FUNDAMENTALS OF PLANT BREEDING

Fundamentals of Plant Breeding

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CHAPTER-01

HISTORICAL DEVELOPMENT, CONCEPT, NATURE AND ROLE OF PLANT BREEDING, MAJOR ACHIEVEMENTS AND FUTURE PROSPECTS

HISTORICAL DEVELOPMENT OF PLANT BREEDING

History of Plant Breeding:

It started when man first chose certain plants for cultivation. There is no recorded history when the plant breeding started.

• As early as 700 BC **Babylonians** and **Assyrians** artificially pollinated the date palm.

• In 1717 **Thomas Fairchild** produced the first artificial hybrid • **Joseph Kolreuter**, a German made extensive crosses in Tobacco and *Solanum* between 1760 and 1766 and studied the progenies in detail.

• **Thomas Andrew Knight** (1759 - 1835) was the first man to produce several new fruit varieties by using artificial hybridisation.

• **Le coutier**, a farmer published his results on selection in wheat in the year 1843. He concluded that progenies from single plants were more uniform

• **Patrick Shireff** a Scotsman practiced individual plant selection in wheat and oats and developed some valuable varieties.

• **Vilmorin** (1857) proposed individual plant selection based on progeny testing. This was known as 'Vilmorins principle of progeny testing'. He proposed this progeny testing in sugar content in sugar beets (*Beta vulgaris*). But this method was ineffective in wheat. This clearly demonstrated the difference between effect of selection in cross and self-pollinated crops.

• **Nilsson** and his associates at Sweedish Seed Association, Svalof Sweeden (1890) refined the single plant selection

• In 1903 **Johansen** proposed the famous 'pure line theory' which states that a pure line is progeny of a single self fertilized homozygous plant. He proposed this theory based on his studies in *Phaseolus vulgaris.*

• **G.H.Shull** work in maize is the fore runner for the present day hybrid maize programme. He described in detail about the effect of inbreeding.

• During 1960's **Norman Borlaug**, the Nobel laureate developed Mexican semi dwarf wheat varieties

which paved the way for green revolution in wheat. The dwarfing gene was isolated from wheat variety **Norin 10.** Later on this Mexican dwarf were introduced in the India by Dr.M.S. Swaminathan and a number of high yielding wheat varieties like Kalyan sona, Sharbathi sonara were developed.

• In rice the identification of dwarf ***Dee- geo- woo- gen*** from a tall rice variety by a Taiwan farmer revolutionized rice breeding. Using this ***DGWG*** at IRRI during 1965 the wonder rice IR 8 was released.

• Nobilisation in sugarcane by **C.A.Barber** and **T.S.Venkatraman** is another monumental work in plant breeding.

History of plant breeding in India

- 1871 – The Government of India created the Department of Agriculture
- 1905 – The Imperial Agricultural Research Institute was established in Pusa, Bihar
- 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. Eg : 70 improved varieties of cotton
- 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
- 1961 - The first hybrid maize varieties released by the project
- ICAR initiated coordinated projects for improvement of the other crops
- 1960 – First Agricultural University established at Pantnagar, Nainital, U.P.

Scientific contributions of eminent scientists

Name of the Scientists	**Contributions**

Allard and Bradshaw	-	G x E interaction
Recurrent Selection for SCA	-	Hull
Recurrent Selection for GCA	-	Jenkins
Dominance hypothesis	-	Davenport
Gene for gene hypothesis	-	Flor
Pureline concept	-	Johannsen
Backcross method	-	Harlan and Pope
Double cross scheme	-	Jones
Cytoplasmic Genetic Male sterility	-	Jones and Davis
Ear to row method	-	Hopkins
Modified Ear to row method	-	Lonnquist
Colchicine	-	Blackslee and Nebel
Single Seed Descent Method	-	Goulden
Self-incompatibility	-	Lewis
Vertifolia effect	-	Van Der Plank
Centres of diversity, Law of homologus series	-	Vavilov
Grater initial capital hypothesis	-	Ashby
Progeny test	-	Vilmorin
First artificial hybrid	-	Thomas Fairchild
Triticale	-	Rimpau
Mutation	-	Hugo de Vries
Sprophytic System of self-incompatibility	-	Hughes and Babcock
Bulk method	-	Nilsson & Ehle
Raphano brassica	-	Karpenchenko
Heterosis	-	Shull
Male sterility	-	Jones and Davis
Father of hybrid rice	-	Yuan Long Ping
Self-incompatibility classification	-	Lewis
Mechanism of insect resistance	-	Painter

Modified bulk method	-	Atkins
Components of genetic variance classification	-	Fischer
Male sterility in maize	-	Rhoades
Microcentre	-	Harlan
Chemical mutagen	-	Aurbach
Multiline concept	-	Jenson
Green revolution in India	-	M.S. Swaminathan
Semidwarf rice varieties at IRRI	-	T.T. Chang
Forage breeder		-G.W. Burton
Forage breeder	-	T.J. Jenkin
Soyabean breeder	-	E.E. Hartwig

Some Indian Plant Breeding

T.S. Venkatraman	-	An eminent sugarcane breeder, he transferred thick stem and high sugar contents from tropical noble cane to North Indian Canes. This process is known as noblization of sugarcane.
B.P. Pal	-	An eminent Wheat breeder,developed superior disease resistant N.P. varieties of wheat.
M.S. Swaminathan	-	Responsible for green revolution in India, developed high yielding varieties of Wheat and Rice
Pushkarnath	-	**F a m o u s** potato breeder
N.G.P. Rao	-	An eminent sorghum breeder
Ramaiah	-	A renowned rice breeder
Ram Dhan Singh	-	Famous wheat breeder
D.S. Athwal	-	Famous pearlmillet breeder
Bosisen	-	An eminent maize breeder
Dharampal Singh	-	An eminent oil-seed breeder

C.T. Patel - Famous cotton breeder who developed world's first cotton hybrid in 1970

V. Santhanam - Famous cotton breeder

Activities in Plant Breeding

(i) Variation : - (A) Naturally existing variability- Domestication
-Germplasm collection
- Introduction

(B) Creation of new variability - Hybridization (Intervarietal, Distant, Somatic)
-Mutation
- Polyploidy
-Somaclonal variation
-Genetic Engineering

(ii) Selection

(iii) Evaluation

(iv) Multiplication

(v) distribution

Concept, nature and Role of Plant Breeding

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.

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

Plant breeding deals with the genetic improvement of crop plants also known as science of crop improvement.

Science of changing and improving the heredity of plants

or

Plant breeding is the current phase of crop evolution-(N. W. Simmonds-1979)

Aim :

Plant breeding aims to improve the characteristics of plants so that they become more desirable agronomically and economically. The specific objectives may vary greatly depending on the crop under consideration.

Objectives of Plant Breeding:

1. **Higher yield:** The ultimate aim of plant breeding is to improve the yield of economic produce. It may be grain yield, fodder yield, fibre yield, tuber yield, cane yield or oil yield depending upon the crop species. Improvement in yield can be achieved either by evolving high yielding varieties or hybrids.
2. **Improved quality**: Quality of produce is another important objective in plant breeding. The quality characters vary from crop to crop. Eg. grain size, colour, milling and backing quality in wheat. Cooking quality

in rice, malting quality in barley, size, colour and size of fruits, nutritive and keeping quality in vegetables, protein content in pulses, oil content in oilseeds, fibre length, strength and fineness in cotton.

3. **Abiotic resistance :** Crop plants also suffer from abiotic factors such as drought, soil salinity, extreme temperatures, heat, wind, cold and frost, breeder has to develop resistant varieties for such environmental conditions.

4. **B iotic resistance :** Crop plants are attacked by various diseases and insects, resulting in considerable yield losses. Genetic resistance is the cheapest and the best method of minimizing such losses. Resistant varieties are developed through the use of resistant donor parents available in the gene pool.

5. **Change in maturity Duration / Earliness** : 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. Development of wheat varieties suitable for late planting has permitted rice -wheat rotation. Thus breeding for early maturing crop varieties, or varieties suitable for different dates of planting may be an important objective. Maturity has been reduced from 270 days to 170 days in cotton, from 270 days to 120 days in pigeonpea, from 360 days to 270 days in sugarcane.

6. **Determinate Growth :** Development of varieties with determinate growth is desirable in crops like Mung, Pigeon Pea (*Cajanus cajan*), Cotton (*Gossypium sp.*), etc.

7. **Dormancy** : In some crops, seeds germinate even before harvesting in the standing crop if there are rains at the time of maturity, e.g., Greengram, Blackgram, Barley and Pea, etc. A period of dormancy has to be introduced in these crops to check loss due to germinatio n. In some other cases, however, it may be desirable to remove dormancy.

8. **Desirable Agronomic Characteristics**: It includes plant height, branching,

tillering capacity, growth habit, erect or trailing habit etc., is often desirable. For example, dwarf ness in cereals is generally associated with lodging resistance and better fertilizer response. Tallness, high tillering and profuse branching are desirable characters in fodder crops.

9. **Elimination of Toxic Substances:** It is essential to develop varieties free from toxic compounds in some crops to make them safe for human consumption. For example, removal of neurotoxin in Khesari (*Lathyruys sativus*) which leads to paralysis of lower limbs, erucic acid from *Brassica* which is harmful for human health, and

   gossypol from the seed of cotton is necessary to make them fit for human consumption. Removal of such toxic substances would increase the nutritional value of these crops.

10. **Non-shattering characteristics:** The shattering of pods is serious problem in green gram. He nce resistance to shattering is an important objective in green gram.

11. **Synchronous Maturity:** It refers to maturity of a crop species at one time. The character is highly desirable in crops like Greengram, Cowpea, and Cotton where several pickings are required for crop harvest.

12. **Photo and Thermo insensitivity**: Development of varieties insensitive to light and temperature helps in crossing the cultivation boundaries of crop plants. Photo and thermo- insensitive varieties of wheat and rice has permitted their cultivation in new areas. Rice is now cultivated in Punjab, while wheat is a major *rabi* crop in West Bengal.

13. **Wider adaptability:** Adaptability refers to suitability of a variety for general cultivation over a wide range of environmental conditions. Adaptability is an important objective in plant breeding because it helps in stabilizing the crop production over regions and seasons.

14. **Varieties for New Seasons:** Traditionally Maize is a *kharif* crop. But scientists are now able to grow Maize as *rabi* and *zaid* crops. Similarly, mung is grown as a summer crop in addition to the main *kharif* crop.

Scope of plant breeding (Future Prospects)

From times immemorial, the plant breeding has been helping the mankind. With knowledge of classical genetics, number of varieties have been evolved in different crop plants. In order to combat the global alarm created by population explosion, the food front has to be strengthened which is serious challenge to those scientists concerned with agriculture. Advances in molecular biology have sharpened the tools of the breeders, and brighten the prospects of confidence to serve the humanity. The application of biotechnology to field crop has already led to the field testing of genetically modified crop plants. Genetically engineered Rice, Maize, Soybean, Cotton, Oilseeds Rape, Sugar Beet and Alfalfa cultivars are expected to be commercialized before the close of 20^{th} century. Genes from varied organisms may be expected to boost the performance of crops especially with regard to their resistance to biotic and abiotic stresses. In addition, crop plants are likely to be cultivated for recovery of valuable compounds like pharmaceuticals produced by genes introduced into them through genetic engineering. It may be pointed out that in Europe hirudin, an anti-thrombin protein is already being produced from transgenic *Brassica napus.*

Undesirable effects

Plant breeding has several useful applications in the improvement of crop plants. However, it has five main undesirable effects on crop plants.

1. **Reduction in Diversity :** Modern improved varieties are more uniform than land races. Thus plant breeding leads to reduction in diversity. The uniform varieties are more prone to the new races of pathogen than land races which have high genetic diversity.

2. **Narrow genetic base:** Uniform varieties have narrow genetic base. Such varieties generally have poor adaptability.

3. **Danger of Uniformity:** Most of the improved varieties have some common parents in the pedigree which may cause danger of uniformity.

4. **Undesirable combinations:** Sometimes, plant breeding leads to undesirable combinations. The examples of manmade crops having undesirable combination of characters are *Raphanobrassica* and Pomato.

5. **Increased susceptibility to minor diseases and pests :** Due to emphasis on breeding for resistance to major diseases and insect pests often resulted in an increased susceptibility to minor diseases and pests. These have gained importance and, in some cases, produced severe epidemics. The epidemic caused by *Botrytis cinerea* (grey mold) in chickpea during 1980-82 Punjab, Haryana. The severe infection by Karnal bunt (*Tilletia sp.*) on some wheat varieties, infestation of mealy bugs in *Bt* cotton.

CHAPTER-02

GENETICS IN RELATION TO PLANT BREEDING, MODE OF REPRODUCTION AND APOMIXES, SELF INCOMPATIBILITY AND MALE STERILITY-GENETIC CONSEQUENCES

GENETICS IN RELATION TO PLANT BREEDING

Genetic and cytogenetic principles and concepts form the basis of planned crop improvement programme, hence their knowledge is essential for a plant breeder. Various Genetic and cytogenetic principles and concepts which play significant role in crop improvement include:

1. Mitosis and Meiosis
2. Chromosomes
3. Structural chromosomal change
4. Numerical change in chromosome
5. Mendel's laws of Inheritance
6. Gene interaction
7. Linkage and crossing over
8. Pleiotropy
9. Penetrance and expressivity
10. Cytoplasmic inheritance
11. Polygenic and oligogenic characters
12. Mutations
13. Population genetics
14. Plant Biotechnology

Mode of reproduction determines the genetic constitution of crop plants, that is, whether the plants are normally homozygous or heterozygous. This, in turn, determines the goal of a breeding programme. If the crop plants are naturally homozygous, *e.g.,* as in self- pollinators like wheat, a homozygous line would be desirable as a variety.

MODES OF REPRODUCTION

The modes of reproduction in crop plants may be broadly grouped into two categories, *asexual* and *sexual.*

Asexual Reproduction

A *sexual reproduction* does not involve fusion of male and female gamentes. New plants may develop from vegetative parts of the plant (*vegetative reprodu ction)* or may arise from embryos that develop without fertilization (apomixis).

Vegetative Reproduction

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

Underground Stems

The underground modifications of stem generally serve as storage organs and contain many buds. These buds develop into shoots and produce plants after rooting. Examples of such modifications are given below.

Tuber: Potato

Bulb: Onion, Garlic

Rhizome: Ginger, Turmeric

Corm: Bunda, arvi

Sub-aerial Stems

These modifications include runner, stolon, sucker etc., Sub-aerial stems are used for the propagation of mint, date plam 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. Scientists are trying to induce bulbil development in plantation crops by culturing young inflorescence on tissue culture media; it has been successfully done in the case of cardamom.

Artificial Vegetative Reproduction

It is commonly used for the propagation of many crop spec ies, although it may not occur naturally in those species. Stem cuttings are commercially used for the propagation of

sugarcane, grapes, roses, etc. Layering, budding, grafting and gootee are in common use for the propagation of fruit trees and ornamental shrubs. Techniques are available for vegetative multiplication through tissue culture in case of many plant species, and attempts are being made to develop the techniques for many others. In many of these species sexual reproduction occurs naturally but for certain reasons vegetative reproduction is more desirable
.

Significance 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.

Apomixis

In apomixis, seeds are formed but the embryos develop without fertilization. Consequently, the plants resulting from them are identical in genotype to the parent plant. In apomictic species, sexual reproduction is either suppressed or absent. When sexual reproduction does occur, the apomixis is termed as *facultative* . But when sexual reproduction is absent, it is referred to as obligate. Many crop species show apomixis, but it is generally facultative. The details of apomictic reproduction vary so widely that a confusing terminology has resulted. A simplified classification of apomixis is given below.

Adventive Embryony

In this case, embryos develop directly from vegerative cells of the ovule, such as nucellus, integument, and chalaza. Development of embryo does not involve production fo embryo sac. Adventive embryony occurs in mango, citrus, etc.

Apospory

Some vegetative cells of the ovule develop into unreduced embryo sacs after meiosis. The embryo may develop from egg cell or some other cell of this embryo sac. Apospory occurs in some species of H*ieraceum, 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.

Significance of Apomixis

Apomixis is a nuisance when the breeder desires to obtain sexual progeny, i.e., selfs or hybrids. But it is of great help when the breeder desires to maintain varieties. thus in breeding of apomictic species, the breeder has to avoid apomictic progeny when he is making crosses or producing inbred lines. But once a desirable genotype has been selected, it can be multiplied and maintained through apomictic progeny. This would keep the genotype of a variety intact. Asexually reproducing crop species are highly heterozygous and show severe inbreeding depression. Therefore, breeding methods in such species must avoid inbreeding.

SEXUAL REPRODUCTION

Sexual reproduction involves fusion of male and female gametes to form a zygote, which develops in to an embryo. In crop plants, male and female gametes are produced in specialised 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, 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), etc. In "crop plants, meiotic division of specific cells in stamen and pistil yields microspores and 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.

SPOROGENESIS

Productions of microspores and megaspores is known as **sporogenesis.** Microspores are produced in anthers (microsporogenesis), while **megaspores** are produced in ovules (megasporogenesis).

Microsporogenesis. Each anther has four pollen sacs, which contain numerous pollen mother cells (PMCs). Each PMC undergoes meiosis to produce four haploid cells or microspores. This process is known as microsporogenesis (Fig. 4.1). The microspores mature into pollen grains mainly by a thickening of their walls.

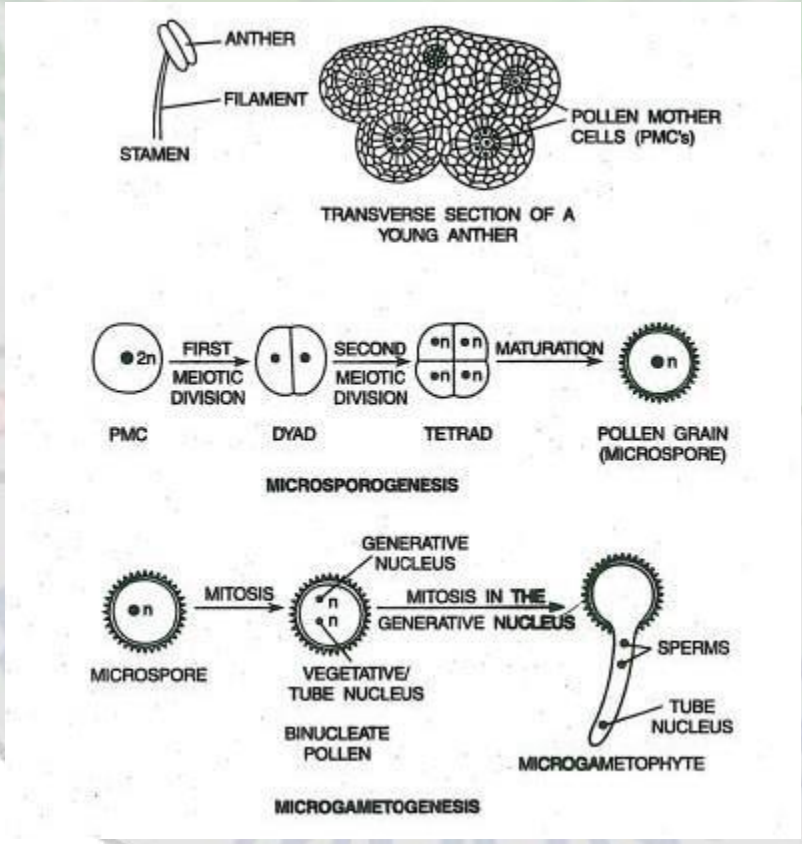
Megasporogenesis. Megasporogenesis occurs in ovules, which are present inside the ovary. A single cell in each ovule differentiates into a megaspore mother cell. The megaspore mother cell undergoes meiosis to produce four haploid megaspores. Three of the megaspores degenerate leaving one functional megaspore per ovule (Fig. 4.2). This completes megasporogenesis.

GAMETOGENESIS

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

Microgameto genesis. This refers to the production of male gamete or **sperm.**

During the maturation of pollen, the microspore nucleus divides mitotically to produce a generative and a vegetative or tube **nucleus.** The pollen is generally released in this binucleate stage. When the pollen lands onto the stigma of a flower, it is known as pollination. Shortly after pollination, the pollen germinates. The pollen tube enters the stigma and grows through the style. The generative nucleus now undergoes a mitotic division to produce two male gametes or sperms. The pollen, along with the pollen tube, is known as microgametophyte . The pollen tube finally enters the ovule through a small pore, micropyle, and discharges the two sperms into the embryo sac.

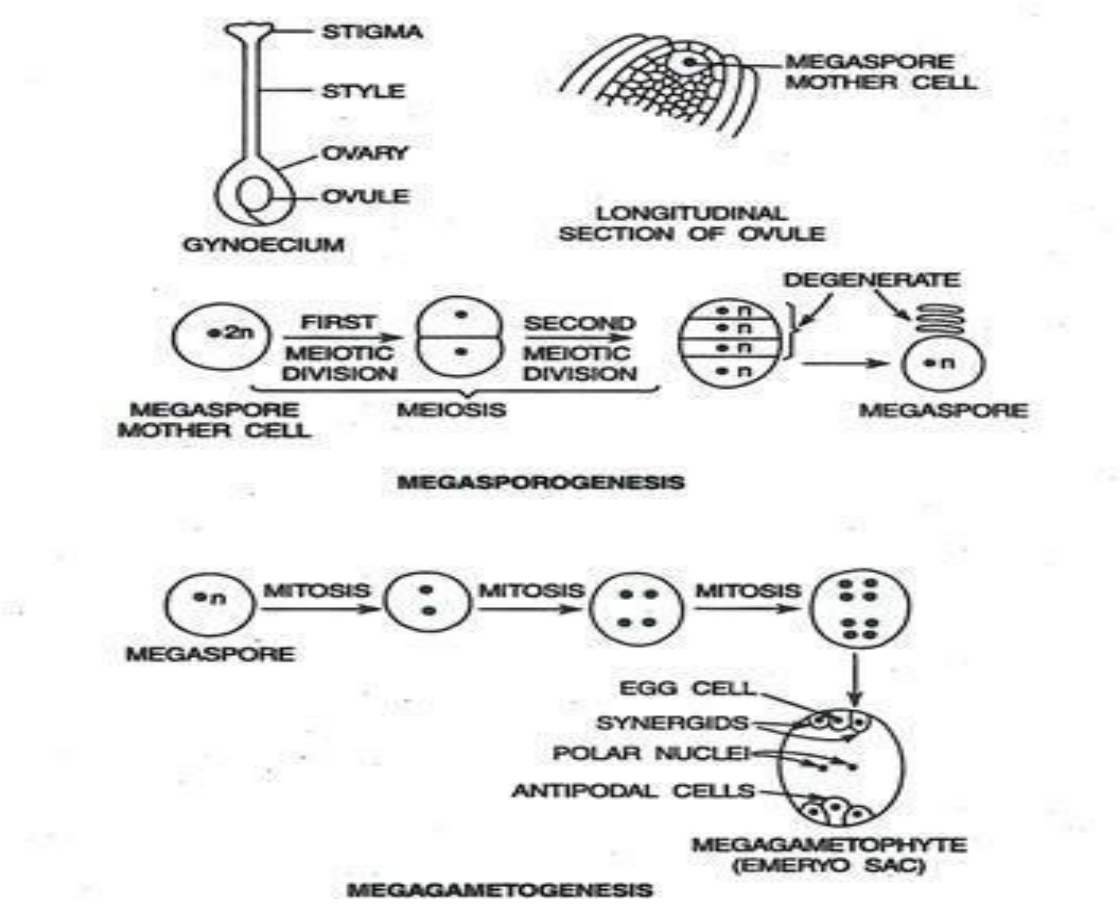


Microsporogenesis and microgametogenesis (a generalized scheme)

Megagametogenesis. The nucleus of a functional megaspore divides mitotically to produce four or more nuclei. The exact number of nuclei and their arrangement vary considerably from one species to another. In most of the crop plants, megaspore nucleus undergoes three mitotic divisions to produce eight nuclei. Three of these

nuclei move to one pole and produce a central egg cell and two synergid cells; one synergid is situated on either side of the egg cell. Another three nuclei migrate to the opposite pole to give rise to antipodal cells. The two nuclei remaining in the centre, the polar nuclei, fuse to form a secondary nucleus. The megaspore thus develops into a mature megagametophyte or embryo sac. The development of embryo sac from a megaspore is known as megagametogenesis. The embryo sac generally contains one egg cell, two synergids, three antipodal cells (all haploid), and one diploid secondary nucleus.

Megasporogenesis and megagametogenesis (generalized scheme)



Significance 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 thr ough 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.

MODES OF POLLINATION

Pollination refers to the transfer of pollen grains from anthers to stigmas. Pollen from an anther may fall on to the stigma of the same flower leading to self- pollination or outogamy. When pollen from flowers of one plant are transmitted to the stigmas of flowers of another plant, it is known as cross-pollination or allogamy. A third situation, geitonogamy, results when pollen from a flower of one plant falls on the stigmas of other flowers of the same plant, e.g., in Maize. The genetic consequences of geitonogamy are the same as those of autogamy.

Self-pollination

Many cultivated plant species reproduce by self- pollination. Self-pollination species are believed to have originated from cross-pollinated ancestors. These 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 environmenta l conditions like temperature and humidity, and location.

Mechanisms promoting self-pollination The various mechanisms that promote self- pollination are generally more efficient than those promoting cross - pollination. These mechanisms are listed below.

1. 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 some varieties of wheat, oats, barley and in a number of other grasses.
2. 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.

3. 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.

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

Genetic Consequences of Self-Pollination

Self-pollination leads to a very rapid increase in homozygosity. Therefore, populations of self-pollinated species are highly homozygous, self-pollinated species do not show inbreeding depression, but may exhibit considerable heterosis. Therefore, the aim of breeding methods generally is to develop homozygous varieties.

Cross-Pollination

In cross- pollinating species, the transfer of pollen from a flower to the stigmas of the others may be brought about by wind (*anemophily).* Many of the crop plants are naturally cross-pollinated (Table 3.1). In many species, a small amount (up to 5- 10 percent) of selfing may occur.

Mechanisms promoting cross pollination There are several mechanism that facilitate cross-

pollination; these mechanisms are described briefly.

1. **Dicliny :** *Dicliny* or *unisexuality* is a condition in which the flowers are either staminate (male) or pistillate (female).

   a) **Monoecy.** Staminate and pistillate flowers occur in the same plant, either in the same inflorescene, *e.g.,* Castor, mango and coconut, or in separate inflorescences, chestnut, strawberries, rubber, grapes and cassava.

   b) **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. Stamens and pistils of hermaphrodite flowers may mature at different times facilitating cross - pollination.

   a) **Protogyny**. In crop species like bajra, pistils mature before stamens.

   b) **Protandry.** in crops like Maize and sugarbeets, stamens mature before pistils.

3. In Lucerne or alfalfa, stigmas are covered with a waxy film. The stigma does not become receptive until this waxy film is broken. The waxy membrane is broken by the visit of honey bees which also effect cross- pollination.

4. A combination of two or more of the above mechanisms may occur in some species.

This improves the efficiency of the system in promoting cross- pollination.

For example, Maize exhibits both monoecy and protandry.

5. **Self-Incompatibility**. It refers to the failure of pollen from a flower to fertilize the same flower or other flowers on the same plant. Self- incompatibility is of two types : sporophytic and gametophytic. In both the cases, flowers do not set seed on selfing. Self- incompatibility is common in several species of Brassica, some species of Nicotiana, radish, rye and many grasses. It is highly effective in preventing self- pollination.

6. **Male Sterility**. Male sterility refers to the absence of functional pollen grains in otherwise hermaphrodite flowers. Male sterility is not common is natural populations. But it is of great value in experimental populations, particularly in the production of hybrid seed. Male sterility is of two types: genetic and Cytoplasmic. Cytoplasmic male sterility is termed Cytoplasmic -genetic when restorer genes are known. In view of the importance of self- incompatibility and male sterility, a more detailed discussion on them follows later.

Genetic Consequences of Cross-Pollination. Cross- pollination preserves and promotes heterozygosity in a population. Cross- pollinated species are highly heterozygous and show mild to severe inbreeding depression and a considerable amount of heterosis. The breeding methods in such species aim at improving the crop species without reducing heterozygosity to an appreciable degree. Usually, hybrid or synthetic varieties are the aim of breeder wherever the seed production of such varieties is economically feasible.

Often Cross-Pollinated Species

In many crop plants (Table 3.1), cross -pollination often exc eeds 5 per cent and may reach 30 per cent. Such species are generally known as often cross - pollinated species, e.g., Jowar, Cotton, arhar, safflower etc. The genetic architecture of such crops is intermediate between self- pollinated and cross- pollinated species. Con-sequently, in such species breeding methods suitable for both of them may be profitably applied. But often hybrid varieties are superior to others.

SELF-INCOMPATIBILITY

More than 300 species belonging to 20 families of angiosperms show self- incompatibility. Self - incompatible pollen grains fail to germinate on the stigma of the flower that produced them. If some pollen grains do germinate, pollen tubes fail to enter the stigma. In many species, the pollen tubes enter the style, but they grow too slowly to effect fertilization before the flower drops. Sometimes, fertilization is effected, but the embryo degenerates at a very early stage. Self-incompatibility appears to be a biochemical reaction, but the precise nature of these reactions is not clearly understood. The genetic control of incompatibility reactions is relatively simple. Lewis (1954) has suggested various classifications of self -incompatibility; a relatively simple classification is as follows; 1. heteromorphic system, 2. homomorphic system, (2a) gametophytic control, and (2b) sporophytic control.

HETEROMORPHIC SYSTEM

In this system, flowers of different incompatibility groups are different in morphology. For example, in Primula there are two types of flowers, pin and thrum. Pin flowers have long styles and short stamens, while thrum flowers have short styles and long stamens. This situation is referred to as distyly. Tristyly is known in some plant species, e.g. Lythrum ; in such cases, the style of a flower may be either short, long or of medium length. In the case of distyly, the only compatible mating is between pin and thrum flowers. This characteristic is governed by a single gene s ; Ss produces thrum, while ss produces pin flowers. The incompatibility reaction of pollen is determined by the genotype of the plant producing them. Allele S is dominant overs. The incompatibility system, therefore, is heteromorphic -sporophytic. The pollen grains produced by pin flowers; would all be s in genotype as well as incompatibility reaction. The pollen produced in thrum flowers would be of two types genotypically, S and s, but all of them would be S phenotypically. The mating

between pin and thrum plants would produce Ss and ss progeny in equal frequencies. This system is of little importance in crop plants ; it occurs in sweet potato and buckwheat.

Homomorphic System

In the homomorphic system, incompatibility is not associated with morphological differences among flowers. The incompatibility reaction of pollen may be controlled by the genotype of the plant on which it is produced or by its own genotype.

