## DEPARTMENT OF GENETICS AND PLANT BREEDING

1. Course No. : GBPR 211
2. Course Title : **Principles of Plant Breeding**
3. Credit Hours : 3 (2+1)
4. General Objective : To impart knowledge to the students on the principles and procedures of plant breeding in self and cross pollinated crops to develop the high yielding varieties / hybrids
5. Specific Objectives

Theory

By the end of the course, the students will be able to

i. learn breeding procedures in self and cross pollinated crops

ii. understand exploitation of heterosis utilizing male sterility and other methods

iii. know about the various population improvement programmes

iv. study about the fundamentals of mutation, polyploidy and wide hybridization and their role in crop improvement

**Theory Lecture Outlines**

1. Definition, aim, objectives and scope of plant breeding
2. History and development of plant breeding – scientific contributions of eminent scientists – landmarks in plant breeding
3. Modes of reproduction – asexual reproduction (vegetative reproduction and apomixis) and sexual reproduction – their classification and significance in plant breeding
4. Modes of pollination – classification of crop species on the basis of mode of pollination – self-pollination – mechanisms promoting self-pollination – genetic consequences of self-pollination – cross-pollination – mechanisms promoting cross-pollination – genetic consequences of cross-pollination – – often cross-pollinated crops
5. Method of plant breeding – classification of plant breeding methods – methods of breeding for self-pollinated, cross-pollinated and asexually propagated species – brief account of breeding methods
6. Plant introduction – primary introduction and secondary introduction – history of plant introduction – plant introduction agencies in India – National Bureau of Plant Genetic Resources (NBPGR) and its activity – procedure of plant introduction – purpose of plant introduction – merits and demerits of plant introduction – germplasm collections – genetic erosion – gene sanctuaries
7. Selection – natural and artificial selection – basic principles of selection – basic characteristics and requirements of selection – selection intensity – selection differential – heritability – genetic advance

8. Mass selection – procedure for evolving a variety by mass selection – modification of mass selection – merits, demerits and achievements
9. Johannsen's pure line theory and its concepts and significance – origin of variation in pure lines – characters of pure lines – progeny test
10. Genetic basis of pure line selection – general procedure for evolving a variety by pure line selection – merits, demerits and achievements – comparison between mass and pure line selection
11. Biometrics – definition – qualitative and quantitative characters – role of environment in quantitative inheritance – biometrical techniques in plant breeding – components of genetic variation i.e. additive, dominance and epistatic variance – differences between additive and dominance variance
12. Hybridization – aims and objectives – types of hybridization – pre-requisites for hybridization – procedure / steps involved in hybridization
13. Handling of segregating generations – pedigree method – procedure – modifications of pedigree method – merits, demerits and achievements
14. Handling of segregating generations – bulk method – procedure – merits, demerits and achievements of bulk method – comparison between pedigree and bulk method – single seed descent method
15. Handling of segregating generations – backcross method of breeding – its requirements and applications – procedure for transfer of single dominant gene and procedure for transfer of single recessive gene
16. Handling of segregating generations – backcross method – applications of back cross method – transfer of a dominant gene – transfer of a recessive gene – transfer of two or more characters into a single recurrent parent (simultaneous transfer, stepwise transfer and simultaneous but separate transfer) – merits, demerits and achievements – comparison between pedigree and backcross method; Multiline variety – definition – characteristics of a good multilane – development of multilane varieties – achievements
17. Self-incompatibility – classification – heteromorphic, homomorphic, gametophytic and sporophytic systems of incompatibility – mechanisms of self-incompatibility
18. Self-incompatibility – relevance of self-incompatibility – methods to over come self-incompatibility – advantages and disadvantages – utilization in crop improvement
19. Male sterility – different types – genetic, cytoplasmic and cytoplasmic genetic male sterility – inheritance and maintenance
20. Male sterility – utilization of male sterile lines in hybrid seed production – their limitations, advantages and disadvantages
21. Hardy Weinberg Law – factors affecting equilibrium frequencies in random mating populations
22. Heterosis – heterosis and hybrid vigour – luxuriance – heterobeltiosis – brief history – heterosis in cross- pollinated and self-pollinated species – manifestations of heterosis
23. Heterosis – genetic bases of heterosis – dominance, over dominance and epistasis hypotheses – objections and their explanations – comparison between dominance and overdomiance

hypotheses – physiological bases of heterosis – commercial utilization

24. Inbreeding depression – brief history – effects of inbreeding – degrees of inbreeding depression – procedure for development of inbred lines and their evaluation

25. Exploitation of heterosis – history of hybrid varieties – important steps in production of single and double cross hybrids – brief idea of hybrids in maize, bajra, sunflower, rice and forage crops

26. Synthetics and composites – production procedures – merits, demerits and achievements – factors determining the performance of synthetic varieties – comparison between synthetics and composites

27. Population improvement – selection without progeny testing – selection with progeny testing – progeny selection – merits and demerits of progeny selection – line breeding – achievements

28. Recurrent selection – different types – detailed procedure of simple recurrent selection and brief description of other recurrent selection methods – conclusion on the efficiency of different selection schemes

29. Methods of breeding for vegetatively propagated crops – clone – characteristics of asexually propagated crops – characteristics of clones – importance of a clone – sources of clonal selection – procedure – advantages and disadvantages – problems in breeding asexually propagated crops – genetic variation within a clone – clonal degeneration – achievements – comparison among clones, purelines and inbreds

30. Mutation breeding – spontaneous and induced mutations – characteristic features of mutations – procedure of mutation breeding – applications – advantages, limitations and achievements

31. Polyploidy – autopolyploids – origin and production – morphological and cytological features of autopolyploids – applications of autopolyploidy in crop improvement – limitations of autopolyploidy – segregation in autotetraploids – allopolyploidy – morphological and cytological features of allopolyploids – applications of allopolyploidy in crop improvement – limitations of allopolyploidy

32. Wide hybridization – history – objectives – barriers to the production of distant hybrids – techniques for production of distant hybrids – applications of wide hybridization in crop improvement – sterility in distant hybrids – cytogenetic, genetic and cytoplasmic bases of sterility – limitations and achievements

**References**


Allard, R.W. 1960. *Principles of Plant Breeding*. John Wiley and Sons, New York.

Phundan Singh, 2006. *Essentials of Plant Breeding* . Kalyani Publishers, New Delhi.

Poehlman, J.M. and Borthakur, D. 1995. *Breeding Asian Field Crops*. Oxford and IBH Publishing Co., New Delhi.

Sharma, J.R. 1994. *Principles and Practice of Plant Breeding*. Tata McGraw Hill, Publishing Company Ltd., New Delhi.

Singh, B.D. 2006. *Plant Breeding: Principles and Methods*. Kalyani Publishers, New Delhi.

Lecture No: 1

# Definition, Aim, Objectives and Scope 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.

or

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

or

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

or

Science of changing and improving the heredity of plants

## 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. **Biotic 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 germination. 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. Hence 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 man made 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.

Lecture No. 2

**History and development of plant breeding**

- The process of bringing a wild species under human management is referred to as **domestication**
- Domestication may be the most basic method of plant breeding
- Domestication continuous today and is likely to continue for some time in future

- Ex : In case of timber trees medicinal plants, microbes
- During the long period of historic cultivation natural selection has definitely acted on the domesticated species.
- Movement of man from one place to another brought about the movement of his cultivated plant species
- 700 BC - Babylonians and Assyrians pollinated date palm artificially
- 17$^{th}$ century - several varieties of **heading lettuce** were developed in France
- **1717 - Thomas Fair Child - produced the first artificial hybrid, popularly known as Fair Child's mule, by using carnation with sweet William**
- 1727 - The first plant breeding company was established in France by the vilmorins.
- 1760-1766 - Joseph koelreuter, a German, made extensive crosses in tobacco.
- 1759-1835 – Knight was perhaps the first man to use artificial hybridization to develop several new fruit varieties.
- Le couteur and Shireff used individual plant selections and progeny test to develop some useful cereal varieties
- 1873 - the work of Patrick Shireff was first published.
- He concluded that only the variation heritable nature responded to selections, and that there variation arose through 'natural sports' (= mutation) and by 'natural hybridization'
  (= recombination during meiosis in the hybrids so produced).
- 1856 - Vilmorin developed the progeny test and used this method successfully in the improvement of sugar beets.
- 1900 - Nilson-Ehle, his associates developed the individual plant selection method in Sweden.
- 1903 - Johannsen proposed the pureline theory that provided the genetic basis for individual plant selection.
- The science of genetics began with the rediscovery of Gregor Johan Mendel's paper in 1900 by Hugo de veris, Tshermark and Correns which was originally published in 1866.
- The modern plant breeding methods have their bases in the genetic and cytogenetic principles.
- Numerous workers who determined the various modes of inheritance have contributed to the development and understanding of plant breeding.

- The discovery of chromosomes as carriers of genes has led to the development of specialized plant breeding methods for chromosome engineering.
- The totipotency of plant somatic and gametic cells allows regeneration of complete plants from single cells. This, coupled with the development of recombinant DNA technology, has enabled the transfer of desirable genes from any organism into plants. Crop varieties developed in this manner are already in cultivation in several countries.

**History of plant breeding in India**

- 1871 – The Government of India created the Department of Agriculture
- 1905 – The Imperial Agricultural Research Institute was establish 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 |
| 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 | - | Famous potato breeder |
| N.G.P. Rao | - | An eminent sorghum breeder |
| K. 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 |

Lecture No: 3

# MODES OF REPRODUCTION

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. But if the plants are heterozygous naturally, *e.g.*, as in cross-pollinators like Maize, a heterozygous population

has to be developed as a variety. Consequently, the breeding methods have to be vastly different for the two groups of crop plants. A knowledge of the mode of reproduction of crop plants is also important for making artificial hybrids. Production of hybrids between diverse and desirable parents is the basis for almost all the modern breeding programmes.

## 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 reproduction)* 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, arwi

### 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 species, 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.

**Diplospo ry**

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 into an embryo. In crop plants, male and female gametes are produced in specialised structures known as flowers.

**Flower**

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 **megs.spores** 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.

