

## **Breeding Methods**

The following are the methods of breeding self-pollinated crop 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

## **Plant introduction**

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**

1. To introduce new plant species there by creating ways to build up new industries.  
E.g. Oil palm
2. To introduce high yielding varieties to increase food production. E.g. Rice and wheat.
3. To enrich the germplasm collection. E.g. Sorghum, Groundnut.
4. 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.
5. 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 travellers, 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 headquarter 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 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.

### **Purpose of plant introduction**

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

#### **To obtain an entirely new crop plant**

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

#### **To serve as new varieties**

Sometimes introductions are directly released as superior commercial varieties. The Mexican semidwarf wheat varieties Sonora 64 and Lerma Rojo, semidwarf rice varieties TN 1, IR-8 and IR-36 are more recent examples of this type.

#### **To be used in crop improvement**

Often the introduced material is used for hybridization with local varieties to develop improved varieties. Pusa Ruby tomato was derived from a cross between Meeruty and Sioux, an introduction from U.S.A.

#### **To save the crop from diseases and pests**

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

#### **For scientific studies**

Collections of plants have been used for studies on biosystematics, evolution and origin of plant species. N.I. Vavilov developed the concept of centres of origin and that of homologous series in variation from the study of a vast collection of plant types.

### **For aesthetic value**

Ornamentals, shrubs and lawn grasses are introduced to satisfy the finer sensibilities of man. These plants are used for decoration and are of great value in social life.

### **Varieties selected from introductions**

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

### **Varieties Developed through Hybridization**

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

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

### **Merits of plant introduction**

1. It provides 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. Factors affecting acclimatization are:

- i. Mode of pollination
- ii. Amount of variability present in original population
- iii. Life cycle of crop plant and
- iv. Mutation

### **SELECTION**

Selection is basic to any crop improvement. Isolation of desirable plant types from the population is known as selection. It is one of the two fundamental steps of any breeding programme viz., 1. creation of variation and 2. Selection. There are two agencies involved in carrying out selection: one is Nature itself (Natural selection) and the other is man artificial selection. Though both may complement each other in some cases, they are mostly opposite in direction since their aims are different under the two conditions (nature and domestication). The effectiveness of selection primarily depends upon the degree to which phenotype reflects the genotype. Before domestication, crop species were subjected to natural selection. The basic for natural selection was adaptation to the prevailing environment. After domestication man has knowingly or unknowingly practiced some selection. Thus, crop species under domestication were exposed to both natural and artificial selection i.e. selection by man. For a long period, natural selection played an important role than selection by man. But in modern plant breeding methods natural selection is of little importance and artificial selection plays an important role.

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

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

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

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

Selection has two basic characteristics *viz.*

1. Selection is effective for heritable differences only,

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

The two basic requirements for selection to operate are:

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

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

### **Mass Selection**

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.

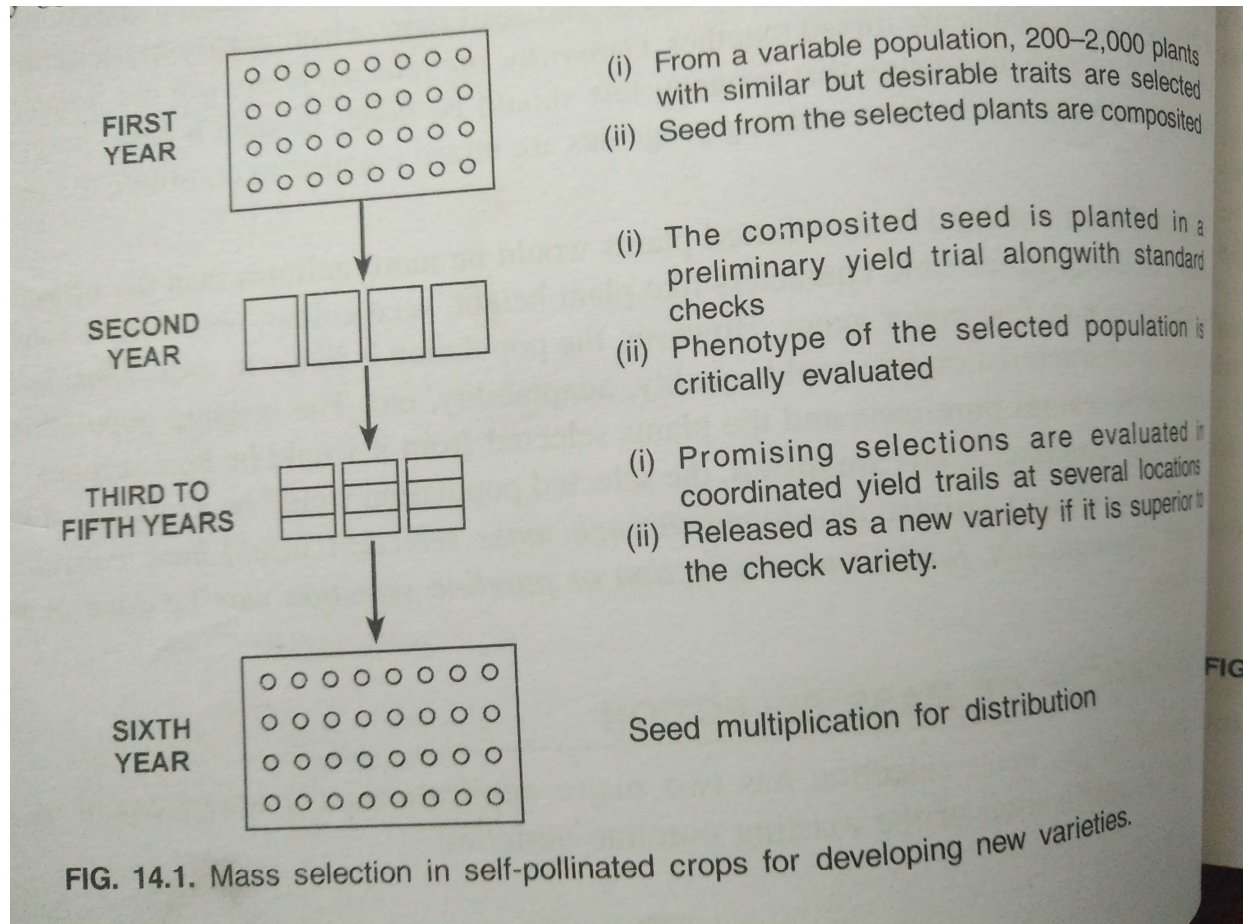
### **Steps:**

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

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

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

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



### Schematic representation of Mass selection

#### Merits of mass selection

1. Since large number of plants are selected, the variety developed has wider adaptability as compared to variety developed through Pureline selection.
2. Often extensive yield trials are not necessary. This reduces the time and cost for developing new variety.
3. Mass selection retain considerable genetic variability in the new variety. Therefore, another round of mass selection after few years would be effective in improving variety further.

4. It is less demanding method so that breeders can devote more time to other breeding programmes.

#### **Demerits of mass selection**

1. The varieties developed through mass selection show variation and are not as uniform as Pureline varieties. Therefore, such varieties are less liked than pureline varieties.
2. Improvement through mass selection is less than that could be achieved through pureline selection, because some progenies will be poorer in performance as compared to the best progeny/pureline present in new variety.
3. In the absence of progeny test it is difficult to find out whether selected plants are homozygous as there may be some degree of cross pollination in self-pollinated crops, some of the selected plants may be heterozygous.
4. Varieties developed through mass selection are more difficult to identify than pureline varieties in seed certification programmes.
5. Mass selection utilizes variability already present in a variety or population, and it can not generate variability.

#### **Pureline theory given by Johannsen (1903)**

- Pureline 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 altogether. He concluded that the market lot of the beans is a mixture of purelines. He also concluded whatever variation observed with in a line is due to environment only.
- Confirmatory evidence was obtained in three ways. In line 13 which is having 450 mg seed weight 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. did not change further. Thus, it was proved that selection within a Pureline is ineffective. In third evidence when parent - offspring regression was worked in line thirteen found be to zero indicating that variation observed is non heritable and it is due to environment only.

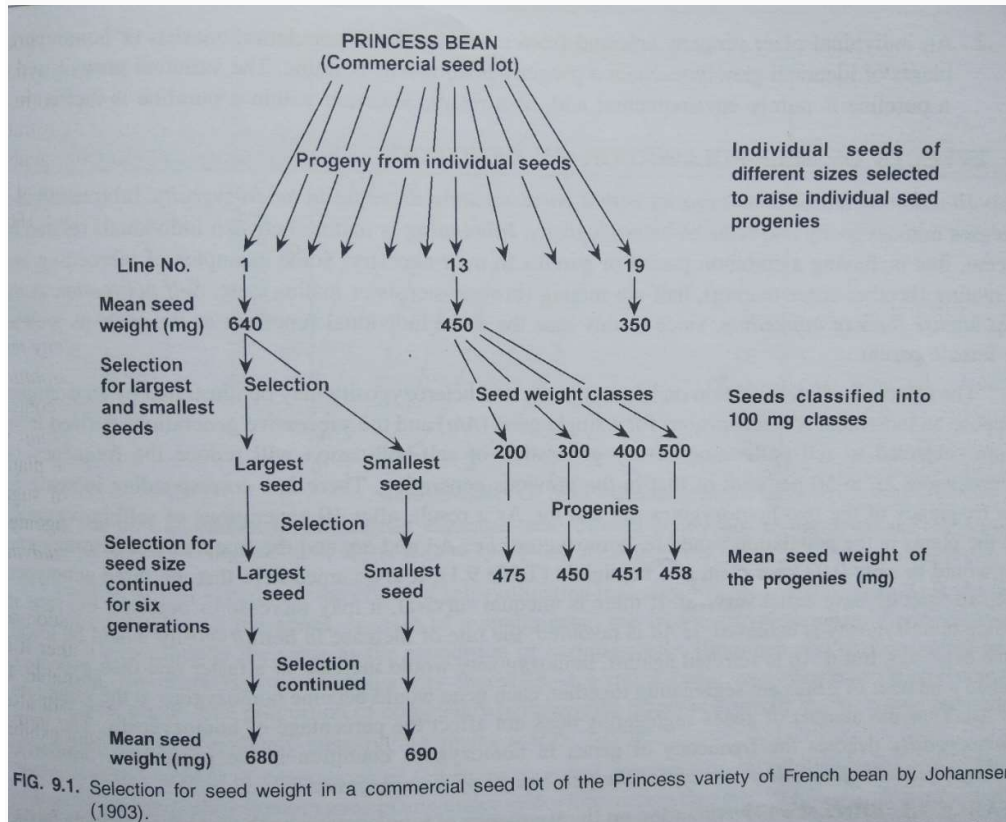


FIG. 9.1. Selection for seed weight in a commercial seed lot of the Princess variety of French bean by Johannsen (1903).

### Confirmatory evidence was obtained in three ways

In the first case, he classified the seed from each pureline into 100 mg classes, and grew them separately. The mean seed weight of progenies from different seed weight class of a single pure line were comparable with each other, and with that of the parent pureline. For example, line no 13 had seed size classes of 200, 300, 400, and 500 mg. The mean seed weights of the progenies derived from these seed weight classes were 475, 450, 451 and 458 mg respectively. The second line of evidence came from selection within a pureline. From each pureline, the largest and the smallest seeds were selected to raise the next generation. In the subsequent generations, large seeds were selected in the progenies obtained from large seeds while in these

from small seeds selection was done from small seeds. Six generations of selection was ineffective in increasing or decreasing the seed size. For example, after 6 generations of selection, the mean seed weight in Line No 1 was 690 and 680 mg in the progenies selected for small and large seeds respectively. Thus, selection within a pureline was ineffective.

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

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

**The two main conclusions from the Johannsen's experiment are:**

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

**Origin of variation in pure lines**

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

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

**Characteristics of purelines**

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

**Progeny test**

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

Isolation principle. Vilmorin worked on sugar beet plants. The progeny test serves two valuable functions:

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

### **Pureline selection**

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

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

### **Procedure of Pureline selection**

#### **First year:**

- An old variety or landrace is used for Pureline selection. Population they selected for pureline selection is homozygous. Single plant is selected and harvested separately superior plants must be selected from the mixed population. About 1000-2000 plants are selected depending on the available resources.

#### **Second year:**

- The individual progenies are grown separately with proper spacing the top 15-20 progenies are selected and they are bulked. Poor, defective, weak and segregating progenies are discarded. Selection should be based on simply inherited character like plant type, Plant height, grain type, flowering and maturity duration disease resistance this process may be repeated

#### **Third year:**

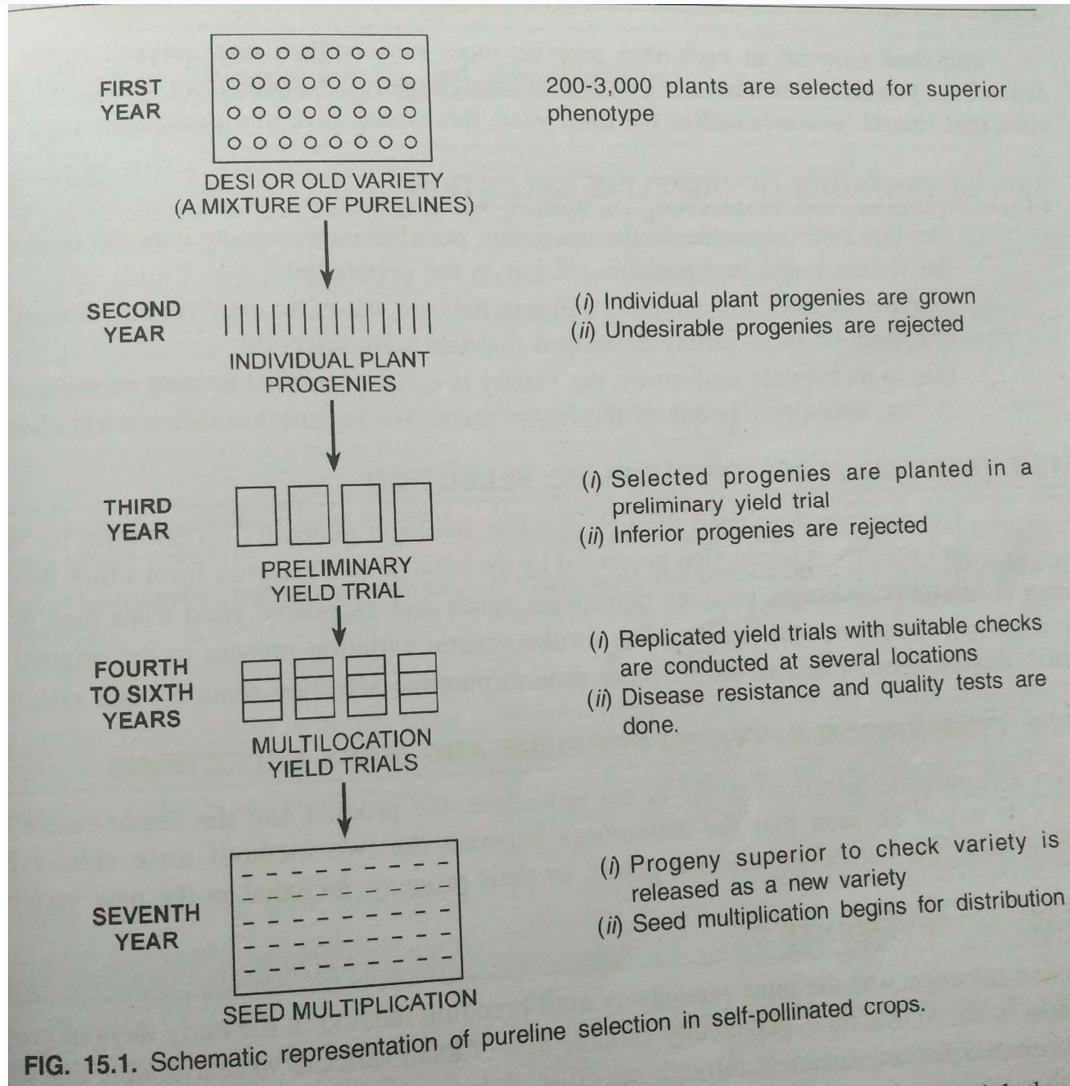
- Seed of the individual plant progenies are not enough to conduct a replication trial. So, they are grown in unreplicated trial with check. Here yield of progenies are taken as a criteria for selection.

#### **Fourth year:**

- Replicated yield trials are conducted using the best available check variety. This may be repeated for 2-3 year. All the observations are recorded

#### **Fifth to Eighth year:**

- Promising strains are evaluated at several locations along with strains or check. The best progeny / strain is released as a new variety and its seed multiplication is initiated for distributed to the farmer.



**Advantages:**

- Maximum possible improvement over original variety.
- Pureline varieties are extremely uniform and therefore, more preferred by farmers and consumers
- Due to uniformity, the variety is easily identified in seed certification programmes.

**Disadvantages:**

- This method can isolate only superior genotypes, it cannot create new genotypes. And not applicable in cross pollinated crop.

- Poor adaptability due to narrow genetic base vulnerable for new diseases and pests.
- Pureline selection requires more time, space and expensive yield trials than mass selection.
- Improvement is dependent on genetic variation present in the original population.
- The breeder has to devote more time to pureline selection than mass selection.

### Comparison between pureline and mass selection

	Pure line selection	Mass selection
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 pureline 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 pureline.
5	Generally, a pureline variety is expected to have narrower adaptation and lower stability in performance than a mixture of purelines.	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.