Gametophytic System

Gametophytic incompatibility was first described by East and Mangelsdorf in 1925 in *Nicotiana sanderae*. The incompatibility reaction of pollen is determined by its own genotype, and not by the genotype of the plant on which it is produced. Generally, incompatibility reaction is determined by a single gene having multiple alleles, e.g., *Trifolium, Nicotiana, Lycoperscion, Solanum, Petunia* etc. If same allele as that of Pollem is present in the stylar tissues, it opposes the growth of pollen tube in the style, so Gametophytic incompatibility is also called as 'oppositional factor system'. Sometimes, Polyploidy may lead to a loss of incompatibility due to a competition between the two S alleles present in diploid pollen. Irradiation of pollen or buds with X-rays or gamma-rays tempor arily suppresses the incompatibility reaction, and thus allows the pollen tube to grow through incompatible style. In some species, e.g., *Phalaris, Physalis* etc., two loci (S and Z) govern incompatibility, while in some others, e.g., *Beta vulgaris and Papaver*, three loci are involved. In these cases, Polyploidy does not affect the incompatibility reaction. Pollen tube grows very slowly in the style containing the same S allele as the pollen, and fails to effect fertilization. Therefore, all the plants are heterozygous at the S locus. In a single gene system, there are three types of mating;

i) Fully incompatible, e.g., S1S2 x S1S2

ii) Fully compatible, e.g., S_1S_2 x S_3S_4

iii) Partially (i.e., 50% of the pollen) compatible, e.g., S_1S_2 x S_2S_3

In some cases, an allele for self -fertility, S*f*, is found (East and Yarnel). Pollen carrying the S*f* alleles does not show incompatibility reaction. Thus, in a plant with the genotype S*f*S1, selfing produces S*f* Sf and S*f* S1 progeny. Mutations for S*f* allele may be induced by irradiating the pollen used for self - pollination. There is another allele, SF, which retards the growth of S*f* pollen tubes, thus enforcing self-incompatibility. The gametophytic system is found in pineapple (2 locus), ryegrass (2 locus), diploid coffee, diploid clovers

Trifolium sp.) etc. In families like *Solanaceae, Rosaseae, Graminae, Leguminoseae, Chenopodiaceae, Ranunculaceae*

Sporophytic System

In the sporophytic system also, the self -incompatibility is governed by a single gene, S, with multiple alleles; more than 30 alleles are known in *Brassica oleracea.* In general, the number of S alleles is considerably larger in the gametophytic than in the sporophytic system. The incompatibility reaction of pollen is governed by the genotype of the plant on which the pollen is produced, and not by the genotype of the pollen. It was first reported by Hughes and Babcock in 1950 in *Crepis foetida*, and by Gerstel in *Parthenium argentatum* (in the same year). In the sporophytic system, the S alleles may exhibit dominance, individual action (codominance) or competition. Consequently, there may be many complex incompatibility relationships. Lewis has summarized the following characteristics of this system.

1. There are frequent reciprocal differences
2. Incompatibility can occur with the female parent
3. A family can consist of three incompatibility groups
4. Homozygotes are a normal part of the system
5. An incompatibility group may contain two genotypes

Sporophytic incompatibility is found in radish (*R.* s*ativus*), diploid *Brassica* crops and *Sinapis*. In many cases, different S alleles vary in their activity leading t o varying degrees of self-incompatibility, e.g., *B. oleracea.* Polygenes

(modifying genes) are known to increase as well as decrease the activities of S alleles both in the gametophytic as well as sporophytic systems.

Mechanism of Self-Incompatibility

The mechanism of self- incompatibility is quite complex and is poorly understood. The various phenomena observed in self- incompatible matings are grouped into three broad categories : (1) pollen-stigma interaction, (2) pollen tube-style interaction, and (3) pollen tube-ovule interaction.

Pollen-Stigma Interaction

These interactions occur just after the pollen grains reach the stigma and generally prevent pollen germination. At the time they reach stigma, pollen grains generally have two nuclei in the gametophytic system, while they have three nuclei in the sporophytic system. This was once considered to be the basis for the two incompatibility systems, but the

available evidence indicates otherwise. However, the structure of stigmatic surface appears to be definitely involved in the differences between the two systems. In the gametophytic system, the stigma surface is plumose having elongated receptive cells and is commonly known as 'wet' stigma. Incompatible pollen grains generally germinate on reaching the stigma; the incompatibility reaction occurs at a later stage. There are clear cut serological differences among the pollen grains with different S genotypes ; such differences have not been observed in the sporophytic system.

In the sporophytic system, the stigma is papillate and dry, and is covered with a hydrated layer of proteins known as 'pellicle'. There is evidence that the pellicle is involved in incompatibility reaction. There are striking differences in the stigma antigens related to the S allele composition. Within few minutes of reaching the stigmatic surface, the pollen releases an exine exudates which is either protein or glycoprotein in nature. This exudates induces immediate callose formation in the papillae (which are in direct contact with the pollen) of incompatible stigma. Often callose is also deposited on the young protruding pollen tubes preventing

any further germination of the pollen. Thus in the sporophytic system, stigma is the site of incompatibility reaction ; once the pollen tube crosses the stigmatic barrier, there is no further inhibition of pollen tube growth. In the homomorphic sporophytic system, the incompatibility reactio n of pollen is probably due to the deposition of some compounds from anther tapetum on to the pollen exine.

Pollen Tube-Style Interaction

In most cases of the gametophytic system, pollen grains germinate and pollen tubes penetrate the stigmatic surface. But in incompatible combinations, the growth of pollen tubes is retarded within the stigma, e.g., in *Oenothera* , or a little later in the style, e.g., in *Petunia, Lycopersicon, Lilium* etc. In the latter case, there is a cessation of protein and polysaccharide synthesis in the pollen tubes, which leads to the degeneration of tube wall and the bursting of pollen tube.

Pollen Tube-Ovule Interaction

In some cases, e.g., Theobromo cacao, pollen tubes reach the ovule and effect fertilization. However, in incompatible combinations, embryos degenerate at an early stage of development.

SELF INCOMPATABILITY

Relevance of Self-Incompatibility

Self- incompatibility effectively prevents self -pollination. As a result, it has a profound effect on breeding approaches and objectives; these are discussed here in some detail.

1. In self - incompatible fruit trees, it is necessary to plant two cross-compatible varieties

   to ensure fruitfulness. Further, cross-pollination may be poor in adverse weather conditions reducing fruit set. Therefore, it would be desirable to develop self-fertile forms in such cases.

2. Some breeding schemes, e.g., development of hybrid varieties etc., initially require some degree of inbreeding. Although sibmating leads to inbreeding, but for the same degree of inbreeding it take twice as much

time as selfing. Further, for the maintenance of inbred lines selfing would be necessary.

3. Self- incompatibility may be used in hybrid seed production. For this purpose, (1) two self - incompatible, but cross-compatible, lines are interplanted ; seed obtained from both the lines would be hybrid seed. (2) Alternatively, a self-incompatible line may be interplanted with a self - compatible line. from this scheme, seed from only the self- incompatible line would be hybrid. (3) Schemes for the production of double cross and triple cross hybrids have also been proposed and their feasibility has been demonstrated in the case of brassicas.

The gametophytic system has been used, to a limited extent, for hybrid seed production in

clover, *Trifolium* (*Leguminosae*). In *Solanaceae*, the cultivated species are generally self- fertile, and self-incompatibility is confined to wild species. The sporophytic system has been exploited for hybrid seed production in brassicas (*C ruciferae*), primarily by the Japanese seed companies. In *Compositae*, another economically important family showing sporophytic self - incompatibility, the cultivated varieties are generally self -fertile.

The use of self- incompatibility in hybrid seed produc tion is hampered by several problems. (1) Production and maintenance of inbred lines by hand pollination is tedium and costly.(2) This raises the cost of hybrid seed. (3) Continued selfing leads to a depression in self - incompatibility, and it unintentionally, but unavoidably, selects for self-fertility. (4) In the gametophytic system, continued inbreeding gives rise to new incompatibility reactions, which may limit the usefulness of such inbreds as parents. (5) Environmental factors, e.g., high temperature and high humidity etc., reduce or even totally overcome self-incompatibility reaction leading to a high (30% or more) proportion of selfed seed. (6) Bees often prefer to stay within a parental line, particularly when the parental lines differ morphologically. This, in turn, increases the proportion of selfed seed. 97) Transfer of S alleles from one variety or, more particularly, species into another

variety or species is tedius and complicated. This has prevented the use of self-incompatibility in hybrid seed production in Solanaceae and Compositae.

Elimination of Self-Incompatibility

In many cases, self-fertile forms will be highly desirable and, in such cases, it would be useful to eliminate self- incompatibility. (1) In the case of single- locus gametophytic system, incompatibility may be eliminated by doubling the chromosome number, e.g., in potato diploidization leads to self-incompatibility. (2) Isolation of self-fertile (S*f*) mutations is a very useful tool in the elimination of self- incompatibility. Flower buds are generally irradiated at the PMC stages, and pollen from these buds is used to pollinated flowers with known S alleles. Generally, selection for S*f* alleles is much more complicated in the sporophytic system than in the gametophytic system due to the temporary loss in incompatibility and pseudofertility in the cases of the former. In Oenothera, S*f* mutations occur spontaneously at the rate of 10^{-8} and the rate of induction with X-rays is 1.6 x 10^{-8} /r unit. Lastly, (3) self-compatibility alleles may be transferred from related species or varieties of the same species, if available, through a backcross programme.

Temporary Suppression of Self-Incompatibility

In many situations, e.g., during the production of inbreds for use as parents in hybrid seed production, it is essential that temporary self-fertility is achieved in a manner so that self-incompatibility is fully functional in the selfed progeny. Such self-fertility is known as **pseudofertility** and is achieved by temporarily suppressing the incompatibility reaction using one of the following techniques.

Bud Pollination

Bud pollination means application of mature pollen to immature nonreceptive stigma, generally 1-2 days prior to the anthesis of flowers. This is the most practicable and successful method both in the gametophytic and sporophytic systems. In some cases, application of the fluid from mature stigmas may improve the success of but pollination.

Surgical Techniques

Removal of the stigmatic surface, the whole of stigma or a part or whole of the style may permit an otherwise incomptible mating. Removal of the stigma is very useful in the sporophytic system, e.g., Brassica, while removal of the style is helpful in some cases of gametophytic incompatibility, e.g., Petunia. In Petunia, the whole of the style may be removed and the pollen grains may be directly dropped on the the ovules in the ovarian cavity.

End-of-Season Pollination

In some species, the degree of incompatibility is reduced towards the end of the flowering season or in mature plants. But there are controversial reports on the usefulness of this technique.

High Temperature

In some species, e.g., Trifolium, Lycopersicon, Brassica, Oenothera etc., exposure of pistils to temperatures upto 60^0 C induce pesudofertility.

Irradiation

In the single-locus gametophytic system, e.g., in Solanaceae, acute irradiation with X- rays or gamma-rays induces a temporary loss of self -incompatibility.

Grafting

Grafting of a branch onto another branch of the same plant or of another plant is reported to reduce the degree of self- incompatibility in Trifolium Pratense. There is only one report on this phenomenon, and the mechanism of this reduction is not known.

Double Pollination

In some species, self- incompatible mating become possible when incompatible pollen in applied as a mixture with a compatible pollen, or it is applied after pollination with a compatible pollen.

Other Techniques

A number of other techniques have been tried with varying degrees of

success, but they are not commonly used. These techniques are : treatment of flowers with carbon monoxide, injecting styles with munosuppressants, application of electrical potential difference of about 100 V between the stigma and pollen grains, treatment of pistil with phytohormone s and with protein synthesis inhibitors, and steel brush pollination.

MALE STERILITY

Male sterility is characterized by nonfunctional pollen grains, while female gametes function normally. It occurs in nature sporadically, perhaps due to mattation. Male sterility is classified into three groups: (1) genetic, (2) Cytoplasmic, and (3) Cytoplasmic - genetic.

Genetic Male Sterility

Genetic male sterility is ordinarily governed by a single recessive gene, ms, but dominant genes governing male sterility are also known, e.g., in safflower. Male sterility alleles arise spontaneously or may be artificially induced. A male sterile line may be maintained by crossing it with heterozygous male fertile plants. Such a mating produces 1:1 male sterile and male fertile plants.

Utilization in Plant Breeding

Genetic male sterility may be used in hybrid seed production. The progeny from ms ms x Ms ms crosses are used as female, and are inerplanted with a homozygous male fertile (Ms Ms) pollinator. The genotypes of ms ms and Ms ms lines are identical except for the ms locus, i.e., they are isogenic; they are known as male sterile (A) and maintainer (B) lines, respectively. The female line would, therefore, contain both male sterile and male fertile plants; the latter must be identified and removed before pollen shedding. This is done by identifying the male fertile plants in seedling stage either due to the pleiotropic effect of the ms gene or due to the phenotypic effect of a closely- linked gene. Pollen dispersal from the male (pollintor) line should be good for a satisfactory seed set in the female line. however, generally pollen dispersal is poor and good, closely- linked markers are rare. Rouging of male fertile plants from the female lines is costly as a result of which

the cost of hybrid seed is higher. Due to these difficulties, genetic male sterility has been exploited commercially only in a few countries. In USA, it is being successfully used in Castor. In India, it is being used for hybrid seed production of arhar by some private seed companies, e.g., Maharashtra Hybrid Seed Co. Ltd., India, produced and sold 50 Q seed of a hybrid variety of arhar, Suggestions have been made for its use in several other crops, e.g., Cotton.,barley, tomato, sunflower, cucurbits etc., but it is not yet practically feasible.

Cytoplasmic Male Sterility

This type of male sterility is determined by the cytoplasm. Since the cytoplasm of a zygote comes primarily from egg cell, the progeny of such male sterile plants would always be male sterile.

Nuclear genotype of male sterile line would be almost identical to that of the recurrent pollinator strain. The male sterile line is maintained by crossing it with the pollinator strain used as the recurrent parent in the backcross programme since its nuclear genotype is identical with that of this new male sterile line. such a male fertile line is known as the maintainer line or B line as it is used to maintain the male sterile line is also known as the A line, there is considerable evidence that the gene or genes conditioning Cytoplasmic male sterility, particularly in Maize, reside in mitochondria, and may be located in a plasmic like elements.

Utilization in Plant Breeding

Cytoplasmic male sterility may be utilized for producing hybrid seed in certain ornamental species, or in species where a vegetative part is of economic value. But in those crop plants where seed is the economic part, it is of no use because the hybrid progeny would be male sterile.

Cytoplasmic -Genetic Male Sterility

This is a case of Cytoplasmic male sterility where a nuclear gene for restoring fertility in the male sterile line is known. The fertility restorer gene, R, is dominant and is found in certain strains of the species, or may be transferred from a related species, e.g., in wheat. This gene restores male fertility in the male sterile line,

hence it is known as restorer gene. The cases of Cytoplas mic male sterility would be included in the Cytoplasmic- genetic system as and when restorer genes for them would be discovered. It is likely that a restorer gene would be found for all the cases of Cytoplasmic male sterility if a thorough search were made. This system is known in Maize, Jowar, bajra, sunflower, rice, wheat, etc.

Plant would be male sterile in the presence of male sterile cytoplasm if the nuclear genotype were rr, but would be male fertile if the nucleus were Rr or RR. New male sterile lines may be developed following the same procedure as in the case of Cytoplasmic system. But the nuclear genotype of the pollinator strain used in such a transfer must be rr, otherwise the fertility would be restored. The development of new restorer strains is somewhat indirect. First, a restorer strain (say R) is crossed with a male sterile line (A). The resulting male fertile plants are used as the female parent in repeated backcrosses with the strain (C) (used as the recurrent parent), into which the transfer of restorer genes(s) is desired. In each generation, male sterile plants are discarded, and the male fertile plants are used as females

for backcrossing to the strain C. This acts as a selection device for the restorer gene R during the backcross programme. At the end of backcross programme, a restorer line isogenic to the stain C would be recovered.

For the production of hybrid seed, removal of anthers before fertilization is essential to avoid selfing. Manually removing of anthers is very te dious and time consuming process in almost all the crops except in Maize and Castor which are monoecious. The pre-requisites for successful hybrid seed production in large quantities are:

1. Existence of male sterility or self-incompatibility through which ha nd emasculation can be avoided.
2. Sufficient cross- pollination should be there to get good seed set.

Male sterility is characterized by non-functional pollen grains while female gametes functions normally. It occurs in nature sporadically due to mutations. MS can be classified into three groups:

1. Genetic 2. Cytoplasmic 3. Cytoplasmic genetic

I. Genetic male sterility: GMS is mostly governed by single recessive gene ms, but dominant genes governing male sterility are also known eg: Safflower, MS alleles arise spontaneously or can be induced artificially. A GMS line can be maintained by crossing it with heterozygous male fertile plant. Such mating produces 50% m.s. & 50% MF plants

msms x Msms
(Male sterile) (Male fertile)

msms : Msms (1:1)
(Male sterile) (Male fertile)

Identifying the male fertile plants from the above progeny is difficult and time consuming. Hence GMS is not commonly used in hybrid seed production.

In USA it is used in Castor. In India it was being used in Redgram, but presently it is being used in safflower.

Marker genes which are linked to male sterility/fertility can be used to

identify the male fertile plants before flowering stage. For example in Maize there is a gene, pigmented hypocotyl(P) and green hypoc otyl (P) which is closely linked with sterility locus

P S - Pigmented & Sterile

P F – Green & Fertile

At seedling stage all the green plants are to be removed and pigmented plants are retained, as they are sterile.

II. Cytoplasmic Male Sterility: In crops like Maize, Bajra and Sorghum, two types of cytoplasms were noticed. One is normal cytoplasm and the other is sterile one which interferes with the formation of normal pollen grains. This follows maternal inheritance therefore all the off springs will be male sterile.

As the F_1 is male sterile, this system cannot be used in crops where the seed is economic part. Hence its utility is confined to certain ornamental species or where a vegetative part is of economic importance. Eg: Onion, Fodder Jowar, Cabbage, Palak etc.

III. Cytoplasmic Genetic Male Sterility System: This is a case of cytoplasmic male sterility where a nuclear gene for restoring fertility in MS line is known. The fertility restorer gene 'R' is dominant and is found in certain strains of species or may be transferred from a related species. This gene restores fertility in the MS line hence it is known as restorer gene. The cytoplasmic MS can be included in CGMS system as and when restorer genes for them are discovered. Restorer gene s can be found for all the cases of cytoplasmic MS if thorough search is made. This system is used in almost all seed crops.

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

lines in the genetic constitution.

Transfer of Male Sterility from Exotic lines to Nature lines:

Most of the times the MS lines obtained from other countries may not be suitable to our condition. Examples are:

Crop	Source of cytoplasm	Drawbacks
Maize	Texas Cytoplasm	Susceptible to *Helminthosporium* leaf blight
Sorghum	Combined kafir	Black glumes and cha lky endosperm
Pearlmillet	Tift 23 A (Tifton)	Susceptible to Green ear & downy mildew
Rice	Wild abortive	Incomplete panicle exertion
Sunflower	*H petiolaris* *H gigantis*	
Tobacco	*Microcephalan*	Reduced vigour in F1 hybrids
Wheat	*Aegilops caudate*	Susceptible to pistiloidy

Due to these drawbacks, the well adapted local lines should be converted into male sterile lines. This can be done by repeated back crossing of the local lines to the exotic MS lines.

Transfer of Male Sterility to a New Strain

Maintenance of Male Sterile Line or A line: Since A line does not produce pollen, seed is not formed for maintaining A line. It has to be crossed with its fertile counterpart having similar nuclear genes with fertile cytoplasm which is known as B-line.

Production of Hybrid seed: For production of hybrid seed, A- line has to be kept as female parent and the pollen parent should possess the restorer genes in order to induce fertility and seed development in the next generation. Such line is known as restorer line and denoted as

'R'line. The A line & R line should be of different genetic constitution and should be able to give maximum heterosis.

Limitations in using Male Sterile Systems:

1. Existence and maintenance of A, B & R Lines is labourious 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 F1 seed will be sterile
7. Isolation is needed for maintenance of parental lines and for producing hybrid seed

CHAPTER-03

DOMESTICATION, PLANT INTODUCTION AND ACCLIMATIZATION

Domestication

Domestication is the process of bringing wild species under human management. The present day cultivated plants have been derived from wild species. Therefore, the first step in the development of cultivated plants was there, domestication. Domestication of wild species is still being done and is likely to continue for a long time in future.

Plant Introduction

Plant introduction consists of taking a genotype or a group of genotypes of plants into new environments where they were not being grown before. Introduction may involve new varieties of a crop already grown in the area, wild relatives of the crop species or a totally new crop species. Mostly materials are introduced from other countries or continents. But movement of crop varieties from one environment into another within a country is also introduction. Some examples of within the country introduction are popularization of grape cultivation in Haryana, Introduction of wheat in West Bengal, Rice in Punjab etc.

Primary Introduction: When the introduced variety is well suited to the new environment, it is released fro commercial cultivation without any alteration in the original genotype, this constitutes primary introduction. Primary introduction is less common, particularly in countries having well organized crop improvement programmes. Introduction of semi dwarf wheat varieties Sonora 64, Lerma Roja and of semi dwarf rice varieties Taichung Native 1 (TN-1), IR- 8 and IR- 36 are some examples of primary introductions.

Secondary Introduction: The introduced variety may be subjected to selection to isolate a superior variety. Alternatively, it may be hybridized with local varieties to transfer one or few characters from this variety to the local ones these processes are known as secondary introduction. Secondary introduction is much more common than primary introduction. Examples of secondary introduction are Kalyan Sona and sonalika wheat varieties selected from material introduced from CIMMYT, Mexico.

History of Plant Introduction: crop plants have traveled ino many new areas from their centres of origin. This movement of plants occurred with the movement of man. Most of these introductions occurred very early in the histoy. For example, mung mustard, pear, apple and walnut were introduced from the Central Asian Center of origin into various parts of India. Similarly, sesame, Jowar, arhar, Asian Cotton and finger millet originated in Africa and traveled to India in the prehistoric period. From this it is clear that plant wealth of various nations is to a large extent the result of plant introductions.

For several centuries A.D. the agencies of plant Introduction wre invaders, settlers, traders, travelles, explorers and naturalists. The plant introduction were made either knowingly or unknowingly. Muslim invaders introduced in India cherries and grapes from Afghanistan by 1300 A.S. In the 16^{th} century A.D. Portugues introduced Maize, groundnut, chillies, potato, sweet potato, guava pineapple, papaya, cashewnut and Tobacco. East India Company brought tea, litchi, and loquat from China. Cabbage, cauliflower and other vegetables from the Mediterranean; annatto and mahogany from West Indies in the last quarter of 18^{th} century.

During 19^{th} century, a number of botanic gardens played an important role in plant introduction. The Calcutta botanic gardens was established in 1781. the Kew botanic gardens, England arranged introduction of quinine and rubber trees from South America into India. During and after the last part of 19^{th} century various agricultural and horticulture research stations were established in the country. These stations introduced horticulture and agriculture plants independent of each other. There was no co-ordination among these agencies regarding their introduction activities.

Plant Introduction Agencies in India

A centralized plant introduction agency was initiated in 1946 at the Indian Agricultural Research Institute (IARI), New Delhi. The agency began as a plant introduction scheme in the Division of Botany and was funded by ICAR. In 1956, during the second five-year plan, the scheme was expanded as the Plant Introduction

and Exploration Organisation. Subsequently in 1961, it was made an independent division in IARI, the Division of Plant Introduction. The division was re organized as National Bureau of Plant Genetic Resources (NBPGR) in 1976. the nature of activities and the functions of the bureau have remained the same, but the scope and scale of its activities have increased considerably. The bureau is responsible for the introduction and maintenance of germplasm of agricultural and horticultural plants.

In addition to the National Bureau of Plant Genetic Resources, there are some other agencies concerned with plant introduction. *Forest Research Institute, Dehradun,* has a plant introduction organization which looks after the introduction, maintenance and testing of germplasm of forest trees. The Botanical Survey of India was established in 1890 ; it was responsible for the introduction, testing and maintenance of plant materials of botanical and medicinal interest. But at present, introduction and improvement of medicinal plants is being looked after by NBPGR. The Central Research Institute for various crops, e.g., tea, coffee, sugarcane, potato, Tobacco, rice etc., introduce, test and maintain plant materials of their interest. But their activities are coordinated by the NBPGR, which has the ultimate responsibility for introduction activities. Plant material may also be introduced by individual scientists, universities and other research organizations. But all the introductions in India must be routed through the NBPGR, New Delhi.

The National Bureau of Plant Genetic Resources. The bureau has it headquarters at IARI, New Delhi. It has four substations for the testing of introduced plant materials. These substations represent the various climatic zones of India, they are listed below.

1. **Simla.** It is situated in Himachal Pradesh and represents the temperate zone; approximately 2,300 m above sea level.
2. **Jodhpur, Rajasthan.** It represents the arid zone
3. **Kanya Kumari, Tamil Nadu.** It represents the tropical zone
4. **Akola, Maharashtra.** It represents the mixed climatic zone. It was recently shifted from Amravati.

In addition, a new substation has recently been established a t Shillong for collection of germplasm from North-east India. This part of the country has a large genetic variability for several crop species, e.g., rice, citrus, Maize etc.

The bureau functions as the central agency for the export and introduction of germplasm of economic importance. The bureau is assisted in its activities by the various Central Research Institutes of ICAR. The activities of the bureau are summarized below.

1. It introduces the required germplasm from its counterparts or other agencies in other countries.
2. It arranges explorations inside and outside the country to collect valuable germplasm.
3. It is responsible for the inspection and quarantine of all the introduced plant materials.
4. Testing, multiplication and maintenance of germplasm obtained through various sources. This may be done by the bureau itself at one of its substations or by one of the concerned Central Institutes of ICAR.
5. To supply, on request, germplasm to various scientists or institutions. The germplasm may be supplied ex-stock or may by procured from outside in case it is not available in the country.
6. Maintenance of records of plant name, variety name, propagating material, special characteristics, source, date and other relevant information about the materials received.
7. To supply germplasm to its counterparts or other agencies in other countries.
8. To publish its exchange and collection lists. An Introduction News Letter containing such lists is being published by the Food and Agriculture Organisation (FAO) since

   1957 at irregular intervals. NBPGR has also published some lists, and is in the process of publishing some other catalogues.
9. To set up natural gene sanctuaries of plants where genetic resources are endangered.
10. Improvement of certain plants like medicinal and aromatic plants.

Procedure of Plant Introduction

Introduction consists of the following steps : Procurement, quarantine, cataloguing, evaluation, multiplication and distribution.

1. Procurement : Any individual or institution can introduce germplasm in India. But all the introductions must be routed through the NBPGR, New Delhi. There are two routes for plant introduction. In first route the individual or the institution makes a direct request to an individual or institution abroad, who has the desired germplasm, to send it through the NBPGR, New Delhi. In second procedure the individual or institute submits his germplasm requirements to the NBPGR with a request for their import.

2. Quarantine : Quarantine means to keep materials in isolation to prevent the spread of diseases etc. All the introduced plant propagules are thoroughly inspected for contamination with weeds, diseases and insect pests. Materials that are suspected to be contaminated are fumigated or are given other treatments to get rid of contamination. If necessary, the materials are grown in isolation for observation of diseases, insect pests and weeds. The entire process is known as quarantine and the rules prescribing them are known as quarantine rules.

3. Cataloguing : When an introduction is received, it is given an entry number. Further, information regarding name of the species, variety, place of origin, adaptation and its various characteristics are recorded. The plant materials are classified into three groups.

1. Exotic collections are given the prefix 'EC'
2. Indigenous collections are designated as 'IC' and
3. Indigenous wild collections are marked as 'IW'

4. Evaluation : To assess the potential of new introductions, their performance is evaluated at different substations of the Bureau. In case of those crops for which Central Research Institutes are functioning, e.g., rice, sugarcane, potato, Tobacco etc., the introduced materials are evaluated and maintained by these institutes. The resistance to diseases and pests is evaluated under environments favouring heavy attacks by them.

Acclimatization : Generally, the introduced varieties perform poorly because they are

often not adapted to the new environment. Sometimes, the performance of a variety in the new environment improves with the number of generations grown there. The process that leads to the adaptation of a variety to a new environment is known as acclimatization.

Acclimatization is brought about by a faster multiplication of those genotypes (present in the original population) that are better adapted to the new environment. Thus acclimatization is essentially natural selection. Variability must be present in the original population for acclimatization to occur. Therefore, land varieties are likely to get acclimatized, while purelines are not likely to.

The extent of acclimatization is determined by (1) the mode of pollination, (2) the range of genetic variability present in the original population, and (3) the duration of life- cycle of the crop. Cross-pollination leads to a far greater gene recombinations than self- pollination. As a result cross- pollination is much more helpful in acclimatisation than self- pollination.

5. Multiplication and Distribution : Promising introductions or selections from the introductions may be increased and released as varieties after the necessary trials. most of the introductions, however, are characterized for desirable traits and are maintained for future use. Such materials are used in crossing programmes and are readily supplied by the bureau on request.

PURPOSE OF PLANT INTRODUCTION

The main purpose of plant introduction is to improve the plant wealth of the country. The chief objectives of plant introduction may be grouped as follows.

To Obtain An Entirely New Crop Plant. Plant introductions may provide an entirely new crop species. Many of our important crops, e.g., Maize, potato, tomato, Tobacco, etc., are introductions. Some recently introduced crops are Soybean, gobhi sarson, oil palm etc.

To Serve as New Varieties. Sometimes introductions are directly released as superior commercial varieties. The Maxican semidwarf wheat varieties Sonora 64 and Lerma Rojo, semidrawf rice varieties TN 1, IR- 8 and IR-36 are more recent examples.

To Be Used in Crop Improvement. Often the introduced material is used for

hybridization with local varieties to develop improved varieties. Pusa Ruby tomato was derived from a cross between Meeruty and Sioux, an introduction from U.S.A.

To Save the Crop from Diseases And Pests. Sometimes a crop is introduced into a new area to protect it from diseases and pests. Coffee was introduced in South America from Africa to prevent losses from leaf rust. Hevea rubber, on the other hand, was brought to Malaya from South America to protect it from a leaf disease.

For Scientific Studies. Collections of plants have been used for studies on biosystematics, evolution and origin of plant species. N.I. Vavilov developed the concept of centres of origin and that of homologous series in variation from the study of a vast collection of plant types. **For Aesthetic Value.** Ornamentals, shrubs and lawn grasses are introduced to satisfy the finer sensibilities of man. These plants are used for decoration and are of great value in social life.

Varieties Selected from Introductions. Many varieties have been developed through selection from introductions. Two varieties of wheat, Kalyan Sona and Sonalika, were selected from introductions from CIMMYT, Mexico.

Varieties Developed through Hybridization. Introductions have contributed immensely to the development of crop varieties through hybridization. All the semidwarf wheat varieties are derived from crosses with Mexican semi-dwarf wheats. All but few semidwarf rice varieties possess the dwarfing gene from Dee-geo-woo -gen through either TN1 or IR 8. Thus, almost all these semi- dwarf wheat and rice varieties have been developed from crosses involving introductions. All the sugarcane varieties have been derived from the introduced noble canes.

Other examples of varieties developed through hybridization with introductions are pusa Ruby tomato obtained from a cross between Meeruti and Sioux ; Pusa Early Dwarf Tomato derived from the cross Meeruti x Red Cloud ; Pusa Kesar carrot, Pusa Kanchan turnip etc.

Merits of Plant Introduction

1. It provides entirely new crop plants.
2. It provides superior varieties either directly or after selection & hybridization.
3. Introduction and exploration are the only feasible means of collecting germplasm and to protect variability from genetic erosion.

4. It is very quick & economical method of crop improvement, particularly when the introductions are released as varieties either directly or after a simple selection.
5. Plants may be introduced in new disease-free areas to protect them from damage, e.g., coffee and rubber.

Demerits of Plant Introduction

The disadvantages of plant introduction are associated with the introduction of weeds, diseases and pests.

GERMPLASM COLLECTIONS

The sum total of hereditary material or genes present in a species is known as the germplasm of that species. Therefore, a germplasm collection is the collection of a large number of genotypes of a crop species and its wild relatives. Germplasm collections are also known as gene banks (or world over the world). Furthe r, germplasm collections furnish the richest s o u r c e of variability. Crop improvement would ultimately depend upon the availability of this variability to be utilized in breeding programmes.

