

## GPBR 211 - PRINCIPLES OF PLANT BREEDING (2+1)

### Theory

Classification of plants, Botanical description, Floral biology, Emasculation and Pollination techniques in cereals, millets, pulses, oil seeds, fibers, plantation crops etc. Aims and objectives of Plant Breeding; Modes of reproduction, Sexual, Asexual, Apomixis and their classification, significance in plant breeding. Modes of pollination, genetic consequences, differences between self and cross-pollinated crops. Methods of breeding – introduction and acclimatization. Selection . Mass selection Johannson's pure line theory, genetic basis, pure line selection. Hybridization – Aims and objectives, types of hybridization. Methods of handling of segregating generations – pedigree method, bulk method, back cross method and various modified methods. Incompatibility and male sterility and their utilization in crop improvement. Heterosis, inbreeding depression, various theories of Heterosis, exploitation of hybrid vigour-development of inbred lines, single cross and double cross hybrids. Population improvement programmes, recurrent selection, synthetics and composites. Methods of breeding for vegetatively propagated crops. Clonal selection. Mutation breeding – Ploidy breeding. Wide hybridization, significance in crop improvement.

### References

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## **01. Aims and objectives of Plant Breeding**

Plant breeding is an art and science, which tells us ways and means to change the genetic architecture of plants so as to attain a particular objective. Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques. Plant breeding has been practiced for thousands of years, since near the beginning of human civilization. It is now practiced worldwide by individuals such as gardeners and farmers, or by professional plant breeders employed by organizations such as government institutions, universities, crop-specific industry associations or research centers.

International development agencies believe that breeding new crops is important for ensuring food security by developing new varieties that are higher-yielding, resistant to pests and diseases, drought-resistant or regionally adapted to different environments and growing conditions.

The objectives may be

- a) Crop improvement
- b) Improved agronomic characters
- c) Resistance against biotic and abiotic stress

### **1. Increased yield**

Majority of our breeding programmes aims at increased yield. This is achieved by developing more efficient genotypes. The classical examples are utilization of Dee Gee Woo Gen in rice and Norin10 in wheat. Identification and utilization of male sterility

### **2. Improving the quality**

- Rice -milling, cooking quality, aroma and grain colour
- wheat- milling and baking quality and gluten content.
- pulses -Protein content and improving sulphur containing amino acids
- oilseeds- PUFA content

### **3. Elimination of toxic substance**

- HCN content in jowar plants

- Lathyrigen content in *Lathyrus sativus* ( $\beta$ N oxalyamine alanine BOAA)
- Erucic acid in Brassicas
- Cucurbitacin in cucurbits

#### **4. Resistance against biotic and abiotic stresses**

- Biotic stress: Evolving pests and diseases resistant varieties there by reducing cost of cultivation, environmental pollution and saving beneficial insects.
- Abiotic stress: It is location specific problem. Soil factors and edaphic factors some times poses severe problems. Breeding resistant varieties is the easy way to combat abiotic stress.

#### **5. Change in maturity duration** – Evolution of early maturing varieties

#### **6. Improved agronomic characters** –Production of more tillers – E.g. Rice, Bajra,

#### **7. Reducing the plant height to prevent lodging** – Rice

#### **8. Photoinsensitivity** – Redgram, sorghum

#### **9. Non-shattering nature** – Green gram, Brassicas

#### **10. Synchronized maturity** – Pulses

#### **11. Determinate Growth habit –determinate growth** – Pulses

#### **12. Elimination or introduction of dormancy** –Groundnut

#### **Scope of plant breeding**

Since the cultivable land is shrinking and there is no scope for increasing the area under cultivation, the only solution to meet the food requirement is by increasing the crop yield through genetic improvement of crop plants. There are two ways by which yield improvement is possible.

#### **1. Enhancing the productivity of crops**

This can be done

- a) By the proper management of soil and crops involving suitable agronomic practices and harvesting physical resources.
- b) By using high potential crop varieties created by appropriate genetic manipulation of crop plants.

## 2. Stabilizing the productivity achieved

This is done by using crop varieties that are bred especially for wide adaptation or for specific crop zones to offset the ill effects of unfavorable environmental conditions prevailing in the areas.

### Plant breeding, the past, present and future scopes

Indian agriculture remained stagnant particularly during early sixties. Long spells of severe drought and serious out break of disease in some parts of the country led some futurologists to state that a possible doom in India by the end of the decade. However, we achieved break through in crops such as rice, wheat, pearl millet, jowar and maize.

The *indica x japonica* cross derivative ADT 27 is the first high yielding rice of Tamil Nadu. The identification of Dee Gee Woo Gen and release of Wonder rice IR 8 (peta x DGWG) changed the scenario from poverty to problem of plenty. Like wide identification of dwarfing gene in Japanese wheat variety Norin-10 by Borlaug and breeding of Mexican dwarf wheat varieties led to the release of wheat varieties like Kalyan sona in India.

In pearl millet, breeding by male sterile line Tift 23A at Tifton, Georgia by Burton and his coworker and later on its introduction to India led the release of hybrid bajra HB1 to HB4, which increased bajra production many fold. In Jowar, breeding of first male sterile line combined kafir 60A and its introduction into India led to the release of first hybrid sorghum CSH 1 (CK 60A x IS 84) during 1970s.

At present we are in search of alternate source of cytoplasm in almost all crops to breed hybrids with new source of cytoplasm to prevent the possibility of appearance of new pest and diseases. Thus, the future of plant breeding is a challenging task. The deployment of innovative breeding techniques will be a new tool to assist the conventional breeding techniques.

### Undesirable effects of Plant Breeding

1. **Genetic erosion:** Disappearance of land races due to introduction of high yielding varieties. Eg. Introduction of IR 20 rice led to disappearance of land races of samba rice.

2. **Narrow genetic base:** Genetic vulnerability to pest and diseases.

Tift 23A - Bajra - Susceptible downy mildew

T cytoplasm - Maize - susceptible to *Helminthosporium*

3. Minor disease and pest become major due to intensive resistance breeding

RTV (Rice Tungro Virus)

Grey mold in Bengalgram

4. **Attainment of yield plateau:** No more further increase in yield.

### **History of Plant Breeding**

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

- As early as 700 BC Babylonians and Assyrians artificially pollinated the date palm.
- In 1717 Thomas Fairchild produced the first artificial hybrid.
- Joseph Kolreuter, a German made extensive crosses in Tobacco and Solanum between 1760 and 1866 and studied the progenies in detail.
- Thomas Andrew Knight (1759-1835) was the first man to produce several new fruit varieties by using artificial hybridization.
- Le Coutier, a farmer published his results on selection in wheat in the year 1843. He concluded that progenies from single plants were more uniform.
- Patrick Shireff a Scotsman practiced individual plant selection in wheat and oats and developed some valuable varieties.
- Vilmorin (1857) proposed individual plant selection based on progeny testing. This was known as “Vilmorins principle of progeny testing’. He proposed this progeny testing in sugar content in sugar beets (*Beta vulgaris*). But this method was ineffective in wheat. This clearly demonstrated the difference between effect of selection in cross and self-pollinated crops.
- Nilsson and his associates in Sweedish Seed Association, Svalof Sweeden (1890) refined the single plant selection.
- In 1903 Johansen proposed the famous ‘pure line theory’ which states that a pure line is progeny of a single self fertilized homozygous plant. He proposed this theory based on his studies in *Phaseolus vulgaris*.
- G.H. Shull work in maize is the forerunner for the present day hybrid maize programme. He described in detail about the effect of inbreeding.
- During 1960’s Norman Borlaug, the Nobel laureate developed Mexican semi dwarf wheat varieties, which paved the way for green revolution in wheat. The dwarfing gene was isolated from wheat variety Norin 10. Later on this Mexican dwarf were introduced

in the India by Dr. M.S.Swaminathan and a number of high yielding wheat varieties like Kalyan Sona, Sharbathi Sonara were developed.

- In rice the identification of dwarf Dee Gee Woo Gen from a tall rice variety by a Taiwan farmer revolutionized rice breeding. Using this DGWG at IRRI during 1965 the wonder rice IR 8 was released.
- Nobilisation in sugarcane by C.a. Barber and T.S.Venkataraman is another monumental work in plant breeding.

## **02. Modes of Reproduction**

Knowledge of the mode of reproduction and pollination is essential for a plant breeder, because these aspects help in deciding the breeding procedures to be used for the genetic improvement of a crop species. Choice of breeding procedure depends on the mode of reproduction and pollination of a crop species.

Reproduction refers to the process by which living organisms give rise to the offspring of similar kind (species). In crop plants, the mode of reproduction is of two types: viz. 1) sexual reproduction and 2) asexual reproduction

### **I. Sexual reproduction**

Multiplication of plants through embryos which have developed by fusion of male and female gametes is known as sexual reproduction. All the seed propagating species belong to this group.

#### **Sporogenesis**

Production of microspores and megaspores is known as sporogenesis. In anthers, microspores are formed through microsporogenesis and in ovules, the megaspores are formed through megasporogenesis.

#### **Microsporogenesis**

The sporophytic cells in the pollen sacs of anther which undergo meiotic division to form haploid i.e., microspores are called microspore (MMC) or pollen mother cell (PMC) and the process is called microsporogenesis. Each PMC produce four microspores and each microspore after thickening of the wall transforms into pollen grain.

#### **Megasporogenesis**

A single sporophytic cell inside the ovule, which undergo meiotic division to form haploid megaspore, is called megaspore mother cell (MMC) and the process is called megasporogenesis. Each MMC produces four megaspores out of which three degenerate resulting in a single functional megaspore.

#### **Gametogenesis**

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

#### **Microgametogenesis**

This is nothing but the production of male gametes or sperm. On maturation of the 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. The reach of pollen over the stigma is called pollination. After the pollination, the pollen germinates. The pollen tube enters the stigma and travels down the style. The generative nucleus at this phase undergoes another mitotic division to produce two male gametes or sperm nuclei. The pollen along with the pollen tube possessing a pair of sperm nuclei is called microgametophyte. The pollen tube enters the embryo sac through micropyle and discharges the two sperm nuclei.

### **Megagametogenesis**

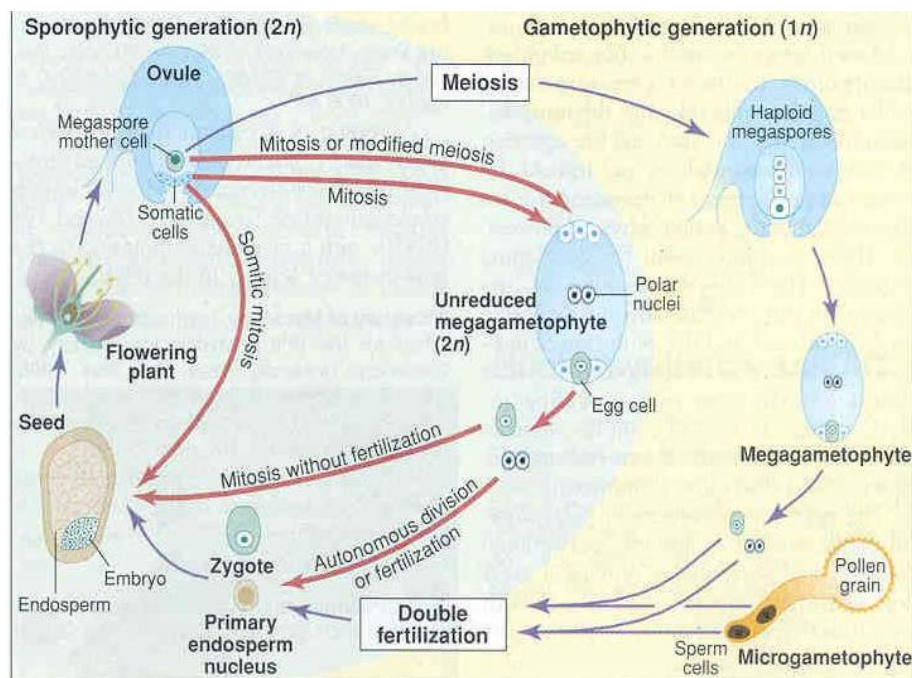
The nucleus of the functional megaspore undergoes three mitotic divisions to produce eight or more nuclei. The exact number of nuclei and their arrangement varies from one species to another. The megaspore nucleus divides thrice to produce eight nuclei. Three of these nuclei move to one pole and produce a central egg cell and two synergid cells on either side. Another three nuclei migrate to the opposite pole to develop into three antipodal cells.

The two nuclei remaining in the center, the polar nuclei, fuse to form the secondary nucleus. The megaspore thus develops into a mature female gametophyte called 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 with the apparent function of guiding the sperm nucleus towards the egg cell and three antipodals which forms the prothalamus cells and one diploid secondary nucleus.

### **Fertilization**

The fusion of one of the two sperms with the egg cell producing a diploid zygote is known as fertilization. The fusion of the remaining sperm with the secondary nucleus leading to the formation of a triploid primary endosperm nucleus is termed as **triple fusion**. The primary endosperm nucleus after several mitotic divisions develops into mature **endosperm**, which nourishes the developing embryo.





## II. Asexual reproduction

Multiplication of plants without the fusion of male and female gametes is known as asexual reproduction. Asexual reproduction can occur either by vegetative plant parts or by vegetative embryos which develop without sexual fusion (apomixis). Thus asexual reproduction is of two types: viz. a) vegetative reproduction and b) apomixis.

Vegetative reproduction refers to multiplication of plants by means of various vegetative plant parts. Vegetative reproduction is again of two types: viz. i) natural vegetative reproduction and ii) artificial vegetative reproduction.

### Natural vegetative reproduction




In nature, multiplication of certain plants occurs by underground stems, sub aerial stems, roots and bulbils. In some crop species, underground stems (a modified group of stems) give rise to new plants. Underground stems are of four types: viz. rhizome, tuber, corm and bulb. The examples of plants which reproduce by means of underground stems are given below:

**Rhizome:** Turmeric (*Curcuma domestica*), Ginger (*Zingiber officinale*)

**Tuber:** Potato (*Solanum tuberosum*)

**Corm:** Arvi (*Colocasia esculenta*), Bunda (*C. antiquorum*)

**Bulb:** Garlic (*Allium sativum*), onion (*A. cepa*)

		
<b>Rhizome: Turmeric</b>	<b>Tuber: Potato</b>	<b>Bulb: Onion</b>

Sub aerial stems include runner, sucker, stolon, etc. These stems lead to vegetative reproduction in mint (*Mentha* sp) rose, strawberry, banana, etc. **Bulbils** are modified forms of flower. They develop into plants when fall on the ground. Bulbils are founding garlic.

### Artificial vegetative reproduction

Multiplication of plants by vegetative parts through artificial method is known as artificial vegetative reproduction. Such reproduction occurs by cuttings of stem and roots, and by layering and grafting. Examples of such reproduction are given below:

**Stem cuttings:** Sugarcane (*Saccharum* sp.) grapes (*Vitis vinifera*), roses, etc.

**Root cuttings:** Sweet potato, citrus, lemon, etc. Layering and grafting are used in fruit and ornamental crops.

### Apomixis

Apomixis refers to the development of seed without sexual fusion (fertilization). In apomixis embryo develops without fertilization. Thus apomixis is an asexual means of reproduction. Apomixis is found in many crop species. Reproduction in some species occurs only by apomixis. This apomixis is termed as **obligate apomixis**. But in some species sexual reproduction also occurs in addition to apomixis. Such apomixis is known as **facultative apomixis**.

There are four types of apomixis: viz.

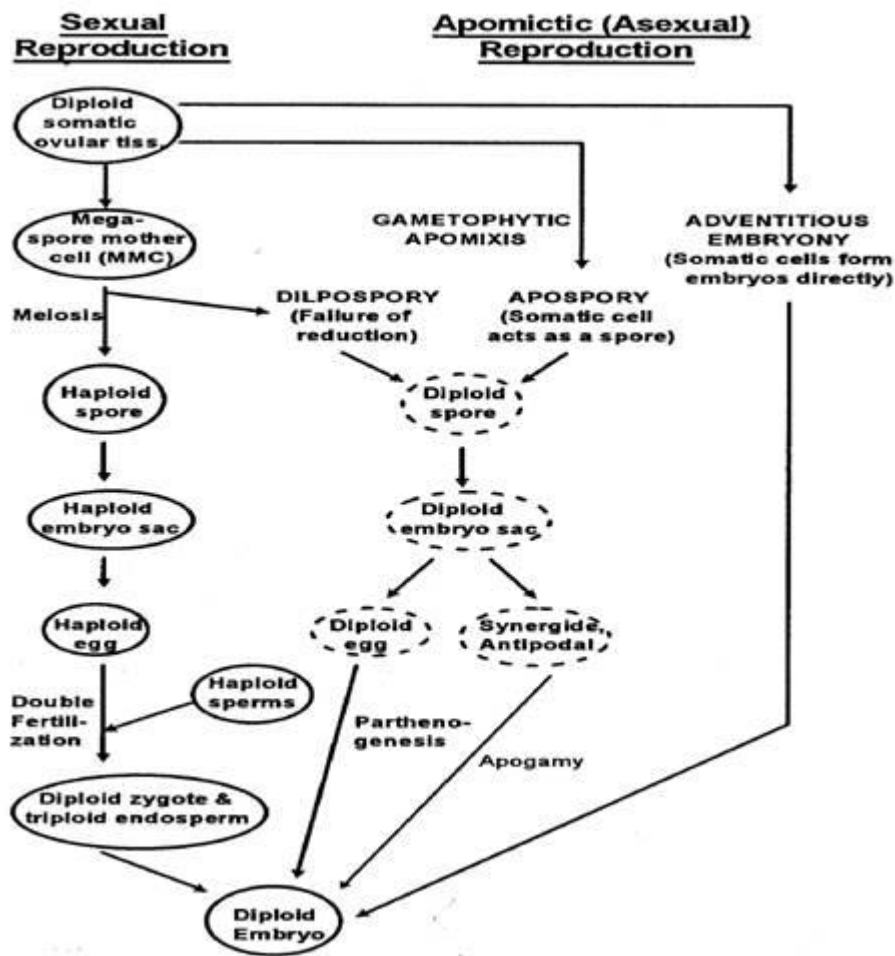
1) parthenogenesis, 2) apogamy, 3) apospory and 4) adventive embryony.

**1. Parthenogenesis.** Parthenogenesis refers to development of embryo from the egg cell without fertilization.

**2. Apogamy.** The origin of embryo from either synergids or antipodal cells of the embryosac is called as apogamy.

**3. Apospory.** In apospory, first diploid cell of ovule lying outside the embryosac develops into another embryosac without reduction. The embryo then develops directly from the diploid egg cell without fertilization.

**4. Adventive embryony.** The development of embryo directly from the diploid cells of ovule lying outside the embryosac belonging to either nucellus or integuments is referred to as adventive embryony.



### **03. Apomixis – classification and significance in plant breeding**

Apomixis, derived from two Greek words "APO" (away from) and "misis" (act of mixing or mingling). It refers to the occurrence of an asexual reproductive process in the place of normal sexual processes involving reduction division and fertilization. In other words apomixis is a type of reproduction in which sexual organs or related structures take part but seeds are formed without union of gametes. Seeds formed in this way are vegetative in origin. When apomixis is the only method of reproduction in a plant species, it is known as obligate apomixis. On the other hand, if gametic and apomictic reproduction occurs in the same plant, it is known as facultative apomixis. The first discovery of this phenomenon is credited to Leuwenhock as early as 1719 in *Citrus* seeds.

Apomixis is widely distributed among higher plants. More than 300 species belonging to 35 families are apomictic. It is most common in Gramineae, Compositae, Rosaceae and Rutaceae. Among the major cereals maize, wheat and pearl millet have apomictic relatives.

#### **Apomixis**

Long back, Winkler (1908) defined apomixis as "the substitution for sexual reproduction or another asexual reproductive process that does not involve nuclear or cellular fusion (i.e. fertilization)". Stebbins (1914) and later Nygren (1954) presented an excellent review on apomixis in angiosperms, which can be referred to for greater details. Here, a brief account of apomixis, is furnished only from the point of view of breeding.

#### **Types of apomixis**

Mainly four types of apomixis phenomenon are suggested by Maheshwari (1954)

##### **1. Recurrent Apomixis**

An embryo sac develops from the MMC or megaspore mother cell (archesporial cell) where meiosis is disturbed (sporogenesis failed) or from some adjoining cell (in that case MMC disintegrates). Consequently, the egg-cell is diploid. The embryo subsequently develops directly from the diploid egg-cell without fertilization. Somatic apospory, diploid parthenogenesis and diploid apogamy are recurrent apomixis. However, diploid parthenogenesis / apogamy occur only in aposporic (somatic) embryo-sacs. Therefore, it is the somatic or diploid apospory that constitutes the recurrent apomixis. Such apomixis occurs in some species of *Crepis*, *Taraxacum*, *Poa* (blue grass), and *Allium* (onion) without the stimulus of pollination. *Malus* (apple), and

*Rudbeckia* where pollination appears to be necessary, either to stimulate embryo development or to produce a viable endosperm.

## **2. Non -recurrent Apomixis**

An embryo arises directly from normal egg-cell (n) without fertilization. Since an egg-cell is haploid, the resulting embryo will also be haploid. Haploid parthenogenesis and haploid apogamy, and androgamy fall in this category. Such types of apomixis are of rare occurrence. They do not perpetuate and are primarily of genetic interest as in com.

## **3. Adventive Embryony**

Embryos arise from a cell or a group of cells either in the nucellus or in the integuments, e.g. in oranges and roses. Since it takes place outside the embryo sac, it is not grouped with recurrent apomixis, though this is regenerated with the accuracy. In addition to such embryos, regular embryo within the embryo sac may also develop simultaneously, thus giving rise to poly-embryony condition, as in *Citrus*, *Opuntia*.

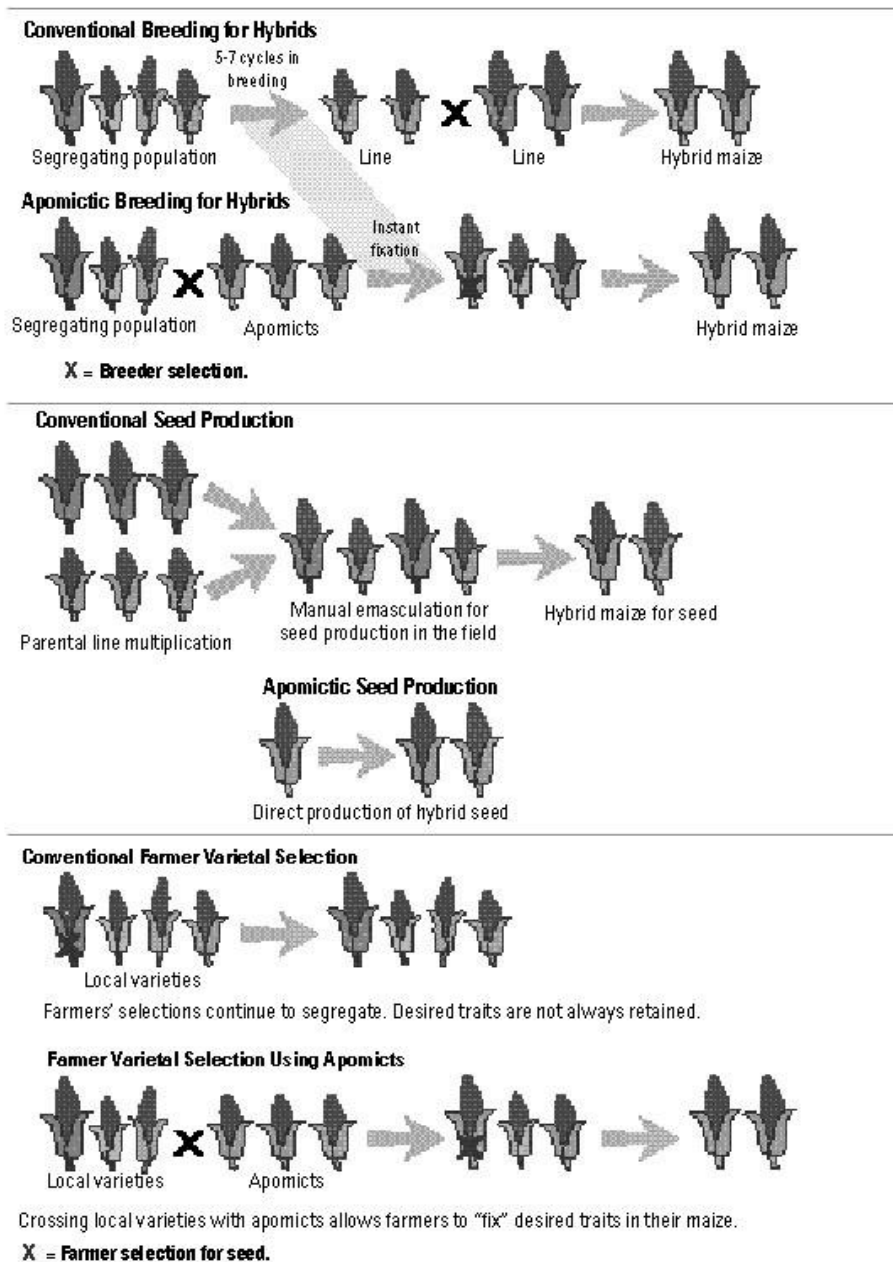
## **4. Vegetative apomixis**

In some cases like *Poa bulbosa* and some *Allium*, *Agave* and grass species, vegetative buds or bulbils, instead of flowers are produced in the inflorescence. They can also be reproduced without difficulty. However, Russian workers do not group this type of vegetative reproduction with apomixis. Now, different apomictic phenomena in each of the recurrent and non-recurrent apomicts are considered in relation to the development of the embryo sac or embryo.

## **Advantages of apomixis in plant breeding**

The two sexual processes, self-and crossfertilization, followed by segregation, tend to alter the genetic composition of plants reproduced through amphimixis. Inbreeding and uncontrolled out breeding also tend to break heterozygote superiority in such plants. On the contrary, apmicts tend to conserve the genetic structure of their carriers. They are also capable of maintaining heterozygote advantages generation after generation. Therefore, such a mechanism might offer a great advantage in plant breeding where genetic uniformity maintained over generation for both homozygosity (in varieties of selfers), and heterozygosity (in hybrids of both selfers and outbreeders) is the choicest goal. Additionally, apomixis may also affect an efficient exploitation of maternal influence, if any, reflecting in the resultant progenies, early or delayed because it causes the perpetuation of only maternal individuals and maternal properties due to

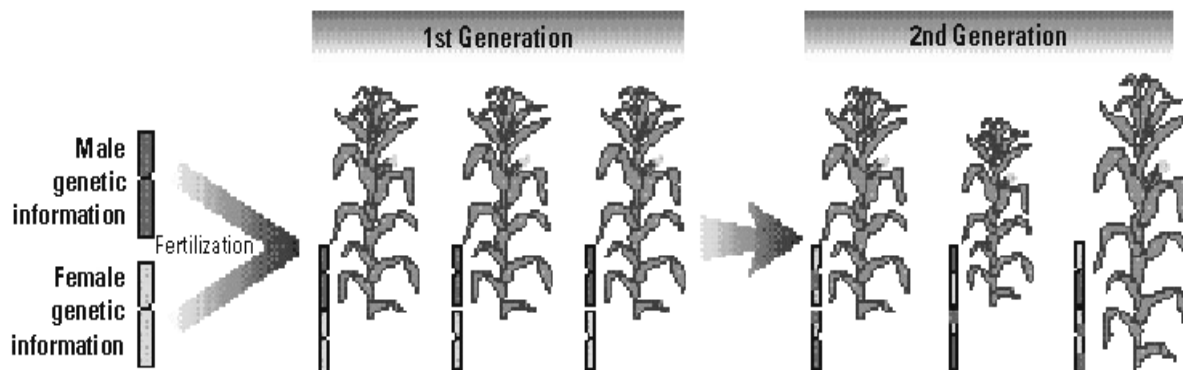
prohibition of fertilization. Maternal effects are most common in horticultural crops, particularly trees and ornamental plants.



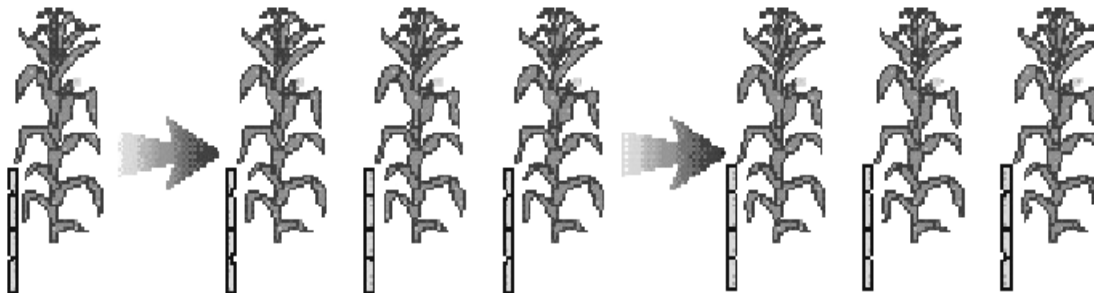
Thus, in short the benefits of apomixis, insofar as their utility in plant breeding is concerned, are:

1. Rapid multiplication of genetically uniform individuals can be achieved without risk of segregation.
2. Heterosis or hybrid vigour can permanently be fixed in crop plants, thus no problem for recurring seed production of F<sub>1</sub> hybrids.

- Efficient exploitation of maternal effect, if present, is possible from generation to generation.
- Homozygous inbred lines, as in corn, can be rapidly developed as they produce sectors of diploid tissues and occasional fertile gametes and seeds.



**Sexual Reproduction:** Hybrid maize that has been produced through sexual reproduction displays identical genetic makeup. The depiction of the second generation represents the use of seed recycled from hybrids, a common practice in many developing countries, and its varied results.



**Apomictic Reproduction:** Hybrid maize that has been produced apomictically (asexually) also displays identical genetic makeup in the first generation, but it *retains* its genetic composition and characteristics through the second generation and beyond.

### Exploitation of apomixis in crop improvement

The use of apomixis in crops in a follow-up process, after a variety or hybrid is evolved, as reflected by the benefits it renders. Therefore, our aim in this section is to deal with only apomixis as a tool to plant breeding. With a view to exploit apomixis in sexual crops, it needs to detect and identify an apomictic phenomenon, occurring spontaneously in any plant, or, to incorporate it artificially, perhaps through hybridization between apomicts and amphimicts.

### Detection of apomixis

Positive evidence for the presence or absence of apomixis can be obtained only from an intensive screening of a large number of plants in a variety/hybrid. The screening involves a

careful and systematic tracing of steps for the development of embryo-sac and embryo, through microtomy of ovule, right from megaspores to embryonic development. as such, therefore, it is a most tedious job requiring a lot of patience and persistence indeed.

It should however be noted that it is only recurrent apomixis, namely diploid forms of apospory / parthenogenesis / apogamy / adventive embryony and vegetative propagation which are beneficial for plant breeding purposes. The simple reason being that it is these which produce viable diploid embryos without fertilization and thus can continue to perpetuate over generations. Nonrecurrent apomixis are of academic use.

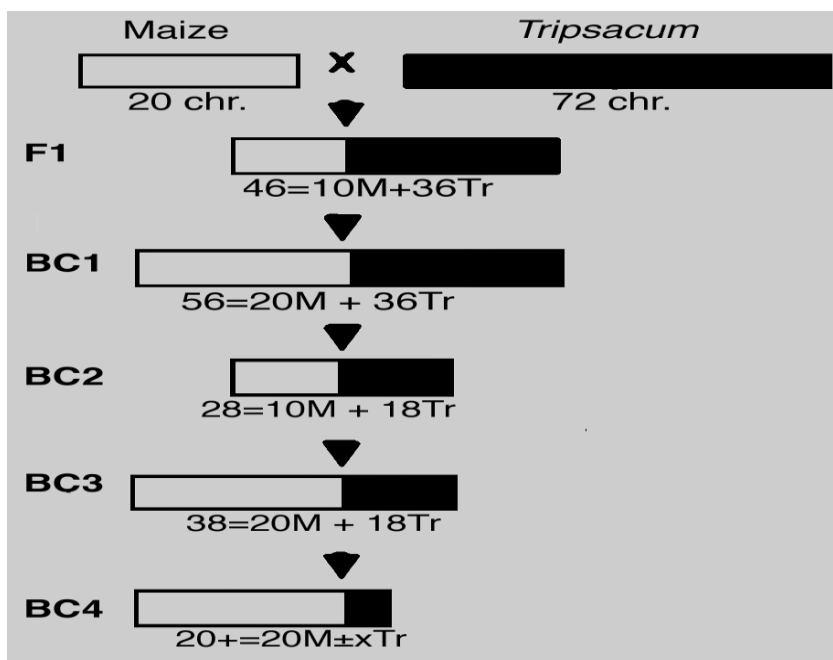
### Maintenance of apomixis

Once an apomict plant is detected its inheritance should promptly be studied by crossing a half or few flowers with the pollen obtained from normal plants and going through the segregation pattern in F2 and onward generations. The remaining flowers may thoroughly be checked and seeds collected on maturity. The true nature of such plants would become distinct only after progeny tests. A true apomictic plant will automatically produce mother apomictic progenies which can be maintained without difficulty.

### Transfer of apomixes

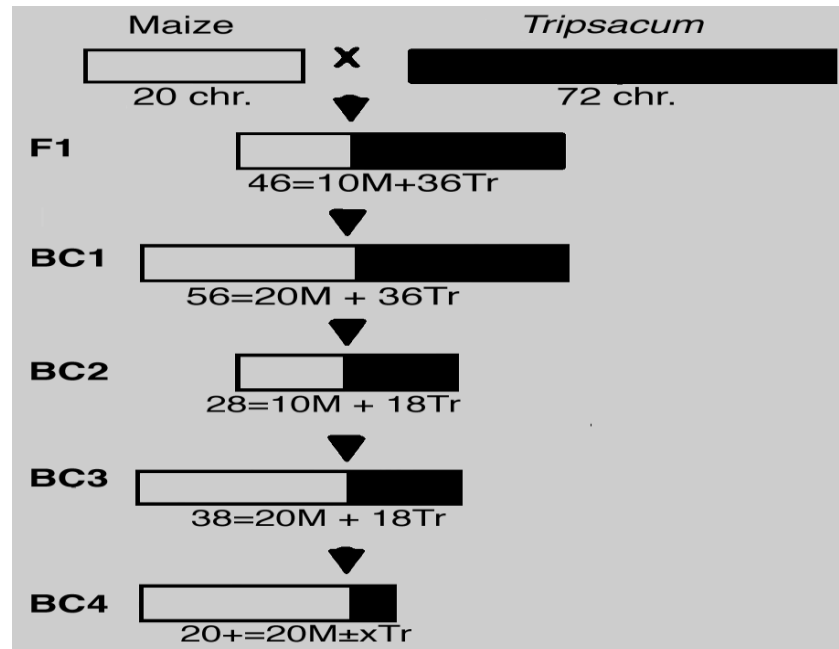
With regard to transfer of apomixis, substantial evidence is available for the hybrid origin of many of the apomicts. Nevertheless, there is no evidence at all the hybridization by itself can induce apomixis (Stebbins, 1950). Situation is further aggravated by the unstable nature of apomicts since there is every likelihood of the breaking down of interacting gene complexes conditioning apomixis, as stated earlier. Therefore, possibilities of introducing apomixis in non-apomicts are the least but not totally absent.

The CIMMYT Apomixis Project was launched in 1989 with the goal of transferring the naturally untapped to maize, that and farmer Building occurring, trait of apomixis an achievement could dramatically directly improve productivity in the developing world. on decades of IRD





(formerly ORSTOM) apomixis research, project scientists are working to create apomictic maize. A hybrid between maize and its wild relative *Tripsacum* is being backcrossed in pursuit of a true apomictic maize variety. This long-term strategy has provided very encouraging results. It was found that all *Tripsacum* chromosomes can be transferred into addition lines and that about 10% of these plants also show maize-*Tripsacum* translocations, suggesting that 20-chromosome recovered maize plants with small *Tripsacum* DNA segments can be produced from the backcross series.



**Production of Maize-*Tripsacum* addition lines:** Four backcross generations are required to produce addition lines from maize-*Tripsacum* F1 hybrids.

## 04. Modes of Pollination

The process by which pollen grains are transferred from anthers to stigma is referred as pollination. Pollination is of two types: viz. 1) Autogamy or self pollination and 2) Allogamy or cross pollination.

### I. Autogamy

Transfer of pollen grains from the anther to the stigma of same flower is known as autogamy or self pollination. Autogamy is the closest form of inbreeding. Autogamy leads to **homozygosity**. Such species develop homozygous balance and do not exhibit significant inbreeding depression.

#### Mechanism promoting self-pollination

##### 1. Bisexuality

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

##### 2. Homogamy

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

##### 3. Cleistogamy

When pollination and fertilization occur in unopened flower bud, it is known as cleistogamy. It ensures self pollination and prevents cross pollination. Cleistogamy has been reported in some varieties of wheat, barley, oats and several other grass species.

##### 4. Chasmogamy

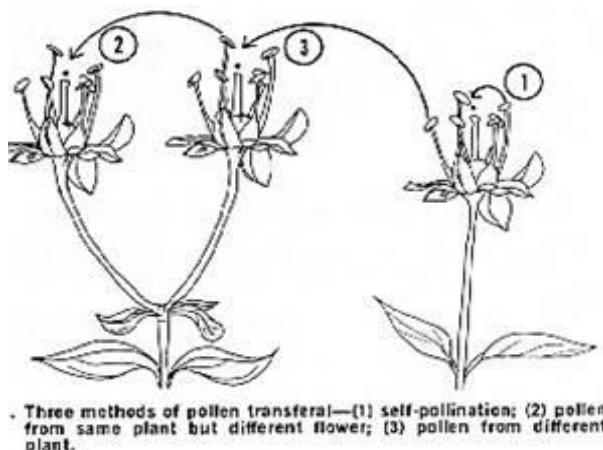
Opening of flowers only after the completion of pollination is known as chasmogamy. This also promotes self pollination and is found in crops like wheat, barley, rice and oats.

##### 5. Position of Anthers

In some species, stigmas are surrounded by anthers in such a way that self pollination is ensured. Such situation is found in tomato and brinjal. In some legumes, the stamens and stigma are enclosed by the petals in such a way that self pollination is ensured. Examples are greengram, blackgram, soybean, chickpea and pea.

## II. Allogamy

Transfer of pollen grains from the anther of one plant to the stigma of another plant is called allogamy or cross pollination. This is the common form of breeding. Allogamy leads to heterozygosity. Such species develop heterozygous balance and exhibit significant inbreeding depression on selfing.



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### Mechanism promoting cross-pollination

#### 1. Dicliny

It refers to unisexual flowers. This is of two types: viz. i) monoecy and ii) dioecy. When male and female flowers are separate but present in the same plants, it is known as **monoecy**. In some crops, the male and female flowers are present in the same inflorescence such as in mango, castor and banana. In some cases, they are on separate inflorescence as in maize. Other examples are cucurbits, grapes, strawberry, cassava and rubber. When staminate and pistillate flowers are present on different plants, it is called **dioecy**. It includes papaya, date palm, spinach, hemp and asparagus.

#### 2. Dichogamy (from the Greek *dikho*-apart and *gamous*-marriage)

It refers to maturation of anthers and stigma of the same flowers at different times. Dichogamy promotes cross pollination even in the hermaphrodite species. Dichogamy is of two types: viz. i) protogyny and ii) protandry. When pistil matures before anthers, it is called **protogyny** such as in pearl millet. When anthers mature before pistil, it is known as **protandry**. It is found in maize, sugarbeet and several other species.

#### 3. Heterostyly

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

#### 4. Herkogamy

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

#### 5. Self incompatibility

The inability of fertile pollens to fertilize the same flower is referred to as self incompatibility. It prevents self-pollination and promotes cross pollination. Self incompatibility is found in several crop species like *Brassica*, *Radish*, *Nicotiana*, and many grass species. It is of two types **sporophytic** and **gametophytic**.

#### 6. Male sterility

In some species, the pollen grains are non functional. Such condition is known as male sterility. It prevents self-pollination and promotes cross pollination. It is of three types: viz. **genetic**, **cytoplasmic** and **cytoplasmic genetic**. It is a useful tool in hybrid seed production.

Study of **floral biology** and aforesaid mechanisms is essential for determining the mode of pollination of various crop species. Moreover, if selfing has adverse effects on seed setting and general vigour, it indicates that the species is cross pollinated. If selfing does not have any adverse effect on these characters, it suggests that the species is self-pollinated.

The percentage of cross pollination can be determined by growing a seed mixture of two different varieties together. The two varieties should have marker characters say green and pigmented plants. The seeds are harvested from the recessive (green) variety and grown next year in separate field. The proportion of pigmented plants in green variety will indicate the percentage of **outcrossing** or cross pollination.

#### Significance of pollination

The mode of pollination plays an important role in plant breeding. It has impact on five important aspects: viz. 1) gene action, 2) genetic constitution, 3) adaptability, 4) genetic purity and 5) transfer of genes.

### Classification of crop plants based on mode of pollination and mode of reproduction

Mode of pollination and reproduction	Examples of crop plants
<b>A. Autogamous Species</b>	
1. Seed Propagated	Rice, Wheat, Barley, Oats, Chickpea, Pea, Cowpea, Lentil, Green gram, Black gram, Soybean, Common bean, Moth bean, Linseed, Sesame, Khesari, Sunhemp, Chillies, Brinjal, Tomato, Okra, Peanut, etc.
2. Vegetatively Propagated	Potato
<b>B. Allogamous Species</b>	
1. Seed Propagated	Corn, Pearl millet, Rye, Alfalfa, Radish, Cabbage, Sunflower, Sugarbeet, Castor, Red clover, White clover, Safflower, Spinach, Onion, Garlic, Turnip, Squash, Muskmelon, Watermelon, Cucumber, Pumpkin, Kenaf, Oil palm, Carrot, Coconut, Papaya, etc.
2. Vegetatively propagated	Sugarcane, Coffee, Cocoa, Tea, Apple, Pears, Peaches, Cherries, grapes, Almond Strawberries, Pine apple, Banana, Cashew, Irish, Cassava, Taro, Rubber, etc.
<b>C. Often Allogamous Species</b>	Sorghum, Cotton, Triticale, Pigeonpea, Tobacco.

