 generation is termed as Syn2, that from Syn2 as syn3 etc. The performance of Syn2 is expected to be lower than that of syn1 due to the production of new genotypes and a decrease in the level of heterozygosity as a consequence of random mating. However, there would not be a noticeable decline in the subsequent generations produced by open-pollination since the zygotic equilibrium for any gene is reached after one generation of random mating. The synthetic varieties are usually maintained by open-pollinated, and may be further improved through population improvement schemes, particularly through
recurrent selection.

Composite variety is produced by mixing the seeds of several phenotypically outstanding lines and encouraging open-pollination to produce crosses in all combinations among the mixed lines. The lines used to produce a composite variety are rarely tested for combining ability with each other. Consequently, the yields of composite varieties cannot be predicted in advance for the obvious reason that the yields of all the F_1's among the component lines are not available. Like synthetics,

composites are commercial varieties and are maintained by open-pollination in
isolation.

Merits of synthetic varieties

Synthetic varieties offer several unique advantages in comparison to hybrid varieties in the exploitation of heterosis. These advantages are listed below.

1. Synthetic varieties offer a feasible means of utilizing heterosis in crop species where pollination control is difficult. In such species, the production of hybrid varieties would not be commercially viable.
2. The farmer can use the grain produced from a synthetic variety as seed to raise the next crop.
3. In variable environments, synthetics are likely to do better than hybrid varieties. This expectation is based on the wider genetic base of synthetic varieties in comparison to that of hybrid varieties.
4. The cost of seed in the case of synthetic varieties is relatively lower than that of hybrid varieties.
5. Seed production of hybrid varieties is a more skilled operation than that of synthetic varieties.
6. Synthetic varieties are good reservoirs of genetic variability. The composites and germplasm complexes also serve as gene reservoirs.
7. There is good evidence that the performance of synthetic varieties can be considerably improved through population improvement without appreciably reducing variability.

Demerits of synthetic varieties

1. The performance of synthetic varieties is usually lower than that of the single or double cross hybrids. This is because synthetics exploit only GCA, while the hybrid varieties exploit both GCA and SCA.
2. The performance of synthetics is adversely affected by lines with relatively poorer GCA. Such lines often have to be included to increase

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the number of parental lines making up the synthetic as lines with outstanding GCA are limited in number.

3. Synthetics can be produced and maintained only in cross-pollinated crop species, while hybrid varieties can be produced both in self- and cross-pollinated crops.

Table. Difference between synthetics and composites

Synthetics	**Composites**
No. of inbred lines are less (6-8)	No. of lines are more (even upto 20)
GCA of parental lines is tested	GCA of parental lines is not tested
Performance can be predicted	Performance cannot be predicted
Synthetic can be reconstituted	Synthetic cannot be reconstituted
Seed replacement after 4-5 years	Seed replacement after 3-4 years

D. POPULATION IMPROVEMENT

POPULATION

In cross-pollinated crops, populations are an alternative to the hybrids owing to the operational simplicity of development of populations. In context of an allogamous crop population refers to a group of freely intermating individuals sharing a common gene pool. Such populations are also referred to as "**Random Mating populations**", "**Panmictic Populations**" or "**Mendelian Populations**". Their genetic structure is governed by **Hardy-Weinberg** law that states that the gene and genotype frequencies of a random mating population remains same generation after

generation provided there are no external forces acting upon the population such as selection, mutation, migration or genetic drift.

POPULATION IMPROVEMENT:

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Sprague (1966) used the term population improvement to denote any breeding procedure aimed at developing improved populations. Specifically applied to cross pollinated crops, such a breeding procedure refers to cyclical improvement in mean performance of a population that has undergone or is undergoing selection but the final product retains high degree of heterozygosity. All population improvement programmes involve three major operations constituting one cycle.

i. Development of progenies.
ii. Evaluation of progenies.
iii. Intermating of selects to generate population for next cycle.

Hallauer (1980) stated that population improvements are cyclical procedures that alternate between these three phases to ensure continuous accumulation of favorable alleles by selective intermating f superior genotypes that are expected to contain favorable alleles.

Genetic Basis of Population Improvement:-

As against hybrid breeding which depends upon exploitation of dominance component of genetic variance, population improvement relies heavily on the additive component of variance. The aim in population improvement programme is to provide for accumulation of favorable alleles for a trait that would improve the mean performance of that trait due to cumulative action of all genes as well as favorable interactions between those genes. A continuous accumulation of such favorably acting and interacting genes leads to an improved population without reaching a dead end. Any material to be used as a base population for population improvement programme must have:

i. Adequate genetic variability for the traits for which improvement is sough
ii. Substantial additive genetic variance plus favorable epistatic interactions.

iii. High heritability of traits.
iv. Absence of undesirable linkages
v. Absence of negative association between the desirable traits.

Advantages of Populations

- They are stable and highly adaptable and can withstand and buffer changes in environment.
- Seed has not been replaced every year and seed production is quite easy as compared to hybrids.
- Populations unlike hybrids are dynamic. The coadapted genotypes provide a barrier for rapid evolution of races in pathogens.
- Populations are amenable for further improvements as they retain sufficient variability after many selection cycles.

TYPES OF POPULATION IMPROVEMENT:-

Intra population Improvement:- Intra population improvement refers to any method that seeks to improve a population per se by exploiting the genetic variability present in the population. Thus the recombination phase involves genotypes carved out of the same population without introgression from other sources. The commonly used procedures are:

Mass Selection:- It is the selection based on phenotype of superior plants and bulking their seeds to raise the next population as discussed in chapter vi, a major limitation of mass selection is that since selection is based on phenotype, it is not known whether the superiority of selected plant is due to genetic factors or effect of environment. In cross pollinated crops, another major drawback of mass selection is lack of pollen control. Moreover, it is efficient in case of traits having high heritability. Lack of control over male parentage has a serious implication on the genetic gain

In order to overcome obvious limitations of mass selection such as lack of control over male parent, low response for traits with low heritability and confusing effects of environment especially soil variability. Gardner, (1961) suggested following modifications:

i. Growing of selected population in isolation to minimize introgression from unknown sources.
ii. Division of plot into subplots or grids and selecting plants from each grid to minimize G x E effects.
iii. Growing of plants at low plant density to allow optimum expression of traits allowing better discrimination of genotypes.

In this modified procedure, known as "Gridded mass selection" or "Stratified mass selection" the seed of selected population is divided into three sets. One is used to grow next generation, other is used to estimate genetic gain while as third set is kept as reserve seed. Faceolus and Boss (1983) suggested another modification of mass selection in which plants are grown in equidistant hexagons in various honey comb designs. Each plant to be selected is compared with surrounding plants that are grown under similar or comparable environment.

Ear-to-Row selection: Ear-to-Row selection was first used by Hopkins,1896 at Illinois to improve oil and protein content in maize. In this method, promising plants are selected based on phenotype, from a population grown in isolation. The selected ears are not bulked as in case of mass selection. The seeds of selected ears are divided into two sets. One set is grown to assess the performance of selected plants and based on the progeny performance, the next cycle is started using the eminent seeds. The seeds of each ear are sown as a progeny row. The method was very popular especially among, maize growers but resulted in low genetic gain for traits with low heritability. Besides, continued ear-to-row selection leads to decline in vigor on account of inbreeding.

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Half-sib progeny selection:- Half-sibs are a family of individuals having either parents as common (male or female). Hallauer and Carena (2009) stated that halfsib family selection is of greater use than any other method in maize breeding. The method implicates use of a tester which is common to the selected plants. The method has variations in terms of the nature of tester used. Jenkins (1940) used source population as a tester whereas, Hull (1945) suggested use of an inbred line or a single cross hybrid as a tester.

Selected plants are selfed and crossed to a tester from the same population. The half-sib families are evaluated in replicated trials. Based on the performance of half-sib families, the selected families are grown from selfed seeds and all possible crosses are made among the plants to ensure recombination of the superior alleles, and a new population is derived to start new cycle of half-sib selection. Half-sib family selection should be clearly differentiated from half-sib test in which the selected plants based on test cross performance, are not grown from selfed seed but the remnant half sib cross seed.

Full-Sib Family Selection:- Full-sibs are the family of individuals having both parents in common. In cross-pollinated plants they are produced by selfing and crossing two plants from the same population. Hallauer and Eberhart (1970) suggested use of prolific plants for making reciprocal crosses between two plants. In practice, Full-sib families are evaluated in replicated trials for yield, disease and pest resistance or any other trait. Based on the performance, best 30% families can be selected and grown in progeny rows for remnant seeds. Crosses are made among
best plants o selected families which constitute next batch of full-sib families.

Selfed Plant Selection:- Selfed plant selection schemes are based on selfed progenies wherein S1,S 2 or an advanced selfed generation is used for evaluation. The progenies that emerge as superior in evaluation are

recombined using the remnant seed. The level of Inbreeding is arbitrary and depends upon availability of time and resources. Thus, use of S1 lines will require three seasons, S2 lines will require four seasons and S_3 lines will require five seasons. However, S3 are seldom uses due to time constraint. A typical S_1 selection involves development of one generation selfed lines, evaluation of lines and recombination of selected lines. Selfed progeny
selection methods usually give higher gains but gains are adversely affected over time when contribution of non-additive variance is increased (Vasal et al, 2004). In order to maintain selection gains over time, Hallauer (1992) suggested that at least 20-50 selfed progenies be recombined. Many workers have reported the non-sustainability of selfed progeny selection over long term basis due to rapid fixation of pollinated crops are adapted to heterozygous balance and do not seem to respond to selfed progeny selection.

Alternate Recurrent Selection:- Dhillon (1991) proposed alternate recurrent selection based on the evaluation of different types of families. Among various schemes, ARS involving S1 and half-sib is the most efficient. It requires four seasons for one cycle. The phenotypically superior plants are selected and selfed to develop S1 families which are evaluated in next season. Based on performance of S1 lines, superior lines are recombined to produce halfsib families. The half-sib families are evaluated in fourth season and based on the performance, selected halfsib families are selfed to produce S1 families for the next cycle of selection..

Inter Population Improvement:- Inter population improvement is the set of breeding procedures aimed at cyclical improvement of cross-bred progenies. These are dual purpose selection schemes that help in improving populations as well as developing hybrid oriented germplasm. There is provision for improvement of two heterotic populations

simultaneously and the emphasis is on combining ability rather than per se performance of selects only.

There are two important operational components of inter population improvement i.e. generation of one-generation selfed lines and crossing of lines to a tester (either OPV or an inbred line). The selfing component helps to expose lines to inbreeding pressure to develop as well as purging them of deleterious alleles while as the test crossing helps to evaluate lines for their nicking ability for suitability as parental lines in hybrid breeding or development of synthetics and composites.

RECURRENT SELECTION

Recurrent selection refers to selection generation after generation with inter-mating of selects

There are four types of recurrent selections.

1. Simple recurrent selection
2. Recurrent selection for GCA
3. Recurrent selection for SCA
4. Reciprocal recurrent selection

1. Simple Recurrent selection :

I year : Several phenotypically superior plants are selected selfed. Harvested separately and evaluated. Seed of superior plants retained and the rest are discarded.

II year : Individual plant progeny rows are raised. The progeny rows are intercrossed in all possible combinations. Equal amounts of seed from each cross is taken and mixed. This forms the source for next selection cycle.

III year: Seed obtained in II year is planted Number of superior plants First selection selection and harvested separately. Seed evaluated. Seeds of superior plants retained and the rest discarded.

IV year : Progeny rows are raised. Inter crossed in all possible ways. Equal amount of seed from each cross is composited. This mixed seed forms the source for next selection cycle.

2. Recurrent selection for general combining ability

Recurrent selection for GCA was first suggested by Jenkins in 1935. in this method a tester with broad genetic base i.e. open pollinated variety or a synthetic or segregating generations is used for evaluating the lines for GCA.

Procedure :

I year : Several phenotypically superior plants are selected from source population. Each selected plant is selfed as well as crossed to a tester with broad genetic base. The selfed seeds are harvested separately and saved for planting in the third year. The test crossed seeds also harvested separately.

II year : A replicated yield trial is conducted using the test crossed seeds. At the end the superior progenies are identified.

III year : Selfed seed (from the first year) of the plants that produced superior progenies on the basis of yield trial of second year is planted in separate progeny rows. These progenies are inter crossed in all possible combinations. Equal amount of seed from each intercross is composited to raise the source population for next selection cycle.

IV year : Source population is raised from the composited seeds. Several phenotypically superior plants are selected. They are selfed and crossed

to a tester (broad genetic base) selfed seed harvested separately and saved for planting in

V year : Test crossed seed also harvested separately.

VI year : Repeat as in third year. This completes the first selection cycle.

The second and third selection cycle may be initiated if necessary. The recurrent selection for GCA.

1. May be used for improving the yielding ability of the population and the end product may be released as a synthetic variety or

2. May be used for increasing the frequency of desirable genes in the population and the population may be used for isolating superior inbreds.

3. Recurrent selection for specific combining ability :

The recurrent selection for SCA was first proposed by Hull in 1945. the objective is the isolate from a population such lines that will combine well with a given inbred useful for selecting lines for SCA. The procedure for recurrent selection for SCA is identical with that of recurrent selection for GCA, expect that the tester used here is an inbred (narrow genetic base)

4. Reciprocal recurrent selection :

Reciprocal recurrent selection was first proposed by Comstock, Robinson and Harvey in 1949. This would be useful.

1. For selecting both for SCA and GCA
2. For improving two source population simultaneously.

Procedure :

I year : Two source populations (A & B) are taken, several phenotypically superior plants are selected from each population. Each of the selected plant is selfed. Each of the selected plant from source A is crossed with random plants from B. Similar each of the selected plants are crossed with random plants of A. plants of a will act as tester for B. The selfed seed is harvested separately and saved for planting in III year. Top crossed seed from each plant is also harvested separately.

II year : Two replicated yield trials are conducted, progeny rows of Test cross seeds of population A in one plot and test cross seeds of population B in another plot are raised. Plants (I year) producing superior progenies (in II year) are identified.

III year : Selfed seed (saved in I year) from plants selected on the basis of evaluation of progeny rows (in the II year) is planted in plant to row progeny in two crossing plots. Seeds of selected plants from population A in one plot and that of the B in another plot. All possible intercrosses among the progeny rows in each plot are made. Equal amount of seed from all intercrosses from the crossing plot A is mixed to raise the source population of 'A' next year. Similarly equal amount of seed from inter crosses of plot B is mixed to raise source population B next year. This completes original selection cycle.

IV year : Source populations of A & B are raised from composited seeds of A & B (III year). Operations of the first year i.e. selection of plants, selfing and crossing with the plants of other population etc. are done.

V year : operations as in second year are repeated.

VI year : Operations as in third year are repeated. This completes first selection cycle. The populations may be subjected to further selection cycles, if necessary by repeating the procedure outlined above.

Reciprocal recurrent selection is a cyclical breeding procedure designed to
improve cross of two populations from different heterotic groups using both SCA and GCA. The individual plants are selected in both populations and recombined to give rise to improved units while as, intra population S1 progenies are used as recombination units (Santos et al, 2005). In RRS, selection intensity is usually high (10-20%) and S1 are used as recombination units which have low population size. High selection intensity and low population size may cause genetic drift that may reduce variability after some cycles of selection.

Factors influencing recurrent selection efficiency

 Base Population: The success of a recurrent selection program rests on the genetic nature of the base population. The base population should be having substantial genetic variability.

 Parental performance: The parents should have high performance regarding the traits of interest and should not be closely related. This will increase the chance of maximizing genetic diversity in the population.

 Number of parents: As many parents as possible in the initial crossing to increase genetic diversity. Crossing provides opportunity for recombination of genes to increase genetic diversity of the population.

 Cycles of mating: More rounds of mating will increase the opportunity for recombination but it increases the duration of the breeding program. The breeder should decide on the number of generations of intermating that is appropriate for a breeding program

E. MULTILINES

They are mixtures of nearly isogenic lines containing different genes of resistance against different races. They are relevant to self-pollinated crops arecan be reconstituted. Similarly, varietal blends are similar to multilines with the difference that the component lines are not isogenic lines but different purelines or varieties. They are relevant to self-pollinated crops are can be reconstituted. Multilines are homozygous and homogenous.

Features of Multiline Breeding:

1. Application: The multiline approach is applicable to self-pollinated species only. Multiline cultivars are commercially used in self/pollinated crops like oat, wheat, soybean, groundnut and many other crops.
2. Genetic Constitution: Multiline cultivars are mixtures of several pure-lines. The pure-lines may be isogenic lines, closely related lines or unrelated lines. Thus, multi-lines are homozygous but heterogeneous populations or genetically diverse populations. The genotypes which are mixed together to constitute a multiline have phenotypic similarities for several characters like height, maturity, grain colour and size etc.
3. Adaptation: Multi-lines are more adaptable to environmental variations than pure-lines by virtue of their genetic diversity. In other words, multi-lines have more buffering capacity to environmental changes than pure lines. The pure lines are adapted to specific environment but have poor adaptability. Multi-lines have broad genetic base which provides them greater adaptability.
4. Disease Control: The use of multiline cultivars is an effective way to minimize the yield losses due to the attack of multiracial disease. In a multiline cultivar, each component genotype has a resistant gene for a different race of a disease. All races of a disease will never appear at a time and all the genotypes of a heterogeneous mixture are never attacked at a time.

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5. Quality of Produce: The produce of multiline cultivars is generally less uniform and less attractive than that of a pure-line, because it is a mixture of several pure lines.

6. Yield: The yield of a multiline would be lesser than that of the most productive cultivar of a pure-line under normal conditions. But under adverse conditions, the yield of a multiline would be much higher than that of most productive pure-line cultivar.

Types of Multiline Breeding:

There are three types of multi-lines: viz.:

(1) Mixtures of isolines,

(2) Mixtures of closely related lines, and

(3) Mixtures of unrelated or distinctly different genotypes.

These are briefly described below:

1. Mixtures of Isolines:

Isolines are genotypes having one gene difference only. Isolines are developed by backcross method. The resistant genes for different races of a disease are transferred into one popular variety by separate backcross programmes. Six to ten isolines are developed and their seeds are mixed in equal quantity to constitute a multiline. Such varieties have been developed in oat, soybean and wheat in USA.

2. Mixtures of Closely Related Lines:

Sometimes, multiline cultivars are constituted by mixing the seed of closely related lines. Closely related lines are developed from crosses having one parent in common. This approach of multi-lines development is being followed now-a-days at important breeding centres like CIMMYT.

A mixture of closely related lines of wheat KSML 3 was released from Ludhiana to provide better resistance to rust disease. The six

components of multiline were derived from crosses with popular cultivar Kalyan Sona as the common parent. Different types of crosses were made to develop each of the components including single crosses and limited backcrossing. In this case the component lines are not isolines. They differ in several characters from each other.

3. Mixtures of Unrelated Lines or Cultivars:
Multi-lines are also constituted from seed mixtures of distinctly different cultivars. Such multi-lines are developed when phenotypic uniformity is not essential. First pure-lines are developed by pedigree, bulk or single seed descent methods. The performance of each pure-line is evaluated and then superior pure-lines each with different gene for resistance are mixed together to constitute multiline. This is almost similar to varietal blends.

Procedure of Developing Multiline Breeding: The development of a multiline consists of four important steps:

(1) Selection of Recurrent Parent: The recurrent parent should be a high yielding popular variety. The recurrent parent should be the best cultivar of a region.

(2) Selection of Donor Parents: Parents with resistance to various races of a disease should be chosen as donor parents. The resistance should be thoroughly examined under artificial epiphytotic conditions before use of the donor parents in the crossing programmes. The donor parents should be adapted varieties as far as possible.
Because in un-adapted parents disease resistance is sometimes linked with several un-desirable characters and transfer of resistant genes from such parents to the recurrent parent becomes difficult task.
Several donor parents are selected to incorporate different resistant genes against various races.

(3) Transfer of Resistance: The resistant genes are transferred from donor parents to the recurrent parent through a series of several separate backcross programmes. Generally 4-5 backcrosses are sufficient to retain the genotype of recurrent parent with added resistance in the backcross derivatives. The backcross derivatives are evaluated for disease resistance during backcrossing and also at the end of backcrossing. The desirable lines from each backcross are mixed to form an isoline.