With the modernization of agriculture, large tracts of land have been put under pureline varieties of self- pollinated crops and hybrid varieties of cross-pollinated species. This has led to a gradual disappearance of local or land varieties (‘desi’ varieties) and open- pollinated varieties -both reservoirs of considerable variability. Cultivation and grazing are gradually destroying many wild species and their breeding grounds. Wild relatives of crops may be eliminated by introduced species of weedy nature or even by the cultivated forms derived from them. ***The gradual loss of variability in the cultivated forms and in their wild relatives is referred to as genetic erosion.*** This variability arose in nature over an extremely long period of time and, if lost, would not be reproduced during a short period.

Most of the countries are greatly concerned about genetic erosion. The establishment of IBPGR to coordinate germplasm conservation activities throughout the world reflects this concern. Germplasm collections are being made and maintained to conserve as many genotype as possible. The germplasm collections contain land varieties, various wild forms, primitive races, exotic collections and

highly evolved varieties. Some of the important germplasm collections are listed below.

1. Institute of Plant Industry, Leningard. It has 1,60,000 entries of crop plants.
2. Royal Botanic Gardens, Kew, England, It has over 45,000 entries.
3. Bellsville, U.S.A., maintains germplasm collections of small grain crops.
4. World collections of some of the crops are maintained at the following places.
    i) Sugarcane. Canal Point, Florida, U.S.A. and Sugarcane Breeding Institute, Coimbatore (2,800 entries).
    ii) Groundnut. Bambey, Senegal (Africa).
    iii) Potato. Cambridge, U.K. and Wisconsin, U.S.A.
    iv) Annual New World Cottons. Near Tashkent, U.S.S.R. v) Coffee. Ethiopis (Africa).
    vi) Sweet Potatoes. New Zealand
5. The National Bureau of Plant Genetic Resources, New Delhi, is maintaining large collections of Sorghum, Pennisetum, wheat, barley, oats, rice, Maize and other agricultural and horticultural crops. For example, groundnut collection is maintained at Junagarh, Cotton at Nagpur, Potato at Simla, Tobacco at Rajahmundhry, tuber crops (other than potato) at Trivandrum etc. The Cotton collection maintained at Central Institute for Cotton Research (CICR, Nagpur) are as follo ws ; Gossypium hirsutum- 4,100 entries ; G. barbadense- 300 entries ; G. arboreum- 1755 entries ; G. herbaceum- 393 entries (1991).
6. IRRI, Philippines, is maintaining 42,000 rice strains and varieties. More than 15,000 entries are maintained at CRRI, Cuttack.
7. The various International Institutes are building up and maintaining collections of many species.

Seeds of most species lose viability quickly. Consequently, germplasm collections have to be grown every few years. (1) Growing, harvesting and storing lar ge collections is a costly affair requiring much time, labour, land and money. (2) There is also risk of errors in labeling. (3) The genotypic constitution of entries may also change, particularly when they are grown in environments considerably different from that to which they are adapted. This is particularly true in case of cross-pollinated species and for local varieties of self- pollinated species. These

difficulties may be considerably reduced by cold storage of seeds. Seeds of most of the plant species can be stored for 10 years or more at low temperatures and low humidity. Thus the entries could be grown every 10 years or so instead of every one or two years. Cold storage facilities are being utilized at Fort Collins, U.S.A and at IRRI, Phillippines, NBPGR has developed cold storage facilities for germplasm maintenance this is known as National Germplasm Repository.

Gene Sanctuaries. It has been proposed that within the centres of origin areas of the greatest diversity should be demarcated and protected from human disturbances. In such areas, the evolutionary potential of the local populations and the environment would be preserved. This would not only preserve variability in these populations, but would also allow evolution to continue and create new types. NBPGR proposes to establish gene sanctuaries in Meghalaya for Citrus, and in the North-Eastern Region for Musa, Citrus, Oryza, Saccharum and Mangifera.

Thus a gene sanctuary may be defined as an area of diversity protected from human interference . A gene sanctuary conserves the germplasm in-situ, within the environment where it naturally grows. This not only conserves the germplasm with very little labour and expense, but also permits evolution to proceed on its natural course. This allows the appearance of new gene combinations and new alleles not present in the preexisting population.

Exploration: Explorations are trips for the purpose of collection of various forms of crop plants and their related species. Explorations generally cover those areas that are likely to show the greatest diversity of forms. The centres of origin are such areas and are often visited by various exploration teams. In addition to wild forms, land races and open- pollinated varieties are also collected. Exploration is the primary source of all the germplasm maintained in germplasm collections.

CHAPTER-04

CENTRES OF ORIGIN/DIVERSITY

Introduction: The cultivation of plants is one of man's oldest occupations and probably began when he selected some plants for his use. One of the old belief regarding to the origin of cultivated plants was that they came to man as a gift from God. By the end of 18th century people started questioning about the origin of cultivated plants.

Darwin (1868) considered that the cultivated plants arose by profound modifications in the wild plant.

Alphonse de Candolle (1863) a Swiss botanist first attempted to solve the mystery about evolution of crop plants. In his " Origin of cultivated plants" he studied 247 plant species of cultivated plants. He classified the economic plants into six classes;

1. Plants cultivated 4000 years ago. 2. Plants cultivated 2000 years ago.
3. Plants cultivated less than 4000 years.
4. Plants cultivated 2000 to 4000 years.
5. Plants cultivated before the time of Columbus.
6. Plants cultivated after the time of Columbus.

It is **N.I.Vavilov** who proposed the concept of 'centres of origin'. He proposed the concept based on his studies of a vast collection of plants at Institute of Plant Industry, Leningrad. The concept is that crop plants evolved from wild species in the area showing great diversity and that place is termed as **primary centre of origin**. Later on from the primary centre the crops moved to other places due to the activities of man. There are certain areas where some crops exhibit maximum diversity of forms but this may not be the centre of origin for that particular crop. Such centres are known as **Secondary centres of origin**. E.g. Sorghum The primary centre of origin for this crop is Africa but India exhibits maximum diversity for this crop.

N.I. Vavilov originally proposed **Eight** main centres of origin.

1. **China**
2. **Hindustan**
3. **Central Asia**
4. **Asia minor**
5. **Mediterranian**
6. **Abyssinya**
7. **Central America**
8. **South America**

1. The China centre: It consists of the mountainous regions of central and western China and the neighbouring low lands. It is the largest and oldest independent centre. The crops originated in this centre are:*I .Primary centre of origin are*: Radish, Soybeans, Proso millet, Opium Brassica, Onion.

ii. Secondary centre of origin are: Maize, Cowpea, Turnip, sesame

2.The Hindustan Centre: This includes Burma, Assam, Malaya, Java Borneo, Sumatra and Philippines, but excludes North West India, Punjab and North Western Frontier Provinces. The crops originated in this centre are: *i. Primary centre of origin are*: Rice, Cucumber, Red gram, Radish, Chickpea, Noble canes, Cowpea, Cotton *(Gossypium arboreum)* ,Greengram, Hemp, Turmeric, Coconut

3.The Central Asia Centre: It includes North West India, all of Afghanistan, the Soviet Republics of Tadjikistan and Tian Shan. It is also known as *the Afghanistan centre of origin.* The crops originated in this centre are: *i. Primary centre of origin are:* Wheat, Rye, Pea, Broad bean, Green gram ,Sesame, Safflower, Cotton*(G.herbaceum),* Onion, Garlic *ii. Secondary centre of origin are*: rye

4.The Asia Minor Centre: This is also known as the *Near East or the Persian Centre* of Origin. It includes the interior of Asia Minor, the whole of Transcaucasia, Iran and Highlands of Turkmenistan. The crops originated in this centre are: *i. Primary centre of origin are:Triticum,rye, alfalfa, carrot, cabbage, oat. ii. Secondary centre of origin are*: rape, black mustard and leaf mustard, turnip and apricot.

5.The Mediterranean Centre: The crops originated in this centre are: *i.Primary centre of origin are:* Many valuable cereals and legumes such as; Durum Wheat, Chickpea, Emmer Wheat, Beets, Barley, Peppermint, Lentil, Pea, Broad bean.

6.The Abyssinian Centre: It includes Ethiopia and hill country of Eritrea. The crops originated in this centre are: *i. Primary centre of origin are: ii. Secondary centre of origin are:* Barley, Broad bean, Sorghum, Pearl millet, Lentil, Khesari, Sunflower, Castor, Coffee, Okra.

7.Central American Centre: This includes South Mexico and Central America. It is also referred to as the *Mexican Centre of Origin*. The crops originated in this centre

are:

i.Primary centre of origin are: Maize, Lima bean, Melons, Pumpkin, Sweet Potato, Arrowroot, Cotton *(G.hirsutum)*

8.The South American Centre: This centre includes the high mountainous regions of Peru, Bolivia, Ecuador, Colombia, parts of Chile, and Brazil and whole of Peraguay. The crops originated in this centre are:*i. Primary centre of origin are:* Potato Maize Lima bean Peanut Egyptian cotton *(G.barbadense)* Tobacco Tapioca Later in, 1935, Vavilov divided the Hindustan Centre of Origin into two centres, viz., *Indo Burma* and *Siam- Malaya-- Java Centre of Origin*. The South American Centre was divided into three centres, namely, *Peru, Chile* and *Brazil-Peraguay Centres of Origin*. At the same time he introduced a new centre of origin, the *U.S.A. Centre of origin*. Two plant species, Sunflower(*Helianthus annuus*) and Jerusalem Artichoke (*H.tuberosus*) osriginated in the U.S.A. Centre of origin. Thus the centres of origin may be more appropriately called the centres of diversity. The centres of origin may not be the centres of origin of the species concerned, but they are the areas of maximum diversity of the species. Within the large centres of diversity, small areas may exhibit much greater diversity than the centre as a whole. These areas are known as *Microcentres* .

OBJECTIONS TO VAVILOV'S THEORY: According to **Vavilov** whenever a crop plant exhibits maximum diversity, that place is the centre of origin for that crop. But this view is no longer valid. E.g. maize and tomato. For maize the centre of diversity is Peru but archeological evidence shows Mexico as centre of origin. For tomato, South America is considered to be primary centre of origin but it is Mexico as per archeological evidence. Secondly Vavilov stated that primary centre is marked by a high frequency of dominant genes in the centre and recessive genes towards the periphery. But it is not so. E.g. Wheat, maize, oil palm Vavilov's claim that centre of origin confined to mountainous regions only. But this is not the case. For E.g. Maize exhibits maximum diversity in plains Many crops have more than one centre of origin E.g. Balsam, Sorghum. In some crops centre of domestication cannot be determined for want of suitable evidence. To counter the objection, **Zhukovsky** student of vavilov has proposed 'mega centre' theory. He divided the world into 12 regions. Mega gene centres were the places where cultivated plant species exhibit diversity and micro gene centre is the place where wild species occur.

Harlan stated that each crop may have been repeatedly domesticated at different times in different locations or may have been brought into cultivation in several regions simultaneously. We cannot pin point a single centre of origin. Harlan developed the idea of '**Centre'** and **'Non- centre'**. According to him 'centre' means places of agricultural origin and 'non centre' where agriculture has been introduced. Harlan divided the world in to three centres and three non centres.

LAW OF HOMOLOGOUS SERIES: This is proposed by N.I Vavilov. According to this law "the characters found in one species also observed in other related species". Thus diploid, tetraploid and hexaploid wheats show a series of identical characters. So also in case of diploid and tetraploid cotton. Similarly genus *Secale* duplicates the variation found in *Triticum*.

CHAPTER-05

COMPONENT OF GENETIC VARIATION: HERITABILITY AND GENETIC ADVANCE

COMPONENTS OF GENETIC VARIATION: The study of variation is an important factor in crop improvement. The breeders who attempt to improve a crop should be able to distinguish the genetic and non genetic parts of the variation occurring in the population. The measurement of variation is statistically expressed in terms of means, variances and covariances. The average of the squared deviations of the individual observations from the mean is the variance. The gross variability in a population is the phenotypic variance (Vp). This is sum total expression of genotype, environment and interaction of both. The variation caused by genotype is expressed as genotypic variance (VG), while variation influenced by the environment is designated as environmental variance (VE).

Genotypic variance It is the most important part of the variation in a breeding material. This is the total variation caused by the segregating genotypes in the population. The genotypic variance is again sub divided into genetic variance, variance due to dominance deviation and variance due to non-allelic interactions. The genetic variance (VD) is ascritable to additive gene action, while the variance due to dominance deviation (VH) is due to interactions between genes of the same locus (intra allelic interaction). The variance caused by the interactions between genes at different loci is called as 'epistasis' and is due to non allelic interaction (inter allelic interaction). The epistatic variance is of three types viz., Additive x Additive, Additive x Dominance and Dominance x Dominance. The numerical examples explaining the interactions are given below. Hence the components of variance can be summarized as follows.

Environmental variance Environmental variance is variation of environmental deviations. Differences in fertility level of plots, moisture contents of soil, seasonal fluctuations etc., contribute to this component of variation. There are several other causes in addition to these, like developmental causes which contribute to the environmental variation but are difficult to be eliminated by choice of appropriate experimental designs. Though it can be reduced by proper experimentation its total elimination is impossible.

Classified by	Type of Genetic variance	Brief description
Fisher, 1918	Additive variance	Average effect of genes at all segregating loci
	Dominance variance	Deviation from mean value due to intrallelic interaction
	Epistatic variance	Deviation from mean value due to non-

		allelic interaction. It is of three types, viz. Additive x Additive, Additive x Dominance and Dominance x Dominance.
Wright, 1935	Additive variance	Same as given by Fisher
	Non- additive	It include dominance and epiststic variances
Mather, 1949	Heritable- fixable	Include Additive variance and Additive x Additive component of epistasis
	Heritable-Non fixable	Include dominance variance and Additive x Dominance and Dominance x Dominance types of epistasis.

C

HERITABILITY AND GENETIC ADVANCE

Introduction: Heritability and genetic advance are important selection parameters. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone.

HERITABILITY

In general sense , heritability specifies the proportion of the total variability that is due to genetic causes, or the ratio of genotypic variance to the total variance. It is generally expressed in percent. Heritability is an index of the transmission of characters from parents to their offspring (Falconer, 1960). The estimates of heritability helps the plant breeder in selection of elite genotypes from diverse genetic populations. Heritability can be estimated as regression of mean phenotypic values of offspring on their corresponding mid-parental value i.e., bop . Therefore, regression of response, which is the mean phenotypic value of offspring expressed as deviation from population mean, on the selection differential is equal to b op and consequently heritability. Thus heritability can also be expressed as

$$H = R/S$$

TYPES OF HERITABILITY

Heritability is of two types, viz.

1. Broad sense heritability
2. Narrow sense heritability

Broad sense heritability is the percentage ratio of genotypic variance to the phenotypic variance, whereas narrow sense heritability is the ratio of additive variance to the phenotypic variance.

BROAD SENSE HERITABILITY

It is the ratio of genotypic variance to total or phenotypic variance. It is calculated from total genetic variance

The broad sense heritability, from different materials is estimated in different ways. From replicated data of several genotypes, heritability is calculated as follows:

$$\textbf{Heritability (bs)} = \frac{V_G}{V_P} 100$$

Where, V_G = genotypic variance, and

V_P = phenotypic variance

From generation mean analysis, the heritability is worked out with the help of following formula:

$$\textbf{Heritability (bs)} = \frac{VF_2 - VF_1}{VF_2} 100$$

Where, VF_1= Variance of F_1 progeny

VF_2= Variance of F_2 progeny

NARROW SENSE HERITABILITY

It is the ratio of additive or fixable genetic variance to the total or phenotypic variance. It plays an important role in the selection process in plant breeding. Ii is calculated from the following formula:

$$\textbf{Heritability} = \frac{1}{2} D/Vp$$

Where,

D is the additive variance and Vp is the phenotypic variance.

Since narrow sense heritability is estimated from additive (heritable fixable) genetic variance, it plays an important role in the selection of elite genotypes from the segregating populations, whereas, broad sense heritability estimates are more useful in selecting superior lines from the homozygous materials.

Difference between Broad sense and Narrow sense heritability

Broad sense heritability	Narrow sense heritability
Estimated from total genetic variance	Estimated from additive genetic variance
Can be estimated from both parental and segregating material	Requires crossing in definite fashion
Useful in selection of elite types from homozygous lines	Useful in selection of elite types from segregating material

As suggested by Johnson et al., (1955a), heritability values are categorized as

follows:

Low	:	Less than 30 %
Moderate	:	30 – 60 %
High	:	More than 60 %

Co-heritability : The analysis of covariance permits estimation of co-heritability for related characters.

Co- heritability between characters *x* and *y* =	Genotypic co-variance ----------100 Phenotypic co-variance

GENETIC ADVANCE

It is a measure of genetic gain under selection. Genetic advance is defined as the difference between the mean genotypic value of the selected lines and the mean genotypic value of the parental population (original population before selection). The expected genetic gain or advance under selection is estimated by the following method suggested by Johnson *et al.*(1955a).

Estimation of Genetic advance

Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. The genetic advance *i.e.,* theexpected genetic gain was worked out by using the formula suggested by **Johnson *et al.* (1955).**

$$G.A. = \frac{\sigma^2 g}{\sigma^2 p} k.\ \sigma p$$

$$= h^2.\ K.\ \sigma p$$

Where,

h^2 = Heritability coefficient

K = Selection differential standard units which is 2.06 for 5% selection intensity

σp = Phenotypic standard deviation

G.A. = Genetic advance

T etic
v der
c etic
advance is usually expressed as percentage of mean.

Genetic advance as per cent of mean (GA % M)

It was calculated by the following formula:

$$\text{Genetic advance percent} = \frac{\text{Genetic Advance}}{\text{Mean}} 100$$

\

The range of genetic advance as percent of mean is classified as suggested by Johnson et al., (1955a).

Low : Less than 10 %
Moderate : 10-20 %
High : More than 20 %

Heritability and genetic advance are important selection parameters. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection (Johnson et al., 1955a).

(i) High heritability accompanied with high genetic advance indicates that most likely the heritability is due to additive gene effects and selection may be effective.
(ii) High heritability accompanied with low genetic advance indicates non-additive gene action and selection for such traits may not be rewarding.
(iii) Low heritability accompanied with high genetic advance reveals that the character is governed by additive gene effects. The low heritability is being exhibited due to high environmental effects. Selection may be effective in such cases.
(iv) Low heritability accompanied with low genetic advance indicates that the character is highly influenced by environmental effects and selection would be ineffective.

CHAPTER-06

SELECTION IN SELF POLLINATED CROPS: To get successful results by selection there are two pre-requisites. a) Variation must be present in the population. b) The variation must be heritable.

HISTORY OF SELECTION: Selection was practiced by farmers from ancient times. During 16th century Van Mons in Belgium, Andrew knight in England and Cooper in USA practiced selection in crop plants and released many varieties. Le coutier, a farmer of island of New Jersey published his results on selection in wheat in the year 1843. He concluded that progenies from single plants were more uniform. During the same period Patrick shireff, a scotsman practiced selection in wheat and oats and developed some valuable varieties. During 1857 Hallet in England practiced single plant selection in wheat, oats and barley and developed several commercial varieties. About this time **Vilmorin** proposed individual plant selection based on progeny testing. This method successfully improved the sugar content in sugar beet. His method was called as vilmorin isolation principle. He emphasised that the real value of a plant can be known only by studying the progeny produced by it. This method was successful in sugar beet but not in wheat. This shows the in-effectiveness of selection in cross pollinated crops. Today progeny test is the basic step in every breeding method.

PURE LINE THEORY A pure line is the progeny of a single self-fertilized homozygous plant. The concept of pureline was proposed by **Johannsen** on the basis of his studies with beans *(Phaseolus vulgaris*) variety called Princess. He obtained the seeds from the market and observed that the lot consisted of a mixture of larger as well as smaller size seeds. Thus there was variation in seed size. Johannsen selected seeds of different sizes and grown them individually. Progenies of larger seeds produced larger seeds and progenies from smaller seeds produced small seeds only. This clearly showed that there is variation in seed size in the commercial lot and it has a genetic basis. He studied nineteen lines al together. He concluded that the market lot of the beans is a mixture of pure lines. He also concluded whatever variation observed with in a line is due to environment only. Confirmatory evidence was obtained in three ways. In line 13 which is having 450 mg seed wt he divided the seeds on weight basis. He divided the line into seeds having 200, 300, 400 and 500 mg weights and studied the progenies. Ultimately he got lines having weight ranging from 458 to 475. Thus the variation observed is purely due to environment. The second evidence was that selection with in a pure line is ineffective. From a pure line having 840 mg selection

was made for large as well as small seeds. After six generations of selection the line for large seed as well as for small seed gave progenies having 680-690 mg. Thus, it was proved that selection within a pure line is ineffective. In third evidence when parent - offspring regression was worked in line thirteen. It worked to zero indicating that variation observed is non heritable and it is due to environment only.

ORIGIN OF VARIATION IN PURELINES

1. Mechanical mixtures.
2. Natural hybridization.
3. Chromosomal aberrations.
4. Natural mutation.
5. Environmental factors.

EFFECT OF SELF-POLLINATION ON GENOTYPE Self-pollination increases homozygosity with a corresponding decrease in heterozygosity. For example an individual heterozygous for a single gene Aa is self-pollinated in successive generations, every generation of selfing will reduce the frequency of heterozygote Aa to 50 percent of that in the previous generation. There is a corresponding increase in homozygotes AA and aa. As a result, after 10 generations of selfing virtually all the plant in the population will be homozygous AA and aa.

This can be calculated by the formulae [2m - 1) / 2m]n where m = No. of generations of self-pollination. n = No. of genes segregating. When number of genes are segregating together, each gene would become homozygous at the same rate as Aa. Thus, the number of genes segregating does not affect the percentage of homozygosity. Similarly, linkage between genes does not affect the percentage of homozygosity in the population.

PURELINE SELECTION

A large number of plants are selected from a self-pollinated crop. The selected plants are harvested individually. The selected individual plants are grown in individual rows and evaluated and best progeny is selected, yield tested and released as a variety.

CHARACTERISTICS OF PURELINES:

1. All plants within a pure line have the same genotype. 2. The variation with in a pureline is environmental and nonheritable. 3. Purelines become genetically variable with time due to natural hybridization, mutation and mechanical mixtures.

General steps for making a pureline selection

First Season : From the base population select best looking plants having the desirable

characters. Harvest them on single plant basis.

Second Season : The selected single plants are grown in progeny rows and estimate the performance. Reject unwanted progenies.

Third Season : Repeat the process of second season.

Fourth Season : Grow the selected single plants in replicated preliminary yield trial along with suitable check or control variety.

Fifth Season : Conduct regular comparative yield trial along with check variety and select the best culture.

Sixth Season : Conduct multilocation trial in different research stations along with local check.

Seventh Season : Conduct Adaptive Research Trial in farmer's field. Fix the best yielder and release it as a variety thro' Variety Release committee.

Advantage of Pureline Selection. 1. Achieves maximum possible improvement over the original variety. 2. Extremely uniform in appearance. 3. Because of the uniformity, a variety is easily identified and seed certification is easy.

Disadvantages :

1. It does not have wide adaptability because improvement is made only in the local variety.
2. Time required for developing a variety is more when compared to mass selection.
3. Depending on the genetic variability present in the base population only the improvement is made. If there is no genetic variability improvement cannot be made.
4. Breeder has to spend more time compared to mass selection.

b) MASS SELECTION Here a large number of plants having similar phenotype are selected and their seeds are mixed together to constitute a new variety. Thus the population obtained from selected plants will be more uniform than the original population. However they are genotypically different.

Steps :

First season : From the base population select phenotypically similar plants which may be 200 - 2000. Harvest the selected plants as a bulk.

Second season : The bulk seed is divided into smaller lots and grown in preliminary yield trial along with control variety. Dissimilar phenotypes are rejected. Higher yielding plots are selected.

Third to Sixth Season : With the selected lots conduct yield trials along with appropriate

check or control. Select the best one and release it as a variety.

Merits of Mass Selection : 1. Varieties developed will be having more adaptability since each plant is genotypicaly not similar. They have buffering action against abnormal environment. 2. Time taken for release of a variety is less. 3. The genetic variability present in the original population is maintained.

Demerits : 1. Compared to pure line variety they may not be uniform.

2. In the absence of progeny test we are not sure whether the superiority of selected plant is due to environment or genotype.

3. May not be as uniform as that of a pureline variety and certification is difficult.

HYBRIDIZATION METHODS

Introduction

In this chapter we will learn about the Meaning of Hybridization Method of Crop Improvement: -

1. Meaning of Hybridization
2. Objectives of Hybridization
3. Types
4. Procedure
5. Hybridization Methods of Plant Breeding in Self-Pollinated Groups

Meaning of Hybridization:

Individual produced as a result of cross between two genetically different parents is known as hybrid. The natural or artificial process that results in the formation of hybrid is known as hybridization.

or

The production of a hybrid by crossing two individuals of unlike genetical constitution is known as hybridization. Hybridization is an important method of combining characters of different plants. Hybridization does not change genetic contents of organisms but it produces new combination of genes.

The first natural hybridization was recorded by Cotton Mather (1716) in corn. The first artificial interspecific plant hybrid was produced by Thomas Fairchild in 1717. It is commonly known as **'Fairchild Mule'**.

Hybridization was first of all practically utilized in crop improvement by German botanist Joseph Koelreuter in 1760. Mendel onward, the hybridization had become the key method of crop improvement. Today, it is the most common method of crop improvement, and the vast majority of crop varieties have resulted from hybridization.

Objectives of Hybridization:

I. To artificially create a variable population for the selection of types with desired combination of characters.

II. To combine the desired characters into a single individual, and

III. To exploit and utilize the hybrid varieties.

Types of Hybridization:

Hybridization may be of following types:

(i) Intra-varietal hybridization:

The crosses are made between the plants of the same variety.

(ii) Inter-varietal or Intraspecific hybridization:

The crosses are made between the plants belonging to two different varieties.

(iv) Interspecific hybridization or intragenric hybridization:

The crosses are made between two different species of the same genus.

v) Introgressive hybridization:

Transfer of some genes from one species into the genome of the other species is known as introgressive hybridization. The crosses between different species of the same genus or different genera of the same family are also known as distant hybridization or wide crossing. Such crosses are called distant crosses.

Procedure of Hybridization:

It involves the following steps:

(i) Selection of parents.
(ii) Selfing of parents or artificial self-pollination.
(iii) Emasculation.
(iv) Bagging
(v) Tagging
(vi) Crossing
(vii) Harvesting and storing the F, seeds
(viii) Raising the F_1 generation.

(i) Selection of parents:

The selection of parents depends upon the aims and objectives of breeding. Parental plants must be selected from the local areas and are supposed to be the best suited to the existing conditions.

(ii) Selfing of parents or artificial self-pollination:

It is essential for inducing homozygosity for eliminating the undesirable characters and obtaining inbreeds.

(iii) Emasculation:

It is the third step in hybridization. Inbreeds are grown under normal conditions and are emasculated. Emasculation is the removal of stamens from female parent before they burst and shed their pollens.

It can be defined as the removal of stamens or anthers or the killing of the pollen grains of a flower without affecting in any way the female reproductive organs. Emasculation is not required in unisexual plants but it is essential in bisexual or self-pollinated plants.

METHODS OF EMASCULATION

Methods used for emasculation are:

(a) Hand Emasculation or Forceps or Scissor Method:

This method is generally used in those plants which have large flowers. In this method the corolla of the selected flowers is opened and the anthers carefully removed with the help of fine-tip forceps.

Following are the important precautions while performing this method:

i. Flowers should be selected at proper stage.

ii. Stigma should be receptive and anthers should not have dehisced.

iii. All the anthers should be removed from the flowers without breaking (Fig. 1).

iv. Stigma and ovary of the flower should not be damaged.

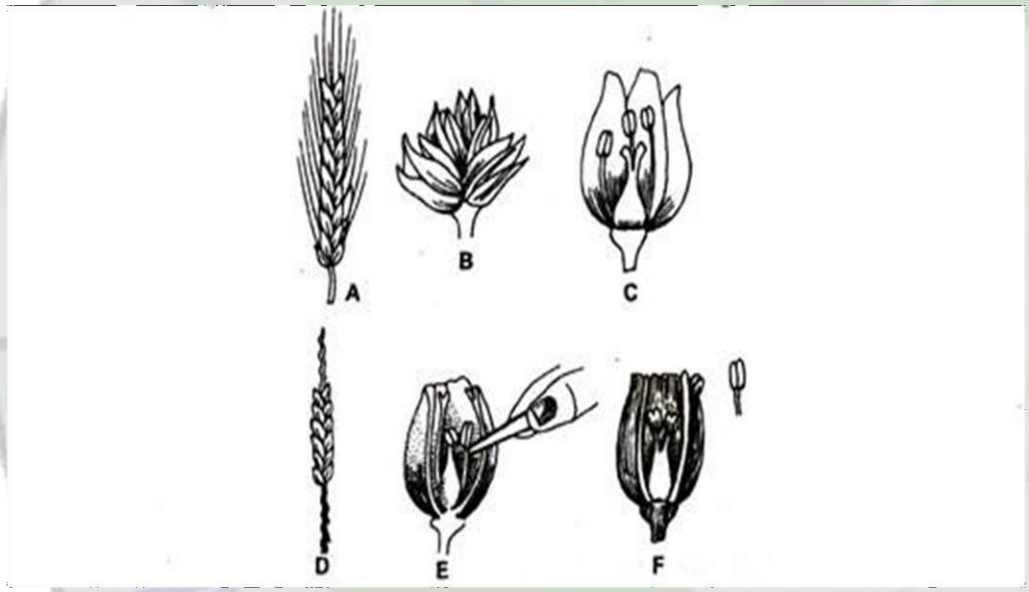
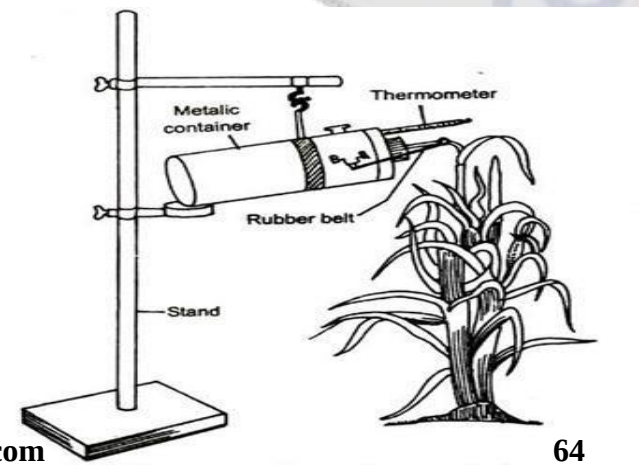


Fig.1 emaculation in wheat (A) Spike of spikelets (B) Spilkelets (C) florets (D) Upper and lower spikelets, awns removed, upper portion of florets cut (E) Anthers removed with help of fine forceps (F) Removed anthers

(b) Hot Water Treatment:

Removal of stamens with the help of forceps is very difficult in minute flowers. In such small hermaphrodite flowers (e.g., Bajra, Jowar) emasculation is done by dipping the flowers in hot water for certain duration (1-10 minutes) of time.



The time varies from species to species. This method Is based on the fact that gynoecia can withstand the hot temperature at which the anthers are killed. In this method an equipment is used which is placed on a simple heavy stand.

It consists of a cylindrical metallic container of 60 cm length, with one

hole of 5 cm to 16 cm diameter on one end to pass over a bajra or jowar head. After inserting the panicle inside the container a cork is fitted in the hole to close it.

A 35 cm long rubber tube or belt is stretched over the side of the container, and when in use this tube is tied around the peduncle of the head. To measure the temperature, in the upper side of the container a thermometer is placed. In the field water is carried in a thermos jug (Fig. 2).

Fig 2. Hot water equipment for emasculation

The panicle is inserted in the container prior to blooming for a particular duration of time. It has been observed that pollen grains of rice are killed by immersing the inflorescence for 5 to 10 minutes in the hot water maintained at 40-44°C in a thermos flask.