### **Genetic consequences of self and cross-pollination**

<b>S.No.</b>	<b>Self-Pollination</b>	<b>Cross-Pollination</b>
1	Self pollination leads to a very rapid increase in homozygosity. Therefore, populations of self – pollinated species are highly homozygous.	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 heterosis.
2	Self pollinated species do not show inbreeding depression, but may exhibit considerable heterosis.	The breeding methods in such species aim at improving the crop species without reducing heterozygosity to an appreciable degree.
3	The aim of breeding methods generally is to develop homozygous varieties. The inbreeding mechanisms are generally under precise genetic control, but can be influenced by both the genetic background as well as the environment.	Usually hybrid or synthetic varieties are the aim of breeder wherever the seed production of such varieties is economically feasible.

## 05. Classification of plants

According to the use of plants and plant products to man the grouping is made.

### Cereals

It is generally applicable to the **grains** obtained from the members of the Family **Poaceae**. Rice, Wheat, Maize, Sorghum, Ragi, Barley, Pearl Millet, Fox tail millet, Rye, Oats etc come under this group. Principal source of food for man and animals. Botanically the characteristic fruit of the family Poaceae is **caryopsis**. Millet is generally used for the member of small grained cereals which are of minor importance as food. Few species of plants other than those of poaceae which produce small grains and used as food as in the of cereals. They are **pseudo cereals**. Buck wheat( *Agropyrum spp* – Chenopodiaceae, grain amaranthus – *Amaranthus spp* Amaranthaceae, Quina, (*Chenopodium quinoa* - Chenapodiaceae). The major cereals are paddy, wheat sorghum, ragi, maize , pearl millet, and the minor millets are fox tail millet, little millet etc . minor cereals are of important space of food in drought prone rural areas. Tillering habit is more common in cereals except in maize and sorghum. The infloresence is panicle, which may be compact or loose. The grain is caryopsis. Cereals supply food to man and straw to animals. Grain contains starch as the major components. Rice is the staple food for nearly half of the world population. Contains large proportion of starch wheat contains good preparation. Bearly is used as malted food and flour is processed in the form of light food.

### Pulses

Seeds of leguminous plants. Pulses supply protein, chief source of in vegetarian food. Seeds are generally used, the whole fruit or pods, with young and mature leguminous plants fix atmospheric Nitrogen in their root nodules by the nitrogen fixing bacteria. The whole plant body in legumes in papilionaceous plants rich in nitrogen and the seeds, the pods and also the leaves and shoots contains a high proportions of protein and are hence used as food. The average per capita consumption of pulses in India is alone one ounce but the minimum require wet is along three ounces, *Cajanus cajan* – Red gram, *Vigna mungo* (black gram) *V. radiata* (green gram)

### Vegetable, Fruits and Nuts

Olericulture deals with vegetables, Pomology deals with fruits and nuts which are rich and valuable sources of food. Horticulture – the branch of Agriculture relating to the cultivation of fruits (Pomology), vegetables (Olericulture) and flowers and oranamental plants (Floriculture)

### Oils and oil seeds

Oil seeds are important both for consumption and for industrial purpose. In human diet, fat is supplied by oils which give the necessary energy for metabolism besides adding taste to the food. Oil is used for medicinal purpose and also for preparation of soap, cosmetics, and lubrication. Castor and coconut oil are the important industrial oil.

### **Sugars and Starch**

The use of sugarcane for the production of jaggery has been in existence for many centuries. In Europe, Canada and USA sugar beet is the source of sugar. Sugar beet was not prominent in tropical countries because sugarcane give high tonnage of yield. The other sources are palmyrah, coconut and date. The tapped juice of the plants are converted into palm gur, the cheap source of sugar to the people. Sugars, besides being used as food sweeteners are rich source of energy.

In Indian diet, cereals supply the bulk of starch as in rice, sorghum, maize and other cereals. Starchy food is also obtained from sweet potato, tapioca, and sago palm. Starch is also an industrial product used in confectionary, textiles, stationary and cosmetic industries.

### **Fibres**

Next to food clothing is the most important one and is obtained from wood pulp for the manufacture of gunny bags, hessian cloths, and packing material. The fibres of Jute and Mesta are of importance. Twines, cordages and ropes needed in daily life are made from them. In carpet, mats, brushes, and for stuffing purpose such as cotton is used.

### **Beverages**

Coffee, tea, cocoa are important beverages and they have stimulating effect. Fruit juices like lemonades, orangeades, apple, pineapple and mango juices constitute the soft drinks. Coffee and tea are commercial crops grown in plantation and exported. Cocoa is gaining importance in beverages and confectionaries.

### **Narcotics, fumigatives and masticatories**

Products from tobacco, ganja, opium which have a stimulating effect on small doses come under narcotics. Narcotics are substances which produce a stimulating or drowsy effect. They relieve pain and produce sleep. Mild stimulating preparations, adjuncts to fermentation, flavouring ingredients to



beverages, and mild poisons are also called narcotics. When substance are smoked because of the stimulating effect of tobacco they are called as fumitories. Substance which are chewed as the betel leaf and arecanut for the masticatories. Tobacco comes as Narcotics, Fumitories and masticatories. The alkaloid present in the plant parts are responsible for creating the effects. Drugs are obtained from large number of plants are called medicinal plants.

### **Species and containments**

A variety of plant products are made use of as food adjuncts to add flavour, aroma and taste, is spices and those give aroma and flavour is condiments. Pepper, cardamom cloves, chillies, turmeric ginger, onion, and garlic. The species and condiments have essential oils which are responsible for the flavour and taste.

### **Rubber**

The rubber plantations in tropical countries, given the species, *Hevea brasiliensis* a plant introduced from Brazil Latex is obtained from the plant and processed as used as rubber. Rubber is also obtained from *Manihot glaziovii* *Cryptostegia* and *Taraxacum*.

### **Forages**

Feed for domestic animals is obtained from grain crops and fodder crops. Generally includes fodder and postarages; Guinea grass Napier grass, Lucerne, fodder cholam, fodder Maize etc., are harvested and fed to animals. The grasses and legumes are grown in arable land and left for grazing of animals come under pastures. The foliage of number of trees and shrubs which are edible to animals form the another source of forage.

### **Green manures and green leaf manures**

Growing of special crops for adding organic matter and Nitrogen to the soil and ploughing them in situ is called green manuring. daincha, sunnhemp, pillipesara, kolingi, indigo and *Sesbania speciosa* the green lopping from shrubs, trees incorporated in the field as from *Ipomoea cornea*, and *Gliricidia* form the green leaf manuring. Usually green manuring plants are popilonacious type which fix nitrogen in the soil by the formation of bacterial nodules and higher 'N' content in leaves and shoots.

## 06. Botanical description and floral biology

### Cereals

#### Characters of Cereals

□ Most of the cereals are herbaceous annuals. □ Stem or culm often erect, cylindrical, hollow except at nodes. Tillering habit, shallow fibrous root system. □ Leaves alternate, distichously with parallel venation and sheathing leaf base. □ Presence of ligules, lodicules □ Inflorescence is panicle or spike □ Stamens usually three (in rice- six). Fruit is a caryopsis.

#### Rice – *Oryza sativa* L. (2n = 24)

##### Systematic Position:

Division : Phanerogams

Sub-Division : Angiosperms

Class : Monocotyledon

Series : Glumacea

Sub class : Glumiflorae

Family : Poaceae

Sub family : Poaideae

Tribe : Oryzeae

**Origin:** India or Africa

#### Putative parents and origin of cultivated rice

There are two divergent views regarding the origin of cultivated rice.

i. **Polyphyletic:** Originated from several species.

According to this theory, the two forms of cultivated rice viz., Asian rice *O. sativa* and African rice, *O. glaberrima* have evolved independently in their respective regions from several species.

ii. **According** to this theory both Asian rice and African rice arose from a common parent. (*O. perennis*). This view is the most accepted one because both Asian rice and African rice are similar except in glume pubescence, ligule size and colour of pericarp which is red in African rice.

### **Species in the genus *Oryza***

According to the latest view the genus *Oryza* include 22 valid species. Out of these, two are cultivated diploids viz. *O. sativa* and *O. glaberrima* and rest are wild species with include both diploid and tetraploid forms.

### **Subspecies in cultivated *Oryza sativa***

Rice has been in cultivation for long period and adapted well under diverse climatic conditions and soils. This has resulted in the evolution of three geographical races which has been given subspecies status. The three subspecies are:

- i. *O. sativa* subsp **indica** : Tall spreading, more tillering, awnless
- ii. *O. sativa* subsp **japonica** : Short, erect, more tillering, awnless
- iii. *O. sativa* subsp **javanica** : Tallest, erect, poor tillering, awned

Marked sterility barriers occur between the subspecies. It ranges up to 80% in case of indica x japonica where as it is less in case of indica x javanica.

### **Wheat – *Triticum* sp. (X = 7)**

Wheat is the most important cereal in the world, giving about one-third of the total production, followed closely by rice. In temperate regions it is the major source of food. The chief use of wheat is the flour for making bread.

#### **Systematic position:**

**Division** : Phanerogams

**Sub-Division** : Angiosperms

**Class** : Monocotyledon

**Series** : Glumacea

**Sub class** : Glumiflorae

**Family** : poaceae

**Tribe** : Triticeae

**Subfamily** : Pooideae

#### **Chromosome number:**

**Diploid** :  $2n = 14$ , **Tetraploid** :  $2n = 28$ , **Hexaploid** :  $2n = 42$

**Place of Origin:****Diploid:** Asia minor, **Tetraploid :** Abyssinia, North Africa, **Hexaploid :** Central Asia**Classification:**

Ploidy level	Species	Common name	Genome
<b>Diploid</b>	<i>T.boeiticum</i>	Wild einkorn	AA
(2n=14) 2 species	<i>(T.aegilopoides)</i>	Einkorn	AA
	<i>T.monococum</i>		
<b>Tetraploid</b>	<i>T.dicoccoides</i>	Wild Emmer	AA BB
(2n=28) 7 species	<i>T.dicoccum</i>	Emmer	AA BB
	<i>T.durum</i>	Macaroni wheat	AABB
	<i>T.persicum</i>	Persian wheat	AABB
	<i>T.turgidum</i>	Rivet wheat	AABB
	<i>T.polonicum</i>	Polish wheat	
	<i>T.timopheevi</i>	-	
<b>Hexaploid</b>	<i>T.aestivum</i>	Common or bread	AABBDD
(2n= 42) 5 species	<i>T.compactum</i>	wheat	AABBDD
	<i>T.sphaerococcum</i>	Club wheat	AABBDD
	<i>T.spelta</i>	Dwarf wheat	AABBDD
	<i>T.macha</i>	Spelt wheat	AABBDD
		Macha wheat	

Fourteen species of wheat are present according to Vavilov



- |                            |                          |
|----------------------------|--------------------------|
| 1. <i>T.boeoticum</i>      | 2. <i>T.monococcum</i>   |
| 3. <i>T.dicoccoides</i>    | 4. <i>T.dicoccum</i>     |
| 5. <i>T.durum</i>          | 6. <i>T.persicum</i>     |
| 7. <i>T.turgidum</i>       | 8. <i>T.polonicum</i>    |
| 9. <i>T.timopheevi</i>     | 10. <i>T.aestivum</i>    |
| 11. <i>T.sphaerococcum</i> | 12. <i>T.compactum</i> , |
| 13. <i>T.spelta</i>        | 14. <i>T.macha.</i>      |

### Origin of diploid wheat:

(Wild einkorn) *T.boeoticum* (*T.aegilopoides*)

Natural mutation and selection  
↓

*T.monococcum*

Cultivated diploid

AA (2n = 14)

*T. boeoticum* is probably the ancestor for all the cultivated wheats

### Origin of Tetraploid wheats:

*T.boeoticum* x *Aegilops speltoides*

AA	↓	BB
2n = 14		2n 14

F<sub>1</sub> Sterile (2n=14) (AB)

Natural mutation and Doubling  
↓

*T.dicoccoides* 2n = 28

Wild emmer AABB

By natural ↓ selection

*T.dicoccum* (Emmer wheat)

### Origin of hexaploid wheats (Fig.2):

*T.dicoccum* x *Aegilops squarrosa*

AABB	↓	DD
2n = 28		2n = 14
F <sub>1</sub>		

ABD(2n = 21)

Sterile

Natural doubling  
↓

*T.aestivum*

AABBDD (2n = 42)

(Cultivated)

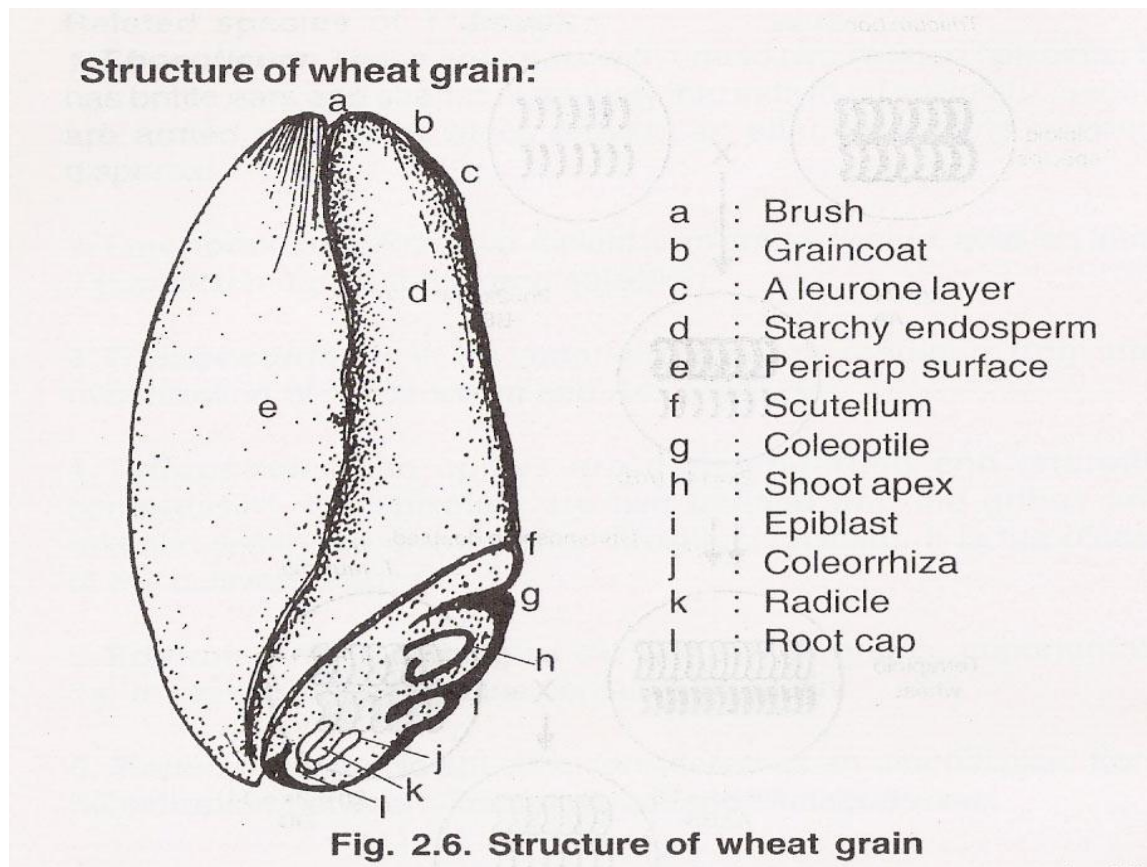
AABB (2n=28) Cultivated

### Structure of Wheat Grain

Fruit is a dry, one seeded indehiscent fruit known as caryopsis. The grain may be either hard or soft in texture with a creamy white, amber, red or purple colour depending upon variety. The dorsal (back side) convex surface of kernal is smooth except at the base where the fruit coat is wrinkled indicating the position of embryo the ventral surface (front side) is flat and characterised by a deep furrow or groove.

**The following 4 structures are recognized in wheat grain**

i. Grain coat, ii. Nucellar epidermis, iii. Endosperm and iv. Embryo.



## Lecture 07

### Maize - *Zea mays* (2n - 20)

Maize is the most important cereal in the world after wheat and rice; it was also most widely distributed. The genus *Zea* is considered to be monotypic previously. Recently *Teosinte* the related genera of *Zea* has been included as *Zea mexicana*.

**Centre of origin :** Southern Mexico.

#### **Systematic Position**

**Division :** Phanerogams

**Sub - Division :** Anageosperms

**Class :** Monocotyledon

**Series :** Glumacea

**Sub class :** Glumiflorae

**Family :** Poaceae

**Sub family :** Poaideae

**Tribe :** Maydeae

***Zea Mexicana*** - The Probable Three Species involved in The evolution ancestor of Maize of Cultivated Maize

#### ***Tripsacum dactyloides* (2n= 36, 72) Gama grass:**

A perennial grass which is used as fodder. Distributed in tropical and subtropical North America.

#### **Origin and putative parent:**

There are three different views about the origin of maize.

1. It originated from *Teosinte* (*Euchlaena mexicana*) (*Zea maxicana*) by direct selection, mutation or hybridization with other grasses.
2. Another theory is that maize originated from a wild pod corn.
3. Another theory is that *teosinte*, *tripsacum* and maize, all descended from a common ancestor by divergent evolution but the ancestor would have been lost.



**Sorghum -*Sorghum bicolor* (L) Moench (2n = 20)**

Sorghum is the fourth important world cereal, following wheat, rice and maize. It is the staple food in the drier parts of tropical Africa, India and China. The threshed grain is ground into a wholemeal flour, and used for making thin porridge or a thick paste or dough by boiling in water.

**Systematic Position:**

**Division :** Phanerogams

**Sub - Division :** Anageosperms

**Class :** Monocotyledon

**Series :** Glumacea

**Sub class :** Glumiflorae

**Family :** Poaceae

**Sub family :** Poaideae

**Tribe :** Andropoganae

**Sub tribe :** Sorgasturn

**Origin:**

Africa in the primary centre. India is the secondary centre of origin.

***Sorghum bicolor* (2n = 20)**

**Origin:** Africa

**Progenitor of sorghum**



1. *S.arundinaceum* 2. *S.verticilliflorum* 3. *S.sudanense* 4. *S.aethiopicum*. The cultivated sorghum *Sorghum bicolor* is divided in to five basic races based the coverage of glume on the grain (Fig 1).

### **Hybrid races:**

This consists of all combinations of the basic races.

1.Guniea	bicolor (GB)	6.Guinea	kaffir (GK)
2.Caudatum	bicolor (CB)	7.Guinea	durra (GD)
3.Kaffir	bicolor (KB)	8.Kaffir	caudatum (KC)
4.Durra	bicolor (DB)	9.Kaffir	durra (KD)
5.Guinea	caudatum (GC)	10.Durra	caudatum (DC)

### **Pearlmillet - *Pennisetum glaucum* L. (2n = 14)**

Pearl millet is the staple food in the drier parts of Tropical Africa and in India, where it is the fourth most important cereal after rice, sorghum and wheat. The grains are also fed to poultry and other livestock. The green plants provide a useful fodder and it is sometimes grown for this purpose. It also plays a major role in fodder improvement by crossing with Napier grass.

### **Systematic Position :**

**Division :** Phanerogams

**Sub – Division:** Anageosperms

**Class :** Monocotyledon

**Series :** Glumacea

**Sub class :** Glumiflorae

**Family :** Poaceae

**Tribe :** Paniceae

**Origin:** Africa

**Distribution :** Africa, India, Pakistan, Bangladesh,

### **Origin and putative parents**

**Stapf** included 32 species is *Penicillaria*. Of these 32 species found in Africa, six annuals are considered wild and probable ancestors of the cultivated one. Pearl millet is a product of **multiple domestication**. They are

1. *Pennisetum perottettii*

2. *P. mollissimum*

3. *P. violaceum*

4. *P. versicolor*

5. *P. adonense*

6. *P. gymnothrix*

The cultivated species of *Pennisetum* is believed to have originated thro' hybridization with in these six species.

### **Characteristics features of Bajra :**

1. Spikelet subtended by involucre of bristles.
2. Lodicules are absent (flower opening does not occur, only androecium and gynoecium protrude out).
3. Pennicillate anthers (anther tip cilliated - charecteristic of the genus *Pennisetum*)
4. Fused style with bifid stigma.
5. Protogynous nature.

### **Ragi - Eleusine coracana Gaertn. (2n = 36)**

Finger millet is an important staple food in parts of East and Central Africa, India, particularly in Karnataka. It is used for malting and brewing.

### **Systematic Position:**

**Division :** Phanerogams

**Sub - Division:** Anageosperms

**Class :** Monocotyledon

**Series :** Glumacea

**Sub class :** Glumiflorae

**Family :** Poaceae

**Tribe :** Eragrostideae

**Place of origin:** India

### **Characters of Eleusine:**

Inflorescence is contracted into a number of digitate spikes of spikelet. Spikelet consists of more than two florets subtended by two glumes.

## Lecture 08

### Small Millets

The grains of small millets are small in size, hence they are called small millets. The characters of small millets are hardy, drought, resistant, with little care it grows and gives some yield, can be grown in sub marginal lands also as a rainfed crop, mostly grown by hill tribes.

### Fox Tail Millet - *Setaria italica* (2n:18)

#### Family: Poaceae

Foxtail millet is the most important millet in India especially in Tamil Nadu, Karnataka and parts of Maharashtra. It is next in importance to Sorghum and finger millet.

#### Botany

Annual grass; seminal roots three followed by numerous thin adventitious roots, culms erect, slender, internodes hollow, tillering; leaf sheath longer than internodes, ligulate; leaf blade linear; tip acuminate; mid rib prominent. Inflorescence spike like panicle, carrying 6-12 two flowered sub sessile spikelets each subtended by 1-3 bristles; stamens three; ovary with two long styles ending in plumose stigma; fruit caryopsis tightly enclosed by lemma and palea.

**Center of origin:** East Asia

#### Wild relatives

*Setaria italica* was probably derived from *S. viridis* a common weed in the old world. It seems that *S. italica* and *Panicum miliaceum* were among the first crops to be domesticated in central eastern Asia. They were widely spread throughout Asia and Eastern Europe in pre historic times.

### Little Millet - *Panicum sumatranse* (*P. miliare*) (2n: 36)

#### Family: Poaceae

#### Botany

An annual tufted grass with slender culms, soft leaves, inflorescence a panicle with erect hairy branches; spikelets in pairs with two glumes; floret with two lemmas, two lodicules, three stamens and ovary with plumose stigma; fruit a caryopsis.

**Centre of origin:** W. Africa

**Distribution :** India, Sri Lanka, parts of China.

**Barn Yard Millet- *Echinochola frumentosa* (2n: 36 and 54)**

**Family: Poaceae**

**Botany**

A robust tufted annual grass; seminal roots followed by adventitious roots; stem smooth, glabrous, producing tillers; internodes hollow; leaf blade linear, lanceolate; tip acute; margin finely toothed. Inflorescence a panicle; spikelet two flowered, awnless, pedicellate, subtended by bristles, two glumes; lower floret sterile with lemma and palea; upper floret hermaphrodite, five nerved lemma and five nerved palea, two lodicules, three stamens, two distinct style with plumose stigma. Fruit a caryopsis enclosed in white shining hardened lemma and palea.

**Center of origin:** E.Asia

Barnyard millet originated either from *E. colona* or *E. crusgalli* and possesses characters intermediate between the two.

**Proso Millet - *Panicum milliaceum* (2n: 36 & 72)**

**Family: Poaceae**

**Center of origin:** Central Asia

**Distribution :** India, Africa, Erope, USA. China, Japan.

**Botany**

A shallow rooted erect annual grass, free tillering, internodes hollow, cylindrical; leaf lamina linear lanceolate. Inflorescence a slender panicle; spikelet with two florets with two glumes; lower floret sterile; upper floret fertile with lemma, palea, two lodicules, three stamens and two styles with plumose stigmas; fruit a caryopsis enclosed by persistent lemma and palea.

**kodo millet - *Paspalum scrobiculatum* (2n: 40)**

**Family: Poaceae**

**Center of origin:** India

**Botany**

An annual tufted grass; leaves in two ranks, stiff, erect. Inflorescence a panicle; 2-8 spikelets in flattened rachis; spikelets usually in two rows; each spikelet has two florets; lower floret sterile, upper bisexual with lemma, palea, two lodicules, three stamens and plumose stigma; grain enclosed in hard horny persistent husk which is difficult to remove.

## Lecture 09

### Pulses

### Fabaceae

(Subfamily – Papilionaceae, caesalpinaceae, mimosceae.)

### Distinguishing characters

Often climbers, bisexual flowers, generally Zygomorphic, sepals 5 with odd sepal anterior, generally more or less united. 5 petals and papilionaceous, stamens mostly 10, mono or diadelphous, carpel one with ventral suture posterior. Fruit mostly a legume.

**Habit:** Mostly herbs, shrubs or climbers wild as well as cultivated

**Root:** Taproot, which are branched and bear nodules containing nitrogen-fixing bacteria.

**Stem:** Erect herbaceous or woody, climbing by means of tendrils.

**Leaf:** Leaves may be simple or compound. Mostly alternate with leafy stipules. The leaves may be modified into tendrils.

**Inflorescence:** Usually racemose but may be Carymbose raceme.

**Flower:** Bracteate, bisexual, complete, Zygomorphic, irregular papilionaceous, and hypogynous.

**Calyx:** Five sepals, gamosepalous, odd sepal anterior with valvate aestivation inferior.

**Corolla:** 5 petals, polypetalous unequal with a descending imbricate aestivation papilionaceous, the outermost (posterior) petal is largest and forms the broad free standard (vexillum). The lateral pair of the side petals, which are also free and generally long clawed, forms the wings, while the anterior pair are closely appressed and often more or less coherent and forms the keel (carina) in which essential organs are closed.

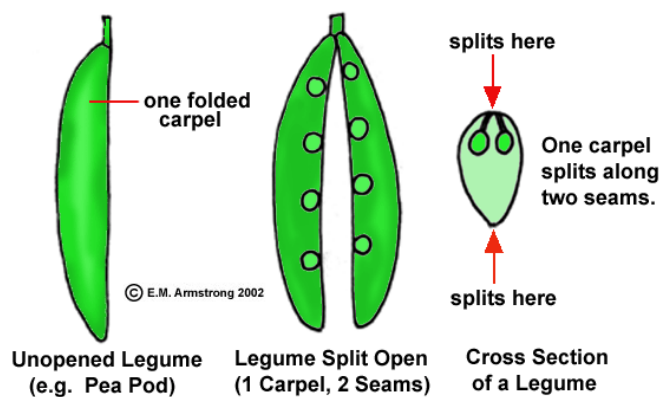
**Androecium:** 10 stamens, diadelphous 9+1

Anthers 2 celled dehiscence by longitudinal, inferior.

**Gynoecium:** Monocarpellary, superior, unilocular with marginal placentation. Style flattened

and hairy with a simple stigma.

**Fruit:** A legume (It develops from a monocarpellary superior ovary with marginal placentation. It dehisces along both the sutures)



**Seeds:** Usually non-endospermic

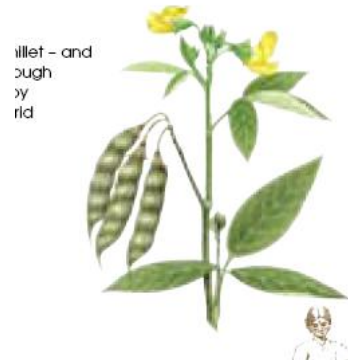
1. **Legume or Pod:** Composed of one carpel.

Note: Some legumes are indehiscent and do not split open.

### **Red Gram - *Cajanus cajan* (2n=22)**

There are two types of redgram varieties. 1) *Cajanus cajan* var, bicolor (Arhar). It is cultivated extensively in N. India. The Plants are woody tall and perennial in habit, large pod, bold grain. 2) *C. cajan* var, flavus. (Tur). It is cultivated in peninsular India. (S. India) Annual crop, medium tall to dwarf type, Medium grain pod. **Fruit** is a **pod**, which is variable in shape size,

constriction color texture, and pubescence. Pods, which have deep constriction, are known as beaded while others are flattish. Seeds may be rounded or lens shaped and varying in color, size and shapes.



and

### **Bengal gram - *Cicer arietinum* (2n= 16)**

In India it is one of the important pulse crop grown throughout the country. It is grown as a cold weather crop both in north and south India. It is drought resistant. There are two races Desi smaller grain and Kabuli bigger grain.

**Fruit** turgid pod normally containing one or two seeds which vary in size and shape and color. The seed coat may be smooth or puckered and wrinkled or roughly granulate. Cotyledons thick and yellowish.



### **Green gram *Vigna radiata* (2n = 22)**

Green gram is indigenous to India and has been in cultivation since prehistoric times. Erect or semi erect herbaceous annual with slight tendency for twining in the upper branches. Leaves trifoliate with long petioles, stipules with basal appendage, stipules minute and leaflets entire ovate, flowers 10 – 20 crowded in axillary racemes on long pedicels, keel spirally coiled, stamens diadelphous (9+1) ovary with long bearded style. Pod longer than in black gram with short hairs.

Seeds globular with many five and wavy ridges on the surfaces, hilum flat cotyledonus yellowish. Pod seed as food for human being, green and dry plant as fodder.

**Black gram *Vigna mungo* (2n = 24)**

Black gram is cultivated in many tropical and subtropical countries in several parts of Asia Africa and C&S America. It is a twining herb, annual plant, densely hairy, stem slightly ridged, leaves alternate, stipulate, petiolate, Pinnately trifoliate. Inflorescence axillary raceme with flowers congested at the top of the peduncle. Flowers 5-6. Shortly pedicelled bisexual, hypogynous, Zygomorphic, Complete. Sepals 5 gamosepalous, imbricate corolla papilionaceous, petals five, polypetalous keel in the form of spiral beak. Androecium diadelphous (9+1) filament alternately long and short. Gynoecium superior ovary, monocarpellary unilocular marginal placentation. Fruit – Legume densely hairy seeds, generally black.

**Soybean - *Glycine max* (2n=40)**

Soybean is one of the most important legume food of the people of far eastern countries like China and Japan and are chiefly used as a pulse. The seeds are rich in protein and are of high biological value. It is also rich in fat and vitamins, being good source of calcium and phosphorus. It has oil content upto 20 % and protein 40 %.

**Cowpea *Vigna unguiculata* (2n=22)**

It is grown in warm parts of the world. Tender leaves are used as greens from the vegetable type cowpea. Sprouted seed as vegetables. Grain as pulses. Whole plant as green fodder. Cow pea and maize green fodder mixture is excellent for cattle. *Vigna unguiculata* sub species *unguiculata* grain cowpea

*Vigna unguiculata* sub species *sinensis* grain cowpea

*V. unguiculata* sub species *sesquipedalis* yard long bean- vegetable cowpea

**Dolichos group of pulses**

The dolichos are twining herbs with stipulate, trifoliate leaves. Flowers are racemose or axillary, calyx tube short, corolla is much exerted petals equal in length keel is obtuse not spiral, stamens are diadelphous. Ovary nearly sessile. The pod is flat linear or oblong.

**Lab-Lab: *Lablab purpureus* 2n=22, 24 (var. typicus)**



Garden or pandal avarai is perennial, but cultivated as annual. The pods are long tapering. It was no oil glands and no smell. Entire pod is edible. *Lablab purpureus* var *lignosus* (Field bean, Mochai) it is a semi bushy type. Podes relatively shorter, oblong, and fibrous seeds. Plant give a mochai odour

**Gingelly - *Sesame indicum* (2n= 26) Pedaliaceae.**

**Botany of Pedaliaceae.**

They are Annual or perennial, leaves opposite or upper alternate, simple, exstipulate, flowers hermaphrodite, Zygomorphic, calyx 5 cleft, corolla gamopetalus 5 lobed, 2 lipped, stamens 4, anthers convenient in pairs, 2 celled, disc hypogynous, ovary superior, 1 celled with 2 intensive parietal placentas, the cell again often divided by superior septa, fruit a capsule or nut. Seeds without endosperm, embryo. Straight with flattened cotyledons

**Distinguishing characters**

Stem quadrangular in shape, Basal leaf opposite, upper leaf alternate. Presence of extra floral nectary gland as peduncle base. Corolla bell shaped, biliped and five lobed. Androecium-epipetalous didynamous stamens. Ovary bicarpellary, by presence of false septa appear as four loculed. Seed color varies from pure white to various shades of brown and gray to black. Seed coat may be rough or smooth.

## Lecture 10. Oil Seeds

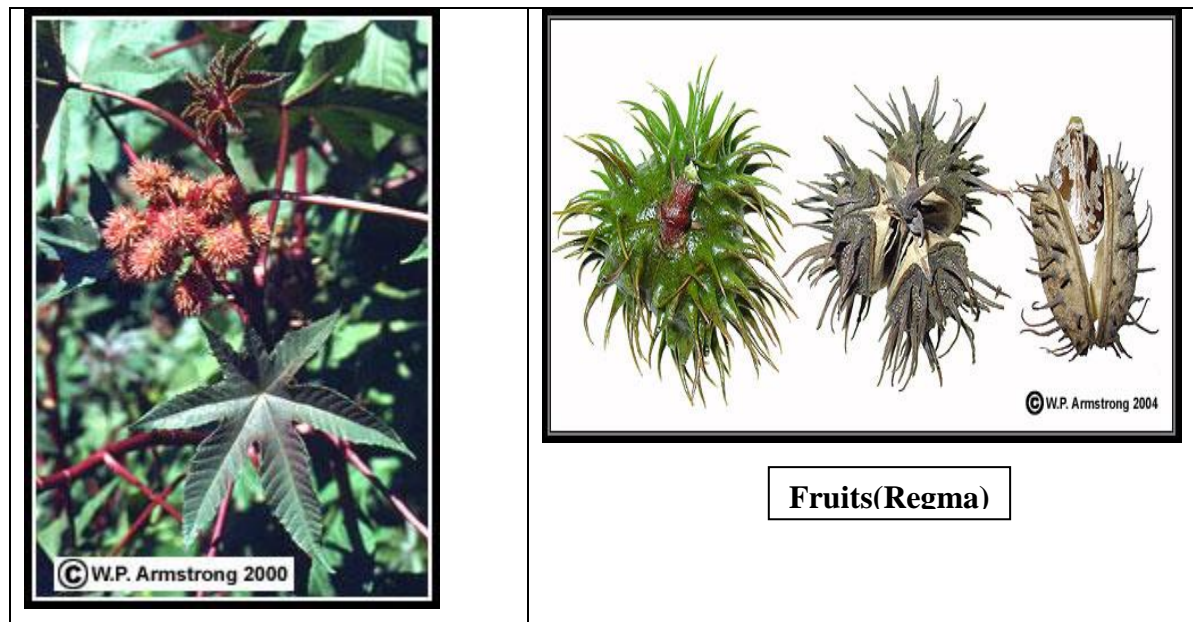
**Castor *Ricinus communis* (2n=20) Euphorbiaceae**

### Botany

Habit varies, mostly herbs but shrubs and trees are also common. Leaves simple or compound stipulate, latex commonly present, inflorescence in its ultimate arrangements is cymose , flowers small, regular, unisexual, perianth usually calycine , rarely petaloid sometimes altogether wanting, male flower –stamens a many as double as many as perianth leaves or numerous of flower or sometimes only one , the male flowers sometimes rudiment of the ovary , female flower ovary generally three celled, ovules one or two in each chamber, styles and stigmas as many as the cells, fruit (rigma) generally a capsule splitting into three coccid that separate from a persistent central column

### Distinguishing characters.

Presence of bloom –Ashy coating on the leaves and stem of the plant. Monoecious condition- unisexual flowers, male at the bottom and female at the top. Androecium – polyadelphous condition, filaments branched. The hilum almost concealed under the caruncle. Presence of thin leaf like cotyledon. Toxic alkaloids like ricin (blood coagulant) ricinin and allergen are present.



## **Fruits (Regma)**

It develops from tricarpeal syncarpous superior trilocular ovary and splits into many parts called cocci. Four distinct size groups of fruits namely 1) very small fruits are found in ornamental types and in some of the wild perennial types. 2) Small and 3) medium types are preferred for cultivation since they have high oil content varying from 45 to 57 %. 4) Big seeds have generally low oil content of less than 40%. Very small seeds are preferred for medicinal purposes.

On the fruit the epicarp may be either smooth or warty or spiny. Attractively colored types of horticultural value with colored inflorescences and fruits have been evolved. The seed color ranges from white to gray deep chocolate, purple and red. Mottling is also much varying. The seed has no dormancy.

## **Sunflower - *Helianthus annuus* (2n= 34) Asteraceae**

### **Botany**

Plants are usually herbs, leaves exstipulate, flowers aggregated into heads, an involucre of bracts surrounds the head or capitulum, calyx reduced to bristles or modified into papas, corolla (5) valvate in all disc florets, but in marginal ray florets which may be sterile or lack stamens and acts as an attraction for insects, inner hermaphrodite bisexual flowers. Stamens epipetalous with syngenesious anthers forming a ring through which the style passes. Stigmas 2. Ovary inferior, one chambered with one anatropous ovule, fruit-achene (A small hard dry indehiscent one seeded fruit. The wall of single seed is free from the hard pericarp or wall of the fruit)

## **Safflower *Carthamus tinctorius* (2n = 24) Asteraceae**

Safflower is an important oilseed crop in India. It is slowly becoming of increasing importance as an oil crop for the drier parts of tropics and subtropics. In India it is cultivated for both oil and reddish dye called safflower dye (cathamin) from florets. At the time of full bloom



flowers collected and corolla lobes removed and dried. Yellow dye is obtained by washing and dissolving it in water. There are two coloring matters. 1. Yellow pigment soluble in water and red color soluble in alkalis. The seed /fruit is **achene**

**Rape and Mustard *Brassica* sp.** (2n=16, 18, 20, 22, 36)

### **Botany of cruciferae.**

Annual, biennial or perennial herbs, a watery sap present, plants emit a sulphurous odour. Stem covered by unicellular stellate hairs. Flowers arranged in typical racemes, usually ebracteate, sepals in two alternating dimerous whorls, petals 4, clawed diagonally placed stamens tetradynamous carpels 2, ovary two chambered, the development of a false septum, ovules numerous on two parietal placentas. Fruit a **Siliqua** (It develops from bicarpellary syncarpous gynoecium with parietal placentation and a false septum. It is a long narrow multiseeded fruit which dehisces from below upwards by both sutures. **Siliqua**. It is a broad flat and shortened form of siliqua.)

The group rape and mustard includes the oil yielding species of *Brassica*. The commercial Indian rape seed and mustard are often mixture of rape seed, mustard and colza in varying proportion. The seeds go by different name in different parts of the country. Generally both colza (sarson) and rape (toria) are called together rape seed. Rai is mustard. Cultivated *Brassica* can be broadly divided into two distinct types.

**1. Vegetable type** - cabbage, (*Brassica oleraceae* - var capitata.) cauliflower (*Brassica oleraceae* var botrytis). Turnip (*Brassica oleraceae* var rapa)

**2. Oil seed type.** 1) **Rape seed** *Brassica campestris* and 2) **Mustard** *Brassica nigra*.

### **Rape seed**

**a) *Brassica campestris*** (2n= 20)

Indian rape seed is self sterile in nature. Important oilseed crop of N.India. There are three cultivated types.

*Brassica campestris* var. **brown sarson**

*Brassica campestris* var. **Yellow sarson**

*Brassica campestris* var. **toria**

**b) *Brassica napus*** 2n=38 European rape seed. Self fertile grown in Europe

### **Mustard**

a) ***B. nigra*** ( $2n=16$ ) Black or true mustard. Banarasi rai contains 28% of fixed oil used as medicine . Oil is pungent due to presence of glucoside sinigrin mostly used as condiments.

b) ***B. alla***  $2n=24$ . White mustard or ujli sarson. Young seedlings used as salads. Seeds yellowish in color contains 30% oil.

c) ***B. juncea***  $2n=36$  Indian mustard.(Brown sarson).Popularly known as rai contains 35% oil. Leaves are used as herbal medicines. Most pungent among cultivated oil seeds. It contains glucoside sinigrin.

The oil producing species of *Brassicca* are all cross fertilized.

### **Key characters**

Leaves two types 1) stem leaves bigger, lance shaped and serrated. Flower leaf small smooth margin. Androecium tetradynamous . Fruit siliqua. The oil content of the seed varies from 30-45% depending on the variety.

### **Fibers**

The fibers are obtained from the sclerenchymatous cells found in the plant body and these fiber cells occur either in groups or bundles. Chemically the fiber cell consists chiefly of cellulose with lignin or semi cellulose or any other substances. The commercial term fiber includes generally all thin and slender substances, which can be spun or made use of as fine stuffing material. Fiber cells are non-living structures, when mature and serve as a purely mechanical function, i.e. they impart strength and rigidity to the plant body.

## Lecture 11

### **Cotton *Gossypium* sp. (2n=26, 52) Malvaceae**

Cotton is the most ancient crop of the tropical and subtropical. It is one of the most important items of export in the developing countries. Cotton fiber is unchallenged natural textile fiber even today. The genus *Gossypium* consists of diploid and tetraploid cultivated cotton.

**Old world cotton or Desi cotton or diploid cotton** 2n=26 are *G. herbaceum* (upam cotton) and *G. arboreum* (Karunkanni cotton)

**New world cotton or American cotton or Tetraploid cotton** 2n=52 are *G. hirsutum*. (Combodia or upland cotton) and *G. barbadense*. (Sea Island cotton)

Cotton fiber is epidermal prolongation of seed coat cells. The longer out growth make **lint** and the shorter one make **fuzz**. In diploid cotton and upland cotton both lint and fuzz are present where as Sea island cotton (*G. barbadense*) only lint are present and such seeds are called **naked seed**.

### **Structure of fiber**

There are five different parts in a mature fiber. 1) The integument or outer layer is also called cuticle or waxy layer .2) Outer cellulose layer which is largely the original cell wall. 3) Layers of secondary deposits. This is nearly pure cellulose. Numerous concentric layers in this portion is recorded. 4) Walls of the lumen, a spiral structure surrounding the central cavity of the fiber and more dense than any other part of the fiber. 5) Substance in lumen is structure less and of a nitrogenous nature.

**Jute - *Corchorus capsularis*, *C. olitorius*.**(2n=14) Tiliaceae.

Jute is a leading crop among all bast fiber. (Stem fiber) plants. It is a typical plant of humid tropics and subtropics. Jute is chiefly raised for the sake of its fiber, which develops in the external part of the stem (in the bark). Individual fibrils are from 5 to 40 mm long. The surface of the jute fiber is smooth and brown in color. Commercial jute fiber is obtained from two species viz. *Corchorus capsularis* (white or bitter jute) and *C. olitorius* (Tossa jute). Mainly grown in W. Bengal, Bangladesh.