(4) Mixing of Isolines: The various isolines developed by various backcrosses are mixed together to constitute a multiline cultivar. Generally 6-10 isolines are mixed to constitute a multiline cultivar.

Merits of Multiline Breeding:

1. Multi-lines are more adaptable to environmental changes than pure-line cultivars due to genetic diversity.

2. They provide better protection form the infection of new race of a disease.

Multiline cultivars have been developed for commercial cultivation in oats, wheat, soybean and peanut in USA. In India three multiline varieties, viz. KSML 3, MLKS 11 and KML 7404 have been released in wheat from Punjab. The first two varieties involve 8 closely related lines and the third one involves 9 closely related lines.

Demerits of Multiline Breeding:

1. The produce of multiline varieties is less attractive and less uniform due to mixture of several pure-lines.

2. Development of multiline cultivars involves several backcrosses and hence is costlier than conventional breeding methods.

Course Name	Principles of Plant Breeding
Lesson 16	Heterosis Concept and Genetic Basis
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the basic concept and genetic basis of heterosis and inbreeding depression
2. Understand genetic implications of heterosis and inbreeding depression

Glossary of terms

Heterosis: Heterosis is defined as the superiority of F_1 over its parents. It is also called as Euheterosis

Luxuriance: Luxuriance is defined as the increased vigour and size of interspecific hybrids. However, Heterosis is accompanied with an increased fertility, while luxuriance is expressed by interspecific hybrids that are generally sterile or poorly fertile.

Relative heterosis: - When F_1 is better than the Mid parental value. Also called as **Mid-parental heterosis**

Better parent heterosis:- When the F_1 is better than the better parent. It is also called as **Heterobeltiosis**

Standard Heterosis: - When the F_1 is better than the best available variety, used as check. It is also called as **Economic heterosis**.

Dominance hypothesis: Dominance hypothesis assumes that heterosis is due to non-expression of deleterious recessive alleles in presence of beneficial dominant alleles in the resulting $F_1$from two parents.

Over-dominance hypothesis: This theory assumes heterozygosity as the major cause of heterosis by providing physiological stimulus to improved development. Also called as '**Single gene heterosis'**; '**Superdominance**' or '**Cumulative action of divergent alleles'**.

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Heterosis

Heterosis is defined as the superiority of F_1 over its parents. The term heterosis was coined by **Shull (1908)**. However, in certain cases, the hybrid may be desired to be inferior to the weaker parent. This is also regarded as heterosis. The term heterosis was coined by Shull in 1912 for quantitative measure of superiority of F_1 over its parents.

The phenomenon of heterosis has been exploited extensively in crop production and has been a powerful force in the evolution of plants. The successful demonstration of yield advantage of hybrid maize in 1930 gave great impetus to breeders of other crops including rice to utilize the principle of hybrid production by exploiting heterosis. Undoubtedly, the exploitation of heterosis has been regarded as the greatest practical achievement of the science of genetics and plant breeding.

Luxuriance

Luxuriance is defined as the increased vigour and size of interspecific hybrids. The principal difference between heterosis and luxuriance lies in the reproductive ability of the hybrids. Heterosis is accompanied with an increased fertility, while luxuriance is expressed by interspecific hybrids that are generally sterile or poorly fertile. In addition, luxuriance may not result from either masking of deleterious genes or from balanced gene combinations brought together into the hybrid. Therefore, luxuriance does not have any adaptive significance.

The concept of heterosis has originally been evolved in maize and later applied to other crops as well. Based on expression, heterosis is classified as True

1. ***Relative heterosis*** or ***Mid-parental heterosis***:- When F_1 is better than the Mid parental value
2. ***Better parent heterosis***:- When the F_1 is better than the better parent. It is also called as **Heterobeltiosis**

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3. **Standard Heterosis**: - When the F_1 is better than the best available variety, used as check. It is also called as Economic heterosis.

Manifestations of Heterosis

Heterosis is the superiority of a hybrid over its parents. This superiority may be in yield, quality, disease and insect resistance, adaptability, general size or the size of specific parts, growth rate, enzyme activity, etc. These various manifestations of heterosis may be summarised as follows.

1. Increased yield. Heterosis is generally expressed as an increase in the yield of hybrids. Commercially, this phenomenon is of the greatest importance since higher yields are the most important objective of plant breeding. The yield may be measured in terms of grain, fruit, seed, leaf, tubers or the whole plant.

2. Increased Reproductive Ability. The hybrids exhibiting heterosis show an increase in fertility or reproductive ability. This is often expressed as higher yield of seeds or fruits or other propagules, e.g., tuber in potato (*S. tuberosum*), stem in sugarcane (*S. officinarum*), etc.

3. Increase in Size and General Vigour. The hybrids are generally more vigorous, i.e., healthier and faster growing and larger in size than their parents. The increase in size is usually a result of an increase in the number and size of cells in various plant parts. Some examples of increased size are increases in fruit size in tomato, mango, brinjal etc., head size in cabbage, cob size in maize, head size in jowar, etc.

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4. Better Quality. In many cases, hybrids show improved quality eg. Tomato, bell pepper, cole crops etc. This may or may not be accompanied by higher yields. For example, many hybrids in onion show better keeping quality, but not yield, than open-pollinated varieties.

5. Earlier Flowering and Maturity. In many cases, hybrids are earlier in flowering and maturity than the parents. This may sometimes be associated with a lower total plant weight. But earliness is highly desirable in many situations, particularly in vegetables.

6. Greater Resistance to Diseases and Pests. Some hybrids are known to exhibit a greater resistance to insects or diseases than their parents.

7. Greater Adaptability. Hybrids are generally more adapted to environmental changes than inbreds. In general, the variance of hybrids is significantly smaller than that of inbreds. This shows that hybrids are more adapted to environmental variations than are inbreds. In fact, it is one of the physiological explanations offered for heterosis.

8. Faster Growth Rate. In some cases, hybrids show a faster growth rate than their parents. But the total plant size of the hybrids may be comparable to that of parents. In such cases, a faster growth rate is not associated with a larger size.

9. Increase in the Number of A Plant Part. In some cases, there is an increase in the number of nodes, leaves and other plant parts, but the total plant size may not be larger.

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GENETIC BASIS OF HETEROSIS

The genetic basis of heterosis has been a topic of contentious debate for almost a century now and is still shrouded in mystery. The earlier plant breeders made attempts to elucidate genetic basis of heterosis based on quantitative genetic models but with the advancements in molecular genetics, we have been able to study this phenomenon in a more refined way. In fact the recent studies in maize and rice to attempt at interpretation of heterosis have been greatly facilitated by molecular markers.

The earlier studies put forth two possible mechanisms of heterosis : (i) **Dominance hypothesis** and (ii) **Over-dominance hypothesis**. Theoretically, the two hypotheses are based on two different genetic phenomenon but under most of the situations, both lead to similar expectations. In either case, inbreeding leads to a decline in vigour while as out-breeding leads to increased vigour. In case of both dominance and over-dominance concepts, the decline in vigour is proportional to decrease in heterozygosity irrespective of the number of dominant and recessive alleles and degree of dominance. The difficulty of precise demarcation of either of two basic assumptions arises due to a number of factors especially the inability of distinction between true over-dominance and pseudo-over dominance. **Linkage disequilibrium** often causes bias in estimation of genetic components arising from repulsion phase linkage or epistasis.

DOMINANCE AS MAJOR GENETIC BASIS OF HETEROSIS:-

The dominance hypothesis was promulgated by **Davenport in 1908, Bruce (1910) and Keeble and Pellew (1910)** and later elaborated by Jones. This hypothesis assumes that heterosis is due to non-expression of deleterious recessive alleles in presence of beneficial

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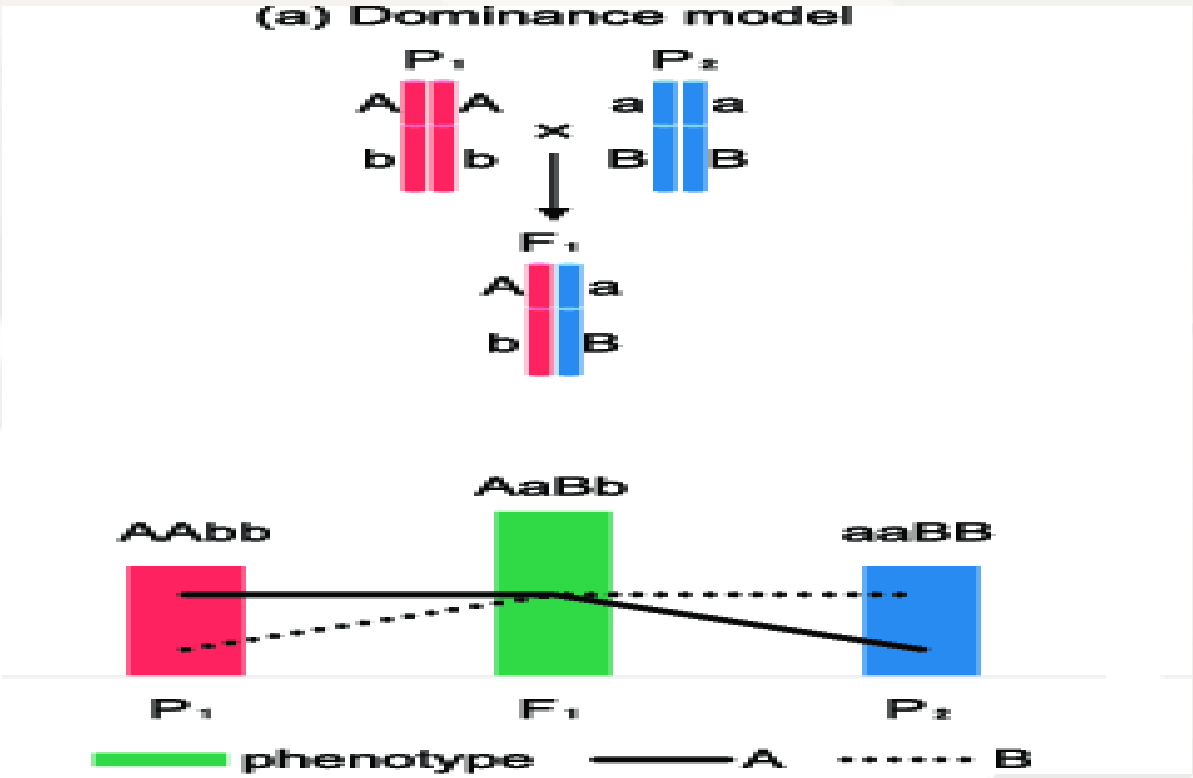
dominant alleles in the resulting F_1 from two parents. Therefore, the F_1 produced from such a cross possess superior characters because of the contribution of dominant alleles from one parent.

Based on the dominance hypothesis, breeders should be able to fix the inbred lines with favourable alleles, and likely produce inbreds equivalent to F_1 hybrids. However, the isolation of such inbreds has been difficult likely due to large number of loci differing between two parents. In fact the opponents of dominance hypothesis put this point as a major evidence against such an explanation of heterosis. This theory is bases on the premise that favourable alleles are dominant while as recessive alleles are deleterious and in F_1, the dominant alleles shield the recessive alleles, hence no deleterious effect.

AABB cc dd x aabb CCDD

Aa Bb Cc Dd



However, there have been various objections to this theory arising out of certain experimental evidences, but explanations have been provided in favour of dominance theory in light of the fact that most of the deviations from expected observations are due to complex inheritance pattern of most economically important traits.

1. **Inability to Isolate Inbreds as Vigorous as Hybrids.** Dominance theory assumes that heterosis is only due to favourable accumulation of dominant alleles and that heterozygote as such has no advantage over homozygotes (Aa = AA). In light of this, it should be possible to develop an inbred line that contains all genes in homozygous dominant form & should be as productive as hybrid, but such a line is not possible to be isolated.

   Jones proposed that, the difficulty in isolating an inbred line contriving all the genes in homozygous dominant condition is due to the large number of genes which deceases the probability of isolation of such line & due to the fact that such a line requires preciously placed crosses, which is impossible under random mating.
2. **Symmetrical Distribution in F_2.** The distribution pattern of F_2 individuals derived from F_1 hybrid is normal as compared to expected skewed which again is due to large number of gene quantitative traits.
3. **Effect of purging:** Since the dominance hypothesis, assumes heterosis to be a result of masking of deleterious alleles which are lethal, with improvement of lines, the magnitude of heterosis should have progressively decreased which has not happened.

Explanations for the Objections.

Jones in 1917 stated that quantitative characters are governed by many genes, and these genes are likely to show linkage. It may be expected that dominant and recessive genes governing a character

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would be linked together. In such a case, inbreds containing all the dominant genes cannot be isolated because this would require several precisely placed crossovers. It would also explain the symmetrical curves obtained in F_2. This phenomenon is also known as **the dominance of linked genes hypothesis.** Similarly, in 1921, **Collins** demonstrated that if the number of genes governing a quantitative character was large, symmetrical distribution would be obtained even without linkage. Further, it is unlikely that a plant containing all the dominant genes would be recovered if the number of genes were large even if they were not linked. This makes distribution curve symmetrical on account of the effects of environment, that is, due to less than 100 per cent heritability.

OVER-DOMINANCE AS MAJOR GENETIC BASIS OF HETEROSIS

The hypothesis advocating over-dominance as major genetic basis of heterosis was first proposed by **Shull (1910) and East (1908).** A similar explanation was later proposed by Gustafsson (1938), Stadler (1939) and Hull (1945). In this theory heterozygosity was proposed as the major cause of heterosis by providing physiological stimulus to improved development. Over-dominance theory is also called as '**Single gene heterosis'**; '**Superdominance**' or '**Cumulative action of divergent alleles'**. Hull (1945) strongly advocated this concept and proposed that F_1 heterosis cannot be accounted for by dominant genes acting additively but can be better explained by over-dominance.

One of the biggest lacuna of this concept is that majority of evidences have been worked out in cases of single locus heterosis while as most of quantitative traits including yield is governed by a number of genes. The overdominance hypothesis for heterosis involves alleles acting in dosage adjustment manner in which neither homozygote is better than heterozygote. With this explanation, it is assumed that

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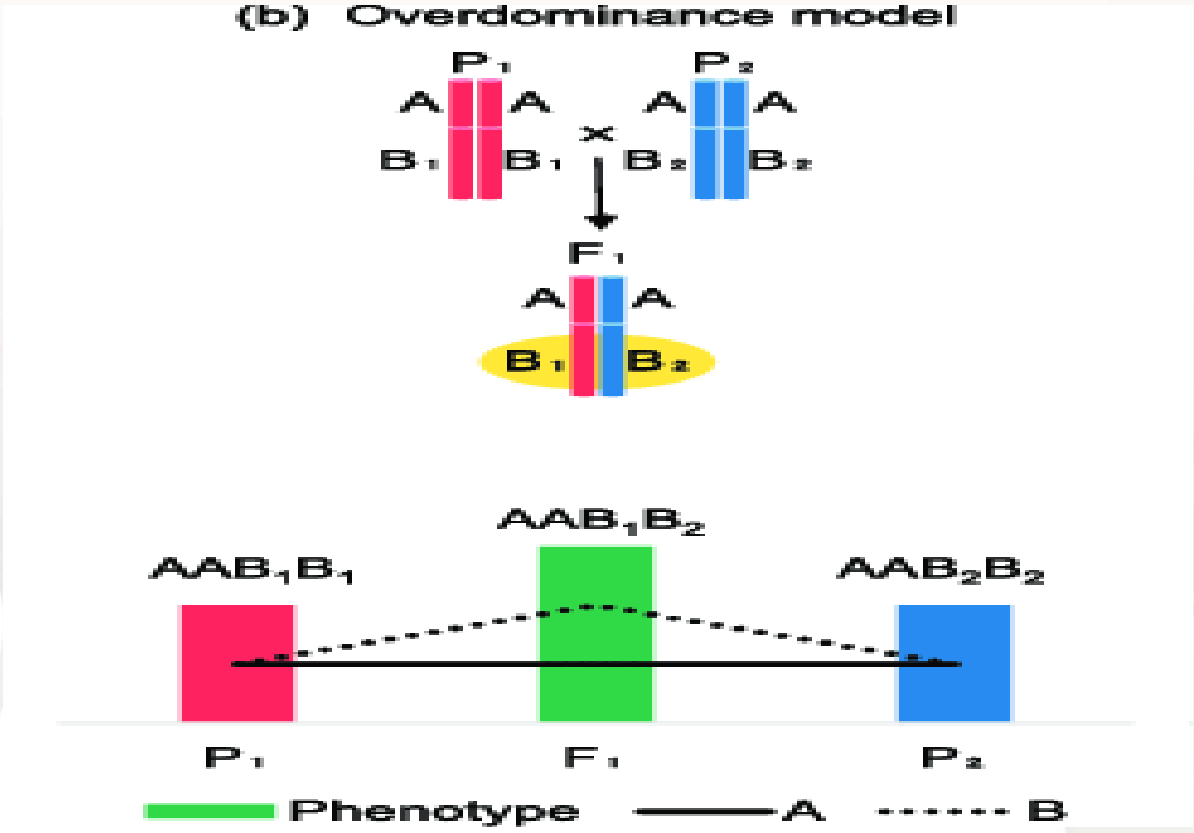
heterozygosity alone is the major genetic basis of heterosis. At the molecular level, the preferable level of gene product by combination effect in heterozygous state results in better catalysis of metabolic pathways that lead to increased growth and yield.

AABB cc dd x aabb CCDD

↓

Aa Bb Cc Dd (F1)



Difficulties in discriminating true overdominance from pseudo-overdominance are major opposition to this hypothesis. Jones (1917) was first to propose that linkage causes great problems in identification of overdominance and in fact pseudooverdominance arising out of repulsion phase linkage may often be misinterpreted as

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true overdominance. In such a situation the pair of linked loci would mimic a single overdominant locus thereby skewing the measure of true overdominance

Jinks (1983) was a strong opponent of overdominance as genetic basis of heterosis in crops like rice where according to him great improvements have been made in performance of inbred lines by alternating cycles of hybridization and re-extraction (pedigree selection). However it is difficult to exclude role of overdominance in heterosis in both autogamous and allogamous crops.

There have also been two major evidences against this hypothesis.

1. **Single locus model:** Most of evidences in support of this theory have been accumulated in case of single locus heterosis whereas most of the quantitative traits are governed by a large number of genes.
2. **Failure to discriminate between true overdominance and pseudooverdominance:** It is different to distinguish between true overdominance and preudo-overdominance resulting on account of repulsion phase linkage.

Similarities between Dominance and Overdominance hypotheses

The two hypotheses have the following similarities.

Inbreeding would produce inbreeding depression.
Outcrossing would restore vigour and fertility.

The degree of heterosis would depend upon the genotypes of the two parents. In general, the greater the genetic diversity between the parents, the higher the magnitude of heterosis.