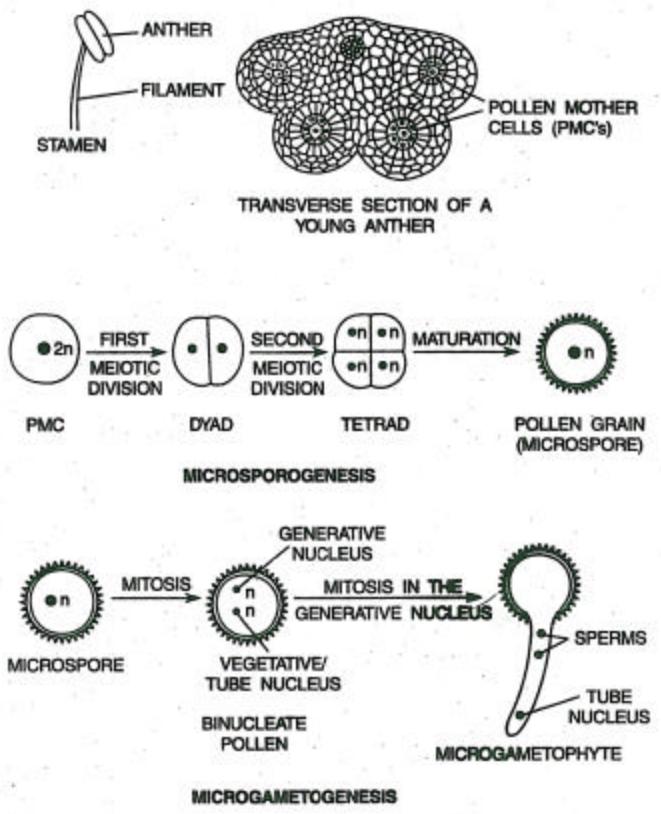
Megasporogenesis. Megasporogenesis occurs in ovules, which are present inside the ovary. A single cell in each ovule differentiates into a megaspore mother cell. The megaspore mother cell undergoes meiosis to produce four haploid megaspores. Three of the megaspores degenerate leaving one functional megaspore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

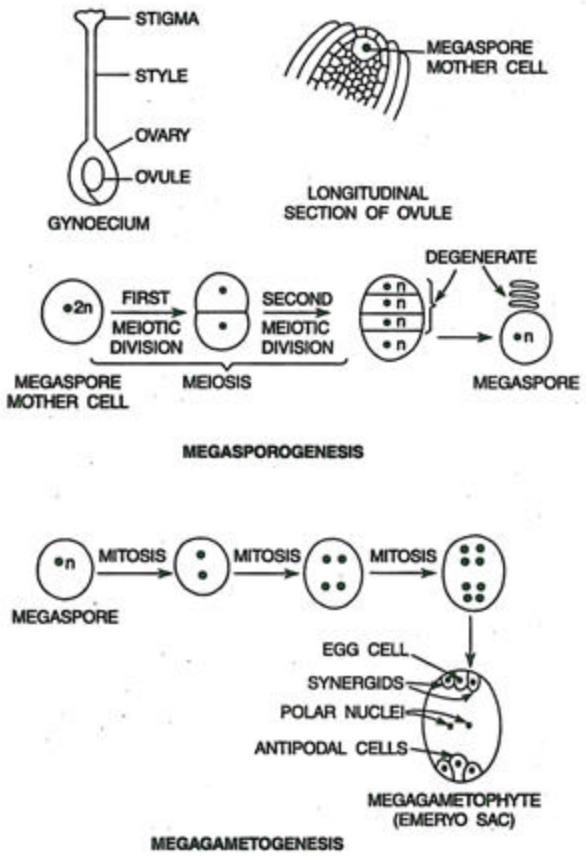
**Microgametogenesis.** This refers to the production of male gamete or **sperm.** During the maturation of pollen, the microspore nucleus divides mitotically to produce a generative and a vegetative or tube **nucleus.** The pollen is generally released in this binucleate stage. When the pollen lands 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.

# Lecture No. 4
# 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 environmental 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 specie. 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 exceeds 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.

Lecture No: 5

### METHODS OF PLANT BREEDING

Various approaches (*viz.,* selection, hybridization, mutation, etc) that are used for genetic improvement of crop plants are referred to as plant breeding methods or plant breeding procedures or plant breeding techniques. The choice of breeding methods mainly depends on the mode of pollination, mode of reproduction, gene action and breeding objective of crop species. Plant breeding methods are generally classified on the basis of application of crop improvement (general methods, special methods and population improvement approaches) and hybridization (methods involving hybridization and methods not involving hybridization).

Various breeding procedures that are more commonly used for the genetic improvement of various crop plants are known as general breeding methods. Such breeding methods include introduction, selection (pure line selection, mass selection, progeny selection), hybridization (pedigree, bulk and backcross methods), heterosis breeding, synthetic and composite breeding. On the other hand, those breeding procedures that are rarely used for improvement of crop plants are referred to as special breeding methods. Such

methods include: mutation breeding, polyploidy breeding, wide crossing or distant hybridization and biotechnology. Four breeding approaches, *viz.*, recurrent selection, disruptive matin g and selection, diallel selective mating system and biparental mating are used mainly for population improvement.

**Classification of Plant Breeding Methods**

| ***Basis of classification and Types of methods*** | ***Breeding methods included*** |
|---|---|
| *A. Application in crop improvement* | |
| (1) General Methods | Plant introduction, Pure line selection, mass selection, progeny selection, pedigree method, bulk method, back cross method, SSD, clonal selection, heterosis breeding, synthetics and composites. |
| (2) Special Methods | Mutation breeding, Polyploidy breeding, transgenic breeding, molecular breeding. |
| (3) Population Improvement | Recurrent selection, disruptive selection, diallel selective approaches mating system, biparental mating. |
| *B. Hybridization* | |
| (1) Methods involving hybridization | Pedigree, bulk, backcross and SSD Methods: heterosis breeding, and population improvement approaches and molecular breeding (marker aided selection). |
| (2) Methods not involving hybridization | Plant Introduction, pureline selection, mass selection, progeny selection, clonal selection, mutation breeding and transgenic breeding. |

There are some differences in the breeding methods used for self pollinated and cross pollinated species. Self pollinated species are homozygous, hence we can start hybridization directly. Cross pollinated species, on the other hand, are highly heterozygous. Hence we can not start hybridization directly. First we have to develop inbred lines by selfing or inbreeding and then only hybridization can be taken up. We have to exploit homozygosity in self pollinated crops and heterozygosity in cross pollinated species. Asexually propagated species such as sugarcane, potato, sweet potato, etc., are highly heterozygous. Hence, $F_1$ hybrids in

such crops exhibit segregation and selection can be practiced in $F_1$ generation. The superior clones are identified and further multiplied. The maintenance or conservation of hybrid vigour is easy in such crops because of asexually propagation.

**Methods of Breeding Autogamous species**

Plant breeding methods that are used for genetic improvement of self pollinated or autogamous species include:

1. Plant Introduction
2. Pureline selection
3. Mass selection
4. Pedigree method
5. Bulk method
6. Single seed descent method
7. Backcross method
8. Heterosis breeding
9. Mutation breeding
10. Polyploidy breeding
11. Distant hybridization
12. Transgenic breeding.

Four breeding approaches, *viz.*, recurrent selection, disruptive selection, diallele selective mating, and biparental mating are used for population improvement.

**Methods or Breeding Allogamous species**

Breeding methods that are used for genetic improvement of cross pollinated or allogamous species include

(1) Plant introduction
(2) Mass and progeny selection
(3) Backcross method
(4) Heterosis breeding
(5) Synthetic breeding
(6) Composite breeding
(7) Polyploidy breeding
(8) Distant hybridization
(9) Transgenic breeding

Mutation breeding is rarely used in allogamous species. Three breeding approaches *viz.,* recurrent selection, disruptive mating and biparental meting are used for population improvement.

**Methods of Breeding Asexually Propagated Species**

Important breeding methods applicable to asexually propagated species are

(1) Plant Introduction
(2) Clonal selection
(3) Mass selection
(4) Heterosis breeding
(5) Mutation breeding
(6) Polyploidy breeding
(7) Distant hybridization
(8) Transgenic breeding.

Mass selection is rarely used in asexually propagated species.

**Brief account of breeding methods**

Plant introduction is applicable to all three groups of crop plants, *viz.,* self pollinated, cross pollinated and asexually propagated species. It is an oldest and rapid method of crop improvement. The introduced material may be used in three ways *viz.,*

(1) Directly as a variety
(2) As a variety after selection
(3) As a parent in the hybridization for development of variety or hybrid

Pureline selection is applicable to self pollinated species. It is also used sometimes in cross pollinated species for development of inbred lines. A single best pure line is released as a variety. Thus a pureline variety is homozygous and homogeneous population.

Mass selection is common in cross pollinated species and rare in self pollinated and asexually propagates species. In self pollinated crops, a mass selected variety is a mixture of several purelines. Thus it is a homozygous but heterogeneous population. In cross pollinated species, a mass selected variety is a mixture of several hetero and homozygotes. Thus, it is a heterozygous and heterogeneous population

Progeny selection is used in cross pollinated species. A variety developed by this method is heterozygous and heterogeneous population because it consists of several hetero and homozygotes.

Pedigree method is applicable to both self and cross pollinated species. In self pollinated crops progeny of a single best homozygote is released as a variety. Thus a variety developed by this method has a homozygous and homogeneous population. In cross

pollinated species, it is used for developed of inbred lines. Bulk nad single seed descent methods are used in self pollinated species. Progeny of a single best homozygote is released as a varie ty by these methods. Thus, varieties developed by these methods are homozygous and homogeneous.

Backcross method is applicable in all three groups of crop species. This method is used for transfer of oligogenic characters from a donor source to a well adapted variety. This method is also used for development of multilines, Isogenic lines and transfer of male sterility. This method is more effective in transferring oligogenic characters than polygenic traits. The end product of backcross method is similar to parent variety expect for the character which has to be transferred from the donor source.

Multiline varieties are developed in self pollinated species. They are mixture of several Isogenic lines, closely related lines or unrelated lines. Thus a multiline variety is a homozygous but heterogeneous population.

Clonal selection is used in asexually propagated species. In this method progeny of a single best clone is released as a variety. Such variety has heterozygous but homogeneous population.

Heterosis breeding is used in/all the three groups. However, it is common in cross pollinated and asexually propagated species and rare in self pollinated species. A hybrid variety has homogeneous but heterozygous population. Synthetic and composite varieties are developed in cross pollinated species. Such varieties consist of several homozygotes and heterozygotes and thus constitute a heterogeneous population.

Mutation breeding is common in self pollinated and asexually propagated species and rare in cross pollinated species. A mutant variety differs from parent variety in one or few characters. A mutant differs from a segregant in two main ways. Firstly, the frequency of segregants is very high and that of mutant is extremely low (0.1%). Secondly, mutant differs from parent variety in one or few characters, where as a segregant differs from parent material in several characters.

Polyploidy breeding is common in asexually propagated species and rare in self and cross pollinated species. A polyploidy variety differs from parent variety in chromosome numbers and exhibit gigant morphological characters.

Distant hybridization is used in all the three types of crop species. However, this method is used for transferring some desirable genes from wild species to the cultivated ones. Generally, backcross method is used for transfer of oligogenic characters and pedigree method for transfer of polygenic characters.

Transgenic breeding is applicable to all three types of crop species. This method is used to solve specific problems which can not be solved by conventional breeding techniques. This method will serve as a tool and can not be used as a substitute for conventional breeding methods.