### **Fiber extraction.**

The ideal phase of harvest is when the plants are in small pods. Harvested plants are bundled and staked for the withering of leaves. After 2 to 4 days the leaves shed and the bundles are then steeped in water.

### **Steeping**

It is a process of immersing the bundles in water. After 2 to 4 days the tissues and cells rupture. This facilitates the entry of micro organism into stem.

### **Retting**

It is a process by which harvested stems are steeped in water so that the fiber in the stem get loosened and separated from the woody stalk due to the removal of protein, gums and other mucilaginous substances by the micro organisms. Fiber yield is 6% of the fresh stem weight.

### **Fiber Quality**

Jute fiber is fine and silky but less stronger than many other fiber.

**Mesta *Hibiscus cannabinus* ( $2n = 36$ ) Malvaceae**

Mesta fiber is a valuable fiber probably next to jute. This crop is successfully grown throughout tropics and subtropics. Bast fiber is obtained from the stem similar to jute.

**Sun hemp. *Crotalaria juncea*.  $2n=16$  Fabaceae**

Sunn hemp is another source of bast fiber, grown in tropical countries. It is also grown as fodder and green manure. The fibers are stronger than jute but lighter in color and more enduring than jute. They are long strands of fiber of about 4 to 5 feet in length and yellow to green in color. Fiber is obtained by retting.

### **Sugars**

**(Sugar cane *Saccharum officinarum* ( $2n=80$ ) Poaceae.)**

Sugar cane is a perennial gigantic grassy plant of Poaceae family. It is extensively grown in India, Cuba, Hawaii W. Indies.

There are 5 species of sugarcane of which three are cultivated and two are wild species.

1. ***Saccharum officinarum***. Noble cane ( $2n=80$ ). Large barreled, low fiber, high sugar content, susceptible to diseases and pests.
2. ***S. barberi***. Indian cane ( $2n=82-124$ ) Intermediate between noble and wild canes. Small barrel, internodes spindle shaped, high fiber content, resistant to diseases.
3. ***S. sinense***. Chinese cane ( $2n=118$ ) Vigorous thin grassy form resistant to drought, pest and diseases. Fair amount of sucrose content.

4. *S. spontaneum*. Wild cane (2n=40-128). Vigorous than grassy form. Virtually no sucrose, resistant to drought, pest and diseases.
5. *S. Robustum*. Wild cane (2n=60-194) Thick stock low sugar content, disease resistant.

The above 5 species are important for the improvement of sugar cane. They all inter cross freely.

**Inflorescence** is panicle. It is also called **Arrow**.

### **Nobilization**

Back crossing of F1 with *S. officinarum* ( noble cane)

### **Forage Crops**

The term **forages** is used broadly to mean all the plant constituents that are eaten by herbivores, including that are grazed (**pastures**) and those that are cut and fed such as **fodder**. Crop residues such as straw and the foliage of trees and shrubs also fall within this broad definition.

**Fodders-** Plants, which are, cultivated as forages crops and they are cut and fed to animals in stalls.

**Pastures-** grasses and legumes are grown in pasture lands where the animals are led to graze.

**Forages** can broadly be classified into three groups. viz. **grasses, legumes and non legumes**.

**Grasses.** Annual grass – Maize, sorghum, and cumbu.

**Perennial grass.**—B.N. and N.B, hybrids.

**Legumes** Annual Cowpea, cluster bean.

Perennial Lucerne, Sirato, Desmanthus

**Non legumes.** – Fodder beet, Fodder radish.

### **Grasses**

**Napier grass - *Pennisetum purpureum***

It is a tall perennial grass forming very thick clumps, tillering is heavy. It comes up well in both under tropical and temperate regions. It comes well in any soil condition and also responds to sewage irrigation 6-8 harvests can be taken in a year. The grass withstands drought for short spell and regenerate with rains.

**Pearl millet** Napier (Bajara - Napier)



They are very vigorous in their growth and adopted for varying climatic and soil conditions. They give heavy yield higher than Napier. They are more nutritious, palatable, succulent, juicy and less fibrous. They tiller profusely have luxuriant growth and responds to higher level of nitrogen.

**Guinea grass- *Panicum maximum***

It is the most popular grass with heavy tillering, forming big clumps with long internodes, slender and glabrous. It comes up well in tropical condition with moist climates. Under cultivation it can be grown in any soil. It requires sufficient moisture but cannot withstand water logging. It responds to sewage irrigation. It can be harvested once in 25 to 30 days interval. The crop can be allowed in the field for several years. Dry matter content is 15- 20% Protein 6-8% free from all toxic principles.

**Buffel grass- *Cenchrus ciliaris***

It is an important perennial pasture grass and grows well in a great variety of soil and climate. It is a perennial grass with underground rhizomes. They are hardy and drought resistant and have quick regeneration capacity. It gives the highest forage yield among the grasses grown under rainfed condition. Aerial branches tufted, leaf sheath compressed with hairs raceme of spikes sessile spikelets, no lodicules.

**Johnson grass- *S. halapense***

It is native of Africa. It was taken by colonel Johnson and hence named after him. In South India it occurs both in  $2n = 20$  &  $40$  forms. Because of rhizomatous condition it will spread easily. Co. 27 fodder cholam (Co 11 x *S. halapense*)

**Legume Fodder**

**Lucerne / Alfalfa (*Medicago sativa*)**

It is also called as **queen of fodder or green gold**. Lucerne is grown for pastuer, hay, dehydrated meal and for medicinal purpose. It is an important leguminaceous fodder grown as a perennial crop in drier regions and as an annual crop in hot humid regions. It is heat and drought resistant.

**Cow pea-*Vigna unguiculata***

It is the most important leguminous fodder crop during summer and rainy seasons mainly due to its quick growing habit, high yielding ability and high protein content.

**Desmodium-** *D. tortuosum* / *intortum* is commonly known as green leaf desmodium, is a large trailing and climbing, perennial rooting at nodes and having a deep tap root. It can be harvested 2-3 times. Shade tolerant green manure cum fodder produces profuse seeds.

Protein 22%. It is a tropical legume. It grows well in acid soils.

***Stylosanthes guianensis***

It is a summer growing perennial pasture fodder legume. *S. hamata* found to thrive in alkaline soils. *S. fruticosa* is from India. It is an herb and small shrub. It thrives in light soils due to its deep rooting system.

**Tree fodder**

**Subabul-** *Leucaena leucocephala*.

Among the browsing leguminous trees it tops the best. It provides economic nutritious and highly palatable forage to livestock and poultry. It has an amino acid mimosin. Excess feeding leads to fall of hairs, thyroid gland swelling and stunted growth. Pods can be fed to cattle. Protein 29%.

***Glyricidia sepium***

It is a medium tall tree grown in tea coffee and cocoa plantation for shade. It is pruned for green manure purpose.

**Agathi / sithagathi-** *Sesbania gandiflora* and *S. sesban*.

It is a fast growing and leaves are rich in protein and very much relished by all types of livestock.

**Erythin-** *Erythrina indica*

Indian coral leaf is a nitrogen fixing cum fodder green manure tree suited even to high acid soil.

**Green manure and green leaf manure**

**Green manure** is generally a leguminous crop raised in a field and incorporated in situ.

E.g. Sunnhemp, daincha.

**Green leaf manure**

It is a practice of cutting and applying them to the fields and ploughing them e.g. Neem, calotropis, glyricidia.

**Sunnhemp.** *Crotalaria juncea*. Erect herbaceous shrub. Cylindrical stem silky appearance pods oblong inflated and hairy.

**Sesbania speciosa.** It was introduced from South Africa. It is a quick growing and attains 3-4m in about 4 months. The crop stands drought and to some extent salinity. The stem is pithy but if allowed To grow for more than 4 or 5 months it becomes woody.

**Daincha.** ( *Sesbania aculeata*)It is aquick growing succulent crop which adpts itself to varying conditions of soil amd clinmates. It can be grown even under adverse drought, water logging condition and salinity.

**Tephrosia pururea noctiflora.** It is a perennial undershrub, growing wild in sandy or gravellywaste places. It ia also grown as and annual crop for green manure

**Neem.** *Azadirachta indica.* Evergreen tree with plenty of foliage. Loppings once or twice a year.

**Pungam.** *Pongamia glabra.* A leguminous ever green tree. Lopping is done once or twice a year.

## **12. Emasculation and Pollination Techniques**

**Rice (*Oryza sativa*) (2n = 24)** (Family – Poaceae)

In rice anthesis commences shortly after emergence of panicle. Spikelets at the tip bloom first and proceed downwards. Anthesis time 8-10 am. Each spikelet remain open 30 minutes and then closes. The anther dehiscence takes place immediately after the opening of the spikelets. Receptivity remains for one day.

### **Emasculation and Crossing techniques**

Emasculation is necessarily followed by controlled pollination. Emasculation is done during early morning between 6 and 8 AM in spikelets, due to open on the same day. Emasculation should be over well ahead of the time of anthesis. Crossing techniques in rice differ based on the method of emasculation. Since maximum number of spikelets open on the 3rd or 4th day of anthesis, panicles of that stage are selected for emasculation. The following methods are widely used for hybridization in rice.

#### **1. Clipping method**

In the previous day evening, top 1/3rd and bottom 1/3rd portions in the panicle of the desired female parent are clipped off by using scissors leaving the middle spikelets. With the help of scissors again, top 1/3 portion in each spikelet is clipped-off in a slanting position. The six anthers present in each spikelet are removed with the help of the needle (Emasculation). Care must be taken during emasculation for not to damage the gynoecium. Then to prevent contamination from the foreign pollen, the emasculated spikelets are covered with a butter paper bag. In the next day morning (usually at 9.00AM), the bloomed panicle from the desired male parent is taken. The top portion of the butter paper bag which was originally inserted in the emasculated female parent is now cut to expose the panicle. The male parent panicle is inserted in an inverted position into the butter paper bag and turned in both ways in order to disperse the pollen. After ensuring the abundant disbursement of pollen, the opened butter paper bag is closed using a pin. Coloured thread may be tied at the base of the panicle to identify the crossed ones. After ensuring pollination, the bag may be removed.

#### **2. Hot water method**

A method of hot water emasculation is used to about the same extent as the clipping method. Panicles in 3rd (or) 4th day of blooming are chosen as female parents. An hour or so before blooming (i.e. normally at 7. A.M.), the panicle is selected and under developed and

opened spikelets are removed. Now, the tiller is bent over (carefully to avoid breaking) and the selected panicle is immersed in hot water contained in a thermos bottle at 40-44°C for a period of 5 to 10 minutes. This treatment causes the florets to open in a normal manner and avoids injury. Then, emasculation is done by removing the six stamens by fine forceps or needles and then dusting should be done.

### **3. Dr. Ramiah method**

Panicles on the 3rd or 4th day of its blooming are selected; top and lower spikelets are removed leaving only the middle. It is covered with a wet cloth and air is blown from mouth. This facilitates opening of spikelets. After 2-3 minutes, wet cloth is removed and spikelets are found to be open. Then, the six anthers are removed.

### **4. Vacuum emasculation method**

This works on the principle of suction pressure. The spikelets are clipped off prior to operation. The minute pipette is to be shown at the point of clipping and pollen is sucked in. Six panicles can be emasculated at a time. By hand emasculation, 100 flowers can be emasculated by a person. With the vacuum emasculator, six persons can operate and emasculate 3000 to 3600 florets/hour.

### **5. Cuttack Method**

The technique was developed by CRRI, Cuttack. The panicle to be emasculated is inserted into hollow piece of bamboo closed at one end and plugged with cotton wool and split cork at the other end. The flowers thus enclosed will open within 5-10 minutes. The anthers are removed.

### **6. Brown paper method**

The panicles are enclosed in a Brown paper cover before a couple of hours of blooming. Heat develops inside due to which the anthers extrude, but do not dehisce. This happens in 15-30 minutes then the anthers are easily clipped off. Stigmatic surface is then dusted with pollen grains collected from the chosen male parent. The crossed panicle is then properly tagged and protected with paper cover which is retained in a position for 7 – 10 days.

### **7. Rhind's method**

In this method hot water is kept in the flask and it is poured outside. After pouring out the water inside of the flask will be warm and humid. The panicle to be emasculated will be inserted into the flask and kept for some time. Due to high temperature and humidity the

spikelets will get opened and the anthers are exposed which can be removed with the help of forceps.

**Wheat (*Triticum aestivum*) ( $2n = 42$  Hexaploid) (Family – Poaceae)**

Much of the pollen grains shed within the floret and the crop is largely self pollinated. The glumes normally open during the flowering process, the anthers protrude from the glumes and part of the pollen grains is shed outside the flowers. Entry of foreign pollen at flower opening may result in a small extent of cross pollination which is normally less than one per cent.

**Selfing**

The inflorescence is covered with a butter paper cover prior to anthesis, and kept undisturbed till the flower opening completed.

**Emasculation**

On emergence of the ear upper 1/3rd of the spikelet is cut and lower spikelets are also removed. Of the remaining spikelets alternate ones on both sides of the axis are removed. The top spikele is held with forceps and pulled downwards and upwards to remove the upper florets of the spikelets. The glumes are separated and anthers left exposed are removed carefully and covered with butter paper cover.

**Crossing**

On the next day earhead selected from the pollen parent are used for crossing. The upper half of the glumes of the few medium spikelets are cut of and the ripened bright yellow anthers are rubbed on the styles of the emasculated florets and then covered.

## Lecture 13

### **Maize (*Zea mays*) ( $2n = 20$ ), Family: Poaceae**

Maize is predominantly cross pollinated. Wind pollination (Anemophily) is the general rule. Pollination by insects also takes place to certain extent. The following are the adaptations for cross pollination, *i.e.*, Monoecious inflorescence, unisexual flower, differences in the time of maturity of the male and female inflorescences, silk receptive on entire length and abundant pollen production. It has protoandry and the tassel anthesis extends 2-14 days. Pollen viability remains for 24 hours. Anthesis of female speakelets starts after the completion of tassel opening and extends up to 2-5 days. The stigma is receptive throughout its length for 14 days.

### **Selfing**

Bag the tassel before anthesis with a paper cover. Bagging of tassels should be done in the previous day evening to avoid contamination from foreign pollen. Cut the tip of the cob before the silks emerge and cover with a paper cover. After 3-4 days, the silks will emerge in the form of a 'sawing brush' in which the silks will be of same height and stand erect. Remove the cover of the tassel containing pollen and insert it over the cob after removing the cob-cover. The inserted cover is then tied.

### **Crossing technique**

#### **Female parent**

- a. Detassel
- b. Cut the tip of the cob before the silks emerge and cover with a butter paper cover.

#### **Male parent**

- a. Cover the tassel before anthesis begins or as soon as the tassel emerges.

When the silks emerge in the female parent in the form of a brush, pollination is done by transferring the freshly shed pollen cover from the male parent and inserting it over the cob of the female parent after removing the cover from the cob.

The details like date of pollination, parentage and breeding programme to be carried out are clearly written by water proof pencil. The date of pollination will be one day later than the date of tasselling. Pollination should be completed within one week of silk emergence. Isolation distance for maize = 400M.

### **Sorghum (*Sorghum bicolor*) ( $2n = 20$ ) Family – Poaceae**

Sorghum is normally self-pollinated but some florets are *protogyny* resulting in cross pollination averaging about 6%. So, it is classified as often cross- pollinated. The amount of natural cross pollination varies from 0.6 to 50 per cent in different varieties and places. The cross pollination is more in loose panicles than in compact ones. Anthesis starts from tip to downwards at the rate of 2-5 cm per day and completes within 7-10 days. Anthesis time 3-6 am. The pollen grains are viable only for short period and stigma is receptive for 8-16 hours.

### **Selfing**

Head bagging becomes efficient for selfing the ear heads. Once the decision to bag heads has been made, all heads in a row should be covered. If a head has already begun to flower, the flowering portion should be cut off. During head bagging, boot leaf of the plant is usually removed prior to placing the bag.

### **Emasculation**

#### **1. Hand emasculation**

Only a part of the panicle is emasculated and the remaining panicle is clipped away. During clipping, flowered tip and the lower panicle branches are removed. About 50 florets which would normally flower the following day are selected for emasculation. The needle is inserted at the middle of the floret and moved across the glumes. The needle is rotated at 90° and three anthers are lifted out. The emasculated panicle is covered by a suitable paper bag.

#### **2. Hot water method**

In this method, in the panicle flowered tip and lower panicle branches are removed. About 50 florets (in clusters of two or three) are immersed in hot water at 48°C for 10 minutes.

#### **3. Plastic bag/ mass emasculation technique**

In this method, sorghum panicle is covered with plastic bag. This creates high humidity inside the bag. Under such humidity, the florets open, the anthers emerge but shed no pollen. The anthers are knocked free of head by tapping. In this method, some selfing occurs. Therefore, marker genes are needed to identify the plants arising from selfed seed.

On a dry morning when pollen shedding is occurring between 6 and 7 A.M., the hand pollination may begin around 9.30 A.M. In rainy days, the operation may be started at 11.30 – 12.30 A.M. The pollen is collected in paper bags. Sorghum pollen kept in bags is viable for 10-20 minutes. For collection, appropriate heads may be selected and bagged in the previous night itself.



The selected male parent panicle will be covered with brown paper bag the previous day evening before dehiscence of anthers. Next day the pollen will be collected by tapping the bag. The collected pollen will be dusted on to the emasculated head and covered with butter paper bag labeled properly. Dusting of pollen is done for two to three days continuously.

### **Cumbu / Pearl Millet (*Pennisetum glaucum*) (2n = 14) Family – Poaceae**

Cumbu (Bajra) is naturally cross pollinated (Allogamous). Wind is the chief agent of pollination (anemophily). Adaptations for cross pollination is Protogyny. Anthesis commence from 1/3rd of the apex of spike and proceeds both ways. Stigma emerges first and anthesis is over within 2-3 days. This is followed by the first male phase in which the anthers from the perfect florets emerge out. On the fifth day of anthesis the 2nd male phase begins in which anthers from the staminate florets emerge. Anthesis time 8 pm -2 am.

#### **Selfing**

To ensure selfing, spikes may be bagged before emergence of the stigmas. As the spike elongates it may be necessary to adjust the bag to cover the lower most spikelets. Another procedure is to enclose within a bag two full spikes from the same plant, one day (or) 2 days older than the other and ready to shed pollen as the stigmas are emerging from the younger spike.

#### **Crossing**

Emasculation in Cumbu is laborious and difficult due to the small size of the flowers and the late maturity of the anthers when compared to the stigma. About four-fifths of the upper portion of the spike is removed and the rest is bagged before the styles appear to prevent contamination. Flowers are pollinated by dusting them with fresh pollen obtained from the desired male plant or by shaking a spike which is shedding pollen over the exposed stigmas.

#### **Controlled cross pollination**

Pearl millet does not require emasculation for making crosses. The female line will be covered before stigma emergence with butter paper bag. Without removing butter paper bag we can see emergence of stigma. After most of the stigma have emerged. Pollen from desired male parent is collected and dusted on to the female line. Pollination is usually made in the morning. Care should be taken to cover pollen parent previous day with butter paper bag. The crossed heads are labeled.

Another method is instead of removing the selfing bag of female and dusting, the top of the cover clipped of desired male parent inflorescence in the process of pollen bursting is inserted to brush the stigma. Then the clipped top of the bag is folded and stapled. The crossed heads can be collected after 30-35 days.

### **Small Millets**

#### **Ragi/ Finger millet (*Eleusine coracana*) (2n:36) Family: Poaceae**

In this crop self pollination is the general rule. The inflorescence takes 7-8 days to complete anthesis. Time of anthesis 1 am – 5am. In each spike the order of opening is from the top to bottom. In each spikelet the opening of the floret is from the base to top and one floret in each spikelet opens a day.

#### **Selfing, emasculation and pollination techniques**

##### **Selfing**

The panicle before commencing anthesis is covered with paper cover and retained till the blooming is over.

##### **Crossing**

Emasculation and crossing are tedious. However, both hand emasculation and hot water treatments are followed. Hand emasculation is done in the evening and pollination is done very early in the morning i.e., before 6 a.m. Hot water technique of emasculation of florets is also successful. Hot water treatment at 52°C for 2 minutes was the best as judged from the percentage of hybrid seed-set. Then the spikelets are pollinated early in the morning.

#### **Approach Method or contact method**

The inflorescence to be opened will be selected and cut with long stalk from the male parent. This is brought to the emasculated flower. The male flower as a whole will be tied round with female flower. Then they are covered with butter paper bag. The cut end of the male inflorescence will be immersed in water kept in a bottle. Natural cross pollination takes place in 2 to 5 days. Marker genes are utilized for identifying the hybrid seedlings in the nursery plot. 60-90% seed set is recorded in both methods.

## Lecture 14

### **Red gram (*Cajanus cajan*) ( $2n = 22$ ) Family – Fabaceae**

Self pollination is the rule in Red gram and natural crossing extents up to 65 per cent. Therefore it is also known as often cross pollinated crop.

#### **Adaptations for self pollination**

1. Bisexual
2. Close proximity of anthers and stigma
3. Simultaneous maturity of anthers and stigma.

### **Selfing, emasculation and pollination techniques in Red gram**

#### **Selfing**

Mature flower buds are to be covered with paper bags for one or two days.

#### **Crossing**

Hand emasculation followed by artificial cross pollination is essential. Emasculation should be done in the previous day evening and the emasculated buds are protected by covers. Early morning on the next day, pollination is done using pollen collected from the protected flowers of the selected male parents.

### **Black Gram (*Vigna mungo*) (Diploid, $2n = 22$ & $24$ ) Family – Fabaceae**

Self pollination is the rule. Here pollination occurs before flower opening (cleistogamous) in night. Anthesis time 1 am – 4 am. The flower opens in the morning at 7 am. The interval between pollination and opening of flower is 4 hours. This ensures self fertilization.

### **Selfing, emasculation and pollination techniques in Black gram**

#### **Selfing**

As in red gram, bagging is done to avoid insect contact.

#### **Crossing**

Young unopened bud is kept between thumb and fore fingers of the left hand. The point of dissecting needle is inserted just under the standard petal in an oblique position along the top of the bud. The left side of the standard and wing petal are pushed outward and held with thumb and left hand. The left side of the keel petal is removed with the forceps. The pistil and stigma are then exposed and the anthers are removed with the forceps. Evening emasculation followed by morning pollination gives best results. Pollination is done by gently rubbing anther of male,

inserting the staminal column and closing it with standard and wing petal. Since flower shedding is common, putting better paper bag is avoided. The emasculated flowers are identified with thread wound round. The crossed pod will be smaller in size with two or three seeds only.

### **Cowpea (*Vigna unguiculata*), Family – Fabaceae (Diploid $2n = 22$ and $24$ )**

#### **Pollination**

Highly self pollinated because of Cleistogamy, Close proximity of the anthers and stigma and Simultaneous maturity of anthers and stigma

#### **Selfing**

Keeping the plants in insect proof cages will lead to selfing. Covering of individual flower buds will lead to poor pod setting.

#### **Crossing**

Select young buds, in an inflorescence and remove all immature buds. Split open the keel petals and remove the stamens one by one holding the filaments. Bring corolla back to position and cover the bud with a folded leaflet. Protection is given by keeping the plants in insect proof cages. Pollination is done on the next day morning by exposing the stigma from the keel petal and brushing it with the pollen collected from the male parent.

Selfing and crossing are the essential procedures in crop improvement process. The exact procedures used to ensure self or cross-pollination of specific plants will depend on the floral structure and normal manner of pollination. Generally effecting cross-pollination in a strictly self-pollinating species is more difficult than vice-versa because for instance preventing self-pollination occurring inside the unopened flowers is cumbersome.

### **Bengal Gram – *Cicer arietinum* ( $2n = 14, 16$ ), (Channa, Chick Pea), Family – Fabaceae**

Chickpea is a self pollinated species with normal out crossing limited to 1.58%. self pollination takes place one or two days before opening up of the flower. The flower open in the morning and close in the afternoon and each flower opens on tow or three successive days. Time of anthesis is 3 AM to 9 AM. For hybridization crossing work should be started when the first pod on the selected plant is already formed. In Northern India, emasculation is done a day prior

to pollination. The pollination is done in the morning hours give better setting. In south India, pollination immediately after emasculation give higher seed setting.

### **Soybean *Glycine max* (2n = 40), Family – Fabaceae**

Flower open early in the morning. The pollen is shed normally shortly before or after the flower opens. But pollen shedding may occur sometimes with in the bud itself. Normally cross pollination does not exceed 1 percent.

### **Emasculation and crossing**

Hand emasculation is the method followed for crop breeding which is tedious since the floral parts are so small and seed set is also less. Emasculation is done in the evening and pollination is done in the morning hours.

### **Groundnut (*Arachis hypogaea*) (2n = 40), Family – Fabaceae**

Self pollination is the rule in groundnut. Anthesis commences at 6 am and continues upto 8 am. Anther dehisces two hours prior to opening of the flower. Twenty four hours before anthesis, the buds are very small. During the day, elongation of calyx, proceeds slowly but process gets accelerated during night.

### **Selfing**

Since cleistogamous condition prevails in groundnut, selfing is most easy in this crop. Usually covering is unnecessary and difficult. Keeping the plants in insect proof cages will ensure self pollination.

### **Crossing**

Mature flower buds which are ready to open in the next day are selected and emasculated in the evening. They can be easily identified by the size and length of calyx tube. The flower bud of groundnut is of crescent shape, being bulged on one side and slightly depressed on the other. The keel petal is located on the bulged side and the standard is present on the depressed side. For emasculation, hold the bud between the thumb and the index finger of the left hand and with the help of a razor blade in the right hand; make a cut on the depressed side at two-thirds the length below the tip so as to cut the standard and a portion of the wing petals. Then gently pull the calyx and corolla by holding at the tip of the flower bud. By doing this, the sepals and the petals except the keel would be removed, with the help of the fine forceps gently liberate the bundle of stamens and the pistil from the keel and nip off the anthers.

With a hand lens, examine the tips of filaments so as to ensure complete removal of the anthers. Take a piece of straw tube (used for sipping cool drinks), 4 to 5 cm long and close one side opening by bending the tip. Cover the emasculated flower bud with the straw tube by slowly inserting calyx tube into it. This would ensure perfect protection to the stigma from any natural cross pollination. The next morning take out the straw tube, dust the stigma with the desired pollen and reinsert the tube. Pollination between 7 and 8 am was found to give more success. If the stigma is found dry, pollinate after smearing it with 2 per cent sucrose solution.

Next day early morning between 7 am and 11 am pollen is collected from mature yellow anthers of the selected male parent and dusted on the receptive stigma. For cross pollination, the selected male flower is held between thumb and the middle finger after the standard and wing petals are removed. The flower with keel protruding is taken to the stigma of the emasculated flower. A gentle push on gently keel by the finger forces lumps of pollen grains of the cover the entire stigmatic surface. Five to seven days after pollination successful crosses will produce gynophores (pegs) with the dried flowers at their tips. These are then introduced into small wire rings of 4 mm diameter which are marked for respective crosses.

**Sesame (*Sesamum indicum*) ( $2n = 26$ ), Family – Pedaliaceae**

Gingelly is a self pollinated (Autogamous) crop. In some varieties cross pollination also takes place to a limited extend of 5-6 per cent. Very high cross pollination between 14 and 65 per cent has been recorded in a few varieties in India. Hence, the crop can be classified as ***often cross pollinated***. Cross pollination may occur due to wind and bee activities. On a bright clear day, the flowers open between 5 and 7 am. In the mature flower bud, just before the flower opens, the four unripe anthers are much below the stigma which at this stage is not receptive. The anthers begin to burst longitudinally after 4am in the next day and commence to liberate their pollen. At this time, the stigma becomes receptive. The plant comes to flowering 4 weeks after sowing.

### **Selfing**

1. **Tieing with thread:** Selfing can be effected by tieing the corolla of the unopened flower which is selected in the previous day evening itself.
2. **Smearing of semi-solid clay:** Selfing can be done by smearing a speck of semi-solid clay, on the upper portion of tubular petals of unopened flowers. The clay while on drying does not allow the tubular petals to open and hence self pollination is the rule.

This method is cheap and less time consuming one. This method is most effective during rainy days. During rainy days, fevicol may be applied on young flower bud to ensure selfing.

## **Crossing**

### **Soda- straw method**

The emasculation technique in sesame is easy for crossing due to epipetalous nature of the stamens. The flower bud which is expected to open in the next day morning is selected in the previous day evening between 3 P.M. and 6 P.M. and emasculated by just removing the corolla tube in which the stamens are attached. Then, the emasculated flower buds are covered with a piece of soda-straw tube, bent at the top in order to avoid contamination from foreign pollens. During the next day morning, between 7 A.M. and 9 A.M., pollen from the desired male parents were dusted gently on the surface of the stigmas of the emasculated flower buds after removing the soda-straw and again covered. The unemasculated flowers are removed in the female parent. Individual crossed flowers are tagged with coloured thread for the identification of crossed capsules. Different coloured threads are used for different type of crosses.

### **Sunflower (*Helianthus annuus*) ( $2n = 20$ ), Family - Asteraceae**

Sunflower is highly *cross pollinated* crop mainly through insects (*Entomophily*) and to a limited extent by wind (Anemophily). The flower opening starts from outside of the head and proceeds towards centre. The head takes 5-10 days for complete blooming depend on size of head and season. Anthesis occurs between 5 to 8 A.M. Pollen viable for 12 hours. Stigma is receptive for 2-3 days. The staminal filaments elongate rapidly and the anthers appear above the top of the corolla. Anthers dehisce early than maturity of stigma. (*Protoandry*). In this crop, the cross pollination occurs due to protandry, limited area of stigmatic surfaces for receptivity, ray floret colour attracts insects and abundance of sweet secretions in the disc florets.

## **Selfing**

The flower head is protected with a suitable cover before the commencement of anthesis in any of the florets and the cover is retained till fertilization is over in all the florets. Artificial self pollination with pollen collected from the same flower or another flower of the same plant using a soft brush will enhance seed settings.

## **Crossing**

### **i. Hand Emasculation**

Emasculation is done in the early morning by removing the anthers of the disc florets in 2 to 3 whorls with forceps and the other florets in the head are removed. About 9-10 am the pollens from desired male parent are collected and dusted on the emasculated head. This process is continued for 2 to 3 days.

### **ii. Without emasculation**

In sunflower, head emasculation is difficult. Considering this difficulty, the heads are pollinated without emasculation. On the basis of hybrid vigour, plants are distinguished from the selfed plants. The presence of marker genes for identifying hybrids is also utilised effectively.

### **iii. Chemical induction of male sterility.**

This is achieved by spraying of 100ppm GA (Gametocide) during bud-initiation stage consecutively for three days in the morning.

Pollination is carried out by collecting pollen from heads which are already bagged prior to flowering. Pollen may be collected from flowering heads into paper bags. Pollination is done in the morning by applying the freshly collected pollen by a small piece of cotton, a hair brush or through fine cloth bag. After each cross, care must be taken to avoid contamination by wiping the hands with alcohol.

### **Castor (*Ricinus communis*) ( $2n = 20$ ), Family – Euphorbiaceae**

Cross pollination is the rule in this crop. It is mainly wind pollinated. But insect activity is also seen to some extent since the young leaves just below the inflorescence exude copious nectar at the time of flower opening cause insect pollination. Unisexual flowers, protogynous, elevated position of female flower in the inflorescence, mechanisms to promote wind pollination and nectar glands to attract insect promotes cross pollination. Here male flowers open first (*protoandry*). After one or two days of male flowers opening, female flowers open. However, *protogyny* is also reported. The opening is between 4.30 and 5.00 A.M. Pollen grains are viable for a 2 days and stigma is receptive for 3 days. Each candle takes 10-12 days to complete flowering.



### **Selfing**

The whole inflorescence is protected with not yet opened are selected. From the selected inflorescence all the male flowers are removed and the female flowers protected with a suitable cover. Artificial cross pollination is done when the stigmas of the retained female flowers become receptive by rubbing the anthers of male flowers collected from the selected male parent. During the rain day old bags are to be replaced with new bags to avoid fungal attack, and free air movement.

### **Crossing**

1. **Emasculation:** It can be achieved by removing or rubbing off the staminate flowers by finger and thumb.
2. **Crossing:** Pollen grains are collected from the desired male parent and are dusted on the stigma of the female parent. Again the inflorescence is covered.

## Lecture 15

### **Sugarcane (*Saccharum officinarum*) ( $2n = 80$ )**

Cross pollination is the rule in sugarcane. Self male and female sterility, protogyny and hanging down of anthers away from the stigma at the time of anthesis promote self pollination. Usually anthesis will be in early morning between 5 am and 6 am. Maximum anthesis between 6 am and 8 am. Stigma protrudes out first and anthers dehisce afterwards. Flower opening will be from top to downwards. It will take about 10 days for complete opening of spikelets. Flowering in sugarcane is location specific and influenced by environment. Natural pollination is by wind (*Anemophily*)

### **Selfing**

Selfing is done by covering the arrow with a bamboo frame work or cage which is covered with muslin cloth or polythene paper. Such a cover is commonly called lantern. It prevents accidental cross pollination. The lantern has to be supported by bamboo poles. The lantern has to be opened once in a day to reduce the temperature that may build up inside during the day time. This is done preferably during the afternoon hours between 12am and 4pm. Usually the cover has to be retained in position till the seeds are set. Within one week or 10 days we can get selfed seeds. This selfing method is followed in Sugarcane Breeding Institute, Coimbatore.

### **Crossing**

Hybridization is very difficult.

1. It is mostly vegetatively propagated. Some varieties seldom flowers outside tropics. Some varieties flowers once in 6 to 8 months. It is highly controlled by photoperiods.
2. Spikelets are minutes. So, hand emasculation is not possible.
3. Self sterility of both pollen and ovule predominates in almost in all the varieties.
4. Hot water treatment can not possible.

### **Hybridization methods**

#### **Coimbatore method**

During flowering period, the sugarcane stem will be cut leaving one or two bud. The cut stem can be transferred to a mud pot having moist mud. Within 10 days the buds will develop into roots and there will be good root system. This can be transferred to the breeding block. In the crossing block, the male and female plants are covered with common **lantern**. Free shedding

pollen over female plant will occur. We can harvest both selfed and crossed seeds from the female parent. The selfed seeds can be identified by chromosome number by raising it in the nursery. Selfed seeds thus removed retaining crossed seeds.

### **Marcotting method**

During flowering, cut around the stem and tie a polythene bag with nutrients (growth medium). The bud near cut end give rise to roots. This can be cut and used for hybridization purposes. This method is called marcotting. Practiced in Sugarcane Breeding Institute, Coimbatore.

### **Lantern method**

Providing Lantern for a female plant before anthesis starts. From the desired male parent cut the arrow. That arrow can be introduced into the Lantern and shaken up and thereby crossing can be effected. This will be repeated for 2-3 days in order to have more seed set.

### **Hawaii method (Sulfurous acid Technique)**

A sulfurous acid solution keeps the inflorescence alive for several weeks. Here, we cut both mal and female flowering arrows along with small portion of stem. These cut end will be immersed in a vessel containing 0.01% sulphuric acid and 0.01% phosphoric acid. The cut end at the lab is brought nearer and effect cross pollination. They absorb the weak acids. We have to add weak solution daily to replace the acid taken by stem. Once in a week we have to completely change the solution. This is done for 20-30 days. During this time, the seed will mature.

In modified method of this, the female plant alone is cut and kept in weak acid at the time of flowering the male parent can be brought nearer and the pollen can be shed by shaking as done in Lantern method.

## Emasculation and Pollination Techniques in Cotton

### Selfing

In the selfing of cross-pollinated species, it is essential that the flower are bagged or otherwise protected to prevent natural cross-pollination. Selfing and crossing are essential in crop breeding. It is important that the breeder, master these techniques in order to manipulate the pollination according to his needs. The exact procedure that he may use to ensure self or cross pollination of specific plants will depend on the particular species with which he is working. The structure of the flowers in the species determine manner of pollination. For these reasons, the breeder should acquaint himself with the **flowering habit** of the crop.



In the case of wheat, rice, barely, groundnut etc., the plant is permitted to have self pollination and the seeds are harvested. It is necessary to know the mode of pollination. If the extent of natural cross pollination is more, then the flowers should be protected by bagging. This will prevent the foreign pollen to reach the stigma. Seed set is frequently reduced in ear heads enclosed in bags because of excessive temperature and humidity inside the bags. In crops like cotton which have larger flowers the petals may fold down the sexual organs and fasten, there by pollen and pollen carrying insects may be excluded.

In certain legumes which are almost insect pollinated, the plants may be caged to prevent the insect pollination. In maize, a paper bag is placed over the tassel to collect pollen and the cob is bagged to protect from foreign pollen. The pollen collected from the **tassel** is transferred to the cob.

### Emasculation

Removal of stamens or anthers or killing the pollen of a flower without the female reproductive organ is known as emasculation. In bisexual flowers, emasculation is essential to prevent of self-pollination. In monoecious plants, male flowers are removed. (castor, coconut) or male inflorescence is removed (maize). In species with large flowers e.g. (cotton, pulses) hand emasculation is accurate and it is adequate.

### Methods of Emasculation

## **1. Hand Emasculation**

In species with large flowers, removal of anthers is possible with the help of forceps. It is done before anther dehiscence. It is generally done between 4 and 6 PM one day before anthers dehisce. It is always desirable to remove other young flowers located close to the emasculated flower to avoid confusion. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed with the help of forceps. Sometimes corolla may be totally removed along with **epipetalous stamens** e.g. gingelly.

In cereals, one third of the empty glumes will be clipped off with scissors to expose anthers. In wheat and oats, the florets are retained after removing the anthers without damaging the spikelets. In all cases, gynoecium should not be injured. An efficient emasculation technique should prevent self pollination and produce high percentage of seed set on cross pollination.

## **2. Suction Method**

It is useful in species with small flowers. Emasculation is done in the morning immediately after the flowers open. A thin rubber or a glass tube attached to a suction hose is used to suck the anthers from the flowers. The amount of suction used is very important which should be sufficient to suck the pollen and anthers but not gynoecium. In this method considerable self-pollination, upto 10% is like to occur. Washing the stigma with a jet of water may help in reducing self-pollination; however self pollination can not be eliminated in this method.

## **3. Hot Water Treatment**

Pollen grains are more sensitive than female reproductive organs to both genetic and environmental factors. In case of hot water emasculation, the temperature of water and duration of treatment vary from crop to crop. It is determined for every species. For sorghum 42-48°C for 10 minutes is found to be suitable. In the case of rice, 10 minutes treatments with 40-44°C is adequate. Treatment is given before the anthers dehiscence and prior to the opening of the flower. Hot water is generally carried in thermos flask and whole inflorescence is immersed in hot water.

## **4. Alcohol Treatment**

It is not commonly used. The method consists of immersing the inflorescence in alcohol of suitable concentration for a brief period followed by rinsing with water. In Lucerne the

inflorescence immersed in 57% alcohol for 10 seconds was highly effective. It is a better method of emasculation than the suction method.

## 5. Cold Treatment

Cold treatment like hot water treatment kills the pollen grains without damaging the gynoecium. In the case of rice, treatment with cold water at 0.6°C kills the pollen grains without affecting the gynoecium. This is less effective than hot water treatment.

## 6. Genetic Emasculation

Genetic/ cytoplasmic male sterility may be used to eliminate the process of emasculation. This is useful in the commercial production of hybrids in maize, sorghum, pearl millet, onion, cotton and rice etc.,

In many species of self-incompatible cases, also emasculation is not necessary, because self-fertilization will not take place. Protogyny will also facilitate crossing without emasculation (e.g.) Cumbu.

## 7. Use of Gametocide

Also known as chemical hybridizing agents (CHA) chemicals which selectively kill the male gamete without affecting the female gamete. e.g., Ethrel, Sodium methyl arsenate, Zinc methyl arsenate in rice, Maleic hydrazide for cotton and wheat.

## Bagging

Immediately after emasculation the flower or inflorescence is enclosed with suitable bags of appropriate size to prevent random cross-pollination.



rice,

size

## Pollination

The pollen grains collected from a desired male parent should be transferred to the emasculated flower. This is normally done in the morning hours during anthesis. The flowers are bagged immediately after artificial crossing.



## Tagging



The flowers are tagged just after bagging. They are attached to the inflorescence or to the flower with the help of a thread. The following may be recorded on the tag with pencil.

1. Date of emasculation
2. Date of pollination
3. Parentage
4. No. of flowers emasculate

## 16. Methods of breeding – introduction and acclimatization

The following are the methods of breeding autogamous plants.

1. Introduction
2. Selection
  - a) Pure line selection
  - b) Mass selection
3. Hybridization and selection
  - i) Inter varietal
    - a) Pedigree Method
    - b) Bulk Method.
    - c) Single Seed Descent Method.
    - d) Modified Bulk Method
    - e) Mass - Pedigree Method.
  - ii) Interspecific hybridization
4. Back cross method
5. Multiline varieties
6. Population approach
7. Hybrids.
8. Mutation breeding
9. Polyploidy breeding
10. Innovative techniques

### I. Plant introduction

#### Definition

Taking a genotype or a group of genotypes in to a new place or environment where they were not grown previously. Thus introduction may involve new varieties of a crop already grown in that area, a wild relative of the crop species or totally a new crop species for that area.

- E.g. a) Introduction of IRRI rice varieties..  
b) Introduction of sunflower wild species *from* Russia  
c) Introduction of oilpalm in to Tamil Nadu.



Plant introduction may be of two types. 1. Primary Introduction and 2. Secondary Introduction

### **1. Primary Introduction**

When the introduced crop or variety is well suited to the new environment, it is directly grown or cultivated without any alteration in the original genotype. This is known as primary introduction. E.g. IR. 8, IR 20, IR 34, IR 50 rice varieties; oil palm varieties introduced *from* Malaysia and Mashuri rice *from* Malaysia.

### **2. Secondary Introduction**

The introduced variety may be subjected to selection to isolate a superior variety or it may be used in hybridization programme to transfer some useful traits. This is known as secondary Introduction. E.g. In soybean EC 39821 introduced from Taiwan is subjected to selection and variety Co 1 was developed. In rice ASD 4 is crossed with IR 20 to get Co 44 which is suited *for* late planting.

### **Objectives of Plant Introduction**

- To introduce new plant species there by creating ways to build up new industries. E.g. Oil palm
- To introduce high yielding varieties to increase food production. E.g. Rice and wheat.
- To enrich the germplasm collection. E.g. Sorghum, Groundnut.
- To get new sources of resistance against both biotic and abiotic stresses.