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Dissimilarities between Dominance and Overdominance hypotheses

Dominance hypotheses	Overdominance hypotheses
Heterozygotes are as good as the dominant homozygote i.e, AA=Aa.	Heterozygotes are superior to the two Homozygotes . According to the overdominance hypothesis i.e, Aa>AA.
Inbreds as vigorous as the F_1 hybrid can be isolated according to the dominance Hypothesis	It will be impossible to derive inbreds as vigorous as the F_1 hybrid.
Inbreeding depression is due to Homozygosity of harmful recessive alleles	Inbreeding depression is due to homozygosity itself.
Heterosis is the result of dominant alleles masking the deleterious effects of their recessive alleles, and heterozygosity itself is not the cause of heterosis.	Heterosis is the consequence of heterozygosity per se

EPISTASIS AS GENETIC BASIS OF HETEROSIS

Dominance and over-dominance (both proposed in 1808) remained the major genetic understandings of the cause of heterosis even though both faced contradictions. The advent of molecular markers made it possible to dissect the loci causing heterosis, in terms of

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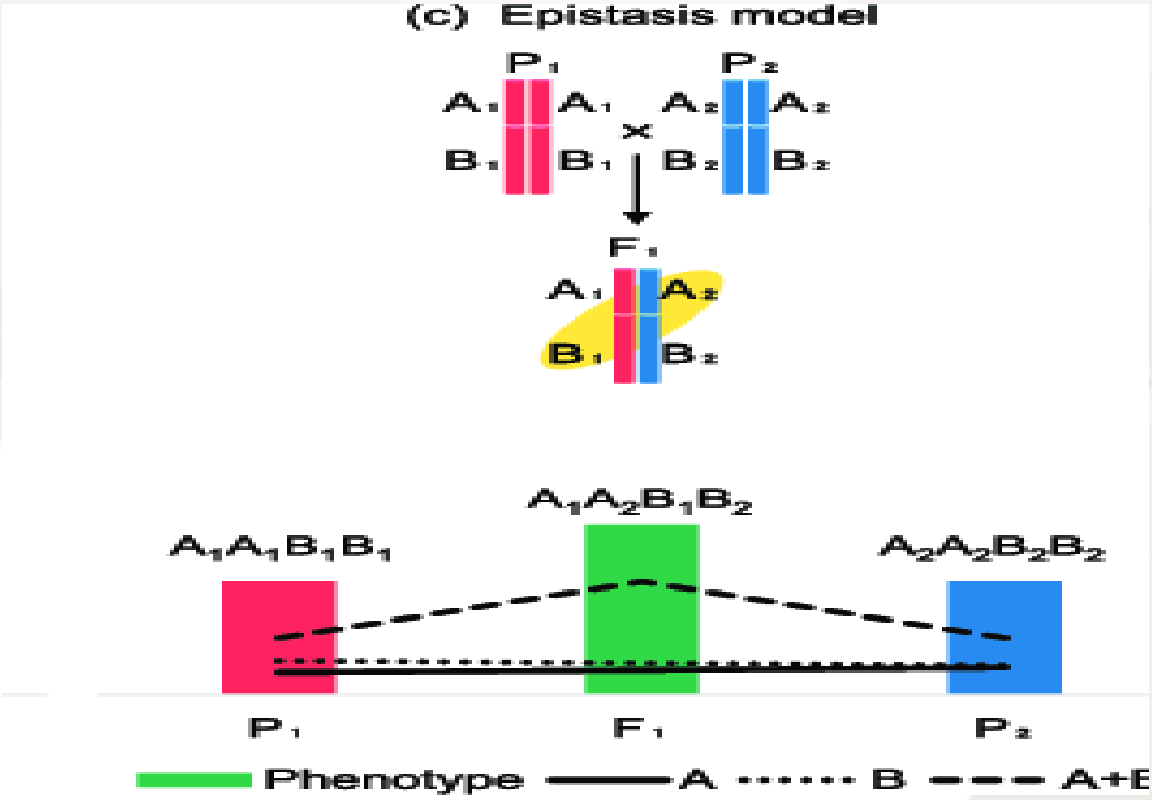
effects and the dominance relationships, with more precision and reliability.

The dominance and over-dominance hypotheses are both based on single-locus model. But Wright (1968) proposed that most of quantitative traits are conditioned by many loci and as such each gene replacement may have effects on many characters because genes invariably do interact with each other. He proposed a “net- like” structure of population genotypes such that the variations of most characters are affected by many loci such that each gene replacement may have effects on many characters. Based on such a premise, epistasis could be regarded as one of the major genetic components in case of quantitative traits. Hallauar and Miranda (1988) also proposed that epistasis should contribute significantly to heterosis.



In 1952, **Gowen** had suggested that influence of one locus on the expression of another may be involved in heterosis. In the following

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years, substantial experimental evidence was accumulated to implicate epistasis as a cause of heterosis. For example, a majority of heterotic crosses show significant epistasis. But all heterotic crosses do not show epistasis, and all crosses that show epistasis are not heterotic. Theoretically, epistatic interactions will lead to the maximum heterosis when the following two conditions are met with.

- First, the epistasis should be predominantly of complementary type, i.e., the estimates of h (dominance effects) and / (dominance x dominance interaction effects) have the same sign so that they do not cancel each other out.

- Second, the interacting pairs of genes should be dispersed in both the parents. It has been suggested that in the absence of overdominance, dispersion (between the two parent's of (hybrids) of genes showing complementary epistasis seems to be the major cause of heterosis.

COMMERCIAL UTILIZATION

Heterosis is observed in almost every crop species studied. Often the degree of heterosis is considerably high to permit its commercial exploitation. Heterosis is commercially used in the form of hybrid or synthetic varieties. Such varieties have been most commonly used in cross-pollinated and often cross-pollinated crop species. In several self pollinated species also hybrid varieties have been commercially used. Attempts have been made to utilize heterosis higher price than in the case of those that fetch a lower price.

The commercial significance of hybrid technology may be illustrated with the singular success of hybrid maize in U.S.A. The yield of open-pollinated maize varieties ranged between -20 and -32 bushels per acre between 1870 and 1930. Around this time, double cross maize hybrids were introduced; their yields increased steadily from -25 bushels per acre during 1935 to -55 bushels/acre during 1960s. The introduction of single cross hybrids around this time marked a quantum jump in maize yields; it started from - 62 bushels/acre in 1960 and rose to -120 bushels/acre by 1990. These data, and those from many other countries, demonstrate the unquestionable superiority of single cross maize hybrids over other varietal forms. Similarly, hybrid rice has become quite popular in China. The first hybrid variety of rice was released in 1976, and by 1997 hybrid rice occupied -54% of the total paddy area and contributed nearly 64% of the total paddy production in China.

Manifestation of Hybrid Vigour in Vegetables

- Higher yield:
  - ✓ In terms of increased weight or size: eg. Cabbage, water melon, onion, pumpkin etc.
  - ✓ Increased number of fruits: Tomato, brinjal, squash, cucumber, okra etc.
  - ✓ Increased number of fruits may be due to early flowering and fruiting and also due to increased branching.
  - ✓ In okra, increased number of fruits are due to increased height and number of nodes so internode length is more important, as flowering / fruiting takes place at every node.
  - ✓ In cucurbits increased vine length may give rise to more fruits but not the case always, as number of fruits depend upon the number of pistilate flowers.
- Uniformity in size: Onion, cabbage, cauliflower, Brussels sprouts. This will influence yield.
- Uniformity in maturity: Onion, sweet corn, cabbage, cauliflower.

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- Early maturity: Cabbage, water melon, M.melon, tomato brinjal, onion.
- Large number of marketable heads / bulbs / roots per unit area : Cabbage, cauliflower, onion, carrot.
- Better resistance to diseases: Tomato, spinach, cucumber, water melon.
- Better resistance to insect pests: Onion against thrips.
- Better resistance to drought: Water melon, sweet corn.
- Better fruit quality (External + Internal): Tomato, Water melon, musk melon. F_1 of water melon have better flavor and in musk melon there is high TSS. In tomato F_1 size is uniform, uniform colour development.
- Better wider adaptability to environment conditions: Sweet corn.

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Course Name	Principles of Plant Breeding
Lesson 17	Estimation of Heterosis , GCA and SCA
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objective

1. Understand the estimation of various types of heterosis
2. Understand the estimation of general and specific combining ability

Glossary of terms

Heterosis: Heterosis is defined as the superiority of F1 over its parents. It is also called as Euheterosis

Relative heterosis :- When F1 is better than the Mid parental value. Also called as **Mid-parental heterosis**

Better parent heterosis:- When the F1 is better than the better parent. It is also called as **Heterobeltiosis**

Standard Heterosis: - When the F1 is better than the best available variety, used as check. It is also called as **Economic heterosis**.

General combining ability: General combining ability (GCA) refers to the ability of a line to produce good cross combinations in a series of crosses with a set of lines. The set of crosses is called as an array.

Specific combining ability: Specific combining ability (**GCA**) refers to the ability of a line to produce good cross combinations in a specific cross in an **array.**

Quantitative estimates of Heterosis

- Mid parental Heterosis
- Better parental heterosis
- Standard Heterosis

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A. Mid parental Heterosis: Heterosis estimated against the average of parents is called as Mid Parental Heterosis. Also called as Relative heterosis

$$\textbf{Mid Parental Heterosis}(\%) = \frac{F1 - MP}{MP} \quad \textbf{X 100}$$

Eg. A cross between parents having yield of 10 and 12 q is 13 q. Estimate MP heterosis

$$\textbf{Mid Parental Heterosis}(\%) = \frac{13 - 11}{11} \quad \textbf{X 100}$$

= (2/11) x 100 = 18.18 %

B. Better parental heterosis: Heterosis estimated against the better parent is called as Better Parental Heterosis. Also called as Heterobeltiosisis

$$\textbf{Better Parental Heterosis}(\%) = \frac{F1 - BP}{BP} \quad \textbf{X 100}$$

Eg. A cross between parents having yield of 10 and 12 q is 13 q. Estimate BPheterosis

$$\textbf{Better Parental Heterosis}(\%) = \frac{13 - 12}{12} \quad \textbf{X 100}$$

= (1/12) x 100 = 8.33 %

C. Standard Heterosis: Heterosis estimated against the Best Check variety is called as Standard Heterosis. Also called as Economic heterosis

$$\textbf{Standard Heterosis}(\%) = \frac{F1 - Check}{Check} \quad \textbf{X 100}$$

Eg. A cross between parents having yield of 10 and 12 q is 15 q. the check variety yield is 13 q Estimate Standard heterosis

$$\textbf{Standard Heterosis}(\%) = \frac{15 - 13}{13} \quad \textbf{X 100}$$

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= (2/13) x 100 = 15.38 %

Estimation of Combining ability

Crossing a line to several others provides the mean performance of the line in all its crosses. Combining ability or productivity in crosses is defined as the cultivars or parents ability to combine among each other during hybridization process such that desirable genes or characters are transmitted to their progenies. In another definition, combining ability is an estimation of the value of genotypes on the basis of their offspring performance in some definite mating design. It can seldom be envisaged only based on parental phenotype and thus it is measured by progeny testing. When parental plants produce potent offspring, they are said to have good combining ability.

From a statistical point of view, the GCA is a main effect and the SCA is an interaction effect. Based on Sprague and Tatum (1942) GCA is owing to the activity of genes which are largely additive in their effects as well as additive × additive interactions.[7] Specific combining ability is regarded as an indication of loci with dominance variance (non-additive effects) and all the three types of epistatic interaction components if epistasis were present. They include additive × dominance and dominance × dominance interactions.

General combining ability: General combining ability (**GCA**) refers to the ability of a line to produce good cross combinations in a series of crosses with a set of lines. The set of crosses is called as an **array.** The concept of **GCA** was given by **Sprague & Tatum (1942)**

In a five parent diallel, the following data was recorded for yield, estimate the GCA of parents

P1 x P2 = 86

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P1 x P3 = 84

P1 x P4 = 98

P1 x P5 = 92

P2x P3 = 91

P2 x P4 = 105

P2 x P5 = 102

P3 x P4 = 87

P3 x P5 = 80

P4 x P5 = 97

Parents	**P1**	**P2**	**P3**	**P4**	**P5**	**TOTAL**	**MEAN (μ)**
P1	-	86	84	98	92	360	90
P2	86	-	91	105	102	384	96
P3	84	91	-	87	80	342	85.5
P4	98	105	87	-	97	387	96.75
P5	92	102	80	97	-	371	92.75
General Mean = 1884/20 = 92.20 GCA is estimated as deviation of array mean from general mean							

GCA estimates of parents is as follows:

P1 = 90 - 92.2 = -2.20
P2 = 96 – 92.2 = 3.80
P3 = 85.5 – 92.2 = -6.7
P4 = 96.75 – 92.2 = 4.55
P5 = 92.75 – 92.2 = 0.55

Thus P2 and P4 are good general combiners and P1 and P3 are very poor combiner

Specific combining ability: Specific combining ability (GCA) refers to the ability of a line to produce good cross combinations in a specific cross in an array. The concept of SCA was given by Sprague & Tatum (1942) SCA of a cross is estimated as deviation of observed performance of cross from the expected performance based on GCA of parents. We can understand the estimation process using the same above data set as follows:

Parents	**P1**	**P2**	**P3**	**P4**	**P5**	**TOTAL**	**MEAN (μ)**
P1	-	86	84	98	92	360	90
P2	86	-	91	105	102	384	96
P3	84	91	-	87	80	342	85.5
P4	98	105	87	-	97	387	96.75

P5	92	102	80	97	-	371	92.75
General Mean = 1884/20 = 92.20 GCA is estimated as deviation of array mean from general mean							

Example SCA of **P1 x P2**

GCA of P1 = -0.22

GCA of P2 = 3.80

Expected performance of P1 x P2 = Mean + GCA P1 + GCA P2
= 92.20 – 2.20 + 3.80 = 93.8

Observed performance of P1 x P2 = 86

SCA of P1 x P2 = 86 – 93.72 = -7.80

Thus P1 x P2 have poor SCA

SCA of **P1 x P4**

GCA of P1 = -2.20

GCA of P2 = 4.55

Expected performance of P1 x P4 = Mean + GCA P1 + GCA P2
= 92.20 – 2.20 + 4.55 = 94.55

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Observed performance of P1 x P4 = 98

SCA of P1 x P2 = 98 – 94.55 = 3.45

Thus P1 x P2 have good SCA

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Course Name	Principles of Plant Breeding
Lesson 18	Inbreeding Depression
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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1. Objectives

1. Understand the genetic consequences of inbreeding depression
2. Understand the characteristics of various degrees of inbreeding depression

Glossary of terms

Inbreeding : Mating is mating between individuals related by descent or ancestry

Inbreeding depression: The decline in performance in progeny of plants under selfing in outcrossed species is called as inbreeding depression

Inbreeding coefficient: The probability that the two genes at any locus in a diploid individual are Identical by descent (i.e., they originated from the replication of one gene in a previous Generation)

Inbreeding load: The extent to which a population is impaired by inbreeding

Homozygous balance: Tendency of self pollinated crops to perform optimally when they are homozygous is called as Homozygous balance

Heterozygous balance: Tendency of cross pollinated crops to perform optimally when they are heterozygous is called as Homozygous balance

INBREEDING DEPRESSION

Inbreeding or consanguineous mating is mating between individuals related by descent or ancestry**.** When the individuals are closely related, e.g., in brother- sister mating or sib mating, the degree of inbreeding is high. The highest degree ofinbreeding is achieved by selfing. The chief effect of inbreeding is an increase in homozygosity in the progeny, which is proportionate to the degree of inbreeding. The degree of inbreeding of an individual is expressed as **inbreeding coefficient (F).**

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The degree inbreeding is proportional to degree of homozygosity. Inbreeding depression may be defined as the reduction or loss in vigour and fertility as a resultof inbreeding.

Inbreeding depression = F1-F2 / F1 x 100

Historical

Inbreeding depression has been recognized by man for a long time. Marriages between closely related individuals has been prohibited since early timein many societies. Because people are aware of the harmful effects of such marriages in the progeny. A systematic observations on effect of inbreeding started during 17th century when inbreeding became a common practice in cattle breeding. In 1876, **Darwin** published his book on **cross and self fertilization in vegetable kingdom**. He concluded that progeny obtained from self fertilization were weaker than those obtained from out crossing. **Darwin** also reported the results from his experiments on self and cross fertilization in maize for the first time.**East (1908) and Shull (1909)** independently showed the effect of inbreeding depression while working in maize. Subsequently scientists reported inbreeding depression in other crop plants. It has become clear that in cross pollinated crops and in asexually propagated species inbreeding has harmful effect which are severe.

Genetic consequences of inbreeding

The genetic consequences of inbreeding arise mainly on account of theincreased tendency towards homozygosity with inbreeding that provides an opportunity for recessive alleles to be homozygous and, hence, expressed. Whereas inbreeding generally has little or no adverse effect in self-pollinated crops, cross- pollinated crops suffer heavily especially when the recessive alleles are less favorable as

compared to the dominant alleles. Inbreeding depression is manifestation of reduced performance, due to the expression of less favourable ordeleterious alleles. Furthermore, the effect of inbreeding is most significant in the first 5–8 generations and negligible after the eighth generation in many cases.

Degrees of inbreeding depression

The severity of inbreeding depression varies among species, being extreme in species such as alfalfa in which inbreeding produces homozygous plants that fail to survive Inbreeding depression may range from very high to very low or it may even be absent. The ID is generally grouped into 4 categories.

High Inbreeding Depression

- Several plant species, e.g. alfalfa (M. sativa), carrot (D. carota), onion, hayfield, tarweed etc show very high inbreeding depression.
- A large proportion of plants produced by selfing shows lethal characteristics and do not survive.
- The loss in vigour and fertility is so great that very few lines can be maintained after 3 to 4 generation of inbreeding.
- The line shows greatly reduced yields, generally less than 25 percent of the yield of open – pollinated varieties.

Moderate Inbreeding Depression

- Many crops species, such as maize, cole crops, radish, jowar, bajra etc. shows moderate inbreeding depression.

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- Many lethal and sublethal types appear in the selfed progeny, but a substantial proportion of the population can be maintained under self-pollination.
- There is appreciable reduction in fertility and many line reproduce so poorly that they are lost.
- However, a large number of inbred lines can be obtained, which yield upto 50 percent of the open- pollinated varieties.

Low Inbreeding Depression

- Several crop plants, e. g., many cucurbits, rye (S. cereale), sunflower (Hannus), hemp etc show only a small degree of inbreeding depression.
- Only a small proportion of the plants show lethal or subvital characteristics.
- The loss in vigour and fertility is small; rarely a line cannot be maintained due to poor fertility.
- The reduction in yield due to inbreeding is small or absent.
- Some of the inbreds lines may yields as much as the open pollinated varieties from which they were developed.

Lack of Inbreeding Depression

- The self- pollinated species do not show inbreeding depression although they do not show heterosis.
- It is because their species reproduce by self – fertilization and as a result, have developed homozygous balance.
- In most of the cross- pollinated species exhibit heterozygous balance.

Homozygous v/s Heterozygous balance

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The concepts of homozygous and heterozygous balance were advanced by Mather to explain the varied responses of different species to inbreeding.

• The species that reproduce by self-fertilization are highly homozygous and adapted to homozygous balance.
The species that reproduce by cross- fertilization are highly heterozygous and adapted to heterozygous balance.
• These species carry a large number of lethal, subvital and other unfavourable recessive genes, which are of little value to the species.
• The sum total of these unfavourable genes constitutes genetic load of these species.
• The harmful effects of such recessive alleles are masked by their dominant allele as result of which they are retained in population.

Effects of inbreeding depression

• **Appearance of Lethal and Sublethal Alleles:** IB results in appearance of lethal; sublethal and subvital characters, e.g : Chlorophyll deficiencies, rootless seedlings, flower deformities. They do not survive, they lost in population.

• **Reduction in vigour** : General reduction in vigour size of various plant parts.

• **Reduction in Reproductive ability**: Reproductive ability of population decreases rapidly. Many lines reproduce purely that they cannot be maintained.

• **Separation of the population into distinct lines**: population rapidly separates into distinct lines i.e. due to increase in homozygosity. This leads to random fixation of alleles in different lines. Therefore lines differ

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in genotype and phenotype. It leads to increase in the variance of the population.

- **Increase in homozygosity**: Each lines becomes homozygous. Therefore, variation within a line decreases rapidly. After 7-8 generations of selfing the line becomes more than 99% homozygous. These are the inbreds. These have to be maintained by selfing.

- **Reduction in yield:** IB leads to loss in yield. The inbreds that survive and maintained have much less yield than the open pollinated variety from which they have been developed.

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Course Name	Principles of Plant Breeding
Lesson 19	Heritability & Genetic Advance
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the genetic consequences of inbreeding depression
2. Understand the characteristics of various degrees of inbreeding depression

Glossary of terms

Heritability

A central question in biology is whether observed variation in a particular trait is due to environmental or to biological factors, most popularly describe as the "**nature versus nurture**" debate. Heritability is a concept that elucidates how much of the variation in a trait is due to variation in genetic factors. Often, this term is used in reference to the resemblance between parents and their offspring and high heritability implies a strong resemblance between parents and offspring with regard to a specific trait, while low heritability implies a low level of resemblance.