Recurrent selection is common in cross pollinated species and rare in other two groups. It is used for accumulating favourable genes in a population *i.e.,* for population improvement. Other approaches which are used for population improvement include disruptive mating, diallel selective mating (DSM) and biparental mating. DSM is used in self pollinated species and other two techniques can be used both in self and cross pollinated species.

## Lecture No: 6

## 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 varietie s 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 16th 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 18th century.

During 19th 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 19th 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 at 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 the 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 isbrought 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 of this type.

**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 source 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 follows ; 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 large 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.

**Lecture No. 7**

## SELECTION

Selection is basic to any crop improvement. Isolation of desirable plant types from the population is known as selection. It is one of the two fundamental steps of any breeding programme viz., 1. creation of variation and 2. Selection. There are two agencies involved in carrying out selection : one is Nature itself (Natural selection) and the other is man artificial selection. Though both may complement each other in some cases, they are mostly opposite in direction since their aims are different under the two conditions (nature and domestication). The effectiveness of selection primarily depends upon the degree to which phenotype reflects the genotype.

Before domestication, crop species were subjected to natural selection. The basic for natural selection was adaptation to the prevailing environment. After domestication man has knowingly or unknowingly practiced some selection. Thus crop species under domestication were exposed to both natural and artificial selection i.e. selection by man. For a long period, natural selection played an important role than selection by man. But in modern plant breeding methods natural selection is of little importance and artificial selection plays an important role.

**Basic Principles of Selection :** Notwithstanding the highly complex genetic situation imposed by linkage and espistasis, there are just three basic principles of selection (Walker, 1969) :

1. **Selection operates on existing variability** : The main function of the selection exercise is to discriminate between individuals. This is possible only when sufficient variation is present in the material subjected to selection pressure. Thus, selection acts on the existing variation it cannot create new variation.
2. **Selection acts only through heritable differences** : only the selected individuals are permitted to contribute to the next genetion / progenies. Therefore, should there be greater influence of non-heritable agencies on the individuals selected, the parent-progeny correlation will be greatly vitiated. Hence the variation among individuals to be selected must be genetic in nature, since it is the genetic variation that tends to close the gap between phenotype and genotype. Environmental variability cannot be of any use under selection.
3. **Selection works because some individuals are favoured in reproduction at the expense of others** : As a consequence of its past evolutionary history and breeding structure, a population or a crop consists of highly genetically variable individuals with regards to such diverse phenomena as differential viability, differential maturity, differences in mating tendencies, fecundity, and duration of reproductive capacity. Hence some individuals tend to become superior to others for some or other traits desirable under domestication. These superior individuals are retained for reproduction while others discarded under selection.

**Selection has two basic characterics viz**.

1. Selection is effective for heritable differences only.
2. Selection does not create any new variation. It only utilizes the variation already present in a population.

**The two basic requirements for select on to operate are** :

1. Variation must be present in the population.
2. The variation should be heritable.

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

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

***Table 1 : Relationship between selection intensity and proportion of population selected***

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

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

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

The I=2.06 to 1.76 (i.e. around 5-10 per cent of individuals selected) has generally been found appropriate in plant populations. However, the limit of selection intensity is set by two factors : (i) population size, and (ii) inbreeding. Under natural selection, selection intensity is expressed as the relative number of offsprings produced by different genotypes, and is termed as selection coefficient.

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

Selection differential (S) S is the average superiority of selected individuals over the mean of population of their origin. It is considered in the same parental generation before selection is made. An arbitrary culling level, k(i.e. I) is fixed for a trait and individuals beyond that level are selected. The average of all such selected individuals can be designated by X. then the mean of selected individuals (X) exceeds the parental population mean by the measure of S.

That is S=X-μ. Therefore, wider the phenotypic variability (i.e. phenotypic variance, and phenotypic standard deviation, that measures variability), greater is the possibility of S being large.

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

$H = Vg / Vp$

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

Vg = genotypic variance

Ve + error variance of environmental variance

Heritability estimated from the above formula is known as the broad sense heritability. This is valid when homozygous lines are studied. But when segregating generations are studied genotypic variance consists of (a) additive variance (b) dominance variance (c) and variance due to epistasis.

Dominance variance is important when we are dealing with hybrids i.e. $F_1$ generations. In self pollinated crops we release varieties only after making them homozygous lines. Hence additive variance is more important in such cases. The proportion of additive genetic variance to the total variance is known as narrow sence heritability.

If heritability is very high for any character it can be improved. Improvement of characters with low heritability is very difficult.

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

Genetic advance(GA) = s P * H * K

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

P = phenotypic standard deviation of the character in the population

H = heritability in broadsense

**Lecture No. 8**

## MASS SELECTION

It is the earliest method of selection. Man has always practiced mass selection consciously or unconciously from the time of domestication. In its most basic form mass selection consists of selecting individuals on the basis of phenotypic superiority and mixing the seeds for using as planting material for next season.

## Procedure for evolving variety by mass selection

**First year :** Large number of phenotypically similar plants having desirable characters are selected. The number may vary from few hundred to few thousand. The seeds from the selected plants are composited to raise the next generation.

**Second year :** composited seed planted in a preliminary field trial along with standard checks. The variety from which the selection was made should also be included as check. Phenotypic characterlistics of the variety are critically examined and evaluated.

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

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

### *Modification of mass selection*

Mass selection is used for improing a local variety. Large number of plants are selected (I year) and individual plant progenies are raised (II year). Inferior, segregating progenies are reflected. Uniform, superior rows are selected and the seed is bulked. Preliminary yield trials are conducted in third year. Fourth to seventh year multilocation tests are conducted and seed is multiplied in eight year and distributed in ninth year. Many other modifications also are followed depending on the availability of time and purpose for ehich it is used.

**Merits of Mass selection :**

1. Can be practiced both in self and cross pollinated crops
2. The varieties developed through mass selection are more widely adopted than pure lines.
3. It retains considerable variability and hence further improvement is possible in future by selection
4. Helps in preservation of land races
5. Useful for purification of pureline varieties
6. Improvement of characters governed by few genes with high heritability is possible.
7. Less time consuming and less expensive.

### *Demerits of mass selection*

1. Varieties are not uniform
2. Since no progeny test is done, the genotype of the selected plant is not known

3. Since selection is based on phenotype and no control over pollination the improvement brought about is not permanent. Hence, the process of mass selection has to be repeated not and then.
4. Characters which are governed by large number of genes with low heritability can not be improved.
5. It can not create any new genotype but utilizes existing genetic variability.

**Achievements**

Mass selection must have been used by pre historic man to develop present day cultivated cross from their wild parents. It was also used extensively before pureline selection came into existence.

Cotton : Dharwad American Cotton

Groundnut : TMV-1 & TMV-2

Bajra : pusa moti, Baja puri, Jamnagar gaint, $AF_3$

Sorghum : R.S. 1

Rice : SLO 13, MTU-15

Potato : K122

## Lecture No 9

# JOHANNSEN'S PURE LINE THEORY

### The pure line theory

A pureline is a progeny of a single homozygous plant of a self-pollinated species. All the plants of a pureline have the same genotype. The phenotypic differences within a pureline is due to environment. Therefore variation within a pureline is not heritable. Hence selection in a pureline is not effective.

The concept of pureline was proposed by Johannsen in 1903 on the basis of his studies with princess variety of beans (*Phaseolus vulgaris*). From a commercial seed lot he selected seeds of different sizes and grew them separately. The progenies differed in seed size. Progenies from larger seeds produced larger seeds than those obtained from smaller seeds. This clearly showed that the variation in seed size in the commercial seed lot of princess variety had a genetic base. As a result selection for seed size was effective.

Johannsen further studied 19 lines, each line was a progeny of a single seed from the original lot. He discovered that each line showed a characteris mean seed weght, rangin from 640mg

in Line No 1 to 350 mg in line No 19. the seed size within a line showed some variation, which was much smaller than that in the original commercial seed lot. Johannsen postulated that the original seed lot was a mixture of purelines. Thus each of the 19 lines represented a pureline, and the variation in seed size within each of the urelines had no genetic basis and was entirely due to environment.

**Confirmatory evidence was obtained in three ways.**

**In the first case**, he classified the seed from each pureline into 100 mg classes, and grew them separately. The mean seed weight of progenies from different seed weight class of a single pure line were comparable with each other, and with that of the parent pureline. For example Line no 13 had seed size classes of 200 , 300, 400, and 500 mg. The mean seed weights of the progenies derived from these seed weight classes were 475, 450, 451 and 458 mg respectively.

**The second line of evidence came from selection within a pureline.** From each pureline, the largest and the smallest seeds were selected to raise the next generation. In the subsequent generations, large seeds were selected in the progenies obtained from large seeds while in these from small seeds selection was done fro small seeds. Six generations of selection was ineffective in increasing or decreasing the seed size. For example, after 6 generations of selection, the mean seed weight in Line No 1 was 690 and 680 mg in the progenies selected for small and large seeds respectively. Thus selection within a pureline was ineffective.

**The third approach was to estimate parent offspring correlation.** The value of parent offspring correlation within line no 13 was $-0.018 \pm 0.038$, that is, zero, while it was $0.336 \pm 0.008$ in the original seed lot of the Princess which is highly significant. The parent-offspring correlation will be zero when the variation is nonheritable, while it will be significantly greater than zero when the variation has a genetic basis, i.e., is heritable.

**These observations reveal that the variation for seed size in the original seed lot of Princess had a genetic basis and was heritable.** But the variation within the purelines obtained from the single seeds selected from this seed lot was purely due to the environment and, therefore, non-heritable.

**The two main conclusions from the Johannsens' experiment are,**

1. A self-fertilized population consists of a mixture of several homozygous genotypes. Variation in such a population has a genetic component, and therefore selection is effective.
2. Each individual plant progeny selected from a self-fertilized population consists of homozygous plants of identical genotype. Such a progeny is known as pureline. The

variation within a pureline is purely environmental and, as a result, selection within a pureline is ineffective.

**The two main conclusions of Johannsen's experiment are**

1. Selection is effective in population since it contains a mixture of several genotypes.
2. Selection is ineffective in a pureline, since it is a progeny of single, self fertilized homozygous individual.