**E.g.** NCAC accessions to have rust resistance in groundnut. Dasal rice variety for saline resistance. Aesthetic value – ornamentals are introduced for aesthetic value.

### **Plant Introduction Agencies**

Most of the introductions occurred very early in the history. In earlier days the agencies were invaders, travelers, traders, explorers, pilgrims and naturalists. Muslim invaders introduced in India cherries and grapes. Portuguese introduced maize, ground nut, chillies, potato, sweet potato, guava, pine apple, papaya and cashew nut. East India Company brought tea. Later Botanic gardens played a major role in plant Introduction.

A centralized plant introduction agency was initiated in 1946 at IARI, New Delhi. During 1976 National Bureau of Plant Genetic Resources (NBPGR) was started. The bureau is responsible for introduction and maintenance of germplasm of agricultural and horticultural plants. Similarly Forest Research Institute, Dehradun has a plant introduction organization, which looks after introduction, maintenance and testing of germplasm of forest trees. Besides

NBPGR the Central Research Institutes of various crops also maintain working germplasm. All the introductions in India must be routed through NBPGR, New Delhi. The bureau functions as the central agency for export and introduction of germplasm.

At International level International Board of Plant Genetic Resources (IBPGR) with head quarters at Rome, Italy is responsible for plant introduction between countries.

### **Procedure for plant Introduction**

The scientist / University will submit the requirement to NBPGR. If the introduction is to be from other countries, NBPGR will address IBPGR for effecting supply. The IBPGR will assign collect the material from the source and quarantine them, pack them issue phytosanitary certificate suitably based on the material and send it to NBPGR. The NBPGR will assign number for the material, keep part of the seed for germplasm and send the rest to the scientist.

There are certain restrictions in plant introduction. Nendran banana from Tamil Nadu should be not be sent out of state because of bunchy top disease. Similarly we cannot import Cocoa from Africa, Ceylon, West Indies, Sugarcane from Australia, Sunflower from Argentina.

### **Functions of NBPGR**

1. Introduction maintenance and distribution of germplasm
2. Provide information about the germplasm through regular publications.
3. Conduct training courses to the scientist with regard to introduction and maintenance of germplasm.
4. Conduct exploratory surveys for the collection of germplasm.
5. To set up Natural gene sanctuaries.

### **Merits of plant introduction.**

1. It provides new crop varieties, which are high yielding and can be used directly
2. It provides new plant species.
3. Provides parent materials for genetic improvement of economic crops.
4. Enriching the existing germplasm and increasing the variability.
5. Introduction may protect certain plant species in to newer area will save them from diseases. E.g. Coffee and Rubber.

### **Demerits**

1. Introduction of new weed unknowingly. E.g. *Argemone mexicana*, *Eichornia* and Parthenium

2. Introduction of new diseases: Late blight of potato from Europe and Bunchy top of banana from Sri Lanka
3. New pests: Potato tuber moth came from Italy
4. Ornamentals becoming weeds: Lantana camara
5. Introduction may cause ecological imbalance E.g.Eucalyptus.

### **Acclimatization**

When superior cultivars from neighbouring or distant regions are introduced in a new area, they generally fail initially to produce a phenotypic expression similar to that in their place of origin. But later on they pick up and give optimal phenotypic performance, in other words they become acclimatized to the new ecological sphere. Thus acclimatization is the ability of crop variety to become adapted to new climatic and edaphic conditions.

The process of acclimatization follows an increase in the frequency of those genotypes that are better adapted to the new environment.

The success of acclimatization depends upon two factors

- i) Place effect
- ii) Selection of new genotypes.

### **Selection, Mass selection, pure line selection and Johannson's pure line theory, genetic basis.**

#### **Selection in Self-Pollinated Crops**

To get successful results by selection there are two pre-requisites.

- a) Variation must be present in the population.
- b) The variation must be heritable.

#### **History of selection**

Selection was practiced by farmers from ancient times. During 16<sup>th</sup> century Van Mons in Belgium, Andrew knight in England and Cooper in USA practiced selection in crop plants and released many varieties.

Le coutier, a farmer of island of New Jersey published his results on selection in wheat in the year 1843. He concluded that progenies from single plants were more uniform. During the same period Patrick Shireff, a Scotsman practiced selection in wheat and oats and developed some valuable varieties. During 1857 Hallet in England practiced single plant selection in wheat,

oats and barley and developed several commercial varieties.

About this time **Vilmorin** proposed individual plant selection based on progeny testing. This method successfully improved the sugar content in sugar beet. His method was called as vilmorin isolation principle. He emphasized that the real value of a plant can be known only by studying the progeny produced by it. This method was successful in sugar beet but not in wheat. This shows the in-effectiveness of selection in cross pollinated crops. Today progeny test is the basic step in every breeding method.

### **Pureline theory**

A pure line is the progeny of a single self fertilized homozygous plant. The concept of pureline was proposed by **Johannsen** on the basis of his studies with beans (*Phaseolus vulgaris*) variety called Princess. He obtained the seeds from the market and observed that the lot consisted of a mixture of larger as well as smaller size seeds.

Thus there was variation in seed size. Johannsen selected seeds of different sizes and grown them individually.

Progenies of larger seeds produced larger seeds and progenies from smaller seeds produced small seeds only. This clearly showed that there is variation in seed size in the commercial lot and it has a genetic basis. He studied nineteen lines all together. He concluded that the market lot of the beans is a mixture of purelines.

He also concluded whatever variation observed with in a pureline is due to environment only. Confirmatory evidence was obtained in three ways. In line 13 which is having 450 mg seed wt he divided the seeds on weight basis. He divided the line into seeds having 200, 300, 400 and 500 mg weights and studied the progenies. Ultimately he got lines having weight ranging from 458 to 475. Thus the variation observed is purely due to environment.

The second evidence was that selection with in a pureline is ineffective. From a pureline having 840 mg selection was made for large as well as small seeds. After six generations of selection the line for large seed as well as for small seed gave progenies having 680-690 mg. Thus it was proved that selection within a pureline is ineffective.

In third evidence when parent - offspring regression was worked in line thirteen. It worked to zero indicating that variation observed is non heritable and it is due to environment only.

### Origin of variation in pure lines

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

### Effect of self-pollination on genotype

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

No. of generations of selfing	Frequency (%)			Frequency (%)	
	AA	Aa	aa	Homozygote	Heterozygote
0	0	100	0	0	100
1	25	50	25	50	50
2	25 + 12.5	25	25 + 12.5	75	25

This can be calculated by the formulae

$[2^m - 1] / 2^m$  where m = No. of generations of self-pollination and  
n = No. of genes segregating.

When number of genes are segregating together, each gene would become homozygous at the same rate as Aa. Thus the number of genes segregating does not affect the percentage of homozygosity. Similarly linkage between genes does not affect the percentage of homozygosity in the population.

### Genetic advance under selection

Normally selection is practiced based on the phenotype of the individual plant. The phenotype in turn is the result of joint action of genotype and environment i.e.,

$$V_P = V_G + V_E \quad \text{Where } P = \text{phenotype; } G = \text{genotype; } E = \text{Environment}$$

The genetic advance is calculated by the following formula.

$$\text{Genetic advance (GS)} = (K) (H) (SD P) \text{ or } GS = (K) (VP)^{1/2} (V_G / V_P),$$

Where GS is the genetic advance under selection, K is the selection differential, SD P is the phenotypic standard deviation of base population and H is the heritability of the character under selection. The estimates of GS have the same unit as that of the mean.

### **Pureline Selection**

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

### **Characteristics of purelines**

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

### **General steps for making a pureline selection**

**First Season:** From the base population select best looking plants having the desirable characters. Harvest them on single plant basis.

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

**Third Season:** Repeat the process of second season.

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

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

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

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

**Advantage of pureline selection.**

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

**Disadvantages**

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

**Mass Selection**

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

**Steps****First season**

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

**Second season**

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

**Third to Sixth Season**

With the selected lots conduct yield trials along with appropriate check or control. Select the best one and release it as a variety.

**Merits of Mass Selection**

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

**Demerits**

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

2. In the absence of progeny test we are not sure whether the superiority of selected plant is due to environment or genotype.
3. May not be as uniform as that of a pureline variety and certification is difficult.

### **Comparison between pure line and mass selections**

	<b>Pureline selection</b>	<b>Mass selection</b>
1.	The new variety is a pureline	The new variety is a mixture of purelines.
2.	The new variety is highly uniform. In fact, the variation within a pureline variety is purely environmental.	The variety has genetic variation of quantitative characters, although it is relatively uniform in general appearance
3.	The selected plants are subjected to progeny test	Progeny test is generally not carried out
4.	The variety is generally the best pureline present in the original population. The pure line selection brings about the greatest improvement over the original variety	The variety is inferior to the best pureline because most of the purelines included in it will be inferior to the best pure line
5.	Generally, a pure line variety is expected to have narrower adaptation and lower stability in performance than a mixture of pure lines	Usually the variety has a wider adaptation and greater stability than a pureline variety
6.	The plants are selected for the desirability. It is not necessary they should have a similar phenotype	The selected plants have to be similar in phenotype since their seeds are mixed to make up the new variety.
7.	It is more demanding because careful progeny tests and yield trials have to be conducted.	If a large number of plants are selected, expensive yield trials are not necessary. Thus it is less demanding on the breeder.



## **17. Hybridization – Aims, objectives and types of hybridization**

### **Objective of hybridization**

The chief objective of hybridization is to create 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 subsequent generations.

The degree of variation produced depends on the number of heterozygous genes in  $F_1$ . The number of heterozygous genes in  $F_1$  in turn depends on number of genes for which the two parents differ. If the parents are not related they may differ for several genes.

### **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. In this approach, increase in yield is obtained by correcting the weaknesses in the yield contributing traits like tiller number, grains per panicle, seed weight of the concerned variety. Example for combination breeding is disease resistance achieved by backcross breeding. Pedigree method is also another example.

### **Transgressive breeding**

Transgressive segregation is the production of plants in  $F_2$  generation that are superior to both the parents for one or more characters. Such plants are produced by the accumulation of favourable genes from both the parents as a consequence of recombination. In this case the parents involved in hybridization must combine well with each other and preferably be genetically diverse. This way, each parent expected to contribute different plus genes which when brought together by recombination gives rise to transgressive segregation. The pedigree method as well as population approach are designed to produce transgressive segregants.

### **Procedure of hybridization**

1. Set up your objective.
2. Selection of parents.
3. Evaluation of parents.
4. Sowing plan.
- s. Emasculation and dusting.
6. Labelling and bagging.

7. Harvesting and storage of seeds.

### **1. Objective**

Based on the requirement, set your objective. Because based on the objective only the selection of parents is done. If it is resistance breeding one of the parents must be a donor.

### **2. Selection of parents**

Normal practice is, the female parent will be a locally adapted one in which we can bring in the plus genes. In case of intervarietal hybridization geographically diverse parents will be selected so as to get superior segregants.

### **3. Evaluation of parents**

In case of parents which are new to the region they must be evaluated for their adaptability. Further to ensure homozygosity, they must be evaluated.

### **4. Sowing plan**

If the flowering duration is same, simultaneous sowing of both the parents can be done. Otherwise staggered sowing is to be followed. The normal practice is to raise the ovule parent in the centre of the plot in rows and on the border pollen parent for each combination.

### **5. Emasculation and dusting**

Emasculation is the removal of immature anthers from a bisexual flower. Depending on the crop the emasculation practice differs. Normal practice of hand emasculation and dusting of pollen is done. Depending on the time of anthesis the time of emasculation differs. For E.g. in rice the anthesis at Coimbatore takes place between 7.00 to 10.00 A.M. So the emasculation is done at around 6.30 A.M. and dusting of pollen is done immediately.

### **6. Labelling and bagging**

Immediately after hybridization put a label indicating the parents and date of crossing. Put appropriate cover to prevent foreign pollen, contamination.

### **7. Harvesting and storage of seeds**

Normally 15-20 days after crossing the seeds will be set. In the case of pulses the crossed pods can be easily identified by the shrunken nature of pod and seed set will be reduced. Harvest of crossed seeds must be done on individual plant basis. Seeds collected from individual plants are to be stored in appropriate containers with proper label and stored.

### **Distant Hybridization**

When crosses are made between two different species or between two different

genera, they are generally termed as **distant hybridization (or) wide hybridization**

### **History**

Thomas Fairchild 1717 was the first man to do distant hybridization. He produced an hybrid between two species of *Dianthus*

*Dianthus caryophyllus* (Carnation) x *D. barbatus* (Sweet william)

**Inter generic hybrid** produced by Karpechenko, a Russian Scientist in 1928. *Raphano brassica* is the amphidiploid from a cross between Radish (*Raphanus sativus*) and cabbage (*Brassica oleraceae*). Triticale was produced by Rimpau in 1890 itself. Triticale is an amphidiploid obtained from cross between wheat and rye. Another example is *Saccharum* nobilisation involving three species.

### **Hybrids in self-pollinated crops - problems and prospects**

Exploitation of heterosis through F I hybrids has hitherto been the prerogative of cross- pollinated crops, chiefly due to their breeding systems favouring allogamy. However, possibilities of working for such a proposition have recently been realized in self-pollinated crops also. Indeed, exploitation of hybrid vigour in autogamous crops is easy and less time-consuming in that homozygous inbreds are already available. There is practically no difference with regard to hybrid breeding between self and cross-pollinated crops. But the prospects of hybrids in selfers is dependant on three major considerations.

1. How high a heterotic effect can be gained under optimal production conditions.
2. In fact, a breeder's main concern is the magnitude rather than the frequency of occurrence of heterosis in crops. Thus the consideration is whether or not it is possible to obtain economically viable heterosis.
3. How much of the yield surplus due to high heterosis can offset the extra seed cost? In major self-pollinated crops like wheat, barley, rice, etc., the seed rate per unit area is exorbitant and hence the hybrid seed requirement is also more.
4. How efficient and effective is the mechanism of cross-pollination in selfers? By nature, self-pollinated crops are shy pollinators with very poor pollen maneuverability (or movability to effect allogamy). Therefore, the efficiency (degree of allogamy) with which cross pollination can take place on a commercial scale is the true determinant of the success of a hybrid programme in selfers.
5. Among self-pollinated crops, FI hybrids have been graduated into the farmer's field in

barely, tomato, Sorghum (often-cross-pollinated) and wheat. Briggie (1963) presented a vivid account of heterosis in wheat. Work in rice is also most encouraging (IRRI, 1972).

### **Methods of handling of segregating generations – pedigree method, bulk method, back cross method and various modified methods**

#### **Pedigree method**

In this method, individual plants are selected from  $F_2$  and subsequent generations and their progenies are tested. During this process details about the plants selected in each generation is recorded in Pedigree Record. By looking into Pedigree record we can know about the ancestry of the selected plants.

For maintenance of pedigree record the basic thing required is Crossing Ledger. This Ledger gives the details about parentage, Season in which the cross is made.

<b>Sl.No.</b>	<b>Cross Number</b>	<b>Parentage</b>
1.	XS 9801	Co2 x MS 9804
2.	X S 9802	. Co4 x C152
3.	X S 9803	Co 1 x Co4

There are several ways to maintain the pedigree Record. The selection of plants starts from  $F_2$  onwards. The details about selected plants can be recorded as follows. E.g.  $F_2$  X S 9801 - 7. Here the 7 denotes seventh plant selected.

In  $F_3$  if selection is made from the 7th plant of cross X S 9801 it can be recorded as  $F_3$  X S 9801 - 7 - 4. The number four indicates that fourth plant of 7th plant of  $F_2$  is selected. This can be followed till  $F_4$  or  $F_5$  generations. After  $F_4$  or  $F_5$  the selected plants are bulked to form a family.

In the pedigree record all the biometrical data like plant height, number of branches, No. of pods / plant, pod length, seeds / pod, pod weight, seed weight are recorded.

#### **Merits of Pedigree Method**

1. Gives maximum opportunity to the breeder to use his skill and judgement for the selection of plants.
2. Well-suited for characters which are simply inherited
3. Transgressive segregants can be easily identified thro' records.

4. Information about inheritance is precisely obtained.

### **Demerits**

1. Maintenance of pedigree record is time consuming and limits handling of larger population.
2. The success in this method is largely dependent on skill of the breeder. There is no opportunity for natural selection.
3. Selection for yield in  $F_2$  and  $F_3$  is ineffective. If care is not taken to maintain larger population, valuable materials may be lost.

### **Pedigree Method Procedure**

#### **$F_1$ Generation**

The  $F_1$  seeds are space planted so that full expression of  $F_1$  can be had. It is advisable to raise the parents involved in the cross to raise as border rows so that dominance and other characters can be studied. The  $F_1$ s are harvested as single plants.

#### **$F_2$ generation**

In  $F_2$ , 2000 to 10,000 plants per cross are planted. About 100 - 500 plants are selected and harvested on single plant basis. The selection in  $F_2$  depends upon the skill of the breeder. The selection intensity may be 5 to 10%.

#### **$F_3$ generation**

Individual plant progenies are space planted. Again desirable plants are selected. From  $F_3$  onwards the term family is introduced. The line selected from each cross is termed as family.

#### **$F_4$ generation**

Similar to  $F_3$ .

#### **$F_5$ generation**

Many families would have attained homozygosity and may be harvested as row bulk.

#### **$F_6$ generation**

The row bulk may be assessed in multi row trial. The families exhibiting segregation may be isolated and studied separately.

#### **$F_7$ generation**

RRYT

#### **$F_8$ generation**

PYT

CYT 3 seasons.

### **Basis of selection**

Depending upon the objective, selection is to be made in segregating generation. For insect and disease resistance part of the seeds may be reserved in segregating generation and the rest may be subjected to epiphytotic conditions. The families exhibiting resistance may be identified and the reserve seeds may be used for further selection and testing.

### **Early generation testing**

If superior families are identified in  $F_3$  or  $F_4$ , they can be tested for desirable characters and this is known as early generation testing.

### **Shuttle breeding**

This is followed especially in disease or insect resistance breeding. For e.g. at Coimbatore YMV in blackgram is in epidemic form during summer season only. Whereas at Vamban (Pudukkottai) the YMV is epidemic during kharif season. So instead of waiting for next summer at Coimbatore the materials can be tested at Vamban during kharif and thus one season is saved.

### **Off season nursery**

Some crops may be season bound. But it may be non - season bound in certain agro - climatic zone. For e.g. *Thalai virichan cholan. (Sroxburghii)* is season bound at Coimbatore. It has to be sown during July - August and harvested during December January. But this *Sroxburghii* is non - season bound in Yercaud. So to save one season, the segregating material can be raised during Rabi summer at Yercaud. This method is otherwise known as rapid generation advancement (RGA).

### **Bulk Method**

In this method  $F_2$  and subsequent generations are harvested as bulk to grow the next generation. The duration of bulking may be 6 - 7 generations. Selection can be made in each generation but harvest is done as bulk. This is similar to mass selection. At the end of bulking period single plant selection is made and tested for yielding ability. If bulking period is long say 20 - 30 seasons, then natural selection acts on the homozygous lines. In this method the breeder uses his skill for selecting the plants and at the same time there is no pedigree record. This saves much time and labour.

### **Merits of bulk method**

1. Simple, convenient and inexpensive
2. By inducing artificial epiphytotic conditions undesirable or weaker genotypes can be eliminated.
3. If bulking period is longer natural selection operates and desirable genotypes are selected.
4. No pedigree record is maintained.
5. Since large population is grown there is chance for appearance of transgressive segregants which will be superior than parents or  $F_2$

### **Demerits**

1. Takes much longer time to develop a new variety.
2. In short term bulk there is no chance for natural selection.
3. A large number of progenies are to be selected in each generation which requires much labour, time and space.
4. We cannot get information on inheritance.

### **Single Seed - Descent Method**

It is the modification of the bulk method. In this method a single seed from each of the  $F_2$  plants is collected and bulked to raise  $F_3$  generation. Similarly single seed from each  $F_3$  plant is collected and carried forward to  $F_4$ . This procedure is followed till  $F_6$  or  $F_7$ . After wards single plant selection is made and studied in progeny rows.

In this Scheme the main features are:

1. Lack of selection till  $F_6$  or  $F_7$  when the population becomes homozygous.
2. Each  $F_2$  plant is represented till  $F_6$  or  $F_7$  generation.
3. In this method there are chances for reduction in population size due to pest, disease or poor germination.
4. Rapid generation advancement (RGA) can be made with the use of glass house or off season nursery.

### **Modified bulk method**

Here selection can be practiced in  $F_2$  and  $F_3$  and subsequent generations. There will not be any pedigree record but superior plants are selected bulked and carried forward. In  $F_4$  superior plants are selected and harvested on single plant basis. In  $F_5$  these single plants are studied in progeny rows and best progenies are selected and harvested. In  $F_6$  PYT can be conducted to select best families. In subsequent generations regular trials can be conducted.

This modification of the bulk method provides an opportunity for the breeder to exercise his skill and judgement in selection. Further there is no maintenance of pedigree record which is another advantage.

### **Mass pedigree method**

This was proposed by Harrington. It is a solution to one of the deficiencies in the pedigree method of breeding. For e.g. if the population is to be subjected to disease resistance screening like YMV and if there is no method to create artificial epiphytotic conditions, it is wasteful to study the population in pedigree method. Instead we can carry the population as a mass and test them when there is occurrence of the disease. When conditions are favourable for the disease, we can terminate the bulking and resort to single plant selection.

### **Comparison between Pedigree and Bulk Methods**

<b>S. No.</b>	<b>Pedigree method</b>	<b>Bulk method</b>
1.	Individual plants are selected in F <sub>2</sub> and the subsequent generations and individual plant progenies are grown.	F <sub>2</sub> and the subsequent generations are maintained as bulks.
2.	Artificial selection, artificial disease epidemics etc., are an integral part of the method	Artificial selection, artificial disease epiphytotics etc., may be used to assist natural selection. In certain cases, artificial selection may be essential
3.	Natural selection does not play any role in the method.	Natural selection determines the composition of the populations at the end of the bulking period.
4.	Pedigree records have to be maintained which is often time consuming and laborious	No pedigree record is maintained.
5.	It generally takes 14-15 years to develop a new variety and to release it for cultivation.	It takes much longer for the development and release of a variety. The bulk population has to be



		maintained for more than 10 years for natural selection to act.
6.	Most widely used breeding method.	Used only to a limited extent.
7.	It demands close attention from the breeder from F <sub>2</sub> onwards as individual plant selections have to be made and pedigree records have to be maintained.	It is simple, convenient and inexpensive and does not require much attention from the breeder during the period of bulking
8.	The segregating generations are space - planted to permit individual plant selection.	The bulk populations are generally planted at commercial planting rates.
9.	The size of population is usually smaller than that in the case of bulk method.	Large populations are grown. This and natural selection are expected to increase the chances of the recovery of transgressive segregants.

## **18. Back cross method and various modified methods**

### **Backcross Method**

In backcross method of breeding, the hybrid and the progenies in subsequent generations are repeatedly backcrossed to one of the parents. As a result, the genotype of the backcross progeny becomes increasingly similar to that of the recurrent parent. The objective of backcross method is to improve one or two specific defects of a high yielding variety.

### **Pre-requisite for back cross breeding**

1. A suitable recurrent parent must be available which lacks in one or two characteristics.
2. A suitable donor parent must be available
3. The character to be transferred must have high heritability and preferably it should be determined by one or two genes.
4. A sufficient number of back crosses should be made so that the genotype of recurrent parent is recovered in full.

### **Application of back cross method**

This method is commonly used to transfer disease resistance from one variety to another. But it is also useful for transfer of other characteristics.

#### **1. Intervarietal transfer of simply inherited characters**

E.g. Disease resistance, seed coat colour

#### **2. Intervarietal transfer of quantitative characters.**

E.g. Plant height, Seed size, Seed shape.

#### **3. Interspecific transfer of simply inherited characters**

E.g. Transfer of disease resistance from related species to cultivated species.

E.g. Resistance to black arm disease in cotton from wild tetraploid species into

*G.hirsutum*

#### **4. Transfer of cytoplasm**

This is employed to transfer male sterility. The female parent will be having the sterile cytoplasm and recurrent parent will be used as male parent.

E.g. *Sesamum malabariucum* x *S.indicum*

Female parent                      Recurrent parent.

## 5. Transgressive segregation

Back cross method may be modified to produce transgressive segregants. The  $F_1$  is backcrossed to recurrent parent for 2 to 3 times for getting transgressive segregants.

## 6. Production of isogenic lines

## 7. Germplasm conversion

E.g. Production of photo insensitive line from photo Sensitive germplasm through back crossing. This was done in the case of sorghum. Popularly known as conversion programme.

### Procedure for backcross method

The Plan of backcross method would depend upon whether the gene being transferred is recessive or dominant. The plan for transfer of a dominant gene is simpler than that for a recessive gene.

First Year	Non-Recurrent		Recurrent
	Parent B	x	Parent A
	Resistant to rust		Susceptible to rust
	$F_1$ Rr	x	rr BC <sub>1</sub>
	Resistant		
rr	Rr	x	rr BC <sub>2</sub>
rr	Rr	x	rr BC <sub>3</sub>
rr	Rr	x	rr BC <sub>4</sub>
rr	Rr	x	rr BC <sub>5</sub>

Back cross upto 6th or 7<sup>th</sup> generation. After 7<sup>th</sup> BC rust resistant lines are self pollinated.

Harvest is done on single plant basis

### 8<sup>th</sup> Season

Individual plant progenies grown

a) Homozygous plants having resistance and resembling parent A are selected harvested and bulked

### 9<sup>th</sup> season

Yield trials.

### 10<sup>th</sup> season

Seed multiplication and distribution

## **Steps**

### **First Season**

#### **Hybridization**

Crossing between parent B donor (Female) and Susceptible parent A recipient (male)

### **Second Season**

Raising the  $F_1$  and backcrossed to recurrent parent A.

### **Third season**

Growing the  $BC_1F_1$ . It will be segregating for 1 susceptible (rr): 1 resistant (Rr). Rust resistant plants are backcrossed with recurrent parent A. This is second backcross.

### **Fourth Season**

Raising  $BC_2 F_1$  will again segregate in the ratio of 1: 1. Third backcross is done with resistant plants.

### **Fifth Season to Eighth Season**

Raising backcross  $F_1$ s and crossing resistant plants with recurrent parent is continued up to 7th backcross

### **Ninth season**

Raising  $BC_7F_1$  and observing resistant lines. The plants resembling parent A coupled with resistance is selected and harvested on single plant basis.

### **Tenth Season**

Growing the progeny row<sup>8</sup> and observing each row for resistance. Best rows are selected and harvest is done on row basis

### **Eleventh Season**

The row bulk is raised in yield trial along with check and the best plots are selected.

### **Twelfth season**

Selected plot seeds are multiplied and released as new variety.

## Back Cross Method - Transfer of Recessive Gene

I Season                      Non recurrent parent B                      Recurrent parent A

Hybridization              Resistant                      Susceptible

$Rr$                        $x$                        $RR$

$F_1$                        $Rr$

II Season                      Grow the  $F_1$

$Rr$

III Season Grow  $F_2$      $RR$  “  $Rr$  “  $rr$   $x$   $RR$      $BC_1$

IV Season Grow  $BC_1F_1$                        $Rr$

V Season Grow  $BC_2F_2$      $RR$  :  $Rr$  :  $rr$   $x$   $RR$      $BC_2$

VI Season Grow  $BC_2F_1$                        $Rr$

VII Season Grow  $BC_2F_2$      $RR$  :  $Rr$  :  $rr$      $x$   $RR$      $BC_3$

VIII Season Raise  $BC_3F_1$

IX Season Raise  $BC_3F_2$  and it will segregate into 1:2:1 with resistant segregant make Backcross 4 ( $BC_4$ )

X Season Do as on VIII Season

XI Season do as in IX season

Continue this process still 7<sup>th</sup> or 8<sup>th</sup> backcross. After studying 8<sup>th</sup>  $BCF_2$  select plants resembling parent B coupled with resistance. Harvest them on single plant basis. Next season raise them in progeny rows and select best progenies. Compare them in yield trial and fix the best culture, multiply it and release it as a variety. While selecting plants artificial bombardment for disease is to be done.

### Steps

**I Season:** Make a cross between donor parent A and recurrent parent B and Harvest the hybrid. The donor parents A is resistant which is governed by recessive genes. The susceptibility is

governed by dominant gene in parent B.

**II Season:** Grow the  $F_1$  which will be susceptible – Harvest them.

**III Season:** Grow  $F_2$  which will be segregating in the ratio of 1:2:1 i.e. 3/4 susceptible and 1/4 resistant. With the resistant lines (rr) make first backcross with parent A having dominant RR gene. Harvest  $BC_1F_1$

**IV Season:** Grow  $BC_1F_1$

**V season:** Grow  $BC_1F_2$  which will be segregating as we saw in III season. Repeat the process of third season. This will give  $BC_2F_1$

**VI Season :** Grow  $BC_2F_1$

**VII season:** Grow  $BC_2F_2$  then repeat the process of V Season. This will give  $BC_3F_1$ .

**VIII Season:** Grow  $BC_3F_1$

**IX Season:** Grow  $BC_3F_2$  and repeat the process of VII Season. Harvest  $BC_4F_1$ .

**X season:** Grow  $BC_4F_1$

**XI Season:** Grow  $BC_4F_2$  and repeat the process of IX Season. Harvest  $BC_5F_1$ .

**XII, XIII & XIV:** Repeat the process and carry out backcross upto 7 time.

**XV Season:** While studying  $BC_7F_2$  select single plants having resistance and resembling parent B.

**XVI Season:** Study the progenies in progeny rows and select best progenies.

**XVII Season:** Conduct yield trial and select best material.

**XVIII Season:** Multiply the seeds and distribute it as improved variety with resistance to disease.

Note: While studying back cross  $F_2$ s they should be bombarded with artificial epiphytotic conditions.

### **Merits of Backcross Method**

- The genotype of the 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.
- It is not necessary to test the variety developed by the back cross 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.
- The backcross programme is not dependent upon 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 generation each year. This would drastically reduce the time required for developing the new variety.

- Much smaller population are needed in the backcross method than in the case of pedigree method.
- 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.
- This is the only method for interspecific gene transfers.
- It may be modified so that transgressive segregation may occur for quantitative' characters.

#### **Demerits of Backcross Method**

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 is often difficult, time taking and costly.
4. By the time the backcross is over, the recurrent parent may have been replaced by other varieties superior in yielding ability and other characteristics.

#### **Number of Plants Necessary in the Backcross Generations**

According to the above schemes, only a few (about 10) seeds are necessary in each backcross generation for the transfer of a character governed by a single gene. This population size would almost certainly have at least one plant with the gene for rust resistance. However, if the character is governed by two or more genes, a larger number.

of backcross progenies would be required. A larger size of backcross population is also desirable to permit an effective selection for the plant type of the recurrent parent, and to increase the probability of recombination between the genes being transferred and the genes tightly linked with it. Therefore, more than 50, preferably 100 or more, plants should be grown in each backcross generation. In F<sub>2</sub> and F<sub>3</sub> generations, the population size should be as large, as

possible.

### **Selection for the Character Being Transferred**

A rigid selection for the character being transferred must be practiced during the backcross and the F<sub>2</sub> generations, otherwise the character is likely to be lost. It is, therefore, essential that the character being transferred must have a high heritability. Although monogenic characters are the easiest to transfer, the number of genes is not as important as the heritability of the character under transfer.

It is desirable that the character should be easily identified either visually or through simple tests. The breeder should try to maintain the character in an intense form through selection. Often the intensity would be lost due to modifying genes in the new genetic background. Therefore, the nonrecurrent parent should be chosen for a high intensity of the character to be transferred.

### **Number of Backcrosses to be made**

In the backcross method, it is essential that the genotype of the recurrent parent should be recovered except for the gene being transferred. The recurrent parent is likely to consist of several closely similar purelines. Therefore, a sufficient number of plants from the recurrent parent should be used for the backcrosses. This would make sure that the new variety will have the same genetic composition as the recurrent parent.

Generally, six backcrosses are sufficient to recover the essential feature of the recurrent parent. Selection for the characteristics of the recurrent parent, particularly in the early backcross generations, may often have the effect of one or two additional backcrosses. Thus six backcrosses along with selection for the recurrent parent plant type in the early backcross generations will be effective in recovering the genotype of the recurrent parent.

### **Modifications of the Backcross Method**

The backcross method may be modified in various ways to suit the needs of the breeder. Following are the three common modifications of the backcross method.

#### **Production of F<sub>2</sub> and F<sub>3</sub>**

The F<sub>2</sub> and F<sub>3</sub> generations are produced after the first and the third backcrosses. A rigid selection for the character being transferred and for the characteristics of the recurrent parent is done in the F<sub>2</sub> and F<sub>3</sub> generations. In the backcross progenies, selection need not be done either for the character being transferred or for the characteristics of the recurrent parent. The fourth,



fifth and sixth backcross are made in succession. For the sixth backcross, a relatively larger number of plants from the backcross progeny is used. This method may be used for the transfer of both dominant and recessive genes. It is believed that an effective selection in  $F_2$  and  $F_3$  generations is equivalent to one or two additional backcrosses.

### **Backcross - Pedigree Method**

In this method, the hybrid is backcrossed 1-2 times to the recurrent parent. Subsequently, the backcross progenies are handled according to the pedigree method. This approach is useful when one of the parents is superior to the other in several characteristics but the non recurrent parent is not desirable agronomically. The superior parent is used as the recurrent parent.

The purpose of the one to two backcrosses is to make sure that the new variety would get a majority of the superior genes from the recurrent parent. It also leaves enough heterozygosity for transgressive segregants to appear. The varieties developed by this method must be put to yield trials as those developed by the pedigree method. The same holds true when two or more recurrent parents are used in the backcross programme.

### **Application of the Backcross Method to Cross Pollinated Crops**

The backcross method is equally applicable to cross-pollinated crops. The method is essentially the same as in the case of self-pollinated crops. The only difference is that in cross-pollinated crops a large number of plants (100-300) from the recurrent parent must be used in each backcross.

This is necessary so that the new variety has the same genetic constitution as the recurrent parent. For example, wilt resistance was transferred to alfalfa variety California Common from the variety Turkestan. Two hundred plants of California Common were used for each backcross. The new variety Calliverde is exactly like California Common except for its wilt resistance.

### Comparison between Pedigree and Backcross Methods

S. No.	Pedigree method	Backcross method
1.	F <sub>1</sub> and the sub sequent generations are allowed to self-pollinate	F <sub>1</sub> and the subsequent generations are backcrossed to the recurrent parent
2.	The new variety developed by this method is different from the parents in agronomic and other characteristics	Usually extensive testing is not necessary before release
3.	The new variety has to be extensively tested before release	Usually extensive testing is not necessary before release
4.	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
5.	It is useful in improving both qualitative and quantitative characters	Useful for the transfer of both quantitative and qualitative characters provided they have high heritability
6.	It is not suitable for gene transfer from related species and for producing substitution or addition lines	It is the only useful method for gene transfers from related species and for producing addition and substitution lines
7.	Hybridization is limited to the production of the F <sub>1</sub> generation.	Hybridization with the recurrent parent is necessary for producing every backcross generation
8.	The F <sub>2</sub> and the subsequent generations are much larger than those in the backcross method	The backcross generation are small and usually consist of 20-100 plants in each generation
9.	The procedure is the same for both dominant and recessive genes	The procedures for the transfer of dominant and recessive genes are

		different.
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### **Multiline Varieties**

Generally, pureline varieties are highly adapted to a limited area, but poorly adapted to wider regions. Further, their performance is not stable from year to year because of changes in weather and other environmental factors. Purelines often have only one or a few major genes for disease resistance, such as, rust resistance, which make them resistant to some races of the pathogen. New races are continuously produced in many pathogens, which may overcome the resistance present in the pureline varieties. For example, Kalyan Sona wheat (*T.aestivum*) originally resistant to brown rust (leaf rust), soon became susceptible to new races of the pathogen.

To overcome these limitations, particularly the breakdown of resistance to disease, it was suggested to develop multiline varieties. Multiline varieties are mixtures of several purelines of similar height, flowering and maturity dates, seed colour and agronomic characteristics, but having different genes for disease resistance. The purelines constituting a multiline variety must be compatible, i.e., they should not reduce the yielding ability of each other when grown in mixture.

In 1954, Borlaug suggested that several purelines with different resistance genes should be developed through back cross programmes using one recurrent parent. This is done by transferring disease resistance genes from several donor parents carrying different resistant genes to a single recurrent parent. Each donor parent is used in a separate backcross programme so that each line has different resistant gene or genes. Five to ten of these lines may be mixed depending upon the races of the pathogen prevalent in the area. If a line or lines become susceptible, they would be replaced by resistant lines. New lines would be developed when new sources of resistance become available. The breeder should keep several resistant lines in store for future use in the replacement of susceptible lines of multiline varieties.

### **Merits of Multiline varieties**

1. All the lines are almost identical to the recurrent parent in agronomic characteristics, quality etc. Therefore, the disadvantages of the pureline mixtures are not present in the multiline varieties.

2. Only one or a few lines of the mixture would become susceptible of the pathogen in anyone season. Therefore, the loss to the cultivator would be relatively low.
3. The susceptible line would constitute only a small proportion of the plants in the field. Therefore, only a small proportion of the plants would be infected by the pathogen. Consequently the disease would spread more slowly than when the entire population was susceptible. This would reduce the damage to the susceptible line as well.

### **Demerits of Multiline Varieties**

1. The farmer has to change the seed of multiline varieties every few years depending upon the change in the races of the pathogen.
2. There is a possibility that a new race may attack all lines of a multiline variety.

### **Achievements**

Multiline variety appears to be a useful approach to control diseases like rusts where new races are continuously produced. In India, three multiline varieties have been released in wheat (*T.aestivum*). Kalyan Sona, one of the most popular varieties in the late sixties, was used as the recurrent parent to produce these varieties. Variety 'KSML 3' consists of 8 lines having rust resistance genes from Robin, Ghanate, KI, Rend, Gabato, Blue Brid, Tobari etc. Multiline 'MLKS 11' is also a mixture of 8 lines; the resistance is derived from E 6254, E 6056, E 5868, Freacor, HS 19, E 4894 etc. The third variety, KML 7406 has 9 lines deriving rust resistance from different sources.

### **Dirty Multiline**

This term is used when a multiline is having one or two susceptible lines also. The idea of including susceptible lines is to prevent race formation.

## 19. Incompatibility and male sterility and their utilization in crop improvement

### Self-Incompatibility

Self-incompatibility and sterility are the two mechanisms, which encourage cross-pollination. More than 300 species belonging to 20 families of angiosperms show self-incompatibility.

#### Definition

In self incompatibility plants, the flowers will produce functional or viable pollen grains which fail to fertilize the same flower or any other flower of the same plant.

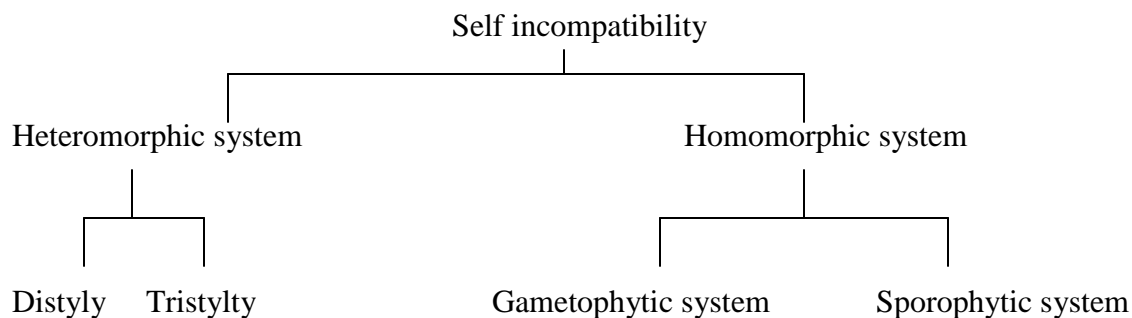
- Self incompatible pollen grain may fail to germinate on the stigmatic surface.
- Some may germinate but fails to penetrate the stigmatic surface.
- Some pollen grains may produce pollen tube, which enters through stigmatic surface, but its growth will be too slow. By the time the pollen tube enters the ovule the flower will drop.
- Some time fertilization is effected but embryo degenerates early.

#### Reason

Self-incompatibility is appeared to be due to biochemical reaction, but precise nature of these is not clearly understood.

#### Classification of self incompatibility

According to Lewis (1954) the self incompatibility is classified as follows:



#### Heteromorphic system

In this case there will be difference in the morphology of the flowers. For example in *Primula sp* there are two types of flowers namely PIN and THRUM. PIN flowers have long style

and short stamens while THRUM flowers have short style and long stamens. This type of difference is known as Distyly. In case of distyly the only compatible mating is between PIN and THRUM. The relative position of anthers is determined by single gene S/s. The recessive *ss* produces PIN and heterozygotes *Ss* produces THRUM.

Homozygous dominant *SS* is lethal and do not exist. The incompatibility reaction of pollen is determined by the genotype of the plant producing them. Allele *S* is dominant over *s*. This system is also known as heteromorphic – sporophytic system. Pollen grains produced by PIN flowers will be all *s* in genotype as well as in incompatibility reaction: Whereas THRUM flowers will produce two types of gametes *S* and *s* but all of them would be *S* phenotypically. The mating between PIN and THRUM would produce *Ss* and *ss* progeny in equal frequencies. This system is of little importance in crop plants. It occurs in sweet potato and bulk wheat.



PIN FLOWER



THRUM FLOWER

Mating		Progeny	
Phenotype	Genotype	Genotype	Phenotype
Pin x Pin	<i>ss</i> x <i>ss</i>	Incom. Mating	-
Pin x Thrum	<i>SS</i> x <i>Ss</i>	1 <i>ss</i> : <i>Ss</i>	1 Thrum 1 Pin
Thrum x Pin	<i>Ss</i> x <i>ss</i>	1 <i>Ss</i> : <i>ss</i>	1 Thrum 1 Pin
Thrum x Thrum	<i>Ss</i> x <i>Ss</i>	Incom. Mating	-

TRISTYLY is known in some plants like *Lythrum salicaria*. In this case the style of the flower may be short, long or medium length

## Homomorphic System

Here the incompatibility is not associated with morphological difference among flower. The incompatibility reaction of pollen may be controlled by the genotype of the plant on which it is produced – (Sporophytic control) or by its own genotype – (Gametophytic control).

## Gametophytic system

First discovered by East and Mangelsdorf in 1925 in *Nicotiana sanderae*. Here the incompatible reaction of pollen is determined by its own genotype and not by the genotype of the plant on which pollen is produced. Genetically the incompatibility reaction is determined by a single gene having multiple allele. Eg. *Trifolium Nicotiana*, *Lycopersicon*, *Solanum*, & *Petunia*. Here codominance is assumed.