(c) Cold Water Treatment:

Like hot water cold water also kills pollen grains without damaging the gynoecium. In rice 0-6°C temperature is maintained to kill the pollen grains. This method is less effective than hot water treatment.

(d) Alcohol Treatment Method:

This method is not commonly used for emasculation because duration of treatment is an important factor since a very short duration is required failing which even the gynoecium may be damaged. Flowers or inflorescences are immersed in alcohol of a suitable concentration for a brief period. In alfa-alfa, a treatment of even 10 seconds with 57 % alcohol is sufficient to kill the pollen grains.

(e) Suction Method:

It is a mechanical method and is suitable for the crops having minute flowers. In this method the amount of pressure is applied in such a way that only anthers are sucked out and other parts of the flower like gynoecium remain intact. However, in this method 10-15% self pollination takes place. It is one of the major drawbacks of this method.

(f) Male Sterility or Self-incompatibility Method:

Emasculation option can be eliminated by the use of male-sterile plants, In some self-pollinated plants for example, Sorghum, Onion, Barley etc. anthers are sterile and do not produce any viable pollens! Similarly self-incompatibility may also be used to avoid emasculation.

(g) Chemical Gametocides:

Certain chemicals are capable of causing male sterility, when sprayed before flowering e.g., 2, 4-D, naphthalene acetic acid (NAA), maleic-hydrazide (MA), tribenzoic acid etc. FW450 in cotton may be used for bringing about emasculation.

(iv) Bagging:

It is the fourth step and completed with emasculation. The emasculated flower or inflorescence is immediately bagged to avoid pollination by any foreign pollen. The bags may be made of paper, butter paper, glassine or fine cloth. Butter paper or vegetable parchment bags are most commonly used.

The bags are tied to the base of the inflorescence or to the stalk of the flower with the help of thread, wire or pins. The bagging is done with the emasculation in bisexual plants and before the stigma receptivity and dehiscence of the anthers in unisexual plants. Both male and female flowers are bagged separately to prevent contamination in male flowers and cross-pollination in female flowers (Fig. 3).

Fig. 3: Different methods of bagging

(v) Tagging:

The emasculated flowers are tagged just after bagging. Generally circular tags of about 3 cm or rectangular tags of about 3 x 2 cm are used. The tags are attached to the base of flower or inflorescence with the help of thread.

The information on tag must be as brief as possible but complete bearing the following information:

(i) Number referring to the field record

(ii) Date of emasculation

(iii) Date of crossing

(iv) Name of the female parent is written first followed by a cross sign (x) and then the male parent, e.g., C x D denotes that C is the female parent and D is the male parent.

(vi) Crossing:

It is the sixth step. It can be defined as the artificial cross-pollination between the genetically unlike plants. In this method mature, fertile and viable pollens from the male parent are placed on the receptive stigma of emasculated flowers to bring about fertilization.

Pollen grains are collected in petridishs (e.g., Wheat, cotton etc.) or in paper bags {e.g., maize) and applied to the receptive stigmas with the help of a camel hair brush, piece of

paper, tooth pick or forceps. In some crops (e.g., Jowar, Bajra) the inflorescences of both the parents are enclosed in the same bag.

(vii) Harvesting and Storing the F_1 Seeds:

Crossed heads or pods of desirable plants are harvested and after complete drying they are threshed. Seeds are stored properly with original tags.

(viii) Raising the F_1 generation:

In the coming season, the stored seeds are sown separately to raise the F_1 generation. The plants of F_1 generation are progenies of cross seeds and therefore are hybrids.

HANDELING OF SEGREGATING POPULATIONS

Introduction

The F_2 and the onward generations obtained through continued selfing of F_1/hybrid between two or more parents is called segregating generations and occurrence of individuals in F_2 and the onward generations exceed either of two parents regarding one or more characters is called transgressive segregation. The breeding method that well exploits transgressive segregation is well known as transgressive breeding.

Breeding methods viz. pedigree, bulk and back cross method are well utilized for handling of segregating generations and objectives of all these methods are to derive pure line varieties which are given below in detail:-

1. Pedigree method or breeding
2. Bulk method or breeding
3. Single seed descent method
4. Back cross method.
5. Multiple cross method

1. Pedigree Method:

Record of the ancestry of an individual selected plant for various generations is known as pedigree. A selection method, which is used in segregating population of self-pollinated species and keeps proper record of plants and progeny selected in each generation is known as pedigree breeding. This method is widely used for the development of varieties in self-pollinated crops.

In this method individual plants are selected till the progenies become homozygous. Selection for plants in the desired combination of characters is started in the F_2 generation and continued in succeeding generations until genetic purity is reached.

The method is as follows (Fig. 9):

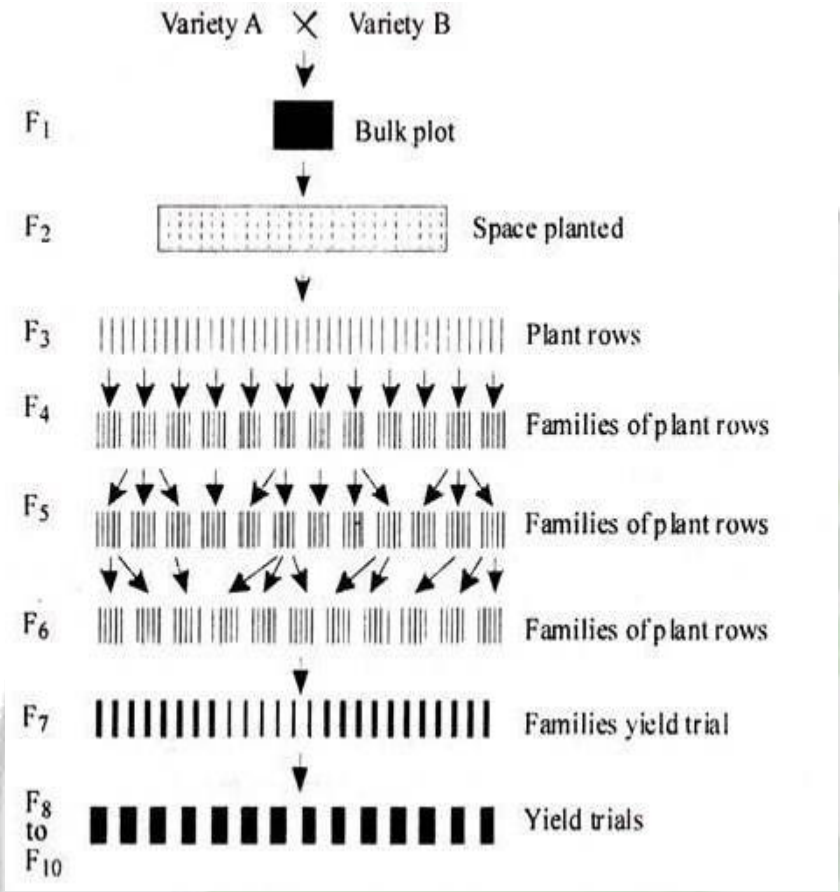


Fig 1. Different steps involved in pedigree methods

I Year:

Plants are chosen for hybridization and F_1 seeds are produced.

II Year (F_1 generation):

F_1 plants are space planted to produce maximum number of F_2 seeds (see Fig.1).

III Year (F_2 generation):

2000-10000 F_2 plants are space planted. About 200-500 desirable superior plants are selected.

IV Year (F_3 generation):

Selected superior plants in III year are space planted to study the individual plant. 3 to 5 best plants in these rows are selected and harvested (F_4)

V Year and VI Year (F_4, F_5 generation):

Process is continued as in F_3 generation. Normally 20-50 families may be retained at the end of F_5 generation.

VII Year (F_6 generation):

Due to successive self-pollination most of the lines become homozygous and uniform. The plants uniform in desired characters are harvested and the seed, bulked together to constitute the variety.

VIII Year (F_7 generation):

Preliminary yield trials are conducted.

IX to XI year (F_8 – F_{10} generation):

Trials of superior lines are confirmed. During the testing period observations are made on height, tendency to lodge, maturity, disease resistance and quality.

XII to XIII Year (F_{10}, F_{11} generation):

Seeds are multiplied and distributed to the farmers.

Merits:

(i) It is the quickest method.

(ii) Plant breeders can also obtain the genetic information.

(iii) There are chances of recovering transgenic segregation by this method.

Demerits:

Maintenance of accurate pedigree record is not easy. It takes much time.

Selected material becomes so large that handling of the same becomes very difficult.

Success of this method depends upon the skill of the breeder.

Mass pedigree method:

It is a modified form of pedigree method in which segregating material is handled by bulk (mass) method when conditions are unfavorable for selection and by pedigree method when conditions are favourable for selection.

2. Bulk Method or Breeding:

A selection procedure which is used in segregating population of self-.pollinated species in which material is grown in bulk plot from $F_2$to F_5 with or without selection, next generation is grown from bulk seed and individual plant selection is practiced in F_6 or later generations is called bulk method or breeding.

This method is also known as the mass or population method. Nilsson-Eule of Sweden was first to use the bulk method and it is in use ever since. This method differs from the pedigree method in that no selection is practiced in F_2-F_5 generations (Fig. 2).

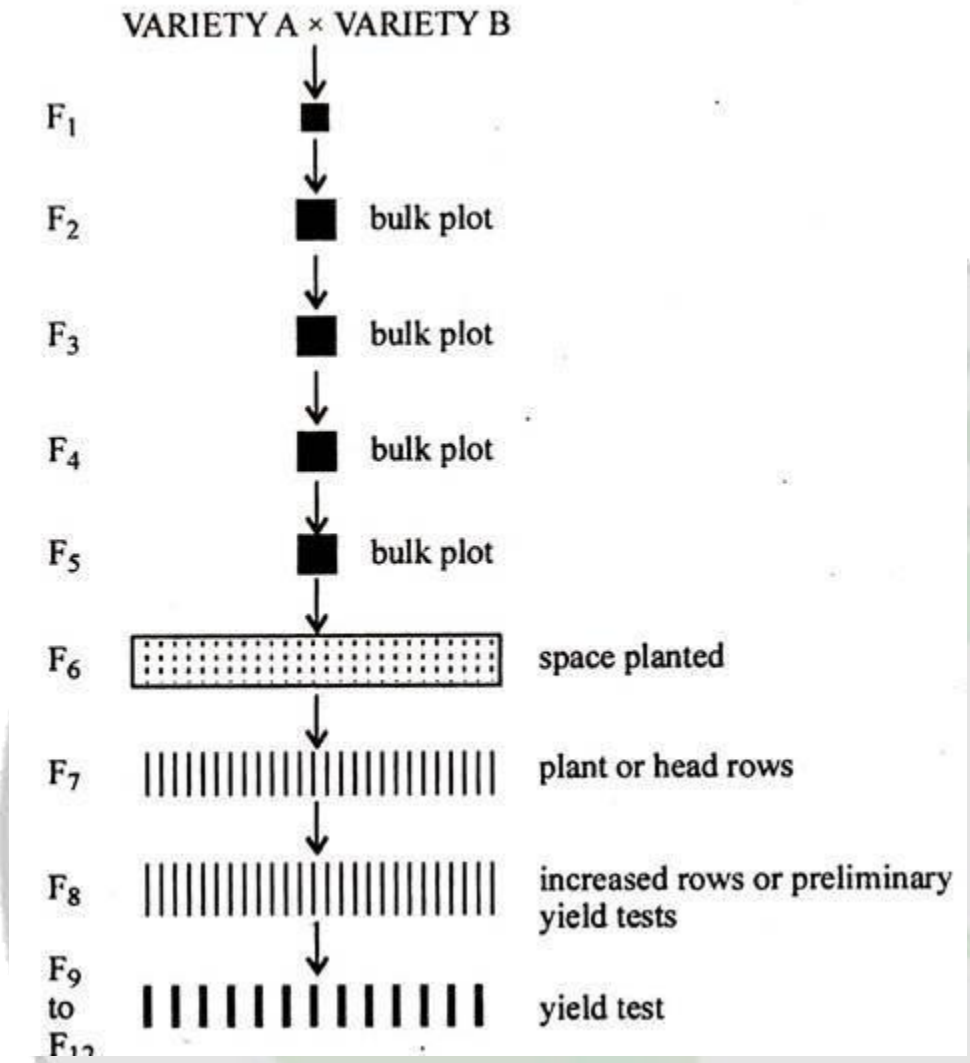


Fig 2. Procedure of bulk breeding method

The method is as follows:

I Year:

Plants are chosen for hybridization and F_1 seeds are produced.

II Year (F_1 generation):

50-100 F_1 plants are grown and their F_2 seeds are harvested in bulk,

III Year (F_2 generation):

F_2 plants are grown and their F_2 seeds are harvested in bulk.

IV Year (F_3 generation):

F_3 plants are grown and their F_4 seeds are harvested in bulk.

V Year (F_4 generation):

F_A plants are grown and their F_5 seeds are harvested in bulk.

VI Year (F_5 generation):

F_5 plants are grown and their F_6 seeds are harvested in bulk. (The process may be repeated until the desired period of homozygosity is achieved. In general bulk period is allowed up to F_5 generation)

VII Year (F_6 generation):

Seeds are space planted and single plant selection is done (F_7generation).

VIII Year (F_7 generation):

The progeny of each single plant is grown separately and superior progeny are selected and isolated (F_8).

IX Year (F_8 generation):

Preliminary yield test are conducted (F_9).

X-XII Year (F_9-F_{12} generations):

Multi-locations field trials are carried out, best performing strain is multiplied for seed distribution.

Merits:

(i) The bulk method is simple, convenient, inexpensive and less labour consuming (no pedigree record is to be kept).

(ii) During early segregating generations, very little work and attention is needed, which gives the breeder more time to concentrate on other breeding projects.

(iii) Selection is done by nature only and it increases the frequency of superior types in the population.

(iv) This method is suitable for studies on the survival of genes and genotypes in populations.

Demerits:

(i) This method takes much longer time to develop a new variety.

(ii) The breeder is enable to exercise his skill and judgement in selection and therefore the method is less satisfying to him.

(iii) Information on the inheritance of characters cannot be obtained.

(iv) This method is totally dependent on natural selection to select the superior types. These types may not be necessarily the best yielding types.

3. Single Seed Descent Method:

This method was suggested by Coulden (1939) for advancing segregating generation of self-pollinated crops. A breeding procedure used with segregating populations of self-pollinated species in which plants are advanced by single seeds from one generation to the next is referred to as single seed descent method.

The procedure is as follows (Fig. 3):

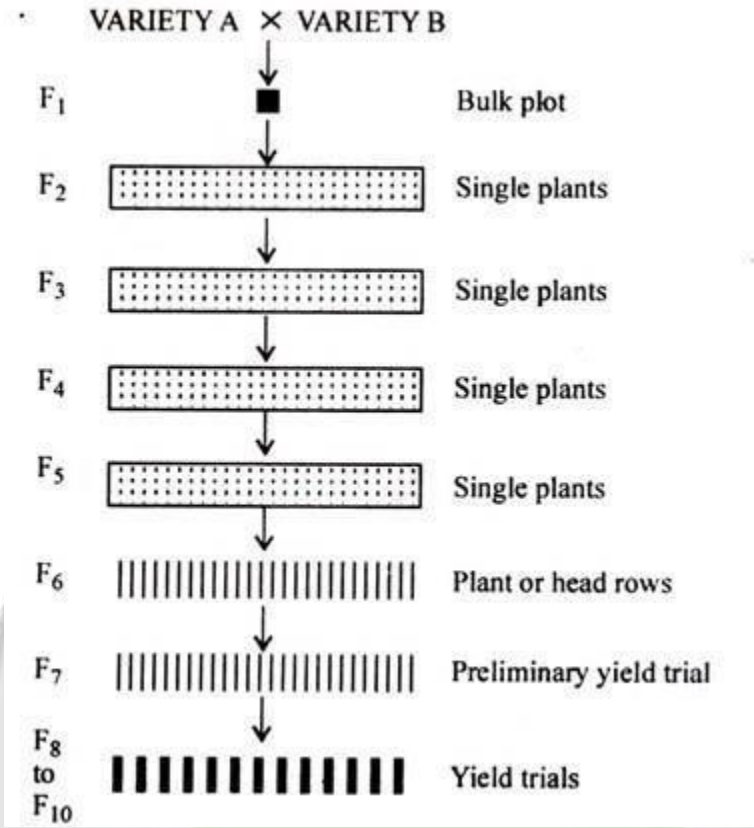


Fig. 3: SSD methods in crop plants

4. Back Cross Method:

This method was first proposed by Harlan and Pope (1922). Now-a-days this method is employed in improvement of both self and cross-pollinated crops where varieties are deficient in one or two aspects. This method is used particularly for transferring a single simply inherited character like disease, frost or drought resistance and earliness from an undesirable variety to a good commercial variety.

The desirable variety is called as recurrent or recipient parent and it is crossed to an undesirable variety, called as donor or non-recurring parent (called donor because the desirable genes are transferred).

F_1 plants instead of permitting to self-pollination as in pedigree or bulk method care crossed with the recurring parent and therefore, it is called as back cross method (A back cross may be defined as a crossing of F_1 hybrid with any of its parents).

The procedure is as follows:

Suppose there are two varieties A and B. A is very good in all characters but disease susceptible and the variety B is disease resistant but very poor in all characters. It is needed to transfer the disease resistance from B to A without adversely affecting the good qualities of A. B is then donor or non-recurring parent and A is recipient or recurring parent.

Selected plants of A and B are crossed to raise the F_1 Here A is treated as the female and B as male. F_1 plant is backcrossed with the recurrent parent; variety A and BC_1 plants (back

cross first generation) plants are raised. Here B is treated as female and A as male. Select the BC_1plants possessing desired characters of A with disease resistance of B.

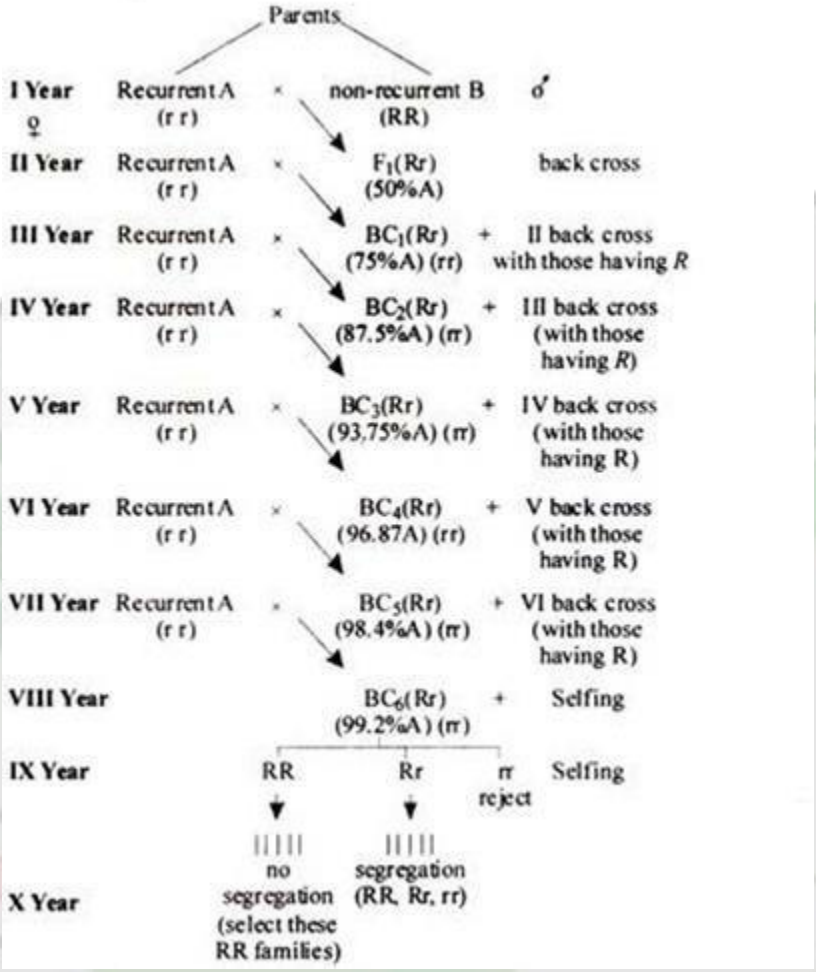


Fig 4. Transfer of dominant gene through backcross method

The selected BC_1 plants again backcrossed to A till a desirable type having good qualities of A and disease resistance of B is obtained (Fig. 4). Disease resistant plants are selfed and individual plant progenies from the selfed seeds of selected plants are grown.

New variety is tested in replicated field trials along with the variety A as check. Seeds are multiplied and released for cultivation. The method is used for the transfer of a dominant gene. In case of recessive gene transfer, a slightly different procedure is followed. It is summarized in Figs. 4 and 5.

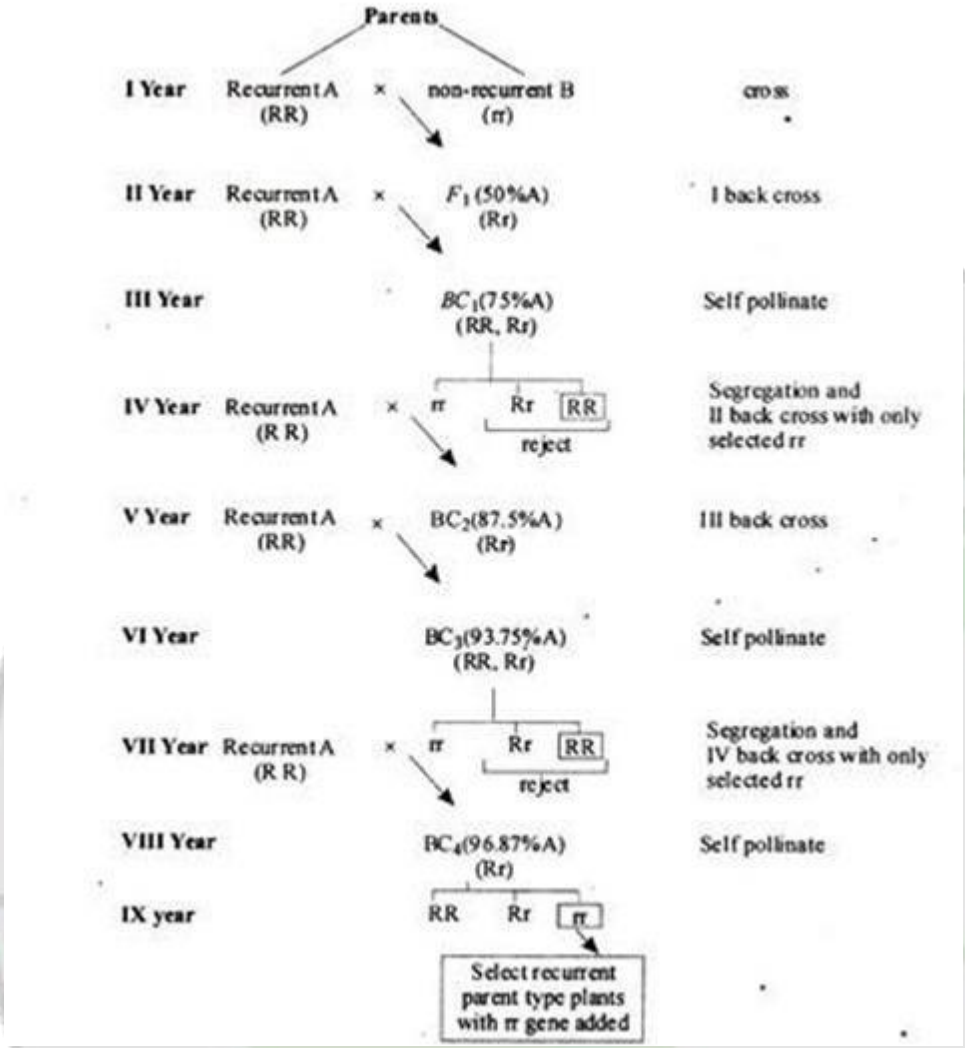


Fig. 5 Transfer of recessive gene through backcross method

Merits:

1. This is the most common method used for transfer for oligogenic character like disease resistance.
2. It is the only method used for the interspecific transfer of characters, transfer of qualitative characters, quantitative characters, transfer of cytoplasm, particularly for male sterility and for the production of isogenics lines.
3. Role of environment is almost negligible. So time required for developing new variety may be drastically reduced because off-season nurseries and greenhouses can be used for developing 2-3 generations each year.
4. Extensive yield tests are not required because the performance of the recurrent parent is already known.
5. Breeder does not require to handle large populations.
6. In this method all desirable characters of a popular variety (genotype known) are retained except the undesirable genes at a particular locus is replaced. So, the outcome of this method is known forehand, and plants can be reproduced any time in future.

Demerits:

(i) The new variety is superior over the already available variety only in the transferred character.

(ii) Hybridization has to be done for each backcross. It requires lot of time and money.

(iii) Sometimes undesirable genes are closely linked with desirable one and get transferred to the new variety.

5. Multiple Cross Method:

A cross-involving more than one inbred line is referred to as multiple cross. It is also known composite cross and is used to combine monogenetic characters from different sources into a single genotype. In this method, several pure lines are crossed together. The selected pure lines are first combined into crosses as A × B, C × D, E × F, G × H and so on. The F_1 plants are mated together as (A × B) × (C × D) and (E × F) x (G × H). Finally, the F_1 plants of double crosses are crossed with each other to produce hybrids [(A × B) x (C x D)] x [(E x F) x (G x H)]. Further breeding in these hybrids is carried out according to either pedigree or bulk method.

Merits:

1. In self-pollinated crops this method is used when three or four monogenic characters scattered in three or four different varieties are to be combined into one.
2. These crosses generally have wider adaptation.

Demerits:

1. These crosses are generally less productive.
2. This method has limited utility except in high risk areas where severe disease damage occurs regularly from a highly specialized disease pathogen.

CHAPTER-08

MULTILINE CONCEPT

Introduction

N. F. Jensen is credited with first using this breeding method in oat breeding in 1952 to achieve a more last- ing form of disease resistance. Multilines are generally more expensive to produce than developing a synthetic cultivar, because each component line must be devel- oped by a separate backcross.

Key features

The key feature of a multiline cultivar is disease protec- tion. Technically, a **multiline** or **blend** is a planned seed mixture of cultivars or lines (multiple pure lines) such that each component constitutes at least 5% of the whole mixture. The pure lines are phenotypically uniform for morphological and other traits of agronomic import- ance (e.g., height, maturity, photoperiod), in addition to genetic resistance for a specific disease. The compon- ent lines are grown separately, followed by compost- ing in a predetermined ratio. Even though the term multiline is often used interchangeably with blend, sometimes the former is limited to mixtures involving **isolines** or near **isogenic lines** (lines that are genetically identical except for the alleles at one locus). The pur- pose of mixing different genotypes is to increase hetero- geneity in the cultivars of self-pollinated species. This strategy would decrease the risk of total crop loss from the infection of one race of the pathogen, or some other biotic or abiotic factor. The component genotypes are designed to respond to different versions or degrees of an environmental stress factor (e.g., different races of a pathogen).

Applications

One of the earliest applications of multilines was for breeding "variable cultivars" to reduce the risk of loss to pests that have multiple races, and whose incidence is erratic from season to season. Planting a heterogeneous mixture can physically impede the spread of disease in the field as resistant and susceptible genotypes intermingle.

Mixtures may be composited to provide stable perform- ance in the face of variable environments. Mixtures and blends are common in the turfgrass industry. Prescribing plants for conditions that are not clear-cut is challenging. Using mixtures or blends will increase the chance that at least one of the component genotypes will match the environment.

In backcross breeding, the deficiency in a high-yielding and most desirable cultivar is remedied by gene sub-stitution from a donor. Similarly, the deficiency of an adapted and desirable cultivar may be overcome by mixing it with another cultivar that may not be as pro- ductive but has the trait that is missing in the desirable cultivar. Even though this strategy will result in lower yield per unit area in favorable conditions, the yield will be higher than it would be under adverse conditions if only a pure adapted cultivar was planted.

Multilines composited for disease resistance are most effective against airborne pathogens with physiological races that are explosive in reproduction. An advantage of blends and mixtures that is not directly related to plant breeding, is marketing. Provided a label "variety not stated" is attached to the seed bag, blends of two or more cultivars can be sold under various brand names, even if they have identical composition.

Procedure

The backcross is the breeding method for developing multilines. The agronomically superior line is the recur- rent parent, while the source of disease resistance con- stitutes the donor parent. To develop multilines by isolines, the first step is to derive a series of backcross-derived isolines or near-isogenic lines (since true isolines are illusive because of linkage between genes of interest and other genes influencing other traits). The results of the procedure are two cultivars that contrast only in a specific feature. For disease resistance, each isoline should contribute resistance to a different physi- ological race (or group of races) of the disease.

The component lines of multilines are screened for disease resistance at multilocations. The breeder then selects resistant lines that are phenotypically uniform for selected traits of importance to the crop cultivar. The selected components are also evaluated for performance (yield ability), quality, and competing ability. Mixtures are composited annually based on disease patterns. It is suggested that at least 60% of the mixture comprises iso- lines resistant to the prevalent disease races at the time. The proportion of the component lines are determined by taking into account the seed analysis (germination percentage, viability).

CHAPTER-09

CONCEPT OF POPULATION GENETICS AND HARDY WEINBERG LAW

HARDY WEINBERG LAW – FACTORS AFFECTING EQUILIBRIUM FREQUENCIES IN RANDOM MATING POPULATIONS

Cross-pollinated crops are highly heterozygous due to the free intermating among their plants. They are often referred to as random mating populations because each individual of the population has equal opportunity of mating with any other individual of that population. Such a population is also known as Mendelian population or panmictic population. A Mendelian population may be though of having a gene pool consisting of all the gametes produced by the population. Thus gene pool may be defined as the sum total of all the genes present in a population. A population, in this case, consists of all such individuals that share the same gene pool, i.e., have an opportunity to intermate with each other and contribute to the next generation of the population. Each generation of a Mendelian population may be considered to arise from a random sample of gametes from the gene pool of previous generation. For this reason, it is not possible to follow the inheritance of a gene in a Mendelian population by using the techniques of classical genetics. To understand the genetic make-up of such populations a sophisticated field of study, population genetics, has been developed. We shall examine the elementary principles of population genetics in order to understand the genetic composition of random mating populations, i.e., cross pollinated crops.

Proof of Hardy-Weinberg law The Hardy-Weinberg law is the fundamental law of population genetics and provides the basis for studying Mendelian populations. This law was independently developed by Hardy (1908) in England and Weinberg (1909) in Germany. The Hardy-Weinberg law states that the gene and genotype frequencies in a Mendelian population remain constant generation after generation if there is no selection, mutation, migration or random drift. The frequencies of the three genotypes for a locus with two alleles, say A and a, would be *p2* A, 2pq Aa, and q2 aa ; where p represents the frequency of A and q represents the frequency of a allele in the population, and the sum of p and q is one, i.e., p+q=1. Such a population would be at equilibrium since the genotypic frequencies would be stable, that is, would not change, from one generation to the next. This equilibrium is known as Hardy-Weinberg equilibrium. A population is said to be at

equilibrium when frequencies of the three genotypes, AA, Aa and aa are p2, 2pq and q2, respectively. Whether a population is at equilibrium or not can be easily determined using a chi-square test. Hardy-Weinberg law can be easily explained with the help of an example. Let us consider a single gene with two alleles, A and a, in a random mating population. There would be three genotypes, AA, Aa and aa, for this gene in the population. Suppose the population has N individuals of which D individuals are AA, H individuals are Aa and R individuals are aa so that D +H + R = N. The total number of alleles at this locus in the population would be 2N since each individual has two alleles at a single locus. The total number of A alleles would be 2D+H because AA individuals have two A alleles each, while each Aa individual has only one A allele. The ration (2D+H)/2N is, therefore, the frequency of A allele in the population, and is represented by p. Similarly, the ratio (2R + H) / 2N is the frequency of allele a, and is written as q. Therefore, p = (2D + H) / 2N or = (D + ½ H) / N and q = (2R + H) / 2N or = (R + ½ H) / N Therefore, p + q = 1 and p = 1 – q, or q = 1 – p The value of p and q are known as gene frequencies. Gene frequency is the proportion of an allele, A or a, in a random mating population. In other words, the proportion of gametes carrying an allele, A or a, is known as gene frequency. The genotype frequency or zygotic frequency is the proportion of a genotype, AA, Aa or aa, in the population. Random mating or random union of the two types of gametes would produce the following genotypes in a ratio proportionate to the frequencies of the gametes that united to produce them.