## Origin of variation in pure lines

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

1. **Mechanical Mixture** : During cultivation, harvesting threshing and storage, other genotypes may get mixed up.
2. **Natural hybridization**: Through pure lines are produced in self pollinated crops, some amount of natural cross pollination occurs in them also can be avoided by isolation and rouging.
3. **Mutation**: occur spontaneously in nature at random

## Characters of purelines

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

## The progeny test

Evaluation of the worth of plants on the basis of performance of their progenies is known as progeny test. This was developed by Louis de vilmorin and so it is also known as the vilmorin Isolation principle. Vilmorin worked on sugar beat plants. The progeny test serves two valuable function;

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

## Genetic basis of pure line

Self pollination increases homozygosity with a corresponding decrease in heterozygosity. The effect of homozygosity and heterozygosity may be illustrated by taking an individual heterozygous for (Aa) a single gene as follows

| No of generations of selfing | Frequency % | | | Frequency % | |
|---|---|---|---|---|---|
| | AA | Aa | aa | Homo-zygosity | Hetero-zygosity |
| 1 | 0 | 100 | 0 | 0 | 100 |
| 2 | 25 | 50 | 25 | 50 | 50 |
| 3 | 37.5 | 25 | 37.5 | 75 | 25 |
| 4 | 43.75 | 12.5 | 43.75 | 87.5 | 12.5 |
| 5 | 46.875 | 6.25 | 46.875 | 93.73 | 6.25 |
| 6 | 48.437 | 3.125 | 48.437 | 96.874 | 3.125 |
| 7 | 49.218 | 1.562 | 49.218 | 98.436 | 1.562 |
| 8 | 49.608 | 0.781 | 49.608 | 99.216 | 0.781 |
| 9 | 49.803 | 0.39 | 49.803 | 99.606 | 0.39 |

Proportion of completely homozygous plants in the population

$[(2^m - 1) / 2^m]^n$

m = No. of generations of self pollination

n = No. of genes segregating

Suppose an individual heterozygous for a single gene (Aa) and the successive generations derived from it are subjected to self-pollination.

Every generation of self-pollination will reduce the frequency of heterozygote Aa to 50 per cent of that in the previous generation.

There is a corresponding increase in the frequency of the two homozygotes AA and aa. As a result, after 10 generations of selfing, virtually all the plants in the population would be homozygous, *i.e.*, AA and a.

On the other hand, the frequency of heterozygote Aa would be only 0.097 per cent, which is negligible. It is assumed here that the three genotypes AA, Aa and aa have equal survival. If there is unequal survival, it may increase or decrease the rate at which homozygosity is achieved. If Aa is favoured, the rate of increase in homozygosity would be lower than expected.

But if Aa is selected against, homozygosity would increase at a faster rate than expected.

## Pureline selection

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

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

## General procedure for evolving a variety by pureline selection

The pureline selection has three steps.

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

**1. Selection**

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

**II. Evaluation :**

**Second year:** Progenies of individual plants selected in 1$^{st}$ year are grown separately with proper spacing (plant to row or head to row). The progenies are evaluated by taking elaborate date on visual characters such as plant height, duration, grain type, ear characters besides yield. The number of progenies should be reduced as much as possible. Disease epiphytotics may be created to test the progenies for disease resistance, poor, weak, diseased, insect attacked and segregating progenies are rejected. The superior progenies are harvested separately. If necessary the process may be repeated for one or more years.

**III. Yield trials :**

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

Superior cultures based on observable characters and yield are selected. The number is drastically reduced.

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

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

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

## *Advantage of pureline selection*

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

## *Limitations or disadvantages of pureline selection*

1. New genotypes are not created by pureline selection
2. Improvement is limited to the isolation of the best genotype present in population. No more improvement is possible after isolation of the best available genotype in the population.
3. Selection of purelines require great skill and familiarity with the crop.
4. Difficult to detect small differences that exist between cultures
5. The breeder has to devote more time

6. Pure lines have limited adaptability hence can be recommended for cultivation in limited area only.

**Achievements :**

Several varieties developed by pureline selection were released in many crops. Some examples are given below

Rice : Mtu-1, Mtu-3, Mtu-7, Bcp-1, Adt-1, 3, 5, and 10

Sorghum : G 1 & 2, M 1 & 2, OO 1, 4 & 5,

Groundnut : TMV 3, 4, 7, 8 and Kadiri 71-1

Redgram : TM-1, ST-1

Chillies : G1 & G2

Ragi : AKP 1 to 7

## Differences between Mass and Pureline selections

| S. No. | Mass selection | Pureline selection |
|---|---|---|
| 1 | Used both in self and cross pollinated crops | Practiced in self pollinated crops only |
| 2 | Large number of plants are selected | Comparatively less number of plants are selected |
| 3 | The produce of the selected plants is mixed and sown as such in next year | Produce of individual plants is kept separate and progeny rows are raised next year |
| 4 | No control of pollination | Pollination is controlled |
| 5 | Variety developed is heterozygous and not uniform | Variety is homozygous homogeneous and uniform |
| 6 | Due to heterozygosity the variety deteriorates quickly | Due to homozygosity the variety lasts long |
| 7 | The method has to be repeated once in 2-3 years to purify the variety | No need to repeat |
| 8 | Wider adaptability due to heterozygosity | Narrow adaptability due to homozygosity |
| 9 | No knowledge of science is required. It is more an art. | Knowledge of science and genetics is required |
| 10 | Selection within a variety is effective | Selection with in a pureline variety is not effective |
| 11 | The variety is relatively difficult to identify | It is relatively easy to identify in seed certification programmes. |

## Lecture No 10

## GENETIC BASIS OF PURE LINE

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9. Selection of purelines require great skill and familiarity with the crop.
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12. Pure lines have limited adaptability hence can be recommended for cultivation in limited area only.

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**Lecture No 11**

## BIOMETRICS – COMPONENTS OF GENETIC VARIATION

**Biometry or biomatrics** is the science thatdeals with the application of statistical procedures to the study of biological problems.

**Biometrical genetics or Quantitative genetics** is that branch of genetics, which attempts to unravel the inheritance of quantitative traits using statistical concepts and procedure.

**INTRODUCTION :**

The two basic requirements of Plant Breeding are the presence of genetic variation and exploitation of this variation through selection. The selection of plants from a population is almost always based on their phenotype. Phenotype has both heritable and nonheritable components. The heritable component is due to the genes present in plants, that is genotype. The non-heritable component consists of the effects of environment. The value of progeny obtained from a selected plant, therefore, would largely depend upon therelative contributions by the heritable and the non-heritable components to its phenotype., clearly, the breeder should be thoroughly familiar with the laws of inheritance and the relative importance of the genotype and the environment in determining the concerned phenotype.

**Qualitative and Quantitative characters :**

Some characters are little affected by other genes, i.e. the genetic back ground, or the environment. Such characters are generally governed by one or few genes with large, easily detectable effects, such genes are known as **oligogenes**. The characters produced by oligogenes show distinct classes and are known as **qualitative characters** or **olygogenic traits**. On the other hand, the development of many characters is very much affected by the genetic background and, more particularly, by the environment. These characters are governed by several genes with small individual effects; these genes one known as **polygenes**. The characters produced by polygenes are referred to as **quantitative characters**, because they do not show clear-cut classes and have to be studied by measurement. They are also called **Polygenic traits** since they are governed by polygenes. The inheritance of both qualitative and quantitative characters follows the laws of Mendel. But the effects of individual genes in the two cases are totally different in magnitude consequently, the techniques used to study the two types of characters are also different.

## Role of the environment in Quantitative Inheritance

Quantitative characters are considerably affected by environment. The main result of this effect is that the relationship between genotype and phenotype is partially or completely hidden i.e. the phenotype doesnot reveal the genotype.

For eg: Phenotype = Genotype+ Environment P=G+E

If environment = 0 then phenotype = Genotype. However the effect of environment is seldem zero. So phenotype is the joint action of Genotype and Environment.

In crop improvement, the breeder selects plants on the basis of their phenotype.

The effectiveness of selection depend on the proportion of phenotype due to the genotype.

Therefore, it is important to know the extent to which environment influences different quantitative characters.

To estimate the effect of environment on a character, large No. of strain / genotypes are grown in a replicated trial and the data is subjected to analysis of variance as per the experimental design used.

The genotype x Environmental interaction signifies that the relative performance of various genotype in effected by the environment. For eg: Performance of genotype 'A' may be superior to the genotype 'B' in one environment but in another environment inferior to that of 'B'

If G X E interaction is absent, genotype 'A' will be superior 'B' in all the environments.

## BIOMETRICAL TECHNIQUES IN PLANT BREEDING

The biometrical techniques are useful to the plant breeders in the following 4 different ways.

1. Assessment of genetic variability present in the population. – It can be assesed by
   Range, variance, standard deviation, coefficient of variation, $D^2$ statistics, metro glyph analysis
2. In the selection of elite genotypes from mixed populations – correlation, path and discriminant function analysis)
3. Selection of parents and breeding procedures – diallel , partial diallel, line x tester, generation means, triallel by parental cross and triple test cross analysis
4. Determining varietal adaptation – Stability analysis
   Finley and Wilkinson (1963)
   Eberhart and Russel (1966)
   Perkins and Jinks (1968)
   Freeman and Parkins (1971)
5. AMMI – Model – Additive main effects and multiplicative interaction

**Components of Genetic variance :**

Phenotypic variance = Genotypic variance + Environment variance

$Vp = Vg + Ve$

Fisher (1918) divided the genotypic variance into three components :

1. Additive
2. Dominance
3. Epistasis

Later Hayman and Mather partitioned the epistatic component into three types of interactions – vig.,
Additive x additive ; additive x dominance and dominance x dominance

1. Additive variance : Arises of from difference between two homozygotes for a gene *i.e.* AA and aa. It is generally represented 'd'
2. Dominance variance or Intra allelic interaction: It is due to the deviation of heterozygote (Aa) phenotype from the average of phenotypic values of the two homozygotes (AA and aa) it is represented by 'h'.
3. Epistasis variance or Inter allelic Interaction : Results from an interaction between two or more genes represented by 'e'.

**Difference between additive and dominance variance**

| | |
|---|---|
| It refers to difference between homozygotes (AA/aa) | It refers to deviation of Aa from the mean of AA and aa |
| Genes show lack of dominance | Genes show incomplete, complete or over dominance |
| Associated with homozygosity and is more in inbreders | Associated with heterozygosity and is more in outbreeders |
| It is fixable | It is non-fixable |
| Selection is very effective as it is fixable | Selection is ineffective as it is non-fixable |
| it is the chief cause of transgressive segregation | It is the chief cause of heterosis |

## Different types of gene actions involving two genes

| Component of genetic variance | Specific symbol | Description | General symbol |
|---|---|---|---|
| Additive | da | The different between AA and aa phynotypic values | d |
| | db | The different between BB and bb phynotypic values | |
| Dominance | ha | The deviation of Aa phenotype from the average of AA and aa phenotypes | h |
| | hb | The deviation of Bb phenotype from the averave of BB and bb phenotypes | |
| Epistasis | da x db | Additive x Additive effect due to interaction between AA & BB | i |
| | da x hb and ha x db | Additive x dominance interaction due to interactions between and AA and Bb and between Aa and BB respectively | j |
| | ha x hb | Dominance x dominance interaction due to interation between Aa and Bb | l |

**Lecture No 12**

## HYBRIDIZATION : TECHNIQUES AND CONSEQUENCES

The mating or crossing of two plants or lines of dissimilar genotype is known as hybridization. In plants, crossing is done by placing pollen grains from one genotype, the male parent, on to the stigma of flowers of the other genotype, the female parent. It is essential to prevent self-pollination as well as chance cross-pollination in the flowers of the female parent. At the same time, it must be ensured that the pollen from desired male parent reaches the stigma of female flowers for successful fertilization. The seeds as well as the progeny resulting from the hybridization are known as hybrid or $F_1$. The progeny of $F_1$, obtained by selfing or intermating of $F_1$ plants, and the subsequent generations are termed as segregating generations. The term cross is often used to denote the products of hybridization, i.e. the $F_1$ as well as the segregating generations.