Heritability was originally defined by Lush as the proportion of phenotypic variance among individuals in a population that is due to heritable genetic effects. This definition is now termed “heritability in the narrow sense” and is designated h^2. The proportion of the phenotypic variance due to genetic effects, is called as “heritability in the broad sense” (H), the proportion of phenotypic variance that is due to all genetic effects. Hanson (1963) proposed that plant breeders should unify the concept of heritability as “the fraction of the selection differential expected to be gained when selection is practiced on a defined reference unit.” Therefore, through- out this review, various heritability estimators are evaluated in terms of response to selection. Heritability has meaning only in reference to defined selection units and response units, and these can vary among breeding schemes.

In crop improvement only the genetic component of variation is important since only this component is transmitted to the next

generation. Heritability is the ratio of genotypic variance to the phenotypic variance. It denotes the proportion of phenotypic variance that is due to genotype i.e., heritable . It is generally expressed in percent (%) and is a good index of transmission of characters from parents to their offspring

Quantifying heritability

Phenotypes that vary between the individuals in a population do so because of both the genes as well as environmental factors that influence traits, as also the interactions between genes and environmental factors. Unless they are genetically identical (e.g., monozygotic twins, inbred lines or clones), the individuals in a population tend to vary in the genotypes they have at the loci affecting particular traits. The combined effect of all loci, including possible allelic interactions within loci (dominance) and between loci (epistasis), constitutes the **genotypic value**. This value creates genetic variation in a population when it varies between individuals. In fact, heritability is formally defined as the proportion of phenotypic variation (VP) that is due to variation in genetic values (VG).

$$H^2 = VG/VP$$

Where VG is the variance in the trait explained by genetics (G), and VP is the total variance of the trait in the population. Two important observations can be made about this definition. First, it's entirely flexible about how specific genetic effects contribute to VP.

The broad-sense H^2 does not care whether VG comes from a single Mendelian variant in just one gene, or the small additive effects from variants in 100 different genes, or complex interactions between every variant in the whole genome. This is an important distinction between broad-sense and some of the other types of heritability.

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Second, broad-sense heritability H^2 is entirely flexible about how VG relates to VP. We can assume that the effects of genes and environment are independent and thus follow the relationship H^2 = VG / VG + VE, where VG is the variance in the trait explained by genetics (G), VE is the environmental variance and VP is the total variance of the trait in the population. But that assumption isn't required. By simply writing the denominator as VP we allow for the possibility that genetic and environmental factors are correlated or interact in some way. This is important since it highlights that the effect of environment on the trait is not simply the "**remainder**" after accounting for all the genetic effects, instead they can overlap and interact in complex ways.

Estimating Heritability

Estimation of heritability in populations depends on the partitioning of observed variation into components that reflect unobserved genetic and environmental factors. Despite recognizing the fact that genetic and/or environmental variation exists, we may not be in a position to assess them directly. However, we still are able to estimate the relative effects of both genes and environment on phenotype. Thus, heritability can be estimated from empirical data on the observed and expected resemblance between relatives. The expected resemblance between relatives depends on assumptions regarding a trait's underlying environmental and genetic causes. Traditionally, heritability was estimated from the correlation of offspring and parental phenotypes. Heritability can also be estimated from the ratio of the observed selection response (R) to the observed selection differential (S) in artificial selection experiments. This relationship is called as the "**Breeder's equation**" viz **$R = h^2S$**.

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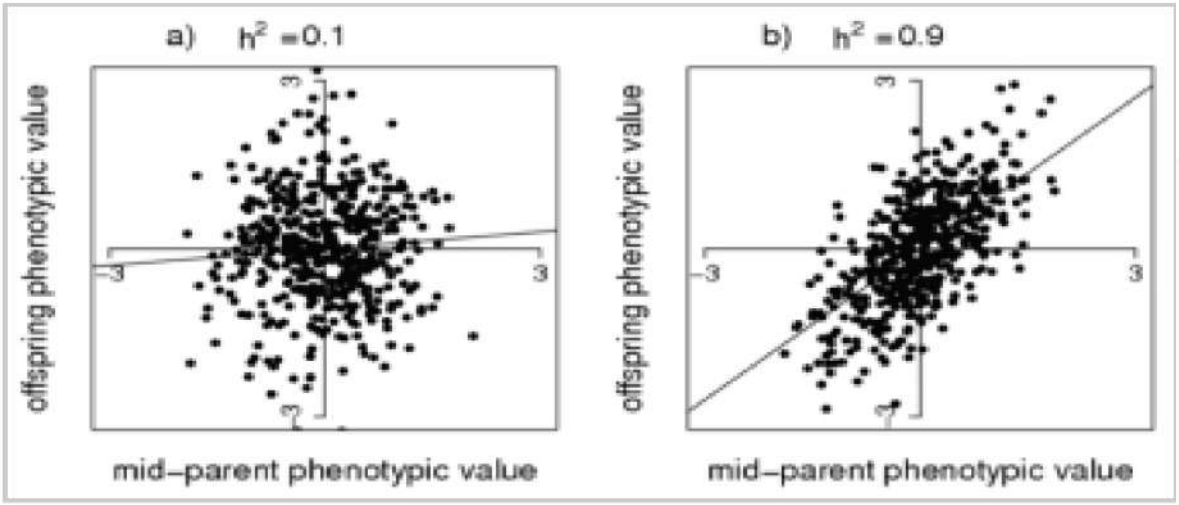

In the above figure shows the scatter plot of progeny phenotypes (y-axis) and the average of two parental phenotypes (x-axis), for traits with high (0.9) and low (0.1) heritability. The straight line is the best-fit linear relationship between y and x, obtained from a statistical technique called linear regression. The slope of the regression line is an estimate of narrow-sense heritability. For the high heritability of 0.9, there still is a lot of variation around the regression line, because the correlation between offspring phenotype and mid-parent value is **√(½) h^2**, which is only 0.64 for h^2 = 0.9.

Even when the heritability is 1.0 (i.e., there is no environmental variation), the phenotypes of offspring and parents are not identical because of random segregation of alleles from parents to progeny. This explains, for example, why human siblings can vary considerably in height, despite the heritability of height being very large. When phenotypic measures are available on individuals with a mixture of relationships, both within and across multiple generations, estimates of additive genetic variance and environmental components are most efficiently calculated via statistical methods that use all data simultaneously

While estimating heritability from the observed and expected resemblance between relatives, a model is necessary to specify the

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expected resemblance in terms of genetic and environmental factors. The observed resemblance between half-sib progenies is solely due to additive genetic factors inherited from the common parent. Typically there are two estimates of heritability:

Heritability (broad sense): The proportion of phenotype of offsprings governed by genetic constitution of parents. It is the portion of variance as accounted for by genes. It is denoted by $\mathbf{H^2}$ and is computed as ratio of genetic variance to total phenotypic variance.

$$\mathbf{H^2} = \sigma^2G / \sigma^2P \times 100$$

Heritability (narrow sense): It is the portion of variance as accounted for by additive genes. It is denoted by $\mathbf{h^2}$ and is computed as ratio of additive genetic variance to total phenotypic variance. It is also called as realized heritability. Since each parent passes a single allele per locus to each offspring, parent-offspring resemblance depends upon the average effect of single alleles. Additive variance represents, therefore, the genetic component of variance responsible for parent-offspring resemblance.

$$\mathbf{h^2} = \sigma^2A / \sigma^2P \times 100$$

Applications of Heritability estimates

As suggested by Holland et al (2003), the main purpose of estimating heritability and the underlying genetic parameters that compose the heritability estimate is to compare the expected gains from selection from alternative selection schemes. The estimates of different types of heritability can have following applications in plant breeding.

- Heritability estimates can be used to predict gain from selection, for example, based on single, un-replicated plot values, and

compares this to gain from selection expected if materials are replicated within and across macro-environments.

- Heritability estimates are also useful for comparing the gain from selection under different experimental designs, and this information coupled with information about the relative costs of additional replications/locations/years within each macro-environment can be used to design optimal breeding strategies.
- In case of multi-environment testing, where G x E interactions cause significant rank changes among families evaluated in different environments, heritability estimates corresponding to response to selection based on means over all environments can be compared with heritability based on means within subsets of local environments to determine the optimal selection strategy (Atlin et al. 2000).
- Heritability based on different family structures derived from the same base population can be compared to determine which family structure is best for maximizing genetic gain over time.
- Heritability may vary among populations, thus, heritability estimates from different populations can be useful for choosing appropriate base populations in which selection will be most effective.
- Heritability varies among traits within a population, as such, the heritability estimates of different traits, coupled with genetic correlation estimates among the traits, can be used to identify indirect selection schemes that are likely to be more effective than direct selection schemes.

Interpretations of heritability

A. Broad sense heritability

- If heritability in broad sense is high, It indicates character are least influenced by environment, selection for improvement of such characters may be useful

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- If heritability in broad sense is low , The character is highly influenced by environmental effects and Genetic improvement through selection will be difficult

B. Narrow sense heritability

- If heritability in narrow sense is high θ characters are govern by additive gene action, Selection for improvement of such characters would be rewarding

- If low heritability in narrow sense, Non additive gene action and Heterosis breeding will be beneficial

Factors affecting heritability

- **Type of genetic material :** the magnitude of heritability is largely governed by the amount of genetic variance present in a population for the character under study

- **Sample size** : Large sample is necessary for accurate estimates

- **Sampling methods :** sampling methods may be Random and Biased . The random sampling methods provide true estimates of genetic variance and hence of heritability

- **Layout or conduct of experiment** : Increasing the plot size and no. of replications we can reduce experimental error and get reliable estimates

- **Method of calculation** : heritability is estimated by several

methods

- **Effect of linkage** : high frequency of coupling phase (AB/ab) causes upward bias in estimates of additive and dominance variances . Excess of repulsion phase linkage (Ab/aB) leads to upward bias in dominance variance and downward bias in additive variances

Genetic Advance

Genetic advance explains the degree of gain obtained in a character under a particular selection pressure and is computed as:

GA= $k \times h^2 \times \sigma P$, where,

k= selection intensity
h^2= heritability (ns)
σP = phenotypic standard deviation

Interpretation of Genetic advance

- If the value of Genetic advance high The character is governed by additive genes and selection will be beneficial for such traits
- If Genetic advance is low The character is governed by non additive genes and heterosis breeding may be useful

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Course Name	Principles of Plant Breeding
Lesson 20	Emasculation and Pollination Techniques
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives of the Lecture in bullets (At least 2).

1. Understand the basic procedures for emasculation and pollination
2. Understand the various precautions of emasculation and pollination in various horticultural crops

Glossary of terms

Emasculation: Removal of anthers by hand or other mechanical means is called emasculation

Pollination: Transfer of pollen from male part of same or different flower to female part to affect fertilization

Self incompatibility: Inability of a pollen to fertilise its own female part

Bagging: Protection of female or emasculated flowers to avoid unwanted contamination

Genetic emasculation: When emasculation is effected by using genetic or cytoplasmic male sterile mechanism, it is referred to as genetic emasculation

Protoandry: Situation where male part matures first and female later

Protogyny: Situation where female part matures first and male later

SELFING AND CROSSING TECHNIQUES IN PLANTS

I.SELFING:

The objective of selfing is to develop homozygous plants that can be used as parents in a crossing programme. The technique of selfing varies from one crop to the other depending upon the mode of reproduction.

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In self-pollinated crops, selfing is the natural mode of reproduction and to ensure selfing no operation is needed. But in legumes like alfalfa, hand tripping of flower is essential for self-fertilization since the stigma has a waxy covering which must be removed to make it receptive. In the case of often-cross pollinated species, the flowers are generally bagged to prevent cross-pollination.

In bisexual flowers or with both male and female flowers in a single inflorescence, bagging the entire inflorescence, or sometimes the whole plant is adequate. In maize, the male and female inflorescences are bagged; the pollen is collected in the tassel bag and dusted on the silk of the female inflorescence. Alternatively, the tassel may be cut and enclosed in the bag-covering cob. The cut end of tassel may be kept in water contained in a small bottle to keep the tassel alike for a longer period.

II.CROSSING

Synchronization of flowering is a prerequisite so that the pollen collected from one variety can be immediately dusted on the stigma of the other variety. If the two parents have different durations to reach flowering, sowing should be staggered suitably so that they reach flowering at the same time. By raising the parents in glass houses under controlled conditions of light, temperature and humidity, synchrony in flowering can be achieved.

Emasculation is the technique of crossing consists of removing the anthers before pollen shedding. The collection of viable pollen from the male parent and transferring it to the receptive stigma of the emasculated flower is called pollination. The removal of the anthers is usually done one day before the pollen is ripe. Hand emasculation can be adopted in crops with large flowers such as cotton. Mass emasculation can be done by the 'hot water method' such as in in rice. [40-44°C for

10 minutes] and Sorghum (42 -48°C for 10 minutes). Cold treatment may also be used for emasculation in rice (0-6°C) and wheat (0-2°C) for a duration of 15-24 hours. Treatment with alcohol (57 per cent) for 10 seconds helps in emasculation in sweet clover. When the stigma becomes receptive pollen collected from the male parent is dusted on the stigma of the emasculated head and the butter- paper bag is replaced immediately.

TYPES OF EMASCULATION

1**. Hand Emasculation:** Hand emasculation is generally carried out between 4 and 6 pm, one day before anthesis. The corolla of the selected flower bud is opened with the help of forceps and needles and the anthers are carefully removed. When the stamens are epipetalous (stamen attached to corolla) corolla may be completely removed eg. Sesamum. The gynoecioum must not be injured during emasculation.

2. Hot water emasculation: As pollen grains are very sensitive to both genetic and environmental factors when compared to female reproductive organs, the treatments with alcohol, cold water, hot water are used for emasculation. The temperature of hot water and the duration of treatment differs from crop to crop. For example, Sorghum requires a temperature of 42-48oC for ten minutes to effect emasculation while in rice, the temperature should be between 40-440C for 10 minutes. Hot water is generally carried in a thermos flask.

3**. Alcohol treatment:** It is not a commonly used method as female reproductive organ is also drastically affected under certain conditions. In this method, the flower bud or the inflorescence is immersed in alcohol for a particular period of time followed by rinsing with water. Eg. In sweet clover and Lucerne, treatment with 57 % alcohol for 10 seconds was highly effective.

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4. Cold treatment: Cold treatment is less effective when compared with hot water treatment. Eg. In rice, cold water treatment at 0.6 C kills the pollen grains without affecting the gynoecium. In wheat, the temperature required is 0.2 C for 15-24 hours.

5. Genetic emasculation: When emasculation is effected by using genetic or cytoplasmic male sterile mechanism, it is referred to as genetic emasculation. In crops like onion, sorghum, maize, pearlmillet, wheat genetic emasculation is widely used. In genetic emasculation the pollen formation is hampered or even when formed itwill be sterile and cannot fertilize. In case of self-incompatible crops eg. Brassica, emasculation is not necessary, because the self-fertilization is ineffective. In some crops with protogyny mechanism (stigma matures earlier than anthers) emasculation is not needed to produce hybrid seeds.

BAGGING

Immediately after emasculation, the flowers or the inflorescence should be covered with butter paper cover to prevent random cross-pollination. In crops like maize, the male inflorescence (tassel) is also bagged to maintain the purity of the pollen used for pollination. TAGGING After bagging, the emasculated flowers are tagged. The plant tags are available in different sizes (3 x 2 or 6x3 cm). The information regarding, date of emasculation, date of pollination and names of the female and male parents should be written on the tag with carbon pencil. The name of the female parent is written first followed by the name of the male parent eg. ADT 43 x ASD 16. ADT 43 is female parent and ASD 16 is male parent.

POLLINATION

Pollination procedure consists of collecting pollen grains from freshly dehisced anthers and dusting this pollen on the stigma of emasculated flowers. In rice, the inflorescence of male parent is shaken over the

emasculated inflorescence. In case of bajra and jowar, the male and female inflorescences are enclosed in the same paper bag to effect crossing. Also, the pollen grains can be collected in a bag or petridish and dusted on the stigma with the help of camel hair brush or forceps or toothpicks.

GARDEN PEA

For emasculation the flower bud chosen should have developed to the stage just before anther dehiscence, indicated by extension of petals beyond sepals. Flowers can be emasculated at any time. The first step in emasculation is to tear away with the forceps the tip of the sepal from in front of the keel. The fore finger is positioned behind the flower and thumb in front and a light pressure is applied. This spreads the standard and wings to expose the keel. The exposed keel is slit-open by tips of forceps. Pressure can be applied by the thumb and finger on keel for increased exposure of the pistil and stamens. The 10 stamens are pulled out.

Pollen can be obtained throughout the day, preferably from a freshly opened flower. For pollen collection, it is more convenient to pick the male flowers, remove the standard and wings, pull back the keel so that the style protrudes and use the pollen covered stylar brush as an applicator to transfer the pollen to the stigma of the emasculated bud. Older flowers and other flower buds not used in crossing are removed the peduncle to increase the pod set after crossing.

FRENCH BEANS

For emasculation an unopened flower bud is selected. The standard is detached from below with a pair of forceps and bent backward. The

keel is pulled in pieces with the forceps. Care should be taken to turn in the same direction as the spiral winding, otherwise the style may break. After pulling off keel, the stamens are removed. For the supply of pollen, freshly opened flowers are collected. The thickly pollinated stigmas emerge as soon as the wings are pressed downward. This stigma is rubbed against the stigma of the emasculated bud. Sometimes, the thickly pollinated stigma of the open flower to be used as male is hooked into the stigma of the flower bud to be pollinated..

Pollination without emasculation has also been suggested. In this method the standard is detached and unfolded. By pressing the left-hand wing downward, the unpollinated stigma emerges. Pollen is rubbed into this stigma. The emasculation and pollinations are done simultaneously in the forenoons.

COWPEA

Cowpea flowers are large and showy. Flowers openonlyoncebetween7and9a.m. On cloudy days the flowers may open even in the afternoon. Though the flowers open late in the morning, the dehiscence of the anthers is much earlier. It may vary farm 10 pm to 12.45 a.m. The dehiscence is influenced by environmental factors like presence of moonlight, a clear sky and a dry warm atmosphere. During dark nights the dehiscence tends to be delayed. Due to dehiscence taking place before the opening of flowers, the cowpea is strictly self-pollinated in nature.

Since the dehiscence of anthers is much in advance of the blooming, the emasculation needs to be carried out in mature flower buds in the preceding evening. The flower buds likely to bloom the next day (recognized by large size, the yellowish colour of the back of the standard petal) is selected for emasculation. The bud is held between the thumb and fore-finger with the keel side uppermost. A needle is

run along the ridge where the two edges of the standard unite. One side of the standard is brought down and secured in position with thumb. Same thing is done with one of the wings. After this the exposed keel is slit on the exposed side, about 1/16 inch from the stigma. A section of keel is also brought down and secured in position under the end of thumb. Now 10 stamens are seen. They are removed with pointed forceps. Afterwards, the disturbed parts of standard, wing and keel are brought in original position as far as possible. To prevent drying out of the emasculated bud, a leaflet may be folded and pinned around the bud. A tissue paper can be used to cover and protect the bud.

Pollination is done next morning from a freshly opened flower. The standard and wings of male flower are removed. By slight depression of the keel, stigma covered with pollen grains protrudes out. This itself can be used as a brush for pollination. Cowpea flowers are highly sensitive and drop off easily with slight mechanical disturbance or injury. Therefore, much labour and time is devoted to get enough crossed seed.

TOMATO

Emasculation is usually done one day prior to anthesis/flower opening. At this stage, the sepals have started to separate and the anthers and corolla are beginning to change from light to dark yellow. The stigma is fully receptive at this stage allowing for pollination even immediately after emasculation.

Anthers are removed as a group with or without the surrounding corolla, by inserting forceps between the sepals to grip the base of the anthers and /or petals which are then removed by a firm but steady pull. If anthers seem reluctant to part company from flower receptacle

as a group, it is advisable to remove a single one first by careful manipulation of the forceps. Following this, the remaining four may be gripped firmly without any fear of damaging the style.

Pollen is be stapplied in experimental crosses by slitting the inside of the anthers of mature flowers of the male parent with the forceps in such a way that a small amount of pollen is collected at the tip of the forceps. This can then be lightly applied to the stigmatic surface and should be visible as a white covering. Forceps should be sterilized by dippingin alcohol or methylated spirit after each pollination. Pollen may be collected in large amounts by inverting the mature flower and tapping pollen into the thumbnail. Protection of pollinated flowers by wrapping with cotton or small pollination bags is essential.