Genotype of plant (Sporophyte)	$S_1S_2$		$S_3S_4$	
Genotype of gametes	$S_1$	$S_2$	$S_3$	$S_4$
	↓	↓	↓	↓
Incompatible reaction of pollen	$S_1$	$S_2$	$S_3$	$S_4$
Incompatible reaction of style	$S_1$	$S_2$	$S_3$	$S_4$
Mating	$S_1S_2 \times S_1S_2$ - Fully incompatible			
	$S_1S_2 \times S_1S_3$ - Partially compatible			
	$S_1S_2 \times S_3S_4$ - Fully compatible			

## **Sporophytic system**

Here also the self incompatibility is governed by a single gene S with multiple alleles. More than 30 alleles are known in *Brassica oleracea*. Here dominance is assumed.

The incompatibility reaction is determined by the genotype of the plant on which pollen grain is produced and not by the genotype of the pollen. This system is more complicated. The allele may exhibit dominance, co-dominance or competition. This system was first reported by Hugues and Babcock in 1950 in *Crepis foetida* and by Gerstl in *Parthenium argentatum*. The sporophytic system is found in radish, brassicas and spinach.

Lewis has summarized the characteristics of sporophytic system as follows :

1. There are frequent reciprocal differences
2. Incompatibility can occur with 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.

## **Mechanism of Self Incompatibility**

This is quite complex and is poorly understood. The various phenomena observed in Self incompatibility is grouped into three categories.

1. Pollen – Stigma interaction
2. Pollen tube – Style interaction
3. Pollen tube – Ovule interaction

### **1. Pollen – Stigma interaction**

This occurs just after the pollen grains reach the stigma and generally prevents pollen from germination. Previously it was thought that binucleate condition of pollen in gametophytic system and trinucleate condition in sporophytic system was the reason for self incompatibility. But later on it was observed that they are not the reason for SI. Under homomorphic system of incompatibility there are differences in the stigmatic surface which prevents pollen germination. In gametophytic system the stigma surface is plumose having elongated receptive cells which is commonly known as wet stigma. The pollen grain germinates on reaching the stigma and incompatibility reaction occurs at a later stage.

In the sporophytic system the stigma is papillate and dry and covered with hydrated layer of protein known as pellicle. This pellicle is involved in incompatibility reaction. With in few



minutes of reaching the stigmatic surface the pollen releases an exine exudates which is either protein or glycerol protein. This reacts with pellicle and induces callose formation, which further prevents the growth of pollen tube.

<b>Gametophytic system</b>	<b>Sporophytic system</b>
Stigma surface Plumose Commonly known as wet Stigma	Stigma surface Papillate and dry. Covered with hydrated layers of protein known as pellicle which involves in incompatibility reaction
Pollen grain germinates and incompatibility reaction occurs at a later stage	Pollen grain releases exine exudates which is protein or Glyco-protein
	This protein reaction with pellicle and induces callose formation and arrests growth of pollen type.

## **2. Pollen Tube – Style interaction**

Pollen grains germinate and pollen tube penetrates the stigmatic surface. But in incompatible combinations the growth of pollen tube is retarded with in the style as in *Petunia*, *Lycopersicon*, & *Lilium*. The protein and poly saccharine synthesis in the pollen tube stops resulting in bursting up of pollen tube and leading to death of nuclei.

## **3. Pollen tube – Ovule interaction**

In *Theobroma cacao* pollen tube reaches the ovule and fertilization occurs but the embryo degenerates later due to some biochemical reaction.

### **Male Sterility**

Male sterility is characterized by nonfunctional pollen grains, while female gametes function normally. It occurs in nature sporadically.

### **Morphological features of male sterility**

The male sterility may be due to mutation, chromosomal aberrations, cytoplasmic factors or interaction of cytoplasmic and genetic factors. Because of any of the above reasons the following

morphological changes may occur in male sterile plants.

- Viable pollen grains are not formed. The sterile pollen grains will be transparent and rarely takes up stain faintly.
- Non-dehiscence of anthers, even though viable pollens are enclosed within. This may be due to hard outer layer, which restrict the release of pollen grains.
- Androecium may abort before the pollen grains are formed.
- Androecium may be malformed, thus there is no possibility of pollen grain formation.

### **Kinds of male sterility, maintenance and uses**

Male sterility may be conditioned due to cytoplasmic or genetic factors or due to interaction of both. Environment also induces male sterility. Depending on these factors male sterility can be classified in to

- Cytoplasmic male sterility (CMS)
- Cytoplasmic-genetic male sterility (CGMS)
- Genetic male sterility (GMS)

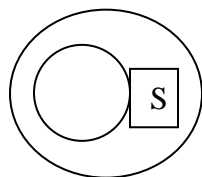
In this there are two categories.

- Environment insensitive genic male sterility- commonly referred as Genetic male sterility.
- Environment sensitive genic male sterility or Environmental induced sterility which is again sub divided in to
  - TGMS (Thermosensitive)
  - PGMS (Photo sensitive)
  - Photo thermo sensitive

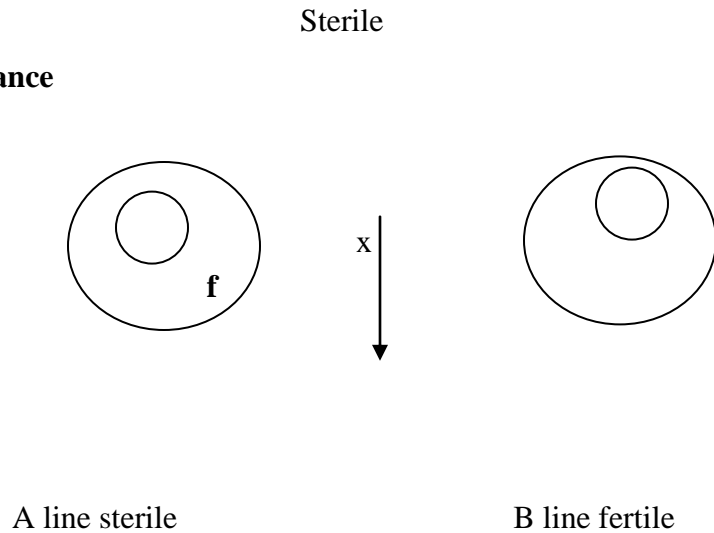
### **1. Cytoplasmic Male Sterility (CMS)**

It occurs due to the mutation of mitochondria or some other cytoplasmic factors outside the nucleus. Nuclear genes are not involved here. There is considerable evidence that gene or genes conditioning cytoplasmic male sterility. Particularly in maize DNA reside in mitochondria and may be located in a plasmid like element.

### **Genetic structure**



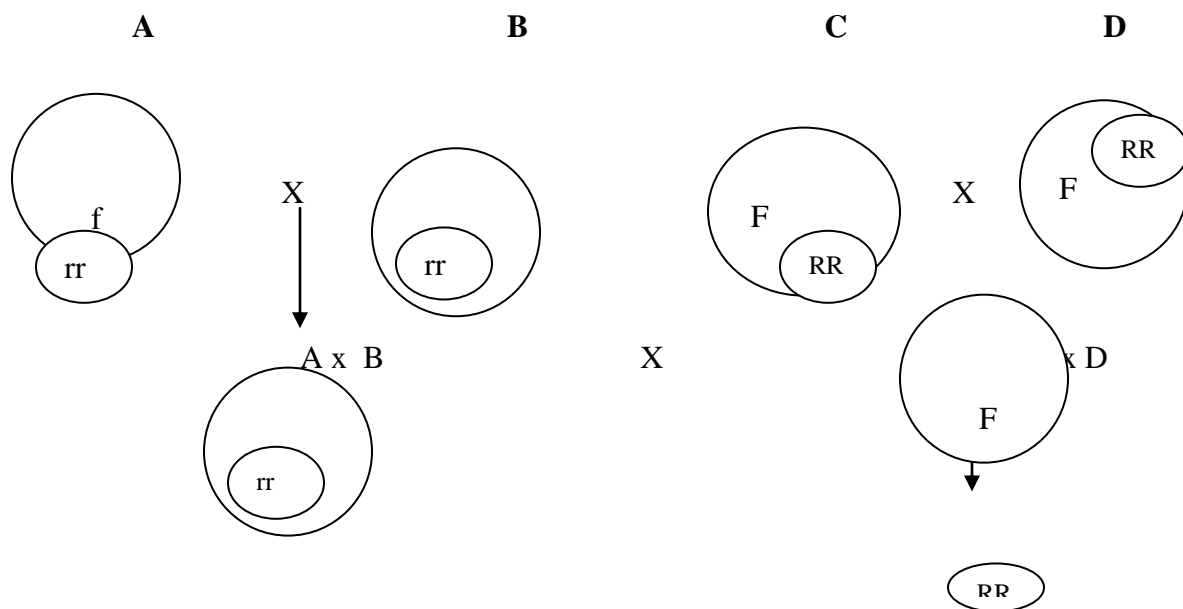
## Maintenance

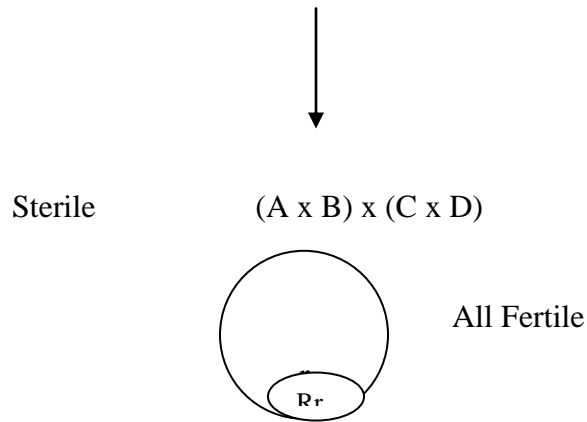


Since mother contributes the cytoplasm to the offspring, the sterility is transferred to the  $F_1$ .

## Uses

Since there are no R lines available, this type of sterility is useful only in crops where seed is not the end product. For example in onion and many ornamental plants the hybrids developed exhibit maximum hybrid vigour with respect to longer vegetative duration and larger flower size and larger bulb size. Cytoplasmic male sterility has successfully been exploited in maize for producing double cross hybrids.

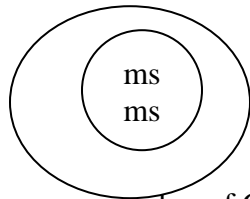




### Genetic Male Sterility (Gms)

Genetic male sterility is normally governed by nuclear recessive genes  $ms\ ms$ . Exception to this is safflower where male sterility is governed by dominant gene  $Ms\ Ms$ . This type of male sterility is used in Redgram and Castor for production of hybrids.

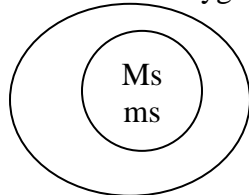
### Genetic structure



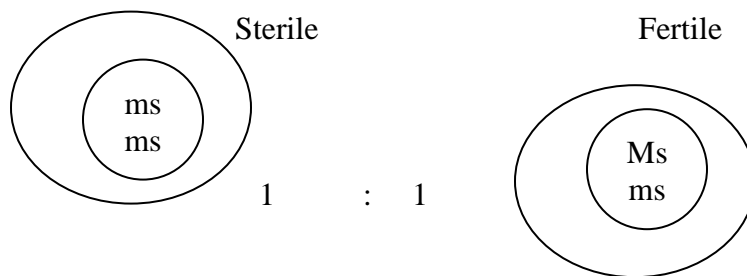
In red gram there are number of GMS lines are available. E.g.  $Ms\ Co\ 5$ ,  $Ms\ T21$

### Maintenance

In genetic male sterility, the sterile line will be maintained from heterozygous condition. The genetic structure of heterozygous line will be.



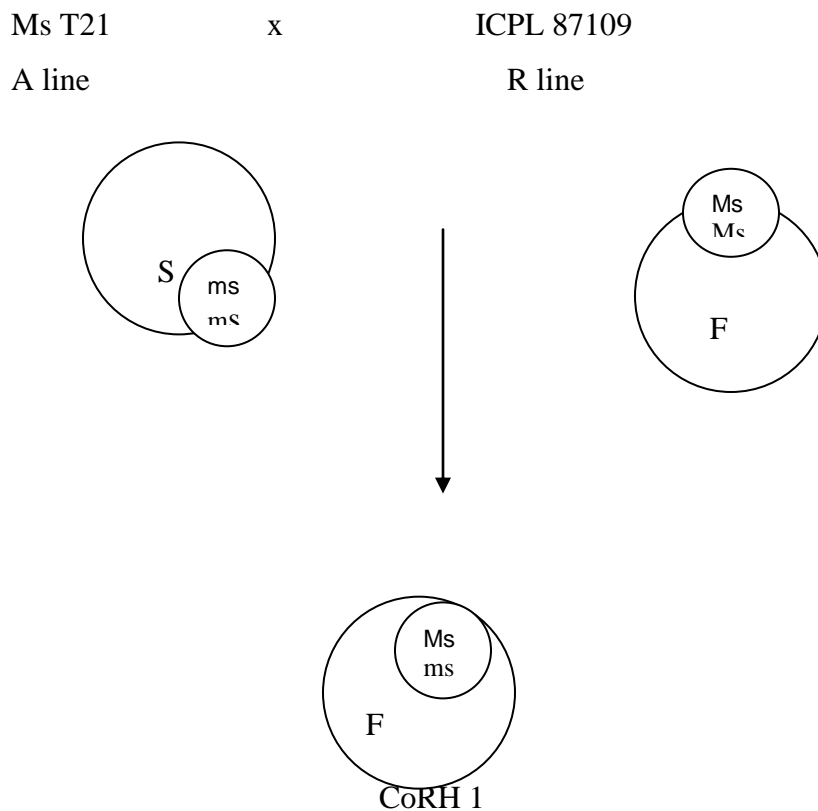
When this heterozygous line is grown in the field it will segregate in the ratio of 1 Fertile : 1 sterile.



The pollen from the Fertile line will pollinate the sterile line and as a result seed set will be there in the sterile line. These seeds are to be harvested and used for hybrid seed production.

For hybrid seed production, the seeds collected from sterile plants will be grown using double the seed rate since it will segregate in the ratio of 1 fertile : 1 sterile line. At the time of flowering, the fertile line will be identified by yellow plumpy anthers and removed from the field. Only the sterile line will remain in field. These will be pollinated by the R line and the R1 obtained will be hybrid redgram.

**Utilisation: Hybrid development. Eg: Redgram**



**Difficulties in use of Gms**

1. Maintenance of GMS requires skilled labour to identify fertile and sterile line. Labelling is time consuming and costly.
2. In hybrid seed production plot identification of fertile line and removing them is costly.
3. Use of double the seed rate of GMS line is costly
4. In crops like castor high temperature leads to break down of male sterility.

**Transgenic Genetic Male Sterility**

A gene introduced into the genome of an organism by recombinant DNA technology or genetic engineering is called transgene. Many transgenes have been shown to produce genetic male sterility, which is dominant to fertility. Consequently, it is essential to develop effective fertility restoration system if these are to be utilized for hybrid seed production. An effective restoration system is available in at least one case called Barnase or Barstar system.

The Barnase gene of *Bacillus amyloliquefaciens* encodes an RNAs. When Barnase gene is driven by TA 29 promoter, it is expressed only in tapetum cells causing their degeneration. Transgenic tobacco and *Brassica napus* plants expressing Barnase were completely male sterile. Another gene, Barstar, from the same bacterium encodes a protein, which is a highly specific inhibitor of Barnase RNase. Therefore, transgenic plants expressing both Barstar and Barnase are fully male fertile.

The Barnase gene has been tagged with bar gene, which specifies resistance to the herbicide phosphinothricin. This male sterile line is maintained by crossing with a male fertile line. The progeny so obtained contain 1 male sterile : 1 male fertile plants; the latter are easily eliminated at seedling stage by a phosphinothricin spray. The male sterile plants are crossed with the Barstar line to obtain male fertile hybrid progeny. This system of male sterility is yet to be commercially used.

Use of TGMS or PGMS eliminates this problem. These male sterile lines are maintained by growing them in a locality where the temperatures and photoperiods during the sensitive developmental stages are such that they exhibit complete male fertility (phenotypically).

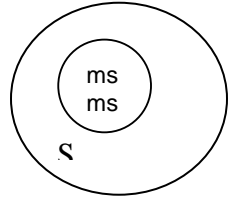
The selfed seeds from such lines are then grown for hybrid seed production in those locations where their ms genes produce complete male sterility due to the prevailing temperature and photoperiod regimes. Clearly, all the plants in the male sterile line will be sterile and no rouging will be required. TGMS and PGMS are being used for hybrid rice development in China.

### **Cytoplasmic Geneic Male Sterility**

This is a case of cytoplasmic male sterility where dominant nuclear gene restores fertility. This system is utilized for the production of hybrids in bajra, jowar, maize, rice, wheat and many other crops.

## Genetic structure

A line



Male sterile

**A line or ms line:** This term represents a male sterile line belonging to any one of the above categories. The A line is always used as a female parent in hybrid seed production.

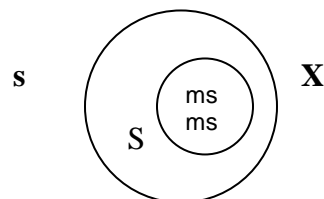
**B line or maintainer line:** This line is used to maintain the sterility of A line. The B line is an isogenic line which is identical for all traits except for fertility status.

**R line and restoration of fertility:** It is otherwise known as Restorer line which restores fertility in the A line. The crossing between A x R lines results in F<sub>1</sub> fertile hybrid seeds which are of commercial value.

## Maintenance

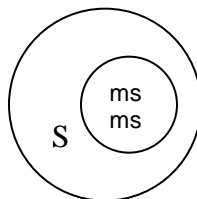
A line

B line



Sterile

Fertile

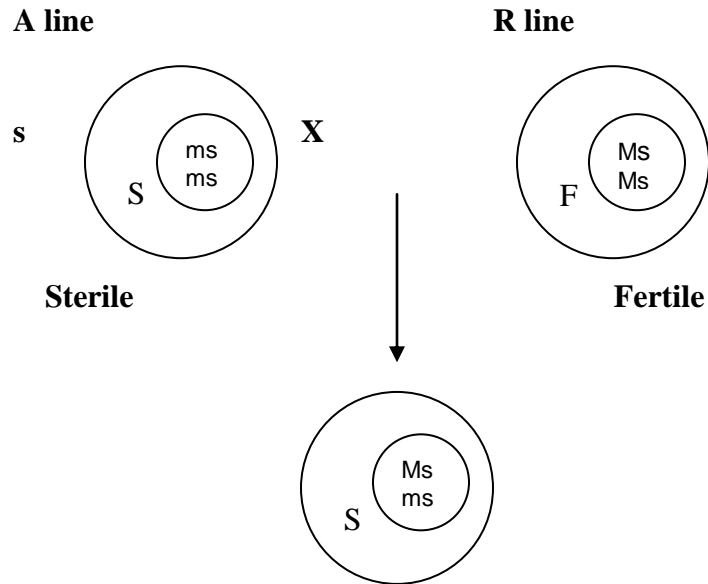


Male sterile line

The A line which is male sterile is maintained by crossing it with isogenic B line which is also known as maintainer line. The B line is similar to that of A line in all characters (isogenic) except fertile cytoplasm.

## Utilisation

The male sterile. A line is crossed with R line (Restorer) which restores fertility in  $F_1$ .



### Hybrid Fertile

#### Limitations of CGMS lines

1. Fertility restoration is a problem. E.g. Rice.
2. Seed set will be low in crops like rice where special techniques are to be adopted to increase seed set.
3. Break down of male sterility at higher temperature
4. In crops like wheat having a polyploidy series it is difficult to develop effective R line.
5. Undesirable effect of cytoplasm. Eg. Texas cytoplasm in maize became susceptible to *Helminthosporium*. In bajra Tift 23 A cytoplasm became susceptible to downy mildew.
6. Modifier genes may reduce effectiveness of cytoplasmic male sterility.



## 20. Incompatibility and male sterility and their utilization in crop improvement

### Self-Incompatibility

Self-incompatibility and sterility are the two mechanisms, which encourage cross-pollination. More than 300 species belonging to 20 families of angiosperms show self-incompatibility.

#### Definition

In self incompatibility plants, the flowers will produce functional or viable pollen grains which fail to fertilize the same flower or any other flower of the same plant.

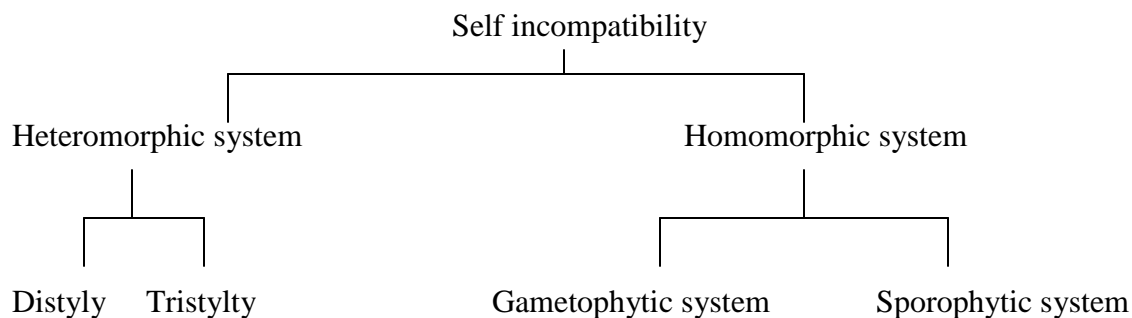
- Self incompatible pollen grain may fail to germinate on the stigmatic surface.
- Some may germinate but fails to penetrate the stigmatic surface.
- Some pollen grains may produce pollen tube, which enters through stigmatic surface, but its growth will be too slow. By the time the pollen tube enters the ovule the flower will drop.
- Some time fertilization is effected but embryo degenerates early.

#### Reason

Self-incompatibility is appeared to be due to biochemical reaction, but precise nature of these is not clearly understood.

#### Classification of self incompatibility

According to Lewis (1954) the self incompatibility is classified as follows:



#### Heteromorphic system

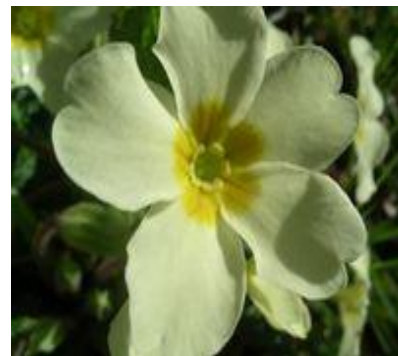
In this case there will be difference in the morphology of the flowers. For example in *Primula sp* there are two types of flowers namely PIN and THRUM. PIN flowers have long style

and short stamens while THRUM flowers have short style and long stamens. This type of difference is known as Distyly. In case of distyly the only compatible mating is between PIN and THRUM. The relative position of anthers is determined by single gene S/s. The recessive *ss* produces PIN and heterozygotes *Ss* produces THRUM.

Homozygous dominant *SS* is lethal and do not exist. The incompatibility reaction of pollen is determined by the genotype of the plant producing them. Allele *S* is dominant over *s*. This system is also known as heteromorphic – sporophytic system. Pollen grains produced by PIN flowers will be all *s* in genotype as well as in incompatibility reaction: Whereas THRUM flowers will produce two types of gametes *S* and *s* but all of them would be *S* phenotypically. The mating between PIN and THRUM would produce *Ss* and *ss* progeny in equal frequencies. This system is of little importance in crop plants. It occurs in sweet potato and bulk wheat.



PIN FLOWER



THRUM FLOWER

Mating		Progeny	
Phenotype	Genotype	Genotype	Phenotype
Pin x Pin	<i>ss</i> x <i>ss</i>	Incom. Mating	-
Pin x Thrum	<i>SS</i> x <i>Ss</i>	1 <i>ss</i> : <i>Ss</i>	1 Thrum 1 Pin
Thrum x Pin	<i>Ss</i> x <i>ss</i>	1 <i>Ss</i> : <i>ss</i>	1 Thrum 1 Pin
Thrum x Thrum	<i>Ss</i> x <i>Ss</i>	Incom. Mating	-

TRISTYLY is known in some plants like *Lythrum salicaria*. In this case the style of the flower may be short, long or medium length

## Homomorphic System

Here the incompatibility is not associated with morphological difference among flower. The incompatibility reaction of pollen may be controlled by the genotype of the plant on which it is produced – (Sporophytic control) or by its own genotype – (Gametophytic control).

## Gametophytic system

First discovered by East and Mangelsdorf in 1925 in *Nicotiana sanderae*. Here the incompatible reaction of pollen is determined by its own genotype and not by the genotype of the plant on which pollen is produced. Genetically the incompatibility reaction is determined by a single gene having multiple allele. Eg. *Trifolium Nicotiana*, *Lycopersicon*, *Solanum*, & *Petunia*. Here codominance is assumed.

Genotype of plant (Sporophyte)	$S_1S_2$		$S_3S_4$	
Genotype of gametes	$S_1$	$S_2$	$S_3$	$S_4$
	↓	↓	↓	↓
Incompatible reaction of pollen	$S_1$	$S_2$	$S_3$	$S_4$
Incompatible reaction of style	$S_1$	$S_2$	$S_3$	$S_4$
Mating	$S_1S_2 \times S_1S_2$ - Fully incompatible			
	$S_1S_2 \times S_1S_3$ - Partially compatible			
	$S_1S_2 \times S_3S_4$ - Fully compatible			

## **Sporophytic system**

Here also the self incompatibility is governed by a single gene S with multiple alleles. More than 30 alleles are known in *Brassica oleracea*. Here dominance is assumed.

The incompatibility reaction is determined by the genotype of the plant on which pollen grain is produced and not by the genotype of the pollen. This system is more complicated. The allele may exhibit dominance, co-dominance or competition. This system was first reported by Hugues and Babcock in 1950 in *Crepis foetida* and by Gerstel in *Parthenium argentatum*. The sporophytic system is found in radish, brassicas and spinach.

Lewis has summarized the characteristics of sporophytic system as follows :

1. There are frequent reciprocal differences
2. Incompatibility can occur with 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.

## **Mechanism of Self Incompatibility**

This is quite complex and is poorly understood. The various phenomena observed in Self incompatibility is grouped into three categories.

1. Pollen – Stigma interaction
2. Pollen tube – Style interaction
3. Pollen tube – Ovule interaction

### **1. Pollen – Stigma interaction**

This occurs just after the pollen grains reach the stigma and generally prevents pollen from germination. Previously it was thought that binucleate condition of pollen in gametophytic system and trinucleate condition in sporophytic system was the reason for self incompatibility. But later on it was observed that they are not the reason for SI. Under homomorphic system of incompatibility there are differences in the stigmatic surface which prevents pollen germination. In gametophytic system the stigma surface is plumose having elongated receptive cells which is commonly known as wet stigma. The pollen grain germinates on reaching the stigma and incompatibility reaction occurs at a later stage.

In the sporophytic system the stigma is papillate and dry and covered with hydrated layer of protein known as pellicle. This pellicle is involved in incompatibility reaction. With in few

minutes of reaching the stigmatic surface the pollen releases an exine exudates which is either protein or glycerol protein. This reacts with pellicle and induces callose formation, which further prevents the growth of pollen tube.

<b>Gametophytic system</b>	<b>Sporophytic system</b>
Stigma surface Plumose Commonly known as wet Stigma	Stigma surface Papillate and dry. Covered with hydrated layers of protein known as pellicle which involves in incompatibility reaction
Pollen grain germinates and incompatibility reaction occurs at a later stage	Pollen grain releases exine exudates which is protein or Glyco-protein
	This protein reaction with pellicle and induces callose formation and arrests growth of pollen type.

## **2. Pollen Tube – Style interaction**

Pollen grains germinate and pollen tube penetrates the stigmatic surface. But in incompatible combinations the growth of pollen tube is retarded with in the style as in *Petunia*, *Lycopersicon*, & *Lilium*. The protein and poly saccharine synthesis in the pollen tube stops resulting in bursting up of pollen tube and leading to death of nuclei.

## **3. Pollen tube – Ovule interaction**

In *Theobroma cacao* pollen tube reaches the ovule and fertilization occurs but the embryo degenerates later due to some biochemical reaction.

### **Male Sterility**

Male sterility is characterized by nonfunctional pollen grains, while female gametes function normally. It occurs in nature sporadically.

### **Morphological features of male sterility**

The male sterility may be due to mutation, chromosomal aberrations, cytoplasmic factors or interaction of cytoplasmic and genetic factors. Because of any of the above reasons the following

morphological changes may occur in male sterile plants.

- Viable pollen grains are not formed. The sterile pollen grains will be transparent and rarely takes up stain faintly.
- Non-dehiscence of anthers, even though viable pollens are enclosed within. This may be due to hard outer layer, which restrict the release of pollen grains.
- Androecium may abort before the pollen grains are formed.
- Androecium may be malformed, thus there is no possibility of pollen grain formation.

### **Kinds of male sterility, maintenance and uses**

Male sterility may be conditioned due to cytoplasmic or genetic factors or due to interaction of both. Environment also induces male sterility. Depending on these factors male sterility can be classified in to

- Cytoplasmic male sterility (CMS)
- Cytoplasmic-genetic male sterility (CGMS)
- Genetic male sterility (GMS)

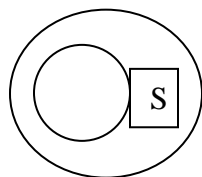
In this there are two categories.

- Environment insensitive genic male sterility- commonly referred as Genetic male sterility.
- Environment sensitive genic male sterility or Environmental induced sterility which is again sub divided in to
  - TGMS (Thermosensitive)
  - PGMS (Photo sensitive)
  - Photo thermo sensitive

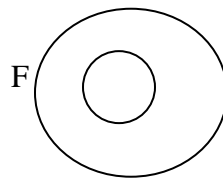
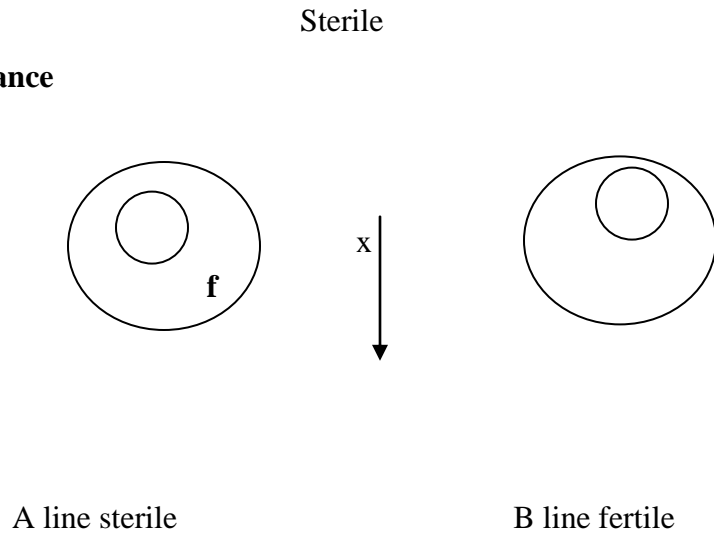
### **1. Cytoplasmic Male Sterility (CMS)**

It occurs due to the mutation of mitochondria or some other cytoplasmic factors outside the nucleus. Nuclear genes are not involved here. There is considerable evidence that gene or genes conditioning cytoplasmic male sterility. Particularly in maize DNA reside in mitochondria and may be located in a plasmid like element.

### **Genetic structure**



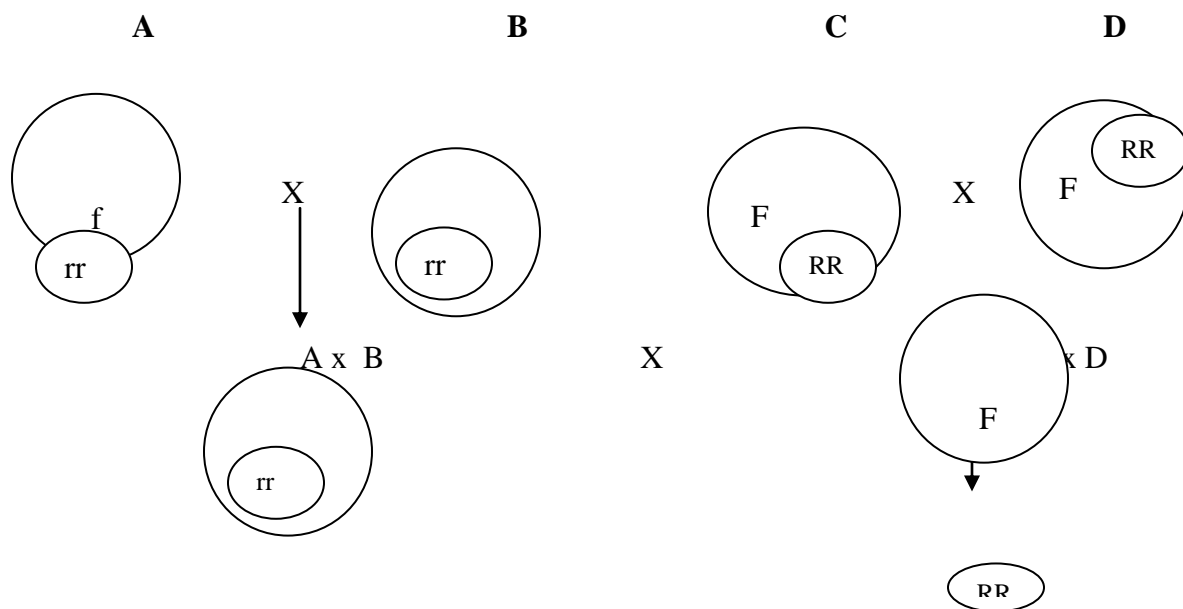
## Maintenance

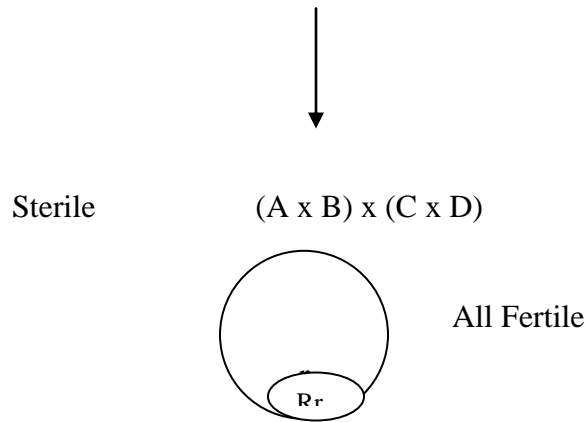


Since mother contributes the cytoplasm to the offspring, the sterility is transferred to the  $F_1$ .

## Uses

Since there are no R lines available, this type of sterility is useful only in crops where seed is not the end product. For example in onion and many ornamental plants the hybrids developed exhibit maximum hybrid vigour with respect to longer vegetative duration and larger flower size and larger bulb size. Cytoplasmic male sterility has successfully been exploited in maize for producing double cross hybrids.

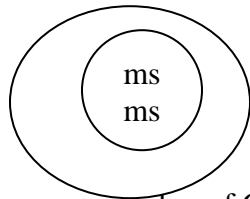




### Genetic Male Sterility (Gms)

Genetic male sterility is normally governed by nuclear recessive genes  $ms\ ms$ . Exception to this is safflower where male sterility is governed by dominant gene  $Ms\ Ms$ . This type of male sterility is used in Redgram and Castor for production of hybrids.

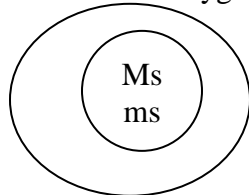
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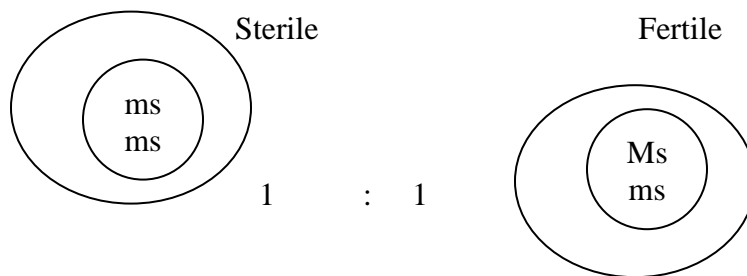
In red gram there are number of GMS lines are available. E.g.  $Ms\ Co\ 5$ ,  $Ms\ T21$

#### Maintenance

In genetic male sterility, the sterile line will be maintained from heterozygous condition. The genetic structure of heterozygous line will be.



When this heterozygous line is grown in the field it will segregate in the ratio of 1 Fertile : 1 sterile.

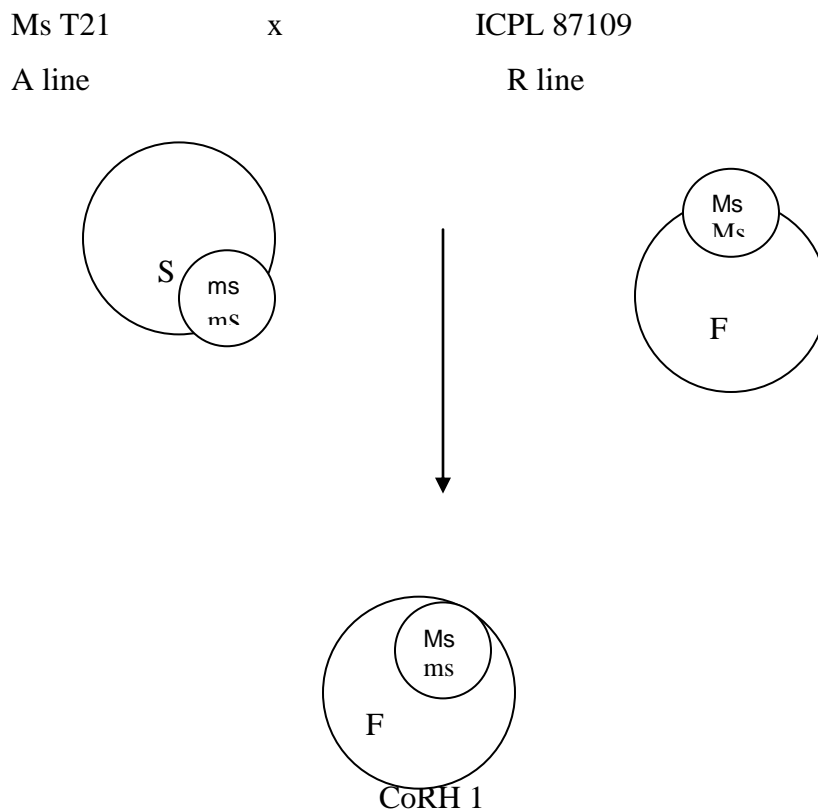




The pollen from the Fertile line will pollinate the sterile line and as a result seed set will be there in the sterile line. These seeds are to be harvested and used for hybrid seed production.

For hybrid seed production, the seeds collected from sterile plants will be grown using double the seed rate since it will segregate in the ratio of 1 fertile : 1 sterile line. At the time of flowering, the fertile line will be identified by yellow plumpy anthers and removed from the field. Only the sterile line will remain in field. These will be pollinated by the R line and the R1 obtained will be hybrid redgram.

**Utilisation: Hybrid development. Eg: Redgram**



**Difficulties in use of Gms**

1. Maintenance of GMS requires skilled labour to identify fertile and sterile line. Labelling is time consuming and costly.
2. In hybrid seed production plot identification of fertile line and removing them is costly.
3. Use of double the seed rate of GMS line is costly
4. In crops like castor high temperature leads to break down of male sterility.

**Transgenic Genetic Male Sterility**

A gene introduced into the genome of an organism by recombinant DNA technology or genetic engineering is called transgene. Many transgenes have been shown to produce genetic male sterility, which is dominant to fertility. Consequently, it is essential to develop effective fertility restoration system if these are to be utilized for hybrid seed production. An effective restoration system is available in at least one case called Barnase or Barstar system.

The Barnase gene of *Bacillus amyloliquefaciens* encodes an RNAs. When Barnase gene is driven by TA 29 promoter, it is expressed only in tapetum cells causing their degeneration. Transgenic tobacco and *Brassica napus* plants expressing Barnase were completely male sterile. Another gene, Barstar, from the same bacterium encodes a protein, which is a highly specific inhibitor of Barnase RNase. Therefore, transgenic plants expressing both Barstar and Barnase are fully male fertile.

The Barnase gene has been tagged with bar gene, which specifies resistance to the herbicide phosphinothricin. This male sterile line is maintained by crossing with a male fertile line. The progeny so obtained contain 1 male sterile : 1 male fertile plants; the latter are easily eliminated at seedling stage by a phosphinothricin spray. The male sterile plants are crossed with the Barstar line to obtain male fertile hybrid progeny. This system of male sterility is yet to be commercially used.

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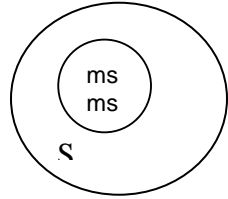
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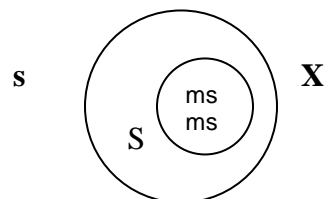
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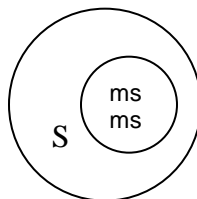
A line

B line



Sterile

Fertile

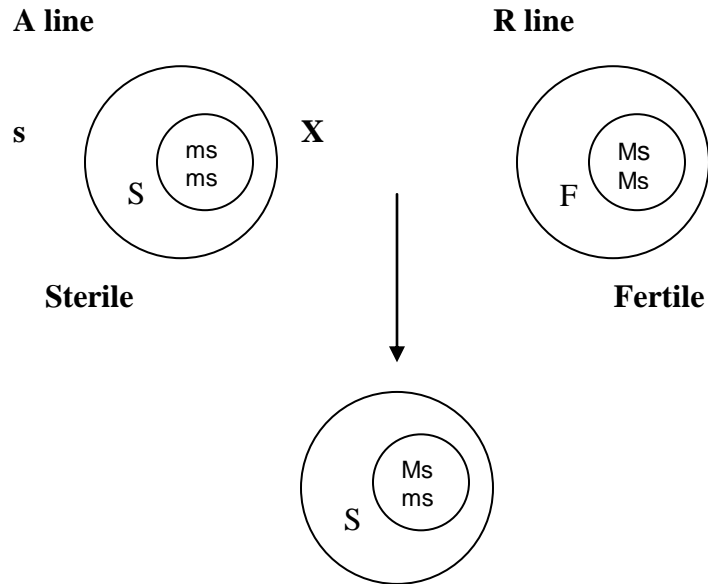


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6. Modifier genes may reduce effectiveness of cytoplasmic male sterility.