## OBJECTIVES OF HYBRIDIZATION

The chief objective of hybridization is to create genetic variation. When two genotypically different plants are crossed, the genes from both the parents are brought together in $F_1$. segregation and recombination produce many new gene combinations in $F_2$ and the later generations, i.e. the segregating generations. The degree of variation produced in the segregating generations would, therefore, depend on the number of heterozygous genes in the $F_1$. This sill, in turn, depend upon the number of the genes for which the two parents differ. If the two parents are closely related, they are likely to differ for a few genes only. But if they are not related, or are distantly related, they may differ for several, even a few hundred, genes. However, it is not likely that the two parents will ever differ for all their genes. Therefore, when it is said that the $F_1$ is 100 per cent heterozygous, it has reference only to those genes for which the two parents differ.

The aim of hybridization may be the transfer of one or few qualitative characters, the improvement in one or more quantitative characters, or use the of $_1$ as a hybrid variety. These objectives are briefly discussed below.

**Combination Breeding :** The main aim of combination breeding is the transfer of one or more characters into a single variety from other varieties. these characters may be governed by oligogenes or polygenes. The intensity of the character in the new variety is either

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

**Transgressive Breeding :** Transgressive breeding aims at improving yield or its contributing characters through transgressive segregation. Transgressive segregation is the production of plants in an $F_2$ generation that are superior to both the parents for one or more characters. Such plants are produced by an accumulation of plus or favourable genes from both the parents as a must combine well with each other, and should preferably be genetically diverse, i.e., quite different. This way, each parent is expected to contribute different plus genes which when brought together by recombination give rise transgressive segregant. As a result, the intensity of character in the transgressive segregant, i.e., the new variety, is greater than that in either of the parents. The pedigree method of breeding and its modifications, particularly the population approach, are designed for the production of transgressive segregants.

**Hybrid Varieties :** In most self-pollinated crops, $F_1$ is more vigorous and higher yielding than the parents. Wherever it is commercially feasible, $F_1$ may be used directly as a variety. In such cases, it is important that the two parents should produce an outstanding $F_1$.

## TYPES OF HYBRIDIZATION

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

1. Intervarietal and
2. Distant hybridization

**Intervarietal Hybridization :** The parents involved in hybridization belong to the same species ; they may be two strains, varieties or races of the same spicies. It is also known as intraspecific hyrbidization. In crop improvement programmes, intervarietal hybridization is the most commonly used. In fact, it is so common that it may often appear to be the only form of hybridization used in crop improvement. an example would be crossing of two

varieties of wheat, rice or some other crop. The intervarietal crosses may be simple or complex depending upon the number of parents involved.

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

A X B → $F_1$ (A X B)

**Complex Cross :** more than two parents are crossed to produce the hybrid, which is then used to produce $F_2$ or is used in a backcross. Such a cross is also known as convergent cross because this crossing programme aims at converging, i.e., bringing together, genes from several parents into a single hybrid. A few examples of convergent cross are described in Fig. 7.1. As

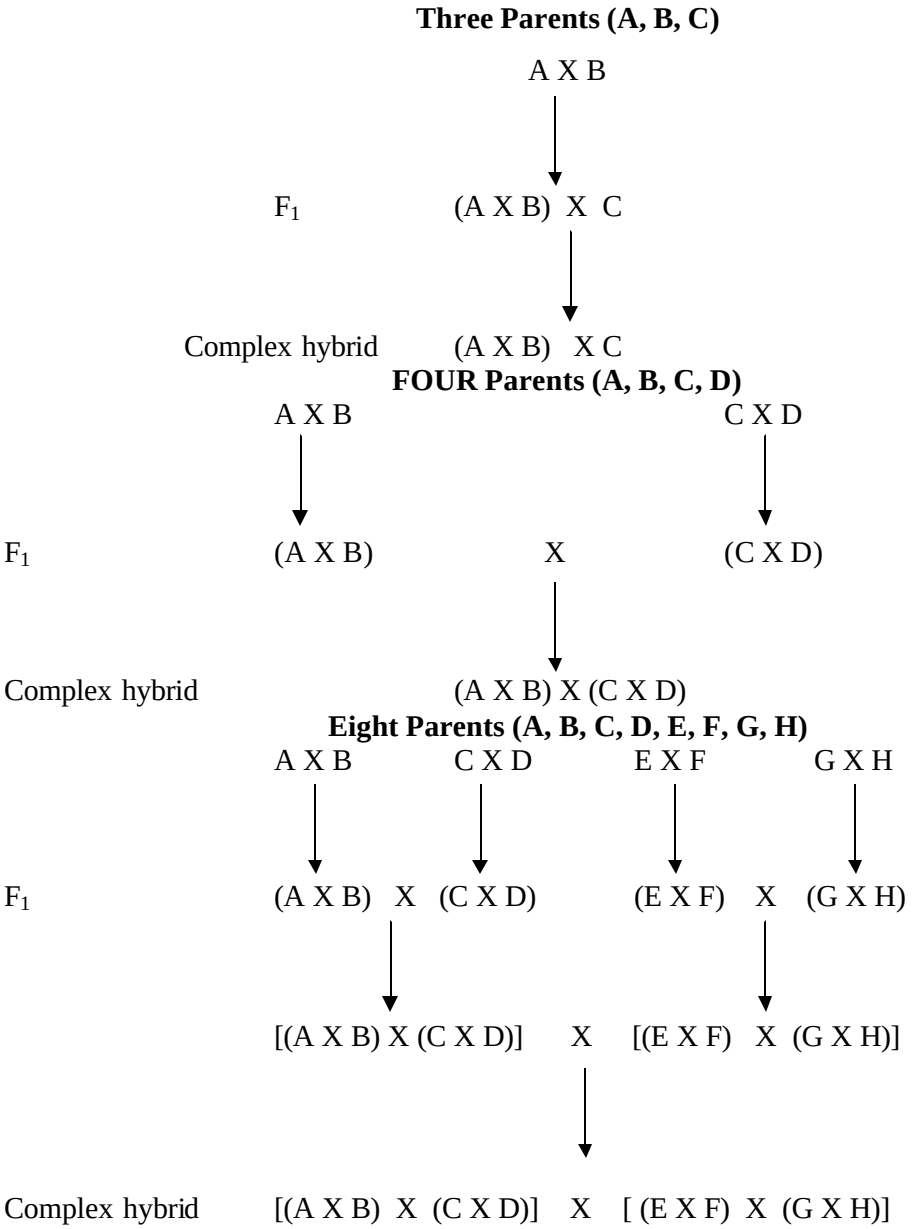

Fig 7.1. Complex crosses involving 3, 4 and 8 parents.

Crop improvement progresses, the crop varieties would accumulate more and more favourable genes. This would lead to greater similarities between even unrelated varieties. In view of this, it may be expected that in future complex crosses would become more and more important. In breeding of highly improved self-pollinated crops like wheat and rice, complex crosses are a common practice today. Complex crosses would become routine in near future in the improvement of other self-pollinated crops with the progress in the level of their improvement.

**Distant Hybridization :** Distant hybridization includes crosses between different species of the same genus or of different genera. When two species of the same genus are crossed, it is known as interspecific hybridization; but when they belong to two different genera, it is termed as intergeneric hybridiztion. Generally, the objective of such crosses is to transfer one or few simply inherited characters like disease resistance to a crop species. Sometimes, interspecific hybridization may be used for developing a new variety, e.g., Clintonoat variety was developed from a cross between Avena sativa x A. byza ntina (both hexaploid oat species), and CO 31 rice variety was developed from the cross Oryza sativa var. indica x O. perennis. Almost al the present-day sugarcance varieties have been developed from complex crosses between Saccharum officinarum (noble canes), S. barberi (Indian canes) and other Saccharum species, e.g., S. spontaneum (Kans.). The improvement in fiber length of Indian Cotton (Gossypium arboreum) has been brought about by crossing it with American cultivated Cotton ; many improved varietie s have resulted from such crosses. Intergeneric hybridization may also be used to develop a new crop species, e.g., Triticale from a cross between Triticum sp. And Secale cereale (rye). Wild species often provide genes which are not present in the cultivated species. For example, many of the genes for rust resistance in wheat are derived from related wild species. Distant hybridization is likely to become increasingly important in the correction of specific defects of crop species. In many cases, wild species may contribute valuable 'yield genes' as well to the cultivated species.

**Pre-requisites for hybridization**

Breeder should have clear knowledge about the following before taking up hybridization.

1. Requirements of the tract
2. Local conditions i.e. soil, climate, Agronomic practices and market requirements
3. Existing varieties of crops both local and introduced
4. Facilities like funds, land, labour and equipment

5. Plant material i.e. germ plasm
6. Objectives : Well set objectives and planning

**Hybridization proce dure or steps involved in hybridization**

Details of the following steps have to be covered in Practical classes

1. Choice or selection of parents
2. Evaluation of parents i.e. by selfing and studying the progeny
3. Emasculation
4. Crossing or pollination
5. Bagging & Labelling
6. Harvesting of $F_1$ seed
7. Raising $F_1$ generation

From $F_2$ onwards the generations are known as segregating generations and they may be handled either by pedigree method of Bulk method or backcross method for evolving new varieties.

**Lecture No 13**

## HANDLING OF SEGREGATING GENERATIONS
## Pedigree Method

In the pedigree method, individual plants are selected from $F_2$ and subsequent generations, and their progenies are tested. During the entire operation a record ofall parent off spring relationships is kept. This is known as pedigree record. Individual plant selection is continued till the progenies show no segregation. At this stage the selection is done among the progenies, multilocation tests are conducted and released as varieties.

The pedigree may be defined as a description of the ancestors of an individual and it generally goes back to some distant ancestors. It is useful to know the relationship of two individuals and useful for selection of parents and prediction of outcome of the cross.

Procedure of pedigree method

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

**2nd year** : Sow the $F_1$ seed giving wide spacing so that each $F_1$ plant produces more seeds. Raise as many $F_1$ plants as possible to produce large number of $F_2$ seeds. Harvest in bulk.