BRINJAL

Flowers generally emerge 40-45 days after transplanting. Anthesis occurs at about 6-8 a. m. in August – September and usually between 9.30 to 11.15 am during winter (December-January).Stigmareceptivityishighestduringanthesisi.e.flower opening. Anthers usually dehisce 15 to 20 minutes after the flowers have opened. The receptivity of the stigma can be observed from its plump and shiny period of effective receptivity ranges from a day prior to flower opening until about four days after opening. Pollen usually remains viable for a day during summerand2-3daysinwinterunderfieldconditions.

For emasculation, a healthy long or medium styled, well developed bud from the central portion of the plant is selected. The bud is opened gently with the help of fine pointed forceps one or two days before the opening of the bud and all the five anthers are carefully removed. For pollination, freshly dehiscing anthers are picked up and are slit vertically with fine needle to get sufficient pollen at the tipof

the needle. Pollen are applied on the stigma of the emasculated flower bud. It is labeled and covered with small pollination bag.

CHILLI

Flowers are emasculated in bud stage. Pollen is transferred to the stigma either rfrom mature undehisced anthers by scooping it out through the lateral sutures with the needle or by touching a freshly dehisced anther to the stigma with the forceps. Hands and tools (a pair of sharp-pointed forceps, a needle, a pair of scissors) are washed with 95% ethyl alcohol. A roll of cheese cloth, some lightweight cotton string and balls of different colours of thread are also needed.

Pollinated flowers are identified by loosely typing coloured thread around the delicate pedicels, preferably enclosing a leaf petiole for protection. Different colours of string can be used for different crosses on the same plant, and white for the selfs. Pollinated flowers are protected from bees by a double layer of cheesecloth, loosely wrapped around the branch, enclosing leaves and flowers, and securely fastened. Appropriately marked plastic labels describing the cross, the date, are attached to a bamboo stake marking the chosen plant. Pollinated flowers are periodically checked and the cheese cloth removed in 4-6 days. Fruits normally mature in about 45 days.

BHINDI

For emasculation, buds likely to open the next day are given as light ring cut at the base of the flower bud with the help of a blade. Petals along with calyx sheath are removed and staminal column and stigma are exposed. The undehisced anthers are removed with a pair of forceps. The emasculated buds are bagged. Next morning, the bags are removed and the emasculated buds are pollinated with the male

flowers bagged a day earlier. Pollinated flowers are bagged and labelled. After a few days the bags may be removed.

CAULIFLOWER

Selfing: The self-compatible varieties of cauliflower can be selfed by simply bagging the flower-stalk. Selfing is also done by caging some plants with flies in cages or by isolation planting of lines having decreased level of self-incompatibility. With self-incompatible plants, bud pollination gives better results. In this system, the pollination is carried out in buds before 2-4 days of opening, with emasculation or without emasculation.

Crossing: The flowers may be emasculated by removing 6 stamens using a pair of forceps. In self-compatible cauliflowers (European types), the stamens are removed before the opening of the buds as the flowers are already fertile in the bud stage, crossing can be done at the same time. In self-incompatible types, emasculation maybe omitted. When pollination cages are available, crosses between self-incompatible types can be made by insects such as honeybees, bumblebees and flies.

CABBAGE

Flowers are slightly protogynous and cabbage is naturally cross-pollinated due to sporophytic self-incompatibility. Pollination is brought about by bees and flies. Bud pollination is effective to achieve selfing. For cross-pollination flower buds expected to open within 1-2 days are emasculated and are pollinated immediately with desired pollen using a brush/flower stamens.

BOTTLEGOURD, RIDGEGOURD and SPONGE GOURD

The flowers of ridge gourd like those of bottlegourd start anthesis (opening) in the evening and remain open throughout the night and are ready for selfing and pollination in the early morning/forenoon. The flowers of sponge gourd open in early morning hours and are suitable for selfing/crossing almost throughout the day.

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Course Name	Principles of Plant Breeding
Lesson 21	Breeding for Biotic and Abiotic Stresses
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
Course Reviewer	Dr. Akhilesh Sharma
University/College Name	Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur

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Objectives

1. Understand the nature of various biotic and abiotic stresses
2. Understand the mechanism of resistance and breeding strategies used

Glossary of terms

Stress: It refers to external conditions that adversely affect growth, development or productivity of plants.

Vertical resistance:-Resistance of a host to the particular race of pathogen; also called as major gene resistance, oligogenic resistance, qualitative resistance.

Horizontal resistance: - Resistance of a host to all the prevalent races of a pathogen; also called as minor gene resistance, polygenic resistance, nonspecific resistance, general resistance

Immune reaction:- When the host does not show the symptoms of disease it is known as immune reaction.

Hypersensitivity:-Upon infection, several host cells surrounding the point of infection are so sensitive that they will die. This leads to the death of the pathogen because the rust mycelium cannot grow through the dead cells..

Tolerance:- Ability of a host to reproduce well despite the establishment of a pathogen in the host tissues or the ability of a variety to produce more yield than susceptible variety at the same level of insect attack.

Avoidance:- Escape of a variety from insect attack either due to earliness or its cultivation in the season when insect population is very low

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Non-preference:- Various features of host plant which make the host undesirable or unattractive to insects for food, shelter or reproduction, a mechanism of insect resistance; also called non-acceptance or antixenosis.

Antibiosis:- Adverse effects of the host on feeding, development and reproduction of insect pest.

Stress in plants refers to external conditions that adversely affect growth, development or productivity of plants. Stresses trigger a wide range of plant responses like altered gene expression, cellular metabolism, changes in growth rates, crop yields, etc. A plant stress usually reflects some sudden changes in environmental condition. However in stress tolerant plant species, exposure to a particular stress leads to acclimation to that specific stress in a time-dependent manner. Plant stress can be divided into two primary categories namely abiotic stress and biotic stress. Abiotic stress imposed on plants by environment may be either physical or chemical, while as biotic stress exposed to the crop plants is a biological unit like diseases, insects, etc. Some stresses to the plants injured them as such that plants exhibit several metabolic dysfunctions. The plants can be recovered from injuries if the stress is mild or of short term as the effect is temporary while as severe stresses leads to death of crop plants by preventing flowering, seed formation and induce senescence. Such plants will be considered to be stress susceptible. However several plants like desert plants (Ephemerals) can escape the stress altogether.

Biotic stress in plants is caused by living organisms, specially viruses, bacteria, fungi, nematodes, insects, arachnids and weeds. The agents causing biotic stress directly deprive their host of its nutrients can lead to death of plants. Biotic stress can become major because of pre- and postharvest losses. Despite lacking the adaptive immune system plants

can counteract biotic stresses by evolving themselves to certain sophisticated strategies. The defense mechanisms which act against these stresses are controlled genetically by plant's genetic code stored in them. The biotic stress is totally different from abiotic stress, which is imposed on plants by non-living factors such as salinity, sunlight, temperature, cold, floods and drought having negative impact on crop plants and also the ability of the crop species to resist that particular type of stress. Many biotic stresses affect photosynthesis, as chewing insects reduce leaf area and virus infections reduce the rate of photosynthesis per leaf area.

Abiotic stresses such as drought (water stress), excessive watering (water logging), extreme temperatures (cold, frost and heat), salinity and mineral toxicity negatively impact growth, development, yield and seed quality of crop and other plants. In future it is predicted that fresh water scarcity will increase and ultimately intensity of abiotic stresses will increase. Hence there is an urgency to develop crop varieties that are resilient to abiotic stresses to ensure food security and safety in coming years. A plants first line of defense against abiotic stress is in its roots. The chances of surviving stressful conditions will be high if the soil holding the plant is healthy and biologically diverse. One of the primary responses to abiotic stress such as high salinity is the disruption of the Na+ /K+ ratio in the cytoplasm of the plant cell. The phytohormone abscisic acid (ABA) plays an important role during plant adaptation to environmental stress such as high salinity, drought, low temperature or mechanical wounding.

Stress is any change in environmental conditions that might reduce or adversely change plant's growth and development (Levitt, 1972).

➢ Adverse force or influence that tends to inhibit normal systems from functioning

➢ Biological stress is an adverse force or a condition, which inhibits the normal functioning and wellbeing of a biological system such as plants.

➢ Types of Stress:-

- Biotic stress
- Abiotic stress

BIOTIC STRESSS

Adverse conditions for crop growth and production caused by biological factors such as diseases, insects and parasitic weeds.

Types of Resistance to biotic stress:-

i) **Vertical resistance**:-Resistance of a host to the particular race of pathogen; also called as major gene resistance, oligogenic resistance, qualitative resistance.

ii) **Horizontal resistance:** - Resistance of a host to all the prevalent races of a pathogen; also called as minor gene resistance, polygenic resistance, nonspecific resistance, general resistance.

Mechanism of Disease resistance:-

Disease escape:- The ability of susceptible host plants to avoid attack of disease due to environmental conditions factors, early varieties, charge in the date of planting, change in the site of planting; balanced application of NPK etc is called disease escape.

Disease endurance or tolerance:- The ability of the plants to tolerate the invasion of the pathogen without showing much damage. This endurance is brought about by the influence of external characters. Generally, tolerance is difficult to measure since it is confounded with partial resistance and disease escape.

Immune reaction:- When the host does not show the symptoms of disease it is known as immune reaction. Immunity may result from prevention of the pathogen to reach the appropriate parts of the host e.g. exclusion of spores of ovary infecting fungi by closed flowering habit of wheat and barley.

Hypersensitivity:-Immediately after the infection several host cells surrounding the point of infection are so sensitive that they will die. This leads to the death of the pathogen because the rust mycelium cannot grow through the dead cells. This super sensitivity (hypersensitivity) behaves as a resistant response for all practical purposes. Phytoalexins are specific polyphenolic or terpenoid chemicals and are produced by the host in response to the infection by a pathogen.

Sources of Disease Resistance:

A known variety:- Disease reactions of most of the cultivated varieties are documented and a breeder may find the resistance he needs in a cultivated variety.

Germplasm collection:- When resistance to a new disease or a new pathotype of a • disease is not known in a cultivated variety germplasm collection should be screened.

Related species:- Often the resistance to a disease may be found in related species and transferred through interspecific hybridization. Eg. Resistance to stem, leaf & stripe rusts of wheat.

Mutation:- Resistance to diseases may be obtained through mutation arising spontaneously or induced through mutagenic treatments. Eg, Resistance to Victoria blight in oats was induced by irradiation with x-rays or thermal neutrons / also produced spontaneously, Resistance to stripe

rust in wheat, Resistance to brown rust in oats, Resistance to mildew in barley. Methods of

Breeding for Disease Resistance:-

Plant Introduction:- Process plants introduced fron their native plac to another place for crop improvement. Eg; Early varieties of groundnut introduced from USA have been resistant to leaf spot (Tikka). Kalyanasona and Sonalika wheat varieties originated from segregating material introduced from CIMMYT, Mexico, were rust resistant. African bajra introductions have been used in developing downy mildew resistant cms lines.

Selection:- Kufri Red potato is selection from Darjeeling Red round , PusaSawani behind (yellow mosaic) selection from a collection obtained from Bihar, MCU I was selection from CO4 for black arm resistance in cotton.

Pedigree method;- In wheat KalyanaSona, Sonalaka, Malvika 12, Malvika 37, Malavika 206, Malavika 234, Laxmi in Cotton (Gadag 1 x CO2) for leaf blight resistance are agronomically highly desirable variety. If the resistant parent is a wholly unadapted variety, backcross method is a logical choice

Budding and Grafting:- The disease resistance in vegetativelypropogated material is transferred by adopting either by budding or grafting. By grafting or budding the resistant material, the resistance can be transferred.

Mutation Breeding:- When adequate resistance is not available in the germplasm. Mutation breeding is resorted to induce resistance. This is also us ed to break the linkages between desirable resistant genes and other desirable genes. Varieties resistant to different diseases crop

variety:- Rice:- Blast Co25, Co26, Wheat:- all three rusts NP 809, Yellow rust NP 785, NM86,Sugarcane : Red rot Co 419, Co 421, Co 527,Cotton :- Wilt Vijay, Kalyan, Suyog, Tikkaleafspot:-Ah 45.

Mechanism of Insect- Pest resistance:

Non-preference:- Various features of host plant which make the host undesirable or unattractive to insects for food, shelter or reproduction, a mechanism of insect resistance; also called non-acceptance or antixenosis.

Antibiosis:- Adverse effects of the host on feeding, development and reproduction of insect pest.

Tolerance:- Ability of a host to reproduce well despite the establishment of a pathogen in the host tissues or the ability of a variety to produce more yield than susceptible variety at the same level of insect attack.

Avoidance:- Escape of a variety from insect attack either due to earliness or its cultivation in the season when insect population is very low.

LIST OF DISEASE/INSECT RESISTANT VARIETIES OF HORTICULTURAL CROPS

Crop	Resistant Variety	Disease/pest
Tomato	Pant Bahar, BSS-20, Roma, Meenakshi, Roza, HS-110, Pan American, Walter, ArkaAlok, ArkaAbha	Fusarium wilt
	H-24, H-36,Hissar Gaurav, HissarAnmol, ArkaAditya	Leaf curl virus
	H-22, H-25, SolanVajar, Kalyanpur No.1	Early blight

	ArkaVishesh	Triple virus resistance
Brinjal	ArkaAnand, ArkaNeelkanth, ARKA NIDHI, Pusa Purple Cluster , ArkaUnnathi, ArkaHarshitha, ArkaAvinash	Bacterial wilt
	Azad Hybrid	Fruit and Shoot borer
Chilli	ArkaHaritha, ArkaMeghana ArkaLohit	Powdery mildew
	PunkjabLal	Virus (CMV)
Capsicum	ArkaGaurav	Bacterial wilt
	Mahabharath	TMV
	Chinense Giant	Fruit rot
	Pant C1, Puri red, Puri Orange	Leaf curl virus
Bitter gourd	KalayanpurBarahmasi, KalyanpurSona, BG-4	Fruit fly
	Pant karela-1	Pumpkin beetle
	BTH-165	Powderyand downy mildew
Onion	IHR 56-1, Red Creole, New Selection and VL-67 , Pusa Red, VL-1, N-2-4-1, Ph. Sel-3, PusaRatnar and Pb. Sel-5 , IHR 25, IHR 471, IHR 500 and ArkaKalyan	Purple blotch
	Telagi Red, White large, Poona Red, Bellary Red, Udaipur 103, Patna Red and N-257-9-1 IHR 141, IHR 506	Stemphylum resistance
	N-2-4-1, Sel-104, PusaRatnar, Sel-171, HR Brown & Sel-1202,	Thrips

	Kalyanpur Red Round, Udaipur 103, SafedGol, No.5, White, Mathewad-1, Shirwal-2, White Creole, Kagar-2, Peth -1	
Potato	KufriSindhuri, JW160, KufriHimalni, KufriShalaija, KufriGirdhari	Late blight
	KufriBadshah	Potato virus-X
	KufriSwarna	Golden cyst Nematode
Bhindi	Sel-7-1, Round selection	Cercospora leaf blight
	Red Ghana, Sel-7-1, BH-27	Damping off
	ParbhaniKranti, Punjab Padmini	YMV
Pea	LMR-4,LMR-10,LMR-20	Leaf miner
	JP 9 ,JP 179, JP JPBatri Brown 3, JP Batri	Bruchids
	ArkaKarthik, ArkaSampoorna, ArkaPriya ArkaPramodh	Powdery mildew
	ArkaApoorva	Rust
Cauliflower	ArkaSpoorthi, ArkaVimal	Downy mildew
Amaranth	ArkaArunima, ArkaSuguna	White rust
Dolichos bean	ArkaPrasidhi	Rust
French bean	ArkaArjun	MYMV
Pumpkin	ArkaSuryamukhi	Fruitfly
Water melon	Arka Manik	Podery mildew
Grapes	Arka Trisha	Anthracnose

Gladiolus	KumKum	Fusarium wilt
Rose	ArkaParimala	Thrips

ABIOTIC STRESSES

Abiotic stresses:-include potentially adverse effects of Salinity, Drought, Flooding, Metal toxicity, Nutrient deficiency, High temperature and Low temperature. In addition, abiotic stresses can include Shade, UV exposure, Photo inhibition, Air pollution, Wind, Hail and Gaseous deficiency which are often sporadic and highly localized in occurrence. Plants can experience abiotic stress resulting from the shortage of an essential resource or from the presence of high concentrations of a toxic or antagonistic substance. In some cases, such as the supply of water, too little (drought) or too much (flooding) can both impose stress on plants.

Major abiotic stresses

Drought stress: Nowadays climate has changed all around the globe by continuously increase in temperature and atmospheric CO2 levels. The distribution of rainfall is uneven due to the change in climate which acts as an important stress as drought. The soil water available to plants is steadily increased due severe drought conditions and cause death of plants prematurely. After drought is imposed on crop plants growth arrest is the first response subjected on the plants. Plants reduce their growth of shoots under drought conditions and reduce their metabolic demands. After that protective compounds are synthesized by plants under drought by mobilizing metabolites required for their osmotic adjustment.

Submergence/ Waterlogging/ Flooding stress : Flooding may be defined as any situation of excess water. Sudden inundation following high rainfall events also poses a severe physiological stress on crops. The

gradual inundation of crop lands that occurs in a more regular cycle of seasonal changes in river levels and associated gradual flooding of crop lands poses a different, but equally challenging, flooding environment to which plants must adapt. As a result, some plants, such as rice, evolve a semiaquatic habit. Flooding stress in terrestrial species is referred to as waterlogging and the damage symptoms caused are primarily due to the prolonged exposure of the plants to hypoxia. The effect of waterlogging of roots and lower stems are apparent as a range of symptoms on the shoots, including rapid wilting and severe physiological disruption. Vast areas of rainfed crops, particularly in South and Southeast Asia, are annually affected by flooding.

Heat stress: Increase in temperature throughout the globe has become a great concern, which not only affect the growth of plants but their productivity as well especially in agricultural crops plants. When plants encounter heat stress the percentage of seed germination, photosynthetic efficiency and yield declines. Under heat stress, during the reproductive growth period, the function of tapetal cells is lost, and the anther is dysplastic.

Cold stress: Cold stress as abiotic stress has proved to be the main abiotic stresses that decrease productivity of agricultural crops by affecting the quality of crops and their post-harvest life. Plants being immobile in nature are always busy to modify their mechanisms in order to prevent themselves from such stresses. In temperate conditions plants are encountered by chilling and freezing conditions that are very harmful to plants as stress. In order to adopt themselves, plants acquire chilling and freezing tolerance against such lethal cold stresses by a process called as acclimation. However many important crops are still incompetent to the process of cold acclimation. The abiotic stress caused by cold affect the cellular functions of plants in every aspect. Several signal transduction pathways are there by which these cold stresses are transduced like

components of ROS, protein kinase, protein phosphate, ABA and Ca2+, etc. and among these ABA proves to be best.

Salt stress: Soil salinity poses a global threat to world agriculture by reducing the yield of crops and ultimately the crop productivity in the salt affected areas. Salt stress reduces growth of crops and yield in many ways. Two primary effects are imposed on crop plants by salt stress; osmotic stress and ion toxicity. The osmotic pressure under salinity stress in the soil solution exceeds the osmotic pressure in plant cells due to the presence of more salt, and thus, limits the ability of plants to take up water and minerals like K+ and Ca2+. These primary effects of salinity stress causes some secondary effects like assimilate production, reduced cell expansion and membrane function as well as decreased cytosolic metabolism.

Toxins: The increased dependence of agriculture on chemical fertilizers and sewage waste water irrigation and rapid industrialization has added toxic metals to agriculture soils causing harmful effects on soil-plant environment system.

Drought:-Drought can be defined as an extended period of deficient rainfall relative to the statistical mean for a region.

- **Meteorological drought** is qualified by any significant deficit of precipitation.

- **Hydrological drought** is manifest in noticeably reduced river and stream flow and critically low groundwater tables.