## **21. Heterosis, inbreeding depression, various theories of Heterosis**

### **Heterosis and Inbreeding Depression**

Cross pollinated species and species reproducing asexually are highly heterozygous. When these species are subjected to selfing or inbreeding they show severe reduction in vigour and fertility. This phenomenon is known as inbreeding depression.

### **Inbreeding**

It is mating between individuals related by descent or having common ancestry. (Brother - Sister mating or sib mating). The highest degree of inbreeding is obtained by selfing.

### **History of inbreeding**

In breeding depression has been recognised by man for a long time. Knowing the consequences of inbreeding many societies have prohibited marriages between closely related individuals.

Darwin in 1876 published a book "cross and self fertilization in vegetable kingdom" in which he concluded that progenies obtained from self fertilization was weaker in maize. Detailed and precise information on inbreeding in maize was published by East in 1908 and Shull in 1909.

### **Effects of inbreeding**

1. Appearance of lethal and sub lethal alleles: Chlorophyll deficiency, rootless seedlings and other malformations.
2. Reduction in vigour : Appearance of dwarf plants.
3. Reduction in reproductive ability - Less seed set, sterility
4. Segregation of population in distinct lines.
5. Increase in homozygosity
6. Reduction in yield.

### **Degrees of inbreeding depression**

Various plant species exhibit different degrees of inbreeding depression. The depression may be from very high to nil. Based on degree of depression, the plant species can be grouped into 4 broad categories.

#### **1. High inbreeding depression**

Inbreeding leads to severe depression and exhibit lethal effects. After 3 or 4 generations of selfing it is hard to maintain lines. E.g. Lucerne, Carrot.

## 2. Moderate inbreeding depression

Though lethal effects are there, lines can be separated and maintained. E.g. Maize, Jowar, Bajra.

## 3. Low inbreeding depression

Only a small degree of inbreeding depression is observed. E.g. Cucurbits, Sunflower.

## 4. No inbreeding depression

The self-pollinated crops do not show inbreeding depression.

## Heterosis

It is defined as the superiority of  $F_1$  hybrid over both the parents in terms of yield or some other characters. The term heterosis was first used by Shull in 1914.

## Types of heterosis

### 1. Average heterosis

It is the heterosis where  $F_1$  is superior to mid parent value. In other words superior to average of two parents.

i) **Average heterodis:** It is the heterosis where  $F_1$  is superior to mid parent value. In other words superior to average of two parents.

$$\frac{F_1 - MP}{MP} \times 100$$

Where,

$$F_1 = \text{Mean of hybrid}$$

$$MP = \text{Mid parental value}$$

$$\frac{(P_1 + P_2)}{2} \quad \text{Where } P_1 = \text{Parent 1}$$

$$MP = \frac{\text{---}}{2} \quad P_2 = \text{Parent 2}$$

This type of heterosis is of no use in agriculture since the superiority is below the better parent value.

**ii) Heterobeltiosis :** Superiority of  $F_1$  over the better parent.

$$\frac{\frac{\text{---}}{2} - \text{---}}{\text{---}} \times 100$$

BP

Where,

---  
BP = Mean of better parent

**iii) Economic heterosis**

Superiority of the  $F_1$  compared to the high yielding commercial variety in a particular crop.

$$\frac{\frac{\text{---}}{2} - \text{---}}{\text{---}} \times 100$$

CV

Where,

---  
CV = Mean of commercial variety

#### 4. Negative heterosis

Performance of  $F_1$  inferior to better parent / mid parent value. – e.g. Duration

#### Heterosis or hybrid vigour

Hybrid vigour is used as synonym of heterosis. Hybrid vigour refers to superiority of  $F_1$  over better parent. In other words hybrid vigour is manifested effect of heterosis. Thus the term hybrid vigour is used to distinguish the  $F_1$  superiority from negative heterosis.

**Manifestation of heterosis May be in the following form.**

1. Increased yield.
2. Increased reproductive ability.
3. Increase in size and vigour.
4. Better quality
5. Greater adaptability.

**Genetic basis of heterosis**

There are two main theories of heterosis and inbreeding depression.

1. Dominant hypothesis
2. Over dominance hypothesis.

**1. Dominant hypothesis**

First proposed by Davenport in 1908. It was later on expanded by Bruce, Keeble and Pellow. According to this hypothesis at each locus the dominant allele has favourable effect, while the recessive allele has unfavourable effect. In heterozygous state, the deleterious effect of recessive alleles are masked by their dominant alleles. Inbreeding depression is produced by the harmful effects of recessive alleles, which become homozygous due to inbreeding. Two objections have been raised against the dominant hypothesis.

**a) Failure of isolation of inbreds as vigorous as hybrids:**

According to dominance hypothesis it is possible to isolate inbreds with all the dominant genes E.g. AA. This inbreed should be as vigorous as that of hybrid. However in practice such inbreds were not isolated.

**b) Symmetrical distribution in  $F_2$**

In  $F_2$  dominant and recessive characters segregate in the ratio of 3: 1. Quantitative characters, according to dominance hypothesis should not show symmetrical distribution. However,  $F_2$  nearly always show symmetrical distribution.

**Explanation for the two objections**

In 1917 Jones suggested that since quantitative characters are governed by many genes, they are likely to show linkage. In such a case inbreds containing all dominant genes cannot be isolated. So also the symmetrical distribution in  $F_2$  is due to linkage. This explanation is often known as Dominance of Linked Genes Hypothesis.



## **2. Over dominance hypothesis**

This hypothesis was independently proposed by East and Shull in 1908. It is also known as single gene heterosis or super dominance theory. According to this hypothesis, heterozygotes or atleast some of the loci are superior to both the homozygotes. Thus heterozygote Aa would be superior to AA and aa.

In 1936 East proposed that at each locus there are several alleles at  $a_1, a_2, a_3$  &I etc, with increasingly different functions. Heterozygotes between more divergent alleles would be more heterotic E.g.  $a_1$  &I will be superior to  $a_1 a_2$  or  $a_2 a_4$ .

### **Evidences for over dominance**

In maize the maturity genes in heterozygous conditions are superior i.e. Ma ma. The heterozygote Mama is more vigorous than MaMa or mama. The human beings sickle cell anaemia is caused by ss which is lethal. But heterozygote individuals having Ss have advantage of having resistance against malaria compared to SS individuals.

### **Physiological basis of heterosis**

Numerous studies were made to find out the physiological basis of heterosis. Earlier studies were related to embryo size, seed size, growth rates at various stages of development, rates of reproduction.

It was suggested that hybrid vigour was resulted from larger embryo and endosperm size of hybrid seeds. This was clearly demonstrated in certain cases only. In 1952 Whaley has concluded that primary heterotic effect is due to growth regulators and enzymes in the F<sub>1</sub>. But all these studies were highly speculative. There was no evidence to point out clearly the possible reasons for heterozygote advantage.

### **Recent studies about heterosis**

#### **1. Reduced amount of single gene product:**

In certain cases the heterozygote produces an intermediate amount of a gene product, which may lead to increased vigour and growth rate.

AA - more gene product

aa - Less gene product

Aa - Intermediate gene product.

This is seen in case of bread mold.

*Neurospora crassa*.

Gene Pab<sup>+</sup> Produces P. amino benzoic acid.

Gene Pab Produces Less P. amino benzoic acid.

Heterozygote Pab + Pab - Intermediate amount of P amino benzoic acid which leads to faster growth of the fungus.

## **2. Separate gene products**

AA - produce protein

aa - Produce protein which is slightly different.

Aa - will have both the Products.

This may have many advantages by having more adaptiveness. Human beings: SS Resistant to sickle cell anaemia

ss - Susceptible

Ss - Resistant to Sickle cell anaemia + malaria.

## **3. Combined gene product**

Otherwise hybrid product. The hybrid may produce an enzyme molecule which may be somewhat different compared to enzymes produced by homozygotes. Such heterozygote enzymes are termed as *Hybrid Substance* which may be the reason for hybrid vigour.

## **4. Effect in two different tissues**

Both homozygotes may produce high levels of an enzyme in two different tissues. But heterozygote may produce intermediate level. E.g. Maize Adh gene for enzyme alcohol dehydrogenase in seeds.

## **22. Population improvement programmes, recurrent selection, synthetics and composites**

### **Population Approach to Breeding of Self -Pollinated Crops**

Self-fertilization of  $F_1$  hybrids leads to a very rapid increase in homozygosity. After several generations of self-pollination, about 94 per cent of the genes would become homozygous. Even in  $F_2$ , half of the genes are in homozygous state. Thus self fertilization quickly separates the progeny from a hybrid into a large number of purelines. As a consequence, selection in such a segregating population only picks out the genes combinations present in the population primarily as a result of recombination in  $F_2$ .

This reduces the chance of recombination between linked, especially tightly linked genes and of recovery of rare transgressive segregants. There is no opportunity for changing the genotype of the plant produced by recombination in  $F_1$ ,  $F_2$  and to some extent, in  $F_3$ . Thus the two obvious limitations of breeding methods based on self-pollination of the hybrid (e.g., pedigree and bulk methods) are: first, the recombination is limited to two or, at the best, three generations, and second, there is no possibility for further changing the genotype of the segregants.

A population breeding approach has been suggested to overcome these problems. In population breeding, outstanding  $F_2$  plants are mated among themselves in pairs or in some other fashion. The intermating of selected  $F_2$  plants restores heterozygosity in the progeny, which provides for a greater opportunity for recombination. This also brings together the desirable genes from different  $F_2$  plants and would help in the accumulation of favourable genes in the intermated population. Thus the chances of the recovery of transgressive segregants would increase considerably. This process may be repeated one or more times.

This procedure is similar to recurrent selection in cross-pollinated crops. A variation of this approach would be to intermate  $F_3$  or later generation progenies. This would allow a more effective selection of desirable progenies than in the case of  $F_2$  where individual plants have to be selected. As noted previously, selection in  $F_2$  based on individual plants is of little value, particularly for characters like yield. Selection based on  $F_3$  or  $F_4$  progenies would be more desirable. Intermating of selected plants may be continued for two or more generations.

This idea of population approach was first suggested by Palmer in 1953. It is not commonly used at present, but may find a greater application in the future, as improvements due to the pedigree method would become less and less marked. Evidently, the population approach

is akin to recurrent selection commonly used in cross-pollinated crops and often it is referred to as such. The chief limitation of recurrent selection in self-pollinated crops is the difficulty in making the large number of required crosses by hand (emasculation and pollination).

This difficulty may be overcome by using genetic or cytoplasmic male sterility. When genetic male sterility is used, selection is confined to the male sterile ( $ms\ ms$ ) plants in each generation. Seeds from the selected male sterile plants are generally harvested in bulk. The progeny from such plants may be expected to have both male sterile ( $ms\ ms$ ) and male fertile ( $Ms\ ms$ ) plants in almost equal proportion. Further, the seeds produced on the male sterile plants would be produced by pollination by the male fertile plants in the population. Thus the use of male sterility effectively ensures intermating among the plants in the population and eliminates the needs for tedious and time-consuming hand emasculation and pollination.

Results from recurrent selection are available in tobacco and soybean. In tobacco, Matzinger and coworkers selected the plants before flowering and intermated them. A linear response of 4.9 and 7 per cent per cycle to selection for decrease plant height and for increased leaf number, respectively, was obtained for five cycles of selection. Further, there was no evidence for a reduction in variability as a result of the selection. Brim and coworkers carried out six cycles of recurrent selection for increased protein content in two segregating populations of soybean and three cycles of selection for yield and three cycles of selection for high oil content in another segregating population. There was an increase of 0.33 and 0.67 per cent / cycle in protein content of the two populations, of 5.3% per cycle in yield and of 0.3% per cycle in oil content. These findings amply demonstrate the effectiveness of recurrent selection in improving yield and yield traits in self-pollinated crops.

In 1970, Jensen proposed a comprehensive breeding scheme which provides for the three basic functions of a versatile breeding programme. Firstly, it allows the development of  $F_2$ ,  $F_3$  etc. (selfing series) at every stage of the breeding programme, which permits the isolation of purelines for use as commercial varieties. Secondly, it requires intermating among the selected plants/ lines in each stage; the progenies from these intermatings form the basis for the next stage of the selfing series in the breeding programme.

Thus the breeding programme progresses in two different directions: (1) Vertically, through the selfing series leading to the isolation of commercial varieties, and (2) horizontally, through intermating among the selected plant / lines; this generates the recurrent selection series.

Thirdly, new germplasm may be introduced at any stage of the programme by intermating it with some of the selected plants of that stage. This permits the retention and or the creation of large amounts of variability for effective selection through several cycles, and the introduction of new genes in the breeding material, if so desired. This breeding scheme is known as Diallel Selective Mating Scheme (DSM) and is designed to serve both short-term and long-term breeding objectives.

A breeder may create more than one such population for a crop, each population being developed to fulfill a specific objective. This scheme has not been widely used primarily due to the difficulties in making the large number of crosses required in this scheme. Jensen has suggested the use of male sterility to overcome this difficulty in the same way as in the recurrent selection scheme discussed earlier. Further, DSM is much more complicated than the simple pedigree method which still is the favourite breeding method for selfpollinated crops.

#### **Merits of population Approach**

1. The population approach provides for greater opportunities for recombination. This is made possible by restoring heterozygosity through intermating of selected plants.
2. This approach helps in the accumulation of desirable genes in the population. This is also brought about by the intermating of selected plants from segregating generation.

#### **Demerits of Population Approach**

1. The success of this approach depends upon the identification of desirable plants in  $F_2$  and the subsequent segregating generations. This is very difficult, if not impossible, for complex characters like yield which show low heritability. This may be avoided to some extent by using later generation ( $F_3$  or  $F_4$ ) progenies; replicated yield data may also be used.
2. Another draw back of this approach is the intermating of selected plants. This may become a major limitation in some crops because crossing in many self-pollinated species is difficult and time consuming.
3. The time taken to develop a new variety through population approach would be always greater than that by the pedigree method.
4. There is no convincing evidence for the benefits from the population approach. It has been argued that increased recombination may be detrimental, as it would break the

desirable linkage. But such a criticism assumes that all or most of the new gene combinations (recombinations) will be inferior to the existing ones. Such an assumption is not entirely valid since crop improvement is based on the creation of new and desirable gene combinations.

### **Breeding Methods for Cross Pollinated Crops**

Populations of cross pollinated crops are highly heterozygous. When inbreeding is practiced they show severe inbreeding depression. So to avoid inbreeding depression and its undesirable effects, the breeding methods in the crop is designed in such a way that there will be a minimum inbreeding. The breeding methods commonly used in cross pollinated crops may be broadly grouped into two categories.

#### **I. Population improvement**

##### **A. Selection**

- a) Mass selection
- b) Modified mass selection
  - Detasseling
  - Panmixis
  - Stratified or grid or unit selection
  - Contiguous control.

##### **B. Progeny testing and selection**

- a) Half sib family selection
  - i) Ear to row
  - ii) Modified ear to row.
- b) Full sib family selection.
- c) Inbred or selfed family selection.
  - i)  $S_1$  self family selection
  - ii)  $S_2$  self family selection.

##### **C. Recurrent selection**

- a) Simple recurrent selection
- b) Reciprocal recurrent selection for GCA
- c) Reciprocal recurrent selection SCA
- d) Reciprocal recurrent selection.

## **D. Hybrids**

## **E. Synthetics and Composites.**

### **Mass selection**

This is similar to the one, which is practiced, in self-pollinated crops. A number of plants are selected based on their phenotype and open pollinated seed from them are bulked together to raise the next generation. The selection cycle is repeated one or more times to increase the frequency of favourable alleles. Such a selection is known as phenotypic recurrent selection.

### **Merits**

- i) Simple and less time consuming
- ii) Highly effective for character that are easily heritable.

Eg. Plant height, duration.

- iii) It will have high adaptability because the base population is locally adapted one.

### **Demerits**

- 1. Selection is based on phenotype only which is influenced by environment
- 2. The selected plants are pollinated both by superior and inferior pollens present in the population.
- 3. High intensity of selection may lead reduction in population there by leading to inbreeding.

To over come these defects modified mass selection is proposed they are

#### **a) Detasseling**

This is practiced in maize. The inferior plants will be detasseled there by inferior pollen from base population is eliminated.

#### **b) Panmixis**

From the selected plants pollen will be collected and mixed together. This will be used to pollinate the selected plants. This ensures full control on pollen source.

#### **c) Stratified mass selection**

### **Unit selection**

Here the field from which plants are to be selected will be divided into smaller units or plots having 40 to 50 plants / plot. From each plot equal number of plants will be selected.

The seeds from selected plants will be harvested and bulked to raise the next generation, by dividing the field into smaller plots, the environmental variation is minimized. This method is

followed to improve maize crop. It is also known as Grid method of mass selection

## **B) Progeny Testing and Selection**

### **a) Half sib family selection**

Half sibs are those, which have one parent in common. Here only superior progenies are planted and allowed to open pollinate.

#### **1. Ear to row method**

It is the simplest form of progeny selection. Which is extensively used in maize. This method was developed by Hopkins

- a) A number of plants are selected on the basis of their phenotype. They are allowed to open pollinate and seeds are harvested on single plant basis.
- b) A single row of say 50 plants i.e. progeny row is raised from seeds harvested on single plant basis. The progeny rows are evaluated for desirable characters and superior progenies are identified.
- c) Several phenotypically superior plants are selected from progeny rows. There is no control on pollination and plants are permitted to open pollinate.

Though this scheme is simple, there is no control over pollination of selected plants. Inferior pollen may pollinate the plants in the progeny row. To overcome this defect, the following method is suggested.

- a) At the time of harvest of selected plants from base population on single plant basis, part of the seed is reserved.
- b) While raising progeny rows, after reserving part of the seeds, the rest are sown in smaller progeny rows.
- c) Study the performance of progenies in rows and identify the best ones.
- d) After identifying the best progenies, the reserve seeds of the best progenies may be raised in progeny rows.
- e) The progenies will be allowed for open pollination and best ones are selected. There are number of other modifications made in the ear to row selection.

For example,

- i. The selected progenies may be selfed instead of open pollination
- ii. The selected plants may be crossed to a tester parent. The tester parent may be an open



pollinated variety, or inbred

iii. The progeny test may be conducted in replicated trial.

#### **b) Full sib family selection**

Full sibs are those which are produced by mating between selected plants in pairs. Here the progenies will have a common ancestry. The crossed progenies are tested.

$$A \times B \qquad B \times A$$

#### **c) Inbred or selfed family selection**

Families produced by selfing.

##### **S<sub>1</sub> family selection**

Families produced by one generation of selfing. These are used for evaluation and superior families are intermated (Simple recurrent selection).

##### **S<sub>2</sub> family selection**

Families obtained by two generations of selfing and used for evaluation. Superior families are intermated.

#### **Merits of progeny testing and selection**

1. Selection based on progeny test and not on phenotype of individual plants.
2. Inbreeding can be avoided if care is taken raising a larger population for selection.
3. Selection scheme is simple.

#### **Demerits**

1. No control over pollen source. Selection is based only on maternal parent only.
2. Compared to mass selection, the cycle requires 2-3 years which is time consuming.

#### **Recurrent selection**

This is one of the breeding methods followed for the improvement of cross pollinated crop. Here single plants are selected based on their phenotype or by progeny testing. The selected single plants are selfed. In the next generation they are intermated (cross in all possible combinations) to produce population for next cycle of selection.

The recurrent selection schemes are modified forms of progeny selection programmes. The main difference between progeny selection and recurrent selection.

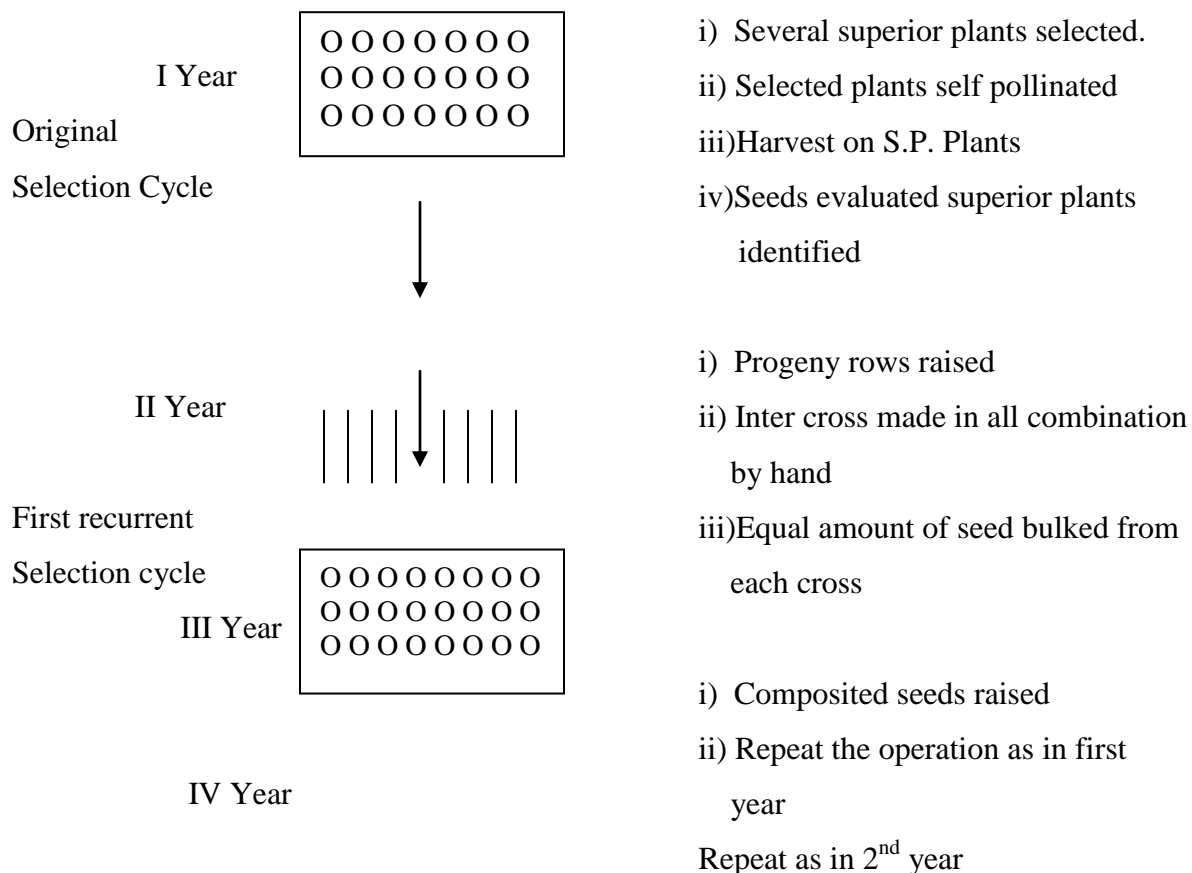
- i) The manner in which progenies are obtained for evaluation.
- ii) Instead of open pollination, making all possible inter crosses among the selected lines.

The recurrent selection schemes are of 4 different types.

### 1. Simple recurrent selection

In this method a number of desirable plants are selected and self pollinated. Separate progeny rows are grown from the selected plants in next generation. The progenies are intercrossed in all possible combination by hand.

Equal amount of seed from each cross is mixed to raise next generation. This completes original selection cycle. From this, several desirable plants are selected and self pollinated. Progeny rows are grown and inter crosses made. Equal amount of seeds are composited to raise next generation. This forms the first recurrent selection cycle.



- Recurrent selection is effective in increasing the frequency of desirable genes in the population
- Most suited for characters having high heritability
- Inbreeding is kept at minimum.

### 2) Recurrent selection for general combining ability

In this case the progenies selected for progeny testing are obtained by crossing the selected plants to a tester parent with broad genetic base.

A tester parent is a common parent mated to a number of lines. Such a set of crosses is used to estimate the combining ability of the selected lines. A tester with broad genetic base means an open pollinated variety, a synthetic variety or segregating generation of a multiple cross.

**Recurrent selection for GCA** can be used for two basically different purposes.

1. It may be used to improve the yielding ability and the agronomic characteristics of a population. In this case the end product will be a synthetic variety.
2. It may be used to concentrate genes for superior GCA. Here the end product will be superior inbreds. Such inbreds can be developed after a few cycles of RSGCA

### **3) Recurrent Selection for Specific Combining Ability**

This is similar to RSGCA except, that in the case of Tester. Here the tester will be an INBRED instead of open pollinated variety. The other operations are similar to RSGCA. The objective of RSSCA is to isolate from population such lines that will combine well with an inbred. These lines are expected to give best hybrids in heterosis breeding.

### **4. Reciprocal recurrent selection**

Proposed by Comstock, Robinson and Harvey. The objective is to improve two different populations in their ability to combine well with each other. In this method we can make selection for both GCA and SCA. Basically two populations A and B are used. Each serve as a tester for the other.

- |                      |   |
|----------------------|---|
| Ist year             | <ol style="list-style-type: none"><li>1. Several plants selected in population A and B.</li><li>2. Selected plants are self pollinated</li><li>3. Selected plant from A is test crossed with plants inB and Vice versa.<br/>Harvest crossed plant on S.P. basis each.</li></ol> |
| 2 <sup>nd</sup> year | <ol style="list-style-type: none"><li>1. Separate yield trials conducted from test cross progenies of A and B</li><li>2. Superior progenies identified</li></ol>  |
| 3 <sup>rd</sup> year | <ol style="list-style-type: none"><li>1. Selfed seed from plants producing superior test cross progenies<br/>planted.</li><li>2. All possible inter crosses made</li><li>3. Seeds harvested and composited</li></ol>  |

4<sup>th</sup> year

5<sup>th</sup> year

6<sup>th</sup> year

### **Use of RRS**

1. Two populations are developed by this method
2. They may be intermated to produce a superior population with broad genetic base. This is similar to a varietal cross but in this case the populations have been subjected to selection for combining ability (GCA and SCA)
3. Inbreds may be developed from populations A and B. These inbreds may be crossed to produce a single cross or double cross hybrids.

## 23. Hybrids

They are the first generation from crosses between two pure lines, inbreds, open pollinated varieties of other populations that are genetically not similar.

Pure line hybrids: Tomato.

Inbred hybrids: Maize, bajra.

### Kinds of hybrids

#### 1. Single cross hybrids

$A \times B$

Crossing two inbreds or pure lines.

#### 2. Three way cross hybrid

$(A \times B) \times C$

A cross between a single cross hybrid and an inbred.

#### 3. Double cross hybrid

$(A \times B) \times (C \times D)$

cross between two  $F_1$ s.

#### 4. Double Top Cross hybrid

Double Cross hybrid crossed with open pollinated variety.

### Operation in production of hybrids.

In production of hybrids inbreds are preferred rather than open pollinated varieties for the following reasons.

1. Inbreds can be maintained without a change in the genotype. Whereas open pollinated variety cannot be maintained pure. They may alter genotypically due to natural selection etc.
2. The hybrids derived from inbreds will be uniform where as it may not be in case, of open pollinated variety.
3. The inbreds are homogenous and their performance can be predicted where as open pollinated variety are heterogenous and their prediction in performance cannot be made.

### Development of inbreds

1. By inbreeding, selfing etc.
2. Development of inbreds from haploids - rice, sorghum, maize.

### Evaluation of inbreds

### a) Phenotypic evaluation

Based on phenotypic performance. Highly suitable for characters with high heritability. .

### b) Top cross test

Top cross test provides a reliable estimate of GCA. The selected inbreds will be crossed to a tester parent with wide genetic base i.e. open pollinated variety. The cross progenies will be evaluated in replicated progeny rows. Based on results better inbreds can be selected.

### c) Single cross evaluation

The developed inbreds can be crossed and the single crosses can be estimated in replicated trial. Outstanding hybrids tested over years in different locations, then released.

### d) Prediction of double cross performance

"The predicted performance of any double cross is the average performance of the four non parental single crosses involving the four parental inbreds".

Inbreds : A, B, C, D.

6 possible single crosses = A x B, A x C, A x D, B x C, B x D, C x D.

From these 3 double crosses produced = (A x B) x (C x D)

(A x C) x (B x D)

(A x D) x (B x C)

The performance of these any one double cross can be predicted from performance of the four single crosses not involved in producing that particular hybrid.

(A x B) x (C x D)

A x C

A x D

B x C

B x D

-----

Average

-----

### Production of Hybrids

#### Methods

I. Hand emasculation and dusting - Cotton, Tomato, Chillies, Bhendi

## 2. Use of male sterile lines

- a) Cytoplasmic male sterility - ornamentals
- b) Genic male sterility - Redgram, Castor.
- c) Cytoplasmic - genic male sterility Jowar, Bajra, Rice

## 3. Use of self in compatibility

By planning cross compatible lines hybrids are produced. Here both are hybrids.

E.g. Brassicas.

### **Success of hybrids**

- a) Easy hand emasculatation
- b) Abundant seed set to compensate cost of hand emasculatation.
- c) Stable male sterile lines.
- d) Effective restorers.
- e) Effective pollen dispersal.

### **Synthetic Varieties**

A synthetic variety is produced by crossing in all combinations a number of inbreds (4-6) that combine well with each other. The inbreds are tested for their GCA. Once synthesised, a synthetic is maintained by open pollination. The lines that make up a synthetic may be usually inbred line but open pollinated variety, or other population tested for general combining ability are also be used.

Synthetic varieties are common in grasses, clover, maize and sugar beets. The normal procedure is equal amounts of seeds from parental lines ( $Syn_0$ ) is mixed and planted in isolation. Open pollination is allowed. The progeny obtained is  $Syn_1$ . This is distributed as synthetic variety or it may be grown in isolation for one more season and  $Syn_2$  is distributed.

### **Merits**

- 1. Less costly compared to hybrids.
- 2. Farmer can maintain his synthetic variety for more seasons which is not possible in hybrids.
- 3. Because of wider genetic base the synthetics are more stable over years and environments.
- 4. Seed production is more skilled operation in hybrids where as it is not so in synthetics.

### **Demerits**

- 1. Performance is little bit lower compared to hybrids because synthetics exploit only

GCA while hybrids exploit both GCA and SCA.

2. The performance may not be good when lines having low GCA are used.

### **Composites**

It is produced by mixing seeds of phenotypically outstanding lines and encouraging open pollination to produce crosses in all possible combinations among mixed lines. The lines used to produce a composite are rarely tested for combining' ability. So the yield of composite varieties cannot be predicted easily. Like synthetics, composites are commercial varieties and are maintained by open pollination.

Composites were released in maize - Amber, Jawahar, Kissan.

#### **Synthetic**

Parental components are generally inbreds tested for their GCA

No of parental lines are limited to 4 - 6 inbreds

Synthetic produced with inbreds can be reconstituted

Yield performance can be predicted

#### **Composite**

It is not so in composite. The lines are not tested for their GCA.

No such limit

It is not possible

Cannot be predicted

### **Poly Cross Test**

This is done to estimate the GCA in crops where production of inbred is not possible. This is followed generally in grasses. Poly cross test is based on seeds obtained by random mating among the clones. Each clone is planted at different date to facilitate random mating. Polycrosses are generally not perfect since mating may not be at random.

### **Combining ability**

Ability of a strain to produce superior progeny when crossed with other strains.

#### **General combining ability**

Average performance of a strain in a series of cross combinations. The GCA is estimated from the performance of  $F_1$  S from the crosses. The tester will have a broad genetic base.

#### **Specific combining ability**

Deviation in performance of a cross combination from that predicted on the basis of general combining ability of the parents involved in the cross. The testing will be on inbred.



## 24. Clonal selection

Some agricultural crops and a large number of horticultural crops are asexually propagated. Some common asexually propagated crops are sugarcane (*S. officinarum*), potato (*S. tuberosum*), sweet potato (*I. batatas*), Colocasia (Taro), Arum, Dioscorea (yams), Mentha, ginger (*Zingiber sp.*), turmeric (*C. domestica*), banana (*Musa paradisiaca*), etc., almost all the fruit trees, e.g., mango (*Mangifera indica*), citrus (*Citrus spp.*), apples (*P. malus*), pears (*P. communis*), peaches (*P. persica*), litchi (*Litchi chinensis*), loquat (*Eriobotrya japonica*), etc., and many ornamentals and grasses. Many of these crops show reduced flowering and seed set, e.g., sugarcane, potato, sweet potato, banana, etc., and some varieties of these crops do not flower at all. But many of these crops flower regularly and show satisfactory seed set. However they are propagated asexually to avoid the ill effects of segregation and recombination, both being the inevitable consequences of sexual reproduction.

Segregation and recombination produce new gene combinations due to which the progeny differ from their parents in genotype and phenotype. Asexual reproduction, on the other hand, produces progeny exactly identical to their parents in genotype because the progeny are derived from vegetative cells through mitosis. The advantage of asexual reproduction is immediately clear. It preserves the genotype of an individual indefinitely. It must be noted that this does not depend on the homozygosity of the genotype of an individual. Any genotype is preserved and maintained through asexual reproduction. In contrast self-pollination preserves and maintains only homozygous genotypes giving rise to purelines.

### Characteristics of Asexually Propagated Crops

- A great majority of them are, perennial, e.g., sugarcane, fruit trees, etc. The annual crops are mostly tuber crops, e.g., potato, cassava (*M. utilissima*), sweet potato, etc.
- Many of them show reduced flowering and seed set. Many varieties do not flower at all. Only the crops grown for fruit, particularly where good fruit set depends upon seed formation, show regular flowering and satisfactory seed set.
- They are invariably cross-pollinated.
- These crops are highly heterozygous and show severe inbreeding depression.
- A vast majority of asexually propagated crops are either polyploids, eg., sugarcane, potato, sweet potato, etc., or have polyploid species or varieties.

- Many species are interspecific hybrid, eg., Banana (*M. paradisiaca*), sugarcane, Rubus, etc.
- These crops consist of a large number of clones, that is, progeny derived from a single plant through asexual reproduction. Thus each variety of an asexually propagated crop is a clone.

## **Clone**

A clone is group of plants produced from a single through asexual reproduction. Thus asexually propagated crops consist of large number of clones, and they are often known as clonal crops. All the members of a clone have the same genotype as the parent plant. As a result, they are identical with each other in genotype. Consequently, the phenotypic differences within a clone do not have a genetic basis and are purely due to the environmental effects. A selection within a clone is thus useless. The various characteristic of a clone are summarised below.

### **Identical Genotype**

All the individuals belonging to a single clone are identical in genotype. This is so because a clone is obtained through asexual reproduction, which involves mitotic cell division only. Genetic variation in the progeny of a plant is produced chiefly by segregation and recombination, which occur during meiosis only. Thus the genotype of a clone is maintained indefinitely without any change.

### **Lack of genetic variation**

The phenotypic variation present within a clone is due to the environment only. This is so because all the individuals belonging to a single clone have the same genotype.

The phenotype of a clone is due to the effects of genotype (G), the environment (E) and the genotype X 'environment interaction (G x E) the population mean. ( $\mu$ ). Thus the phenotype (P) of a clone may be expressed as follows.

$$P = \mu + G + E + GE$$

Thus the phenotypic differences among clones would be partly due to E and GE components. Hence the efficiency of selection among clones, as among purelines, would depend upon the precision with which the E and GE components of phenotype are estimated.

### **Immortality**

Theoretically, clones are immortal i.e., a clone can be maintained indefinitely through asexual reproduction. But clones usually degenerate due to viral or bacterial infection. A clone

may become extinct due to its susceptibility to diseases or insect pests. Further, genetic variation may arise within a clone changing its characteristics.

### **Severe Inbreeding Depression**

Generally, clones are highly heterozygous and show severe loss in vigor due to inbreeding.

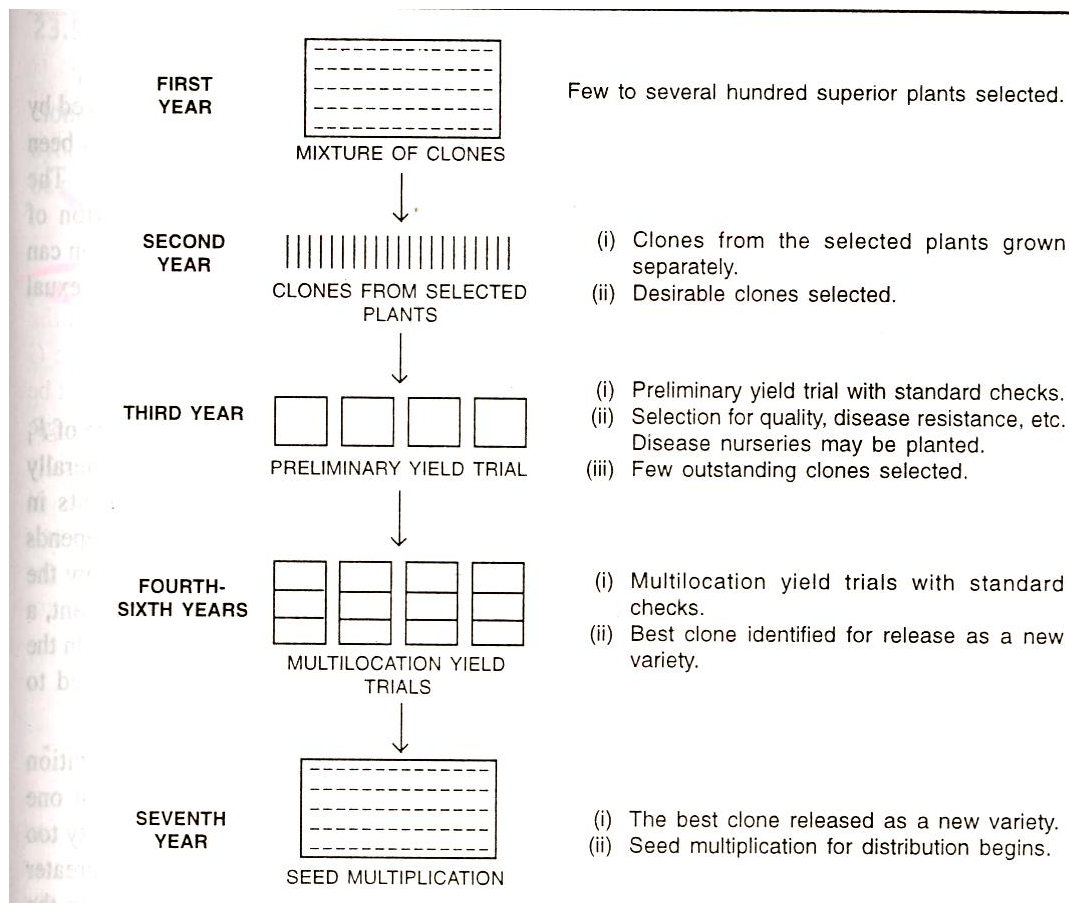
### **Clonal Selection**

The phenotypic value of a plant or clone is due to the effects of its genotype (G), the environment (E) and genotype x environment (G x E) interaction. Of these, only the G effects are heritable. The environmental and interaction effects are non heritable and cannot be selected for. Therefore, a selection for quantitative characters based on observations on single plants is highly unreliable. In fact, plants selected in this way may be no better than a random sample.

Further, a selection for characters like yielding ability, etc. on the basis of unreplicated clonal plots would often be misleading and unreliable. Therefore, the value of a clone can be reliably estimated only through replicated yield trials. However, selection for highly heritable characteristics, such as plant height, days to flowering, color, disease resistance, etc., are easy and effective even on the basis of individual plants or single plots. Clearly, these situations are the same as those in the case of sexually reproducing crops.

### **Selection Procedure**

In view of these considerations, in the earlier stages of clonal selection, when selection is based on single plants or single plots, the emphasis is on the elimination of weak and undesirable plants or clones. The breeder cannot reasonably hope to identify superior' genotypes at this stage. In the later stages, when replicated trials are the basis of selection, the emphasis is to identify and select the superior clones. The various steps involved in clonal selection are briefly described below and are depicted in Fig.



### 1. First Year

From a mixed variable population, few hundreds to few thousand desirable plants are selected. A rigid selection can be done for simply inherited characters with high heritability. Plants with obvious weaknesses are eliminated.

### 2. Second Year

Clones from the selected plants are grown separately, generally, without replication. This is done in view of the limited supply of the propagating material for each clone, and because of the large number of clones involved. The characteristics of clones will be more clear now than in the previous generation when the observations were based on individual plants. The number of clones is drastically reduced and inferior clones eliminated. The selection is based on visual observations and on the basis of clonal characteristics. Fifty to one hundred clones may be selected on the basis of clonal characteristics.

### 3. Third Year

Replicated preliminary yield trial is conducted. Suitable checks included for comparison. Few superior performing clones with desirable characteristics selected for

multilocation trials. At this stage, selection for quality is also done. If necessary, separate disease nurseries may be planted to evaluate the disease resistance of selected clones.

#### **4. Fourth to Sixth Years**

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

#### **5. Seventh Year**

The superior clone is multiplied released as a new variety.

#### **Merits of Clonal Selection**

- It is the only method of selection applicable to clonal crops. It avoids inbreeding depression, and preserves the gene combinations present in the clones.
- Clonal selection, without any substantial modification, can be combined with hybridization to generate the variability necessary for selection.
- The selection scheme is useful in maintaining the purity of clones.

#### **Demerits of Clonal Selection**

- This selection method utilizes the natural variability already present in the population; it has not been devised to generate variability.
- Sexual reproduction is a prerequisite for the creation of variability through hybridization

## **25. Hybridization**

Clonal crops are generally improved by crossing two or more desirable clones, followed by selection in the F1 progeny and in the subsequent clonal generations. Once the F1 has been produced, the breeding procedure is essentially the same as clonal selection. The improvement through hybridization involves the following three steps:

1. Selection of parents,
2. Production of F1 progeny, and
3. Selection of superior clones.

Hybridization can be used only in such crops, which can reproduce sexually. In case of those crops where sexual reproduction is lacking, mutagenesis or biotechnological approaches can be applied.

### **Selection of Parents**

Selection of the parents to be used in hybridization is very important since the value of F1 progeny would depend upon the parents used for producing the F1. Parents are generally selected on the basis of their known performance both as varieties and as parents in hybridization programmes. The performance of a strain in hybridization programmes depends on its prepotency and general combining ability. It would be highly desirable to know the relative values of CGA and SCA in the crop to be improved. If GCA is more important, a small number of parents with good should be used in hybridization programmes. On the other hand, when SCA is more important, a large number of parents should be used to produce a large number of F1 families. In an effort to find some outstanding crosses.