### Hybridization

- Natural variability in self-pollinated population is exhausted during selection, for further improvements new genetic variability has to be created by crossing two different

pure lines. Hybridization means the mating or crossing of two plants or lines of dissimilar genotypes.

- The seeds as well as the progeny resulting from the hybridization are known as hybrid or F<sub>1</sub>. The progeny of F<sub>1</sub> obtained by self or inter mating of F<sub>1</sub> plants and the subsequent generations are called segregating generations. Today hybridization is the most common method of crop improvement and the majority of the crop varieties have originated from hybridization.
- One of the objectives of hybridization is to create genetic variation. Two genotypically different plants are crossed together to obtain F<sub>1</sub> generation. F<sub>1</sub> is advanced to generate F<sub>2</sub> generation. The degree of genetic variation in F<sub>2</sub> and subsequent generation depend on number of heterozygous genes in F<sub>1</sub>.

### **Aims of hybridization**

1. To transfer of one or few qualitative characters.
2. Improvement in one or more quantitative character.
3. F<sub>1</sub> Hybrid as variety.

#### **I. Combination breeding:**

This method is used for the transfer of one or more character into or single variety from another variety. Eg: improving the yield by correcting the defect. i.e. disease resistance. The other parent selected for hybridization must have a sufficient intensity of a character under transfer.

#### **II. Transgressive breeding:**

It aims at improving yield or its contributing character through transgressive segregation. It refers to the appearance of such plants in F<sub>2</sub> generation that are superior to both the parents for one or more character. It is due to accumulation of plus or favourable genes from the parents as a consequence of recombination. The parents used for crossing must combine very well and are genetically diverse. So, pedigree breeding followed by population approach are designed for production of transgressive segregants.

#### **III. Hybrid varieties:**

In self-pollinated crops F<sub>1</sub> is more vigorous and high yielding than the parents. Two parents should combine well to produce outstanding F<sub>1</sub> hybrid.

### **Types of hybridization**

- Inter-varietal Hybridization / Intra specific: Parents involved in hybridization belong to the same species. They may be two strains, varieties or races.
- Varietal crosses may be simple crosses or complex crosses
  - a.) Simple crosses: Two parents are crossed to produce  $F_1$  (A x B)
  - b.) Complex crosses: More than two parents are crossed to produce the hybrid (A x B) x C x  $F_1$

### **Procedure of hybridization:**

The breeder has clear cut objective in developing the variety. He has to select the variety accordingly.

1. Choice of parents: One of the parents involved in crosses should be a well-adapted and proven variety in the area. The other variety should be having the character that are absent in this variety. Combining ability of the parents serves as useful guides in the selection of parents, which produce superior  $F_1$  and  $F_2$ .
2. Evaluation of parents: Parents are evaluated for their combining ability.
3. Emasculation: The removal of stamens/anther without affecting the female reproductive organs, hand emasculation is mostly followed.
4. Bagging: Immediately after emasculation the flowers are enclosed in suitable bags to prevent cross pollination.
5. Tagging: The emasculated flowers are tied with a thread. The information on date of emasculation, date of pollination, names of female and male parents are recorded in the tag with pencil. The name of the female parent is written first then male parent.
6. Pollination: Mature fertile and viable pollen from the male parent should be placed on receptive stigma of emasculated flowers to bring about fertilization. Pollen grain is collected, allowed for dehiscence and pollination is carried out with camel hair brush.
7. Harvesting and storing of  $F_1$  seeds: The crossed heads/pods should be harvested and threshed. The seeds should be dried and properly stored to protect them from storage pests.
8. Raising the  $F_1$  generation: Identify the selfed seeds in the  $F_1$  generation by using dominant marker gene. Larger  $F_1$  population is desirable, because both the genes are present in heterozygous condition.
9. Selfing: To avoid cross pollination and to ensure self-pollination. In often cross-pollinated crops, they are bagged to prevent cross pollination.

### **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 a 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 oleracea*). Triticale was produced by Rimpau in 1890 itself. Triticale is an amphidiploid obtained from cross between wheat and rye. Another example is *Saccharum* noblisation involving three species.

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

Exploitation of heterosis through F1 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/ self-pollinated crops is easy and less time consuming as 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 self-pollinated crops are dependent 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 self-pollinated crops? By nature, self-pollinated crops are shy pollinators with very poor pollen manoeuvrability (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 self-pollinated crops.
5. Among self-pollinated crops, F<sub>1</sub> hybrids have been graduated into the farmer's field in rice, barely, tomato, Sorghum (often-cross-pollinated) and wheat.

## **Pedigree method of handling segregating generations**

- In pedigree method individual plants are selected from F<sub>2</sub> and their progenies are tested in subsequent generations. A record of the entire parent off spring relationship is maintained

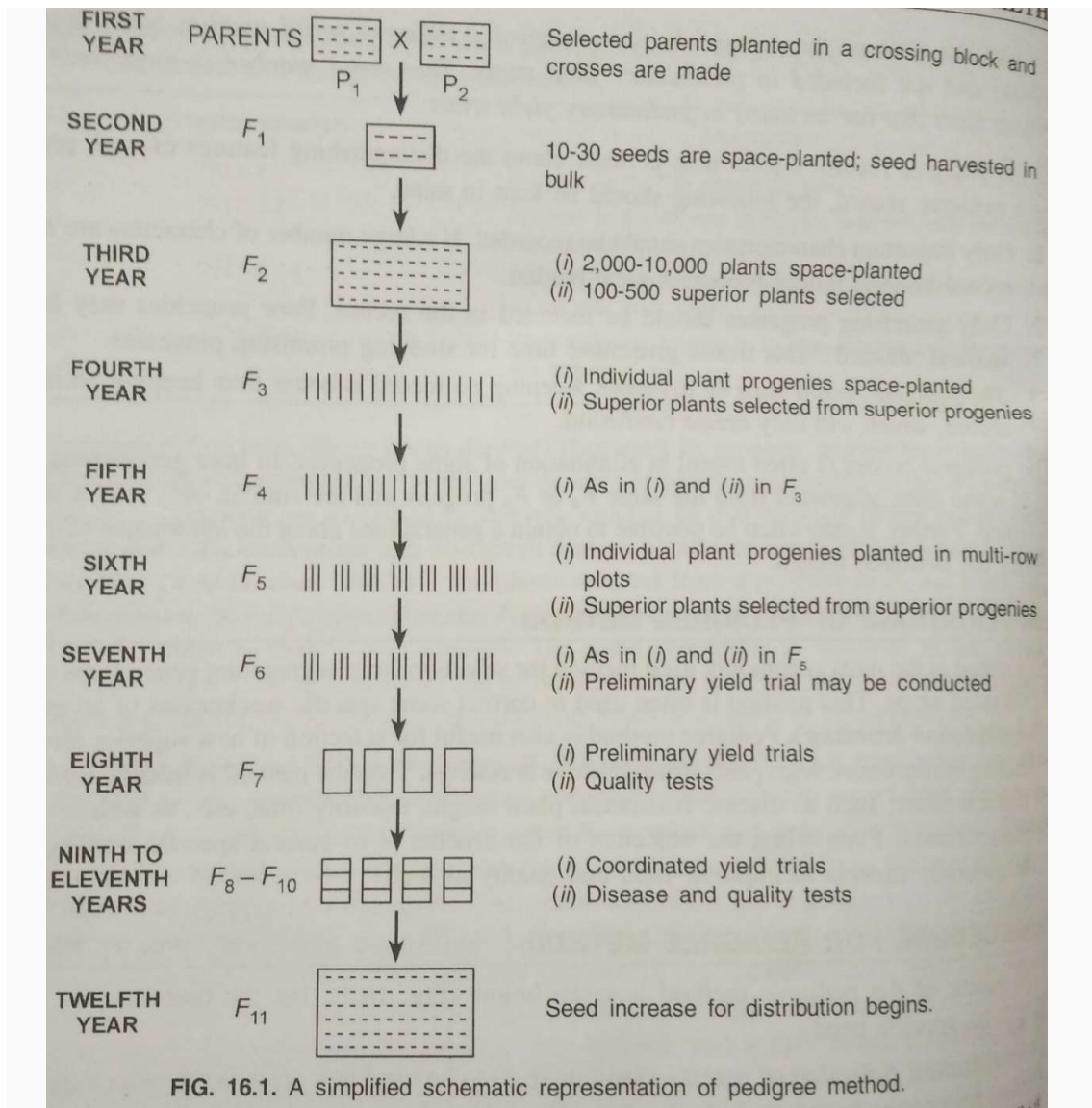


and known as pedigree record. The pedigree may be defined as a description of the ancestor of an individual and it generally goes back to some distant ancestor. So, each progeny in every generation can be traced back to the  $F_2$  plant from which it is originated.

- This method used for selection from segregating population of crosses in self-pollinated crops. It is used for combination or transgressive breeding.

**Procedure:**

1. Hybridization: The selected parents are crossed to produce a simple / complex cross ( $F_1$  seed)
2.  $F_1$  generation:  $F_1$  seeds are space planted to each produces maximum number of  $F_2$  seed. 15-30  $F_1$  plants are sufficient to produce good  $F_2$  populations.
3.  $F_2$  generation: 200-10000 plants are space planted and 100-500 plants are selected and their seeds are harvested separately. He should select as many as  $F_2$  plants as he can handle efficiently. The selection depends on skill of the breeder and his ability to judge to select  $F_2$  which produce good progeny.
4.  $F_3$  generation: Individual plant progeny are space planted. Individual plant with desirable characters from superior progenies is selected.
5.  $F_4$  generation: Individual plants progenies are space planted desirable plants are selected undesirable progenies are rejected. Progenies are compared visually and more plants are selected from superior progenies. Selection of desirable plants from superior progenies selection is practiced within / between family.
6.  $F_5$  generation: Many families have reached homozygous and may be harvested in bulk. The breeder has to assess the yielding potential of progenies, 25-100 progenies are advanced and tested in preliminary yield trial.
7.  $F_6$  generation: Multi row plots and evaluated visually progenies harvested bulk and they have become homozygous.
8.  $F_7$  generation: Preliminary yield trail with replication to identify the superior progenies. Progenies are evaluated for other component character 2-5 outstanding lines superior to check are advanced to multi location testing.
9.  $F_8 - F_{10}$  generation: Replicated yield trial at several locations. They are tested for yield as well as for resistance.
10.  $F_{11}$  generation: Seed multiplication and release.



**Merits:**

1. Maximum opportunity for the breeder to use his skill and judgment for the selection of plants in segregating generation.
2. It provides information about the inheritance of qualitative character from the pedigree record.
3. Chances of recovering transgressive segregants is more.
4. Plants and progenies with visible defects and weaknesses are eliminated at an early stage.
5. Well suited for the improvement of easily identified and simply inherited characters.

**Demerits:**

1. Maintenance of accurate pedigree record is tedious and takes up valuable time

2. Selection of progenies in every generation laborious, time consuming. Difficult to handle many crosses.
3. No opportunity for natural selection.
4. Possibility of losing the valuable genotype is early segregating generation.
5. The success of this method largely depends on skill of the breeder.

### **Bulk method**

Bulk method was first used by Nilsson Ehle in 1908. F<sub>2</sub> and the subsequent generations are harvested as bulks to raise the next generation. At the end of bulking period individual plants are selected and evaluated in a similar manner as in the pedigree method. The duration of bulking may vary from 7-30 generation artificial selection may seldom be practiced

#### **1. Isolation of homozygous lines**

It is used for the isolation of homozygous lines with a minimum of effort and expense. The population is carried to F<sub>6</sub>-F<sub>7</sub> as Bulk, where it reaches homozygosity. Individual plants are selected and evaluated to derive pure line. So preliminary yield trails are conducted to derive homozygous lines.

#### **2. Waiting for the opportunity for selection:**

Selection for resistance to disease, lodging and cold depends upon the presence of suitable environmental conditions favouring epidemic. Waiting till such environment do occur so the segregating generations are carried as bulk in such environment. Individual plants are selected and handled as in pedigree method. The duration of bulking depends upon the occurrence of the concerned environment. This is known as mass pedigree method of Harlan.

#### **3. Opportunity for natural selection:**

Maintenance of bulk is inexpensive and without much efforts. Some bulk populations are carried up to F<sub>20</sub> to F<sub>30</sub> to provide an opportunity for natural selection to act on their composition. Up to F<sub>6</sub> generation the population is heterozygous and after F<sub>7</sub> generation natural selection to act on homozygous plants and would change the frequency of homozygous genotypes present in the population. It is assumed that natural selection would favour higher yielding genotypes and eliminate poorer genotypes.

#### **Procedure of bulk method:**

1. Hybridization: Parents are selected and crossed
2. F<sub>1</sub> generation: F<sub>1</sub> is space planted more than 200 F<sub>1</sub> plants
3. F<sub>2</sub>-F<sub>6</sub> Generation: Planted at commercial seed rate, spacing and harvested as bulk, during this period. Frequency of population changes due to outbreak of disease or pest.

4. F<sub>7</sub> generation: 50000 plants are space planted about 1000-5000 plants with phenotype is selected and the seeds are harvested separately.
5. F<sub>8</sub> generation: Individual plant progenies are single/multi row plants, since progenies are homozygous and harvested in bulk weak and inferior progenies are rejected and 100-300 individual plant progenies with desirable characters.
6. F<sub>9</sub> generation: Preliminary yield trial with standard check, yield and quality parameter is taken for selection.
7. F<sub>10</sub>---F<sub>12</sub> generations: Replicated yield trails are conducted. Yield and its component characters are evaluated along with the check. Superior progenies are released as variety
8. F<sub>13</sub> generation: Seed multiplication of the newly released variety and distribution to farmers.

### **Merits**

1. Simple, convenient and inexpensive method
2. Natural selection is likely to increase the frequency of superior genotypes in the population. Therefore, progenies selected from long term bulks are likely to be superior to those selected from F<sub>2</sub> or short bulks.
3. Little work and attention are required in F<sub>2</sub> and subsequent generations, and no pedigree record is to be kept. This save time and labour, and the breeder can concentrate more on other breeding projects.
4. Since large populations are grown, chances of getting transgressive segregants are more.
5. Individual plant selection is done when population has become homozygous. Therefore, selection is expected to be more effective than in F<sub>2</sub> and F<sub>3</sub> generations.
6. Particularly suited to small grain crops grown in high crop densities.
7. Natural selection is expected to improve characters like adaptation to prevailing environment which are otherwise difficult to assess and select for.

### **Demerits**

1. The major disadvantage of bulk method is longer time taken to develop a new variety. Natural selection becomes important only after F<sub>10</sub> generation and bulking may have to be done up to F<sub>20</sub> or more which is considerably longer than the time taken in pedigree method
2. In short term bulks, natural selection has little effect on the genetic composition of populations. But short-term bulks are useful for isolation of homozygous lines.
3. It provides little opportunity to breeders to exercise their skill in selection.
4. A large number of progenies have to be handled at the end of the bulking period.
5. Information on inheritance of characters cannot be obtained

6. Off season and green house facilities cannot be used to advance the generation since environment at such locations may be markedly different from that in target location.

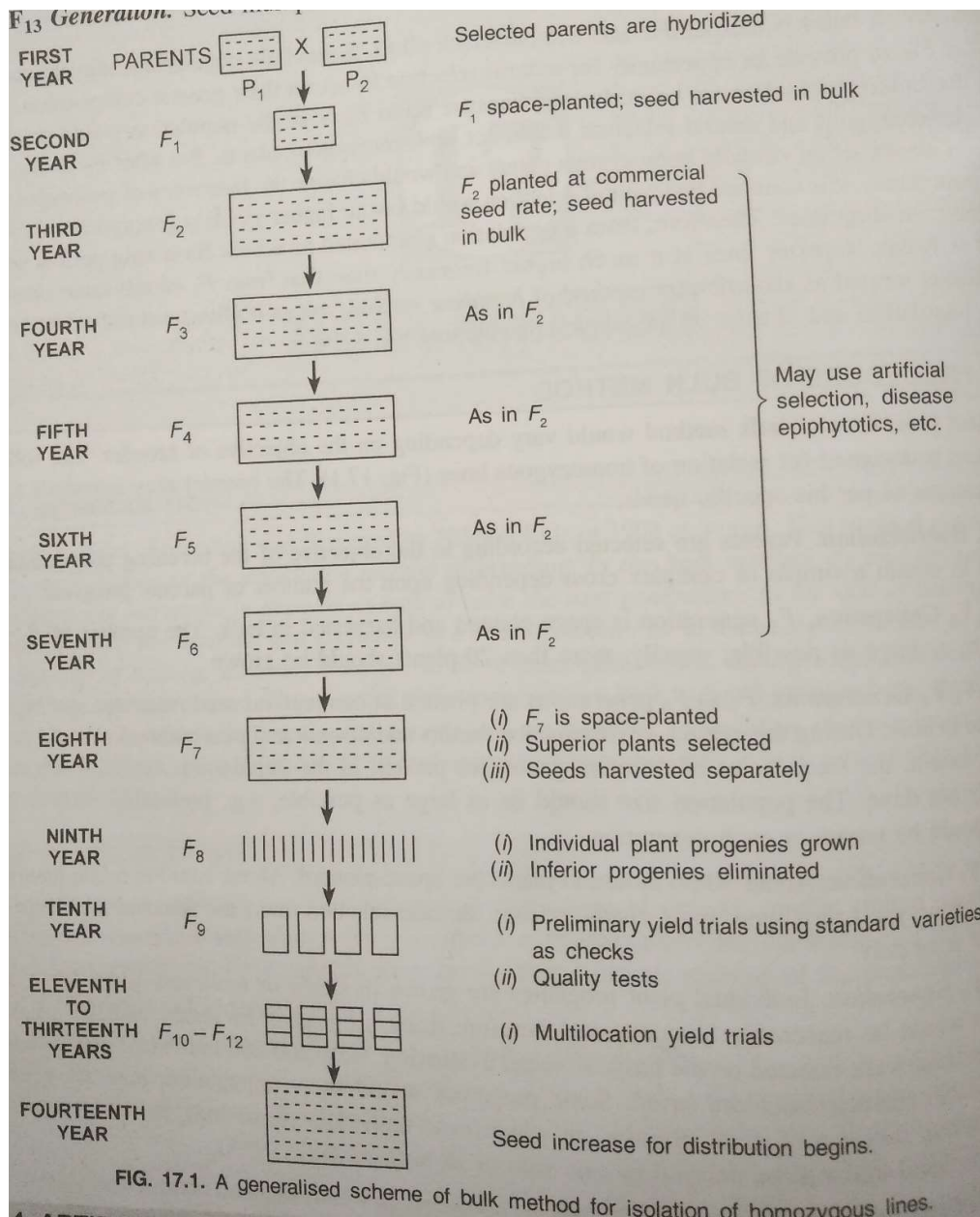


FIG. 17.1. A generalised scheme of bulk method for isolation of homozygous lines.