Factors affecting equilibrium frequencies The equilibrium in random mating populations is disturbed by (1) migrations, (2) mutation, (3) selection and (4) random drift. These factors are also referred to as evolutionary forces since they bring about changes in gene frequencies, which is essential for evolution to proceed. Obviously, a population in which gene and genotype frequencies remain constant over generations cannot evolve any further, unless its gene and genotype frequencies are disturbed.

Migration Migration is the movement of individuals into a population from a different population. Migration may introduce new alleles into the population or may change the frequencies of existing alleles. The amount of change in gene frequency q will primarily depend upon two factors ; first, the ratio of migrant individuals to those of the original population and second, the magnitude of difference between the values of q in the population and in the migrants. In plant breeding programmes, migration is represented by

inter varietal crosses, polycrosses, etc., wherein the breeder brings together into a single population two or more separate populations.

Mutation Mutation is a sudden and heritable change in an organism and is generally due to a structural change in a gene. It is the ultimate source of all the variation present in biological materials. Mutation may produce a new allele not present in the population or may change the frequencies of existing alleles. However, since the mutation rate is generally very low, i.e., approximately 10-6, the effects of mutation on gene frequency would be detectable only after a large number of generations. Therefore, in breeding populations such effects may be ignored. A desirable mutation may prove very useful when it is discovered. But a routine use of mutations in crop improvement would not be feasible until techniques for directed mutagenesis have been perfected. Directed mutagenesis implies that the experimenter should be able to induce a high frequency of the desired mutations through certain techniques. At present, directed mutagenesis is an ideal, which is yet to be achieved even partially.

Random drift

Random drift or genetic drift is a random change in gene frequency due to sampling error, Random drift occurs in small populations because sampling error is greater in a smaller population than in a larger one. Ultimately, the frequency of one of the alleles becomes zero and that of the other allele becomes one. The allele with the frequency of one is said to be fixed in the population because there would be not further change in its frequency. It may be expected that in a small population all the genes would become homozygous, or would be fixed in due course of time. Breeding populations are generally small, hence a certain amount of genetic drift is bound to occur in them. The breeder cannot do anything to prevent this genetic drift, except to use very large populations, which is often not practicable. Alternatively, he may resort to phenotypic disassortative mating, which would again require time, labour and money.

Inbreeding Mating between individuals sharing a common parent in their ancestry is known as inbreeding. In small populations, a certain amount of inbreeding is bound to occur. Inbreeding reduces the proportion of heterozygotes or heterozygosity and increases the frequency of homozygotes or Homozygosity. The rate of decrease in heterozygosity is equal to ½ N (N=number of plant in the population) per generation in monoecious or

hermaphrodite species. In dioecious species and in monoeicious species where selfpollination is prevented, the decrease in heterozygosity is somewhat lower ; it is equal to ½(N=1) per generation. Thus in small populations, even with strict random mating or even with strict cross-pollination the frequency of homozygotes increases, while that of heterozygotes decreases due to inbreeding.

Selection Differential reproduction rates of various genetopes is known as selection. In crop improvement, selection is very important because it allows the selected genotypes to reproduce, while the undesirable genotypes are eliminated. Thus the breeder is able to improve the various characteristics by selecting for the desirable types. In a random mating population, if plants with AA or aa genotypes are selected, the frequency of A allele in the selected population would be 1 or 0, respectively. It is assumed in this case that AA and aa genotypes would be identified without error. In the next generation, therefore, only A or a allele would be present, i.e., the alleles would be fixed. Here selection against the remaining genotypes is complete, that is, these genotypes are not allowed to reproduce. In such cases, the disadvantage in reproduction, i.e, selection differential (=s) is 1 and the fitness is zero for the remaining genotypes. The fitness of a genotype may be defined as its reproduction rate in relation to that of other genotypes. Generally, s has values less than one. Further, often it is not possible to identify the genotypes with certainty. The identification of genotypes is made difficult by dominance and due to less than 100 per cent heritability. This is particularly true for quantitative characters. As a result, selection is expected to change gene frequencies rather than to eliminate one or the other allele **Numericals** There are two equations necessary to solve a Hardy-Weinberg Equilibrium in question:

1) $p + q = 1$ and 2) $p2 + 2pq + q2 = 1$

   p is the frequency of the dominant allele. q is the frequency of the recessive allele. p 2 is the frequency of individuals with the homozygous dominant genotype. 2pq is the frequency of individuals with the heterozygous genotype. q 2 is the frequency of individuals with the homozygous recessive genotype.

CHAPTER-10

POPULATION IMPROVEMENT SCHEMES

Introduction: Cross pollinated crops are highly heterozygous and heterogeneous. Consequently, they show varying degrees of inbreeding depression. Therefore, inbreeding should be avoided or kept to a minimum in cross pollinated crops. Individual plants are heterozygous and their progeny would be heterogeneous and usually different from the parent, due to segregation and recombination. Therefore, desirable genes can be seldom fixed through selection in cross pollinated crops except for highly heritable qualitative characters. Hence, the breeder aims of increasing the frequency of desirable alleles in the population. In cross pollinated crops, the genotype of the individual plants is generally of little importance, especially in population improvement programmes but, the frequency of desirable alleles or genes in the population as a whole is more important.

The population improvement methods may be grouped into two general classes.

1. **Selection without progeny testing** : Plants are selected on the basis of their phenotype, and no progeny test is carried out. Eg : Mass selection.
2. **Selection with progeny testing** : The Plants are initially selected on the basis of their phenotype, but the final selection of the plants that contribute to the next generation is based on progeny testing. The methods are eg :
    a. Progeny selection or ear to row method
    b. Line breeding
    c. Recurrent selection

Progeny selection

This method was developed by Hopkins in 908 and used extensively in maize. In its simplest form the ear-to-row method of selection is as follows

1. Number of plants (50-100) are selected on the basis of phenotypic superiority. They

are allowed to open pollinate and the seed is harvested separately.

2. Progeny rows are grown (each10- 50 plants) from the selected plants. They are evaluated for desirable characters and superior progenies are identified.
3. From the superior progenies several superior plants are selected based on

phenotypic characters. Plants are allowed to open pollinate. Plants are harvested separately.

4. Small progeny rows are grown and the process of selection and raising progeny rows is repeated till superior population is obtained. May be for 2 or 3 selection cycles. At the end superior plants from superior families are selected and composited to produce a new variety.

Several modifications of ear to row method are available and many more may be developed to suit the needs of the breeder.

Merits of progeny selection

1. Selection is based on progeny test and not phenotype as in mass selection. 3-8%

improvement is possible per each selection cycle.

2. Inbreeding is avoided by selection of large number of plants.
3. Method is relatively simple and easy.

Demerits of progeny selection

1. No. control on pollination and thus selection is based on maternal parent only.
2. Each selection cycle takes 2 years in many cases.

Line breeding

It is a variation of progeny selection in which one or several cycles of selection is carried out on the basis of progeny tests. At the end of the selection process i.e. in the last selection cycle, the superior lines or progenies are composited to produce a new variety. Hence, it is known as line breeding in contrast to progeny selection where superior plants from superior progenies are composited at the end. The merits and demerits are almost same in the case of progeny selection.

Achievements

Mass and progeny selections have been extensively used for the improvement of cross – pollinated crops. The early varieties of bajra were developed through mass selection; some of the examples are; Bajapur i, Jamnagar Giant, AF 3, S 530 and Pusa Moti; all these varieties were isolated from African introductions. Mass selection improved the yielding ability of toria by 30% and oil content by 56%; a further increase of 16% in yield was obtained by using mass-pedigree method.

RECURRENT SELECTION

History: Breeding schemes similar to recurrent selection were first suggested in 1919 by Hayes and Garber and independently by East and Jones in 1920. First detailed description of this type of breeding method was published by Jenkins in 1940 as a result of his experiments with early testing for GCA in maize. The method acquired its name in 1945 when Hull suggested detailed scheme of recurrent selection for SCA, Hull (1952) defined recurrent selection as "Method which involves reselection generation after generation with interbreeding of selects to provide for genetic recombination".

The advantages of recurrent selections are

1. The rate of inbreeding can be kept at low level
2. The frequency of favourable genes in the population will be increased and so
3. The chance of obtaining satisfactory individuals from the population will be increased because greater opportunity for recombination is present.

The are four types of recurrent selections.

1. Simple recurrent selection
2. Recurrent selection for GCA (RSGCA)
3. Recurrent selection for SCA (RSSCA)
4. Reciprocal recurrent selection (RRS)

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.	Original selection cycle
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	
III year:	Seed obtained in II year is planted Number of superior	First selection
	selection and harvested separately. Seed evaluated. Seeds of	cycle
IV year :	Progeny rows are raised. Inter crossed in all possible ways. Equal amount of seed from each cross is composited. This mixed	

The procedure ma y be repeated for another one or two selection cycles. The effectiveness of the simple recurrent selection was published with data by Sprague and Brimhall (1950).

This is useful for characters that can be measured on individual plants and having high heritability. The procedure is to be modified suitably for characters which can not be messured on individual plants.

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.

Year : Repeat as in second year

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 simultanniously.

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 crossin g 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 ra ise the source population of 'A'

next year. Similarly equal amount of seed from inter crosses of plot B is mixed to raise sourc
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.

Conclusion on the efficiency of different Recurrent selection Schemes :

1. If dominance is incomplete

   Recurrent selection for GCA and reciprocal recurrent selection are equal but both are superior to recurrent selection for SCA.
2. If dominance is complete the three methods are equal
3. If overdominance is present

   Reciprocal recurrent selection and recurrent selection for SCA are equally effective both are superior to recurrent selection for GCA.

CHAPTER-11

HETEROSIS AND INBREEDING DEPRESSION, DEVELOPMENT OF INBRED LINES AND HYBRID, COMPOSITES AND SYNTHETIC VARIETIES

HETEROSIS :

The term heterosis was first used by Shull in 1914. **Heterosis** may be defined as the superiority of an F, hybrid over both its parents in terms of yield or some other character. Generally, heterosis is manifested as an increase in vigour, size, growth rate, yield or some other characteristic. But in some cases, the hybrid may be inferior to the weaker parent. This is also regarded as heterosis; Often the superiority of F, is estimated over the average of the two parents, or the mid - parent. If the hybrid is superior to the mid - parent, it is regarded as heterosis (average heterosis or relative heterosis). However, in practical plant breeding, the superiority of F, over mid-parent is of no use since it does not offer the hybrid any advantage over the better parent. Therefore, average heterosis is of little or no use to the plant breeder. More generally, heterosis is estimated over the superior parent; such an estimate is sometimes referred to as **heterobeltiosis.** The term heterobeltiosis is not commonly used since most breeders regard this to be the only case of heterosis and refer to it as such i.e., heterosis. In 1944, Powers suggested that the term heterosis should be used only when the hyb rid is either superior or inferior to both the parents. Other situations should be regarded as partial or complete dominance. However, the commercial usefulness of a hybrid would primarily depend on its performance in comparison to the best commercial variety of the concerned crop species. In many cases, the superior parent of the hybrid may be inferior to the best commercial variety. In such cases, it will be desirable to estimate heterosis in relation to the best commercial variety of the crop; such an estimate is known as economic, standard or

useful heterosis. Economic heterosis is the only estimate of heterosis, which is of commercial or practical value.

Estimation of Heterosis:

Average heterosis: When the heterosis is estimated over the mid parent, i.e., mean value or average of the two parents, it is known as average heterosis/ mid parent heterosis.

Averge heterosis=[(F1-MP)/MP]x100

where, F1 is the mean value of F1 and MP is the mean value of the two parents involved in the cross.

Heterobeltiosis: When the heterosis is estimated over the better parent/ superior parent.

Heterobeltiosis=[(F1-BP)BP]x100

where, F1 is the mean value of F1 and BP is the mean value(over replication) of the better parent of the particular cross.

Standard/ Useful heterosis/ Economic heterosis: It refers to the superiority of F1 over the standard commercial check variety.

Standard /Useful heterosis=[(F1-CC)CC]x100

Where, CC is the mean value over replications of the local commercial cultivar.

Heterosis and Hybrid Vigour

Hybrid vigour has been used as a synonym of heterosis. It is generally agreed that hybrid vigour describes only the superiority of hybrids over their parents, while heterosis describes other situations as well. But a vast majority of the cases of heterosis are cases of superiority of hybrids over their parents. The few cases where F_1 hybrids are inferior to their parents may also be regarded as cases of hybrid vigour in the negative directions. For example, many F_1 hybrids in tomato are earlier than their parents. Earliness in many crops is agriculturally desirable. It may be argued that the earliness of F_1 hybrids exhibits a faster development in them so that their vegetative phase is replaced by the reproductive phase more quickly than in their parents. Therefore, the use of heterosis and hybrid vigous as synonym seems to be reasonably justified.

Luxuriance

Luxuriance is 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.

Historical

Hybrid vigour in artificial tobacco (Nicotiana spp.) hybrids was first reported by **Koelreuter** in 1673. Subsequently, many workers reported hybrid vigour in a large numbe r of plant species. These hybrids were produced from interspecific as well as intraspecific crosses. In 1876, **Darwin** concluded that hybrids from unrelated plant types were highly vigorous. Most of our present knowledge on heterosis comes from the work on maize. Maize is perhaps the most extensively studied crop species with respect to heterosis and inbreeding depression. **Beal** studied the performance of intervarietal hybrids between 1877 and 1882. He reported that some hybrids yielded as much as 40 per cent more than the parental varieties. From subsequent studies on intervarietal crosses in maize, it became clear that some of the hybrids showed heterosis, while others did not. Crosses between distinct types, i.e., genetically diverse varieties, exhibited greater heterosis than those involving closely related varieties.

The genetic hypotheses to account for heterosis were first advanced during 1908. The dominance hypothesis was proposed **Davenport** in 1908 (it was later elaborated by **Keeble and Pellew** in 1910) , while the overdominance hypothesis was put forth by **East and Shull** in the same year, i.e., 1908. In 1912, **East and Hays** advocated heterosis breeding as an alternative plant breeding strategy. The concept of double cross hybrids was proposed by Jones in 1917, while that of top cross hybrids was advanced by Davis in 1927.

Heterosis in Cross - and Self-Pollinated Species

In general, cross- pollinated species show heterosis, particularly when inbred lines are used as parents. In many cross- pollinated species, heterosis has been commercially

exploited, for example, in maize, bajra, jowar, cotton, sunflower, onion (A. cepa), alalfa, etc. Many crosses in self - pollinated species also show heterosis, but the magnitude of heterosis is generally smaller than that in the case of cross- pollinated species. But in some self - pollinated crops, heterosis is large enough to be used for the production of hybrid varieties. Hybrid varieties are commercially used in some vegetables, such as tomato, where a single fruit produces a large number of seeds, and in crops like rice. The chief drawback in the use of hybrid varieties in self-pollinated crops is the great difficulty encountered in the production of large quantities of hybrid seed.

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, head size in cabbage, cob size in maize, head size in jowar, etc.

4. **Better Quality.** In many cases, hybrids show improved quality. 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 Flowerin g 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. Many tomato hybrids are earlier than their parents.
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. Such hybrids are known in beans (P. vulgaris) and some other crops.

These are some of the characteristics for which heterosis is easily observed. Many other characters are also affected by heterosis, e.g. enzyme activities, cell division, vitamin content (vit. C content in tomato), other biochemical characteristics, etc., but they are not so readily

observable.

Fixation of heterosis: There are four principles ways for fixation of heterosis

(1) Asexual reproduction (2) Apomixis (3) Polyploidy (4) balanced lethal system

GENETIC BASES OF HETEROSIS AND INBREEDING DEPRESSION

Heterosis and inbreeding depression are closely related phenomena. In fact, they may be regarded as the opposite sides of the same coin. Therefore, genetic theories that explain heterosis also explain inbreeding depression. There are three main theories to explain heterosis and, consequently, in breedings depression: (1) dominance, (2) over dominance, and (3) epistatis hypotheses.

Dominance Hypothesis

The dominance hypothesis was first proposed by **Davenport** in 1908. It was later expanded by Bruce, and by Keeble and Pellew in 1910. In simplest terms, this hypothesis suggests that at each locus the dominant allele has a favourable effect, while the recessive allele has an unfavourable effect. In heterozygous state, the deleterious effects of recessive alleles are masked by their dominant alleles. **Thus heterosis results from the masking of harmful effects of recessive alleles by their dominant alleles. Inbreeding depression, on the other hand, is produced by the harmful effects of recessive alleles, which become homozygous due to inbreeding.** Therefore, according to the dominance hypotheses, heterosis is not the result of heterozygosity; it is the result of prevention of expression of harmful recessives by their dominant alleles. Similarly, inbreeding depression does not result from homozygosity per se. but from the homozygosity of recessive alleles, which have harmful effects. **Objections.** Two objections have been raised against the dominance hypothesis. The first objection relates to the failure in isolation of lines homozygous for all the dominant genes The second objection is directed at the symmetrical distributions obtained in F_2 populations.

1. Failure in the Isolation of Inbreds as Vigorous as Hybrids. According to the dominance hypothesis, it should be possible to isolate inbreds with all the dominant genes. Such inbreds would be as vigorous as the F_X hybrids. However, such inbreds have not been isolated in many studies. But in some studies, it has been possible to recombine

genes so that inbred lines as good as or superior to the heterotic hybrids were isolated.

2. **Symmetrical Distribution in F_2.** In F_2 , dominant and recessive characters segregate in the ratio of 3 : 1. According to the dominance hypothesis, quantitative characters, therefore, should not show a symmetrical distribution in F_2. This is because dominant and recessive phenotypes would segregate in the proportion $(3/4 + 1/4)^n$, where n is the number of genes segregating. However, F_2's nearly always show a symmetrical distribution.

Explanations for the Objections. In 1917, **Jones** suggested that since quantitative characters are governed by many genes, these genes are likely to show linkage. It may be expected that dominant and recessive genes governing a character 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 explanation is often known as **the dominance of linked genes hypothesis.**

Later in 1921, **Collins** showed 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. The distribution curve would further become symmetrical due to the effects of environment, that is, due to less than 100 per cent heritability.

Overdominance
Hypothesis

This hypothesis was independently proposed by **East and Shull** in 1908. This is sometimes known as single gene heterosis, superdominance, cumulative actio n of divergent alleles, and stimulation of divergent alleles. The idea of superdominance, i.e., heterozygote superiority, was initially put forth by Fisher in 1903; it was elaborated by East and Shull in 1908 to explain heterosis. According to overdominanc e hypothesis, heterozygotes at atleast some of the loci are superior to both the relevant homozygotes. Thus heterozygote Aa would be superior to both the homozygotes AA and aa. Consequently, heterozygosity is essential for and is the cause of heterosis, while homozygosity resulting from inbreeding produces inbreeding depression. It would, therefore, be impossible to isolate inbreds as vigorous as F_X hybrids if heterosis were the consequence of overdominance.

In 1936, East proposed that at each locus showing overdominance, there are several alleles, e.g., a_h a2, a^, a 4....., etc., with increasingly different functions. He further proposed that heterozygotes for more divergent alleles would be more heterotic than those involving less divergent ones. For example, a_Xa4 would be superior to a_Xa2 , a2a- x, or a^a^. It is assumed that the different alleles perform somewhat different functions. The hybrid is, therefore, able to perform the functions of both the alleles, which is not possible in the case of two homozygotes.

Evidence for Overdominance. There are not many clear- cut cases where the heterozygote is superior to the two homozygotes; in fact, overdominance has not been demonstrated unequivocally for any polygomic trait (see, Banga and Banga, 1998). This has be en the biggest objection to the general acceptance of overdominance hypothesis. But there is no doubt that in the case of some oligogenes, heterozygotes are superior to the homozygotes. In case of maize, gene ma affects maturity. The heterozygote Ma ma is more vigorous and later in anthesis and maturity than the homozygotes Ma Ma and ma ma. Gustafsson has reported two chlorophyll mutants in barley that produce larger and more number of seeds in the heterozygous state than

do their normal homozygotes. Simila rly, heterozygotes for the hooded gene in barley show a higher rate of photosynthesis than the two homozygotes.

In human beings *(Homo sapiens)*, sickle cell anaemia is produced by a recessive gene *s* which is lethal in the homozygous state. In Africa, the heterozygotes Ss are at a sel active advantage over the normal SS individuals because they are more resistant to malaria. Ai other case of heterozygote advantage is reported in Neurospora crassa (bread mold). Gene pab is concerned with the synthesis of/7-aminobenzoic acid. The heterozygote pab^{+} pab is more vigorous and shows a faster growth rate than the two homozygotes pab pab and pab^{+} pab^{+}.

But the number of such genes where heterozygote superiority has been established beyond doubt is limited. There is a large number of cases, however, where heterozygotes for chromosome segments, e.g., inversions, etc., or complex loci are known to be superior to the homozygotes, However, the superiority of heterozygotes need not be a result of overdominance. It could more easily be due to linkage in the repulsion phase or epistatic effects, i.e., an interaction between two or more nonalleles.

Comparison between Dominance and Overdominance Hypotheses

The two hypotheses lead to similar expectations, but they do differ from each other with respect to some expectations. The similarities and differences between them are listed below (Table 13.2).

Similarities. The two hypotheses have the following similarities.

1. Inbreeding would produce inbreeding depression.
2. Outcrossing would restore vigour and fertility.
3. 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. **Differences.** The chief differences between the two hypotheses are

1. Heterozygotes are superior to the two homozygotes according to the overdominance hypothesis, while according to the dominance hypothesis they are as good as the dominant homozygote.
2. Inbreds as vigorous as the F, hybrid can be isolated according to the dominance

hypothesis, but it will be impossible according to the overdominance hypothesis.

3. According to dominance hypothesis, inbreeding depression is due to homozygosity of harmful recessive alleles, while as per overdominance hypothesis, it is due to homozygosity itself.

4. According to the overdominance hypothesis, heterosis is the consequence of heterozygosity per se. But as per dominance hypothesis it is the result of dominant alleles masking the deleter ious effects of their recessive alleles, and heterozygosity itself is not the cause of heterosis.

TABLE 13.2

A comparison between dominance and overdominance hypotheses of heterosis

Feature	*Hypothesis of heterosis*	
	Dominance	*Overdominance*
Similarities		
Inbreeding leads to	Reduced vigour and fertility	Reduced vigour and fertility
Out-crossing leads to	Heterosis	Heterosis
Degree of heterosis increases with	Genetic diversity between parents	Genetic diversity between parents
Differences		
Inbreeding depression is the results of	Homozygosity for deleterious recessive alleles	Homozygosity itself
Heterosis is the result of	Masking of the harmful effects of recessive alleles by their dominant alleles.	Heterozogosity itself
The phenotype of heterozygote is	Comparable to that of the dominant homozygote	Superior to both the homozygotes
Inbreds as vigorous as the F_1 hybrid	Can be isolated	Can not be isolated

Epistasis Hypothesis

In 1952, **Gowen** had suggested that influence of one locus on the expression of another may be involved in heterosis. Subsequently, considerable data has 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. In many cases, the effects of a single homozygous successive allele is epistatic to almost the whole genetic make up of an inbred. When the effects of such an allele are masked by its dominant allele, the effects on heterosis are usually dramatic (Stuber 1994).

However, epistatic variance usually forms only a much smaller component of the total

genetic variance than do additive and dominance variances.

Theoretically, epistatic interactions will lead to the maximum heterosis when the following two conditions are met with. (1) 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 parents of hybrid s) of genes showing complementary epistasis seems to be the major cause of heterosis. In many experiments, multiplicative interaction has been reported as a cause of heterosis; it was concluded that in such cases, epistatic effects are nonlinear functions of the one-locus involve several mutually interacting genes.

Conclusion

In spite of the large experimental evidence accumulated, it is not possible to conclusively accept or reject one or the other hypothesis. There are definitely some genes that show heterozygote superiority. But the number of such genes appears to be rather small, and even these cases could be due to linkage in repulsion phase or epistasis or both. It is generally accepted that heterosis, to a large extent, is due to dominance gene action, but epistasis and overdominance are also involved (both in self- and cross -pollinated crops; see, banga and Banga, 1998). The relative importance of these phenomena is, however, not clearly understood. Recent evidence accumulated with maize seems to suggest that overdominance may not be the primary cause of heterosis. Overdominance is easily imitated by epistasis and linkage, and that most reported cases of overdominance may not represent true overdominance.

Molecular markers linked to quantitative trait loci (QTLs) have been used to investigate the relative significance of dominance, overdominance and epistasis in heterosis. Some workers have reported important overdominance, others have observed preponderance of dominance and some others have found extensive epistasis in various crops. For example, in one elite rice hybrid (Zhenshan 97 x Minghin 63) overdominance was observed for most of the QTLs for yield, but the overall heterozygosity was of little significance to heteroris. Digenic epistasis was frequent and widespread even between such loci that did not show overdominance. Thus single - locus overdominance (but not the overall heterozygosity) and epistasis appeared to be important contributors to heterosis in this rice hybrid (Yi etai, 1997). But as pointed out earlier, a QTL may consist of more than one polygene, and repulsion

phase linkage and or epistasis could easily mimick overdominance.

PHYSIOLOGICAL BASES OF HETEROSIS

Early studies on the physiological basis of heterosis related to embryo and seed sizes, growth rates in the various stages of development, rates of reproduction and of various assimilation activities. It was suggested that hybrid vigour resulted from larger embryo and endosperm sizes of the hybrid seeds as compared to those of the inbreds. As a result, the rate of growth in the seedling stage may by expected to be greater in the hybrids than in the inbreds. But these relationships were clearly demonstrated in some cases, while in other cases they were not detectable. There is evidence that increased size of hybrids is a result of an increase both in the size and the number of cells. This and other observations indicate a basic difference in the metabolic activities of hybrids and inbreds. In 1952, Whaley concluded that the primary heterotic effect concerns growth regulators and enzymes. He suggested that the hybrid embryo would be able to mobilize stored food materials earlier than those of the inbreds due to a more efficient enzyme system. This, in turn, would lead to the superiority of hybrids at least in the early seedling stages.

Khanna-Chopra et al. (1993) concluded that heterotic hybrids generally show a faster growth rate, higher leaf area index and greater biomass production than do their parents, but their harvest index (HI) is comparable to those of their parental inbreds (see, Verma et al., 1993). In case of plants, the major components of total biomass are net assimilation rate and leaf area index. Heterotic hybrids are generally earlier in flowering mainly due to their faster initial growth rate.

Net Assimilation Rate

Some heterotic hybrids show heterosis for photosynthesis at the seedling stage (Sinha and Khanna, 1975). Subsequently, heterosis for $C0_2$ exchange rate in wheat and for photosynthetic efficiency in some hybrids of rice were reported. It has been suggested that heterosis for photosynthetic efficiency was associated with an increased N content in tissues of rice and that this association results in the inconsistencies observed for heterosis for photosynthesis. It has been suggested that most likely heterosis for photosynthesis is not related to heterosis for yield (see, Banga and Banga, 1998).

Leaf Area Index (LAI)

The total area of leaf produced per square meter of a crop is known as leaf area index (LAI). Hybrids in various crops show distinct advantage over their parents in terms of LAI, especially during the early growth phases. Studies in cotton and rice demonstrate that heterosis in leaf area index during the early seedling stages is likely be manifested as significant advantage during the later stages of crop growth (see, Banga and Banga, 1998). However, it must be realized that heterosis for yield results from an increase in both source and sink capabilities; increase in one without a corresponding increase in the other will only be a wasteful exercise.

Root Growth

Many hybrids show heterosis for root growth. Root growth depends on shoot growth and roots serve as 'sinks' for the photosynthates till such time when the 'sinks' contributing to economic yield start developing. The enhanced photosynthetes produced by hybrids due to their higher LAI, etc. may lead to production of longer root systems.

Hormone Balance

It has been suggested that heterosis is the consequence of a superior hormone balance (relative concentrations of various plant hormones) in hybrids as compared to those of their parents. In case of maize, inbreds contain lower levels of GA_3 and respond to exogenous GA_3 application (in terms of shoot growth acceleration), while the hybrids contain relatively higher endogenous GA_3 levels. But these studies were based on seedling growth, which does not appear to be correlated with final yield in the majority of crops. Therefore, critical evidence in support of hormone balance hypothesis is lacking.

Metabolic Concept

Yield can be viewed as the end product of a series of reactions controlled by many rate-limiting specific enzymes. It has been suggested that inbreds have an unbalanced metabolic system in which certain enzyme are present in rate- limiting concentrations. Different enzymes may be rate- limiting in different inbreds so that when two such inbreds that complement each other in terms of their rate -limiting enzymes are crossed, a heterotic F\ hybrid is obtained.

Mitochondrial Complementation

It was proposed by Sarkissian in 1972, and elaborated by Srivastava in 1975, that mitochondrial and chloroplast heterogeneity may be the cause of hybrid vigour. It has been shown that mitochondria isolated from seedlings of the hybrids and their parents show different efficiencies of oxidative phosphorylation and of respiration rates. However, the efficiency of oxidative phosphorylation in mitochondria of heterotic hybrids is often comparable to that of mitochondria from nonheterotic hybrids. In addition, heterosis in mitochondrial activity in maize could not be correlated with grain weight/ear in maize. In conclusion, there is little concrete evidence in support of organelle activities being the basis of heterosis.

In 1998, Banga (See, Banga and Banga, 1998) concluded that "the understanding regarding the exact role of the many physiological contributions for the expression of heterosis still remains unclear."

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. Further, the quantum of additional production will increase with the level of useful heterosis,and also with the average yield/ha of the standard varieties of the crop. Therefore, the level of heterosis required to generate a given quantum of additional yield will depend on the average yield of crop; it will be lower in crops having higher yields than in crops having lower yields.

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 pe r 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.

A list of some examples of plant and animal species where heterosis is being commercially exploited

Category	*Examples*
Crop species	1. *Asexually propagated species* 2. *Cross-pollinated species*: maize, jowar, bajra, sugarbeets, sunflower, forage grasses, castor, forage legumes, and cotton 3. *Self-pollinated crops*: rice, pigeonpea (India)
Vegetable crops	Tomato, brinjal (*Solanum melongena*), onion, Brussel's sprouts, Watermelon, pepper, winter sqash, muskmelon, cabbage, broccoli, spinach, red beets, carrot cauliflower, celery, asparagus
Fruit trees	In almost all the fruit trees
Animals	Silkworm, poultry, cattle, swine

Lec. No. 25 **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 of inbreeding 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).**

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 result of inbreeding.

Inbreeding depression = F_1-F_2 / F_1 x 100

Historical

Inbreeding depression has been recognized by man for a long time.

Marriages between closely related individuals has been prohibited since early time in many societies. Because people are aware of the harmful effects of such marriages in the progeny.

A systematic observation on effect of inbreeding started during 17^{th} 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.