A recent suggestion is to partially inbreed the parents to be used in hybridization programmes. Clonal crops show severe inbreeding depression, but it is expected that one generation of selfing or 2-3 generations of sib-mating may not reduce vigour and fertility too severely. Inbreeding may enable the breeder to identify plants that would have a greater concentration of desirable alleles. These plants may be more prepotent as parents than the highly heterozygous clones. The practice is gaining some favour with plant breeders.

### **Production of F1 progeny**

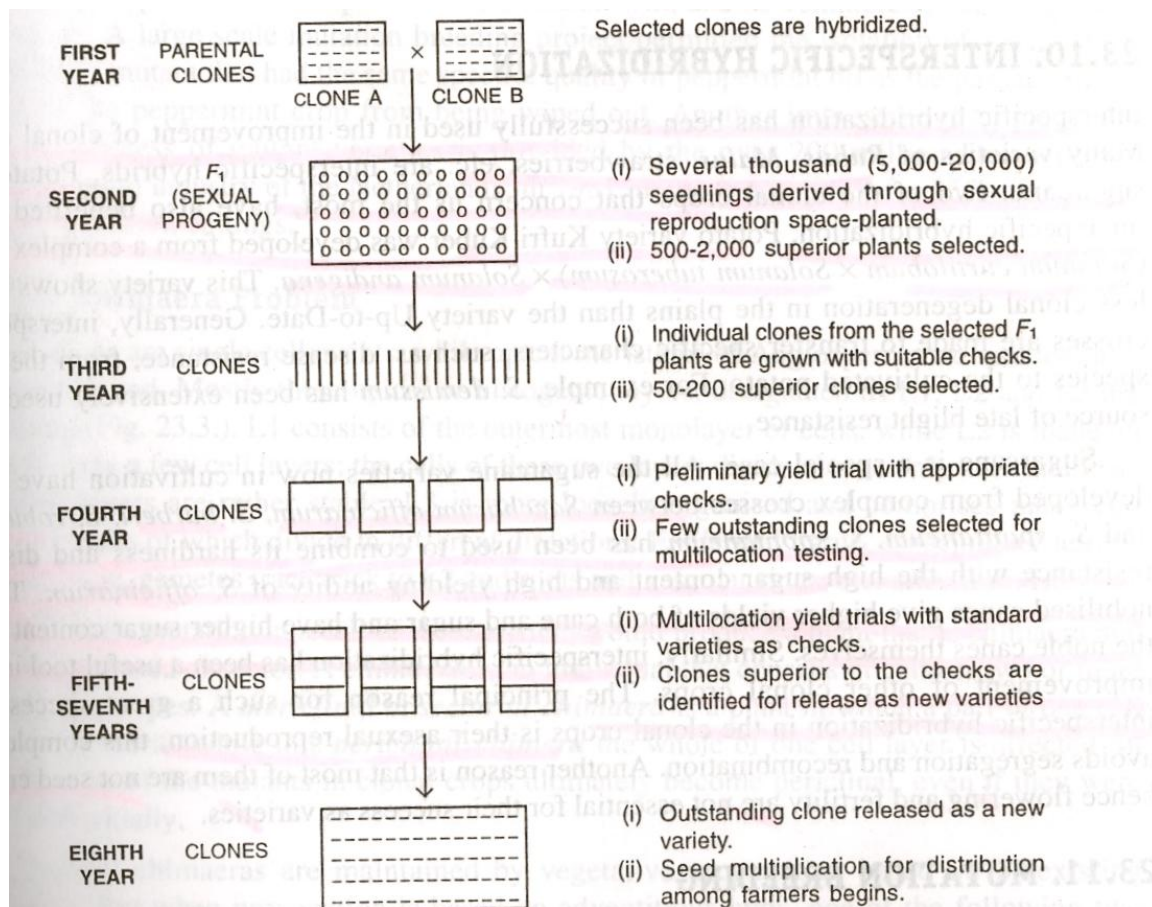
Generally, clonal crops are cross-pollinated and they may show self-incompatibility. The selected parents may be used to produce single crosses involving two parents or an equivalent of a polycross involving more than two parents.

### **Selection among FI Families**

When the breeding value of parents is not known, and the relative contributions of GCA and SCA is not available, a large number of crosses have to be made in order to ensure that at least some of the crosses would produce outstanding progeny in  $F_2$ . This is particularly true in a species where crop improvement has not been done or has been done at a small scale. In such cases, it would be cumbersome to evaluate a large number of  $F_2$  progeny in detail. To avoid this, generally small samples of several  $F_2$  populations are grown. The general worth of individual  $F_2$  populations is estimated visually. The presence of outstanding individuals in the  $F_2$  populations is also noted, and inferior  $F_1$ 's are eliminated. Promising  $F_1$ 's with outstanding individuals are then grown at a much larger scale for selection. The procedure is designed to save time, space and labor by planting only small populations of a large number of crosses at the preliminary stage.

### **Selection within FI Families**

The selection procedure within  $F_2$  populations is essentially the same as that in the case of clonal selection. The various steps involved in the breeding of clonal crops through hybridization are briefly described below. From second year onward, these should be read along with the steps described in clonal selection.



### First Year

Clones to be used as parents are grown and crosses are made to produce  $F_1$  progeny.

### Second Year

Sexual progeny from the cross, i.e., seedlings obtained from seeds, are grown. Undesirable plants are eliminated. Few hundred to few thousand desirable plants are selected.

### Third Year

Clones from the selected individual plants are grown separately. Poor and inferior clones are eliminated. Up to 200 superior clones may be selected for preliminary yield trial.

### Fourth Year

A replicated preliminary yield trial is conducted in which suitable checks are included for comparison. Few outstanding clones are selected for trials at several locations.

### Fifth to seventh year



Replicated yield trials are conducted at several locations. Suitable checks are included for comparison. One or a few outstanding clones are identified and realised as new varieties.

**Eighth year**

The clones released as varieties are multiplied and distributed among farmers.

## 26. Mutation Breeding

Mutation is a sudden heritable change in a characteristic of an organism. This definition requires that the change in the characteristic be heritable, but it does not state the genetic basis of the heritable change. Clearly, a mutation (as defined above) may be the result of a change in a gene, a change in chromosome(s) that involves several genes or a change in a plasmagene (genes present in the cytoplasm, *e.g.*, in chloroplasts, mitochondria, etc., which have circular naked DNA as chromosomes). Mutations produced by changes in the base sequences of genes (as a result of base pair transition or transversion, deletion, duplication or inversion, etc.) are known as gene or point mutations.

Gene mutations can be easily and clearly shown by fine genetic analysis techniques available with microorganisms. Some mutations may be produced by changes in chromosome structure, or even in chromosome number; they are termed as chromosomal mutations. Gross chromosomal changes, *e.g.*, changes in chromosome number, translocations, inversions, large deletions and duplications are detectable cytologically under the microscope. But small deletions and duplications can rarely be detected, and would be considered as gene mutations.

This is particularly so in higher organisms where the techniques of genetic analysis are not yet as refined as those in the case of microorganisms. Thus what we refer to as gene mutation in plants is likely to include a fair number of small chromosomal changes. In clonal crops, mutations may even include gross changes in chromosome structure, sometimes even in number, unless cytological analyses are performed. Therefore, in this chapter, the word mutation would be used without a reference to the change in gene or chromosome (but easily detectable chromosome changes are not included) because in most of the cases the site of change is not known. When the mutant character shows cytoplasmic or extranuclear inheritance, it is known as cytoplasmic or plasmagene mutation. Another term bud mutation or somatic mutation, is used to denote mutations occurring in buds or somatic tissues, which are used for propagation, *e.g.*, in clonal crops.

### SPONTANEOUS AND INDUCED MUTATIONS

Mutations occur in natural populations (without any treatment by man) at a low rate; these are known as spontaneous mutations. The frequency of spontaneous mutations is generally

one in 10 lacs, i.e.,  $10^{-6}$  but different genes may show considerably different mutation rates. For example, R locus in maize mutates at a frequency of  $4.92 \times 10^{-4}$ , Su at  $2.4 \times 10^{-6}$ , while Wx appears to be highly stable. Spontaneous mutation rates of genes may be considerably affected by the genetic background; some mutator genes may promote mutation of other genes. Mutations may be artificially induced by a treatment with certain physical or chemical agents; such mutations are known as induced mutations, and the agents used for producing them are termed as mutagens. The utilization of induced mutations for crop improvement is known as mutation breeding.

Mutation induction rarely produces new alleles; it produces alleles, which are already known to occur spontaneously or may be discovered if an extensive search were made. It is reasonable to say that induced mutations are comparable to spontaneous mutations in their effects and in the variability they produce. But the induced mutations have a great advantage over the spontaneous ones; they occur at a relatively higher frequency so that it is practical to work with them. Mutations have certain general characteristics; those that concern us the most are summarized below.

- Mutations are generally recessive but dominant mutations also occur.
- Mutations are generally harmful to the organism. Most of the mutations have deleterious effects, but a small proportion (Ca 0.1%) of them are beneficial.
- Mutations are random. i.e., they may occur in any gene. However, some genes show higher mutation rates than others.
- Mutations are recurrent that is, the same mutation may occur again and again.
- Induced mutations commonly show pleiotropy, often due to mutations in closely linked genes.

### **Mutagens**

Agents that induce mutations are known as mutagens. Mutagens may be different kinds of radiation (physical mutagens) or certain chemicals (chemical mutagens). The different mutagens may be grouped as follows.

A. Physical mutagens (all of them are various kinds of radiation)

1. Ionizing radiation

a. Particulate radiation. Eg.  $\alpha$  – rays (DI),  $\beta$ - rays (SI), fast neutrons\* (DI), and thermal neutrons (DI)

b. Non Particulate radiation (electromagnetic radiation), eg., X- rays\* (SI), and  $\gamma$ - rays (SI).

2. Non Ionizing radiation. Eg. UV radiation.

#### B. Chemical mutagens

1. Alkylating agents, e.g., sulphur mustards, nitrogen mustards, epoxides, imines, (e.g., ethylene imine or EI)\*, sulphates and sulphonates, diazoalkanes, nitroso compounds, e.g., N-methyl- N- nitro-N-nitroso-guanidine or MNNG).

2. Acridine dyes, e.g., acriflavine, proflavine, acridine orange, acridine yellow, ethidium bromide.

3. Base analogues, e.g., 5- bromouracil, 5-chlorouracil.

4.others, e.g., nitrous acid, hydroxyl amine, sodium azide\*.

(\* denotes that these agents are commonly used in mutation breeding. DI denotes densely ionizing and SI denotes sparsely ionizing radiations.)

#### Beta-Rays

Beta rays are high energy electrons produced by the decay of radioactive isotopes, e.g.,  $^3\text{H}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , etc. High energy electrons are slowed down by positively charged molecules in and they have very little penetrating power as compared to X-rays. Beta-rays transfer energy to electrons of the atoms in their path causing these electrons to fly away from their orbit leaving the nucleus positively charged; this is known as ionisation. When the amount of energy transferred to an electron is not sufficient to cause ionisation, the electron is pushed to an outer orbit representing a higher level of energy, thus producing excitation. Electrons are easily deflected by atoms in their path, hence they move in a zig-zag line. After energy is spent, electrons attach to an atom making it negatively charged. Beta-rays may interact with the nuclei of atoms to produce electromagnetic radiation similar to X-rays. Electrons liberated as a result of ionisation also produce ionisation and excitation, that is, they behave like beta-rays.

#### Alpha-Rays

The alpha particles making up alpha-rays have two protons and two neutrons each; thus the alpha-particles have double positive charge. Alpha-particles are produced by fission of radioactive isotopes of heavier elements. Since they are heavy particles, they move in a straight line. Alpha-particles have a strong attraction for electrons and pull them away from the nuclei of atoms in their path. Alpha-rays produce both ionisation and excitation. After energy, each alpha-particle captures two electrons and produces an atom of helium. As  $\alpha$ -particles move away from

their source, they slow down and produce dense ionisation. Alpha particles are much less penetrating than neutrons and even beta-rays.

### **Fast and Thermal Neutrons**

Fast Neutrons are produced in cyclotrons or atomic reactors as a result of radioactive decay of heavier elements. The velocity of fast neutrons is reduced by graphite or heavy water to generate thermal or slow neutrons. Neutrons are uncharged particles, and are highly penetrating in biological tissues. They are not repelled by nuclei of atoms, and move in a straight line. They do not cause ionisation directly. Ionisation is produced by (1) elastic scattering, in which nuclei, of atoms are kicked away by the neutron; these nuclei then cause ionisation, and (2) production of gamma-rays as thermal neutrons are captured by atomic nuclei, which then become unstable and give off gamma-rays. Fast and thermal neutrons are densely ionising radiations.

### **X-rays and Gamma Rays**

X-rays and gamma-rays are nonparticulate electromagnetic radiation with a wavelength of  $10^{-11}$  to  $10^{-7}$  cm. These are high energy radiation and consist of photons, i.e., small packets of energy. The physical properties and the biological effects of X-rays and gamma-rays are similar, but they differ in the source of their origin. X-rays are produced by X-ray tubes, while gamma-rays are produced by radioactive decay of certain elements, e.g., radium,  $^{14}\text{C}$ ,  $^{60}\text{Co}$ , etc.  $^{60}\text{Co}$  is the common Source of gamma-rays used for biological studies. X-rays are often referred to as hard ( $0.1\text{-}0.001\text{\AA}$ ) or soft ( $10\text{-}1\text{\AA}$ ) depending upon their wavelength. X-rays and gamma-rays are highly penetrating and sparsely ionising. The electromagnetic radiations produce the following effects.

### **Photoelectric Effects**

Low energy photons transfer all their energy to individual electrons, which are kicked off as high energy electrons ( $e^-$ ) from their orbit producing ionisation. These high energy  $e^-$  produce secondary ionisations, which are of greater significance than the primary ones.

### **Compton Scattering**

A high energy photon transfers a part of its energy in kicking away an  $e^-$  of an atom from its orbit producing ionisation. The wavelength of such a photon becomes increasingly longer as it loses energy in repeated ionisations.

### **Pair Production**

A high energy photon passing close to the nucleus of an atom may be completely

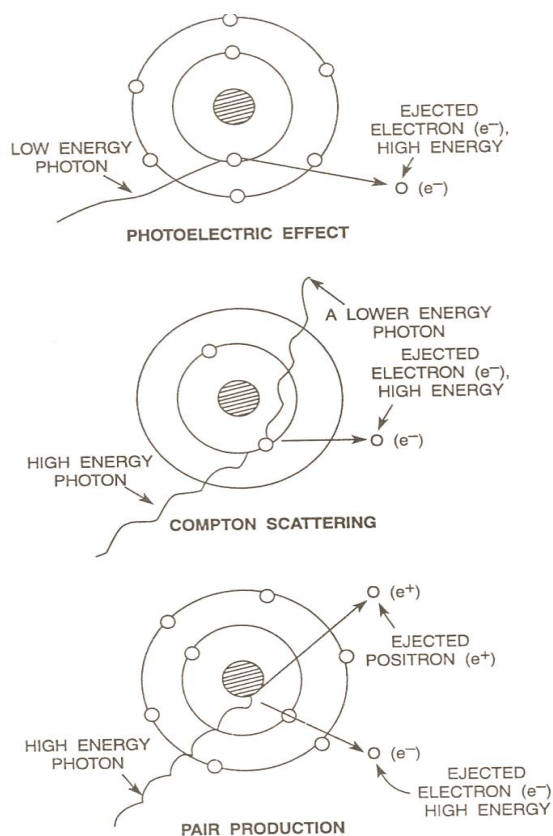
absorbed accompanied with the ejection of a high energy  $e^-$  and a high energy positron ( $e^+$ ); the high energy  $e^-$  and  $e^+$  produce ionisation and excitation.

## Ultraviolet Radiation

Ultraviolet (UV) rays have a wavelength of 100 to 3,900 Å (10-390 nm). UV is present in solar radiation and is produced by mercury vapour lamps or tubes. UV is a low energy radiation; it does not cause ionisation and has a very limited penetrating capacity (usually limited to one or two cell layers). UV rays generally produce dimers of thymine, uracil and, sometimes, cytosine present in the same strand of DNA. It also produces addition of a molecule of water to the 5, 6 double bond of uracil and cytosine, which promotes deamination of cytosine. The mutagenic action of UV is most likely due to dimer formation and deamination. The most effective wavelength of UV is 2,540 Å since DNA bases show the maximum absorption at this wavelength.

UV is commonly used in microorganisms since penetration presents no problem in that system. But in higher organisms, poor penetration of UV has limited its use to

irradiation of pollen grains (in plants) and of small eggs (e.g., in *Drosophila*). In plants, pollen grains may be irradiated and used for pollination. But the difficulty in collecting large quantities of pollen grains in most of the crop species, except in maize and similar crops, and the limited duration of pollen viability have prevented the use of UV in crop improvement.



Ion production by X-ray and gamma-ray photons. The large central solid circle represents the nucleus of an atom, the small open circles on the periphery denote electrons ( $e^-$ ), and the particle  $e^+$  is a positron. The atom pictured here is an oxygen atom.

## **27. PROCEDURE FOR MUTATION BREEDING**

Treating a biological material with a mutagen in order to induce mutations is known as mutagenesis. Exposure of a biological material to a radiation like X rays, gamma - rays, etc. is known as irradiation. When mutations are induced for crop improvement, the entire operation of the induction and isolation, etc. of mutants is termed as mutation breeding. A mutation breeding programme should be clearly planned and should be large enough with sufficient facilities to permit an effective screening of large populations. The various steps involved in mutation breeding are briefly discussed below.

### **Objectives of the Programme**

A mutation breeding programme should have well defined and clear-cut objectives. If the experimenter starts a mutagenesis programme just with the hope that he will discover something useful, he is most likely wasting his time and resources. This is because the ratio of beneficial to useless mutations is very small (1 in 800 mutations, that is, about 0.1 % of the mutations), and identifying desirable mutations from among the undesirable ones is a very difficult task indeed. Further, if a character governed by oligogenes is to be improved, the procedure for the handling of treated populations would be different from that when a polygenic trait is the target for improvement.

### **Selection of the Variety for Mutagen Treatment**

Generally, the variety selected for mutagenesis should be the best variety available in the crop. This is particularly so when polygenic traits are to be improved. It serves no purpose to isolate desirable mutants in a less adapted inferior variety only to discover that the mutant lines have no agricultural worth, or that the mutants have to be used in a hybridization programme for transferring the mutant characteristics to a superior variety. In certain situations; however, it may be desirable to isolate mutants in varieties other than the best one. For example, an extensive search is being made for alternative dwarfing genes in cereals, particularly in wheat and rice (*O. sativa*). In this situation, dwarf and semi dwarf mutants would have to be isolated from tall varieties, which obviously would not be the best varieties of these crops.

### **Part of the Plant to be Treated**

Seeds, pollen grains or vegetative propagules (buds and cuttings) or even complete plants may be used for mutagenesis. Which plant part should be used for mutagen treatment depends primarily on whether the crop is sexually or asexually propagated and on the mutagen to be used.

In sexually propagated crops, seeds are the most commonly used plant part. Dry dormant seeds are biologically almost inert and they can stand a range of extreme environmental conditions, such as, soaking, desiccation, heating, freezing, oxic or anoxic regimes, etc. Mutagenic treatment of seeds is essentially a treatment of embryo meristems .

Since mutation is a single cell event, the M1 plants will carry an induced mutation only in parts of the shoot, i.e., they will be chimaeras. Pollen grains may be used, but they are infrequently used because (1) it is difficult to collect large quantities of pollen grains in most crop species, (2) hand pollination (with treated pollen) is difficult, and (3) pollen life is relatively short. Pollen grains are the only plant part, which can be successfully treated with UV radiation. A pollen monolayer is exposed to UV rays of 250 to 290 nm; of the biological effects induced by UV rays are almost comparable to those produced sparsely ionizing radiation. In case of clonal crops, buds or cuttings are used for mutagenesis.

Radiation (except UV) is suitable for use with all the three plant parts and even with the plants. Whole plants are generally irradiated during the flowering stage so that it is equivalent to the irradiation of pollen grains and egg cells. However the treatment of whole requires special facilities (a gamma garden) and is possible in a few places only. Chemical mutagens are best used with seeds, but some workers have used them with vegetative propagules as well.

### **Dose of the Mutagen**

The usefulness of a mutagen and the type of treatment required to obtain a high efficiency pendent upon specific properties of the mutagenic agent employed (its effectiveness, effect relationship and mode of application) as well as on specific characteristics of the biological system to be treated (the sensitivity of the treated tissues depending upon anatomical, physiological, biochemical and genetic peculiarities). The most appropriate plant or stage to be treated requires a thorough knowledge of the organisms and a clear definition of experimental objectives.

Mutagen treatments reduce germination, growth rate, vigour and fertility (pollen as well lie). There is considerable killing of plants during the various stages of development after mutagen treatment; thus survival is reduced considerably. Mutagens generally induce a high frequency of chromosomal changes and mitotic and meiotic irregularities. Usually, the damage increases with the mutagen dose, but it may not necessarily be proportional. An optimum dose is the one, which produces the maximum frequency of mutations and causes the minimum killing.



The dose required for high mutagenic efficiency depends on the properties of the mutagenic agent, of the solvent medium and of the biological system. Many workers think that a dose close to LD<sub>50</sub> should be the optimum. LD<sub>50</sub> is that dose of a mutagen, which would kill 50 per cent of the treated individuals. LD<sub>50</sub> varies with the crop species and with mutagen used. A preliminary experiment is generally conducted to determine the suitable mutagen dose. In general, an overdose is likely to kill too many treated individuals, while an underdose would produce too few mutations.

Dose of the mutagen may be varied by varying the intensity or the treatment duration. In case of radiation, intensity may be varied by changing the radiation source or by changing the distance from the radiation source of the material being irradiated. Intensity in the case of chemical mutagens may be varied by changing the concentration of mutagens.

### **Giving Mutagen Treatment**

The selected plant part is exposed to the desired mutagen dose. In case of irradiation, the plant parts are immediately planted to raise M<sub>1</sub> plants from them (pollen grains are used for pollination). In case of chemical mutagens, seeds are usually presoaked for a few hours to initiate metabolic activities, exposed to the desired mutagen and then washed in running tap water to remove the mutagen present in them. The treated seeds are, usually, immediately planted in the field to raise the M<sub>1</sub> generation. M<sub>1</sub> is the generation produced directly from the mutagen-treated plant parts without a recourse to sexual or asexual reproduction. But when pollen grains are treated, the generation resulting from the seeds that were produced by pollination with the treated pollen grains would be the M<sub>1</sub> generation. M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub>, etc. are the subsequent generations derived from M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, etc. plants through selfing or clonal propagation.

### **Handling of the Mutagen-Treated Population**

Treatment of seeds and vegetative propagules commonly produces chimaeras. A chimera is an individual with one genotype in some of its parts and another genotype in the others. Shoot-tip meristem usually has three functional layers as follows: (1) L<sub>1</sub> gives rise to epidermis, (2) L<sub>2</sub> produces a part of leaf mesophyll and gametes, and (3) L<sub>3</sub> yields the rest of plant body. When the whole of L<sub>1</sub>, L<sub>2</sub> or L<sub>3</sub> layer is affected, the chimera is known as periclinal chimera, while in a sectorial chimera only a part of L<sub>1</sub>, L<sub>2</sub> or L<sub>3</sub> layer is affected.

In sexually reproducing species, only the L2 chimaera (periclinal or sectorial) will be transmitted to the next generation; other chimaeras will not be recovered since these layers do not contribute to the production of gametes. In clonal crops, however, all chimaeras can be utilized either as periclinal chimaeras or by producing homogeneous individuals through sexual reproduction (only if the L2 layer is affected), tissue culture or certain other horticultural manipulations, e.g., wounding, etc., which induce production of adventitious shoot buds (all chimaeras are utilized). Sectorial chimaeras are unstable in clonal crops and have to be made periclinal through successive clonal propagation and selection for stability.

Mutations usually occur in small sectors of the meristem and, as a result, only a part of the plant is affected. One or more sexual or clonal generations coupled with selection are necessary to obtain a stable mutant phenotype. Mutant alleles are generally recessive, but some dominant mutations may also occur. In case of sexually reproducing crops, mutation breeding utilizes both recessive and dominant mutations and, in addition, excellent opportunities exist for mutation breeding for polygenic traits. Mutation breeding in clonal crops, however, primarily depends on dominant mutations; recessive mutations may also be utilized provided the clone used for mutagen treatment was heterozygous for the gene in question. For example, if recessive mutant allele *a* is to be useful in a clonal crop, the clone used for mutagenesis has to have the genotype *Aa*. Such situations are, however, rare; more frequently, the mutants useful in the improvement of clonal crops contain dominant mutations, and they may even include changes in chromosome structure or even number.

Mutations are called macro or micro-mutations depending on the magnitude of phenotypic effect produced by them. A macromutation produces a large phenotypic effect recognizable on individual plant basis; obviously, such mutations are oligogenic in nature and can be easily selected in the M2 generation. In contrast, a micromutation has a small phenotypic effect that cannot be recognised on individual plant basis; it can be detected only in a group of plants and often statistical treatment of data may be necessary. Obviously, macromutations are polygenic in nature and selection for them is delayed till M3 or a later generation.

The detailed procedure for handling of M2, M3, etc. generations will differ depending mainly on the oligogenic or polygenic trait to be improved and on the mode of reproduction crop species. The following discussion is based on sexually reproducing species, more particularly, self-pollinated species. Since dominant mutations are able to express themselves in heterozygous

state, mutant plants are selected in M1 and often in M 2 and M3, individual plant progenies are raised and homozygous mutants are selected. Selection for recessive mutations, however, can be taken up in M2 only, but the mutant allele will be homozygous in the M2 itself. Selection for polygenic traits is delayed till M3 generation, and is based on individual plant progenies rather than on individual plants. Generalized schemes handling the mutagen treated populations for oligogenic and polygenic traits are outlined in the next section.

## **28. APPLICATIONS OF MUTATION BREEDING**

Mutation breeding has been used for improving both oligogenic as well as polygenic characters. It has been employed to improve morphological and physiological characters, disease resistance and quantitative characters including yielding ability. The various applications of mutation breeding may be briefly summarized as under.

Induction of desirable mutant alleles, which may not be present in the germplasm or which may be present, but may not be available to the breeder due to political or geographical reasons. To some extent, mutation breeding relieves the complete dependence of breeders on the natural germplasm. But it should be remembered that mutation breeding cannot minimise the necessity of germplasm collections; it only serves as a useful supplement to the available germplasm.

It is useful in improving specific characteristics of a well adapted high Yielding variety. This is particularly so in the case clonal crops due to their highly heterozygous nature; in such a case, mutagenesis is the only method available to improve the specific characteristics of clones without changing their genetic make up.

In self-pollinated species, mutagenesis is useful in improving the specific characteristics of otherwise adapted and superior varieties. However, in such species mutagenesis may not be simpler or quicker than the standard backcross procedure if the characteristic is available in a variety. This is more so because the desirable mutations are often associated with undesirable side effects due to other mutations, chromosomal aberrations, sterility, etc. As a result, one or few backcrosses with the parent variety may be necessary to bring the desirable mutant allele in an acceptable genetic background.

Mutagenesis has been successfully used to improve various quantitative characters, including yield. Several varieties have been developed by this technique. However, there is no critical comparison available to show that the same improvement would not have been brought about by the conventional hybridization programmes.

F1 hybrids from intervarietal crosses may be treated with mutagens in order to increase genetic variability by inducing mutations and by facilitating recombination among linked genes. But this method has not been widely used.

Irradiation of interspecific hybrids has been done to produce translocations. This is done to transfer a chromosome segment carrying a desirable gene from the alien chromosome to the chromosome of a cultivated species. This illustrates another application of irradiation in crop

improvement, but this does not constitute mutation breeding.

In developing countries, mutation breeding is widely used, but in Europe it is mainly confined to clonal and ornamental crops. For example, mutagenesis is the principal source of genetic variation in chrysanthemum and banana breeding programmes. This is because most breeders believe that the characteristics of mutation breeding, viz., (1) the need for large ( $10^5$  to  $10^6$ ) M<sub>2</sub> populations, (2) associated detrimental effects of mutations, and (3) the existence in germplasm of the so called 'novel' mutant alleles, mitigate against the incorporation of this technique into conventional breeding programmes. In addition, the yields of new varieties released over a period of years (developed through conventional breeding approaches) show an average increase of -1 % in case the major field crops. Development of a new variety using mutagenesis would require about 7 years; therefore the mutant variety must show an increase of - 7% in yield over the parent variety. An increase of this magnitude is unlikely from modification of a single gene or trait unless it is critical for plant performance, e.g., disease or insect resistance.

#### **LIMITATIONS OF MUTATION BREEDING**

The experience with mutation breeding has brought out certain limitations of the technique; these limitations are summarized as under.

1. The frequency of desirable mutations is very low, about 0.1 per cent of the total mutations. Therefore, large M<sub>2</sub> and subsequent populations have to be grown and carefully studied. This involves considerable time, labour and other resources.
2. The breeder has to screen large populations to select desirable mutations. Therefore, efficient, quick and inexpensive selection techniques are required to screen large populations. Mutation breeding is more easily applied to such characters where quick screening techniques are available, e.g., disease resistance. But in the case of characters where elaborate tests are required, e.g., quality characteristics, mutation breeding is virtually impractical. For this reason, mutation breeding has been more successful with those characteristics where the mutant phenotype is distinct and easily detectable.
3. Desirable mutations are commonly associated with undesirable side effects due to other mutations, chromosomal aberrations, etc. The mutant lines often have to be backcrossed to the respective parent varieties to remove these defects. This increases the time requirement of mutation breeding programmes and involves additional labour, time and

expenditure.

4. Often mutations produce pleiotropic effects. The chief procedure for reducing or eliminating pleiotropic effects is to transfer the gene into different genetic backgrounds by hybridizing the mutant with a randomly selected range of elite varieties. Alternatively, when the pleiotropic effect is on a specific trait, e.g., delayed flowering, appropriate genes for correction of the defect, e.g., genes for early flowering, can be introgressed into the mutant strain.
5. Mutations in quantitative traits are usually in the direction away from the selection history of the parent variety; this conclusion was reached by Brock in 1965 and is generally regarded as valid. This may tend to limit the degree of improvement attainable in a quantitative trait that has been the object of selection for a long period of time, e.g., yield.
6. There may be problems in the registration of a mutant variety since it may be difficult to convincingly demonstrate the new variety to be distinct from the parent variety; the PBR laws, where they exist, require a new variety to be distinct, uniform and stable (the DOS requirement). Such a variety may also attract a royalty liability in case the PBR title of the parent variety was held by another breeder/organization.
7. Most of the mutations are recessive; detection of recessive mutations is almost impossible in clonal crops and is difficult in polyploidy species. Consequently, in polyploidy species, larger population have to be grown and larger doses of mutagens have to be applied. Mutagenesis has been most commonly applied to diploid species that reproduce sexually, more particularly to self pollinated species.

## **ACHIEVEMENTS**

The year 1969 is widely regarded as the year of transition from mainly fundamental investigations to practical mutation breeding; upto this time, only 77 varieties had been developed through mutagenesis. In 1983, this number rose to 337, and by 1989, 1322 mutant varieties had been released. This number rose to 1542 by 1990, and to 1737 by 1992; at this rate, the number would be well over 2,000 by now. Of the 1542 varieties (up to 1990), 1019 varieties were in seed propagated crops (609 direct releases of mutants, and 410 varieties were derived from crosses involving mutants as, at least, one of the parents).

In contrast, only 523 varieties were developed in vegetatively propagated crops. Bulk of

the varieties (1029) were due to direct release of mutants and only a small proportion (Ca. 23%) were obtained by using mutants in hybridization programmes. Among seed propagated crops, the largest number of variety have been developed in rice (278), closely followed by barely (229) and wheat (113), etc. Of these, China has developed the largest number (281) of mutant varieties, followed by India (116), USSR (82) and Japan (65).

## **29. Ploidy breeding**

The mitotic and meiotic divisions are very precise as a result of which the chromosome numbers of different species are highly stable. But a low frequency of irregularities do occur both during mitotic and meiotic divisions. These irregularities give rise to individuals with chromosome numbers different from the normal somatic chromosome number of the concerned species. Changes in chromosome number (some types) have contributed greatly to crop evolution, and (all the types) are of much use in plant breeding. In this chapter, we shall discuss in some detail the types of changes in chromosome number, their characteristics, production and applications in crop improvement.

### **TYPES OF CHANGES IN CHROMOSOME NUMBER**

The Somatic chromosome number of any species, whether diploid or polyploid, 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. Individuals carrying chromosome numbers other than the diploid ( $2x$ , and not  $2n$ ) number are known as heteroploids, and the situation- is known as heteroploidy. The terminology of heteroploidy is summarised in Table.

The change in chromosome number may involve one or a few chromosomes of the genome; this is known as aneuploidy. The aneuploid changes are determined in relation to the somatic chromosome number ( $2n$  and not  $2x$ ) of the species in question. Therefore, the terminology for aneuploid individuals arising from diploid and polyploid species is the same. Heteroploidy that involves one or more complete genomes is known as euploidy. By definition, therefore, the chromosome numbers of euploids are an exact multiple of the basic chromosome number of the concerned species, while those of aneuploids are not.



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**A summary of the terms used to describe heteroploidy**  
**(variation in chromosome number)**

<i>Term</i>	<i>Type of change</i>	<i>Symbol*</i>
<b>Heteroploid</b>	A change from $2x$	—
<b>A. Aneuploid</b>	One or a few chromosomes extra or missing from $2n$	$2n \pm \text{few}$
Nullisomic	One chromosome pair missing	$2n - 2$
Monosomic	One chromosome missing	$2n - 1$
Double monosomic	One chromosome from each of two different chromosome pairs missing	$2n - 1 - 1$
Trisomic	One chromosome extra	$2n + 1$
Double trisomic	One chromosome from each of two different chromosome pairs extra	$2n + 1 + 1$
Tetrasomic	One chromosome pair extra	$2n + 2$
<b>B. Euploid</b>	Number of genomes or copies of a single genome more or less than two	
Monoploid	One copy of a single genome	$x$
Haploid	Gametic chromosome complement	$n$
Polyploid	More than 2 copies of one genome or 2 copies each of 2 or more genomes**	
1. Autopolyploid	Genomes identical with each other	
Autotriploid	Three copies of one genome	$3x$
Autotetraploid	Four copies of one genome	$4x$
Autopentaploid	Five copies of one genome	$5x$
Autohexaploid	Six copies of one genome	$6x$
Autooctaploid	Eight copies of one genome	$8x$
2. Allopolyploid	Two or more distinct genomes (generally each genome has two copies)**	$(2x_1 + 2x_2)^{**}$
Allotetraploid	Two distinct genomes	$(2x_1 + 2x_2 + 2x_3)^{**}$
Allohexaploid	Three distinct genomes	$(2x_1 + 2x_2 + 2x_3 + 2x_4)^{**}$
Allooctaploid	Four distinct genomes	

\* $2n$  = Somatic chromosome number (and complement) and  $n$  = gametic chromosome number (and complement) of the species, whether diploid or polyploid.

$x$  = The basic chromosome number (and complement) or genomic number.

$x_1, x_2, x_3, x_4$  = Distinct genomes from different species.

\*\*In general, this condition occurs; other situations may also occur.

Aneuploid individuals from which one chromosome pair is missing ( $2n - 2$ ) are termed as nullisomic, while those lacking a single chromosome ( $2n - 1$ ) are known as monosomic. A double monosomic individual has two chromosomes missing, but the two chromosomes belong to two different chromosome pairs ( $2n - 1 - 1$ ). An individual having one extra chromosome ( $2n + 1$ ) is known as trisomic, and that having two extra chromosomes each belonging to a different chromosome pair is called double trisomic ( $2n + 1 + 1$ ). When an individual has an extra pair of chromosomes, it is known as tetrasomic ( $2n + 2$ ).

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

polyploid species are identical, it is known as autopolyploid and the situation is termed as autopolyploidy. But in case of allopolyploids, two or more distinct genomes are present. Euploids may have 3 (triploid), 4 (tetraploid), 5 (pentaploid), 6 (hexaploid), 7 (heptaploid), 8 (octaploid) or more genomes making up their somatic chromosome number.

In case of autopolyploidy, they are known as autotriploid, autotetraploid, autopentaploid, autohexaploid, autoheptaploid, auto - octaploid and so on, while in the case of allopolyploidy they are termed as allotriploid, allotetraploid, allopentaploid, allohexaploid, alloheptaploid, allooctaploid, 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.

### **ANEUPLOIDY**

Of the various aneuploids, monosomics (in polyploid species, such as, tobacco, wheat and oats) and trisomics [in diploid species, *e.g.*, *Datura*, maize, bajra, tomato (*L. esculentum*), rye (*S. cereale*), pea (*P. sativum*), spinach (*S. oleracea*), etc.] are the most commonly used in genetic studies. Nullisomics are viable in a few highly polyploid species only, *e.g.*, wheat and oats; they are not viable even in tobacco, which is an allotetraploid.

A trisomic is known as primary trisomic if the extra chromosome is the same as one of the haploid genome, that is, it is not modified. In a secondary trisomic, the additional chromosome is an isochromosome. In an isochromosome, the two arms of the chromosome are identical. A tertiary trisomic has a translocated chromosome as the extra chromosome. For the present, we shall confine ourselves to primary trisomics.

### **Applications in Crop Improvement**

Aneuploids are useful in the studies on effects of loss or gain of an entire chromosome or a chromosome arm on the phenotype of an individual. Their study has clearly demonstrated that character expression is governed by a balance between a large number of genes present in the genome, that is, a *loss* or a *gain* of chromatin upsets the normal development.

Aneuploids are useful in locating a linkage group and a gene to a particular chromosome. B) using a secondary or tertiary trisomic, the gene may be located to one of the two arms of a chromosome, or even to a part of the chromosome arm. The most important application of aneuploids is in locating genes on particular chromosomes; this will be considered in some detail.

Study of aneuploids has shown the homoeology between A, B and D genomes of wheat (*T. aestivum*), since a chromosome of A genome does compensate for the loss of the corresponding chromosome from genome B or D. For example, tetrasomic condition of 2B compensates for the nullisomic condition of 2A or 2D so that a tetra- 2A nulli-2B or 2D appears normal.

Aneuploids are useful in identifying the chromosomes involved in translocations. They are useful in the production of substitution lines. Chromosome substitution may be desirable for studying the effects of individual chromosomes of a variety or for the transfer of the genes carried by specific chromosomes or a variety into another one.

### **Limitations of Aneuploid Analyses**

It is necessary to produce and maintain a complete set of aneuploids. Production, identification and maintenance of aneuploids require elaborate cytogenetic analysis, which is difficult, time consuming and requires considerable skill.

Maintenance of aneuploids is complicated by the phenomenon of univalent shift. Univalent shift denotes that some of the progeny of an aneuploid plant would become aneuploid for a different chromosome as compared to the parent plant. Univalent shift generally occurs in monosomic lines and is a result of univalent formation in a chromosome other than that for which they are monosomic. Therefore, cytological analysis and testing must form an integral part of the aneuploid programmes.

During aneuploid analysis and chromosome substitution, cytological analysis must be carried out for accuracy. This involves a considerable cytological work and makes aneuploid analysis a time consuming and tedious task.

### **MONOPOIDS AND HAPLOIDS**

Monoploids and haploids are weaker than diploids and are of little agricultural value directly. But they are of great interest because they offer certain unique opportunities in crop improvement. (1) They are used for developing homozygous diploid lines, following chromosome doubling in two years. This greatly reduces the time and labor required for the isolation of inbreds and purelines. (2) They may be useful in the isolation of mutants because the mutant allele (even if it is recessive) expresses itself in M, due to a single dose of the gene in somatic tissues. Chromosome number of mutants may be doubled to produce homozygous mutant lines in a single generation. (3) Since desirable gametes are more frequent ( $=p$ , that is, the frequency of desirable allele in the population) than desirable zygotes ( $=p^2$ ), selection based on haploids or doubled

haploids may be expected to be more efficient than that based on diploid (zygote-derived) plants. There is some evidence that this may be so. And (4) in autotetraploids like potato, breeding is relatively much easier at the haploid ( $2x$ ) level than at the tetraploid level ( $4x$ ). For comparison, consider segregation in an autotetraploid and in a diploid. There is an increasing tendency to breed potato varieties at the haploid level and then double their chromosome number to obtain tetraploid varieties.

In monoploids and most haploids, the chromosomes do not pair and their distribution at anaphase I is random leading to an almost complete sterility. Some functional gametes with  $n$  chromosomes may be produced, which may give rise to  $2n$  progeny. Monoploids and haploids occur spontaneously in low frequencies, may be induced from pollen grains or haploid cells of unfertilized ovaries through callus formation or embryo production and by chromosome elimination in certain interspecific crosses, e.g., *Hordeum bulbosum* X *H. vulgare*. In the first method, the recovery of haploids is generally very low (1 in 1,000 plants or lower). But the latter two methods produce a relatively high frequency of haploids in case of those species for which appropriate techniques are available. It may be pointed out that the latter two methods are not applicable to many crop species as yet.

### **AUTOPOLYPLOIDY**

In autopolyploidy are included triploidy, tetraploidy and higher levels of ploidy. Autopolyploids are produced directly or indirectly through chromosome doubling.

### **Origin and Production of Doubled Chromosome Numbers**

Cells/individuals having doubled chromosome numbers may originate in one of the following several ways: (1) spontaneous, (2) due to treatment with physical agents, (3) regeneration in vitro, (4) colchicine treatment, and (5) other chemical agents.

#### **Spontaneous**

Chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are also produced in low frequencies. Production of unreduced gametes is promoted by certain genes, e.g., genes causing complete asynapsis or desynapsis and, more particularly, Such mutant genes as those producing parallel spindle (ps) and omission of second division (os) in potato.

#### **Production of Adventitious Buds**

Decapitation in some plants leads to callus development at the cut end of stem. Such a callus has some polyploid cells, and some of the Shoot buds regenerated from the callus may be

polyploid. This is of common occurrence in Solanaceae where 6-36 per cent of adventitious shoot buds are reported to be tetraploid. The frequency of polyploidy buds may be increased by the application of 1% IAA at the cut ends as it promotes callus development.