- **Agricultural drought:** indicates an extended dry period that results in crop stress and crop yield. The impact of drought on agriculture is due to a deficit of moisture in the soil, when the moisture in the soil is no longer sufficient to meet the needs of growing crops.

Effects of drought stress on crops:-

- Reduced seed germination and seedling development.
- Poor vegetative growth.
- Impaired Reproductive growth
- .
- Plant height and leaf area reduced.
- Significantly reduction in leaf weight.
- Reduced photosynthesis.
- Reduced stomatal conductance.
- Reduced total dry matter.

Effects of Heat stress on crops

- Seedling establishment is hampered
- Drying of leaf margins and scorching effect on leaves
- Reduced in plant growth
- Impaired Pollen development
- Alteration in photosynthesis
- Reduced total biomass

- Spikelet sterility

- Impaired grain and fruit development and quality

Drought resistance:- The ability of crop plants to grow, develop and reproduce normally under moisture deficit conditions is referred to as drought resistance.

Mechanisms of Drought Resistance:

- Drought escape:- Plant avoid the injury of stress by regulating its life cycle to avoid meeting with stress. This is not the kind of resistance – some short-lived, desert ephemeral plants germinate, grow and flower very quickly following seasonal rains. They thus complete their life cycle during a period of adequate moisture and form dormant seeds before the onset of dry season. •

- Drought avoidance:-Drought avoidance refers to ability of the plant to maintain a favourable internal water balance under moisture stress. In other words, plants which avoid drought retain high water contents in their tissues. Drought avoidance can permit a longer growth period in the crop through reduced water use or increased water uptake

- Drought tolerance :-The ability of crop plants to withstand low tissue water content is referred to as drought tolerance. Drought tolerance is more desirable because the crop can produce more yield at lower water potential.

- Drought resistance:-Drought resistance is the sum of drought avoidance and drought tolerance. It refers to the ability of crop

plants to give higher yield under moisture stress conditions or survival of plants under water deficit conditions without injury.

Source of Drought Resistance in Plant Breeding:

- **Cultivated varieties:-** Transfer of drought resistance is easy from cultivated variety and germplasm of cultivated species, because such material can be easily used in the breeding programmes. Moreover, there is no problem of cross incompatibility

- **Germplasm collections**

- **Wild relatives and wild species:-** When the source of drought resistance is a wild species, the transfer of resistance poses several problems such as cross incompatibility, hybrid inviability, hybrid sterility and linkage of several undesirable genes with desirable ones. Wild sources of drought resistance have been reported in wheat, sugarcane, tomato, and several other crops.

Plant Breeding Methods for Drought Resistance:-

Breeding methods for drought resistance are the same as for yield and other economic characters. Breeding for drought resistance refers to breeding for yield under soil moisture stress condition. Four breeding methods, viz.;

- Introduction,
- Selection ,
- Hybridization ,
- Mutations are commonly used for development of drought resistant crop cultivars.

Salt tolerance: Ability of plants to prevent, reduce or overcome injurious effects of soluble salts present in their root zone. Salinity can be overcome by

- Soil reclamation: costly, time consuming and short lived .
- Resistant varieties: less costly, more effective, long lasting.

Classification of plants based on salt tolerance:-

- Highly tolerant crops: Sugar beet, barley, cotton, date palm, asparagus.
- Moderately tolerant: Barley, rye, sorghum, wheat, safflower, Soya been.
- Moderately sensitive: Rice, corn, foxtail millet, cow pea, peanut, sugar cane, tomato, potato, radish, cabbage.
- Extremely sensitive: Citrus, strawberry, melon, peas, carrot, okra, onion.

Mechanism of salt tolerance:-

- **Salt tolerance**: By accumulating salt, generally in their cells or glands & roots. Halophytes show tolerance by ion accumulation mechanism .
- **Salt avoidance**: By maintaining their cell salt concentration unchanged either by water absorption (e.g. Rice, chenopodiaceae) or by salt exclusion (e.g. tomato, Soya bean, citrus, wheat grass)

Glycophytes (nonhalophytes) owe their resistance primarily to avoidance e.g. barley

Breeding strategies:- Breeding for yield potential should have greater emphasis than breeding for salt resistance. Selection should be done in stresses target environments Screening techniques 1. Sand culture by using nutrient solution in sand & irrigation with saline water. 2. Solution culture by using solution culture tanks 3. Micro plot techniques by using small micro plots.

➢ Achievements:- Rice : Mohan, pokkali, SR23b, SR26b, CSR-2, CSR-3, CSR-6. Onion: Hissar-2, pb selection, Karchia, Karna-92. Okra: PusaSawani Barley: Ratna, RS-16, Karan18, 19, 92 Sugar cane: Co7717, Co1148, Bo9.

Chilling tolerance:-

Chilling: When temp remain above freezing i.e. >0°C to < 10-15°C.

Freezing: When temp remains below freezing i.e. < 0^{O}C

Genetic resources for freezing tolerance:-

- Cultivated varieties,
- Germplasm lines,
- Induced mutations ,
- Related wild species e.g. Wheat: Agropyron sp., Rye Oats: Avenasterilis Transgenes: e.g. chemical synthesized anti freeze protein gene, ala3 in tobacco

Selection criteria: Freezing test in laboratory, Cryo freezing, Osmoregulation, Field survival.

Mechanism of freezing resistance:-

Freezing avoidance : The ability of plant tissues / or genes to avoid ice formation at sub zero temperature Super cooling is a mechanism of freezing avoidance which is controlled by Lack of ice nucleators, Small cell size, Little or no intercellular space, Low moisture contents, Barriers against external nucleators

Freezing tolerance: Ability of plants to survive the stress generated by extra cellular ice formation and to recover and re grow after thawing Components of freezing tolerance, Osmotic adjustment , Amount of bound water, Plasma membrane stability, Cell wall components properties, Cold responsive proteins, e.g. ABA

Genetic resources for freezing tolerance:-

- Cultivated varieties,
- Germplasm lines,
- Induced mutations ,
- Related wild species e.g. Wheat: Agropyron sp., Rye Oats: Avenasterilis Transgenes: e.g. chemical synthesized anti freeze protein gene, ala3 in tobacco

Selection criteria: Freezing test in laboratory, Cryo freezing, Osmoregulation, Field survival.

LIST OF ABIOTIC RESISTANT VARIETIES IN HORTICULTURAL CROPS

Crop	Resistant Variety	Disease/pest
Tomato	Pusa hybrid-1	**Heat stress**

	PusaSadabahar	**Cold stress**
	SabourSuphala	**Salt stress**
	ArkaVikas	**Drought stress**
Potato	H92, H621	**Heat and drought**
Custard Aple	ArkaSahan	**Drought**
Grapes	Doridge	**Drought**

Course Name	Principles of Plant Breeding
Lesson 22	Polyploidy Breeding
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
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Designed and developed under the aegis of NAHEP Component-2 Project "Investments In ICAR Leadership In Agricultural Higher Education"
Division of Computer Applications, ICAR-Indian Agricultural Statistics Research Institute

1. Objectives

1. Understand characteristics of auto and allo-polyploids
2. Application of polyploids in crop improvement

Glossary of terms

Polyploid: Individuals having multiple sets of chromosome number

Haploid: An individual carrying the gametic chromosome number, n, is known as haploid.

Monoploid: A monoploid has the basic chromosome number, x.

Autopoplyloids: A polyploid organism that originates by the multiplication of a single genome of the same species

Allopolyploids: Plants with more than two sets of chromosomes that originate from two or more
parents; the sets contain at least some nonhomologous chromosomes

POLYPLOIDY

The somatic chromosome number of any species, whether diploid or polyploidy, is designated as 2n, and the chromosome number of gametes is denoted as n. An individual carrying the gametic chromosome number, n, is known as haploid. A monoploid, on the other hand, has the basic chromosome number, x. In a diploid species, n=x; one x constitutes a genome or chromosome complement. The different chromosomes of a single genome are distinct from each other in morphology and or gene content and homology; members of a single genome do not show a tendency of pairing with each other. Thus a diploid species has two, a triploid has 3 and a tetraploid has 4 genomes and so on.

In euploids, the chromosome number is an exact multiple of the basic

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or genomic number. Euploidy is more commonly known as polyploidy. When all the genomes present in a polyploidy species are identical, it is known as autopolyploid and the situation is termed as autopolyploidy. In the case of allopolyploids, two or more distinct genomes are present. Euploids may have 3 (triploid), 4(tetraploid), 5 (pentaploid), or more genomes making up their somatic chromosome number. In caseof autopolyploidy, they are known as autotriploid, autotertaploid, autopentaploid, and so on, while in the case of allopolyploidy they are termed as allotriploid, allotetraploid, allopentaploid, etc. Amphidiploid is an allopolyploid that has two copies of each genome present in it and, as a consequence, behaves as a diploid during meiosis. A segmental allopolyploid contains two or more genomes, which are identical with each other, except for some minor differences.

Examples of polyploidy crops

Triploid crops: Banan, Apple, Ginger, Watermelon, Citrus
Tetraploid crops: Potato, Cabbage, Leek, Tobacco, Peanut, Kinnow, Pelargoinium
Hexaploid crops: Chrysanthemum, Bread wheat, Triticale, Oats, Kiwi fruit
Octaploid crops: Strawberry, Dahilia, Pansy, Sugarcane

Types of Polyploids

A. **Aneuploids:** Those polyploids in which the increased chromosome number is not an exact multiple of basic chromosome number. Various types of aneuploids are:

   Monosomics : Organisms having one chromosome less than the normal multiple of basic chromosome number (2N-1)

   Trisomics: Organisms having one chromosome more than the normal multiple of basic chromosome number (2N + 1)

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Tetrasomics: Organisms having one chromosome pair of same homologous group more than the normal multiple of basic chromosome number (2N + 1)

Nullisomics: Organisms having one chromosome pair of same homologous group less than the normal multiple of basic chromosome number (2N - 2)

Double monosomics: Organisms having two chromosomes less than the normal multiple of basic chromosome number (2N – 1 - 1)

Double Trisomics: Organisms having two chromosomes less than the normal multiple of basic chromosome number (2N + 1 + 1)

Origin of Aneuploids

(1) Mitotic or meiotic, abnormalities, e.g., lagging chromosomes resulting in the nuclei/cells with hypoploid chromosome numbers.

(2) Hypo- and hyper-ploid nuclei may be formed due to chromosome/chromatid nondisjunction during mitosis or meiosis. Such gametes would, on union with normal (n) gametes, give rise to aneuploid progeny.

(3) Polyploids, with an odd number of chromosome sets, e.g., triploid, pentaploid etc., result in univalents during meiosis.

(4) Multipolar mitosis where distribution of chromosomes in the daughter cells is irregular. Such aneuploid cells may possess varying numbers of chromosomes and may occur in the same tissue forming chromosome mosaicism. Such type of aneuploidy is called as multiform-aneuploidy.

(5) Nondisjunction during postmeiotic divisions, such as during the formation of microgametophytes and megagametophytes.

B. **Euploids**: Polyploids having a chromosome number which is exact

multiple of basic chromosome number such as Triploids (3), Tetraploids (4), Pentaploids (5), Hexaploids (6) and so on.

Origin Autopolyploids

1. **Spontaneous**: chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are produced in low frequencies.

2. **Production of adventitious buds** : decapitation in some plants leads to callus development at the cut ends of the stem. Such a callus has some polyploid cells and some of the shoot buds regenerated from the callus may be polyploid. In solonaceae 6-36% of adventitious buds are tetraploids. The frequency of ploypoid buds may be increased by the application of 1% IAA at the cut ends as it promotes callus development.

3. **Treatment with physical agents**: Heat or cold treatment centrifugation , x-ray or gamma ray irradiation may produce polyploidy. Exposing the plants or ears of maize to a temperature of 38-45 ^{0}C at the time of the first division of zygote produce 2-5 % tetraploid progeny.

4. **Regeneration in vitro**: polyploidy is a common feature of the cells cultured in- vitro.

5. **Colchicine treatment**: Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling.

Autopolyploidy

In autopolyploidy, triploidy, tetraploidy and higher levels of ploidy are

included.

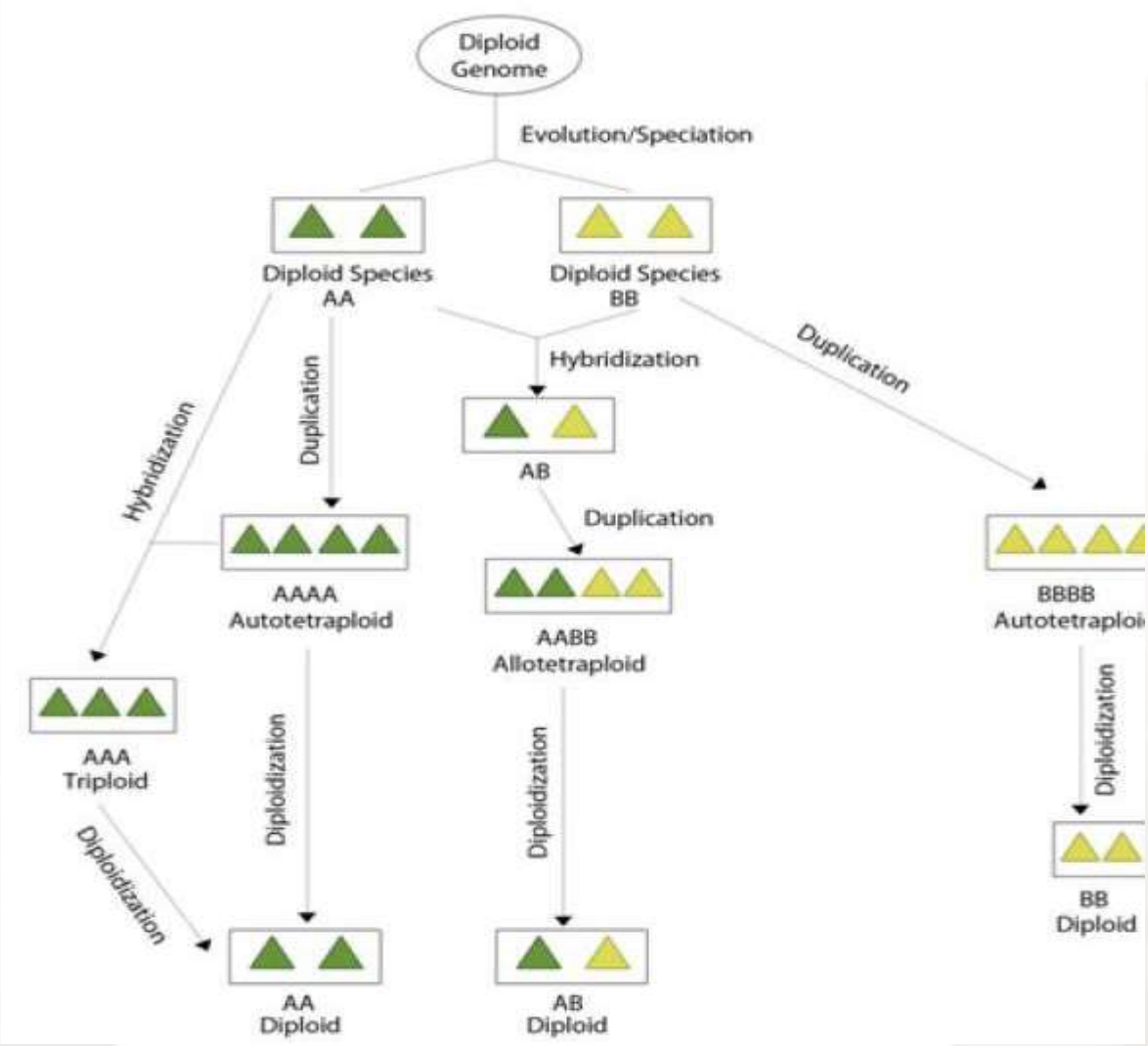

Fig : Major pathways of polyploid formation

Morphological and cytological features of auto polyploids :
The general features are summarized below.

1. Polyploids have larger cell size than diploids. Guard cells of stomata are largerthe number of stomata per unit area is less in polyploids than diploids.

2. Pollen grains of polyploids are generally larger than those

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of the correspondingdiploids.

3. Polyploids are generally slower in growth and later in flowering.

4. Polyploids usually have larger and thicker leaves, and larger flowers and fruitswhich are usually less in number than in diploids.

5. Polyploids generally show reduced fertility due to irregularities during meiosisand due to genotypic imbalance leading to physiological disturbances.'

6. In many cases autopolyploidy leads to increased vigour and vegetative growth.

7. Different species have different levels of optimum ploidy. For sugarbeet theoptimum level is 3x, sweet potato 6x while for timothy grass it is between 8-10x.

8. Autopolyploids generally have lower dry matter content than diploids.

Application of Autopolyploidy in Crop improvementTriploids

Triploids are produced by hybridization between tetraploid and diploid strains.They are generally highly sterile, except in a few cases. This feature is useful in the production of seedless watermelons. In certain species, they may be more vigorous than the normal diploids, e.g., in sugarbeets. These two examples are described in some detail.

Seedless watermelons are produced by crossing tetraploid (4x, used as female) anddiploid (2x, used as male) lines, since the reciprocal

cross (2x x 4x) is not successful. The triploid plants do not produce true seeds; almost all the seeds are small, white rudimentary structures like cucumber (*Cucumis sativus*) seeds. But few normal size seeds may occur which are generally empty. For good seed setting pollination is essential. For this purpose diploid lines are planted in the ratio 1 diploid : 5 triploid plants. There are several problems viz. genetic instability of 4x lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in trioploid seed production.

Triploid sugarbeets : Among root crops triploid sugar beets apparently representthe optimum level of polyploidy because 3n plants have longer roots than diploid andalso yield more sugar per unit area.

Tetraploid rye : the advantage of tetraploid over its diploid counterpart are large kernel size, superior ability to emerge under adverse condition and higher protein content.tetraploid rye varieties have been released for cultivation. Eg. Double steel, Tetra petkus.

Limitations of autoployploidy:

1. Larger size autoployploiods generally contain more water and produce less dry matter content than diploids

2. High sterility with poor seed setting is observed

3. Due to complex segregation, progress through selection is slow

4. Monoploids and triploids cannot be maintained except through clonal propagation

5. The varieties cannot be produced at will

6. Effects of autopolyploidy cannot be predicted.

Segregation in Auto tetraploids

Segregation in autotetraploids is much more complex than in diploids. Depending upon the number of dominant alleles present, they are referred as simplex (Aaaa), duplex (AAaa), triplex (AAAa), Quadruplex (AAAA) and nulliplex (aaaa). On selfing asimplex will produce two types of gametes Aa and aa in 1:1 ratio due to random chromosome segregation. Self pollination of such a simplex would produce three genotypes AAaa, Aaaa and aaaa in the ratio 1:2:1 giving the phenotypic ratio of 3 : 1. While as a duplex will produce 3 types of gametes viz., AA, Aa and aa in the ratio of 1:4:1.

Allopolyploidy :

Allopolyploids have genomes from two or more species production of allopolyploids has attracted cons iderable attention; the aim almost always was creation of new species. Some success has been evident from the emergence of triticale. Raphano brassica and allopolyploids of forage grasses.

Morphological features of allopolyploids

1. Allopolyploids combine the morphological and physiological characteristics of the parent species but it is very difficult to predict the precise combination of characters that would appear in the new species.

2. Many allopolyploids are apomictic Ex : Tulips, Solanum

3. The chromosome pairing in the new species depends upon the

similarities between the chromosomes of the parental species. Chromosomes with such similarities are known as homoeologous chromosomes. After chromosome doubling, the allopolyploid would have two homeolegous chromosomes for each chromosome present in the F1 hybrid, comparable to the diploid species. Such allopolyploid is referred as amphidiploid or Allotetraploid.