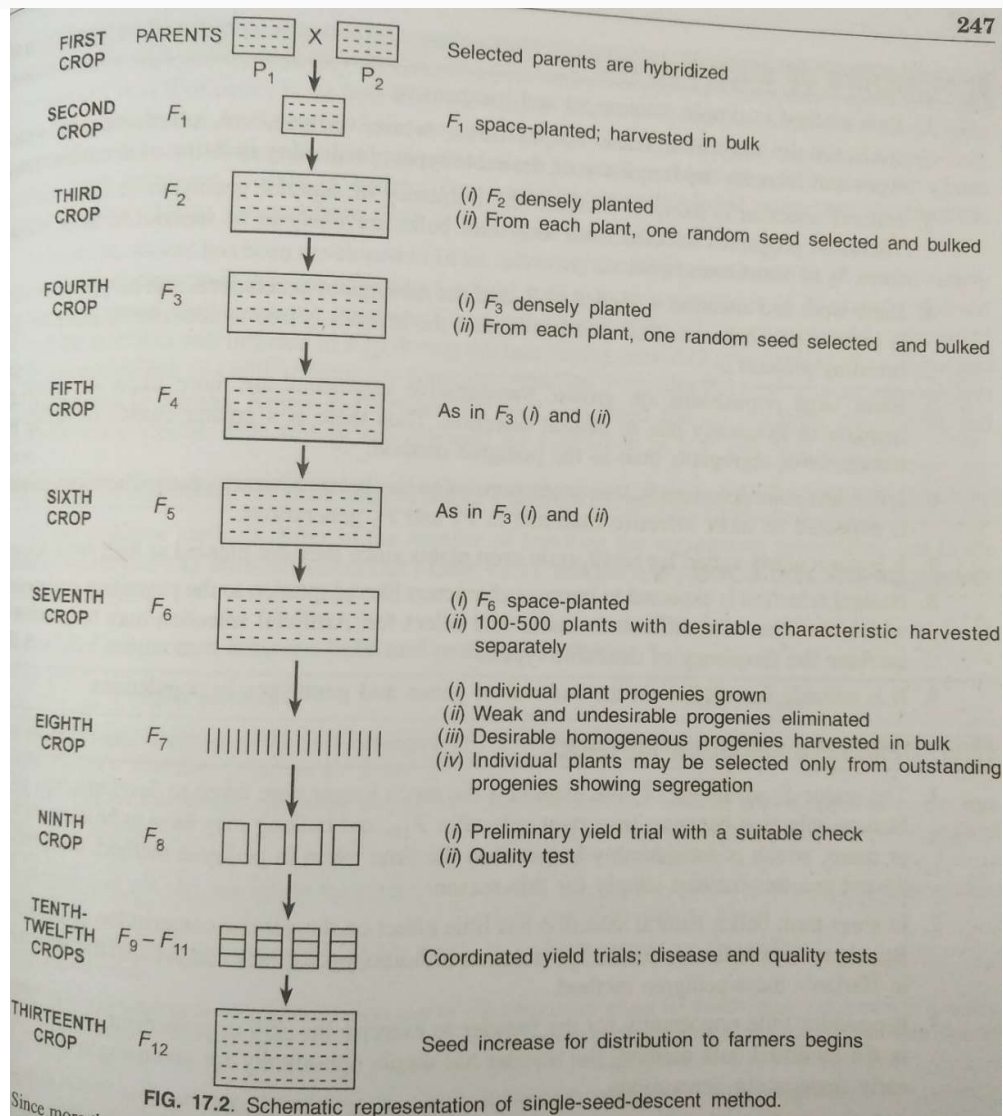
### Single seed decent method

- Single seed descent method This method is a modification of bulk method
- Single seed from each F<sub>2</sub> plants is bulked to raise the F<sub>3</sub> generation. Similarly, F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub> generation when the plants are homozygous plant progenies are advanced to next generation. Selection is done mainly among the progenies and number of progenies is sufficiently reduced to permit replicated trail. Individual plants may be selected from

outstanding families showing segregation. So preliminary yield trial and quality tests begin in  $F_7$  to  $F_8$ .

### Objectives

1. Rapidly advance of generation of crosses.
2.  $F_2$  and subsequent generation are grown with a very high plant density.
3.  $F_2$  plant is represented equally in the end population.
4. Off season nursery/green house facilities are utilized.
5. Maximum possible speed.
6. Require very little space/effort/ labour.
7. Do not permit any form of selection during the segregating generation.
8. In each successive generation the population size become small due to poor generation and death of plants due to disease/pest.



<b>Pedigree Method</b>	<b>Bulk Method</b>
Individuals plants are selected in F <sub>2</sub> and Segregation generation and individual plant progenies are grown	F <sub>2</sub> and the subsequent generation are maintained as bulk
Artificial selection, Artificial disease epidemics are an integral part	Artificial selection, artificial disease epidemic can be created to assist natural selection.
No role of Natural section	Natural selection determines the composition of population.
Pedigree record have to be maintained which is laborious and time consuming	No pedigree records are maintained
It takes 12 years to develop new variety	More than 12 years bulk population > 10 years for effective natural selection
Widely used method	Limited use
Needs close attention from F <sub>2</sub> onwards	It is simple convenient.
Segregating generation are space planted to permit individual plant selection	Bulk population are planted at commercial planting rate
Population size is small	Larger population are grown and the natural selection expected to the increase the chances recovery of transgressive segregants

### **Back cross method**

A Crossing between a F<sub>1</sub> hybrid or its segregating generation with one of its parents is known as Back cross. The hybrid and its progenies in the subsequent generations are repeatedly back crossed to one of their parents. As a result, the genotype of back cross progeny becomes increasingly similar to that parent to whom the back crosses are made. At the end of 6-8 back crosses, the progeny would be almost identical with the parent involved in back crossing.

#### **Objective:**

1. To improve one or two specific defects of a high yielding variety and a well-adapted variety with desirable character.
2. The characters lacking in this variety are transferred to it from a donor parent without changing the genotype of this variety except for the genes being transformed.

#### **Requirements of back cross breeding**

1. Suitable recurrent parent must be available which lacks in one or two characteristics.
2. A suitable donor parent must be available; the character must be highly intense.

3. The character(s) to be transferred must have high heritability and preferably, should be governed by one or few genes

4. A sufficient number of back crosses should be made so that genotype of recurrent parent is recovered in full. Ordinarily, 6-7 backcrosses are sufficient for the purpose.

#### **Applications of back cross breeding**

1. Inter varietal transferring of simply inherited traits. Characters governed by one or two genes like disease resistance are successful.
2. Inter varietal transfer of quantitative characters and highly heritable quantitative characters like earliness, plant height, seed size and seed shape are transferred.
3. Inter specific transfer of simply inherited characters: Disease resistance is transferred from related species to cultivated species. Inter specific transfer of genes are easy when the chromosome of the two species pair regularly.
4. Transferring of cytoplasm: wild species cytoplasmic are transferred to cultivated species transfer of male sterility. The variety or species from which the cytoplasm is to be transferred is used as the female parent. The parent to which the cytoplasm is to be transferred is used as the male parent in the original cross and back cross. After 6-8 back crosses the progeny would have the nuclear genotype of the recurrent parent and the cytoplasm from the donor parent.
5. Transgressive segregation: F1 is back crossed to one or two times to the recurrent parent leaving much heterozygosity for transgressive segregation to appear. In the second modification two or more recurrent parent may be used in the back-cross progeny to accumulate genes from them into the back cross. Progeny of the new variety is not exactly like any one of the recurrent parents.
6. Production of isogenic lines: Isogenic lines are identical in their genotype except for one gene
7. Germplasm conversion: When valuable germplasm cannot be utilized in breeding programmes and may be used as recurrent parent in separate back cross programme these lines are called converted lines.

#### **Procedure for transfer to dominant gene**

E.g. High yielding and widely adapted wheat variety A is susceptible to stem rust another variety B is resistance to stem rust. Stem rust is dominant to susceptibility.

1. Hybridization: Variety A is crossed to variety B. Generally, variety A should be used as female parent. This would help in identification of selfed plants.



2. BC<sub>1</sub> generation: F<sub>1</sub> plants are back crossed to variety A. Since all the F<sub>1</sub> are heterozygous for rust resistance, selection for rust resistance is not necessary.
3. First BC<sub>1</sub> generation: Half of the plants in BC<sub>1</sub> generation are resistant and the remaining half would be susceptible to stem rust. Rust resistant plants are selected and back crossed to variety A.
4. BC<sub>2</sub> to BC<sub>5</sub> generation: Segregation would occur for rust resistance. Rust resistant plants are selected and back crossed to variety A.
5. BC<sub>6</sub> generation: BC<sub>6</sub> plants will have 99 percent genes from variety A. Rust resistant plants are selected and selfed, their seeds are harvested separately.
6. BC<sub>6</sub>F<sub>2</sub> generation: Individual plants progeny from the selfed seeds of the selected plants are grown. Rust resistant plant similar to the plant type of variety A are selected and they are selfed. Seeds are harvested separately.
7. BC<sub>6</sub> F<sub>3</sub> generation: Individual plants progeny are grown. Progenies homozygous for rust resistant and similar to plant type of variety A are harvested in bulk. Several similar progenies are usually mixed to constitute the new variety.
8. Yield tests and seed multiplication: New variety is tested in replicated yield trails along with variety A as check. Ordinarily, the new variety would be identical to variety A in performance, detailed yield trials are therefore, generally not required.

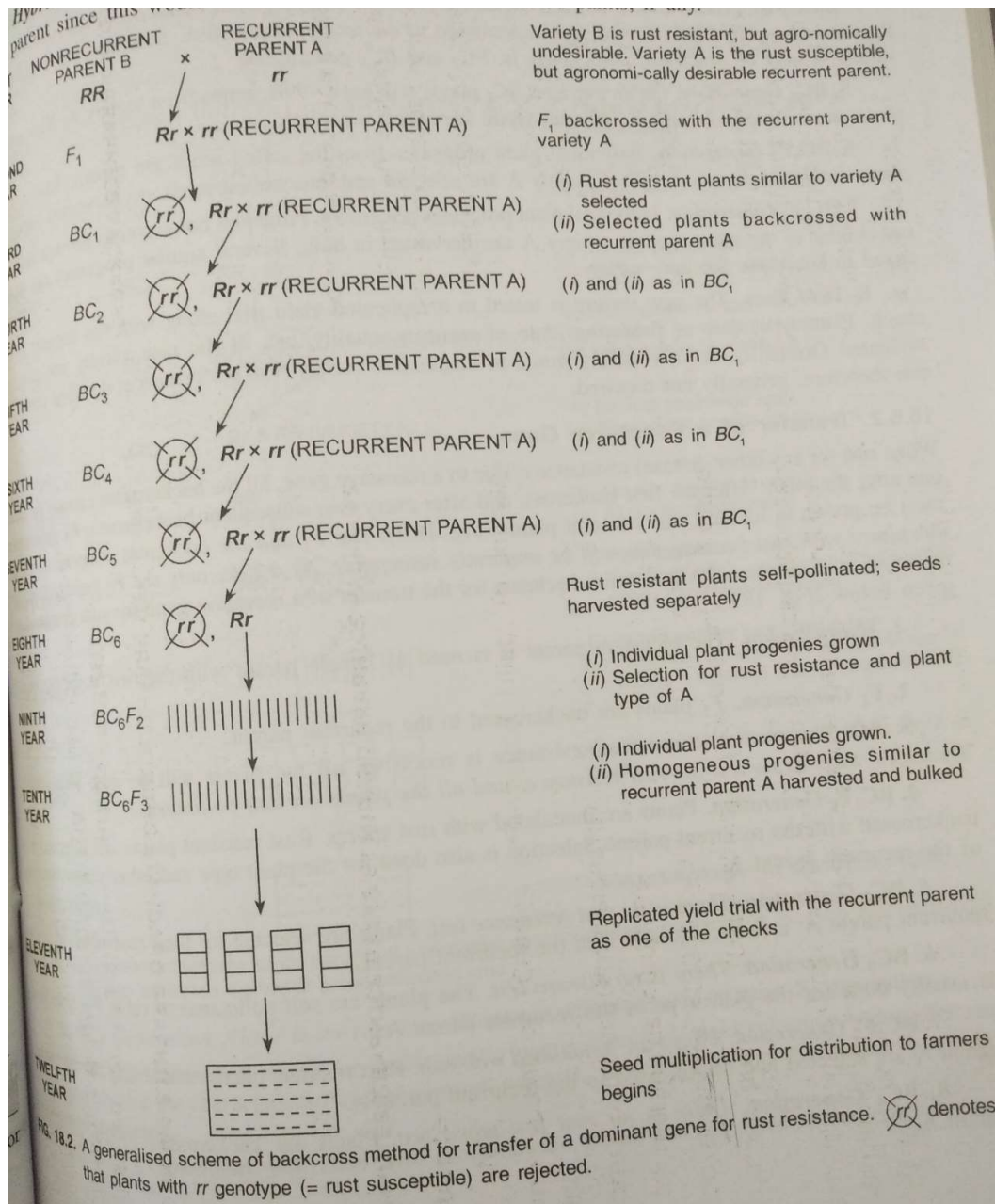
#### **Procedure for transfer to recessive gene**

1. Hybridization: Variety A, recurrent parent is crossed as female to variety B, donor parent for rust resistance.
2. BC<sub>1</sub> generation: F<sub>1</sub> plants are back crossed to variety A. Since all the F<sub>1</sub> are heterozygous for rust resistance, selection for rust resistance is not necessary.
3. First BC<sub>1</sub> generation: Since rust resistance is recessive, all plants will be susceptible to rust. Therefore, there is no test for rust resistance and all plants are self-pollinated.
4. BC<sub>1</sub>F<sub>2</sub> generation: Plants are inoculated with rust spores and rust resistant plants are selected and back crossed with recurrent parent. Selection will be done for plant type like recurrent parent A.
5. BC<sub>2</sub> generation: There is no rust resistance test. Plants are selected for resemblance to recurrent parent A and back crossed with recurrent parent A.
6. BC<sub>3</sub> generation: There is no disease test. The plants are self-pollinated to raise F<sub>2</sub>. Selection is done for plant type of recurrent parent A.
7. BC<sub>3</sub>F<sub>2</sub> generation: Plants are inoculated with rust spores. Rust resistant plants resembling recurrent parent A are selected and back crossed to recurrent parent A.

8. BC<sub>4</sub> generation: No rust resistance test and plants are back crossed to recurrent parent A.
9. BC<sub>5</sub> generation: There is no rust resistance test. Plants are self-pollinated to raise F<sub>2</sub> generation.
10. BC<sub>5</sub>F<sub>2</sub> generation: Plants are inoculated with rust spores and rigid selection is done for resistance and for the characteristics of recurrent parent A. Selfed seeds from selected plants are harvested separately.
11. BC<sub>5</sub>F<sub>3</sub> generation: Individual plant's progeny from the selfed seeds of the selected plants are grown and subjected to rust epiphytotic. A rigid selection is done for rust resistant plant similar to the plant type of variety A. Seeds from several similar rust resistant homogenous progenies are usually mixed to constitute the new variety.
12. Yield tests and seed multiplication: New variety is tested in replicated yield trails along with variety A as check. Ordinarily, the new variety would be identical to variety A in performance, detailed yield trials are therefore, generally not required.

**Merits:**

1. Back cross method retains all desirable character of a popular adapted varieties and replaces undesirable allele at particular locus
2. Useful for the transfer of disease resistance and incorporation of quality traits into a variety
3. This is used for the development of isogenic lines,
4. Extensive tests are not required 2-3 generation can be raised in off season nurseries greenhouses; it would save time.
5. This is the only method for the inter specific gene transfer and transfer of cytoplasm.
6. Male sterility and fertility restoration genes can be transferred to various back ground.



### Demerits:

1. New variety cannot be superior to recurrent parent except for the character transferred
2. It involves lot of crossing work. 6-8 back cross is often difficult and time consuming.
3. Sometime undesirable gene linked with desirable also may be transferred.
4. By the time the back cross programme the recurrent parent may have been replaced by other varieties superior in yield and other character.

### 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, Kl, Rend, Gabato, Blue Brid, Tobar, 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.