### **Physical Agents**

Heat or cold treatments, centrifugation and X-ray or gamma ray irradiation may produce polyploids in low frequencies. Tetraploid branches were produced in *Datura* in response to cold treatment. Exposure of maize plants or ears to a temperature of 38-45°C at the time of the first division of zygote produces 2-5 per cent, tetraploid progeny. Heat treatment has been successfully used in barley, wheat, rye and some other crop species.

### **Regeneration *in Vitro***

Polyploidy is a common feature of the cells cultured *in vitro*. Some of the plants regenerated from callus and suspension cultures may be polyploids. Plants of various ploidy have been regenerated from callus cultures of *Nicotiana*, *Datura*, rice (*D. sativa*) and several other species.

### **Colchicine Treatment**

Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling. It has been used with great success in a large number of crop species belonging to both dicot and monocot groups. Colchicine is a poisonous chemical isolated from seeds (0.2-0.8%) and bulbs (0.1-0.5%) of autumn crocus (*Colchicum autumnale*). It is readily soluble in alcohol, chloroform or cold water, but is relatively less soluble in hot water. Pure colchicine is C<sub>22</sub>H<sub>25</sub>O<sub>6</sub>N. It blocks spindle formation and thus inhibits the movement of sister chromatids to the opposite poles. The resulting restitution nucleus includes all the chromatids; as a result, the chromosome number of the cell is doubled. Since colchicine affects only dividing cells, it should be applied to a shoot-tip meristem only when its cells are actively dividing.

At any given time, only a small proportion of the cells would be in division; therefore, repeated treatments should be given at brief intervals to double the chromosome number in a large number of cells of the shoot apex. The polyploid and diploid cells present in a shoot-tip compete with each other and diploid cells may often out compete the polyploid ones. The degree of competition varies from species to species and even among varieties within species.

## **Applications of Autopolyploidy in Crop Improvement**

Autopolyploidy has found some valuable applications in crop improvement. These are briefly summarised below.

### **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 grown commercially in Japan. They 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 sativus*) seeds. But a few normal sized seeds may occur, which are generally empty. For good fruit setting, pollination is essential. For this purpose, diploid lines are planted in the ratio 1 diploid: 5 triploid plants. There are several problems, viz., genetic instability of 4x lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production (by hand-pollination). Recently, some diploid hybrids of watermelon ('ice-box type') have been developed that produce seedless fruits (all their seeds are like cucumber seeds).

Triploid sugarbeet produce larger roots and more sugar per unit area than do diploids, while tetraploids produce smaller roots and lower yields than diploids. Apparently, 3x is the optimum level of ploidy in sugarbeets. Triploid sugarbeet varieties have been grown commercially in Europe and Japan, but their popularity is declining rapidly. The triploid varieties are mixtures of triploid, diploid and other ploidy level plants. Seed production of triploid sugarbeet is difficult because the beet flower is small. Triploid seed may be produced in one of the following two ways: (1) using 4x plants as female and 2x as male or (2) using 4x as male and 2x as female. The first combination gives lower seed yield but a higher proportion of triploids, while the second gives a higher seed yield but a lower proportion of triploids. Commercial triploid sugarbeet seed is produced by interplanting 4x and 2x lines in the ratio 3: 1, and seeds from both 4x and 2x plants are harvested. This seed consists of about 75% triploid (3x) seeds. Triploid sugarbeet may give 10-

15 per cent higher yields than diploids.

A triploid (3x) clone of tea (*Cameiia assamica*) has been recently released by the Tea Research Association, India for commercial cultivation in the Northern parts of the country. The triploid cultivar, TV29, produces larger shoots and, thereby, biomass, yields more cured leaf per unit area and is more tolerant to drought than the available diploid cultivars. The quality of the triploid clone is comparable to that of diploid cultivars used for making CTC (curl-tear-cut) tea.

### **Tetraploids**

Autotetraploids have been produced in a large number of crop species and have been extensively studied in several cases. Tetraploids may be useful in one of the following ways: (1) in breeding, (2) improving quality, (3) overcoming self incompatibility, (4) making distant crosses and (5) used directly as varieties.

In banana (*M. sapientum*), autotetraploids are inferior to triploids in that they have weaker leaves and increased fertility. But they offer the only available chance of adding disease resistance to commercially successful varieties. In banana, autoteraploids are produced by chance fertilization of an unreduced triploid egg (AAA) by a haploid pollen from a disease resistant diploid parent. A large number of such tetraploids have been produced, but they have not yet gained any commercial success. This is an unusual case where auto tetraploidy is the only practical approach to breeding an otherwise successful triploid crop species.

Some autotetraploids may be superior in some quality characters to their respective diploids, e.g., tetraploid maize has 43% more carotenoid pigment and vitamin A activity than the diploid. Some tetraploids may be more hardy than diploids. However, it is impossible to predict the performance of tetraploids, and a superior diploid may not necessarily produce a superior tetraploid. The tetraploids may be superior, inferior or comparable to the corresponding diploids in quality and hardiness; the actual response has to be determined ex.p-erimentally.

Autotetraploidy is able to overcome self-incompatibility in certain cases, e.g., some genotypes of tobacco and white clover (*Trifolium repens*), Petunia, etc. Certain distant crosses are not successful at the diploid level, but are relatively successful at the autotetraploid level, e.g., 4x *Brassica oleracea* x *B. chinensis* is successful, but when *B. oleracea* is diploid it is unsuccessful. Similarly, autotetraploids of certain Solanum species produce hybrids with *S. tuberosum*, while the diploids do not.

Autotetraploids are larger in size and are more vigorous than diploids. Autotetraploid

varieties of forage crops have been considerably successful. The most successful examples are, tetraploid red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). Other examples are tetraploids of alsika clover (*Trifolium hybridum*, Variety Tetra) and berseem (*Trifolium alexandrinum*, variety Pusa Giant Berseem). Autotetraploid red clover and ryegrass are more vigorous, more digestible and palatable, and have greater resistance to nematodes as compared to the diploids. Autotetraploid turnips (*B. rapa*) and cabbage (*B. oleracea*) are larger in size, but they also have more water content than the diploids; thus they are not commercially attractive. Many ornamentals are autotetraploids. In cases of ornamentals, increased flower size, and longer flowering duration of the tetraploids are desirable.

Pusa Giant Berseem is the first autopolyploid variety released for general cultivation in India. It yielded 20--30 per cent more green fodder than the diploid berseem varieties. Some autotetraploid varieties of medicinal plants have been released and adopted. Variety HMT-1 of *Hyoscyamus niger* is an autotetraploid; it gives 15% more biomass and 36% greater crude drug yield than the diploid parent. Similarly, Sugandha is an autotetraploid variety of vetiver (*Vetiveria zizanoides*); it gives 11 % more oil yield than the control.

In case of crops where seed is the commercial product, autotetraploidy has been much less successful. The chief difficulty is the high sterility and genetic instability of autotetraploids. Fertility can be improved through breeding and selection, but the progress is slow. Autotetraploids have been explored in several crop species but the most successful case that of rye (*S. cereale*) where tetraploid varieties have been released for cultivation (e.g., Double Steel, Tetra Petkus). Other extensive programmes on autotetraploidy are on crops like barley (*H. vulgare*) and jowar (*S. bicolor*) where larger grains, increased protein content and larger yields are the objective. After many years of extensive breeding, some success in achieving these goals has been realized. Based on the experience so far, the following generalizations may be made about autopolyploidy.

- Autopolyploidy is more likely to succeed in species with lower chromosome numbers than in those with higher chromosome numbers.
- Cross-pollinating species are generally more responsive than self pollinating species.
- Crops grown for vegetative parts are more likely to succeed as polyploids than those grown for seeds.

### **Limitations of Autopolyploidy**

The larger size of autopolyploids is generally accompanied with a higher water content. As



a result, autopolyploids of the crop species grown for vegetative parts do not always produce more dry matter than the respective diploids. For example, tetraploid turnip (*B. rapa*) and cabbage (*B. oleracea*) out yield the diploids in fresh weight, but are comparable, or even inferior, to them in terms of dry matter production.

In crop species grown for seed, autopolyploids show high sterility accompanied with poor seed set. Consequently, the larger seed size of autotetraploids does not generally lead to an increased seed yield per unit area. Fertility in autotetraploids can be increased by hybridization and selection at the tetraploid level. But due to the complex segregation in autotetraploids, progress under selection is slow. It would take many years to raise the fertility to acceptable levels.

Triploids cannot be maintained except through clonal propagation. The progeny of triploids and tetraploids are variable in chromosome number since they produce aneuploid gametes as well. Triploids have to be regularly produced by crossing  $4n \times 2n$  plants. Maintenance of tetraploids is somewhat less difficult. Thus genetic instability of autotriploids and autotetraploids makes their maintenance difficult, and commercial seed production presents many problems.

The hope that polyploidy would help to create new agricultural types at will was entirely misplaced. New polyploids (raw polyploids) are always characterized by a few or more undesirable features, e.g., poor strength of stem in grapes, irregular fruit size in watermelons, etc. Thus new polyploids can rarely be used directly in crop production. A considerable improvement through hybridization and selection is essential to remove these defects.

## **ALLOPOLYPLOIDY**

Allopolyploids have genomes from two or more species. Several of our crop plants are allopolyploids. Production of allopolyploids has attracted considerable attention; the aim almost always was the creation of new species. Some success has been obtained as is evident from the emergence of Triticale as a new crop species in some areas, and the promise shown by some other allopolyploids, e.g., *Raphanobrassica* and some allopolyploids of forage grasses.

### **Applications of Allopolyploidy in Crop Improvement**

Allopolyploidy has three major applications in crop improvement: (1) as bridging species in the transfer of character from one species into another. (2) In the production of new crop species, and (3) for widening the genetic base of existing allopolyploid crop species.

### **Utilization as a Bridging Species**

Amphidiploids serve as a bridge in the transfer of characters from one species to a related species, generally from wild species to cultivated species. The use of an amphidiploid as a bridging species becomes necessary when the hybrid between the cultivated species (recipient species) and the wild species (donor species) is sterile. The sterility of F<sub>1</sub> hybrid makes it impossible to cross the recipient species with the F<sub>1</sub> and this does not permit the transfer of characters from the donor to the recipient species. In such cases, chromosome number of the F<sub>1</sub> interspecific hybrid is doubled to produce an amphidiploid, which is a novel species; it will be generally reasonably fertile and can be crossed to the recipient species. Progeny from the cross between the recipient species and the amphidiploid would have the somatic (2n) chromosome complement of the recipient species and one genome from the donor species. As a result, they would be sufficiently fertile to be used in backcross with the recipient species. From such a programme, alien addition and alien substitution lines are recovered, which are used in the transfer of genes, groups of genes or of small chromosome segments to the recipient species.

An example of the use of an amphidiploid as a bridging species is the use of synthetic *N. digluta* (allohexaploid) for the transfer of resistance to tobacco mosaic virus from *N. sylvestris* to *N. tabacum*. The F<sub>1</sub> obtained from the cross *N. tabacum* x *N. sylvestris* is sterile. Chromosome doubling of the F<sub>1</sub> hybrid produces the synthetic allohexaploid *N. digluta*, which is reasonably fertile. *N. digluta* is backcrossed to the recipient species (*N. tabacum*) to produce a pentaploid having the complete somatic (2n) chromosome complement of *N. tabacum* and one genome of *N. sylvestris*. The pentaploid is sufficiently fertile to be backcrossed to *N. tabacum*. In the progeny, *N. tabacum* like plants resistant to tobacco mosaic are reselected and cytologically analysed. From among the backcross progeny, both alien addition and alien substitution lines can be recovered.

Other examples of the use of an amphidiploid as a bridging species are in the cases of transfer of genes from *G. thurberi* to *G. hirsutum* and of chromosomes from *Haynaldia villosa* to *T. aestivum*.

### **Creation of New crop species**

It was once hoped that allopolyploidy would enable man to create new species at will, and that these species would be superior to the existing crop species. This hope was based on the fact that some of the present-day important crop species are allopolyploids, and that the existing as well as new allopolyploids can be synthesized in the same manner as they would have been produced in the nature. Thus it was expected that a duplication of the nature's own methods would lead to the

creation of new and superior crop species as it had occurred in the nature.

This hope, however, did not take into account the following facts. (1) Allopolyploidy itself has not enabled a species to become successful as a crop; in fact, many allopolyploids are weedy wild species, e.g., *S. spontaneum* and *S. robustum* are noxious weeds. (2) The natural allopolyploids have evolved over a long period to achieve their present-day forms. Newly synthesized allopolyploids, therefore, could hardly be expected to become successful as crops. (3) An allopolyploid that would be superior to the existing diploid species would have already been produced and refined by the natural forces. Consequently, the allopolyploids that are not already existing may be expected to be inferior to the diploid species. These present a discouraging picture of the possibilities of using new allopolyploids as crop species, which seems to have been confirmed by the experience with synthetic allopolyploids.

Triticale is the most successful synthetic allopolyploid produced by crossing wheat (tetraploid or hexaploid) with rye. Triticales derived from tetraploid wheats have been the most successful, but those from hexaploid wheats may also become a successful crop species. At present, triticales are being grown commercially in some parts of the world, e.g., in Canada, and the yields of triticales are comparable to those of the best wheat varieties. The desirable features of triticales are that they combine the yielding ability and grain qualities of wheat with the hardiness (tolerance to adverse environment) of rye. But the development of such superior lines of triticales has taken 50 years of intensive research. The newly synthesized triticales were of low yielding ability due to high sterility, poor seed set and poor and variable development of grains.

Triticales also show cytogenetic and genetic instability due to meiotic irregularities and produce some aneuploid progeny. In Sweden, the raw triticales yielded about 50 per cent of the standard varieties of wheat. The yielding ability of triticales increased under selection to about 90 per cent of the yield of wheat varieties in 15 years. Extensive breeding work on triticales is going on at CIMMYT, Mexico. The breeding strategy involves (1) production of a large number of triticales strains using different combinations (varieties as well as species) of wheat and rye, (2) hybridization of these triticales strains among themselves, and (3) improvement of the defects of the triticales through selection. The results from such breeding programmes have been spectacular and have led to the release of several commercial varieties of triticales, which yield as much as the best varieties of common wheat.

Triticale varieties are being cultivated mainly in Poland (the largest area), Germany and

France; the area of cultivation is around 2.6 million hectares with an annual production of ~8 million tons. In India, three varieties of Triticales have been released; these are TL419, TL1210 and DT46 (amber color grains). The chief drawbacks of triticales is their deep grain colour. As a result, TL1210 is mainly grown as a fodder crop in Punjab although its grain yield is comparable to that of the best wheat varieties. Indian breeders have been successful in developing amber coloured triticales by using white-seeded rye as one of the parents of the triticales.

Some other promising allopolyploids are Raphanobrassica, the triploid (AAC) obtained by crossing *B. napus* (AACC) with *B. campestris* (AA), allopolyploid clovers, Festuca-Lolium hybrids and some species hybrids in Rubus and Jute (*Corchorus sp.*).-In Raphanobrassica, the breeding objectives are to combine the hardiness of *B. oleracea* with quick growth and disease resistance of fodder radish. The problems of Raphanobrassica are the same as those of triticales, i.e., low fertility, cytogenetic and genetic instability and leafy rape-like plants that do not produce bulbs. There is evidence that hybridization and selection at the polyploid level would be effective in improving Raphanobrassica.

The amphidiploid *B. napus* (AACC) crosses very easily with *B. campestris* (AA) to produce the triploid (AAC), which has some desirable features. The triploid is produced so easily that it may be used as a hybrid variety, a special case of hybrid varieties produced by crossing two different species. Varalakshmi, a hybrid variety of cotton is also an interspecific hybrid between *G. hirsutum* (American cotton) and *G. barbadense* (Egyptian cotton); several other such hybrid varieties have been released in cotton.

### **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. oleracea*, 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**

- 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.

- Newly synthesized allopolyploids have many defects, low fertility, cytogenetic and genetic instability, other undesirable features, etc.
- The synthetic allopolyploids have to be improved through extensive breeding at the polyploid level. This involves considerable time, labour and other resources.
- Only a small proportion of allopolyploids are promising; a vast majority of them are valueless for agricultural purposes (except for their use as a bridging species). Thus a costly trial and error has to be done before one is likely to come across a promising allopolyploid combination that can be improved through breeding to yield a new crop species.

### **30. Ploidy breeding**

The mitotic and meiotic divisions are very precise as a result of which the chromosome numbers of different species are highly stable. But a low frequency of irregularities do occur both during mitotic and meiotic divisions. These irregularities give rise to individuals with chromosome numbers different from the normal somatic chromosome number of the concerned species. Changes in chromosome number (some types) have contributed greatly to crop evolution, and (all the types) are of much use in plant breeding. In this chapter, we shall discuss in some detail the types of changes in chromosome number, their characteristics, production and applications in crop improvement.

#### **TYPES OF CHANGES IN CHROMOSOME NUMBER**

The Somatic chromosome number of any species, whether diploid or polyploid, 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. Individuals carrying chromosome numbers other than the diploid ( $2x$ , and not  $2n$ ) number are known as heteroploids, and the situation- is known as heteroploidy. The terminology of heteroploidy is summarised in Table.

The change in chromosome number may involve one or a few chromosomes of the genome; this is known as aneuploidy. The aneuploid changes are determined in relation to the somatic chromosome number ( $2n$  and not  $2x$ ) of the species in question. Therefore, the terminology for aneuploid individuals arising from diploid and polyploid species is the same. Heteroploidy that involves one or more complete genomes is known as euploidy. By definition, therefore, the chromosome numbers of euploids are an exact multiple of the basic chromosome number of the concerned species, while those of aneuploids are not.

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**A summary of the terms used to describe heteroploidy  
(variation in chromosome number)**

<i>Term</i>	<i>Type of change</i>	<i>Symbol*</i>
<b>Heteroploid</b>	A change from $2x$	—
<b>A. Aneuploid</b>	One or a few chromosomes extra or missing from $2n$	$2n \pm \text{few}$
Nullisomic	One chromosome pair missing	$2n - 2$
Monosomic	One chromosome missing	$2n - 1$
Double monosomic	One chromosome from each of two different chromosome pairs missing	$2n - 1 - 1$
Trisomic	One chromosome extra	$2n + 1$
Double trisomic	One chromosome from each of two different chromosome pairs extra	$2n + 1 + 1$
Tetrasomic	One chromosome pair extra	$2n + 2$
<b>B. Euploid</b>	Number of genomes or copies of a single genome more or less than two	
Monoploid	One copy of a single genome	$x$
Haploid	Gametic chromosome complement	$n$
Polyploid	More than 2 copies of one genome or 2 copies each of 2 or more genomes**	
1. Autopolyploid	Genomes identical with each other	
Autotriploid	Three copies of one genome	$3x$
Autotetraploid	Four copies of one genome	$4x$
Autopentaploid	Five copies of one genome	$5x$
Autohexaploid	Six copies of one genome	$6x$
Autooctaploid	Eight copies of one genome	$8x$
2. Allopolyploid	Two or more distinct genomes (generally each genome has two copies)**	$(2x_1 + 2x_2)^{**}$
Allotetraploid	Two distinct genomes	$(2x_1 + 2x_2 + 2x_3)^{**}$
Allohexaploid	Three distinct genomes	$(2x_1 + 2x_2 + 2x_3 + 2x_4)^{**}$
Allooctaploid	Four distinct genomes	

\* $2n$  = Somatic chromosome number (and complement) and  $n$  = gametic chromosome number (and complement) of the species, whether diploid or polyploid.

$x$  = The basic chromosome number (and complement) or genomic number.

$x_1, x_2, x_3, x_4$  = Distinct genomes from different species.

\*\*In general, this condition occurs; other situations may also occur.

Aneuploid individuals from which one chromosome pair is missing ( $2n - 2$ ) are termed as nullisomic, while those lacking a single chromosome ( $2n - 1$ ) are known as monosomic. A double monosomic individual has two chromosomes missing, but the two chromosomes belong to two different chromosome pairs ( $2n - 1 - 1$ ). An individual having one extra chromosome ( $2n + 1$ ) is known as trisomic, and that having two extra chromosomes each belonging to a different chromosome pair is called double trisomic ( $2n + 1 + 1$ ). When an individual has an extra pair of chromosomes, it is known as tetrasomic ( $2n + 2$ ).

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

polyploid species are identical, it is known as autopolyploid and the situation is termed as autopolyploidy. But in case of allopolyploids, two or more distinct genomes are present. Euploids may have 3 (triploid), 4 (tetraploid), 5 (pentaploid), 6 (hexaploid), 7 (heptaploid), 8 (octaploid) or more genomes making up their somatic chromosome number.

In case of autopolyploidy, they are known as autotriploid, autotetraploid, autopentaploid, autohexaploid, autoheptaploid, auto - octaploid and so on, while in the case of allopolyploidy they are termed as allotriploid, allotetraploid, allopentaploid, allohexaploid, alloheptaploid, allooctaploid, 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.

### **ANEUPLOIDY**

Of the various aneuploids, monosomics (in polyploid species, such as, tobacco, wheat and oats) and trisomics [in diploid species, *e.g.*, *Datura*, maize, bajra, tomato (*L. esculentum*), rye (*S. cereale*), pea (*P. sativum*), spinach (*S. oleracea*), etc.] are the most commonly used in genetic studies. Nullisomics are viable in a few highly polyploid species only, *e.g.*, wheat and oats; they are not viable even in tobacco, which is an allotetraploid.

A trisomic is known as primary trisomic if the extra chromosome is the same as one of the haploid genome, that is, it is not modified. In a secondary trisomic, the additional chromosome is an isochromosome. In an isochromosome, the two arms of the chromosome are identical. A tertiary trisomic has a translocated chromosome as the extra chromosome. For the present, we shall confine ourselves to primary trisomics.

### **Applications in Crop Improvement**

Aneuploids are useful in the studies on effects of loss or gain of an entire chromosome or a chromosome arm on the phenotype of an individual. Their study has clearly demonstrated that character expression is governed by a balance between a large number of genes present in the genome, that is, a *loss* or a *gain* of chromatin upsets the normal development.

Aneuploids are useful in locating a linkage group and a gene to a particular chromosome. B) using a secondary or tertiary trisomic, the gene may be located to one of the two arms of a chromosome, or even to a part of the chromosome arm. The most important application of aneuploids is in locating genes on particular chromosomes; this will be considered in some detail.



Study of aneuploids has shown the homoeology between A, B and D genomes of wheat (*T. aestivum*), since a chromosome of A genome does compensate for the loss of the corresponding chromosome from genome B or D. For example, tetrasomic condition of 2B compensates for the nullisomic condition of 2A or 2D so that a tetra- 2A nulli-2B or 2D appears normal.

Aneuploids are useful in identifying the chromosomes involved in translocations. They are useful in the production of substitution lines. Chromosome substitution may be desirable for studying the effects of individual chromosomes of a variety or for the transfer of the genes carried by specific chromosomes or a variety into another one.

### **Limitations of Aneuploid Analyses**

It is necessary to produce and maintain a complete set of aneuploids. Production, identification and maintenance of aneuploids require elaborate cytogenetic analysis, which is difficult, time consuming and requires considerable skill.

Maintenance of aneuploids is complicated by the phenomenon of univalent shift. Univalent shift denotes that some of the progeny of an aneuploid plant would become aneuploid for a different chromosome as compared to the parent plant. Univalent shift generally occurs in monosomic lines and is a result of univalent formation in a chromosome other than that for which they are monosomic. Therefore, cytological analysis and testing must form an integral part of the aneuploid programmes.

During aneuploid analysis and chromosome substitution, cytological analysis must be carried out for accuracy. This involves a considerable cytological work and makes aneuploid analysis a time consuming and tedious task.

### **MONOPOLOIDS AND HAPLOIDS**

Monoploids and haploids are weaker than diploids and are of little agricultural value directly. But they are of great interest because they offer certain unique opportunities in crop improvement. (1) They are used for developing homozygous diploid lines, following chromosome doubling in two years. This greatly reduces the time and labor required for the isolation of inbreds and purelines. (2) They may be useful in the isolation of mutants because the mutant allele (even if it is recessive) expresses itself in M, due to a single dose of the gene in somatic tissues. Chromosome number of mutants may be doubled to produce homozygous mutant lines in a single generation. (3) Since desirable gametes are more frequent ( $=p$ , that is, the frequency of desirable allele in the population) than desirable zygotes ( $=p^2$ ), selection based on haploids or doubled

haploids may be expected to be more efficient than that based on diploid (zygote-derived) plants. There is some evidence that this may be so. And (4) in autotetraploids like potato, breeding is relatively much easier at the haploid ( $2x$ ) level than at the tetraploid level ( $4x$ ). For comparison, consider segregation in an autotetraploid and in a diploid. There is an increasing tendency to breed potato varieties at the haploid level and then double their chromosome number to obtain tetraploid varieties.

In monoploids and most haploids, the chromosomes do not pair and their distribution at anaphase I is random leading to an almost complete sterility. Some functional gametes with  $n$  chromosomes may be produced, which may give rise to  $2n$  progeny. Monoploids and haploids occur spontaneously in low frequencies, may be induced from pollen grains or haploid cells of unfertilized ovaries through callus formation or embryo production and by chromosome elimination in certain interspecific crosses, e.g., *Hordeum bulbosum* X *H. vulgare*. In the first method, the recovery of haploids is generally very low (1 in 1,000 plants or lower). But the latter two methods produce a relatively high frequency of haploids in case of those species for which appropriate techniques are available. It may be pointed out that the latter two methods are not applicable to many crop species as yet.

### **AUTOPOLYPLOIDY**

In autopolyploidy are included triploidy, tetraploidy and higher levels of ploidy. Autopolyploids are produced directly or indirectly through chromosome doubling.

### **Origin and Production of Doubled Chromosome Numbers**

Cells/individuals having doubled chromosome numbers may originate in one of the following several ways: (1) spontaneous, (2) due to treatment with physical agents, (3) regeneration in vitro, (4) colchicine treatment, and (5) other chemical agents.

#### **Spontaneous**

Chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are also produced in low frequencies. Production of unreduced gametes is promoted by certain genes, e.g., genes causing complete asynapsis or desynapsis and, more particularly, Such mutant genes as those producing parallel spindle (ps) and omission of second division (os) in potato.

#### **Production of Adventitious Buds**

Decapitation in some plants leads to callus development at the cut end of stem. Such a callus has some polyploid cells, and some of the Shoot buds regenerated from the callus may be

polyploid. This is of common occurrence in Solanaceae where 6-36 per cent of adventitious shoot buds are reported to be tetraploid. The frequency of polyploidy buds may be increased by the application of 1% IAA at the cut ends as it promotes callus development.

### **Physical Agents**

Heat or cold treatments, centrifugation and X-ray or gamma ray irradiation may produce polyploids in low frequencies. Tetraploid branches were produced in *Datura* in response to cold treatment. Exposure of maize plants or ears to a temperature of 38-45°C at the time of the first division of zygote produces 2-5 per cent, tetraploid progeny. Heat treatment has been successfully used in barley, wheat, rye and some other crop species.

### **Regeneration *in Vitro***

Polyploidy is a common feature of the cells cultured *in vitro*. Some of the plants regenerated from callus and suspension cultures may be polyploids. Plants of various ploidy have been regenerated from callus cultures of *Nicotiana*, *Datura*, rice (*D. sativa*) and several other species.

### **Colchicine Treatment**

Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling. It has been used with great success in a large number of crop species belonging to both dicot and monocot groups. Colchicine is a poisonous chemical isolated from seeds (0.2-0.8%) and bulbs (0.1-0.5%) of autumn crocus (*Colchicum autumnale*). It is readily soluble in alcohol, chloroform or cold water, but is relatively less soluble in hot water. Pure colchicine is C<sub>22</sub>H<sub>25</sub>O<sub>6</sub>N. It blocks spindle formation and thus inhibits the movement of sister chromatids to the opposite poles. The resulting restitution nucleus includes all the chromatids; as a result, the chromosome number of the cell is doubled. Since colchicine affects only dividing cells, it should be applied to a shoot-tip meristem only when its cells are actively dividing.

At any given time, only a small proportion of the cells would be in division; therefore, repeated treatments should be given at brief intervals to double the chromosome number in a large number of cells of the shoot apex. The polyploid and diploid cells present in a shoot-tip compete with each other and diploid cells may often out compete the polyploid ones. The degree of competition varies from species to species and even among varieties within species.

## **Applications of Autopolyploidy in Crop Improvement**

Autopolyploidy has found some valuable applications in crop improvement. These are briefly summarised below.

### **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 grown commercially in Japan. They 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 sativus*) seeds. But a few normal sized seeds may occur, which are generally empty. For good fruit setting, pollination is essential. For this purpose, diploid lines are planted in the ratio 1 diploid: 5 triploid plants. There are several problems, viz., genetic instability of 4x lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production (by hand-pollination). Recently, some diploid hybrids of watermelon ('ice-box type') have been developed that produce seedless fruits (all their seeds are like cucumber seeds).

Triploid sugarbeet produce larger roots and more sugar per unit area than do diploids, while tetraploids produce smaller roots and lower yields than diploids. Apparently, 3x is the optimum level of ploidy in sugarbeets. Triploid sugarbeet varieties have been grown commercially in Europe and Japan, but their popularity is declining rapidly. The triploid varieties are mixtures of triploid, diploid and other ploidy level plants. Seed production of triploid sugarbeet is difficult because the beet flower is small. Triploid seed may be produced in one of the following two ways: (1) using 4x plants as female and 2x as male or (2) using 4x as male and 2x as female. The first combination gives lower seed yield but a higher proportion of triploids, while the second gives a higher seed yield but a lower proportion of triploids. Commercial triploid sugarbeet seed is produced by interplanting 4x and 2x lines in the ratio 3: 1, and seeds from both 4x and 2x plants are harvested. This seed consists of about 75% triploid (3x) seeds. Triploid sugarbeet may give 10-

15 per cent higher yields than diploids.

A triploid (3x) clone of tea (*Cameiia assamica*) has been recently released by the Tea Research Association, India for commercial cultivation in the Northern parts of the country. The triploid cultivar, TV29, produces larger shoots and, thereby, biomass, yields more cured leaf per unit area and is more tolerant to drought than the available diploid cultivars. The quality of the triploid clone is comparable to that of diploid cultivars used for making CTC (curl-tear-cut) tea.

### **Tetraploids**

Autotetraploids have been produced in a large number of crop species and have been extensively studied in several cases. Tetraploids may be useful in one of the following ways: (1) in breeding, (2) improving quality, (3) overcoming self incompatibility, (4) making distant crosses and (5) used directly as varieties.

In banana (*M. sapientum*), autotetraploids are inferior to triploids in that they have weaker leaves and increased fertility. But they offer the only available chance of adding disease resistance to commercially successful varieties. In banana, autoteraploids are produced by chance fertilization of an unreduced triploid egg (AAA) by a haploid pollen from a disease resistant diploid parent. A large number of such tetraploids have been produced, but they have not yet gained any commercial success. This is an unusual case where auto tetraploidy is the only practical approach to breeding an otherwise successful triploid crop species.

Some autotetraploids may be superior in some quality characters to their respective diploids, e.g., tetraploid maize has 43% more carotenoid pigment and vitamin A activity than the diploid. Some tetraploids may be more hardy than diploids. However, it is impossible to predict the performance of tetraploids, and a superior diploid may not necessarily produce a superior tetraploid. The tetraploids may be superior, inferior or comparable to the corresponding diploids in quality and hardiness; the actual response has to be determined ex.p-erimentally.

Autotetraploidy is able to overcome self-incompatibility in certain cases, e.g., some genotypes of tobacco and white clover (*Trifolium repens*), Petunia, etc. Certain distant crosses are not successful at the diploid level, but are relatively successful at the autotetraploid level, e.g., 4x *Brassica oleracea* x *B. chinensis* is successful, but when *B. oleracea* is diploid it is unsuccessful. Similarly, autotetraploids of certain Solanum species produce hybrids with *S. tuberosum*, while the diploids do not.

Autotetraploids are larger in size and are more vigorous than diploids. Autotetraploid

varieties of forage crops have been considerably successful. The most successful examples are, tetraploid red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). Other examples are tetraploids of alsika clover (*Trifolium hybridum*, Variety Tetra) and berseem (*Trifolium alexandrinum*, variety Pusa Giant Berseem). Autotetraploid red clover and ryegrass are more vigorous, more digestible and palatable, and have greater resistance to nematodes as compared to the diploids. Autotetraploid turnips (*B. rapa*) and cabbage (*B. oleracea*) are larger in size, but they also have more water content than the diploids; thus they are not commercially attractive. Many ornamentals are autotetraploids. In cases of ornamentals, increased flower size, and longer flowering duration of the tetraploids are desirable.

Pusa Giant Berseem is the first autopolyploid variety released for general cultivation in India. It yielded 20--30 per cent more green fodder than the diploid berseem varieties. Some autotetraploid varieties of medicinal plants have been released and adopted. Variety HMT-1 of *Hyoscyamus niger* is an autotetraploid; it gives 15% more biomass and 36% greater crude drug yield than the diploid parent. Similarly, Sugandha is an autotetraploid variety of vetiver (*Vetiveria zizanoides*); it gives 11 % more oil yield than the control.

In case of crops where seed is the commercial product, autotetraploidy has been much less successful. The chief difficulty is the high sterility and genetic instability of autotetraploids. Fertility can be improved through breeding and selection, but the progress is slow. Autotetraploids have been explored in several crop species but the most successful case that of rye (*S. cereale*) where tetraploid varieties have been released for cultivation (e.g., Double Steel, Tetra Petkus). Other extensive programmes on autotetraploidy are on crops like barley (*H. vulgare*) and jowar (*S. bicolor*) where larger grains, increased protein content and larger yields are the objective. After many years of extensive breeding, some success in achieving these goals has been realized. Based on the experience so far, the following generalizations may be made about autopolyploidy.

- Autopolyploidy is more likely to succeed in species with lower chromosome numbers than in those with higher chromosome numbers.
- Cross-pollinating species are generally more responsive than self pollinating species.
- Crops grown for vegetative parts are more likely to succeed as polyploids than those grown for seeds.

### **Limitations of Autopolyploidy**

The larger size of autopolyploids is generally accompanied with a higher water content. As

a result, autopolyploids of the crop species grown for vegetative parts do not always produce more dry matter than the respective diploids. For example, tetraploid turnip (*B. rapa*) and cabbage (*B. oleracea*) out yield the diploids in fresh weight, but are comparable, or even inferior, to them in terms of dry matter production.

In crop species grown for seed, autopolyploids show high sterility accompanied with poor seed set. Consequently, the larger seed size of autotetraploids does not generally lead to an increased seed yield per unit area. Fertility in autotetraploids can be increased by hybridization and selection at the tetraploid level. But due to the complex segregation in autotetraploids, progress under selection is slow. It would take many years to raise the fertility to acceptable levels.

Triploids cannot be maintained except through clonal propagation. The progeny of triploids and tetraploids are variable in chromosome number since they produce aneuploid gametes as well. Triploids have to be regularly produced by crossing  $4n \times 2n$  plants. Maintenance of tetraploids is somewhat less difficult. Thus genetic instability of autotriploids and autotetraploids makes their maintenance difficult, and commercial seed production presents many problems.

The hope that polyploidy would help to create new agricultural types at will was entirely misplaced. New polyploids (raw polyploids) are always characterized by a few or more undesirable features, e.g., poor strength of stem in grapes, irregular fruit size in watermelons, etc. Thus new polyploids can rarely be used directly in crop production. A considerable improvement through hybridization and selection is essential to remove these defects.

## **ALLOPOLYPLOIDY**

Allopolyploids have genomes from two or more species. Several of our crop plants are allopolyploids. Production of allopolyploids has attracted considerable attention; the aim almost always was the creation of new species. Some success has been obtained as is evident from the emergence of Triticale as a new crop species in some areas, and the promise shown by some other allopolyploids, e.g., *Raphanobrassica* and some allopolyploids of forage grasses.

### **Applications of Allopolyploidy in Crop Improvement**

Allopolyploidy has three major applications in crop improvement: (1) as bridging species in the transfer of character from one species into another. (2) In the production of new crop species, and (3) for widening the genetic base of existing allopolyploid crop species.

### **Utilization as a Bridging Species**

Amphidiploids serve as a bridge in the transfer of characters from one species to a related species, generally from wild species to cultivated species. The use of an amphidiploid as a bridging species becomes necessary when the hybrid between the cultivated species (recipient species) and the wild species (donor species) is sterile. The sterility of F<sub>1</sub> hybrid makes it impossible to cross the recipient species with the F<sub>1</sub> and this does not permit the transfer of characters from the donor to the recipient species. In such cases, chromosome number of the F<sub>1</sub> interspecific hybrid is doubled to produce an amphidiploid, which is a novel species; it will be generally reasonably fertile and can be crossed to the recipient species. Progeny from the cross between the recipient species and the amphidiploid would have the somatic (2n) chromosome complement of the recipient species and one genome from the donor species. As a result, they would be sufficiently fertile to be used in backcross with the recipient species. From such a programme, alien addition and alien substitution lines are recovered, which are used in the transfer of genes, groups of genes or of small chromosome segments to the recipient species.

An example of the use of an amphidiploid as a bridging species is the use of synthetic *N. digluta* (allohexaploid) for the transfer of resistance to tobacco mosaic virus from *N. sylvestris* to *N. tabacum*. The F<sub>1</sub> obtained from the cross *N. tabacum* x *N. sylvestris* is sterile. Chromosome doubling of the F<sub>1</sub> hybrid produces the synthetic allohexaploid *N. digluta*, which is reasonably fertile. *N. digluta* is backcrossed to the recipient species (*N. tabacum*) to produce a pentaploid having the complete somatic (2n) chromosome complement of *N. tabacum* and one genome of *N. sylvestris*. The pentaploid is sufficiently fertile to be backcrossed to *N. tabacum*. In the progeny, *N. tabacum* like plants resistant to tobacco mosaic are selected and cytologically analysed. From among the backcross progeny, both alien addition and alien substitution lines can be recovered.

Other examples of the use of an amphidiploid as a bridging species are in the cases of transfer of genes from *G. thurberi* to *G. hirsutum* and of chromosomes from *Haynaldia villosa* to *T. aestivum*.

### **Creation of New crop species**

It was once hoped that allopolyploidy would enable man to create new species at will, and that these species would be superior to the existing crop species. This hope was based on the fact that some of the present-day important crop species are allopolyploids, and that the existing as well as new allopolyploids can be synthesized in the same manner as they would have been produced in the nature. Thus it was expected that a duplication of the nature's own methods would lead to the



creation of new and superior crop species as it had occurred in the nature.

This hope, however, did not take into account the following facts. (1) Allopolyploidy itself has not enabled a species to become successful as a crop; in fact, many allopolyploids are weedy wild species, e.g., *S. spontaneum* and *S. robustum* are noxious weeds. (2) The natural allopolyploids have evolved over a long period to achieve their present-day forms. Newly synthesized allopolyploids, therefore, could hardly be expected to become successful as crops. (3) An allopolyploid that would be superior to the existing diploid species would have already been produced and refined by the natural forces. Consequently, the allopolyploids that are not already existing may be expected to be inferior to the diploid species. These present a discouraging picture of the possibilities of using new allopolyploids as crop species, which seems to have been confirmed by the experience with synthetic allopolyploids.

Triticale is the most successful synthetic allopolyploid produced by crossing wheat (tetraploid or hexaploid) with rye. Triticales derived from tetraploid wheats have been the most successful, but those from hexaploid wheats may also become a successful crop species. At present, triticales are being grown commercially in some parts of the world, e.g., in Canada, and the yields of triticales are comparable to those of the best wheat varieties. The desirable features of triticales are that they combine the yielding ability and grain qualities of wheat with the hardiness (tolerance to adverse environment) of rye. But the development of such superior lines of triticales has taken 50 years of intensive research. The newly synthesized triticales were of low yielding ability due to high sterility, poor seed set and poor and variable development of grains.

Triticales also show cytogenetic and genetic instability due to meiotic irregularities and produce some aneuploid progeny. In Sweden, the raw triticales yielded about 50 per cent of the standard varieties of wheat. The yielding ability of triticales increased under selection to about 90 per cent of the yield of wheat varieties in 15 years. Extensive breeding work on triticales is going on at CIMMYT, Mexico. The breeding strategy involves (1) production of a large number of triticales strains using different combinations (varieties as well as species) of wheat and rye, (2) hybridization of these triticales strains among themselves, and (3) improvement of the defects of the triticales through selection. The results from such breeding programmes have been spectacular and have led to the release of several commercial varieties of triticales, which yield as much as the best varieties of common wheat.

Triticale varieties are being cultivated mainly in Poland (the largest area), Germany and

France; the area of cultivation is around 2.6 million hectares with an annual production of ~8 million tons. In India, three varieties of Triticales have been released; these are TL419, TL1210 and DT46 (amber color grains). The chief drawbacks of triticales is their deep grain colour. As a result, TL1210 is mainly grown as a fodder crop in Punjab although its grain yield is comparable to that of the best wheat varieties. Indian breeders have been successful in developing amber coloured triticales by using white-seeded rye as one of the parents of the triticales.

Some other promising allopolyploids are Raphanobrassica, the triploid (AAC) obtained by crossing *B. napus* (AACC) with *B. campestris* (AA), allopolyploid clovers, Festuca-Lolium hybrids and some species hybrids in Rubus and Jute (*Corchorus sp.*).-In Raphanobrassica, the breeding objectives are to combine the hardiness of *B. oleracea* with quick growth and disease resistance of fodder radish. The problems of Raphanobrassica are the same as those of triticales, i.e., low fertility, cytogenetic and genetic instability and leafy rape-like plants that do not produce bulbs. There is evidence that hybridization and selection at the polyploid level would be effective in improving Raphanobrassica.

The amphidiploid *B. napus* (AACC) crosses very easily with *B. campestris* (AA) to produce the triploid (AAC), which has some desirable features. The triploid is produced so easily that it may be used as a hybrid variety, a special case of hybrid varieties produced by crossing two different species. Varalakshmi, a hybrid variety of cotton is also an interspecific hybrid between *G. hirsutum* (American cotton) and *G. barbadense* (Egyptian cotton); several other such hybrid varieties have been released in cotton.

### **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. oleracea*, 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**

- 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.

- Newly synthesized allopolyploids have many defects, low fertility, cytogenetic and genetic instability, other undesirable features, etc.
- The synthetic allopolyploids have to be improved through extensive breeding at the polyploid level. This involves considerable time, labour and other resources.
- Only a small proportion of allopolyploids are promising; a vast majority of them are valueless for agricultural purposes (except for their use as a bridging species). Thus a costly trial and error has to be done before one is likely to come across a promising allopolyploid combination that can be improved through breeding to yield a new crop species.