Effects of inbreeding

Inbreeding is accompanied with a reduction in vigour and reproductive capacity i.e. fertility. There is a general reduction in the size of various plant parts and in yield. In many species, harmful recessive alleles appear after selfing; plants or lines carrying them usually do not survive. The different effects of inbreeding are :

1. **Appearance of Lethal and Sublethal Alleles** : IB results in appearance of lethal;

   sublethal and subvital characters. Eg : Chlorophyll deficiencies, rootless seedlings, flower deformities – They do not survive, they lost in population.

2. **Reduction in vigour** : General reduction in vigour size of various plant parts.
3. **Reduction in Reproductive ability** : Reproductive ability of population

decreases rapidly. Many lines reproduce purely that they cannot be maintained.

4. **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 in genotype and phenotype. It leads to increase in the variance of the population.

5. **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.

6. **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.

Degrees of inbreeding depression

Inbreeding depression may range from very high to very low or it may even be absent. The ID is grouped into 4 categories.

1. **High inbreeding depression:** Eg : alfalfa and carrot show very high ID. A large proportion of plants produced by selfing show lethal characteristics and do not survive.
2. **Moderate inbreeding depression:** Eg : Maize, Jowar and Bajra etc. show moderate ID. Many lethal and sublethal types appear in the selfed progeny, but a substant ial proportion of the population can be maintained under self-pollination.
3. **Low inbreeding depression:** Eg : Onion, many Cucurbits, Rye and Sunflower etc. show a small degree of ID. 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.
4. **Lack of inbreeding depression:** The self-pollinated species do not show ID, although they do show heterosis. It is because these species reproduce by self- fertilization and as a result, have developed homozygous balance.

Procedure for development of inbred lines and their evaluation

1. Development of inbred lines:

Inbred lines are developed by continues self fertilization of a cross-pollinated species. Inbreeding of an OPV leads to many deficiencies like loss of vigour, reduction plant height, plants become susceptible to lodging, insects and pests and many other undesirable characters appear. After each selfing desirable plants are selected and self-pollinated or sib pollinated. Usually, it takes 6- 7 generations to attain near homozygosity. An inbred line can be maintained by selfing or sibbing. The purpose of inbreeding is to fix the desirable characters in homozygous condition in order to maintain them without any genetic change.

The original selfed plants is generally referred as S_0 plant and the first selfed progeny as S_1 second selfed progeny as S_2 as so on. The technique of inbreeding requires careful attention

to prevent natural crossing. The inbred lin es are identified by numbers, letters or combination of both. In India inbred lines are developed are released through co-ordinate maize improvement scheme and are designated as CM (Co-ordinate maize), CS (Co-ordinate sorghum) etc.

CM-100-199 - Yellow flint
CM-200-299 - Yellow
Dent CM-300- 399 - White Flint
CM-400-499 - White Dent
CM-500-599 - Yellow
CM-600-699 - White

2. Evaluation of inbred lines: After an inbred line is developed, it is crossed with other inbreds and its productiveness in single and double cross combination is evaluated. The ability or an inbred to transmit desirable performance to its hybrid progenies is referred as its combining ability.

General combining ability (GCA) : The average performance of an inbred line in a series of crosses with other inbred lines is known as GCA.

Specific combining ability (SCA) : The excessive performance of a cross over and above the excepted performance based on GCA of the parents is known as specific combining ability

Thus GCA is the characteristic of parents and SCA is characteristic of crosses or hybrids.

Top Cross: A cross between an inbred line and an open pollinated variety, also known as inbred variety cross.

Single cross hybrid: The hybrid progeny from across between two inbreds viz. (AxB).

Double cross hybrid: The hybrid progeny from a cross between two single crosses, i.e., (AxB) x (CxD).

Three way cross hybrid :The hybrid progeny between a single cross and an inbred, i.e., (AxB) X C.

Double Top cross : A cross between a single cross and an open pollinated variety, i.e., (AxB) X O.P.V.

The inbreds are evaluated in following way.

a. Phenotypic evaluation: It is based on phenotypic performance of inbreds themselves. It is effective for characters, which are highly heritable i.e. high GCA. Poorly performing inbreds are rejected. The performance of inbreds is tested in replicated yield trials and the inbreds showing poor performance are discarded.

b. Top Cross test: The inbreds, which are selected on phenotypic evaluation, are crossed to a tester with wide genetic eg. An OPV, a synthetic variety or a double cross. A simple way of producing top cross seed in maize is to plant alternate rows of the tester and the inbred line and the inbred line has to be detasselled. The seed from the inbreds is harvested and it represents the top cross seed. The performance of top cross progeny is evaluated in replicated yield trains preferably over locations and years. Based on the top cross test about 50% of the inbred are eliminated. This reduces the number of inbreds to manageable size for next step. Top cross performance provides the reliable estimate of GCA.

c. Single cross evaluation: Out standing single cross combinations can be identified only by testing the performance of single cross. The remaining inbred lines after top cross test are generally crossed in diallel or line x tester mating design to test for SCA. A single cross plants are completely heterozygous and homogenous and they are uniform. A superior single cross regains the vigour and productivity that was lost during inbreeding and can be more vigorous and productive than the original open pollinated variety. The performance of a single cross is evaluated in replicated yield train over years and location and the outstanding single cross identified and may be released as a hybrid where production of single cross seed is commercially feasible.

In case of maize the performance of single cross is used to predict the double cross performance.

Number of single crosses with reciprocals = n (n- 1)

Number of single crosses without reciprocals = n (n-)/2

Hybrid varieties:

When F_1 generation from a cross between two or more purelines, inbreds, clones or other genetically dissimilar populations / lines is used for commercial cultivation, it is called **hybrids varieties**.

History

Hybrid varieties were first commercially exploited in maize. **Beal in 1878** suggested that heterosis can be exploited by growing hybrids in maize. He suggested that in maize certain varietal crosses showed substantial heterosis upto 52%. Later **Shull in 1909** suggested that inbreds can be developed from open pollinated varieties by continuous self fertilization and then inbreds could be combined to produced superior hybrids (single cross hybrid).

Shull scheme could not be exploited commercially because of the following reasons :

1. Superior inbreds were not available in those days.
2. Since the female parent was an inbred the amount of hybrid seed produced per acre was low (30- 40% of the open pollinated varieties). Therefore, the hybrid seed was more expensive.
3. The male parent was also was an inbred so there is poor pollen availability. Therefore, more area has to be maintained under male parent. Hence hybrid seed production became more expensive.
4. The hybrid seed was often poorly developed as it was produced by an inbred and had a relatively poor germination. So it needs higher seed rate.

The last three difficulties are overcome by double cross scheme proposed by **Jones** in 1918. Since in double cross the male and female parents are single cross hybrid, the pollen production and seed production are abundant, seed quality and germination are high, and as a result hybrid seed production was not expensive / it is low.

First double cross hybrid in U.S.A. was **Burr Leaming Dent** in 1922 in maize.

In India hybrid maize began in 1952 under AICRP on maize improvement. In collaboration with Rock Feller Foundation. Four hybrids were released in 1961 Ganga-1, Ganga-101, Rangit and Deccan. The first hybrid Jowar was released in 1964 i.e. CSH-1 while that of Bajra HB-1.

Hybrid maize could not become popular in India because 1) Intensive management

and Input requirement for hybrids 2) need for changing of the seed every year. Hence development of composite varieties in maize during 1967 namely Vija y, Kisan, Amber etc. **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) Top-cross testing (one cross per inbred) to know the breeding value to each of the four inbreds (total 4 top-crosses per double cross).
b) Mean of the four non- parental single crosses involved in (AXB) X (CXD) double cross, viz., (AXC), (AXD), (BXC) and (BXD) (total 4 non- parental single crosses per double cross).
c) Average yield performance of all possible six crosses [n(n- 1)/2], namely AXB, AXC, AXD, BXC, BXD and CXD (total six crosses per double cross).

d) Average progeny- performance of each inbred can be de termined by the mean performance of each inbred in all possible single crosses where it occurs (n- 1 crosses per inbred). For instance, the mean performance of AXB, AXC and AXD will determine the average breeding value of the inbred A. Similarly, the mean of AXB, BXC and BXD will indicate the potential of the inbred B and so on (total 12 crosses per double cross).

These procedures of predicting the performance of double cross hybrids have been extensively investigated long ago. The available evidence shows that the method (b), i.e., mean performance of non- parental single crosses, is the most adequate and effective, since there is a close correspondence between predicted and realized yields of double crosses in maize. Fortunately, the total number of crosses required to be sampled per double cross is also the minimum, thus greatly facilitating the testing programme.

All possible single crosses (in a diallel mating) = n(n-1) Where, n = number of inbred lines

All possible 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

SYNTHETICS AND COMPOSITES VARIETIES

The possibility of commercial utilization of synthetic varieties in maize was first suggested by Hayes and Garber in 1919. synthetic varieties have been of great value in the breeding for those cross-pollinated crops where pollination control is difficult, e.g., forage crop species, many clonal crops like cacao, alfalfa (M.Sativa), clovers (Trifoulim sp.) etc. The maize improvement programme in India now places a considerable emphasis on synthetic varieties. The maize programme of CIMMYT. Mexico, is based on population improvement; the end - product of such a programme is usually a synthetic variety.

DEFINITIONS

A synthetic variety is produced by crossing in all combinations a number of lines that combine well with each other. Once synthesized, a synthetic is maintained by open- pollination in isolation. Some breeders use the terms synthetic variety in a restricted sense: a synthetic variety is regularly reconstructed from the parental lines and is not maintained by open-pollination.

A 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 F1's among the component lines are not available. Like synthetics, composites are commercial varieties and are maintained by open- pollination in isolation.

Germplasm complexes are produced by mixing seeds from several lines or populations of diverse genetic origin. The objective of germplasm complexes is to serve as reservoirs of germplasm. Germplasm complexes are experimental populations and they are not commercial varieties.

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 a 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 is 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., sugarbeets, 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 syn 3 etc. The performance of Syn 2 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.

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 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.

Factors determining performance of synthetic varieties

The yield of syn_2 would be less than that of syn_1 due to loss in heterozygocity as a result of random mating.

The decrease in yield ability of syn_2 would depend on

1. The number of inbred lines and
2. On the difference in the yielding abilities of syn_1 and syn_0 generations.

Sewall wright, 1922 suggested the formula for predictin g the performance of syn_2. $syn_2 = syn_1 (syn_1 - syn_0) / n$

where n = number of parental lines

How to improve the performance of syn_2 ? there 3 ways

1. By increasing the number of lines
2. By increasing the performance of syn_1
3. By improving the performance of parental lines

Difference between synthetics and composites

	Synthetics	**Composites**
1.	No. of inbredlines are less (6-8)	No. of lines are more (even upto 20)

2.	GCA of parental lines is tested	No. testing
3.	Performance can be predicted	Cannot be predicted
4.	Broad based	More broad based
5.	Synthetic can be reconstituted	Cannot be reconstituted at a later date
6.	Seed replacement after 4-5 years	After 3-4 years

Achievements

Synthetic varieties have been widely used in forage crops and in crops where pollination control is difficult. The maize breeding programme at CIMMYT, Mexico and the pearlmillet programme at ICRISAT, Hyderabad are based on synthetic varieties generally developed through population improvement. The maize breeding programme in India is placing increasingly greater emphasis on the production of synthetic or composite varieties.

In India, the first composite varieties were released in 1967; the six maize composites were, Ambar, Jawahar, Kisan, Vikaram, Sona and Vijay.

Composite 1, has been evolved in *Brassica campestris* Var. *toria.* It was developed by compositing 10 *elite toria* strains; it matures in 100 days, exhibits profuse branching and
yields about 11 q/ha seed, which contains about 40% oil.

CHAPTER-12

METHODS OF BREEDING FOR VEGETATIVELY PROPAGATED CROPS

Clone : A clone is a group of plants produced from a single plant through asexual reproduction.

The crop plants can either be propogated by seeds or by vegetative parts. The vegetative propogation is resorted due to :

1. Lack of seed : Eg. Ginger, termiric
2. There is short viability of seed : Eg. Sugarcane
3. The seed production is very rare : Eg. Banana
4. Seeds are produced under special conditions only : Eg. Sugarcane, potato

Characteristics of Asexually propagated crops :

1. Majority of them are perennials: Eg . Sugarcane, fruit trees.
   The annual crops are mostly tuber crops: Eg. Potato, cassava, sweet potato
2. Many of them show reduced flowering and seed set
3. They are invariably cross pollinated
4. These crops are highly heterozygous and show severe inbreeding depression upon selfing.

1. Majority of asexually propagated crops are polyploids : Eg. Sugarcane, Potato, Sweet, Potato
2. Many species are interspecific hybrids. Eg. Banana, Sugarcane

Characteristics of a clones :

1. All the individual belonging to a single clone are identical in genetype
2. The phenotypic variation within a clone in due to environment only
3. The phenotype of a clone is due to the effects of genotype(g), the environment(e) and the genotype x environment interaction (GxE), over the pop.mean(M)
4. Theoratically clones are immortal. They deteriorate due to viral/bacterial infection and mutations.
5. Clones are highly heterozygous and stable
6. They can be propagated generation after generation without any change.

Importance of a clone

1. Owing to heterozygosity and sterility in many crops clones are the only means of propagation.
2. Clones are used to produce new varieties.
3. Clones are very useful tools to preserve the heterozygosity once obtained. In many crops the superior plants are maintained. (Mango, orange, apple, sugarcane)

Sources of clonal selection :

1. Local varieties
2. Introduced material
3. Hybrids and
4. Segregating populations

Clonal selection

The various steps involved in clonal selection are briefly mentioned below.

First year: From a mixed variable population, few hundred to few thousand desirable plants are selected. Rigid selection can be done for simply inherited characters with high heritability. Plants with obvious weakness are eliminated.

Second year: Clones from the selected plants are grown separately, generally without replication. This is because of the limited supply of propagating material for each clone, and because of the large number of the clones involved.

Characteristics of the clones will be more clear now than in the previous generation. Based on the observations the inferior clones are eliminated. The selection is based on visual

observations and on judgement of the breeder on the value of clones. Fifty to one hundred clones are selected on the basis of clonal characteristics.

Third year : Replicated preliminary yield trial is conducted. A suitable check is included for

comparison few superior performing clones with desirable characteristics are selected for multilocation trials.

At this stage, selection for quality in done. If necessary, separate disease nurseries may be planted to evaluate disease resistance of the clone s.

Fourth to eighth years : Replicated yield trials are conducted at several locations along with suitable check. The yielding ability, quality and disease resistance etc. of the clones are rigidly evaluated. The best clones that are superior to the check in one or more characteristics are identified for release as varieties.

Ninth year : The superior clones are multiplied and released as varieties.

Advantages

1. Varieties are stable and easy to maintain
2. Avoids inbreeding depression
3. Clonal selection, combined with hybridization generates necessary variability for several selections.
4. Only method to improve clonal crops
5. Hybrid vigour is easily utilized selection may be used in maintaining the purity of clones.

Disadvantages:

1. Selection utilizes the natural variability already present in the population.
2. Sexual reproduction is necessary for creation of variability through hybridization.
3. Applicable only to the vegetatively propogated crops.

Problems in Breeding asexually propogated crops

1. Reduced flowering and fertility
2. Difficulties in genetic analysis
4. Perennial life cycle.

Genetic variation within a clone: may arise due to

1. Mutation
2. Mechanical mixture
3. Sexual Reproduction

1. **Mutation :** The frequency is generally very low (10^{-5} to 10^{-7}). Ordinarily dominant mutations would be expressed in the somatic tissue. A mutant allele would be homozygous only when.

   i) both the alleles in a cell mutate at the same time producing the same mutant allele or

   ii) The mutant allele is already in heterozygous condition in the original clone. Though rare, both these events are possible. Bud mutations may aften produce chimeras i.e. an individual containing cells of two or more genotypes.

   But mutations make possible selection of buds to establish new desirable clones,

   the process being known as Bud selection. It is of some importance in improvement of perennial crops like fruit trees or of those crops where flowering does not take place. It requires large number of plants to be observed and several trained persons to detect the mutant buds. Hence the bud selections are practiced in commercial plantations.

2. **Mechanical mixtures :** Mechanical mixtures produces genetic variation within a clone much in the same way as in the case of purelines.

3. **Sexual reproduction :** Occasional sexual reproduction would lead to segregation and

   recombination. The seedlings obtained from sexual reproduction would be genotypically different from the asexual progeny. It is evident that only clones would tend to became variable atleast in annuals and biennials. Eg. Potato

Clonal degeneration : The loss in vigour and productivity of clones with time is known as clonal degeneration and results due to :

1. Mutation
2. Viral diseases
3. Bacterial diseases

Achievements

I. Through clonal selection :

Potato : 1.Kufri Red from Darjeeling Red Round

2. Kufri Safed from phulwa

3. Bombay Green banana is a bud selection from dwarf Cavendish : pidi monthan from Monthan

II. Through hybridization

Potato : Kufri Alankar, Kufri Kuber, Kufri Sindhuri, Kufri Kundan, Kufri Chamatkar, Kufri Jyothi (late blight resistant), Kufri Sheetman (frost resistant) Sugarcane : Co 1148, Co 1158, CoS 510, Co 975, Cos 109, Co 541

MAINTENANCE OF BREEDING RECORDS AND DATA COLLECTION

Introduction

Development of a suitable variety for a particular region depends upon the proper evaluation in a number of environments and location. The breeder has to evaluate thousands of strains of a particular species every year to identify a suitable variety for a particular region. Therefore, the maintenance of complete and accurate record becomes necessary for successful handling of thousand strains of number of species every year.

There are different types of records:

- Accession records
- Project book
- Sowing /planting plan
- Planting list,
- Record book of crosses and
- Field book.

Maintenance of Records:

- Accession Register
- Germplasm Bank
- Descriptive blank Register
- Crossing programme
- Single plant selection register
- Row test and Replicated row test
- Preliminary & Initial evaluation trail
- Comparative yield & yield evaluation trail
- Multillocation I & II trail
- Quality observation note book
- Record of crosses
- Handling of segregating generation note book

Accession Register

This will contain the details of seeds/planting material with regard to receipt date, source their number, assigned at the receiving unit, short description of the planting material to whom sent for evaluation, date, feed back information about the genotypes. Accession number governed by the serial number followed by the year of entry i.e. serial 154 in 2016. Then accession number will be 16154 or 16154. It will be mentioned as EC = exotic collection and IC = Indigenous collection

Performa of Accession Register

Acce ssio	Name of	Date of	Sourc e of	Sour ce	Pedi gree	Descr ipti on of	How dispo sed to	Feed back infor mati	Remarks
1	2	3	4	5	6	7	8	9	10

Besides, there are breeding records which are very much essential for breeders and

geneticists. Any system of record keeping should be as simple as possible, otherwise the details can become so time consuming that the breeder may fail to maintain up to date records. Because large number of crosses generated in a breeding programme will be discarded and detailed pedigree need not to be recorded each time a cross is made. However, adequate information should be provided in some type of permanent records so that ancestral pedigree of a particular strain can be ascertained if desired.

Breeders use simple accession numbers in a breeding nursery beginning with 1 each year. The number for current year is listed in the first column and the number of the previous year in the second column. Other information follows in later columns, such as amount of selfing and backcrossing. For example, the accession number 97-35 x 21 written on each seed packet means, the cross was made between 35 and 21 and grown in the year, 1997. Generally, most of the breeders list the female parent first. Simple accession number system enables the breeder to check the pedigree when desired.

Another system of accession number (Fehr and Headly, 1980) followed by the breeders is two digit number for the year followed by three digit cross number. This may be preceded by the letter(s) denoting the state or a specific location. For example DGS- indicate cross number 56 made at Dhiansar in 1999. As the generation are advanced in the pedigree system, a selection number is added in each generation. DGS 99056-6-9-5 denotes selfed seed from F3 plant (5) which resulted from the selfed progeny of plant number 9 in an F2 row which is turn resulted from selfed progeny of plant number 6 in an F1 row. If open pollinated seed is saved, a zero precedes the selection number. Then the zero (0) is added before the 5 in the above selection number (DGS 99056 -6-9-05). This number now denotes open pollinated seed harvested from plant number 6. If the selfed seeds in row are bulked, DGS 99056-6-9-B would be designated and if the open pollinated seed is bulked then the selection number would be written as DGS-6-7-0B.

CROSS DESIGNATION

The standard practice is to first list the pistillate parent followed by the staminate parent in across. Other than the normal practice, there are many variations of abasic system of designating crosses. All have certain advantage but some are than others. The following are two systems that have been used to designate crosses in plant breeding programmes. The first system was used for decade but was replaced by the second system, which is very simple and easy.

Cross	First system	Second system
Single cross	A x B	A / B
Back cross	A/2 x B	A^2 / B
Three-way cross	(A x B) x C	A / B // C
Four way cross	(A x B) x (c x D)	A/B // C/D
Compound cross	(([(A x B) x C] x D x E))x	A / B //C D/4 /E /5

	F	

The second system replaces the symbol x with the symbol / .

Chronological order of crosses	System
1	/
2	//
3	///
4	/4/
5	/5/
Backcross	Exponent

The systems of designating crosses are worth mentioning. One is used by the International Centre for the Improvement of Maize and wheat (CIMMYT) and the other by United States Department of Agriculture (USDA).

Cross	CIMMYT System	USDA System
Single cross	A-B	A / B
Back cross	A2 – B	A*2 / B
Three way cross	A – B x C	A / B // C
Four way cross	A – B x C – D	A / B // C / D
Compound cross	[(A – B) x C / D) E] F	A / B // C /// D / 4 / E 5 / F

The CIMMYT system is easy to read and write while the USDA system is compatible with computers.

At IRRI, the breeders have adopted the USDA system. The new IRRI system differs from the previous one only in ther designation of the backcrosses.

Cross	Previous IRRI System	New IRRI system (USDA)
Single cross	A2 / B	A*2 / B
Back cross	A /B^2	A/^{2}* B

A /2 * B // C means that A was crossed with B, the F1 was backcrossed with B, the F1 was backcrossed to B and the progeny was backcrossed with C.

Minnesota Method

This method is very easy and, for comparison, notations for introductions, selections and hybrids included.

I – 97 -1 Selection

II – 97 -1 Crosses

III – 97 -1 New Introduction

In this method , I, II and III stands for individual plant selections, crosses and introductions, respectively. 97 represents the year in which the selection, cross or introduction was made and the final number represents the particular selection, cross or introduction.

Crosses are given a selection number only after having shown to be homozygous. Parental and Fn populations are numbered by carrying row numbers for the current and proceeding season until homozygosity is reached. The method often used by the USDA is given here:

Where, F1 = A

F2 = A -1, A -2 etc.

F3 = A -1, A -2 etc .according to the number of selections grown.

First Year = II 18, A

Second Year = II – 18 , A -1, A -2 etc.

Third Year = II -18, A -1 -1, A -1 -2 etc.

Selection of crosses, when given series number after reaching homozygosity are designated II – 18 -1, II – 18 2 etc. according to the selection grown.

Alberta System

This is the modification of Minnesota system

I = Introduction

S = Selection

H = Hybrid

Crossing book

This is the register where the crosses made during the year are listed in chronological order mentioning the following information.

- Crossing book are prepared in triplicate and new pages are added for each group of crosses.
- Always store all the three copies in different places to avoid against the loss.
- Record the following information for each cross:
  - ✓ Cross number
  - ✓ Varietal names or parents
  - ✓ Pedigree of both the pistillate and staminate parent
  - ✓ Put identification number of both parents
  - ✓ Number of crossed seed obtained

CHAPTER-14

WIDE HYDRIDIZATION OR DISTANT

HYBRIDIZATION Introduction

When individuals from two distinct species of the same genera are crossed, it is known as **inter specific hybridization.**

Eg.Inter specific hybridization : e.g., Oryza sativa x O. perennis

When individuals being crossed belong to two different genera, it is referred to as **inter generic hybridization**. e.g. Wheat x rye.

Hybridization between individuals from different species belonging to the same genus or tw o different genera, is termed as distant hybridization or wide hybridization, and such crosses are known as distant crosses or wide crosses.

1. History : The first distant hybridization; hybrid between carnation (*Dianthus caryophyllus*) and sweet willian (*Dianthus barbatus*) by Thomas Fairchild in 1717 and the hybrid is called as fairchilds mule
2. Most of the interspecific hybrids were of no agricultural value many interspecific hybrids particularly in case ornamentals, served as commercial varieties.
3. An interesting inter generic hybrid *Raphano brassica* was an amphidiploid cross between radish and cabbage but it was useless.

The first inter generic hybrid with a great potential was TRITICALE

Objectives:

1. To transfer some desirable character from wild relatives that are not available in cultivated varieties.

Eg. Many disease resistance and, insect resistance genes

Wide adaptability : (i.e. drought-resistance, cold tolerance etc.) Quality improvement (Eg. Cotton (fibre) Tobacco (leaf)

Yield improvement (Eg. Oats, Tobacco, Maize, S. cane)

Other characters (E g. CMS, Earliness, dwarfness morphological characters)

2. Exploitation of luxuriance (heterosis) in vegetatively propagated / ornamental crops.

Prolonged vegetative period, Prolonged blooming period

3. Creation of Novel genotypes: new species or F1 hybrids hither to non – existent in nature.

BARRIERS TO THE PRODUCTION OF DISTANT HYBRIDS

1. **Failure of zygote formation / Cross incompatibility**
2. **Failure of zygote development / Hybrid inviability**
3. **Failure of F1 seedling development / Hybrid sterility**

A variety of mechanisms may be responsible for each of these three difficulties:

1. **<u>Failure of zygote formation / cross incompatibility.</u>**

Inability of the functional pollens of one species or genera to effect fertilization of the female gametes of another species or genera is referred to as **cross incompatibility**.

It may be due to – 1. failure of fertilization, because the pollen may not germinate.

2. Pollen tube is unable to reach to embryo sac and hence sperms are not available for fertilization –

3. Pollen tube may burst in the style of another species Eg. Datura.

4. The style of the female parent may be longer than the usual length of the pollen tube growth therefore the pollen does not reach the embryo sac. Eg. Zea mays and *Tripsacum sp*.

5. Pollen tubes of polyploidy species are usually thicker than those of diploid species.

6. When a diploid is used as female and a polyploidy as male, the polyploidy pollen tube grows at a slower rate in the diploid style than it would be in a polyploid style.

These barriers are known as pre-fertilization barriers.

Techniques to make wide crosses successful

1. Removal or scarification of stigma
2. Using short styled parent as female.
3. Using the diploid species as the male parent.

2. Failure of zygote Development / Hybrid inviability

The inability of a hybrid zygote to grow into a normal embryo under the usual conditions of development is referred to as **hybrid inviability.** This may be due to : **Lethal genes :** some species carry a lethal gene, which causes death of the interspecific hybrid zygote during early embryonic development.

Eg. 1. *Aegilops umbellulata* carries a lethal gene with 3 alleles against diploid wheats.

2. Genetic Dishormony between the two parental genomes.

The genetic imbalance between the two parental species may cause the death of embryos.

Eg. Cotton - G.gossypioides x other G. sps.

Brassica – B.napus x B.oleracea

3. Chromosome elimination : In some cases of distant hybridization, chromosomes are gradually eliminated from the zygote. This generally does not prevent embryo development, but the resulting embryo and the F_1 plants obtained from such crosses are not true interspecific hybrids since they do not have the two parental genomes in full. Generally. Chromosomes from one are successively eliminated due to mitotic irregularities.

Eg. Hordeum bulbosum x H. vulgare

Hordeum bulbosum x Triticum aestimum Triticum aestivum x Zea mays

4. Incompatible cytoplasm : Embryo development may be blocked by an incompatibility between cytoplasm of the species used as female and the genome of the species used as male. Such an interaction, more generally, leads to hybrid weakness and male sterility in the hybrids or may some times leads to failure of embryo developments.

Endosperm Abortion : Seeds from a large number of distant crosses are not fully developed and are Shrunken due to poorly developed endosperm. Such seeds show poor germination, and may often fail to germinate. When the endosperm development is poor or is blocked, the condition is generally known as endosperm abortion.

Eg. 1. Triticum x secale – Triticale . In this case the endosperm aborts at a much later stage so that a small frequency of viable seed is obtained.

2. *Hordium bulbosum x H. vulgare* – the endosperm aborts at an early stage so that viable seeds are not produced.

In case of endosperm abortion - embryo rescue culture is practiced.

3. Failure of Hybrid seedling development / Hybrid sterility

Some distant hybrids die during seedling development or even after initiation of flowering. The mechanisms involved in the failure of seedling development most likely involve complementary lethal genes.

Eg. 1. In cotton-certain interspecific hybrids appear normal, but die in various stages of seedling growth; some plants die at flowering.

2. Interspecific and intergeneric F_1 hybrids of wheat show both chlorosis and necrosis;

Hybrid sterility : Hybrid sterility refers to the inability of a hybrid to produce viable off spring. The main cause of hybrid sterility is lack of structural homology between the chromosomes of two species.

Techniques for production of distant hybrids

1. **Choice of parents :** Genetic differences exist among parents in a species for cross compatibility. More compatible parents should be selected for use in wide crosses.
2. Pollina ting sufficiently large no. of flowers.
3. **Reciprocal crosses :** it is better to attempt reciprocal crosses when distant crosses are not successful.

   Eg. : *Phaseolus aureus* and *p.mungo* are crossable only when *P. aureus* in used as

   female and *P. mungo* as male.
4. Determine the barrier and then take measures to

   overcome it: Longer style sps - cut the style

   Use more than one strain of each sps for lethal genes

   Autopolyploidy (B.olerecia x B. compestris)

   Manipulation of ploidy – when two species of a cross differ in chromosome number, it is necessary to manipulate their ploidy

   a. Direct crossing-use higher ploidy sp as female parent

b. Chromosome no. of the wild species or of the interspecies hybrd (F_1) may be doubled to overcome sterility of the hybrid.

5. **Bridge crosses :** Some times, two species say 'A' and 'C' do not cross directly. In such case a third species say 'B' which can cross with both 'A' and 'C' is chosen as a bridge species. First 'B' is crosses with 'C' and then the amphidiploid is crossed with

'A'. Bridge crosses have been used in Tobacco and wheat.

Eg. *Nicotiana repanda* can cross with *N. sylvstis* but not with *N. tabacum N. sylvestris* crosses with both *N. repanda* and *N.tabacum*. For transfer of genes from *N. repanda to N.tabacum N. sylvestis* is used as bridge species.

6. **Use of pollen mixtures :** Cross incompatibility results due to unfavourable

interaction between the protein of pistil and pollen which inhibits normal germination and growth of pollen tube. This problem can be overcome by using the mixture of pollen from compatible (self) and incompatible parents.

7. **Manipulation of pistil :** In some cases, pollen tube is short and style is very long, due to species difference. Thus pollen tube cannot reach ovule to effect fertilization. In such situation either reciprocal cross should be made or the style should be cut to normal size before pollination. This technique is successful in maize – Tripsacum crosses, where maize style remains receptive even after cutting.

8. **Use of growth regulation :** Some times, the pollen tube growth is so slow that the eggcell dies or the flower aborts before the male gametes reach the ovary. In such cases, growth regulators should be used to accelerate the pollen tube growth or to prolong the viability of the pistil.

Use of grow th regulators such as IAA; NAA; 2,4-D and GA_3 etc; are promising in some wide crosses.

9. **Large number of crosses :** The success of seed set is generally very low in wide crosses. Hence, large no. of crosses should be made to obtain crossed seeds.

10. **Protoplast fusion :** The wide crosses can be obtained through protoplast fusion, when it is not possible to produce such crosses through sexual fusion.

11. **Embryo culture :** This technique is being used widely to obtain viable interspecific

or intergeneric hybrids. This is used when hybrid zygote is unable to develop. This technique has been successfully used is *Triticum, Hordeum, Phaseolun, Nicotiana, Gossypium, Lycopersicon, Trifolium, Cucumbita* etc.