**3rd year** : Grow 2000-10000 plants of $F_2$ giving wide spacing for full expression of the characters in $F_2$ generation plants. Grow parents for comparision. Depending upon the facilities and objectives of the programme about 100-500 superior plants are selected. The

value of selection depend on the skill of the breeder. He has to judge which $F_2$ plant will produce superior progeny for characters under consideration. The breeder develops this skill through close study of the crop for many generations. The selection in $F_2$ is done for simply inherited characters like head type disease resistance etc. and selection for characters governed by many genes like yield will be reserved for later generations. The selected plants are harvested separately and given serial numbers and description entered in pedigree registers.

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

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

**6th year** : $F_5$ plants raised in 3 row plots i.e. seeds of each selected plant sown in 3 rows. By this time many families might have become reasonly homozygous. For comparision check variety is grown for every 3 or 5 block. Progenies are evaluated for yield and the inferior ones are rejected. The number should be reduced to 25-50. superior plants from superior progenies are selected. Plants from each progeny are bulked.

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

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

**9th, 10th & 11th year :** selected lines are tested in several localities for 2 or 3 years for adaptation tests. Lines are evaluated for all characters mainly yield and disease resistance. A line that is superior to commercial variety in yield and other characters is selected. 11th and **12th year** : Selected superior lines is named, multiplied and released as a new variety. Number of year can be reduced if generations are advanced during off seasons either in green house or under irrigated conditions.

Several modifications for the above described pedigree method are followed by breeders depending upon the crop, time and availability of funds and facilities like labour, land etc.

**Early generation tests :**

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

**$F_2$ progeny testing :** Another modification for pedigree method. In $F_2$ make as many single plants selections as possible. From $F_3$ to $F_6$ advance the progenies in bulk making selections of the progenies as a wholw and discarding the inferior progenies. Thus each of the progeny is derived from the single plant selected in $F_2$ generation. In $F_6$ make single plant selections in each of the progeny. Compare the yields of the single plants with progenies from which they are selected. Select superior single plant progenies and advance to preliminary yield trials, multilocation tests etc. There are two advantages 1. No. of crosses can be handled simultaneously 2. Natural selection operates from $F_3$ to $F_6$ since they are advanced in bulk.

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

**Merits of pedigree method :**

1. It gives maximum opportunity for the breeder to use his skill and judgement in selection of plants
2. It is well suited for the improvement of characters which can be easily identified and are simply inherited.
3. Transgressive segregation for yield and other quantitative characters may be recovered.
4. Information about the inheritance of characters and pe digree of lines can be obtained.

5. Inferior plants and progenies are eliminated in early generations.
6. It takes less time than bulk method to develop new variety.

**Demerits of pedigree method :**

1. Valuable genotypes may be lost in early generations, if sufficient skill and knowledge are lacking in the breeder, at the time of selection.
2. No opportunity for natural selection
3. Difficult to handle many crosses
4. Maintenance of records, selections, growing progeny rows etc are time consuming and laborious.

**Achievements :** Large number of varieties have been developed by pedigree method in many crops.

A few examples are

Wheat – NP-52, 120,125, 700 and 800 series

Rice – ADT – 25, Jaya, Padma

Cotton – Lakshmi, Digvijay, Sorghum – Co 18, RS 610 etc., Tobacco – NP 222

Sorghum – Co 18, Rs 610, Tobacco – NP 222

Lecture No. 14

# BULK METHOD

The bulk method was first proposed by Nilsson Ehle in 1908 at Svalof. This method is also known as **mass method** 'or' Population method of breeding

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

## *THE PROCEDURE FOR BULK METHOD*

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

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

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

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

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

**$F_8$ Generation :** Individual plant progenies are grown in single or multi-row plots. Most of the progenies would be reasonably homozygous and are harvested in bulk. Weak and inferior progenies are rejected on the basis of visual evaluation. Only 100-300 plant progenies with desirable characteristics are saved.

Some progenies which show segregation are generally rejected unless they are of great promise. In promising pr ogenies, individual plants may be selected ; preliminary yield trial will be delayed for one year in such cases.

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

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

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

## *MERITS OF BULK METHOD*

1. The bulk method is simple, convenient and less expensive.
2. Since, each $F_2$ plant is equally represented till $F_6$, no chance of elimination of good genotypes in early generations.

3. Artificial or natural disease epiphytics, winter killing high temperature etc. eliminates undesirable types and increases the frequency of desirable type. Thus isolation of desirable types becomes easier.
4. Progenies select from long term bulks are superior than the selection from $F_2$ or short term bulk.
5. Since, little work and attention is needed in $F_2$ and subsequent generation more no. of crosses can be handled.
6. No pedigree records which saves time
7. Since large population are grown, transgressive segregants are more likely to appear and increase due to natural selection. Hence, there is a greater chance to isolate good segregants than pedigree method.

**DEMERITS OF BULK METHOD**

1. The major disadvantage of bulk method is that it takes a much longer time to develop a new variety. Natural selection becomes important only after $F_8$ or $F_{10}$, and bulking may have to be done upto $F_{20}$ or more. Thus the time required is considerably longer, and most breeders do not use the bulk method simply for this reason.
2. In short-term bulks, natural selection has little effect on the genetic composition of populations. But short-term bulks are useful for the isolation of homozygous lines and for specific objectives as in Harlan's mass-pedigree method.
3. It provides little opportunity for the breeder to exercise his skill or judgement in selection. But in the modified bulk method, the breeder has ample opportunity for practicing selection in the early segregating generations.
4. A large number of progenies have to be selected at the end of the bulking period.
5. Information on the inheritance of characters cannot be obtained which is often available from the pedigree method.
6. In some cases, at least, natural selection may act against the agronomically desirable types.

**Comparison between bulk and pedigree method.**

| S. No. | PEDIGREE METHOD | S. No. | BULK METHOD |
|---|---|---|---|
| 1 | Most widely used Breeding method | 1 | Used only to a limited extent |
| 2 | Individual plants are selected in $F_2$ and subsequent generations and individual plant progenies are grown | 2 | $F_2$ and subsequent generations are grown in bulk |
| 3 | Artificial selection ; artificial disease epidemics etc. are an integral part of the method | 2 | Mainly natural selection. In certain cases artificial selection may be essential |

| | | | |
|---|---|---|---|
| 3 | Natural selection does not play any role | 3 | N.S. determines the composition of the pop n at the end of the bulking period |
| 4 | Pedigree Records have to be maintained which is often time consuming and laborious | 4 | No pedigree records are maintained |
| 5 | Generally its taken 12-13 years to release a new variety | 5 | Takes more than 15 years. |
| 6 | Requires close attention of breeder from $F_2$ onwards | 6 | It is quite simple and does not require much attention |
| 7 | Planting (spacing) the segregating generations are space planted to permits effective individual plant selection | 7 | The bulk populations are generally planted at commercial planting rates |
| 8 | Population size is small in comparison to bulk | 8 | The population size is large |

Much improvement in crop plants could not be done through this method reason being.

1. Long time required for Natural Selector
2. Lack of opportunity for the breeder to use his skills
3. Lack of facilities to raise large population

**Achievements of bulk method:**

The method has been used to a limited extent is Barley breeding in U.S.A. and more than 50 varieties were developed. They are : ARIVAL, BEECHER, GLACIER, and GEM. Originated from a cross : Atlas x Vaughn. The bulk was maintained for 7 to 8 months.

**SINGLE-SEED-DESCENT METHOD**

Another modification of the bulk method is the single-seed-descent method, which is becoming increasingly popular. In this method, a single seed from each of the one to two thousand $F_2$ plants is bulked to raise the $F_3$ generation. Similarly, in $F_3$ and the subsequent generations one random seed is selected from every plant present in the population and planted in bulk to raise the next generation. This procedure is followed till $F_5$ or $F_6$ when the plants would have become nearly homozygous. In $F_5$ or $F_6$, a large number (1 to 5 hundred) of individual plants are selected and individual plant progenies are grown in the next generation. Selection is done mainly among the progenies, and the number of progenies is sufficiently reduced to permit replicated trial in the next generation. Individual plants may be selected only from outstanding families not showing segregation. Thus preliminary yield trials and quality tests begin in $F_7$ or $F_8$ and coordinated yield trials in $F_8$ or $F_9$.

The objective of single-seed-descent method is to rapidly advance the generations of crosses ; at the end of the scheme, a random sample of homozygous or near homozygous genotypes/lines is obtained. $F_2$ and the subsequent generations are grown at very high plant densities as vigour of individual plants is not important. In each year, 2-3 generations may be raised using off-season nurseries and greenhouse facilities. The important features of this scheme are : (1) lack of selection, natural or artificial, till $F_5$ or $F_6$ till the population is reasonably homozygous , and (2) raising of $F_3$ and later generations from a bulk of one seed from each $F_2$ and the subsequent generation plant in order to ensure that each $F_2$ plant is represented in the population. As a result of the speed and economy, the single-seed-descent scheme is becoming increasingly popular with the breeders.

The single-seed-descent scheme (1) advances the generation with the maximum possible speed in a conventional breeding method; (2) requires very little space, effort and labour ; (3) Makes the best use of greenhouse and off-season nursery facilities; and (4) ensures that the plants retained in the end population are random sample from the $F_2$ population. However, (1) it does not permit any form of selection (which is implied in the scheme) during the segregating generations; and (2) in each successive generation, the population size becomes progressively smaller due to poor germination and death of plants due to diseases, insect pests and accidents. In some crops, e.g., pulses, plant loss may be one of the most serious problems of the scheme.

Lecture No. 15

# BACK CROSS

Breeders of early $20^{th}$ century engaged in the development of disease resistant varieties observed that pureline selections with genes for resistance from intra-or inter-specific hybridization were inferior to the generally acceptance superior parent in yield or quality characteristic. To overcome this problem, (Harlan and Pope (1922) suggested the back cross method by which an undesirable allele at a particular locus is replaced by the desirable allele in otherwise elite variety. In other words, B.C. procedure conserves all good characteristics of a popular adapted variety and incorporates a desirable character from another variety.

**Back cross :** A cross between a hybrid ($F_1$ or a segregating generation) and one of its parents is known as backcross.

**Back cross method :** In the B.C. method, the hybrid and the progenies in the subsequent generations are repeatedly back crossed to one of their parents.

**Objective :** To improve or correct one or two specific defects of a high yielding variety, which is well adapted to the area and has other desirable characteristics.

**Recipient parent :** Well adapted, high yielding variety, lacking one or two characters and hence receives these genes from other variety.

**Donor parent** : The variety which donates one or two useful genes.

**Recurrent parent :** Since the recipient parent is repeatedly used in the backcross programme, it is also known as the recurrent parent.

**Non-recurrent parent :** The donor parent, on the other hand, is known as the non-recurrent parent because it is used only once in the breeding programme (for producing the $F_1$ hybrid).

## REQUIREMENTS OF A BACK CROSS PROGRAMME

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

**Applications of Back Cross method**

B.C. method in applicable to both S.P. & C.P. crops.