### **Hardy Weinberg Law**

- Cross pollinated crops are highly heterozygous due to the free inter mating among them so these are random mating populations. Because each individual of the population has equal opportunity of mating with any other individual. It is also known as mendelian/panmictic population. A Mendelian population may be thought of having a gene pool consisting of all gametes produced by the population. So, gene pool may be defined as the sum total of all genes present in the population. A population consists of all such individuals that share the same gene pool has an opportunity to inter mate with each other and contribute to the next generation of the population.
- Each generation of a Mendelian population may be considered to arise from a random sample of gametes from the gene pool of previous generation. Hence, it is not easy to follow the inheritance of a gene in a Mendelian population. It cannot be estimated by using the techniques of classical genetics. So, to understand the genetic makeup of such population a population genetics has been developed.
- This law is independently developed by Hardy (1908) in England and Weinberg (1909) in Germany. The law states that the gene and genotype frequencies in a Mendelian population remain constant from generation after generation if there is no selection, mutation, migration or random drift.

- The frequencies of these genotypes for a locus with two alleles A and a would be  $P^2(AA)$ ,  $2pq(Aa)$  and  $q^2(aa)$

Where,  $p$  = Frequency of 'A' allele in the population.

- $q$  = Corresponding frequency 'a' allele in the population the sum of  $p+q$  is equal=1

Such a population would be at equilibrium since the genotypic frequencies would be stable, that is, would not change from one generation to the next. This equilibrium is known as Hardy Weinberg equilibrium.

**Migration:** Migration is the movement of individual into a population from a different population. Migration may introduce new alleles into the population or may change the frequency of existing allele. The amount of change in gene frequency 'q' will primarily depend upon two factors.

- a. Ratio of migrant individuals to those of the original population.
  - b. The Magnitude of difference between the values of q in the population and in the migrants.
- So, in plant breeding migration is by inter varietal crosses or poly crosses.

**Mutation:** mutation is a sudden heritable change in an organism and is generally due to a structural change in a gene. It may produce a new allele not present in the population or may change the frequency of existence allele that  $10^{-6}$  mutation is detected. So, such effects in breeding population may be ignored.

**Random drift or genetic drift:** It is a random change in gene frequency due to sampling error. Random drift is more in small population than larger. Ultimately, the frequency of one of the alleles becomes zero and that of the other allele becomes one. The allele with the frequency one is fixed in the population because there would be no change in the frequency. So, all genes become homozygous. The genetic drift can be reduced by handling large population.

**Inbreeding:** Mating between individuals sharing a common parent in their ancestry inbreeding reduces the proportion of the heterozygosity and increase the frequency of homozygosity by the rate of decrease in heterozygosity is equal to  $\frac{1}{2N}$  (N- Number of plants in the population) per generation in monoecious or hermaphrodite species. In dioecious species and monoecious species where self-pollination is prevented the decrease in heterozygosity is low. In small population, even with strict random mating / strict cross pollination the frequency of homozygotes increases while that of heterozygotes decreases due to inbreeding

**Selection:** The selection in a random mating population is highly effective in increasing or decreasing the frequency of allele, but it is unable to either fix or eliminate them. However, in combination with a system of inbreeding, selection is highly efficient in the fixation and elimination of an allele.

## **Inbreeding**

It is mating between individuals related by descent or having common ancestry. The highest degree of inbreeding is obtained by selfing. Inbreeding depression refers to decrease in fitness and vigour due to inbreeding. The degree of inbreeding is measured by the inbreeding coefficient.

### **History of inbreeding:**

Inbreeding 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 were weaker in maize. Detailed and precise information on inbreeding in maize was published by East in 1908 and Shull in 1909.

### **The different effects of inbreeding are:**

- 1. Appearance of Lethal and Sublethal Alleles:** IB results in appearance of lethal; sublethal and sub vital characters. e.g.: Chlorophyll deficiencies, rootless seedlings, flower deformities – They do not survive, they lost in population.
- 2. Reduction in vigour:** General reduction in vigour size of various plant parts.
- 3. Reduction in Reproductive ability:** Reproductive ability of population decreases rapidly. Many lines reproduce purely that they cannot be maintained.
- 4. Separation of the population into distinct lines:** Population rapidly separates into distinct lines i.e. due to increase in homozygosity. This leads to random fixation of alleles in different lines. Therefore, lines differ in genotype and phenotype. It leads to increase in the variance of the population.
- 5. Increase in homozygosity:** Each line becomes homozygous. Therefore, variation within a line decreases rapidly. After 7-8 generations of selfing the line becomes more than 99% homozygous. These are the inbreds which have to be maintained by selfing.
- 6. Reduction in yield:** Inbreeding leads to loss in yield. The inbreds that survive and maintained have much less yield than the open pollinated variety from which they have been developed.

### **Degrees of inbreeding depression**

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

**2. Moderate inbreeding depression:** E.g. Maize, Jowar, Bajra. Though lethal effects are there, lines can be separated and maintained.

**3. Low inbreeding depression:** E.g. Cucurbits, Sunflower. Only a small degree of inbreeding depression is observed.

**4. No inbreeding depression:** The self-pollinated crops do not show inbreeding depression.

### **Heterosis**

The term heterosis was first used by Shull in 1914. Heterosis may be defined as the superiority of an F1 hybrid over both of its parents in terms of yield or some other character. Generally, heterosis is manifested as an increase in vigour, size, growth rate, yield or some other characteristic.

### **History**

Koelreuter is first reported hybrid vigour in tobacco produced artificial hybrids. In 1876, Darwin concluded that hybrids from unrelated plant type were highly vigorous. Most of our present knowledge on heterosis comes from the work on maize. Crossing inbred lines rather than open pollinated varieties produces the commercial maize hybrids. Hybridization between inbreds developed from the same variety or from closely related varieties produced only a small degree of heterosis.

- But a vast majority of the cases of heterosis are cases of superiority of hybrids over their parents. Hybrid vigour describes only superiority of hybrids over the parents. The few cases where F1 hybrids are inferior to their parents may also be regarded as cases of hybrid vigour in the negative direction.

### **Heterosis is the superiority of a hybrid over its parents.**

1. **Increased yield:** Heterosis is generally expressed as an increase in the yield of hybrids. The yield may be measured in terms of grain, fruit, seed, leaf, tubers or the whole plant.
2. **Increased reproductive ability:** More number of flowers/fruits/seeds. **Increase in Size and General Vigour:** The hybrids are generally more vigorous, i.e., healthier and faster growing and larger in size than their parents.
3. **Better quality:** In many cases, hybrids show improved quality. For example, many hybrids in onion show better keeping quality, but not yield, than open-pollinated varieties.



4. Earlier flowering and maturity: In many cases hybrids are earlier in flowering and maturity than the parents. But earliness is highly desirable in many situations particularly in vegetables.
5. Greater resistance to disease and pest: Some hybrids are known to exhibit a greater resistance to insect or diseases than their parents.
6. Greater adaptability: Hybrids are generally more adapted to environmental changes than inbreds.
7. Faster growth rate: In some cases, hybrids show a faster growth rate than their parents. But the total plant size of the hybrids may be comparable to that of parents. In such cases, a faster growth rate is not associated with a larger size.
8. Increase in the number of a plant part: In some cases there is an increase in the number of nodes, leaves and other plant parts, but the total plant size may not be larger.

### **Estimation 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.

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

Where  $F_1$  = Mean of hybrid

MP = Mid parental value.

$$MP = \frac{(P_1 + P_2)}{2}$$

where  $P_1$  = Parent 1;  $P_2$  = Parent 2

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

#### **2. Heterobeltiosis:**

Superiority of  $F_1$  over the better parent.

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

Where BP = Mean of Better Parent.

#### **3. Economic heterosis:**

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

$F_1 - CV$

----- x 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.

## **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. To isolate plants with superior genotypes. Individual plants are highly heterozygous. The progeny from such plants are highly heterogeneous and usually different from the parent plant due to segregation and recombination. This would increase the frequency of desirable genes combinations. As a result, the phenotype of the population would be favorably changed. In cross pollinated species genotype of the individual plants is of little importance but it is the frequency of desirable genes or alleles in the population as a whole that determines the value of a population.

The breeding methods commonly used in cross pollinated crops may be broadly grouped into two categories.

### **1. Population improvement**

#### **2. Hybrids**

#### **3. Synthetics and Composites**

### **Population Improvement**

#### **a) Intra-population improvement**

In cross pollinated crops, population improvement is used to enhance the frequency of desirable alleles in a population. It may be intra or interpopulation improvement. Requirement of intra-population improvement:

- Presence of genetic variation for different traits
- Presence of additive genetic variation with partial dominance in population
- High heritability of traits
- Absence of any undesirable linkages
- There should not be negative association between desirable traits
- Population under improvement should be grown to fairly large plant population size in selection blocks.
- Improvement is expected to be high for characters which can be easily and precisely rated particularly before flowering.

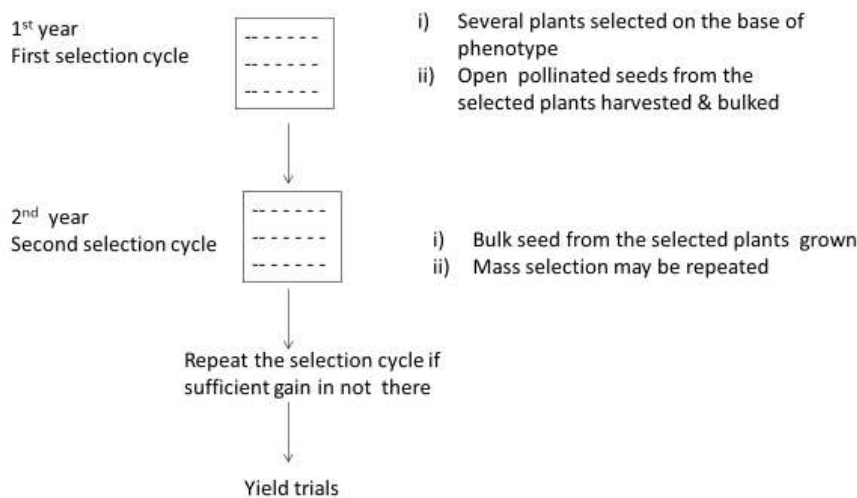
Here mass selection or its modification are used to increase the frequency of desirable alleles thus improving the characteristics of the population.

1. **Mass selection without progeny testing:** Plants are selected on the basis of their phenotype and no progeny test carried out
2. **Mass selection with progeny testing:** Initial selection on the basis of phenotype but final selection on the basis of progeny test. This includes the ear-to-row method and recurrent selection

### 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. Mass selection is a form of maternal selection as there is no control on pollination.

#### Mass Selection (Selection without Progeny Testing)



**Note:** Sufficiently large no. of plants are to be selected in each population so as to check inbreeding

### 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 overcome 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.

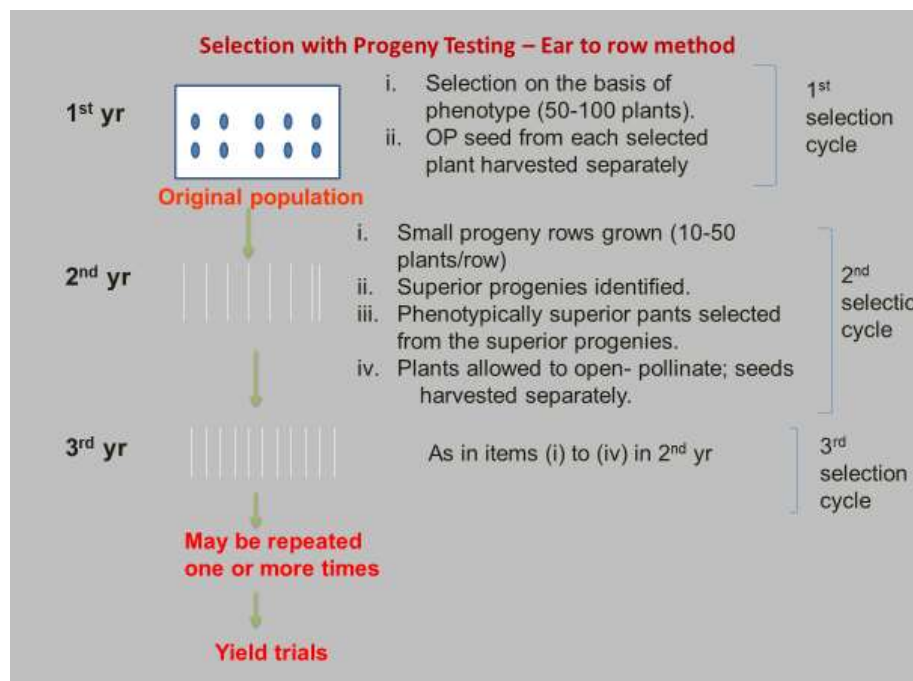
c) Stratified mass selection: Here the field from which plants are to be selected will be divided into smaller units or plots having 40 to 50 plants / plot. From each plot equal number of plants will be selected. The seeds from selected plants will be harvested and bulked to raise the next generation, 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) Family selection

Selection based on means of the individual plant progenies or families.

**I) 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 (1908).



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.

**II) 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 x B   B x A

**III) Inbred or selfed family selection:** Families produced by selfing.

a) 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).

b) S<sub>2</sub> family selection: Families obtained by two generations of selfing and used for evaluation. Superior families are intermated.

### **Merits of Family 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.

#### **b) Inter -population improvement**

Suggested by Hayes and Garber (1919) and independently by East and Jones (1920) and breeding schemes developed during 1940s

#### **Recurrent selection**

This is one of the breeding methods followed for the improvement of cross-pollinated crop. The different Recurrent selection schemes are variations of progeny selection, the difference lying in the manner of obtaining the progeny for evaluation, and in making the all possible inter crosses among the selected lines in place of open pollination.

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:

- ii. Simple Recurrent Selection
- iii. Recurrent Selection for GCA
- iv. Recurrent Selection for SCA
- v. Reciprocal Recurrent Selection

#### **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.

First Year:

- i) Several superior plants are selected.

- ii) Selected plants selfed.
- iii) Harvest the single plants.
- iv) Seeds are evaluated, superior plants are identified.

Second Year:

- i) Progeny rows raised
- ii) Inter crosses are made in all combination by hand.
- iii) Equal amount of seed bulked from each cross.

Third Year:

- i) Compositeds seeds raised
- ii) Repeat the operation as in first year

Fourth Year:

Repeat as in second year.

- i) Recurrent selection is effective in increasing the frequency of desirable genes in the population
- ii) Most suited for characters having high heritability.
- iii) Inbreeding is kept at minimum.

Eg: oil content, protein content and high heritability traits are effective for increasing the frequency of desirable genes in the selected populations

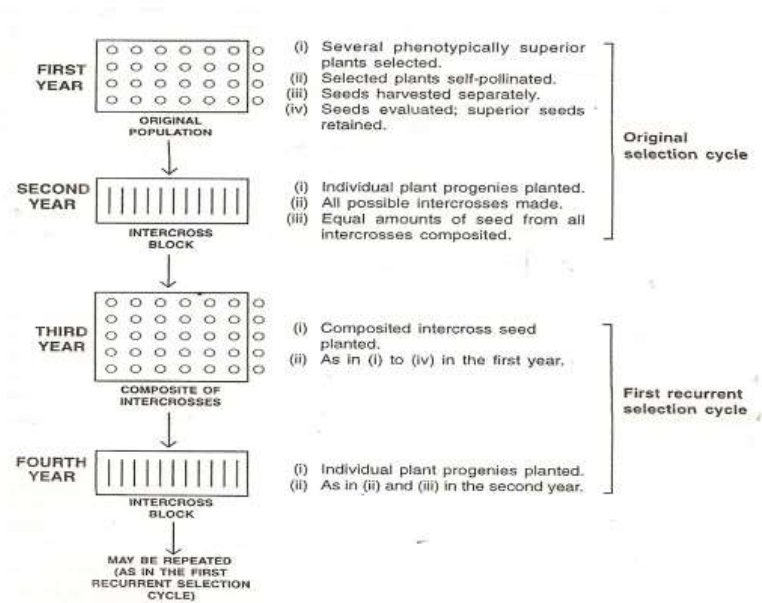


Fig: Simple Recurrent Selection schematic representation



### **Merits of recurrent selection**

- i) Recurrent selection is effective in increasing the frequency of desirable genes in the population
- ii) Most suited for characters having high heritability
- iii) Inbreeding is kept at minimum.

### **2. Recurrent selection for general combining ability**

It is the modification of the early testing suggested by the Jenkins, 1935. 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. The gametes of the tester would be variable the difference between population and tester is primarily due to general combining ability (GCA). 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

The steps involved in recurrent selection for GCA are outlined below:

#### First year

A number of phenotypically outstanding plants are selected from source population. Each selected plant is selfed and crossed (as male) to a number of plants selected from tester with broad genetic base. Tester with broad genetic base implies a population that has a large genetic variation. e.g. an open pollinated variety/synthetic/segregating population. The selfed seeds are harvested separately and saved for planting in third year. The test crossed (seed from plant x tester cross) from each selected plant is harvested separately and used for replicated yield trial in second year.

#### Second year

A replicated yield trial is conducted using plant x tester seeds, and plants producing superior test cross progeny are identified.

#### Third year

Selfed seeds (from first year) from those plants producing superior test cross progenies are planted in separate progeny rows in a crossing block. The progenies are crossed in all possible

combinations, and equal amounts of seeds from all intercrosses are composited to obtain the next generation. This completes the original selection cycle.

Fourth year

The composited seed (from all the intercrosses) is planted and used as the source population for first cycle of recurrent selection. All operations of first year are carried out.

Fifth year

Operations of second year are repeated.

Sixth year

Operations of third year are repeated. This completes the First recurrent selection cycle.

Seventh year

The second recurrent cycle may be initiated, and in this manner, several cycles of selection may be carried out.