12. **Grafting :** Grafting of interspecific hybrid on to the cultivated species helps in making the cross successful.

Applications of wide hybridization in crop improvement

1. **Alien addition lines:** Carries one chromosome pair from a different species in addition to somatic chromosome complement. For Eg. Disease resistance in Wheat, oats, tobacco
2. **Alien substitution lines :** has one chromosome pair from different species in place of the chromosome pair of the recipient parent.
3. **Introgression of genes** : Transfer of small chromosome segments with desirable genes.

Eg. A. Disease resistance :

In Cotton transfer of black arm disease resistance from *G. arboreium to G. barbadense*

B. Wider adaptation : Cold tolerance has been transferred from wild relatives to

Wheat, onion, potato, tomato and grape.

C. Quality : Oil quality in oil palm was impr oved by genes from wild

relatives. D. Changing the mode of reproduction :

1. Self- incompatibility : S.I. genes from B.campestis to self compatible

B.napus for hybrid seed production.

E. Yield :
F. Other characters :

4. **Development of New crop species :**

Eg. Triticale

5. **Utilization as New hybrid varieties :**

Eg. F1 hybrids in cotton Varalaxmi cotton (G.hirsutum x G. barbadense)

Sugarcane : All the present day commercial varieties are complex interspecific hybrids involving S. officinarum & S. spontanium

Sterility in distant hybrids :

Distant Hybrids show variable sterility ranging from complete fertility to complete sterility

For eg. *L.esculentum x L.pimpinellifolium* hybrid in completely fertile while sugarcane maize hybrid is completely sterile.

Distant Hybrids are of two broad groups : The first group includes those Distant hybrids that exhibit atleast some fertility so that than can be maintained by selfing, intercrossing among them selves or backcrossing to the parental species.

The second group consists of those hybrids that are completely sterile and have to be maintained clonally or by doubling their chromosome number.

The sterility of distant hybrids may be caused by cytogenetic, genetic or cytoplasmic factors.

Cytogenetic Basis of sterility : Most of the interspecific hybrids show reduced chromosome

pairing and in extreme cases all the chromosomes may be present as univalents.

The distribution of chromosome in such cases is irregular, and it leads to the formation of unbalanced gametes resulting in partial to complete sterility.

Inter specific crosses also show rings and chains at metaphase -I (indicating translocations). Bridges and fragments at anaphase-I (indicating invessions)

Loops at pachytene (indicating duplications or deletions). These cytological aberrations also

reduce fertility. Fertility in such hybrids is improved by doubling their chromosome number, that is by producing amphidiploids from them.

Genetic Basis of sterility :

Chromosome pairing in some interspecific hybrid is regular, but they show variable sterility which is due to genes.

Eg. The F1 hybrid between foxtail millet, *setaria italica* and its wild relative *S.viridis* showed normal pairing and regular formation of bivalents. But pollen and ovule sterilities were 70 and 50% respectively.

Cytoplasmic Basis of sterility :

In some interspecific hybrids, sterility is produced by the cytoplasm. In such cases, the reciprocal crosses produce fertile hybrids. Clearly, in such cases sterility is produced by the cytoplasm such instances of hybrid sterility are known is *Epilobium, Oenothera* . **Limitations of Distant Hybridization :**

1. Incompatible Crosses

2. F_1 Sterility
3. Problems in Creating New species
4. Lack of Homoeology between Chromosomes of the Parental Species
5. Undesirable Linkages
6. Problems in the Transfer of Recessive Oligogenes and Quantitative Taits
7. Lack of Flowering in F_1
8. Problems in using Improved varieties in Distant Hybridization
9. Dormancy

Achievements

Wehat, Tobacco, Cotton

Parbhani Kranthi : Derived fr om A. esculentus C.V. Pusa Sawani x A. Manihot – Resistant to yellow mosaic vein virus, yield – Kharif : 110- 120 q/ha, Summer : 85-90 q/ha

Pusa Kranthi : Kharif 105-110 q/ha, Summer 75-80 q/ha

CHAPTER-15

MUTATION BREEDING

The term mutation was coined by Hugo de Vries in 1900 for the first time and the 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's concept of mutation develops into 4 eras:

(i) 1900-1927 : First era deals with concept of mutation.

(ii) 1927-1945 : artificial production of mutation by X-rays. Mutagenic action of X-rays was discovered by Muller in 1927 in Drosophila . Stadler discovered mutagenic action in 1929 in barley and it was observed that mutation can be enhanced by X-rays.

(iii) 1945-1953 : Chemical mutagens. Auerbach and Robson showed that nitrogen mustard produced mutation in Drosophila.

(iv) 1953 onwards : After proposing double helical structure of DNA in 1953 people started working on molecular mechanism of mutation. Combined effect of irradiation and chemical mutagens on the frequency of mutation was studied.

Mutation may be the result of a change in a gene, a change in chromosome that involves several genes or a change in plasmagene.

Mutations produced by changes in the base sequence of genes are known as gene or point mutations some mutations may be produced by changes in chromosome structure or even in chromosome number they are termed as chromosomal mutation.

There are three types of mutations based on genetic basis of heritable change:

1. Gene mutations: These are produced by change in the base sequence of genes. The change may be due to base substitutions, deletion or addition.
2. Chromosomal mutation: These arise due to change in chromosome number that may leads to polyploidy or aneuploidy or change in chromosome structure that result in deletions duplication, inversion and translocation.
3. Cytoplasmic or plasmagene mutation: These are due to change in the base sequence of plasma genes. The plasma genes are present in mitochondria or

4. chloroplast. Here the mutant character occurrs in buds or somatic tissues which are used for propagation in clonal crops.

Classification of mutations :

Based on origin, the mutations are classified as spontaneous and induced mutations.

1. **Spontaneous mutations :** Mutations occur in natural populations at a low rate (10^{-6}) but different genes may show different mutation rates. Here the different genes show different mutation rate. For example : in maize R- locus mutates at the frequency of
   4.92×10^{-4} i.e. (1 in 20000 population), when as Su locus at 2.4×10^{-6} (1 in 25 lakhs).

The Wx locus considered to be highly stable.

The difference in mutation rate may be due to a) Genetic back gr ound i.e. presence of mutator genes b) Genes them selves c) Environment

2. **Induced mutation:**
   Mutations may be artificially induced by treatment with certain

physical or chemical agents. Available evidence indicates that induced mutation rarely produces new alleles they produce alleles which are already known to occur spontaneously. Induced mutations are comparable to spontaneous mutations in their effects and in the variability they produce. Induced mutation occur at a relatively higher frequency so that it is practical to work with them.

3. **Based on magnitude of phenotypic effects mutation as classified as**

Macro mutations : Oligogenic Mutation – Large phenotypic effect and recognizable on individual plant basis and can be seen easily in M2 generations – Eg. Ancon breed in sheep, pod maize to cob maize

Micro mutations : Polygenic mutations – Small phenotypic effect which can not be recognized on individual plant basis but can be recognize only in a group of plants. Selection should be done in M3 or later generations.

Micro mutations are of economic value in plant breeding.

Depending upon the effect on the survival of an individual, induced mutations are of four types

(1) Lethal (all the individuals carrying such mutations are killed)

(2) Sub-lethal (kill more than 50% of the individual)

(3) Sub-vital (kill much less than 50% of the individual)

(4) Vital mutations do not reduce the viability of the individuals carrying them. Obviously, crop improvement can utilize such mutations.

Characteristic feature of mutations

1. Mutations are generally recessive but dominant mutations also occur
2. Mutations are generally harmful to the organism. Most of the mutations have deleterious effects but small proportion (0.1%) of them are beneficial.
3. Mutations are random i.e. they may occur in any gene. However some genes show high mutation rates than the others.
4. Mutations are recurrent
5. Induced mutations commonly show pleiotropy often due to mutation in closely linked genes.

MUTAGENS :

Agents which induce mutation is known as mutagens.

A. **Physical mutagens** (all of them are various kind of radiation)
   1. Ionizing radiation
      (a) Particulate radiation, e.g. alpha-rays (DI), Beta-rays (SI), fast neutrons (DI) and thermal neutrons (DI).
      (b) Nonparticulate radiation e.g., X-rays (SI) and **Gamma rays** (SI)
   2. Non Ionizing radiation,e.g., ultra violet (UV) radiation

B. Chemical mutagens
   1. Alkylating agents, e.g., sulphur mustard, nitrogen mustard, ethylene-imines (e.g., **EMS** (etylmethane sulphonate), MMS (methylmethane sulphonate).
   2. Acridine dyes, e.g. acriflavin, proflavin, acridine orange, acridine yellow, ethidium bromide.
   3. Base analogues e.g., 5-bromouracil, 5-chlorouracil.
   4. Others, e.g., nitrous acid, hydroxyl amines, sodium azide.

Mutagenesis : Treating of a biological material with a mutagen in order to produce mutation is known as mutagenesis.

Irradiation : Exposure of a biological material to one of the radiation is known as irradiation.

A mutation breeding programme should have well defined and clear cut objective because the ratio of beneficial to useless mutation is very less, e.g. 1 in 800 (only 0.1% mutation is desirable)

Procedure for irradiation : The plant material may be treated in any of the following

source. 1. Seeds, 2. Seedlings, 3. Flowers, 4. Cuttings

1. **Seeds :** Seeds are used after soaking to get greater frequency of induced mutations than air dried.
2. **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.
3. **Flowers :** Meiotic cells have been found more sensitive than the mitotic cells and therefore plants are irradiated in the flowering stage in order to affect the developing gametes.
4. **Cuttings :** In case of fruit tree when they are propagated by clones – the desirable cuttings are exposed to irradiation.

Selection of the variety for mutagen treatment

The variety selected for mutagenesis should be the best available in the crop.

Dose of the Mutagen

An optimum dose of the mutagen should be used. An optimum dose is the one which produces the maximum frequency of mutations and causes the minimum killing. Many workers feel that a dose close to LD50 should be optimum. LD50 is that dose of a mutagen, which would kill 50% of the treated individuals.

Mutation Breeding for oligogenic traits

The handling procedure described here is based on the selection for a recessive mutant allele of an oligogene.

1. **M1.** Several hundred seeds are treated with a mutagen and are speceplanted. 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; this can be achieved either by planting the M1 population in isolation or by bagging the inflorescenes of M1 plants or even the whole M1 plants. M1 plants will be chimeras for the mutations present in heterozygous state. About 20 to 25 seeds from each M1 spike are harvested separately to raise the M2 progeny rows.

2. **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 beca use the observations are based on single plants. All the plants in M 2 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. However, most of the mutations will be useless for crop improvement. Only 1-3 per cent of M2 rows may be expected to have beneficial mutations.

Alternatively, M2 may be grown as a bulk produced by compositing one or more, but equal number of, seeds from each M1 spike/fruit/branch. Individual plants are then selected in M2 and individual plant progenies are grown in M3.

3. **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 rows are harvested in bulk for a preliminary yield trial in M4.

4. **M4.** A preliminary yield trial is conducted with a suitable check, and promising mutant

lines are selected for replicated multilocation trials.

5. **M5-M7**. Replicated multilocation yield trials are conducted. The out-standing line may be released as a new variety. The low yielding mutant lines, however, should be retained for use in hybridization programmes.

Mutation breeding for polygenic traits : Mutagenesis does produce genetic variation in polygenic traits; this variation is usually as much as 50% of that generated in F2

generation, but sometimes it may be as much as or even greater than the latter.

1. **M_1 and M_2.** M_1 and M_2 are grown in the same way as in the case of oligogenic traits.

   In M_2, vigorous, fertile and normal looking plants that do not exhibit a mutant phenotype are selected and their seeds are harvested separately to raise individual plant progeny rows in M_3.

2. **M_3.** Progeny rows from individual selected plants are grown. Careful observations are made on M_3 rows for small deviations in phenotype from the parent variety. Inferior rows are discarded. Few rows may be homogeneous and would be harvested in bulk. Selection in done in M_3 rows showing segregation; a majority of M_3 rows would show segregation. Intensive and careful evaluation of a large number of M_3 progeny rows allows identification of mutants with altered quantitative traits, e. g., partial or horizontal disease resistance. Such mutants occur in high frequencies that approach 1% or even high, so that their isolation becomes quite cost effective.

3. **M_4.** Bulked seed from homogeneous M_3 rows may be planted in a preliminary yield trial with a suitable check; superior progenies are selected for replicated multi- location yield trials. Individual plant progenies from M_3 are critically observed. Progenies showing segregation may be subjected to selection only if they are promising. Super ior homogeneous progenies are harvested in bulk for preliminary yield tests in M_5.

4. **M_5-M_8.** Preliminary yield trials and / or multi- location trials are conducted depending upon the stage when the progenies become homogeneous. Outstanding progenies may be released as new varieties.

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 yielding variety.

3. Mutagenesis has been successfully used to improve various quantitative characters including yield.

4. F_1hybrids 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.

Advantages :

1. Mutation create inexhaustible variation.

2. Mutation breeding is more effective for the improvement of oligogenic characters such as disease resistance than polygenic traits.

3. Mutation breeding is the simple, quick and best way when a new character is to be induced in vegetatively propagated crops.

4. When no improvement is possible this method has to be adopted.

Limitations :

1. Frequency of desirable mutations is very low about 0.1 percent. To detect the desirable one in M_2 considerable time, labour & other resources are to be employed.

2. To screen large population, efficient quick and unexpensive selection techniques are needed.

3. Desirable mutations may be associated with undesirable side effects due to other mutations thus extending the mutation breeding programme.

4. 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.

Achievements :

a) Natural mutants :

Rice : GFB 24 – arose as a mutant from Konamani variety Dee – Gee – Woo – Gen – Arose as a mutant from rice in China

MTU 20 – arose as a mutant from MTU-3

Sorghum Co. 18 – arose as a mutant from Co. 2

Cotton : DB 3-12 from G. heroaccum variety Western 1

b) Induced mutants :

Rice : Jagannath-gamma ray induced mutant from T.141

Wheat : Sarbati Sonora Gamma radiation from Sonora 64

NP 836 mutants, through irradiation from NP 709

Cotton : Indore 2 Induced from Malwa upland 4

MLU 7 gamma ray induced mutant from culture 1143 EE MLU 10 gamma ray induced mutant from MLU 4

Mustard : Primax whicte (1950)
Summer Pope seed Regina I (1953)
Sugarcane : Co.8152 gamma ray induced mutant from Co. 527
Groundnut : NC 4
Cas tor : Aruna (NPH1) – Fast neutrons induced mutant from HC 6

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 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 case of 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.

Autopolyploid

Origin and production of doubled chromosome numbers:

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 poluploid clls and some of the shoot buds regenerated from the callus may be polyuploid. In solanaceae 6- 36% of adventitious buds are tetraplods. 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 tretment centrifugation , x-ray or gamma ray irradiation may produce polyplods. Exposing the plants or ears of maize to a temperature of 38- 45 ^{o}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.

Autopolyplody: In autopolyploidy, triploidy, tetraploidy and higher levels of ploidy are included.

Morphological and cytological features of auto

polyploids :

The general features are summerised below.

1. Polyploids have larger cell size than diploids. Guard cells of stomats are larger the number of stomata per unit area is less in polyploids than diploids.
2. Pollen grains of polyploids are generally larger than those of the corresponding diploids.
3. Polyploids are generally slower in growth and later in flowering.
4. Polyploids usually have larger and thicker leaves, and larger flowers and fruits which are usually less in number than in diploids.
5. Polyploids generally show reduced fertillility due to irregularities during meiosis and due to genotypic imbalance leadint 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 the optimum level is 3x, sweetpotato 6x while for timothy grass it is between 8-10x.
8. Autopolyploids generally have a lower dry matter content than diploids.

Application of Autopolyploidy in Crop Improvement

Triplods:

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) and diploid

(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 stivus*) 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 problem viz. genetic instability of 4x lines, irregular fruit shape, a tendency towars hollowness of fruits, production of empty seeds and the labour involved in trioploid seed production.

1. **Triploid sugarbeets** : Among root crops triploid sugar beets apparently represent the optimum level of polyploidy because 3n plants have longer roots than diploid and also yield more sugar per unit area.
2. **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 maiontained 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 a simplex 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. There genotypes AAaa, Aaaa and aaa in the ratio 1:2:1 giving the phenotypic ratio of 3 : 1. while produces 3 types of gametes viz., AA, Aa and aa in the ratio of 1:12:15 due to random chromatid segregation selfing of a simplex in such a case is expected produce the following progeny.

Quadraplix	AAAA	1
Triplex	AAA a	24
Duplex	AAaa	174
Simplex	Aaaa	360
Nulliplex	aaaa	225
		784

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 and extological 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 chracters 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 hhomoeologous chromosomes. After chromosome doubling, the allopolyploid would have two homelegous chromosomes for each chromosome present in the F_1 hybrid, comparable to the diploid species. Such allopolyploid is referred as amphidiploids or Allotetraploid.
4. Fertility of Allopolyploids can be improved by hybridization and selection.

Application of Allopolyploidy in crop Improvement:

1. **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 the use of synthetic N.digluta or transfer of resistance to tobacco mosaic virus from N.glutinosa to N.tabacum. The F_1 hybrid from the cross N.tabacum x N.glutinosa is sterile. Chromosome doubling of the F_1 hybrid pr oduces 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.

2. **Creation of New crop species**

Ex : Triticales, Raphanobrassica

Triticum turgidum xsecale cereale.

3. **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 s pecies. 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.

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.

CHAPTER-16

BREEDING FOR ABIOTIC STRESS RESISTANCE (Drought, Cold, Salinity and alkalinity)

1. Temperature stress

a. Cold resistance / tolerance: This is applicable in case of rice grown in Gudalur taluk of Nilgiris and Cumbum valley. Numerous methods have been developed for the evaluation of cold hardiness. This included artificial low temperature and freeze tests. However, none them is useful for single plant selection. This is a handicap for the breeder. Testing the segregating lines under field condition is the most suitable one. But this will be time consuming and often favorable conditions may not be a available.

b. High temperature: Due to high temperature seed set may be affected. In case of male sterile lines, the sterility may be broken down. In this case also testing single plants for high temperature resistance is time consuming and skill is required. Tests like heat test with leaf discs and desiccation tolerance test are followed.

. Water stress

a. Low water i.e., Drought resistance: This is more important for all the dry land crops. 75% of area is cultivated under rainfed conditions and drought tolerance is more important. Drought resistance in crop plants can be divided in to three categories. i. Drought escape - ability of a plant to complete its life cycle before serious soil and plant water deficit occurs. ii. Drought tolerance with high tissue water potential iii. Drought tolerance with low tissue water potential Drought resistance in crop plants are more due to physiological conditions of plant like stomatal aperture and photosynthetic rates, root characteristics. Various techniques have been developed to test drought resistance. One e.g. is accumulation of proline in leaves. Because of the high skill needed in evaluating the single plants the process is tedious.

b. Excess water: This is the case in places like tail end areas of Cauvery delta. here the paddy varieties must have long stem - ie., deep water paddy. The screening procedure is done both under field conditions and laboratory conditions.

3. Chemical stress

a. Salinity and alkalinity : Screening for salinity and alkalinity can be done more successfully by in vitro techniques. Raising the seedling in test tube containing different concentration of salt is done in case of rice. This is followed in case of pesticide and herbicide tolerance also.

4. Difficulties in abiotic stress breeding

i. Screening techniques require high skill and they are time consuming

ii. Creation of artifical conditions is expensive.

Under field screening, nature may or may not provide optimum condition for screening. iv. In

many cases *in vitro* techniques are to be followed which is expensive. v. Abiotic stress breeding depends mostly on physiological traits which are often not stable.
B. Breeding for Drought resistance variety High yield x High cuticular wax content (Poor cuticular Transpiration) F1 (F1 tested under moisture stress condition) F2

1. Progeny rows screened in moisture stress nursery in two locations

1. Selection based on cuticular wax and no agronomic characters are considered F3 Selected single plants - Screened under normal conditions for yield and then associated characters F4 Selected single plants - Screened under stress situation F5 - Normal condition - yield F7/F8 Homogeneity with relative resistance to drought and with considerable yield

2. Converge genes for yield and drought resistance

C. Breeding for Drought Resistance 1. Breeder search for a source for Drought resistance

2. Yield should be a secondary character Economic Parts

3. Partitioning of photosynthates Vegetative Parts Total Dry matter should be taken as a criterion for selection.

Drought Resistance

Drought avoidance

Drought tolerance

1. Xeromorphic traits 1. Root Growth 2. Stomal control 3. Cuticular resistance 108 (water permeability of leaf cuticle 4. Stomatal No. (transpiration low, low stomatal frequency and high photosynthetic rate) 5. Cell tungor (Inhibit plant growth) (root water absorption ⇔ stomatal water loss)
D. Screening for salt tolerance Rice varieties of differing : IR.20 & IR.50 (susceptible) salt tolerance level : Co 43 & Manoharsali (Moderately tolerant) : Dasal & Pokkali (highly tolerant)
1. **Salinized soil method** Crosses were made between suceptible and moderately tolerant; suceptable x highly tolerant; and moderately x highly tolerant types. The parents along with F1 progenies and subsequent segregating progenies have to be screened for their tolerance. Plastic tubs (45 x 30 x 45 cm) with 10kg of soil was taken one with normal soil and others salinized wit 6 liters of 0.3 % NaCl solution, so that the electrical conductivity was raised to 4.9 M m/cm uniformly in all the tubs. Then the plant materials (labeled 20 day old seedlings) to be tested are planted in the tub with a spacing of 15 x 10 cm so that each tub caries 6 seedlings. Normal cultural practices were followed and irrigated daily to maintain a water level of 1 cm above the soil level. Once a week the soil between the plants was carefully racked to facilitate mixing and aeration. The plants were grown to maturity and data were recorded for yield characters. The cultivar which recorded a grain yield on par with culture in control is selected as tolerant.

CHAPTER-17

DNA Markers and Marker Assisted Selection

Introduction

Markers are any trait of any organism that can be identified with confidence and relative ease which can be also followed in mapping population. In general, markers are heritable entities associated with economically important trait under the control of polygenes. In particular, molecular markers were used in genome scans to select those individuals that contain the greatest proportion of favorable alleles from the recurrent parent genome. Molecular breeding is currently standard practice in many crops. Molecular breeding may be defined as genetic manipulation performed at DNA molecular levels to develop characters of interest in plants, including genetic engineering or gene manipulation, molecular marker-assisted selection, genomic selection, etc. However, molecular breeding also implies molecular marker-assisted breeding (MAB) which may be defined as the novel application of molecular markers, in permutation with linkage maps and genomics, to alter and improve plant traits on the basis of genotypic assays.

In the current scenario, the DNA markers become the marker of choice for improving genetic diversity of crops and had revolutionised the plant biotechnology. Gradually, methods are developing more specifically, rapidly and cheaply to assess genetic variation. Results obtained after successful usage of molecular markers indicated that when inbreed lines were unrelated, a measurement of relative relationship based on proportion of homomorphic marker loci, was significantly correlated with a measure of relationship based on yield. Conversely, when lines were related the correlations were low. With the rise of DNA marker technology, several types of DNA markers and molecular breeding strategies are now available to plant breeders and geneticists, helping them to over- come many of the problems faced during conventional breeding. In this chapter, basic qualities of molecular markers, their characteristics, usage and their applications will be discussed.

GENETIC MARKERS: CONCEPT AND TYPES

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 chromosome or a gene. Genetic markers that are positioned in near vicinity to genes may be stated as gene ‘tags’.

Such markers do not intrude the phenotype of the trait of interest as they are located only adjacent or 'linked' to genes regulating the trait. All genetic markers occupy definite genomic positions within chromosomes (like genes) called 'loci' (singular 'locus'). The quality of a genetic marker is typically measured by its heterozygosity in population of interest.

Genetic markers may differ in key features like genomic abundance, level of polymorphism, locus specificity and reproducibility. No marker is superior to all others for a wide range of applications. The selection of appropriate marker depends on specific application, presumed polymorphism level, economical variability. Genetic markers were originally used in genetic mapping to determine the order if genes along the chromosomes. In 1913, Alfred Sturtevant generated the first genetic map using sex morphological traits (termed 'factors') in fruit fly *Dorsophila melanogaster* and soon after, Karl sax produced evidence for genetic linkage in common bean . Since these pioneer studies, genetic markers have evolved from morphological markers through isozymes markers to DNA markers. Today, genetic markers are used in both basic plant research, for gene isolation, plant breeding for characterizations of germplasm, for marker assisted introgression of favourable alleles and for variety protection.

Genetic markers used in genetics and plant breeding can be classified into two categories: classical markers and DNA markers.

Classical markers are further classified as morphological markers, cytological markers and biochemical markers.

DNA markers developed into many systems based on different polymorphism detecting techniques or methods (southern blotting, PCR , and DNA sequencing), such as RFLP, AFLP, RAPD, SSR, SNP, etc.

Classical Markers

Morphological Markers

Use of markers as a supportive tool for selection of plants with preferred traits had started in plant breeding long time ago. In early history of plant breeding, markers were used usually for visual phenotype character identification such as leaf shape, flower color, pubescence color, pod color, seed shape, hilum color, type and length, fruit shape, etc. These morphological markers mostly signify genetic polymorphisms which can be simple to identify and manipulate.

Therefore, they are usually used in construction of linkage maps by classical two- and/or three- point tests. Some of these markers which are linked with other agronomic traits and thus can be used as indirect selection criteria in practical breeding. In scientific terms, **morphological markers are those traits that are scored visually, or morphological markers are those genetic markers whose inheritance can be followed with the naked eye**.

The main **advantages** of using morphological markers are easily monitoring and economically viable. However, molecular markers are severely affected by the external environment. Such markers regularly cause major alternations in the phenotype which leads to failure of breeding programs. Morphological markers are also limited in number and appears late in plant development which makes scoring almost impossible. Also, Morphological markers are dominant in nature.

The best example for successful usage of morphological marker that could be considered is selection of semi dwarfism in rice and wheat leading to high yield cultivation of crop. This could be regarded as a suitable illustration for effective use of morphological markers to modern breeding.

Cytological markers: Cytological markers are used for the identification of structural features of chromosomes. Cytological markers can be revealed by chromosome karyotypes and bands. The distributional differences of euchromatin and hetrochromatin in chromosomes including its color, width, order and position in banding patterns can be studied using cytological markers. For example, Q bands are formed by quinacrine hydrochloride, G bands are visualized by Giemsa stain, and R bands are the reversed G bands. The applications of identified chromosome landmarks are not only for classification of normal chromosomes and uncovering of chromosome mutation, but also broadly used in physical mapping and linkage group identification. The physical maps created using combination of morphological and cytological markers lays a perfect basis for genetic linkage mapping with the assistance of modern molecular techniques. However, direct usage of cytological markers has been very limited in genetic mapping and plant breeding.

Molecular markers: Molecular markers have become the marker of choice for the study of plant genetic diversity. Molecular markers are heritable differences in nucleotide sequences of DNA at the corresponding position on homologous chromosome of two dfferent individuals,

W hich follow a simple Mendelian pattern of inheritance. Over the last two decades, the advent of molecular markers has revolutionized the entire scenario of biological sciences. DNA-based molecular m a r k e r s are a versatile tool in the fields of taxonomy, physiology,embryology, genetic engineering, etc. They are no longer looked upon in simple DNA fingerprinting markers in variability studies or in mere forensic tools. Ever since the development of molecular markers, these are constantly being modified to enhance the utility and to bring about automation in the process of genome analysis.
The discovery of PCR (polymerase chain reaction) was a landmark in this effort and proved to be a unique process that brought about a new class of DNA profiling markers. This facilitated the development of marker- based gene tags, genetic mapping, map-based cloning of agronomically important genes, genetic diversity studies, phylogenetic analysis, and marker-assisted selection of desirable genotypes etc. Thus, giving new dimensions to breeding and marker-aided selection, that can reduce the time span of developing new and improved varieties and the dream of super varieties come true. These DNA markers offer several advantages over traditional phenotypic markers, as molecular markers are not environmentally influenced and provides data that can be analyzed objectively. The existence of various molecular techniques and differences in their principles and methodologies require careful consideration in choosing one or more of such marker.

Types of Molecular Markers

Due to the rapid developments in the field of molecular genetics, varieties of different techniques have emerged to analyze genetic variation during the last few decayed. These genetic markers may differ with respect to important features, such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements and financial investment. No marker is superior to all others for a wide range of applications. The most appropriate genetic marker has depend on the specific application, the presumed level of polymorphism, the presence of sufficient technical facilities and knowhow, time constraints and financial limitations.

A. Biochemical Marker - Allozymes (Isozyme)

Isozymes analysis has been used for over 60 years for various research purposes in biology, viz. to delineate phylogenetic relationships, to estimate genetic variability and taxonomy, to study population genetics and developmental biology, to characterization in plant genetic resources management and plant breeding. Isozymes were defined as structurally

different molecular forms of an enzyme with, qualitatively, the same catalytic function. Isozymes originate through amino acid alterations, which cause changes in net charge, or the patial structure (conformation) of the enzyme molecules and also, therefore, their electrophoretic mobility. After specific staining the isozyme profile of individual samples can be observed (Hadacová & Ondrej 1972, Vallejos 1983, Soltis & Soltis 1989).

Allozymes are allelic variants of enzymes encoded by structural genes. Enzymes are proteins consisting of amino acids, some of which are electrically charged. As a result, enzymes have a net electric charge, depending on the stretch of amino acids comprising the protein. When a mutation in the DNA results in an amino acid being replaced, the net electric charge of the protein may be modified, and the overall shape (conformation) of the molecule can change. Because of changes in electric charge and conformation can affect the migration rate of proteins in an electric field, allelic variation can be detected by gel electrophoresis and subsequent enzyme-specific stains that contain substrate for the enzyme, cofactors and an oxidized salt (e.g. nitro-blue tetrazolium). Usually two, or sometimes even more loci can be distinguished for an enzyme and these are termed isoloci. Therefore, allozyme variation is often also referred to as isozyme variation isozymes have been proven to be reliable genetic markers in breeding and genetic studies of plant species, due to their consistency in their expression, irrespective of environmental factors.

Advantages:

Isoenzymes/ allozymes markers are the oldest among the molecular markers and has following advantages-

i. Doesn't require DNA extraction or the availability of sequence information, primers or probes, they are quick and easy to use.

ii. Simple analytical procedures, allow some allozymes to be applied at relatively low costs, depending on the enzyme staining reagents used.

iii. Allozymes are codominant markers that have high reproducibility.

Disadvantages: The main weakness of allozymes is

i. relatively low abundance and low level of polymorphism.

ii. Like phenotypic markers they may be affected by environmental conditions.

iii. Stage and tissue specific.

B. DNA based Molecular Markers: A molecular markers a DNA sequence that is readily

detected and whose inheritance can be easily be monitored. The uses of molecular markers are based on the naturally occurring DNA polymorphism, which forms basis for designing strategies to exploit for applied purposes. A marker must to be polymorphic i.e. it must exit in different forms so that chromosome carrying the mutant genes can be distinguished from the chromosomes with the normal gene by a marker it also carries. Unlike protein markers, DNA markers segregate by following simple Mendelian Genetics and they are not affected by the environment. DNA is easily extracted from plant materials and its analysis can be cost and labour effective.

Characteristics of ideal DNA marker

1) Highly polymorphic in nature: It must be polymorphic as it measures for various genetic diversity studies.

2) Codominant inheritance: determination of homozygous and heterozygous states of diploid organisms.

3) Frequent occurrence in genome: A marker should be evenly and frequently distributed throughout the genome.

4) Selective neutral behaviours: The DNA sequences of any organism are neutral to environmental conditions or management practices.

5) Easy access (availability): It should be easy, fast and cheap to detect.

6) Easy and fast assay

7) High reproducibility

8) Easy exchange of data between laboratories.