1. Inter varietal transfer of simply inherited characters : characters governed by one or two major genes – Eg. disease resistance, used color.

   Linkage drag : Failure of transfer of simply inherited characters like disease resistance by B.C. method due to a tight linkage between the gene being transferred and some other undesirable gene.
2. Inter varietal transfer of Quantitative characters : Quantitative characters with high heritability can be transferred.

   Eg. Early ness, Pl. height seed size, seed shape.

3. Inter specific transfer of simply inherited characters : Mostly disease resistance from related species into a cultivated species.

   Eg. 1. Leaf and stem rust resistance from *Triticum timopheevii*

   *T. monococcum, Aegilops speltoides and rye (*S. cereale*) to T. aestivum*

   2. Black arm resistance from several *Gossypium* species to *G. hirsutum*

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

   E. Transfer of *T. timopheevii* cytoplasm to T. aesticem

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

   I. The $F_1$ may be Back crossed only 1 or at most 2 times to the recurrent parent leaving much heterozygosity for transgressive segregants to appear.

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

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

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

**Transfer of a Dominant Gene**

Let us suppose that a high yielding and widely adapted variety A is susceptible to stem rust. Another variety B is resistant to stem rust, and that resistance to stem rust is dominant to susceptibility. A generalized scheme of the backcross programme for the transfer of rust resistance from variety B to variety A is given below.

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

**$F_1$ Generation :** $F_1$ plants are backcrossed to variety A. Since all the $F_1$ plants will be heterozygous for rust resistance, selection for rust resistance is not necessary.

**First Backcross Generation ($BC_1$) :** half of the plants would be resistant and the remaining half would be susceptible to stem rust. Rust resistant plants are selected and backcrossed to variety A. $BC_1$ plants resistant to rust may be selected for their resemblance to variety A as well.

**$BC_2$-$BC_5$ Generations :** In each backcross generation, segregation would occur for rust resistance. Rust resistant plants are selected and backcrossed to the recurrent parent A. Selection for the plant type of variety A may be practiced, particularly in $BC_2$ and $BC_3$.

**$BC_6$- Generation :** On an average, the plants will have 98.4 per cent genes from variety A. Rust resistant plants are selected and selfed; their seeds are harvested separately.

**$BC_6$ $F_2$ Generation :** Individual plant progenies are grown. Progenies homozygous for rust resistance and similar to the plant type of variety A are harvested in bulk. Several similar progenies are mixed to constitute the new variety.

**Yield Tests :** The new variety is tested in a replicated yield trial along with the variety A as a check. Plant type, date of flowering, date of maturity, quality etc. are critically evaluated. Ordinarily, the new variety would be identical to the variety A in performance. Detailed yield tests are, therefore, generally not required and the variety may directly be released for cultivation.

**Transfer of a Recessive Gene**

When rust resistance is due to a recessive gene, all the backcrosses cannot be made one after the other. After the first backcross, and after every two backcrosses, $F_2$ must be grown to identify rust resistant plants. The $F_1$ and the backcross progenies are not inoculated with rust because they would be susceptible to rust. Only the $F_2$ is tested for rust resistance. A generalized scheme for the transfer of a recessive gene for rust resistance is given below.

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

**$F_1$ Generation :** $F_1$ plants are backcrossed to the recurrent parent.

**$BC_1$ Generation :** Since rust resistance is recessive, all the plants will be rust susceptible. Therefore, there is no test for rust resistance. All the plants are self-pollinated.

**$BC_1$ $F_2$ Generation :** Plants are inoculated with rust spores. Rust resistant plants are selected and backcrossed with the recurrent parent. Selection is done for the plant type and other characteristics of the variety A.

**$BC_2$ Generation :** There is no rust resistance test. Plants are selected for their resemblance to the recurrent parent A, and backcrossed with the recurrent parent.

**$BC_3$ Generation :** There is no disease test. The plants are self-pollinated to raise $F_2$. selection is usually done for the plant type of variety A.

**$BC_3F_2$ Generation :** Plants are inoculated with stem rust. Rust resistant plants resembling variety A are selected and backcrossed to variety A. Selection for plant type of A is generally effective.

**$BC_4$ Generation :** There is no rust resistance test. Plants are back-crossed to variety A.

**$BC_5$ Generation :** There is no rust test. Plants are self-pollinated to raise $F_2$ generation.

**$BC_5F_2$ Generation :** Plants are subjected to rust epidemic. A rigid selection is done for rust resistance and for the characteristics of variety A. Selfed seeds from the selected plants are harvested separately.

**$BC_5F_3$ Generation :** individual plant progenies are grown and subjected to rust epiphytotic. A rigid selection is done for resistance to stem rust and for the characteristics of variety A. Seeds from several similar rust resistant homogeneous progenies are mixed to constitute the new variety.

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

Lecture No: 16

# BACK CROSS

**Transfer of Two or More Characters Into a Single Recurrent Parent**

When two of more characters are to be transferred into the same variety, one of the following three approaches may be used.

**Simultaneous Transfer :** Genes for the different characteristics may be transferred simultaneously in the same backcross programme. The characters to be transferred are bought together into the hybrid by successively crossing each of the non-current parents to the recurrent parent or the hybrid thus produced. But in such a case, a larger backcross population would be needed than in the case of transfer of a single character. Further, the breeding programme may be delayed because the conditions necessary for the selection of all the characters may not occur every year. Sometimes, the two genes under transfer may be linked. In such a case, the transfer becomes very easy, and selection for only one gene may be necessary. some examples of such a favourable linkage are; between the genes Lr 24 and Sr 24, Lr 19 and Sr 25, and Lr 26 and Sr 31.

**Stepwise Transfer :** The recurrent parent is first improved for one character. The improved recurrent parent is then used as the recurrent parent in a backcross programme for the transfer of the second character. If additional characters are to be transferred, they are transferred one

at a time in a stepwise fashion. This approach takes much longer time for the transfer of two or more characters.

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

**Merits**

1. The genotype of new variety is nearly identical with that of the recurrent parent, except for the genes transferred. Thus the outcome of a backcross programme is known beforehand and it can be reproduced any time in the future.
2. It is not necessary to test the variety developed by the backcross method in extensive yield tests because the performance of the recurrent parent is already known. This may save upto 5 years' time and a considerable expense.
3. The backcross programme is not dependent upon the environment, except for that needed for the selection of the character under transfer. Therefore, off-season nurseries and green-houses can be used to grow 2-3 generations each year. This would drastically reduce the time required for developing the new variety.
4. Much smaller populations are needed in the backcross method than in the case of pedigree method.
5. Defects, such as susceptibility to disease, of a well-adapted variety can be removed without affecting its performance and adaptability. Such a variety is often preferred by the farmers and the industries to an entirely new variety because they know the recurrent variety well.
6. This is the only method for inter-specific gene transfers, and for the transfer of cytoplasm.
7. It may be modified so that transgressive segregation may occur for quantitative characters.

**Demerits**

1. The new variety generally cannot be superior to the recurrent parent, except for the character that is transferred.
2. Undesirable genes closely linked with the gene being transferred may also be transmitted to the new variety.

3. Hybridization has to be done for each backcross. This often difficult, time taking and costly.
4. By the time the backcross programme improves it, the recurrent parent may have been replaced by other varieties superior in yielding ability and other characteristics.

**Achievements**

1. Two cotton varieties 170-Co-2 and 134 – Co 2m were developed
2. Kalyana sona susceptible to leaf rust. Resistant has been transferred from several diverse sources *i.e.*, Robin, K1, Blue bird, Tobari, Frecor and HS-19
3. Tift 23A is susceptible to downy mildew. The line backcrossed with MS-521A, MS-541 A, MS-570A resistant hybrids were produced.

## Comparison between backcross and pedigree methods

| Pedigree method | Backcross method |
|---|---|
| $F_1$ and the subsequent generations are allowed to self-pollinate | $F_1$ and the subsequent generations are backcrossed to the recurrent parent |
| The new variety developed by this method is different from the parents in agronomic and other characteristics | The new variety is identical with the recurrent parent, except for the character under transfer |
| The new variety has to be extensively tested before release | Usually extensive testing is not necessary before release |
| The method aims at improving the yielding ability and other characteristics of the variety | The method aims at improving specific defects of a well adapted, popular variety |
| It is useful in improving both qualitative and quantitative characters | It is useful for the transfer of both quantitative and qualitative characters provided they have high heritability |
| It is not suitable for genes transfer from related species and for producing substitution of addition lines | It is the only useful method for gene transfers from related species and for producing addition and substitution lines |
| Hybridization is limited to the production of the $F_1$ generations | Hybridization with the recurrent parent is necessary for producing every backcross generation |
| The $F_1$ and the subsequent generations are | The backcross generations are small and |

| | |
|---|---|
| much larger than those in the backcross method | usually consist of 20-100 plants in each generation |
| The procedure is the same for both dominant and recessive genes | The procedures for the transfer of dominant and recessive genes are different |

Multiline variety are mixtures of several purelines of similar height, flowering and maturity dates, seed colour and agronomic characters of each of which has a different gen for resistance to the given disease.

**Characteristics of a good Multiline**

1. Its genetic diversity for vertical resistance genes for the concerned disease
2. The vertical resistance genes should be strong enough
3. It should have normal resistance to other diseases
4. Components of multiline should be uniform for agronomic and other features.
5. It should have yield advantage

**Development of multiline varieties**

A multiline variety is usually created by mixing the seeds of several lines that are similar in appearance but have different genes for resistance to a given disease. There are two main steps in the development of multilines:

1. Development of component lines
2. Evaluation and grouping of the components.

**Development of component lines**

The resistance genes are incorporated in an elite variety or line to produce as many near-isogenic lines as there are distinct R genes. This is done through a conventional backcross programme (5-6 backcrosses), a limited backcrossing (2-3 backcrosses, followed by pedigree selection) or by making double or multiple crosses. The lines obtained from the last two approaches are likely to differ for agronomic and other features as well; therefore, a detailed evaluation of such lines is essential.

**Evaluation and grouping of the components**

The number of component lines should be large, 15-20 according to Borlaug (1959), if durability of resistance is desired. But if a reduced level of disease is the objective, a rather small number of component lines would be adequate.

**Achievements**

Multiline variety appears to be a useful approach to control disease like rusts where new races are continuously produced. In India, four multiline varieties have been released in

wheat. Kalyan Sona and Sonalika, one of the most popular varieties during the late sixties were used as the recurrent parent to produce these varieties. Variety 'KSML3' consists of 8 lines having rust resistance genes from Robin, Ghanate, K1, Rend, Gabato, Blue Bird, Tobari, etc. Multiline 'MLKS11' is also a mixture of 8 lines ; the resistance was derived from E6254, E6056, E5868, Frecor, HS19, E4894, etc. The third variety, KML 7406, has 9 lines deriving rust resistance from different sources. In addition, Sonalika Multiline-1 was released for cultivation in Punjab state (six component lines).