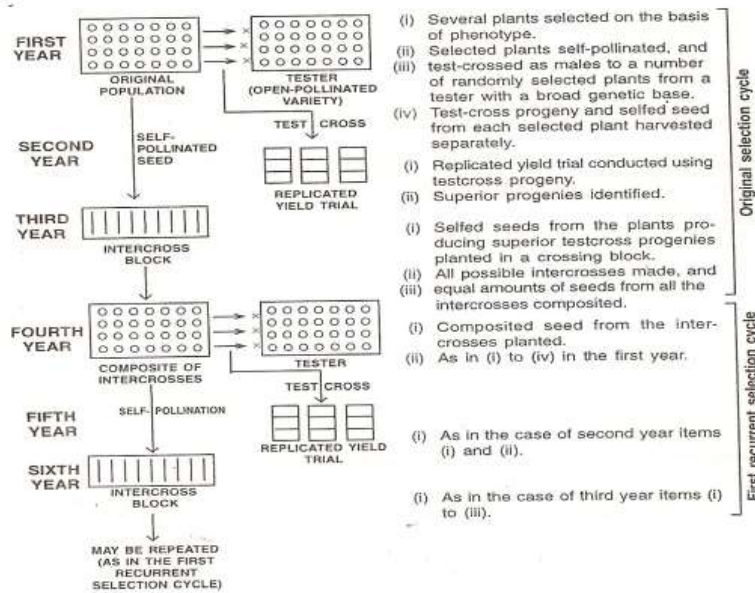


Fig: Schematic representation of Recurrent Selection for GCA

### 3) Recurrent Selection for Specific Combining Ability

Recurrent selection for SCA was first proposed by Hull in 1945. 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 (1949), 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.

First year

1. Several plants selected in population A and B.
2. Selected plants are self-pollinated
3. Selected plant from A is test crossed with plants in B and Vice versa. Seeds from testcross of each selected plant is harvested separately.

Second year

1. Separate yield trials conducted from test cross progenies of A and B
2. Superior progenies identified

Third year

1. Selfed seed from plants producing superior test cross progenies planted.
2. All possible inter crosses made 3. Seeds harvested and composited

Fourth year

Populations A and B are planted from the composited seed produced in the third year, and operations of first year are repeated.

Fifth year

Operations of the second year are repeated

Sixth year

Operations of third year are repeated. This completes the first recurrent selection cycle. The population may be subjected to further selection cycles, if desired, by repeating the operations of first recurrent selection cycle.

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.
4. Production of synthetic variety

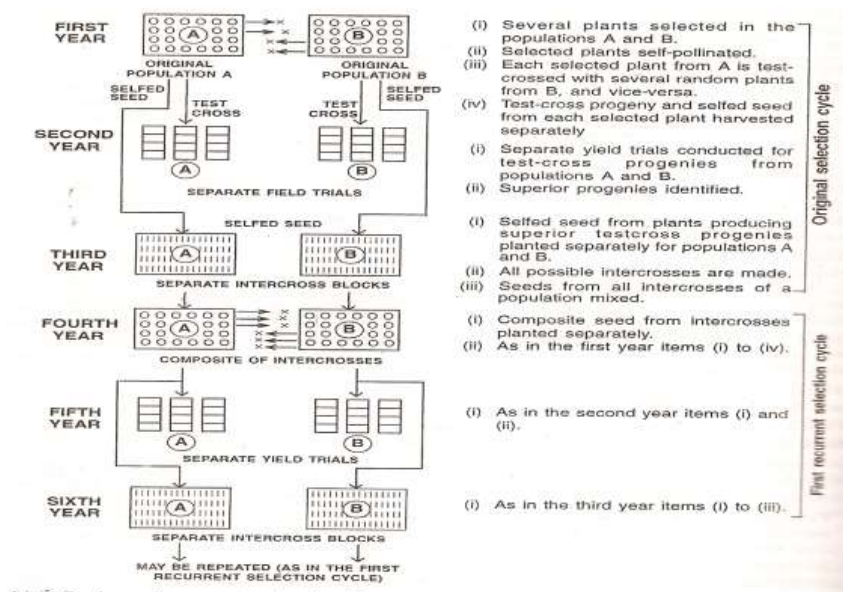


Fig: Schematic representation of Reciprocal Recurrent Selection

## 2. 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 x B)

Crossing two inbreds or pure lines.

2. Three-way cross hybrid (A x B) x C

A cross between a single cross hybrid and an inbred.

3. Double cross hybrid (A x B) x (C x D)

cross between two F<sub>1</sub>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 whereas it may not be in case of open pollinated variety.

3. The inbreds are homogenous and their performance can be predicted whereas open pollinated varieties 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.

$$(A \times B) \times (C \times D) = [(A \times C) + (A \times D) + (B \times C) + (B \times D)]/4$$

### **Production of Hybrids**

#### **Methods**

I. Hand emasculation and dusting - Cotton, Tomato, Chillies, Bhendi

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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 planting cross compatible lines hybrids are produced. Here both are hybrids. E.g. *Brassicas*.

#### **Success of hybrids**

- a) Easy hand emasculating
- b) Abundant seed set to compensate cost of hand emasculating.
- c) Stable male sterile lines.
- d) Effective restorers.
- e) Effective pollen dispersal.

### **3. 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.

### **4. Composite varieties**

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.

<b>Synthetic variety</b>	<b>Composite variety</b>
Parental components are generally inbreds tested for their GCA	It is not so in composite. The lines are not tested for their GCA.
No of parental lines are limited to 4 - 6 inbreds	No such limit
Synthetic produced with inbreds can be reconstituted	It is not possible to reconstitute composite variety
Yield performance can be predicted	Yield performance cannot be predicted

### **Combining ability**

Ability of a strain to produce superior progeny when crossed with other strains.

#### **General combining ability (GCA)**

Average performance of a strain in a series of cross combinations. The GCA is estimated from the performance of  $F_{1S}$  from the crosses. The tester will have a broad genetic base.

#### **Specific combining ability (SCA)**

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.

## **Breeding methods for vegetatively propagated crops**

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 is 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. utilisissima*), 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 a group of plants produced from a single through asexual reproduction. Thus, asexually propagated crops consist of a 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 characteristics 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**

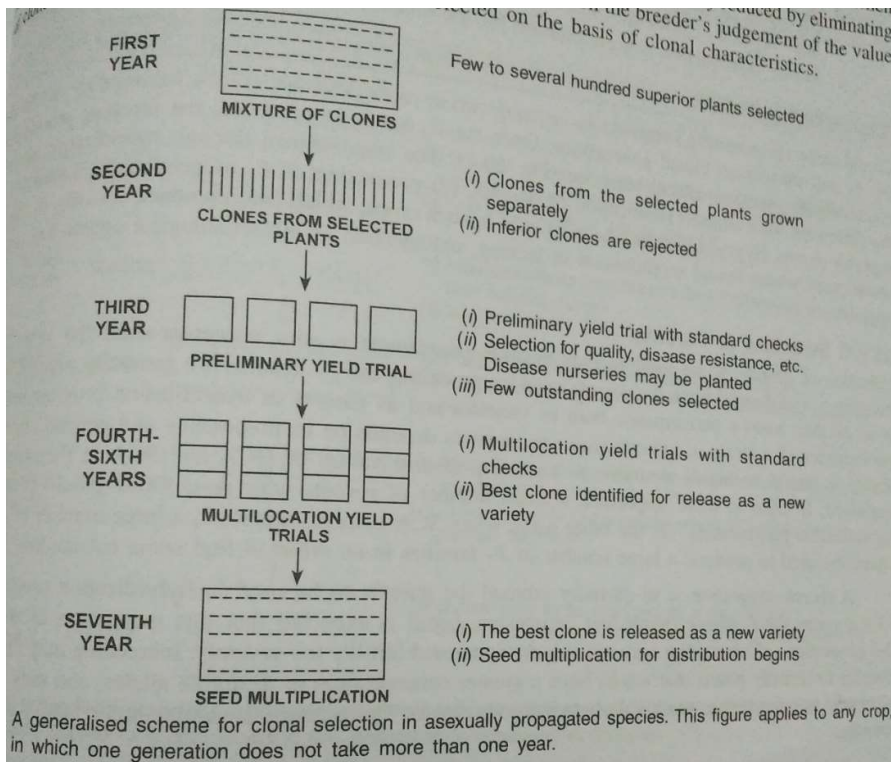
In view of these considerations, in the earlier stages of clonal selection, when selection is based on single plants or single plots, the emphasis is on the elimination of weak and undesirable plants or clones. The breeder cannot reasonably hope to identify superior' genotypes at this stage. In the later 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 clearer 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

### **Clonal Hybridization**

Clonal crops are generally improved by crossing two or more desirable clones, followed by selection in the F<sub>1</sub> progeny and in the subsequent clonal generations. Once the F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> progeny would depend upon the parents used for producing the F<sub>1</sub>. 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 F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> 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<sub>1</sub>. 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<sub>1</sub> progeny in detail. To avoid this, generally small samples of several F<sub>1</sub> populations are grown. The general worth of individual F<sub>1</sub> populations is estimated visually. The presence of outstanding individuals in the F<sub>1</sub> populations is also noted, and inferior F<sub>1</sub>'s are eliminated. Promising F<sub>1</sub>'s with outstanding individuals are then grown at a much larger scale for selection. The procedure is designed to save time, space and labour by planting only small populations of a large number of crosses at the preliminary stage.

### **Selection within F<sub>1</sub> Families**

The selection procedure within F<sub>1</sub> 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<sub>1</sub> 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.

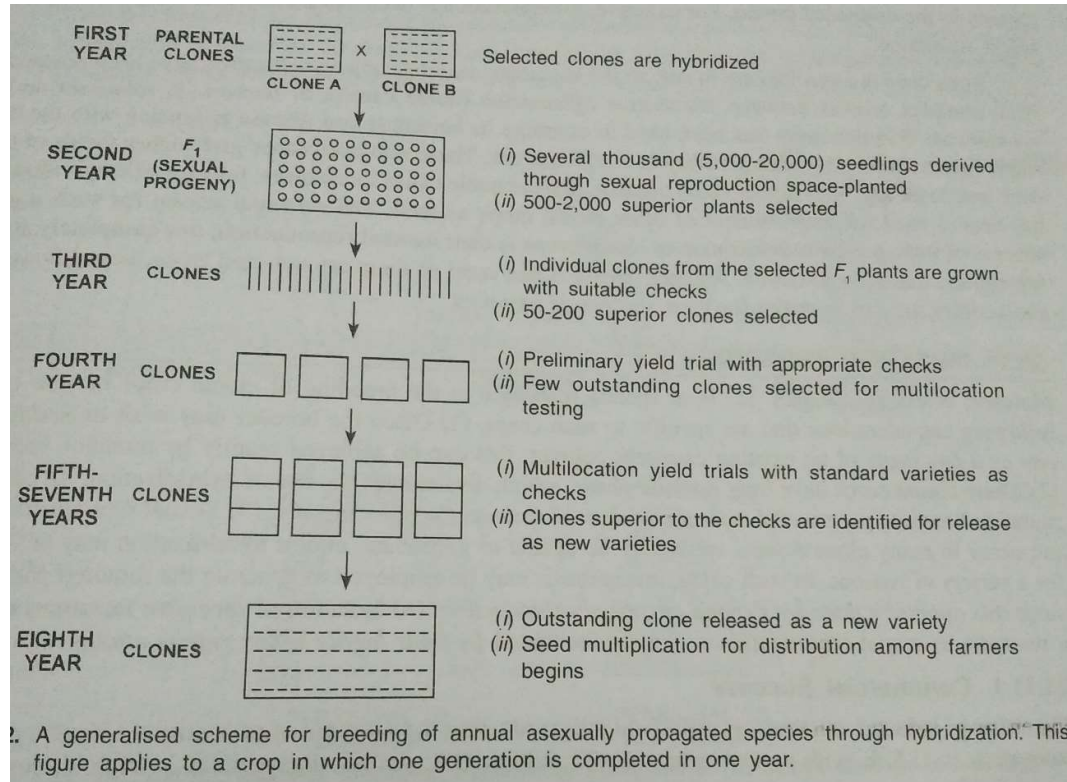
Fourth Year

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 released as new varieties.  
Eighth year

The clones released as varieties are multiplied and distributed among farmers.



## MUTATION BREEDING

The term mutation was coined by Hugo De Vries in 1900 for the first time and the word is derived from the latin word 'MUTARE' means to change. Mutation is the sudden heritable change other than the Mendelian segregation and gene recombination in an organism.

Mutation may be the result of a change in a gene, a change in chromosome that involves several genes or a change in plasma gene. Mutations produced by changes in the base sequence of genes are known as gene or point mutations some mutations may be produced by changes in chromosome structure or even in chromosome number they are termed as chromosomal mutation. There are three types of mutations based on genetic basis of heritable change:

1. Gene mutations: These are produced by change in the base sequence of genes. The change may be due to base substitutions, deletion or addition.
2. Chromosomal mutation: These arise due to change in chromosome number that may leads to polyploidy or aneuploidy or change in chromosome structure that result in deletions duplication, inversion and translocation.
3. Cytoplasmic or plasma gene mutation: These are due to change in the base sequence of plasma genes. The plasma genes are present in mitochondria or chloroplast. Here the mutant character occurs in buds or somatic tissues which are used for propagation in clonal crops.

### Classification of mutations:

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

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

- a) Genetic back ground i.e. presence of mutator genes
- b) Genes themselves
- c) Environment

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

B. Based on magnitude of phenotypic effects mutation as classified as

**Macro mutations:** Oligogenic mutation – Large phenotypic effect and recognizable on individual plant basis and can be seen easily in M<sub>2</sub> generations. e.g. Ancon breed in sheep, pod maize to cob maize

**Micro mutations:** Polygenic mutations – Small phenotypic effect which cannot be recognized on individual plant basis but can be recognize only in a group of plants. Selection should be done in M<sub>3</sub> or later generations.

### **Characteristic features of mutations**

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

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

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

1. Seeds: Seeds are used after soaking to get greater frequency of induced mutations than air dried.

2. Seedlings: At any stage of life cycle can be subjected to radiation but usually seedlings neither too young nor too old are irradiated due to their convenience in handling in pots transportation from nursery easily.

3. Flowers: Meiotic cells have been found more sensitive than the mitotic cells and therefore plants are irradiated in the flowering stage in order to affect the developing gametes.

4. Cuttings: In case of fruit tree when they are propagated by clones – the desirable cuttings are exposed to irradiation.

### **Selection of the variety for mutagen treatment**

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

### **Dose of the Mutagen**

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

### **Mutation Breeding for oligogenic traits**



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

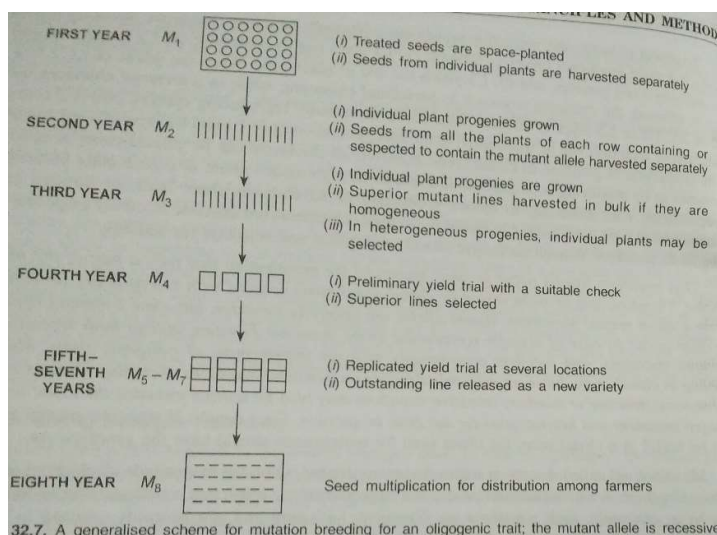
1.  $M_1$  generation: Several hundred seeds are treated with a mutagen and are space planted. In general, the number of treated seeds is so adjusted as to give rise to 500 fertile  $M_1$  plants at the harvest. Care should be taken to avoid outcrossing; this can be achieved either by planting the  $M_1$  population in isolation or by bagging the inflorescence of  $M_1$  plants or even the whole  $M_1$  plants.  $M_1$  plants will be chimeras for the mutations present in heterozygous state. About 20 to 25 seeds from each  $M_1$  spike are harvested separately to raise the  $M_2$  progeny rows.

2.  $M_2$  generation: About 2,000 progeny rows are grown. Careful and regular observations are made on the  $M_2$  rows. But only distinct mutations are detected in  $M_2$  because the observations are based on single plants. All the plants in  $M_2$  rows suspected of containing new mutations are harvested separately to raise individual plant progenies in  $M_3$ . If the mutant is distinct, it is selected for multiplication and testing. However, most of the mutations will be useless for crop improvement. Only 1-3 per cent of  $M_2$  rows may be expected to have beneficial mutations.

3.  $M_3$  generation: Progeny rows from individual selected plants are grown in  $M_3$ . Poor and inferior mutant rows are eliminated. If the mutant progenies are homogeneous, two or more  $M_3$  progenies containing the same mutation may be bulked. Mutant  $M_3$  rows are harvested in bulk for a preliminary yield trial in  $M_4$ .

4.  $M_4$  generation: A preliminary yield trial is conducted with a suitable check, and promising mutant lines are selected for replicated multilocation trials.

5.  $M_5$ - $M_7$  generations: Replicated multilocation yield trials are conducted. The out-standing line may be released as a new variety. The low yielding mutant lines, however, should be retained for use in hybridization programmes.