4. Fertility of Allopolyploids can be improved by hybridization and selection.

APPLICATION OF ALLOPOLYPLOIDY IN CROP IMPROVEMENT:

Utilization as a Bridging species : Amphidiploids serve as a bridge in transfer of characters from one species to a related species, generally from a wild species to cultivated species. An example of use of an amphidiploid as a bridging species in theuse of synthetic *N.digluta* or transfer of resistance to tobacco mosaic virus from *N.glutinosa* to *N.tabacum*. The F1 hybrid from the cross *N.tabacum* x *N.glutinosa* is sterile. Chromosome doubling of the F1 hybrid produces the synthetic allehexaploid *N.digluta* which is reasonably fertile. *N digluta* is backcrossed to the recipient species (*N.tabacum*) to produce a pentaploid having complete somatic chromosome complement of *N.tabacum* and one genome of *N.glutinosa*. The pentaploid is sufficiently fertile to be backcrossed to *N.tabaccum*. In the progeny N.tabacum like plants resistant to tobacco mosaic are selected and cytologically analysed.

1. Creation of New crop species Ex : Triticales, Raphanobrassica Triticum turgidum x Secale cereale.

2. Widening the genetic base of existing Allopolyploids : The genetic base of some natural allopolyploids may be narrow, and it

may be useful to introduce variability in such cases by producing the allopolyploids afresh from their parental species.*B.napus* is a case in point; the genetic variability of this species is narrow and the only recourse available is to synthesize new allopolyploid *B. napus* to widen its genetic base. This is being done by crossing *B. campestris* (n=10, AA) with *B. oleracea* (n=9, CC), the parental diploid species, to produce the amphidiploid *B.napus* (n=19, AACC). The two species, *B.campestris* and *B.olerancea*, have to be crossed as autotetraploids; the cross is very difficult and embryo culture has to be used; somatic hybridization is being used to get around these problems.

Application of Anueploids

1. The aneuploids have not been much direct use in plant breeding. Used to incorporate alien addition of complete chromosomes or preferably segments of chromosomes.
2. Monosomics has been used in gene mapping.
3. Aneuploids are used in the genetic studies to identify the chromosome or arm of the chromosome on which a particular gene is located.
4. Nullisomic lines have also been used to assign genes to a particular chromosome but the nullisomics are less vigorous and less fertile than monosomics.
5. Monosomics are also very useful for intervarietal chromosome substitution.

Limitations of Allopolyploidy

1. The effects of allopolyploidy cannot be predicted. The allopolyploids have some features from both the parental species, but these features may be the undesirable ones, e.g., Raphanobrassica, or the desirable ones, e.g., Triticale.

2. Newly synthesized allopolyploids have many defects, e.g., low fertility,cytogenetic and genetic instability, other undesirable features etc.

3. The synthetic allopolyploids have to be improved through extensive breeding at the polyploidy level. This involves considerable time, labour and other resources.

4. Only a small proportion of allopolyploids are promising; a vast majority of them are valueless for agricultural purposes.

Varieties developed by polyploidy in Horticultural crops

Crop	Variety	Trait
Apple	Hanfu Gala	Salt tolerance Salt tolerance
Grapes	Wanheibao Shenfeng	Berry size Berry size
Ber	Chenguang	Fruit size
Pear	Beure Diel	Fruit size
Pineapple	Gigante de Tarauca	Fruit size
Guava	Pusa Sirjan	Seedless
Watermelon	Sugar Baby Swarna Shonima	Seedless Seedless Seedless

Induction of polyploidy

Polyploidy does not exist naturally in all plant genera, so it has been artificially induced in many economically important crops. Formation of induced polyploids is an effective way to develop genetic variation for study of genetics and plant breeding. Polyploidy can be artificially

induced through interspecific hybridization, in vitro endosperm culture or somatic cell doubling through colchicine.

There are two different ways to induce polyploidy artificially:

(i) Meiotic (sexual): In meiotic polyploidization, *2n* pollens (gametes) are produced. Naturally produced sexual polyploids, particularly triploids produced through formation of *2n* gametes, have been studied as important features in the evolution of flowering plants. Although, artificially induced sexual polyploids are less frequently used for breeding purpose

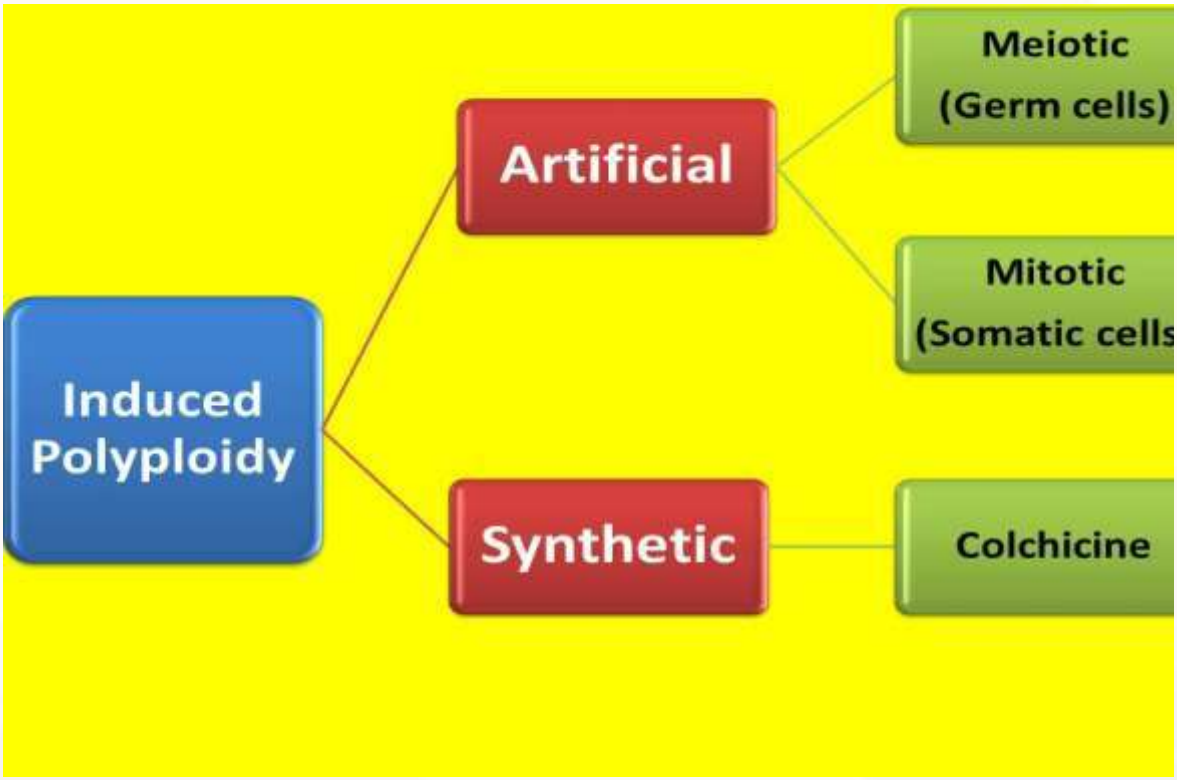
(ii) Mitotic (somatic): In mitotic polyploidization, the duplication of chromosome is done in somatic cells. This type of chromosome duplication is widely used to induce tetraploids through anti-mitotic chemicals. However, chromosome duplication of somatic cells form additional copies of chromosome and existing genes but many variations occur after the chromosome doubling which leads to variations in plant phenotype.

Synthetic polyploidy: Colchicine induced

Colchicine is a poisonous alkaloid derived from bulbs (0.1–0.5%) and seeds (0.2%–0.8%) of Autumn crocus or Meadow saffron (*Colchicum autumnale*). Colchicine works by preventing the microtubules formation and doubles the number of chromosomes. It is commonly used to develop polyploid plants and functions as a mitotic poison and induces polyploidy by preventing the segregation of chromosomes during meiosis that results into half of the gametes (sex cells) containing double the chromosome number than usual.

Colchicine is commonly applied in a form of an aqueous solution; however, it is unstable in water. Therefore, it is advisable to make a fresh aqueous solution before treatment. Colchicine concentrations for

seed treatment usually range from 0.1%–0.8%, but high doses cause malformation and reduce the production of tetraploids plants.



HAPLOID PRODUCTION

In-vivo Approaches

1. **Androgenesis**: This is a spontaneous process of *in vivo* development of a male derived haploid embryo from a fertilized egg in the absence of, or after the elimination of, the female nucleus. Now, advances in research have generated several approaches to perform the procedure under in vitro conditions.

2. **Gynogenesis**: This is a process of development of an embryo from unfertilized eggs or ovaries. In vivo, it occurs when the zygote formation/fertilization event does not occur but the cell division of the egg is initiated. This condition forms haploid seeds of completely maternal origin.

3. **Distant hybridization**: This is defined as a cross between species of the same or two different genera. So, it's a selective elimination of chromosomes of one parent for haploid production.

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4. **Irradiation effects**: Ionizing (x-ray and gamma-ray) and non-ionizing radiation are used to treat pollens employed in the pollination of normal plants. The process leads to the destruction of the germination of pollens but doesn't affect the capability of the egg cell. This induces parthenogenetic embryo development in plants.

The first study of radiation-induced haploid production was reported in Triticum monococcum and later experimented in several commercial crops. The process is affected by several factors, including genotype, physiological status of parents, source, and the dose of irradiation.

5. **Chemical treatments**: Chemicals like colchicine, nitrous oxide, and maleic hydrazide are used to produce haploids. However, some mitotic inhibitors have proved to be very helpful in the production of double-haploids (the number of chromosomes is doubled). These include: colchicine, oryzalin, amiprophosmethyl, trifluralin, and pronamide.

In-vitro approaches

1. **Androgenesis**: The production of haploids through anther or pollen culture has been reported in 135 species. The principle involved in the process is to halt the development of pollen cells into a gamete and induce it in a suitable environment to develop into a haploid plant. The two types of androgenesis include:

Direct androgenesis: Embryo directly from pollen or microscope without callus.

Indirect androgenesis: Embryo with an intermediary callus stage.

2. **Gynogenesis**: The process of development of haploids through ovary or ovule culture is known as gynogenesis. It was first reported in *Hordeum vulgare* (Barley) by San Norm in 1976, and later the technique was used for haploid production in wheat, maize tobacco, sunflower, sugarbeet, and other economically important plants. This approach is used where androgenesis is not effective in producing haploids. To-date, haploids have been produced in about 19 species

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Course Name	Principles of Plant Breeding
Lesson 23	Mutation Breeding
Content Revisor	Dr. Parvaze Ahmad Sofi
University/College Name	Junagadh Agricultural University, Junagadh
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Objectives

1. Understand genetic basis and role of mutations in crop improvement
2. Understand operational procedures and impacts of mutation breeding in crops

Glossary of terms

Mutation: A sudden heritable change other than the Mendelian segregation and gene recombination in an organism that results in altered phenotype.

Diplontic selection: It refers to competitive disadvantage of mutant cells to normal cells. The normal cells proliferate rapidly and thus restrict the mutant cells to a small region that may be excluded from contribution in gamete formation.

Haplontic selection: It refers to the competitive disadvantage of mutated germ cells to their normal counterparts.

Mutant Cleaning: A method whereby a desirable mutatnt having an undesirable mutation is corrected by backcrossing with normal variety.

TILLING: Induction of a series of alleles in a target locus, providing that its sequence is known using mutations.

Eco-TILLING: Mining of naturally occurring allele variants in germplasm without mutations.

Breeders often face situations where variability for a trait is absent in cultivated or wild germplasm or is too low in magnitude to be of any practical value. In such situations breeders look to use various out of turn methods either to createvariability or to enhance its magnitude

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through creation of better alleles of endogenous genes. One of the approaches for such methods is the use of induced mutations. Breeding methodologies that use mutations at any stage of breeding are called as “Mutation Breeding”.

Over a period of time since the discovery of mutagenic effect of X-ray by Muller in 1920, Mutation breeding has been used practically to develop crop cultivars with better expression of traits for which improvement was sought. The achievements made through mutation breeding in crop improvement have been spectacular in view of the following facts:

1. Not many breeders use mutation breeding due to under-appreciation ofmethod and lack of capacity and resources.
2. Mutant varieties have been the leading national varieties e.g., Yuanjengzao rice variety in China (1970-80), Zheju-802, Niab-78 cotton in Pakistan.
3. Several mutant genes have been incorporated in crop varieties such as Sd1 gene in Reimei in Japan and Calrose-76 in USA, mlo gene in barley, o-2 gene in maize.
4. Mutations have been used to improve almost all traits.
5. Mutation breeding can be used to change a trait keeping other traits unchanged.
6. Less than 1% of induced mutations have been chosen for further investigation out of which only 0.5-1% have passed further necessary trials until they were found to be of commercial value. Thus in plant breeding average proportion of useful mutants to useless mutants is 1:10000.

Historical Background

1. The term mutation was coined by Hugo Devries in 1900 for the first time and then word is derived from the latin word

'MUTARE' means to change. Mutation is the sudden heritable change other than the Mendelian segregation and gene recombination in an organism. Mutation may be the result of a change in a gene, a change in chromosome that involves several genes or a change in plasmagene.

2. In 1907, Cramer published extensive examples of spontaneous mutants in crop plants
3. In 1927, Muller working with Drosphila provided proof of mutation induction by X-rays.
4. In 1928, Stadler published the results of mutation experiments
5. In 1936, First mutant variety of tobacco, Chlorina released using X-rays
6. In 1936, First report of induced disease resistance in a crop plant; X-ray induced mildew resistance in barley (Freisleben and Lein)
7. In 1946, First report of chemically induced mutation in Drosophilla by Auerbach and Robinson
8. In 2000, TILLING tools were developed for creating desirable mutants.

CAUSES OF MUTATIONS

Errors in DNA Replication: On very, very rare occasions DNA polymerase will incorporate a non-complementary base into the daughter strand. During the next round of replication the mis-incorporated base would lead to a mutation. This, however, is very rare as the exonuclease functions as a proofreading mechanism recognizing mismatched base pairs and excising them.

Errors in DNA Recombination: DNA often rearranges itself by a process called recombination which proceeds via a variety of mechanisms. Occasionally DNA is lost during replication leading to a mutation.

Chemical Damage to DNA: Many chemical mutagens, some exogenous, some man-made, some environmental, are capable of damaging DNA.

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Many chemotherapeutic drugs and intercalating agent drugs function by damaging DNA.

Radiation: Gamma rays, X-rays, even UV light can interact with compounds in the cell generating free radicals which cause chemical damage to DNA

CLASSIFICATION OF MUTATIONS

A. On the basis of source

- Natural or spontaneous mutations- caused by natural forces and occur at a very low frequency.
- Induced or artificial mutations- induced by artificial forces such as chemicals and radiations that cause changes in DNA structure and occur at an enhanced frequency.

B. On the basis of direction

- Forward mutation-from wild type to mutant
- Reverse or supressor mutation- from mutant to wild type

C. On the basis of effect on DNA structure

- Point mutations- involve changes at specific sites in a gene
- Frameshift mutations-involve the addition or deletion of a number of nucleotides
- Base substitution, in which one base is substituted for another
- Transition- change of a pyrimidine to another pyrimidine, such as C to T, or a purine to

another purine

- Transversion-in which purines and pyrimidine are interchanged
- Deletion mutations — loss of some portion of DNA.
- Insertion mutations — addition of one or more extra nucleotides.

D. **On the basis of effect on Code**

- Silent mutations-those that do not alter the amino acid sequence
- Missense mutations- those causing base substitutions in which an amino acid change occur
- Nonsense mutations-involve a change from a normal codon to a stop codon**.** When a nonsense mutation occurs in a bacterial operon, it may also inhibit the expression of downstream genes. This phenomenon is termed **polarity.**

E. On the basis of site of effect

- Gene mutations- mutations in the coding regions of genes
- Promoter mutations- mutation within non-coding sequences altering the sequence within the core promoter of a gene. Promoter mutations that increase transcription are termed up promoter mutations making a sequence more like the consensus sequence In contrast, a down promoter mutation causes the promoter to become less like the consensus sequence, decreasing its affinity for regulatory factors and decreasing the transcription

F. On the basis of effect on organism

- Neutral mutation- does not alter protein function, so it does not affect survival or
- reproductive success
- Deleterious/ sub-lethal mutation- decreases the chances of survival and reproduction
- Beneficial mutation- enhances the survival or reproductive success of an organism
- Conditional mutation- affect the phenotype only under a defined set of conditions

G. **On the basis of tissue**

- Somatic mutation- occurs within a somatic tissues
- Germ-line mutation- occur directly in a sperm or egg cell, or it can occur in a precursor

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cell that produces the gametes

H. On the basis of expression

- Dominant mutations- can show effect in both homozygous and heterozygous state
- Recessive mutations -express only when homozygous

I. On the basis of recurrence

- Recurrent mutations- occur repeatedly or again and again in different individuals of the
- same population
- Non-recurrent mutation- do not occur repeatedly or again and again in different individuals of the same population

J. On the basis of survival

- Lethal mutations-that cause death in all individuals carrying mutation
- Sub-Lethal mutations- when more than 50% individuals carrying the mutation die
- Sub- vital - when less than 50% individuals carrying the mutation survive
- Vital- when all the mutants survive

H. On the basis of character

- Morphological-effects morphological traits
- Biochemical-effects biochemical processes

I. On the basis of Effect on Gene Function:

 Loss-of-function mutation — a mutation that results in a lack of gene function, this can result from a number of different types of mutations and is recessive in nature.

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 Gain-of-function mutation — a mutation that results in a new or different gene function; this can result from a number of different types of mutations and is dominant in nature.

PHYSICAL MUTAGENS

Mutagen	Characteristics
X- rays	Electromagnetic radiation; penetrate tissues from a few millimeters to many centimeters first discovered by Roentgen 1895 wavelength varies from 10-11 to 10-7. Highly penetrating they break chromosomes and produce all types of mutations in the nucleotides like addition, deletion, inversion, transition and transversion.
Gamma rays	Electromagnetic radiation produced by radioisotopes and nuclear reactors; very penetrating into tissues; sources are Co60 and Ce137 largely used in crop plants
Neutrons	A variety exists (fast, slow, thermal); produced in nuclear reactors; uncharged particles; penetrate tissues to many centimeters; source is U235
Beta particles	Produced in particle accelerators or from radioisotopes; are electrons; ionize; shallowly penetrating; sources include P32 and C14
Alpha particles	Derived from radioisotopes; a helium nucleus capable of heavy ionization; very shallow penetrating

Protons	Produced in nuclear reactors and accelerators; derived from hydrogen nucleus penetrate tissues up to several centimeters
Non ionizing radiations	Non-ionizing radiation produced from mercury vapor lamps or tubes. They penetrate one or two cell layers eg. Pollen.

CHEMICAL MUTAGENS

Mutagen group	Examples
Base analogues	5-bromouracil, 5-bromodeoxyuridine
Related compounds	Maleic hydrazide, 8-ethoxy caffeine
Antibiotics	Actinomycin D, Mitomycin C, Streptonigrin
Alkylating agents	Sulfur mustards Ethyl–2-chloroethyl sulfide Nitrogen mustards 2-chloroethyl-dimethyl amine Epoxides Ethylene oxide Ethyleneimines Ethyleneimine Sulfonates, etc. Ethyl methane sulfonate (EMS), Diethylsulfonate (DES)
Diazoalanes Nitroso compounds Azide Hydroxylamine Nitrous acid Acridines	Diazomethane *N*-ethyl-*N*-nitroso urea Sodium azide Hydroxylamine Nitrous acid Acridine orange

GENETIC BASIS OF MUTATION BREEDING:-

Mutation breeding aims at exploiting the changes in expression of traits caused by change in allele structure. The variation thus induced is brought under selection pressure to isolate the desirable mutants. Genetically, the source of variation are the new alleles arising spontaneously or created artificially from endogenous genes, However, as against the recombination, where there is no selective transmission but co-segregation of linked alleles are subjected to haplontic and diplontic selection. The gene is usually recessive. Any mutation either spontaneous or induced has to pass through following severs before it can express as a phenotypic variation.

1. DNA repair through which various changes in DNA structure are enzymatically restored back to original condition.
2. Diplontic selection which refers to competitive disadvantage of mutant cells to normal cells. The normal cells proliferate rapidly and thus restrict the mutant cells to a small region that may be excluded from contribution in gamete formation.
3. Haplontic selection which refers to the competitive disadvantage of mutated germ cells to their normal counterparts.
4. The expression of mutant allele may be strongly influenced by environment. The environment requirements of mutant allele may not be same as that of normal allele and thus expression may not be realized in same environment as that of normal allele

MUTATION BREEDING: WHEN & WHERE

Mutation breeding cannot act as principle breeding

methodology for crop improvement but can be a useful complement to an integrated approach. However, there are certain situations where it can be of greater value than other breeding approaches (Sharma & Chopra, 2000).