Types of DNA markers:

1. Hybridization based DNA marker
2. PCR based DNA marker
3. Sequencing based DNA marker

1. Hybridization based DNA marker:

a) Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of particular Restriction Endonucleases, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species

(and even strains) from one another. This technique is mainly based on the special class of enzyme i.e. Restriction Endonucleases. In RFLP analysis, restriction enzyme-digested genomic DNA is resolved by gel electrophoresis and then blotted (Southern 1975) on to a nitrocellulose membrane. Specific banding patterns are then visualized by hybridization with labeled probe.

Advantages:

i) RFLPs, being co-dominant markers, can able to detect homozygous *vs* heterozygous.

ii) RFLPs are generally found to be moderately polymorphic.

iii) High reproducibility

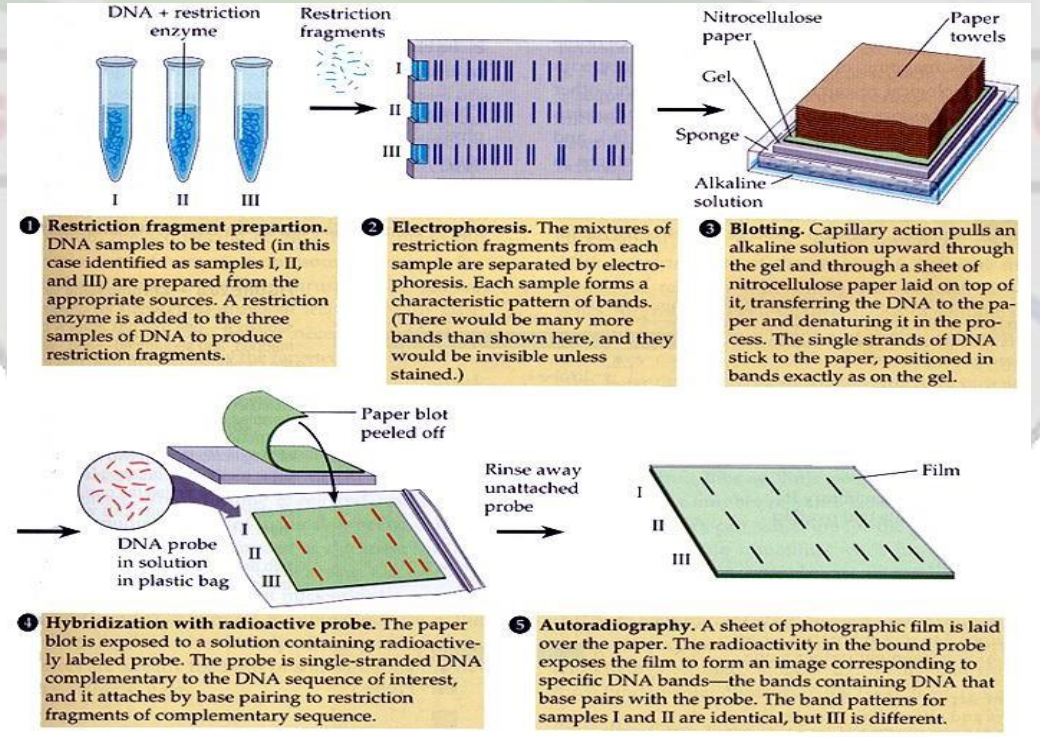
Disadvantages:

i) Requirement of large quantities (1–10 μg) of purified, high molecular weight DNA

ii) The requirement of radioactive isotope makes the analysis relatively expensive and hazardous.

iii) The assay is time-consuming and labourintensive.

iv) Their inability to detect single base changes restricts their use in detecting point mutations occurring within the regions at which they are detecting polymorphism.



1 Restriction fragment prepartion. DNA samples to be tested (in this case identified as samples I, II, and III) are prepared from the appropriate sources. A restriction enzyme is added to the three samples of DNA to produce restriction fragments.

2 Electrophoresis. The mixtures of restriction fragments from each sample are separated by electrophoresis. Each sample forms a characteristic pattern of bands. (There would be many more bands than shown here, and they would be invisible unless stained.)

3 Blotting. Capillary action pulls an alkaline solution upward through the gel and through a sheet of nitrocellulose paper laid on top of it, transferring the DNA to the paper and denaturing it in the process. The single strands of DNA stick to the paper, positioned in bands exactly as on the gel.

4 Hybridization with radioactive probe. The paper blot is exposed to a solution containing radioactively labeled probe. The probe is single-stranded DNA complementary to the DNA sequence of interest, and it attaches by base pairing to restriction fragments of complementary sequence.

5 Autoradiography. A sheet of photographic film is laid over the paper. The radioactivity in the bound probe exposes the film to form an image corresponding to specific DNA bands—the bands containing DNA that base pairs with the probe. The band patterns for samples I and II are identical, but III is different.

PCR based DNA marker

a) Random Amplified Polymorphic DNA (RAPD)

RAPD is a PCR-based technology. The method is based on enzymatic amplification of target or random DNA segments with arbitrary primers. In 1991 Welsh and McClelland developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD). This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer anneals to the genomic

DNA at two different sites on complementary strands of DNA template. If these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through thermo cyclic amplification. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals (William et al.1993). RAPDs are DNA fragments amplified by the PCR using short synthetic primers (generally 10 bp) of random sequence. These oligonucleotides serve as both forward and reverse primer, and are usually able to amplify fragments from 1–10 genomic sites simultaneously. Amplified products (usually within the 0.5–5 kb size range) are separated on agarose gels in the presence of ethidium bromide and view under ultraviolet light and presence and absence of band will be observed. These polymorphisms are considered to be primarily due to variation in the primer annealing sites, but they can also be generated by length differences in the amplified sequence between primer annealing sites.

Advantages:

i) The main advantage of RAPDs is that they are quick and easy to assay.

ii) Low quantities of template DNA are required, usually 5–50 ng per reaction.

Since random primers are commercially available, no sequence data for primer construction are needed.

Disadvantages:

i) The main drawback of RAPDs is their low reproducibility

ii) The inherent problems of reproducibility make RAPDs unsuitable markers for transference or comparison of results among research teams working in a similar species and subject.

iii) RAPD, being dominant markers, can't able to detect homozygous *vs* heterozygous.

iii)

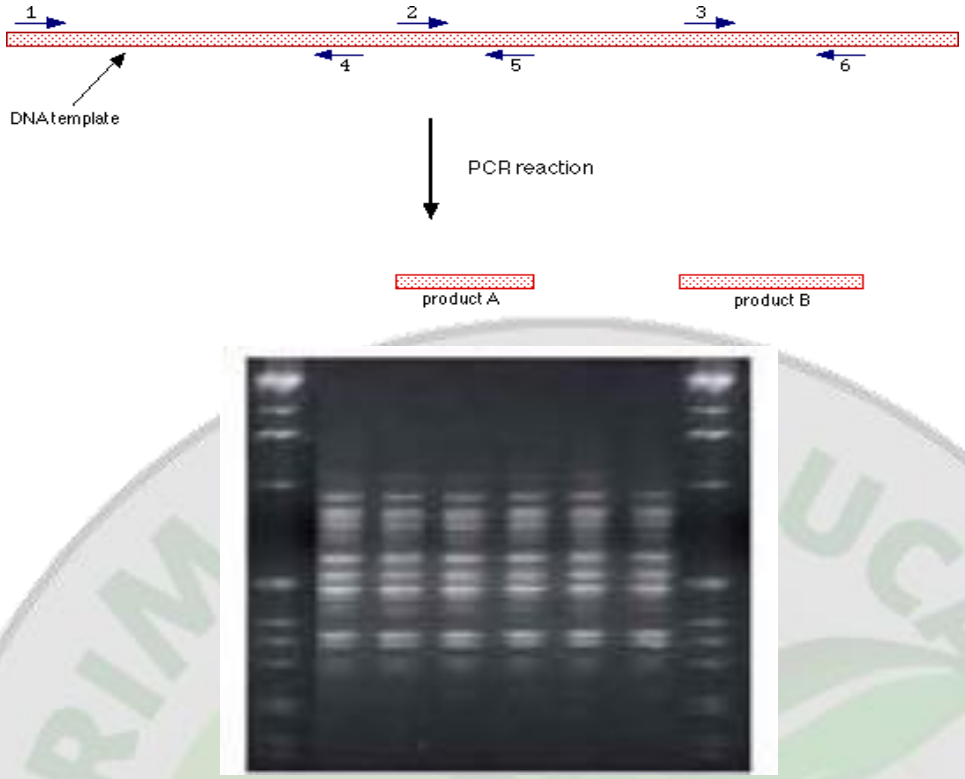


Figure 2. RAPD analysis. In the upper panel binding of random primes to the double stranded DNA has been shown. Gel picture showing the RAPD pattern.

b) AFLP (Amplified Fragment Length Polymorphism)

Amplified fragment length polymorphism (AFLP), which is essentially intermediate between RFLPs and PCR. AFLP is based on a selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases. Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE) (Matthes et al. 1998)or by capillary electrophoresis. The technique involves four steps: (1) restriction of DNA and ligation of oligonucletide adapters; (2) preselective amplification; (3) selective amplification; (4) gel analysis of amplified fragments. AFLP is a DNA fingerprinting technique, which detects DNA restriction fragments by means of PCR amplification. AFLP involves the restriction of genomic DNA, followed by ligation of adaptors complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. These fragments are viewed on denaturing polyacrylamide gels either through autoradiographic or fluorescence methodologies. AFLPs are DNA fragments (80–500 bp) obtained from digestion with restriction enzymes, followed by ligation of oligonucleotide adapters to the digestion products and selective amplification by the PCR.

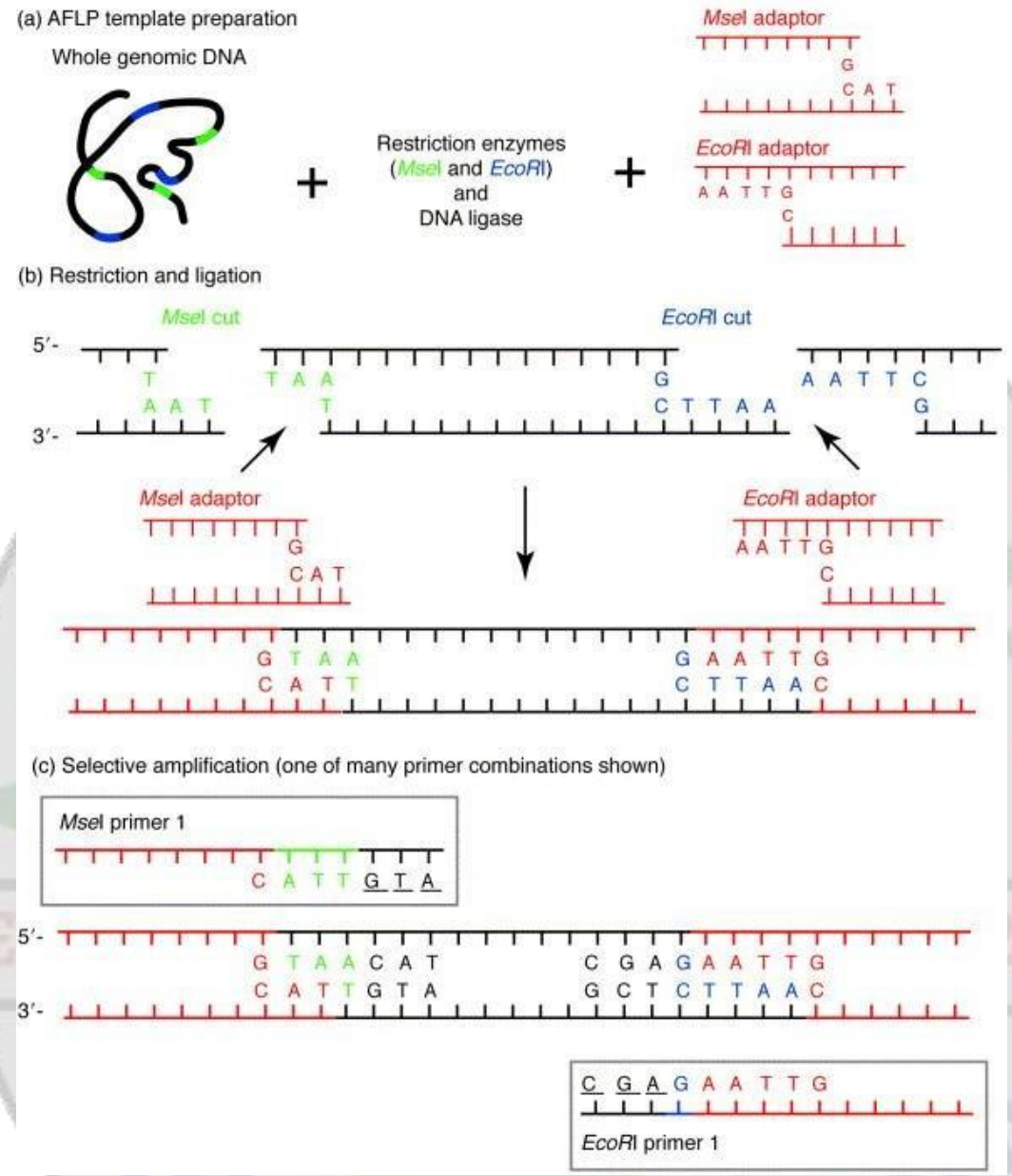


Figure 3: Schematic representation of the AFLP analysis principle.

Advantages:

i) high genomic abundance and considerable reproducibility in nature

ii) no sequence data for primer construction are required

iii) high number of polymorphic bands will be formed by single reaction

Disadvantages:

i) Dominant in nature

ii) Technically demanding

iii) Need to use kits

Minisatellites, Variable Number of Tandem Repeats (VNTR)

These loci contain tandem repeats that vary in the number of repeat units between genotypes and are referred to as variable number of tandem repeats (VNTRs) (i.e. a single locus that contains variable number of tandem repeats between individuals) or hypervariable regions (HVRs) (i.e. numerous loci containing tandem repeats within a genome generating high levels of polymorphism between individuals). Minisatellites are a conceptually very different class of marker. They consist of chromosomal regions containing tandem repeat units of a 10–50 base motif, flanked by conserved DNA restriction sites. A minisatellite profile consisting of many bands, usually within a 4–20 kb size range, is generated by using common multilocus probes that are able to hybridize to minisatellite sequences in different species.

Advantages:

i) The main advantages of minisatellites are their high level of polymorphism and high reproducibility.

ii) No sequence information is required for designing primers.

iii) Co-dominant in nature

iv) Occurs throughout the genome

Disadvantages:

i) Require synthesis of large number of primers

Sequence based molecular marker

Simple sequence repeats (SSRs), also known as microsatellites were recognized and presented a preference for many genetic researcher for its low to high-throughput approaches. They are random tandem repeats of short nucleotide motifs (2 - 6 bp). SSRs are high frequency polymorphic sequences present in animals and plants, and utilized to study relationship between inherited traits within a species. Microsatellite markers are the sequences from noncoding regions of genome.

Polymorphism is based on the variation in the number of repeats in different genotypes. In recent years, SSR markers can easily be developed *in silico* due to the availability of large-scale gene (expressed sequence tag) EST sequence information for many plant species. The high degree of polymorphism as compared to RFLPs and RAPDs, their co-dominant nature and locus

specific make them the markers of choice for a diversity of purposes including practical plant breeding. Therefore, (SSRs) have become a marker of choice for an array of applications in plants due to extensive genome coverage and hyper variable nature.

Inter Simple Sequence Repeats (ISSRs)

It includes the enhancement of DNA fragments present at an amplifiable separation in the middle of two indistinguishable microsatellite rehash locales situated in inverse bearings. The procedure utilizes microsatellites as basis as a part of a solitary preliminary PCR response focusing on numerous genomic loci to increase basically ISSR of diverse sizes. The microsatellite repeats utilized for ISSRs can be dinucleotide, trinucleotide, tetranucleotide, or pentanucleotide. This primer utilized can be either unanchored or all the more for the most part secured at 3` or 5` end with one to four ruffian bases reached out into the flanking groupings. ISSRs utilize longer primer (15-30 mers) when contrasted with RAPD (10 mers), which allow the resulting utilization of a high annealing temperature prompting higher stringency. In addition, ISSRs, as RAPDs, may have reproducibility issues. ISSR examination can be connected in studies including hereditary character, parentage, clone and strain recognizable proof, and taxonomic investigations of firmly related species and in addition in quality mapping studies.

3. Sequencing based DNA marker

Single Nucleotide Polymorphisms (SNPs)

SNP is a single nucleotide polymorphism in which single base difference between two DNA sequences of samples, SNPs are typically biallelic and generated due to substitutions/point mutations (transversion and transition) or as a result of deletion/insertion of nucleotides.

SNPs provide the simplest and ultimate form of molecular markers as a single nucleotide base is the smallest unit of inheritance, and therefore they can provide a great marker density. High density of SNPs over other markers makes it advantageous to find more probability of polymorphisms in a target gene at best closely linked to a locus of interest. Typically, SNP frequencies are in a range of one SNP 100 - 300bp in plants. SNPs are present within coding, non-coding regions or in the intergenic regions between genes at different frequencies in different chromosomal segments.

SNP discovery methods are broadly categorized into four segments: hybridization with

allele-specific oligonucleotide probes, oligonucleotide ligation, enzymatic cleavage, and single nucleotide primer extension. In principle, the SNP methods show differences between a probe of known sequence and a target DNA containing the SNP site. The target DNA sections are typically PCR products and mismatches with the probe reveal SNPs within the amplified target DNA segment. The mismatched DNA can be sequenced to identify SNP polymorphisms.

Now a day, it is very gainful and easier to quickly identify a large number of SNPs within limited time frame in any plant species. This is due to the emergence of the third generation DNA sequencing technologies. The advantage of this new sequence technology are expected to further reduction in sequencing costs to $1 per mega base compared to $60, $2, and $1 expected costs for sequences generated by next generation sequencing (Thudi et al., 2012).

Table. Comparison of Molecular Markers

Feature	RFLP	RAPD	AFLP	SSRs	SNPs
DNA Require (µ g)	10	.02	.5-1.0	.05	.05
DNA quality	High	High	Moderate	Moderate	High
PCR based	No	Yes	YES	YES	YES
No. of Polymorph loci analyzed	1-3	1.5-50	20-100	1-3	1
Ease of use	Not Easy	Easy	Easy	Easy	Easy
Amenable to automation	Low	Moderate	Moderate	High	High
Reproducibility	High	Unreliable	High	High	High
Development Cost	Low	Low	Moderate	High	High
Type of probes/primers	Low copy DNA or cDNA clones	10 bp random nucleotides	Specific sequence	Specific sequence	Allelespecific PCR primers
Effective multiplex ratio	Low	Moderate	High	High	Moderate to high
Marker index	Low	Moderate	Moderate to high	High	Moderate
Genotyping throughput	Low	Low	High	High	High
Primary application	Genetics	Diversity	Diversity and genetics	All purposes	All purposes

MARKER-ASSISTED SELECTION (MAS)

MAS procedure and theoretical and practical considerations

Marker-assisted selection (MAS) refers to such a breeding procedure in which DNA marker detection and selection are integrated into a traditional breeding program. Taking a single cross as an example, the general procedure can be described as follow:

Select parents and make the cross, at least one (or both) possesses the DNA marker allele(s) for the desired trait of interest.

a) Plant F_1 population and detect the presence of the marker alleles to eliminate false hybrids.
b) Plant segregating F_2 population, screen individuals for the marker(s), and harvest the individuals carrying the desired marker allele(s).
c) Plant F2:3 plant rows, and screen individual plants with the marker(s). A bulk of F3 individuals within a plant row may be used for the marker screening for further confirma- tion in case needed if the preceding F2 plant is homozygous for the markers. Select and harvest the individuals with required marker alleles and other desirable traits.
d) In the subsequent generations ($F_4$and F5), conduct marker screening and make selection similarly as for F2:3s, but more attention is given to superior individuals within homozygous lines/rows of markers.

f. In F5:6 or F4:5 generations, bulk the best lines according to the phenotypic evaluation of target trait and the performance of other traits, in addition to marker data.

g. Plant yield trials and comprehensively evaluate the selected lines for yield, quality, re- sistance and other characters of interest.

In MAS, phenotypic evaluation and selection is still very helpful if conditions permit to do so, and even necessary in cases when the QTLs selected for MAS are not so stable across environments and the association between the selected markers and QTLs is not so close. Moreover, one should also take the impact of genetic background into consideration. The presence of a QTL or marker does not necessarily guarantee the expression of the desired trait. QTL data derived from multiple environments and different populations help a better understanding of the interactions of QTL x environment and QTL x QTL or QTL x genetic

background, and thus help a better use of MAS. In addition to genotypic (markers) and phenotypic data for the trait of interest, a breeder often pays considerable attention to other important traits, unless the trait of interest is the only objective of breeding.

There are several indications for adoption of molecular markers in the selection for the traits of interest in practical breeding. The situations favorable for MAS include:

The selected character is expressed late in plant development, like fruit and flower fea- tures or adult characters with a juvenile period (so that it is not necessary to wait for the plant to become fully developed before propagation occurs or can be arranged)

• The target gene is recessive (so that individuals which are heterozygous positive for the recessive allele can be selected and/or crossed to produce some homozygous offspring with the desired trait)

• Special conditions are required in order to invoke expression of the target gene(s), as in the case of breeding for disease and pest resistance (where inoculation with the disease or subjection to pests would otherwise be required), or the expression of target genes is highly variable with the environments.

• The phenotype of a trait is conditioned by two or more unlinked genes. For example, selection for multiple genes or gene pyramiding may be required to develop enhanced or durable resistance against diseases or insect pests.

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CHAPTER-18

Intellectual Property and its Protection

Intellectual property (IP) refers to creations of the mind, such as inventions; literary and artistic works; designs; and symbols, names and images used in business and commerce. As compared to movable and immovable property the characteristic feature of Intellectual property which differentiates it from other types of properties is that it is "intangible" in nature, that is, it cannot be defined or identified by its own physical parameters. It must be expressed in some discernible way to be protectable. Examples of intellectual properties include – patents, trademarks, copyrights, and trade secrets, geographical indications, plant varieties, semi-conductors and integrated circuits etc.

IP is protected in law by, for example, patents, copyright and trademarks Acts, which enable people to earn recognition or financial benefit from what they invent or create. By striking the right balance between the interests of innovators and the wider public interest, the IP system aims to foster an environment in which creativity and innovation can flourish.

Intellectual property rights are customarily divided into two main areas

i) Copyright and rights related to copyright

The rights of authors of literary and artistic works (such as books and other writings, musical compositions, paintings, sculpture, computer programs and films) are protected by copyright, for a defined period after the death of the author.

Copyright is a legal term used to describe the rights that creators have over their literary and artistic works. Works covered by copyright range from books, music, paintings, sculpture and films, to computer programs, databases, advertisements, maps and technical drawings.

Also protected through copyright and related (sometimes referred to as "neighbouring") rights are the rights of performers (e.g. actors, singers and musicians), producers of phonograms (sound recordings) and broadcasting organizations. The main social purpose of protection of copyright and related rights is to encourage and reward creative work.

ii) Industrial property

Industrial property can further be divided into two main areas

a) One area can be characterized as the protection of distinctive signs, in particular trademarks (which distinguish the goods or services of one undertaking from those of other undertakings) and geographical indications (which identify a good as originating in a place where a given characteristic of the good is essentially attributable to its geographical origin).

A trademark is a sign capable of distinguishing the goods or services of one enterprise from those of other enterprises. Trademarks date back to ancient times when craftsmen used to put their signature or "mark" on their products.

The protection of such distinctive signs aims to stimulate and ensure fair competition and to protect consumers, by enabling them to make informed choices between various goods and services. The protection may last indefinitely, provided the sign in question continues to be distinctive.

Geographical indications and appellations of origin are signs used on goods that have a specific geographical origin and possess qualities, a reputation or characteristics that are essentially attributable to that place of origin. Most commonly, a geographical indication includes the name of the place of origin of the goods.

b) Other types of industrial property are protected primarily to stimulate innovation, design and the creation of technology. In this category fall inventions (protected by patents), industrial designs and trade secrets.

A patent is an exclusive right granted for an invention. Generally speaking, a patent provides the patent owner with the right to decide how - or whether - the invention can be used by others. In exchange for this right, the patent owner makes technical information about the invention publicly available in the published patent document.

An industrial design constitutes the ornamental or aesthetic aspect of an article. A design may consist of three-dimensional features, such as the shape or surface of an article, or of two-dimensional features, such as patterns, lines or color.

Trade secret: The Trade Related Aspects of Intellectual Property Rights (TRIPS) lays down three essential conditions which are to be fulfilled by any information before it can be considered undisclosed information (trade secret), they are

- Such information must be secret, i.e., not generally known or readily accessible to "persons within the circles that normally deal with the kinds of information in question."
- The information must have commercial value because it is secret
- The information must be the subject of reasonable steps by its owners to keep it secret

The social purpose is to provide protection for the results of investment in the development of new technology, thus giving the incentive and means to finance research and development activities.

What is Patent?

A patent is an exclusive right granted by a country to the owner of the invention for a limited period to make, use, manufacture and market the invention, provided the invention satisfy the criteria of novelty, non-obviousness and usefulness.

1. Novelty means- not known in prior-art
2. Non-obviousness means- the invention must involve inventive steps
3. Usefulness means- the invention must have industrial application
4. In case of varieties for registration at PPV and FR Authority office, the varieties must fulfill the criteria of distinctiveness, uniformity and stability (DUS).
5. Distinctiveness means- the variety must be distinct from the previously known varieties.
6. Uniformity means- the display of same traits in each plant of the variety.
7. Stability means- the retention of essential characteristics on reproduction.

Importance of patenting

The patent system originated on the basis of various considerations, its principle motive being to stimulate innovation and encourage investment in scientific research and progress.

By issuing patent protections, the government makes it possible that individuals engaged in scientific research are provided access to the necessary financial means to conduct the research and a return of the costs borne, which would not be possible if uncontrolled copying of inventions were permitted. In this way, the patent system ensures an appropriate sharing of costs among all those who will benefit from the technology covered by the patent. Because profits from patents depend on meeting market demands, this encourages potentially useful scientific

research and the introduction of these results into practice.

Protection of plant varieties system in India

Establishment of World Trade organization (WTO) at Geneva replaced the GATT (General Agreement on Tariffs and Trade), established in the year 1944 was the only legal rules for international trades. Eight round of multilateral trade negotiation under GATT was concluded with the constitution of WTO in 1994, a more extensive regulation than GATT. TRIPS (Agreement on Trade Related Aspect of Intellectual Property Rights) was administered by WTO which maintain minimum standard for many forms of Intellectual property. As a member nation of WTO, each country has to protect their plant varieties either by patent or any effective *sui generis* system as stated in the TRIPS article 27.3(b). *sui generis* means a system developed by your own. India adopted the *sui generis* system for the protection of plant varieties of our country as a result the **Protection of Plant Varieties and Farmers' Rights Act** came into force in the year 2001. The act covers almost all types of plants except microorganisms.

To whom exclusive rights are given under the act

There are several important provisions are kept in the act which makes it distinct and unique from other existing act of related kind. A variety is protected for 15 or 18 years depending upon the species for the owner or breeder of that variety. Beside the owner, provisions are also kept for other parties like researchers and farmers. These are briefly described below-

a. Breeders' rights

An exclusive right given to the breeders upon registration to produce, sell, market, distribute, import or export of the variety [Section 28 (1)]. The exclusive rights of the breeder is heritable to his successor or his agent or licensee.

b. Researchers' right

In the section 30 of this act is mentioned as to access of protected varieties by any researchers for research purposes. This Section states, 'Nothing contained in this act shall prevent (a) the use of any variety registered under this act by any person using such variety for conducting experiments or research; and (b) the use of a variety by any person as an initial source of a variety for the purpose of creating other varieties provided that the authorization of the breeder of a registered

variety is required where the repeated use of such variety as a parental line is necessary for commercial production of such other newly developed variety'.

c. Farmer's Rights

An exclusive right given to farmers in this act as to keep the interest of the farmers for continuing conservation and development of germplasm in the farmers field. The role of farmers in the current agricultural growth and development is enormous. Most the varieties released in the latter half of last century was through direct selection from the farmers' materials or improved through mass selection of available high yielding landraces. Following rights have been given to a farmer in this act-

1. A farmer has the right to save, use, reuse, exchange and sell of its farm produce including seed of a registered varieties.
2. Farmers can claim compensation from the breeder of a registered variety if it fails to do expected performance as mentioned at the time of registration.
3. Farmers are exempted from any fee for registration and in any proceedings under the act before registered or Authority or Tribunal or High Court.
4. If a variety is evolved, cultivated and conserved by a group of farmers or by a community, can be registrable for the group or for the community.
5. Farmers are entitled for benefit sharing if their genetic materials are used for development of new registered variety; he or she can apply for benefit sharing.
6. If a registered variety is unable to meet the requirement of the farmers with reasonable price than he or she can apply for compulsory license, where the right for seed production and marketing of that variety will be given to other agencies.
7. Farmers are exempted from the compensation if the registered variety is fails to do expected performance under suitable condition.
8. Farmers are protected from the innocent infringement if proven that at the time of infringement he or she was unaware about the act.
9. Farmers are entitled for recognition and rearward as Plant Genome Savoir Farmer Reward & Recognition if their conserved materials are used as donor for genes in the varieties registrable under this act. When a farming community is engaged in conservation, improvement and preservation of genetic resources of economic plant or

their wild relatives; the community would be eligible for the Plant Genome Savior Community Award.

Table 1: Duration of Protection of Plant Varieties under the Act.

Period of protection (in Years)			
Total	**Initial**		**Extended**
Trees & Vines	18 from date of registration	9	9
Other Crops	15 from date of registration	6	9
Extant Notified varieties	15 from date of notification of variety under the Seeds Act, 1966 by the Central Govt.		

Participatory Plant Breeding

What is PPB?

Broadly, PPB is the development of a plant breeding program in collaboration between breeders and farmers, marketers, processors, consumers, and policy makers (food security, health and nutrition, employment).

In the context of plant breeding in the developing world, PPB is breeding that involves close farmer-researcher collaboration to bring about plant genetic improvement within a species.

Developing a clear vision together with the stakeholders in the breeding process is important.

What are the goals do PPB?

- Increase production and profitability of crop production through the development and enhanced adoption of suitable, usually improved, varieties.
- Provide benefits to a specific type of user, or to deliberately address the needs of a broader range of users.
- Build farmer skills to enhance farmer selection and seed production efforts.

Why focus on PPB? Traditional breeder-directed breeding programs are very effective at developing varieties that can be used in farming systems that are fairly homogeneous, but less effective when the reality of the farmer is more complex and risk-prone. Sources of genetic variation and the maintenance and use of genetic diversity.

Functional Participation

- Plant breeders can direct their research according to the needs of the specific groups of farmers (women, men, rich, poor). The physical and economic resource bases of different people necessitate tailored research approaches.
- Farmers can assure plant breeders that they are assessing tradeoffs among traits correctly.
- On-farm research assures that varieties will produce well under “real life” conditions. On-farm research can be managed by the researcher, by the farmer, or by both.
- PPB ensures greater success of adoption of innovation by the farmers.

Empowering Participation

- Increasing farmer knowledge and skills so that farmers can participate more fully in the collaborative breeding efforts and be better at their own, personal efforts.

What activities can PPB include?

- Identifying breeding objectives
- Generating genetic variability (including the provision of plants to be included in breeding program)
- Selecting within variable populations to develop experimental varieties
- Evaluating experimental varieties (PVS - participatory variety selection)
- Variety release
- Popularization (diffusion of information about new variety and and how it is managed)
- Seed production

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