32.7. A generalised scheme for mutation breeding for an oligogenic trait; the mutant allele is recessive.

**Mutation breeding for polygenic traits:** Mutagenesis does produce genetic variation in polygenic traits; this variation is usually as much as 50% of that generated in F<sub>2</sub> generation, but sometimes it may be as much as or even greater than the latter.

1. M<sub>1</sub> and M<sub>2</sub> generations: M<sub>1</sub> and M<sub>2</sub> generations are grown in the same way as in the case of oligogenic traits. In M<sub>2</sub> generation, vigorous, fertile and normal looking plants that do not exhibit a mutant phenotype are selected and their seeds are harvested separately to raise individual plant progeny rows in M<sub>3</sub>.

2. M<sub>3</sub> generation: Progeny rows from individual selected plants are grown. Careful observations are made on M<sub>3</sub> rows for small deviations in phenotype from the parent variety. Inferior rows are discarded. Few rows may be homogeneous and would be harvested in bulk. Selection is done in M<sub>3</sub> rows showing segregation; a majority of M<sub>3</sub> rows would show segregation. Intensive and careful evaluation of a large number of M<sub>3</sub> progeny rows allows identification of mutants with altered quantitative traits, e.g., partial or horizontal disease resistance. Such mutants occur in high frequencies that approach 1% or even high, so that their isolation becomes quite cost effective.

3. M<sub>4</sub> generation: Bulk seed from homogeneous M<sub>3</sub> rows may be planted in a preliminary yield trial with a suitable check; superior progenies are selected for replicated multilocation yield trials. Individual plant progenies from M<sub>3</sub> are critically observed. Progenies showing segregation may be subjected to selection only if they are promising. Superior homogeneous progenies are harvested in bulk for preliminary yield tests in M<sub>5</sub>.

4. M<sub>5</sub>-M<sub>8</sub> generations: Preliminary yield trials and / or multi-location trials are conducted depending upon the stage when the progenies become homogeneous. Outstanding progenies may be released as new varieties.

### **Applications of Mutation Breeding**

Mutation breeding has been used for improving both oligogenic as well as polygenic characters. Mutagenesis has been used to improve morphological and physiological characters including yielding ability. Various applications of mutation breeding are:

1. Induction of desirable mutant alleles which may not be available in the germplasm
2. It is useful in improving specific characteristics of a well-adapted high yielding variety.
3. Mutagenesis has been successfully used to improve various quantitative characters including yield.
4. F<sub>1</sub> hybrids from intervarietal crosses may be treated with mutagens in order to increase genetic variability by inducing mutation and to facilitate recombination of linked genes.

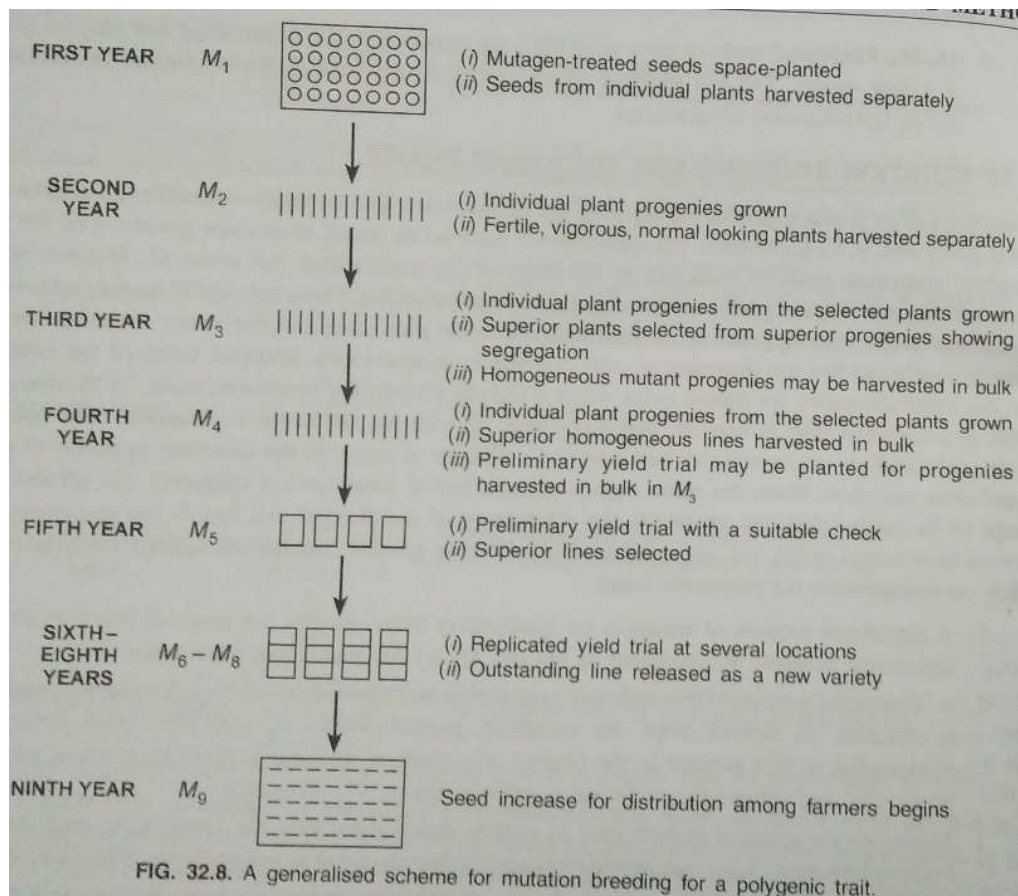
5. Irradiation of interspecific (distant) hybrids has been done to produce translocations.

**Advantages:**

1. Mutation create inexhaustible variation.
2. When no improvement is possible this method has to be adopted.

**Limitations:**

1. Frequency of desirable mutations is very low about 0.1 percent. To detect the desirable one in  $M_2$  considerable time, labour & other resources are to be employed.
2. To screen large population, efficient quick and inexpensive selection techniques are needed.
3. Desirable mutations may be associated with undesirable side effects due to other mutations thus extending the mutation breeding programme.
4. Detection of recessive mutations in polyploids and clones is difficult and larger doses of mutagen have to be applied and larger populations are to be grown.



## **Polyploidy Breeding**

The somatic chromosome number of any species, whether diploid or polyploidy, is designated as  $2n$ , and the chromosome number of gametes is denoted as  $n$ . An individual carrying the gametic chromosome number,  $n$ , is known as haploid. A monoploid, on the other hand, has the basic chromosome number,  $x$ . In a diploid species,  $n = x$ , one  $x$  constitutes a genome or chromosome complement. The different chromosomes of a single genome are distinct from each other in morphology and or gene content and homology; members of a single genome do not show a tendency of pairing with each other. Thus, a diploid species has two, a triploid has 3 and a tetraploid has 4 genomes and so on.

**In euploids**, the chromosome number is an exact multiple of the basic or genomic number. Euploidy is more commonly known as polyploidy.

When all the genomes present in a polyploidy species are identical, it is known as autopolyploid and the situation is termed as autopolyploidy.

In the case of allopolyploids, two or more distinct genomes are present.

Euploids may have 3(triploid), 4(tetraploid), 5 (pentaploid), or more genomes making up their somatic chromosome number.

In case of autopolyploidy, they are known as autotriploid, autotetraploid, autopentaploid, and so on, while in the case of allopolyploidy they are termed as allotriploid, allotetraploid, allopentaploid, etc.

**Amphidiploid** is an allopolyploid that has two copies of each genome present in it and, as a consequence, behaves as a diploid during meiosis.

**A segmental allopolyploid** contains two or more genomes, which are identical with each other, except for some minor differences.

### **Autopolyploids**

Origin and production of doubled chromosome numbers:

1. Spontaneous: chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are produced in low frequencies.
2. Production of adventitious buds: decapitation in some plants leads to callus development at the cut ends of the stem. Such a callus has some polyploid cells and some of the shoot buds regenerated from the callus may be polyploid. In Solanaceae 6-36% of adventitious buds are tetraploids.

3. Treatment with physical agents: Heat or cold treatment, centrifugation, x-ray or gamma ray irradiation may produce polyploids. Exposing the plants or ears of maize to a temperature of 38-45°C at the time of the first division of zygote produce 2-5 % tetraploid progenies.

4. Regeneration *in vitro*: polyploidy is a common feature of the cells cultured *in-vitro*.

5. Colchicine treatment: Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling.

#### Autopolyploidy

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

#### **Morphological and cytological features of auto polyploids:**

The general features are summarised below:

1. Polyploids have larger cell size than diploids. Guard cells of stomata are larger and the number of stomata per unit area is less in polyploids than diploids.
2. Pollen grains of polyploids are generally larger than those of the corresponding diploids.
3. Polyploids are generally slower in growth and later in flowering.
4. Polyploids usually have larger and thicker leaves, and larger flowers and fruits which are usually less in number than in diploids.
5. Polyploids generally show reduced fertility due to irregularities during meiosis and due to genotypic imbalance leading to physiological disturbances.
6. In many cases autopolyploidy leads to increased vigour and vegetative growth.
7. Different species have different levels of optimum ploidy. For sugar beet the optimum level is 3x, sweet potato 6x while for timothy grass it is between 8-10x.
8. Autopolyploids generally have a lower dry matter content than diploids.

#### **Application of autopolyploidy in crop improvement**

##### **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 sugar beets. These two examples are described in some detail. Seedless watermelons are produced by crossing tetraploid (4x, used as female) and diploid (2x, used as male) lines, since the reciprocal cross (2x x 4x) is not successful. The triploid plants do not produce true seeds; almost all the seeds are small, white rudimentary structures like cucumber (*Cucumis sativus*) seeds. But few normal size seeds may occur which are generally empty. For good seed setting pollination is essential. For this purpose, diploid lines are planted in the ratio 1 diploid: 5 triploid plants. There are several problems *viz.* genetic instability of 4x lines, irregular fruit

shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production.

1. Triploid sugar beets: Among root crops triploid sugar beets apparently represent the optimum level of polyploidy because  $3n$  plants have longer roots than diploid and also yield more sugar per unit area.
2. Tetraploid rye: The advantage of tetraploid over its diploid counterpart are large kernel size, superior ability to emerge under adverse condition and higher protein content. Tetraploid rye varieties have been released for cultivation. e.g. Double steel, Tetra petkus.

#### **Limitations of autopolyploidy:**

1. Larger size autopolyploid generally contain more water and produce less dry matter content than diploids
2. High sterility with poor seed setting is observed
3. Due to complex segregation, progress through selection is slow
4. Monoploids and triploids cannot be maintained except through clonal propagation
5. The varieties cannot be produced at will
6. Effects of autopolyploidy cannot be predicted.

**Allopolyploidy:** Allopolyploids have genomes from two or more species production of allopolyploids has attracted considerable attention; the aim almost always was creation of new species. Some success has been evident from the emergence of triticale. *Raphano brassica* and allopolyploids of forage grasses.

#### **Morphological and cytological features of allopolyploids**

1. Allopolyploids combine the morphological and physiological characteristics of the parent species but it is very difficult to predict the precise combination of characters that would appear in the new species.
2. Many allopolyploids are apomictic eg: *Tulips, Solanum*
3. The chromosome pairing in the new species depends upon the similarities between the chromosomes of the parental species. Chromosomes with such similarities are known as homoeologous chromosomes. After chromosome doubling, the allopolyploid would have two homeologous chromosomes for each chromosome present in the  $F_1$  hybrid, comparable to the diploid species. Such allopolyploid is referred as amphidiploid or Allotetraploid.
4. Fertility of Allopolyploids can be improved by hybridization and selection.

#### **Application of allopolyploidy in crop improvement:**

1. Utilization as a Bridging species: Amphidiploids serve as a bridge in transfer of characters from one species to a related species, generally from a wild species to cultivated species. An

example of use of an amphidiploid as a bridging species in the use of synthetic *N. digluta* or transfer of resistance to tobacco mosaic virus from *N. glutinosa* to *N. tabacum*. The F<sub>1</sub> hybrid from the cross *N. tabacum* x *N. glutinosa* 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 complete somatic chromosome complement of *N. tabacum* and one genome of *N. glutinosa*. 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.

2. Creation of new crop species: Triticales, Raphanobrassica

3. Widening the genetic base of existing allopolyploids: The genetic base of some natural allopolyploids may be narrow, and it may be useful to introduce variability in such cases by producing the allopolyploids afresh from their parental species. *B. napus* is a case in point; the genetic variability of this species is narrow and the only recourse available is to synthesize new allopolyploid *B. napus* to widen its genetic base. This is being done by crossing *B. campestris* (n=10, AA) with *B. oleracea* (n=9, CC), the parental diploid species, to produce the amphidiploid *B. napus* (n=19, AACCC). 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**

1. The effects of allopolyploidy cannot be predicted. The allopolyploids have some features from both the parental species, but these features may be the undesirable ones, e.g., Raphanobrassica, or the desirable ones, e.g., Triticale.
2. Newly synthesized allopolyploids have many defects, e.g., low fertility, cytogenetic and genetic instability, other undesirable features etc.
3. The synthetic allopolyploids have to be improved through extensive breeding at the polyploidy level. This involves considerable time, labour and other resources.
4. Only a small proportion of allopolyploids are promising; a vast majority of them are valueless for agricultural purposes.

## Wheat Breeding

Wheat - *Triticum spp.* ( $x=7$ )

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. Basic chromosome no  $x=7$ , chromosome number: Diploid:  $2n = 14$  Tetraploid:  $2n = 28$  Hexaploid:  $2n = 42$

### Place of origin:

Diploid: Asia minor

Tetraploid: Abyssinia, North Africas

Hexaploid: Central Asia

Classification:

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

There are fourteen species of wheat according to Vavilov.



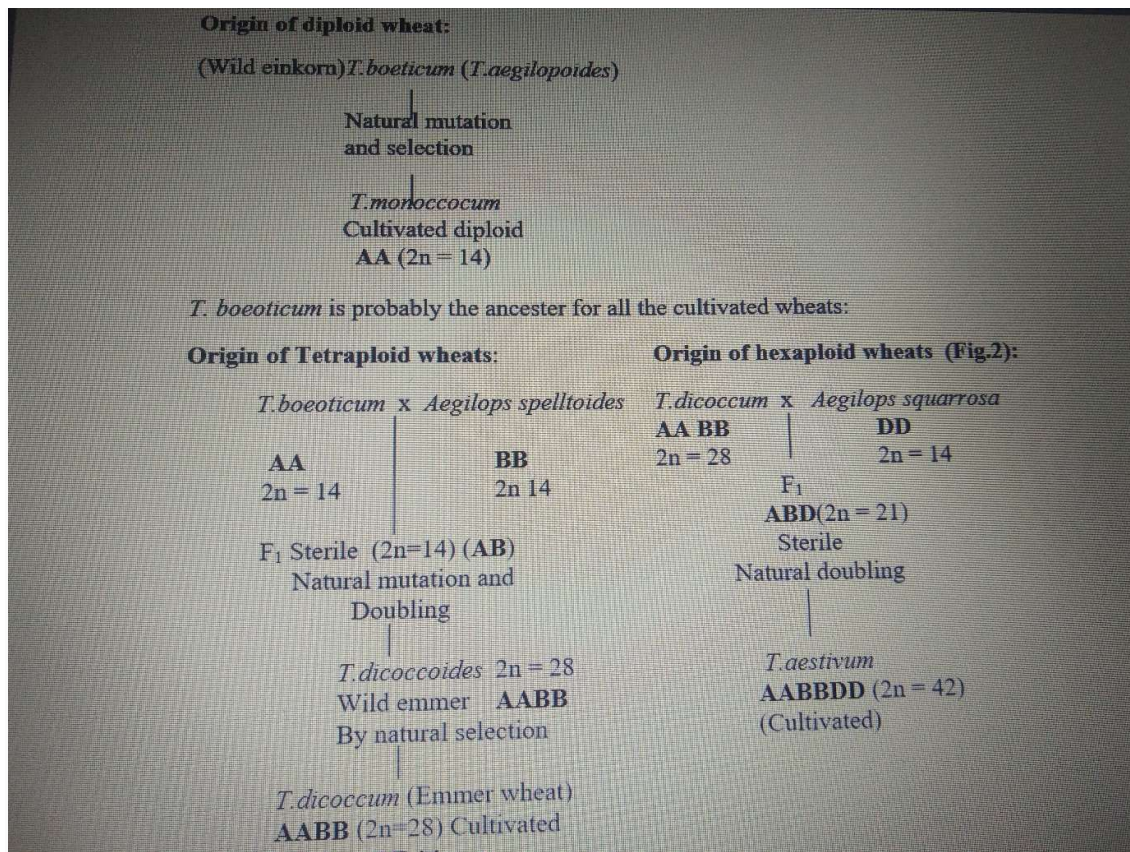


Fig:1 Origin of diploid, tetraploid and hexaploid wheat

Related Species of *Triticum*:

1. *T. boeoticum*: forms with one to two seeded spikelets occur. The brittle ears shatter at maturity into individual spikelets armed with awns which provide an effective means of seed dispersal.
2. *T. monococcum*: Primitive diploid form domesticated, evolved from *T. boeoticum* by mutation and selection.
3. *Aegilops speltoides*: (2n=14; B genome). It is naturally cross-pollinating. It is the recognized donor of the B genome.
4. *T. dicoccoides*: It is an amphidiploid form resulting from the hybridization of *T. boeoticum* and *Ae. speltoides*.
5. *T. dicoccum*: The spikes are dense, bearded and laterally compressed, the spikelets are two grained and the grains are retained within the glumes after threshing (speltoid). It is the oldest of the cultivated wheat.
6. *T. durum*: Free threshing wheat with naked grains, important of the tetraploid wheats. Grains contain high gluten.