1. When natural variability is not present or is very limited for a trait, especially in cases where recombination is precluded by sterility as in case oftriploids e.g. Saffron or predominance of vegetative propagation e.g. Fruits &Trees.
2. When it is desired to break a tight linkage between a desirable and undesirable gene.
3. When a simply inherited defect is to be corrected in otherwise agronomicallysuperior varieties. The defects can be disease susceptibility, quality or plant architecture, etc.
4. In case of situations, where it is desired that changes in one trait will not be accompanied by drastic changes in rest of the genetic makeup of a variety.
5. In some cases blocks at specific points of a biochemical pathway are required to enhance the quality either by increasing a particular nutritional component or reduce a certain anti-nutritional compound. As in case of oil seed brassica, the fatty acid biosynthesis pathway is complex and in order to reduce the erucic acid content only one or very few steps have to be blocked rather than entire pathway, i.e. conversion of Linoleic acid to Ecosenic acid.
6. Where distributions of co-adapted gene complexes lead to a critical disadvantage and impose limitations on recombination breeding.
7. Where the novelty value of a phenotypic change has a distinct economic premium as in case of ornamental plants where colour variants and variegation of flower and leaves have great novelty value.
8. In case of plantation or fruit crops where juvenile phase is

very long and changes can be suitably induced through mutagenesis.

9. In case of crops where industrial and consumer quality characteristics are very rigid.

Economic Impact of Mutations:-

The crop improvement using induced mutations began after reports of mutagenic effect of X-rays in Drosophila by Miller in 1927 and in maize, barley and wheat by Stadler in 1928-30. As of 2020, about 3222 have been officially released in 180 species in 60 countries with mostof the varieties developed through use of physical mutagen (88%), while as chemical& other mutagens have been used in 10% & 2% of varieties respectively.(IAEA, 2020). Most of the varieties have been released in Asia (>1900) followed by Europe (953) and North America (200). Among countries most mutant varieties have been developed in China (816) followed by Japan (417) and India (341). Among crops, most varieties have been developed in cereals (1954) followed by ornamentals (709) and legumes (432).

Mutation Breeding in India

India holds second rank in terms of the number of varieties developed and released through mutation breeding. The major Institutions involved in mutation research areIARI, TNAU, and NBRI. Up to 2000, as many as 341 mutant varieties have been released in India (Kharkhival et al, 2004), out of which 103 have been released in ornamentals, 75 in cereals, 55 in legumes, 30 in oil seeds, 24 in cash crops and 12 in vegetables. Among Ornamentals, the crop with largest number of mutation derived varieties has been Chrysanthemum (46), among cereals- rice (39), among pulses- moong bean(13), among cash crops-cotton(8), among the vegetables- Tomato(8).Among the largest mutant varieties that had the largest economic impact are K-84, Jaganath, IIT-60, Sattari, Khesari, PNR-102, and PNR- 382 in rice; TAU-1 & TPU4 of black gram;

TG-1, TG-17, TAG-24 of Castor; Co-2 of Groundnut; PUSA- 408,PUSA-413& PUSA-417 of Chick pea; Co-4, Pant Moong-2, MUM-2, TARM-1 of moong bean and Maru Moth-1 of Moth bean. In India, most of the mutant varieties have been developed by using physical mutagens especially ɣ-rays (169) followed by X-rays (26) followed by chemicals such as Ethyl Methane Sulphonate (15). The major traits targeted for improvement have been plant architecture, yield, maturity, grain quality, disease resistance etc.

ASPECTS OF MUTATION BREEDING:-

Plant breeders use the genetic variation created through mutations for improving upon a trait by selecting the desirable mutant. However, there are certain key aspects for using mutations that determine the amount of success that is likely to be achieved through it. These key aspects are:

Objective:- In mutation breeding, a clear objective regarding the trait to be altered and level of alteration is must that would determine the success of subsequent steps. Usually a simply inherited trait is easy to be manipulated through mutagenesis than complex quantitative traits such as grain yield.

Choice of Material: The objective of induced mutations determines the plant material. Even though homogenous and homozygous lines are invariably preferable, various breeding materials have been successfully used for mutagenic treatment to obtain improved cultivars. Usually the released varieties with minor defects are mutagenized, to improve one or two characters that can significantly increase their agronomic value. It would be ideal to use a variety that is already adapted and has superior agronomic characters with some minor defects that can

be corrected through mutations. This would ensure that once a desirable mutant for a trait is identified, the genotype has not to be passed through other cycles of selection.

Choice of explants:

Seeds: Seeds are used after soaking to get greater frequency of inducedmutations than air dried.

Seedlings : At any stage of life cycle can be subjected to radiation but usually seedlings neither too young nor too old are irradiated due to their convenience in handling in pots transportation from nursery easily.

Flowers : Meiotic cells have been found more sensitive than the mitotic cells andtherefore plants are irradiated in the flowering stage in order to affect the developing gametes.

Cuttings : In case of fruit tree when they are propagated by clones – the desirable cuttings are exposed to irradiation.

Choice of Mutagen:- Mutagens both physical and chemical vary greatly In terms of their suitability, efficiency and effectiveness on different plant systems. The physical and chemical mutagens can be either used singly or in combination to enhance the recovery of desirable mutants. Among ionizing radiations ɣ-rays are usually used in most of the cases (From cobalt-60 or Caesium-137) and causebiological effects though direct and indirect actions. Among chemical mutagens, the alkylating agents such as Ethyl methane suphonate (EMS), Nitroso-Methyl urea or Nitroso-ethyl urea are usually used. The choice of mutagen is usually based on its effectiveness in a a particular plant systems.

Chemical mutagens used most commonly in plant mutagenesis

Name	Abbreviation	Molecular weight
1 Ethyleneimine	EI	43.07
Dimethyl sulfate	DMS	126.13
Diethyl sulfate	dES (DES)	154.19
Ethyl methanesulphonate	EMS	124.20
N-ethyl-N-nitrosourea	ENU (ENH)	117.11
N-methyl-N-nitrosourea	MNU (MNH)	103.08
N-methyl-N1-nitro-N-nitrosoguanidine	MNNG	147.09
Sodium azide	NaN	3 65.01

Dosage of Mutagen:- Plants may respond differently to different dose rates of mutagens (Dose and duration of application). Thus the dose rate of mutagen is decided on the basis of its ability to induce sufficient changes in DNA or chromosomes that have obvious biological effects without causing appreciable cell deaths and loss of reproducibility.

An optimum dose of the mutagen which produces the maximum frequency of mutations and causes the minimum killing. LD50 is that dose of a mutagen, which would kill 50% of the treated individuals. There are several laboratory tests to help define a critical dose for both physical and chemical mutagens. The term 'critical dose' implies the dose of a mutagen beyond which the somatic effects in the M1 generation are too high. In sexually propagated crops, doses of LD50 and above are definitely considered to be critical doses in current approaches to induced mutations for breeding purposes.

Experimental Conditions:- The level of Oxygen and moisture status of the plant tissues used in mutation induction greatly influences the ability of mutagens to cause damage. The

damage is usually higher at higher moisture and oxygen levels. The change in the mutgenic effect with oxygen level of tissue is called “Oxygen Enhancement ratio”. In order to enhance the mutagenic effect of chemical mutagens oxygen is usually bubbled through the solution. Other actors that potentially influence the effect of mutagens are temperature and pH.

Size of M1 Population:- An optimal size of M1 population derived from mutation treatment of various explants materials is a key element in recovering a desirable mutation. The size of M1 is essentially governed by the inheritance pattern of the trait. In case of simply inherited traits, M1 is usually smaller than those of quantitative traits controlled by many epistatic genes (Sharma and Chopra, 2000). It is very important to calculate the size of the M1 generation which is largely defined by survival and fertility reduction for a particular dose.

STEPS IN CHEMICAL MUTAGEN TREATMENT

The steps generally followed in mutagenic treatment of seeds with chemical mutagens are:

General precautions

1. All steps of mutagenic treatment should be done using glass beakers to avoid any interaction of chemical mutagens with even trace quantities of metallic cations or other active reagents.
2. The beakers with pre-soaked seeds should be gently shaken a few times to remove air bubbles, which can block access of mutagen to embryos. Duration of presoaking depends on the biology of germination of a particular crop species.

Presoaking:

1. Seeds for each dose of mutagenic treatment (M0 generation) and for the untreated control—usually the parent variety—are put into beakers that are visibly labelled with the applied concentration of mutagen. As dry seeds are usually used for treatment, pre-soaking in distilled water should be applied to activate seeds physiologically before treatment.
2. Pre-soaking significantly reduces the somatic effect of chemical mutagen. Short washing, 2–3 times in room-temperature tap water should be applied after soaking to remove water-soluble substances leaching from the seed. Such prepared seeds are ready for mutagenic treatment.

3. Pre-soaking is done overnight and mutagenic treatment in the early morning. This allows the M1 seeds to be sown the same day.

Mutagen treatment

1. It is advisable to use three doses of mutagen for a large-scale field experiment. This is especially desired for regions with very variable and unpredictable weather conditions during the growing period of mutagenetically treated material.

2. A temperatureof mutagenic solution of 22–24°C is most often applied for the seed treatment of various crop species..

3. To obtain equal penetration of a mutagen through the cells of a seed embryo, it is necessary to treat seeds in a water solution of the mutagen for 3 to 5 hours. Similar to the pre-soaking, the treatment should be done with a significant

surplus of mutagenic solution..

4. The concentration of the mutagen should be considered, together with duration of the treatment. A shorter treatment time with higher concentration of mutagen can increase somatic effects and could be insufficient to penetrate equally all cells in the plant material.

Post-treatment rinsing

Extensive post-treatment rinsing several times in room-temperature tap water is necessary to stop action of the mutagen and to remove its residues from the surface of the seeds. To facilitate sowing, the treated seeds canbe dried on filter paper under a fume hood.

Size of the M1, M2 and M3 populations

M1 generation: The size of the M1 population is rather small in comparison with thefollowing generations. In cereals, a few thousand seeds per treatment dose should be enough to obtain 10 000 to 30 000 seeds for the M2 generation, if the applied mutagen dose was not too high.

M2 and M3 generation: The field size of M2 and M3 generations determines the cost of the programme. It should be noted that mutagenic treatment generates mutations in many other genes in the genome of each cell, not only at the desired locus. Low doses of mutagen decrease the frequency of mutations and in consequence a larger population of M2 or M3 is necessary tofind the most desirable mutants in a

promising genetic background. It is a good breeding practice to cross a selected mutant with its parent variety and select desired recombinant from the segregating F2 generation. This approach, known as the **'cleaning method'**, helps in elimination of undefined deleterious mutations from a mutated genotype..

Somatic effects of mutations

The somatic effects of mutations are calculated as follows:

$$\text{Emergence reduction (\%)} = \frac{100 - \text{Avg. emergence in dose}}{Avg.emergence\ in\ control} X\ 100$$

$$\text{Seedling growth reduction (\%)} = \frac{100 - \text{Avg. Seedling height in dose}}{Avg.seedling\ height\ in\ control} X\ 100$$

$$\text{Frequency of mutations (\%)} = \frac{\text{Number of Mutated}}{Number\ of\ seedlings} X\ 100$$

Handling mutated generations and mutant selection

M1 generation: Several hundred seeds are treated with a mutagen and are space planted. In general, the number of treated seeds is so adjusted as to give rise to - 500 fertile M1 plants at the harvest. Care should be taken to avoid outcrossing; thiscan be achieved either by planting the M1 population in isolation or by bagging the

inflorescenes of M1 plants or even the whole M1 plants. About 20 to 25 seeds from each M1 spike are harvested separately to raise the M2 progeny rows. Mutagen-treated seeds should be sown in fertile soil and grown under good management practices, including the use of fertilizers.

M2. About 2,000 progeny rows are grown. Careful and regular observations are made on the M2 rows. But only distinct mutations are detected in M2 because the observations are based on single plants. All the plants in M2 rows suspected of containing new mutations are harvested separately to raise individual plant progenies in M3. if the mutant is distinct, it is selected for multiplication and testing.

M3. Progeny rows from individual selected plants are grown in M3. Poor and inferior mutant rows are eliminated. If the mutant progenies are homogeneous, two or more M3 progenies containing the same mutation may be bulked. Mutant M3 rowsare harvested in bulk for a preliminary yield trial in M4.

M4. A preliminary yield trial is conducted with a suitable check, and promising mutant lines are selected for replicated multilocation trials.

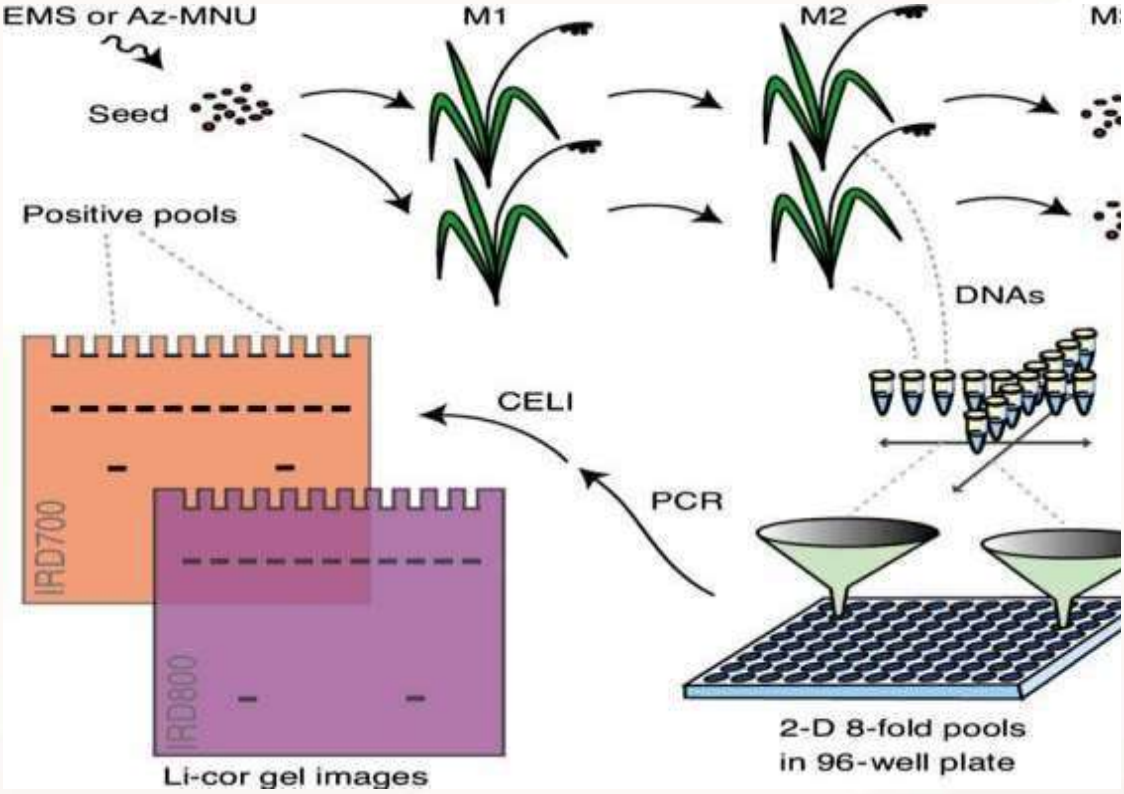
M5-M7. Replicated multilocation yield trials are conducted. The outstanding line may be released as a new variety. The low yielding mutant lines, however, should be retained for use in hybridization programmes.

INDUCED MUTATIONS IN MOLECULAR BREEDING – TILLING

Recent advances in plant genomics, especially large-scale genome

sequencing, have opened new possibilities for application of mutation techniques in crop improvement. Using the reverse genetic strategy called TILLING (Targeting Induced Local Lesions In Genomes), it is possible to induce a series of alleles in a target locus, providing that its sequence is known (McCallum et al., 2000). The TILLING strategy was initially developed for model plant and animal species as a discovery platformfor functional genomics, but soon it became a valuable tool in crop breeding as an alternative to the transgenic approach. The TILLING technique relies on a high frequency of mutations induced by chemical mutagenesis, combined with a high throughput screening method for single nucleotide polymorphisms (SNPs) in the targeted sequence. The basic TILLING methodology has the following steps:

5. Creation of a mutated population (M2);
6. Isolation of the DNA from M2 plants;
7. PCR amplification of the targeted DNA segment using pooled DNA from M2 plantsas a template;
8. Denaturation and re-annealing of PCR products to form heteroduplexes between mutated and wild-type DNA strands;
9. Detection of mismatches in the heteroduplex using different procedures, e.g. cleavage by the specific endonuclease or denaturating high performance liquid chromatography (DHPLC); and
10. Sequencing the targeted DNA region in M2 individuals composing the positive pool,for detection of the mutant.



Characteristics of Mutations

1. Mutations are mostly recessive.
2. Most mutations have harmful effects (99.9%).
3. Due to a change in a gene, a group of genes or in entire chromosome.
4. If non-lethal, the mutant individuals may survive.
5. If mutation occurs at both loci simultaneously, the mutants can be identified in M1 generation.
6. Many of the mutants show sterility.
7. Most mutants are of negative selection value.
8. Mutation for altogether new character generally does not occur.
9. Mutations are random i.e. they can occur in any tissue or cell of an organism.
10. Mutations can be sartorial. mutated sector show mutant characters.

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11. Mutations are recurrent i.e. the same mutation may occur again and again.
12. Induced mutations commonly show pleiotropy often due mutation in closely linked genes.

Applications of Mutation Breeding

Mutation breeding has been used for improving both oligogenic as well as polygenic characters. Mutagenesis has been used to improve morphological and physiological characters including yielding ability. Various applications of mutation breeding are :

1. Induction of desirable mutant alleles which may not be available in the germplasm
2. It is useful in improving specific characteristics of a well adapted high yieldingvariety.
3. Mutagenesis has been successfully used to improve various quantitativecharacters including yield.
4. F1hybrids from intervarietal crosses may be treated with mutagens in order to increase genetic variability by inducing mutation and to facilitate recombination of linked genes.
5. Irradiation of interspecific (distant) hybrids has been done to produce translocations.

Advantage of Mutations

1. Only method of creating variation in vegetatively propagated and sterile crops
2. Useful method for simply inherited traits
3. Can be used in both sexual and asexual crops
4. Limited breeding effort required

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5. Can produce novel variation

Limitations:

1. Most mutations are lethal
2. Frequency of desirable mutations is very low about 0.1 percent. To detect the desirable one in M2 considerable time, labour & other resources are to be employed.
3. To screen large population, efficient quick and unexpensive selection techniques are needed.
4. Desirable mutations may be associated with undesirable side effects due to other mutations thus extending the mutation breeding programme.
5. Detection of recessive mutations in polyploids and clones is difficult and larger doses of mutagen have to be applied and larger populations are to be grown.
6. Can have negative plieotropic effects
7. Many mutations are not heritable (Freaks)

Table: Mutant varieties of different horticultural crops

Crop	Mutant variety	Mutation type
Okra	EMS8	EMS
French Bean	Pusa Parvati Selection-3	X-ray Spontanous Mutation
Tomato	S12, Maruthan, PKM-1, Pusa Lal Meeruti	X-ray
Chilli	MDU-1	Gamma Ray
Hyacinth bean	Co-10	Gamma Ray

Palak	Jobner Green	Spontanous mutation
Pea	L-166 Hans	- Early maturity
Mango	Rosica Hayden	Bud sport
Banana	Gros michel Poovan	Bud sport
Grapefruit	Hudson	Bud sport
Pear	Starkrimson Kotobuki shinsu	Bud sport Gamma rays
Orange	Baianinha Navelina, Navelate, Marrs, Leng, Autumn Gold, Powell Summer, Winter Red	Bud sport
Almond	Supernova	Gamma rays
Grape	Fikreti	Gamma rays
Sweet Cherry	Burlat C1 Ferrovia spur Compact Stella 35B11	Gamma rays X rays X rays