## Lecture No. 17
## 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 over s. 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 temporarily 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., $S_1S_2$ x $S_1S_2$

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 $S_fS_1$, selfing produces $S_f S_f$ and $S_f S_1$ progeny. Mutations for $S_f$ allele may be induced by irradiating the pollen used for self-pollination. There is another allele, $S_F$, 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. sativus*), diploid *Brassica* crops and *Sinapis*. In many cases, different S alleles vary in their activity leading to 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 reaction 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.

*Lecture No: 18*

# *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 (*Cruciferae*), 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 production 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 $1.6 \times 10^{-8}$/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.

# Lecture No 19 & 20
# 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 genes, 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. Cytoplasmic male sterility is known in many plant species, some of wihc are crop plants

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 Cytoplasmic 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 genes 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 chalky endosperm |
| Pearlmillet | Tift 23 A (Tifton) | Susceptible to Green ear & downy mildew |
| Rice | Wild abortive | Incomplete panicle exertion |
| Sunflower | *H petiolaris*<br>*H gigantis* | |
| Tobacco | *Microcephalan* | Reduced vigour in $F_1$ hybrids |
| Wheat | *Aegilops caudata* | 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 counter part 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 posses 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 $F_1$ seed will be sterile
7. Isolation is needed for maintenance of parental lines and for producing hybrid seed

Lecture No. 21

## 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 $p^2$ A, 2pq Aa, and $q^2$ 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 $p^2$, 2pq and $q^2$, 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 umber 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,

$$p = (2D + H) / 2N \text{ or}$$
$$= (D + ½ H) / N \text{ and}$$

$$q = (2R + H) / 2N \text{ or}$$

$$= (R + ½ H) / N$$

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 intervarietal 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 self-pollination 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.

Lecture No. 22

## 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 hybrid 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.

**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 number 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 Flowering and Maturity.** In many cases, hybrids are earlier in flowering and maturity than the parents. This may sometimes be associated with a lower total plant weight. But earliness is highly desirable in many situations, particularly in vegetables. 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.

Lecture No: 23

## 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)", 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 action 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., $a_h$ $a_2$, 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, $a_x a_4$ would be superior to $a_x a_2$, $a_2$a-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 been 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 deleterious 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 hybrids) 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 per acre between 1870 and 1930. Around this time, double cross maize hybrids were introduced; their yields increased steadily from -25 bushels per acre during 1935 to -55 bushels/acre during 1960s. The introduction of single cross hybrids around this time marked a quantum jump in maize yields; it started from -62 bushels/acre in 1960 and rose to -120 bushels/acre by 1990. These data, and those from many other countries, demonstrate the unquestionable superiority of single cross maize hybrids over other varietal forms.

Similarly, hybrid rice has become quite popular in China. The first hybrid variety of rice was released in 1976, and by 1997 hybrid rice occupied -54% of the total paddy area and contributed nearly 64% of the total paddy production in China.

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

| *Category* | *Examples* |
|---|---|
| Crop species | 1. *Asexually propagated species*<br>2. *Cross-pollinated species*: maize, jowar, bajra, sugarbeets, sunflower, forage grasses, castor, forage legumes, and cotton<br>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. 24

## 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 observations 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 can not 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 substantial 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 $S_o$ 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 lines 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.

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

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. 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-1)/2

Lecture No: 25

## Exploitation of Heterosis

**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 Vijay, 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 determined 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 = $n(n-1)(n-2)(n-3)/8$

**Lecture No. 26**

## 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 $F_1$'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 $Syn_1$ 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 $syn_1$ 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 $Syn_1$ generation.

The open-pollinated progeny from the $Syn_1$ generation is termed as $Syn_2$, that from $Syn_2$ as $syn_3$ etc. The performance of $Syn_2$ is expected to be lower than that of $syn_1$ 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 predicting 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 at a later date | Cannot be reconstituted |

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.

Lecture No 27

## POPULATION IMPROVEMENT

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. 38% 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.

**Lecture No 28**

## 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. critical data were not given by the above scientist. 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
3. Recurrent selection for SCA
4. Recuprocal recurrent selection

**1. Simple Recurrent selection :**

| | | |
|---|---|---|
| I year : | Several phenotypically superior plants are selected selfed. Harvested separately and evaluated. Seed of superior plants retained and the rest are discarded. | 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 source for next selection cycle. | |
| III year: | Seed obtained in II year is planted Number of superior plants | First selection |

| | | |
|---|---|---|
| | selection and harvested separately. Seed evaluated. Seeds of superior plants retaine d and the rest discarded. | cycle |
| IV year : | Progeny rows are raised. Inter crossed in all possible ways. Equal amount of seed from each cross is composited. This mixed seed forms the source for next selection cycle. | |

The procedure may be repeated for another one or two selection cycles. The affectiveness 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 crossing plots. Seeds of selected plants from population A in one plot and that of the B in another plot. All possible intercrosses among the progeny rows in each plot are made. Equal amount of seed from all intercrosses from the crossing plot A is mixed to raise the source population of 'A'

next year. Similarly equal amount of seed from inter crosses of plot B is mixed to raise source population B next year. This completes original selection cycle.

IV year : Source populations of A & B are raised from composited seeds of A & B ( III year). Operations of the first year i.e. selection of plants, selfing and crossing with the plants of other population etc. are done.

V year : operations as in second year are repeated.

VI year : Operations as in third year are repeated. This completes first selection cycle. The populations may be subjected to further selection cycles, if necessary by repeating the procedure outlined above.

**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.

Lecture No. 29

## 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.

5. Majority of asexually propagated crops are polyploids : Eg. Sugarcane, Potato, Sweet, Potato
6. 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 clones.

**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 propagative crops.

**Problems in Breeding asexually propagated crops**

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

**Genetic variation within a clone**

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

Mango : Pedda Neelam, Chinna Suwarnarekha

Bana : High gate from Gross Michel

Citrus : Robertson Navel Orange

Sweet oranges : Yuvaraj blood Red

Turmeric : Kesari, Kasturi

## Comparison among clones, purelines and inbreds

| No. | Particular | Clone | Pureline | Inbre |
|---|---|---|---|---|
| . | Mode of pollination in crop species where they occur | Cross-pollination | Self-pollination | Cross-pollination |
| ! | Natural mode of reproduction in such species | Asexual (in most of the cases) | Sexual | Sexual |
| 3 | Genetic make-up of the plants in natural population of such species | Heterozygous | Homozygous | Heterozygous |
| 4 | Obtained through | Asexual reproduction from a single plant | Natural self-pollination from a single homozygous plant | Artificial self-poll other form of inbr selection for sever generation |
| 5 | Maintained through | Asexual reproduction | Natural self-pollination | Artificial self-po close inbreeding |
| 6 | All the plants in a single entity are genetypically | Indentical | Identical | Almost identical |
| 7 | Used directly as a variety | Yes | Yes | No (Used in devel hybrid or synthetic |
| 8 | The genetic make-up of plants within a variety | Heterozygous | Homozygous | Almost homozygo |
| 9 | Organism where found | Plants | Plants | Plants and animals |

Lecture No. 30

### MUTATION BREEDING

The term mutation was coined by Hugo Devries 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.

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 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 $4.92 \times 10^{-4}$ i.e. (1 in 20000 population), when as Su locus at $2.4 \times 10^{-6}$ ( 1 in 25 lakhs). The Wx locus considered to be highly stable.

   The difference in mutation rate may be due to a) Genetic back ground 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 produce 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 $M_2$ 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 $M_3$ or later generations.

**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.

**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 $LD_{50}$ should be optimum. $LD_{50}$ 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. **$M_1$.** 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 $M_1$ plants at the harvest. Care should be taken to avoid outcrossing; this can be achieved either by planting the $M_1$ population in isolation or by bagging the inflorescenes of $M_1$ plants or even the whole $M_1$ plants. $M_1$ plants will be chimeras for the mutations present in heterozygous state. About 20 to 25 seeds from each $M_1$ spike are harvested separately to raise the $M_2$ progeny rows.
2. **$M_2$.** About 2,000 progeny rows are grown. Careful and regular observations are made on the $M_2$ rows. But only distinct mutations are detected in $M_2$ because 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 $M_3$. 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 $M_2$ rows may be expected to have beneficial mutations.

   Alternatively, $M_2$ may be grown as a bulk produced by compositing one or more, but equal number of, seeds from each $M_1$ spike/fruit/branch. Individual plants are then selected in $M_2$ and individual plant progenies are grown in $M_3$.
3. **$M_3$.** Progeny rows from individual selected plants are grown in $M_3$. Poor and inferior mutant rows are eliminated. If the mutant progenies are homogeneous, two or more $M_3$ progenies containing the same mutation may be bulked. Mutant $M_3$ rows are harvested in bulk for a preliminary yield trial in $M_4$.
4. **$M_4$.** A preliminary yield trial is conducted with a suitable check, and promising mutant lines are selected for replicated multilocation trials.
5. **$M_5$-$M_7$.** 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 $F_2$ 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. Superior 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. $F_1$hybrids 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. 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
**Castor :** Aruna (NPH1) – Fast neutrons induced mutant from HC 6

**Lecture No. 31**

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.

**Autopolyploids**

## 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.

**Autopolyploidy**

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 mattercontent than diploids.

**Application of Autopolyploidy in Crop improvement**

**Triploids**

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 amphidiploid or Allotetra ploid.
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 produces the synthetic allehexaploid N.digluta which is reasonably fertile. N digluta is backcrossed to the recipient species (N.tabacum) to produce a pentaploid having complete somatic chromosome complement of N.tabacum and one genome of N.glutinosa. the pentaploid is sufficiently fertile to be backcrossed to N.tabaccum. in the progeny N.tabacum like plants resistant to tobacco mosaic are selected and cytologically analysed.

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 species. B.napus is a case in point; the genetic variability of this species is narrow and the only recourse available is to synthesize new allopolyploid B.napus to widen its genetic base. This is being done by crossing B.campestris (n=10, AA) with B.oleracea (n=9,

CC), the parental diploid species, to produce the amphidiploid B.napus (n=19, AACC). The two species, B.campestris and B.olerancea, have to be crossed as autotetraploids; the cross is very difficult and embryo culture has to be used; somatic hybridization is being used to get around these problems.

**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.

Lecture No: 32

## 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 : Eg. Oryza sativa x O. perennis

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

Hybridization between individuals from different species belonging to the same genus or two 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.

4. 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 (Eg. 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 $F_1$ 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 $F_1$ seedling development / Hybrid sterility**

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

1. **Failure of zygote formation / cross incompatibility.**

   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.

**5. 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. Pollinating 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 growth 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 improved 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. $F_1$ 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 $F_1$ 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**

What, Tobacco, Cotton

Parbhani Kranthi : Derived from 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