7. *Ae. squarrosa*: ( $2n=14$ ; D genome) It is the source of D genome in the cultivated hexaploid wheat, high adaptability.

8. *T. spelta*: Hexaploid species, considered an amphidiploid from hybridization between *T. dicoccoides* and *Ae. squarrosa*.

The most important of all the hexaploid wheat is the common bread wheat, *T. aestivum* grown in all parts of the tropics and sub tropics. This hexaploid wheat from which most modern wheats have been developed. It exhibits an extremely wide range of morphological and physiological variation and ecological adaptation.

Breeding objectives

1. High yield: High yield depends on

- a) The number of heads / unit area
- b) The number of grains /head.
- c) The average weight of grain

While breeding for high yielding varieties all the above three components must be looked into. Omitting any one of them may not yield results. Further while breeding for high yield it is necessary to combine into a variety a favourable combination of genes influencing all yield process.

2. Breeding non- lodging varieties: This is achieved after the identification of dwarfing gene in Japanese variety Norin 10. Most of our dwarf wheats are two gene dwarfs. E.g. Sonora 63, Sonora 64, Kalyan Sona. Emphasis is now on triple gene dwarfs.

3. Breeding for disease resistance: Rust is the major disease. Both stem rust and leaf rust are important ones. There are different races of rust. So, while breeding for rust resistance horizontal resistance is to be looked into. Back cross method of breeding and development of multi lines are the methods.

4. Breeding for insect resistance: Hessian fly is the major pest. Resistance in most varieties is through antibiosis.

5. Breeding for quality. Different wheat varieties vary greatly in their chemical composition which is considerably influenced by environment. The varieties of hard wheat or bread wheat which have higher gluten content. The soft wheat contains lesser gluten content which is suitable for cake making, pastries. The durum wheats are unsuited for either cakes or bread but they are suitable for making macaroni. So, depending upon the use the quality breeding objective is to be fixed.

**Methods of breeding:**

**1. Introduction:** Semi dwarf wheat introduced from Mexico, Sonora 63, Sonora 64, Mayo 64, Lerma Roja 64

**2. Pure line selection:** Earlier varieties like P4, P6, P12 evolved at Pusa Institute are result of pure line selection from local population.

**3. Hybridisation and selection**

a) Inter varietal: A number of successful derivatives were developed at IARI New Delhi and Punjab. NP 809 - New Pusa multiple cross derivative. However, all these varieties were lodging and poor yielder when compared to other countries. Hence the wheat hybridization programme was changed by Dr. M.S. Swaminathan during 1963. Dr. N. E. Borlaug was invited to our country and he suggested for introduction of semi dwarf varieties from Mexico. As a result, four commercial spring wheat varieties viz., Sonara 63, Sonara 64, Mayo 64 and Lerma Roja 64 were introduced. However, they had red kernel hard wheats. These were utilised in our breeding programme and amber colour wheat varieties like Kalyan Sona, Safed Lerma, Sharbati Sonora were released, these are double gene dwarfs.

b) Inter specific crosses: To get resistance against Hessian fly and also for rust resistance.

c) Back cross method of breeding: Rust resistance in Chinese spring wheat was introgressed from Thatcher.

**4. Hybrid wheat:** At Kansas Agri. Expt. Station USA, male sterile lines were identified by crossing *T. timophevi* x *T. aestivum* Bison variety

By repeated back crossing, a male sterile line resembling Bison variety was developed. At present USA and Canada are doing work on this.

**5. Mutation breeding:** Dr. M. S. Swaminathan did extensive work on this with gamma rays. Sharbati Sonora with increased protein content was evolved.

**6. Development of multiline varieties:** Borlaug developed multiline varieties against rust. MLKS 15 was developed at IARI. Multiline variety is a mixture of pure lines which are phenotypically similar but genotypically dissimilar. Each line is produced by separate back cross method of breeding. Each line having resistance against a particular race of a disease.

**7. Wheat genetic engineering:** Genetic engineering opened up new opportunities for plant breeders by enabling them to incorporate genes isolated from organisms outside the gene pools to which they usually have access. Despite huge promise and potential of transgenic wheat, the approach to transgenic wheat commercialization by private sector multinational seed companies appears to be cautious.

**8. Molecular markers and wheat breeding:** Resistance to Fusarium head blight is governed by QTL *Fhb1* and near-isogenic lines carrying a resistant allele at QTL *Fhb1* have

been developed using the resistant cultivar “Sumai3,” which showed a 23% reduction in disease severity. A marker linked to the “Sumai3” allele at *Fhb1* showed a large phenotypic effect on resistance across different populations (Pumphrey et al., 2007) and was used in wheat breeding programs across the world (Haberle et al., 2009).

### **International Programme**

International Centre for Maize and Wheat Improvement (CIMMYT) is an international non-profit, scientific research and training organization at Mexico and offices at 20 different locations all over the world operates a global programme on maize, wheat and triticale improvement, investigates economic issues related to these crops and supports national programmes in developing countries.

### **National Programme**

All India Coordinated Wheat Improvement Project established in 1965, was upgraded to Project Directorate in 1978. The headquarter of Directorate of Wheat Research (DWR) was at IARI, New Delhi till 1991 and afterwards shifted to Karnal, now known as Indian Institute of Wheat and Barley Research. The project undertakes integrated, multidisciplinary and multilocational research programmes to increase overall productivity of wheat at national level. This is sought to be achieved by developing high yielding varieties with crop production and protection technologies.

## **Barley Breeding**

Barley (*Hordeum vulgare* L.) is one of the oldest of the cultivated cereals and is widely grown in many climates of the world. Barley ranks fourth among the cereals worldwide in the production of grain, after wheat, corn, and rice. The major production areas are northern and western Europe and the USSR, where seasons are too short or too dry to grow feed grains such as corn or sorghum. Barley is tolerant of alkali, drought, or frost. Barley grows in the arid climates of the Sahara, the high plateaus of Tibet, and the tropical plains of India, but it produces best where fertility is favourable and the spring seasons are long and cool. It does not mature well in hot humid weather. Barley has developed a diversity of head and seed types, disease resistance, and quality characteristics. Many improvements have been made in the cultivated varieties of barley by breeding. Barley is mostly used for feed and malt industry as it can not be used for bread making because of low gluten content.

## **Origin**

The Near East is considered as the origin of barley. Barley together with emmer wheat was the first cereal to be domesticated in the Middle East, at least 9000 years ago. The first archeological material of barley was two rowed barley which is closely resemble with some races of wild barley, i.e. *Hordeum spontaneum*. This wild species crosses readily with cultivated barley and is the progenitor species of *H. vulgare* (Helback, 1966; Harlan, 1975).

## **Taxonomy**

The genus *Hordeum* contains 28 species with a basic chromosome number of  $x=7$ . Depending upon the level of ploidy, the species are grouped as diploid ( $2n=2x=14$ ), tetraploid ( $2n=2x=28$ ) and hexaploid ( $2n=2x=42$ ).

The diploid species of barley:

Cultivated species: *H. vulgare*

Wild species: *H. chilense*, *H. comosum*, *H. muticum* and *H. pusillum*

Tetraploid barley species: *H. capense* and *H. secalinum* (all wild spp.)

Hexaploid species: *H. arizonicum* and *H. lechleri* (all wild spp.)

A species named as *H. bulbosum* exists both as diploid and polyploid and used to produce haploid barley embryos.

*H. vulgare* is only cultivated species with two phenotypic forms, viz. two rowed and six rowed barley based on ear morphology. Earlier these two forms were grouped into two separate species but now these has been grouped into single species *H. vulgare* because these two have same chromosome number, intercross freely and produce fertile hybrids.

## **Cytology**

There are seven pairs of chromosomes in cultivated barley whereas in wild species, chromosome no is 7, 14 and 21 pairs. Chromosomes are relatively large, easily distinguished at metaphase of mitosis. Chromosome 1 is the longest and chromosome 5 is the shortest chromosome, chromosome 6 and 7 are satellite chromosomes. Chromosome 6 has larger satellite and 7 has shorter satellite. The chromosomes are median to submedian (Nilan, 1974).

Kasha and Kao (1970) reported the use of wild cross-pollinated barley species *H. bulbosum* for producing haploids in common barley. The crosses are made between *H. vulgare* and *H. bulbosum* and shortly after fertilization the embryo is removed and cultured on artificial medium in a test tube. The chromosomes of wild species are selectively eliminated, resulting thereby in a haploid embryo which is sterile unless diploid chromosome number is restored by treating the haploid seedlings with a 0.1 percent colchicine solution for five hours in the two to three leaf stage.

Three complete sets of primary trisomics ( $2n+1$ ) have been developed in barley (Nilan, 1974). These trisomics offer one of the most effective methods of associating genes with their respective chromosomes.

### **Breeding objectives**

- **Yield improvement:** To enhance the production, breeding for high yield is the first and foremost objective of barley breeders.
- **Increased adaptability:** Barley does not perform well in hot and humid weather. So, to increase adaptability, barley varieties are to be developed for hot and humid weather conditions.
- **Resistance to yellow rust, aphid and nematode:** Breeding for biotic stresses put major focus on developing varieties resistant to major diseases and pests of barley. Due to various races of yellow rust, rust resistant varieties should be developed to increase the production. Corn leaf aphid is the major pest of barley and resulted in yield reduction upto 25 per cent. Due to the development of hull less varieties of barley, this problem is expected to aggravate further as hull less varieties are damaged more as compared to hulled varieties.
- **Improvement in nutritional quality:** Developing varieties with high lysine and crude protein content is one of the breeding objective of barley breeders. The correlations between crude protein-grain yield and lysine- grain yield were negative.
- **Improvement in traits related to malt industry:** Barley is preferred grain for malt production due to tightly adhering lemma and pericarp which protects coleoptile during malting process which prevents damage to tender shoot, hulls form natural filter and aid in

separating soluble materials from undissolved particles in the production of wort, due to firm texture grains can be handled with less damage at high moisture level and conversion of starch to dextrins and fermentable sugars is more efficient due to the combined presence of alpha and beta amylases.

### **Methods of breeding**

#### **1. Introduction:**

Direct Introduction: Clipper

Secondary Introduction (Selection in other collections): Dolma, Sonu (HBL 87), LSB2

**2. Pure line selection in local material:** Earlier varieties like NP13, NP21, NP100, T4, T5, K12, K14, C50, C84, CN292, CN294 are result of pure line selection from local population.

#### **3. Hybridisation and selection**

a) Inter varietal: i.) A number of successful derivatives were developed by intercrossing within indigenous germplasm (tall types). BR32, DL157, K18, K19, K24, K70, K71, K125, K141, K169, K225

ii.) Tall type varieties developed through intercrossing between indigenous and exotic germplasm include BG25, BG105, BG108, C144, C155, C164, DL100, DL260, DL349, DL353, NP103, NP104, NP105, NP106, NP 109

iii.) Semidwarf varieties developed by crossing indigenous and exotic germplasm. e.g. DL70, DL85, DL88, DL171, P100, P103, P267, RD118

iv.) Semidwarf varieties were developed by crossing within exotic germplasm. e.g. BH87

b) Back cross method: Backcrossing had historical significance as its first application was in breeding of smooth awned varieties of barley.

**5. Mutation breeding:** Barley responds favourably to mutagenic treatments due to its diploid nature and low chromosome number. The old classical experiment of Stadler on induction of mutation was on barley. Use of mutations in barley is now limited to resistance to diseases and simply inherited traits like earliness and dwarfness etc. Barley varieties developed through mutation breeding included PL56, DL253 and RDB1

**6. Molecular markers and barley breeding:** In barley, MAS has been employed for the management of stripe rust caused by *Puccinia striiformis* Westend. f. sp. *hordei*, an important disease of barley worldwide. Several qualitative and quantitative genes conferring resistant against barley stripe rust have been reported by many workers. Three QTLs (QTL4, QTL5, and QTL7) were identified on chromosomes 4, 5, and 7, respectively. Castro et al. (2003) pyramided these three QTLs and studied their effect on resistance against the disease at the seedling stage. The parents used in the study were Orca, Harrington, and D1-72. Orca,

a barley cultivar derived from the cross between Calicuchima-sib and Bowman, was the source of QTL4 and QTL7. Harrington is a two-row malting barley cultivar. D1-72 is a line derived from the cross between Shyri and Galena population that has a resistance allele at QTL5 tracing to Shyri. MAS was performed for resistance alleles at QTL4 and QTL7 in the BC<sub>1</sub> generation (HarringtonOrca). Four BC<sub>1</sub> plants with Orca alleles at marker loci flanking QTL4 and QTL7 were crossed with D1-72. DH lines were derived from the F<sub>1</sub> plants of these crosses. Phenotyping of the parents and the DH lines was done using three races of the fungus: PSH-13, PSH-14, and PSH-71. Genotypic screening for the polymorphism was done using 14 SSR markers. The absence of resistance alleles at both QTL4 and QTL5 was associated with the susceptible phenotype, which validated the effect and location of two QTLs, QTL4 and QTL5, each tracing from a different parent. However, QTL7 had no effect on barley stripe resistance at the seedling stage.

### **International Programme**

Barley is mandated crop of the International Centre for Agricultural Research in the Dry Areas (ICARDA), located at Aleppo, Syria. It was established in 1976 with purpose to increase and stabilize food production in developing countries of temperate zones having arid and semi-arid climate.

### **National Programme**

Till mid-sixties, barley improvement programme in India was carried out by individual states, organizations and the scientists independently with little chance of mutual cooperation and the evaluation of varieties of one state into other states. In order to rectify these limitations, ICAR launched All India Coordinated Barley Improvement Project in 1967. It is mandated crop of Indian Institute of Wheat and Barley.



### **Chickpea breeding**

Chickpea (*Cicer arietinum* L.) is cultivated in almost all parts of the world covering Asia, Africa, Europe, Australia, North America and South America continents. Chickpea, a member of Fabaceae, is a self-pollinated true diploid ( $2n = 2x = 16$ ) with genome size of 738 Mbp (Varshney et al. 2013). It is an ancient cool season food legume crop cultivated by man and has been found in Middle Eastern archaeological sites dated 7500–6800 BC (Zohary and Hopf, 2000). Its cultivation is mainly concentrated in semiarid environments (Saxena, 1990). India ranks first in the world's production and area by contributing around 70.7 % to the world's total production (FAOSTAT, 2011). It is one of the most important food legume plants in sustainable agriculture system because of its low production cost, wider adaptation, ability to fix atmospheric nitrogen and fit in various crop rotations (Singh, 1997) and presence of prolific tap root system. It also helps in enhancing the soil quality for subsequent cereal crop cultivation by adding organic matter for the maintenance of soil health and ecosystem.

It is a rich source of quality protein (20–22 %), has the highest nutritional compositions and free from anti-nutritive components compared to any other dry edible grain legumes, and thus, it is considered a functional food or nutraceutical. Besides proteins, it is rich in fibre and minerals (phosphorus, calcium, magnesium, iron and zinc), and its lipid fraction is high in unsaturated fatty acids (Williams and Singh, 1987). It has no anti-nutritional factors (Mallikarjuna et al., 2007) and contains higher amounts of carotenoids like  $\beta$ -carotene than genetically engineered 'golden rice' (Abbo et al. 2005). This plant holds a good reputation in 'Ayurvedic' and 'Unani' systems of medicine. In India, acid exudates from the leaves were used medicinally for aphrodisiac, bronchitis, cholera, constipation, diarrhoea, dysentery, snakebite, sunstroke and warts. It also has the property to act as hypo-cholesteremic agent; germinating chickpea is believed to reduce the blood cholesterol level. Sprouted seeds are eaten as a vegetable or salad. Young leaves and stems and green pods are eaten like vegetables. Leaves yield an indigo-like dye. The dried seeds may be used in soups or after grinding as flour. Grain husks, stems and leaves may be used in livestock feed. In the USA and Europe, chickpeas are marketed dried, canned or in various vegetable mixtures. Mashed chickpea mixed with oils and spices (hummus) is a popular hors d'oeuvre in the Mediterranean Middle East.

### **Origin**

Vavilov (1926) supported the idea of Southwest Asia and the Mediterranean region being the primary centres of origin, with Ethiopia as the secondary centre. van der Maesen (1987)

suggested that Anatolia in Turkey was the area where chickpea was believed to have originated because three wild species of *Cicer* closely related to chickpea are found here namely *C. bijugum* K. H. Rech, *C. echinospermum* P. H. Davis, *C. reticulatum* Lad. *C. reticulatum* can be regarded as probable progenitor of chickpea or may be presumed to have common ancestor with chickpea.

### **Taxonomy**

The *Cicer* genus belongs to family Leguminosae, subfamily Papilionaceae and tribe Cicereae Alef. The *Cicer* genus currently comprises 43 species, out of which 9 are annual and 34 are perennial species (Muehlbauer et al. 1994). Most of these species are found in West Asia and North Africa, covering Turkey in the north to Ethiopia in the south and Pakistan in the east to Morocco in the west. Of the 9 annual *Cicer* species, *C. arietinum* is the only cultivated species. The eight **other annual *Cicer* species** are *C. reticulatum*, *C. echinospermum*, *C. pinnatifidum*, *C. judaicum*, *C. bijugum*, *C. cuneatum*, *C. chorassanicum* and *C. yamashitae*.

The wild annual progenitor of chickpea has been identified as *C. reticulatum* L. (Ladizinsky and Adler, 1976), and the perennial progenitor is proposed as *C. anatolicum* (Tayyar and Waines, 1996). The *Cicer* species, including cultivated and wild, have been **classified into four sections based on their geographical distribution, life cycle and morphological characteristics** (van der Maesen, 1987).

**Monocicer section:** The 8 annual species, namely, *C. arietinum*, *C. reticulatum*, *C. echinospermum*, *C. pinnatifidum*, *C. bijugum*, *C. judaicum*, *C. yamashitae* and *C. cuneatum*, were grouped in this section. These species are annual having small flowers with firm, erect to prostrate stems.

**Chamaecicer section:** This section includes annual or perennial shrubby species with thin creeping branches. e.g. *C. chorassanicum* and *C. incisum* (perennial species) in,

**Polycicer section:** 23 perennial, rather large flowered species with imperripinnate leaves or rachis ended in tendril and

**Acanthocicer section:** The remaining 7 woody perennial species with large flowers and persistent spiny leaf rachis and spiny calyx teeth.

Based on seed size and colour, cultivated chickpea are of two types (Cubero, 1987):

- **Macrosperma (kabuli type):** Large seeded (100 seed weight >25g), round or ramhead, cream coloured. The plant is medium to tall in height, with large leaflets and white flowers, and contain no anthocyanin.

- **Microsperma (desi type):** Seeds of this type are small and angular in shape. Seed colour varies from cream, black, brown, yellow to green. There are 2-3 ovules per pod but on an average 1-2 seeds per pod produced. The plants are short with small leaflets and purplish flowers and contain anthocyanin.

Two types of chickpea cultivars are recognized globally – kabuli and desi. The kabuli types are generally grown in the Mediterranean region including Southern Europe, Western Asia and Northern Africa, and the desi types are grown mainly in Ethiopia and Indian subcontinent.

### **Cytology**

Chickpea has  $2n=16$ . All *Cicer* species except *C. pungenis*, *C. montbretii*, *C. songaricum* and *C. anatolicum* have  $2n=16$ . The diploid chromosome number was reported to be 14 in *C. pungenis*, both 14 and 24 in *C. montbretii*, and 14 and 16 in *C. songaricum* and *C. anatolicum*. Gupta and Bahl (1983) reported that chickpea has a pair of very long chromosome, distinctly satellite and sub metacentric. Six pairs of metacentric and submetacentric chromosomes and a pair of very short metacentric chromosomes.

### **Genepool**

van der Maesen et al. (2007) proposed recent classification in which **primary gene pool** consists of cultivated species and landraces. The **secondary gene pool** consists of the progenitor species, *C. reticulatum* and *C. echinospermum*, the species that are crossable with *C. arietinum* but with reduced fertility of the resulting hybrids and progenies; nevertheless, both are cross-compatible with the cultigen and do not need *in vitro* interventions to produce hybrids. The **tertiary gene pool** consists of all the annual and perennial *Cicer* species that are not crossable with cultivated species.

### **Breeding objectives**

**Increased seed yield:** Developing high yielding varieties is the main objective of crop improvement programme. The yield potential of chickpea is almost static for the last decade. To increase productivity varieties with higher yield potential are required to be developed.

**Increase biomass, tall, erect and compact cultivars:** Increased biomass is resulted in increased yield due to more photosynthesis. Similarly, with the development of compact and erect varieties, planting density can be increased and such varieties are also suitable for mechanical harvesting.

Resistance to diseases: *Fusarium* wilt is the most devastating disease of chickpea and yield losses upto 90% has been reported in susceptible cultivars. So, developing varieties resistant to *Fusarium* wilt is major objective of chickpea breeding programme.

Another disease *Ascochyta* blight is major disease of chickpea prevalent in north west India. Developing *Ascochyta* blight resistant varieties to enhance the productivity is one of the objectives of chickpea improvement programmes. Botrytis grey mold is an important foliar disease of chickpea and three types of root rot diseases affect chickpea in dry areas.

Resistance to insects: Gram pod borer is the major insect pest of chickpea globally due to high mobility, polyphagy, high reproductive rate and wide host range. Yield losses due to this pest ranges from 18% to 24%. Sources of resistance are lacking in cultivated chickpea germplasm. Resistance sources are available in germplasm belonging to tertiary gene pool i.e. in *C. bijugum* which can be used provided cross ability barriers overcome.

Breeding for abiotic stress tolerance: Drought and salinity are major abiotic stresses in chickpea cultivation. developing drought and salinity tolerant varieties is important objective of chickpea breeding programmes.

Breeding methods: Being highly self-pollinated crop, the breeding method used in developing improved varieties will be same as used in other self-pollinated crops like wheat, barley etc.

### **Commercial cultivars**

In the 1970s, most of the commercial varieties were developed through selection from landraces. The major emphasis was given on increasing yield potential.

During the 1980s, the focus was laid on breeding for disease resistance. Consequently, several varieties (Avarodhi, JG 315, Pusa 209, GNG 16, Pusa 212, Pusa 240, Pusa 244, Pusa 256, Pusa 413, ICCV 10, ICCV 37, Phule G 5, Phule G 12, etc.) resistant/tolerant to *Fusarium* wilt were developed and released for their cultivation in different regions of the country (Chaturvedi et al. 2003).

Similarly, genotypes tolerant to *Ascochyta* blight, namely, C235, Gaurav, H 75-35, BG 261, PBG 1, GNG 146 and PBG 5, were developed for North West Plain Zone (Delhi, Punjab, Haryana, North Western Rajasthan and Western UP) and G 543 and PBG 5 for the state of Punjab (Sandhu et al. 2004).

However, during the 1990s, the major thrust was given on breeding for multiple disease resistance and high-input responsive varieties. Sources for drought tolerance (RSG 44, RSG 963, RSG 888, ICC 4958, ICCV 10, Vijay, GL 769, GPF 2, PDG 3, PDG 4, Phule G 5), cold tolerance (ICCV 88506, ICCV 88503, Phule G 96006, ICC 8923, PDG 84-10, GL 28008,

GL 28028) and salt tolerance (CSG 88101, CSG8962) were identified for their use in breeding programme. As a result, multiple disease-resistant varieties, namely, Bharati, Pusa 372, Pusa 362, BG 391, KWR 108 and GNG 1581 against wilt and root rot and GNG 469 against *Ascochyta* blight and root rot, and high-input responsive variety like DCP 92-3 were released for cultivation. Rice fallows (about 11.0 m ha) in Eastern India (eastern UP, Bihar, West Bengal, Orissa, Jharkhand and Assam) and Central India (eastern MP and Chhattisgarh) provide opportunities for horizontal expansion of area under chickpea.

The development of short-duration varieties like ICCV 2, JG 74, Vijay, JG 11, JG 16, JAKI 9218 and KAK 2 was the major catalyst for the expansion of chickpea area in Southern and Central India. In spite of reduction in duration, the yield potential of these early maturing varieties remains almost unaffected, thus improving per day the productivity of the crop.

Presently emphasis has been laid on the development of extra-large seeded kabuli chickpea varieties (>50 g/100 seed weight).

A major breakthrough has been witnessed in developing large seeded kabuli varieties with high- yield potential such as KAK 2, BG 1003, BG 1053, JGK 1, Phule G 95311, IPCK 2002-29, IPCK 2004-29, L 555 and HK 05-169 (Chaturvedi et al. 2010). Similarly, prominent large seeded desi varieties, viz. BG 256, Phule G 5, BGM 391, K 850, Radhey, Gujarat Gram 2 and L 556, were also developed.

### **International Programme**

Two international crop research institutes viz., ICRISAT (International Crop Research Institute for Semi-Arid Tropics) at Patancheru, India and ICARDA (International Centre for Agricultural Research in Dry Areas) at Aleppo, Syria are engaged in research on chickpea improvement. Desi type chickpea comes under ICRISAT and kabuli type is under both ICRISAT and ICARDA.

### **National Programme**

All India Coordinated Pulses Improvement Project including chickpea was started in 1967-68 which was upgraded to Project Directorate in 1977 and to Directorate of Pulsed Research (DPR), at Kanpur in 1984. Now DPR is known as Indian institute of Pulses Research (IIPR) w.e.f. 1993. An independent all India coordinated project on chickpea was started in 1993.

## Lentil breeding

Among the cool season legume crops, lentil production has displayed the greatest increase, while cropping of many other grain legumes is actually in decline. This increase is most likely due to a convenient fast cooking coupled to a saving of fuel and time – dehulled lentils cook even faster than milled rice. Worldwide lentil consumption has augmented more than twice the rate of human population growth, with lentil consumption over the past 40 years having increased more than any other food crop. Therefore, continued genetic improvement of lentil is essential in all production regions; nevertheless, without access to and use of diverse germplasm, prospects for yield genetic gain in lentil may be difficult to accomplish. Wild *Lens* accessions represent less than 1 % of the world germplasm collection of this genus, this small fraction likely representing the greatest untapped pool of genetic variation in lentil. Germplasm evaluation together with a successful and targeted hybridization allows for an efficient breeding and selection of varieties adapted to specific environments.

## Origin

The oldest remains of wild lentils have been found in Mureybit (Syria) dated around 10,000 BP, while those of the cultigens have been dated around 9,000 BP and were discovered in aceramic Neolithic layers located in the Near East (see Cubero et al. 2009 for archaeological data). Given the coexistence of the wild and domesticated forms which is not found elsewhere, coupled with archaeological evidence, the Fertile Crescent is the most likely candidate to be the center of origin of the cultivated lentil. Ladizinsky (1999) also suggested the Near East as the center of origin based on the polymorphism found in wild accessions of *ssp. orientalis* and the monomorphism of *culinaris*. Yadun et al. (2000) suggested a lentil domestication site close to or overlapping the area where einkorn and emmer wheats had been domesticated in the Fertile Crescent, concretely, the Karacadag/ Diyarbakir region as the domestication center of lentil.

Furthermore, two centers of diversity for *L. culinaris ssp. orientalis* have been described: (1) Southeast Turkey and Northwest Syria and (2) West and North Jordan and southern Syria (Ferguson et al. 1998).

Compared to *L. orientalis*, cultivated lentils have a greater stem and rachis length, more leaflets per leaf, a greater leaf area, an increased number of flowers per peduncle, as well as an increased number of pods and seeds. In addition, peduncles of the cultivated forms are generally shorter or equal in length to the rachis when compared to the wild forms. Aside from all of the above, pod indehiscence of the cultivated forms in contrast to the dehiscent mode of the wild species, together with the erect growth habit of the cultivated lentil

compared to the wild procumbent and prostrate plant structure, typifies the most noticeable differences between the domesticated and wild relatives, being traits of great economic value and with many implications on yield and ease of harvesting. All of the above mentioned characters are strongly associated with increased yield levels, in a similar way as observed for the other domesticated food legumes. Lentil cultigens show a higher frequency of white flowers compared to the dominant purple-colored inflorescences of the wild relatives, most probably a character associated to a better culinary quality and fixed by indirect selection of lighter-colored seed coats (Fratini et al. 2011).

### **Taxonomy**

Taxonomic analyses based on morphological and/or biochemical markers ranged from four species in 1979, namely, *L. culinaris*, *L. orientalis*, *L. nigricans*, and *L. ervoides*, to two species in 1984: *L. culinaris* (with subspecies *culinaris* (the cultigen), *orientalis*, and *odemensis*) and *L. nigricans* (with *ssp. nigricans* and *ervoides*); again to four species in 1993, i.e., *L. culinaris* (*ssp. culinaris* and *orientalis*), *L. odemensis*, *L. nigricans*, and *L. ervoides*; and to the previous four species plus the addition of *L. tomentosus* and *L. lamottei* to comprise a total of six species in 1997.

The number of six species is now widely accepted: *L. culinaris* (*ssp. culinaris*, the cultigen and *ssp. orientalis* (Boiss.) Ponert, the wild ancestor of the cultivated forms), *L. odemensis* (Godr.) Ladiz., *L. tomentosus* Ladiz., *L. nigricans* (Bieb.) Godr., *L. ervoides* (Bring.) Grande., and *L. lamottei* Czfr. The geographical distribution of the *Lens* species which are all self-pollinated is described by Cubero et al. (2009).

Cultivated lentil (*L. culinaris ssp. culinaris*): The cultivars are grouped into two intergrading clusters- small seeded lentil (microsperma) with small pods and seeds diameter 3-6mm and large seeded lentils (macrosperma) with large pods and seeds size of 6-9mm.

Wild species: are delicate, small flowered annuals distributed over south west Asia and Mediterranean basin.

### **Cytology**

All *Lens* species possess the same chromosome number ( $2n=14$ ) and share similar karyotypes, which consists of three pairs of metacentric and submetacentric chromosomes, three pairs of acrocentric chromosomes, and a pair of metacentric chromosomes with the nucleolar organizing region (NOR) secondary constriction site proximal to the centromere location (Ladizinsky 1993), although some karyotype variants have been described (Balyan et al., 2002).

### **Gene pools**

Besides of the forms of the cultigen (*L. culinaris ssp. culinaris*), *L. culinaris ssp. orientalis* obviously belongs to the primary gene pool and *L. odemensis* to the secondary, although success of *L. odemensis* crosses with the cultigen may or may not require embryo rescue depending on the specific accessions used. The tertiary gene pool is composed of *L. tomentosus*, *L. lamottei*, *L. nigricans*, and *L. ervoides*, but these can become part of the secondary gene pool by means of embryo rescue. In any case, further hybridization studies are needed to truly establish whether *L. tomentosus* and *L. lamottei* belong definitely to the secondary or tertiary gene pool (Cubero et al. 2009).

### **Breeding Objectives**

- **High yield:** Plant height is strongly associated to yield index. Plants of erect and a very tall habit tend to lodge as they approach maturity; thus, erect varieties of medium height should be expected to be more lodging resistant, which results in higher yields and also to a more amenable mechanical harvesting. Flower number per peduncle in lentil may retain significance in relation to productivity potential.

- **Bold seed size, high protein and less cooking time**

Seed size, protein content and cooking time are the most important quality parameters of lentil. There is negative correlation between yield and protein content and a positive correlation between seed size and cooking time. Seed size can be used to predict cooking quality and close relationship between seed size and cooking time eliminates the need for screening early generation lentil genotypes for cooking time (Erskine et al., 1985).

According to Sharma (2011), in most crops, especially cereals, protein content is invariably negatively correlated to seed size; however, lentil is possibly an exception and protein content has been claimed to be positively (although mildly) correlated to seed size.

- **Early maturity:** Earliness is a desirable trait ensuring completion of the crop cycle in a relatively short period of time, thereby making more efficient use of resources as well as avoiding losses due to high temperatures during crop maturation.

- **Resistance to diseases: *Ascochyta* blight, rust and wilt**

*Ascochyta* blight, rust and wilt are the serious diseases of lentil in India. A number of resistance sources for these diseases have been identified and multiple disease resistant varieties have been developed. Pant L406 is resistant to wilt and rust.

- **Resistance to insects: Pod borer, aphids and cutworms**

Pod borers, cut worms and aphids are occasionally serious pest of lentil. A few lines are reported to be free from pod borer infestation are P202, P248, P262, P772, P803, P927.



- Resistance to shattering

Reduced shattering is a trait of great economic value as pod dehiscence can cause significant losses before or during harvest; the trait is considered to have played a major role in lentil domestication.

- Tolerance to drought: Lentil has been traditionally grown in semiarid regions under rainfed conditions; thus, it combines a high degree of drought resistance and a low water requirement; in fact, excessive water supply is damaging to the crop.

### **Breeding methods**

Breeding methods used for developing improved varieties of lentil are similar to those of other self-pollinated crops.

**Selection:** Breeding history of released varieties showed that majority of these varieties are selection from germplasm and it is evident that Pureline selection among locally adapted germplasm lines has been very successful.

**Hybridization:** Varietal hybridization followed by application of pedigree, bulk methods or their modifications are now increasingly being used. The trends are in favour of bulk and SSD methods where segregating populations are handled in bulk till F<sub>5</sub>/F<sub>6</sub> generations and single plant selections are made in F<sub>5</sub>/F<sub>6</sub> to be grown as individual plant progeny row followed by further selection among rows. The bulk harvest of selected progeny rows (each row to be separately bulked) are evaluated in yield trails.

#### **Interspecific hybridization**

Artificial cross-pollination in a highly self-pollinated crop species, such as lentil, is important to increase genetic variability. Wide crosses to yield interspecific hybrids allow for the introgression of important alleles of agricultural interest from wild species to cultivars, as, for instance, the resistance or tolerance to abiotic and biotic stresses (Erskine et al. 1994; Ocampo et al. 2000; Davis et al. 2007; Cubero et al. 2009; Pérez de la Vega et al. 2012). Lentil is generally described as a strictly self-pollinated species holding cleistogamous flowers (Wilson 1972; Kumar and Singh 1998); nonetheless, recent studies point out that natural outcrossing rates of lentil can be relatively high (Horneburg 2006).

In the case of interspecific hybridization, crossing efficiencies are much lower than intraspecific ones, for instance, out of a total of 1,707 pollinations, six interspecific hybrids with *L. odemensis*, two with *L. nigricans*, and one with *L. ervoides* were recovered using embryo rescue (Fratini and Ruiz, 2006). Therefore, a large number of manual pollinations are needed to recover each single interspecific hybrid.

With regard to inter-subspecific hybrids of lentil, it has been reported that the domesticated lentil is readily crossable with subspecies *orientalis* (Ladizinsky 1979; Muehlbauer et al. 1989; Vandenberg and Slinkard 1989; Vaillancourt and Slinkard 1992; Fratini et al. 2004), although the fertility of the hybrids depends on the chromosome arrangement of the wild parent (Ladizinsky 1979; Ladizinsky et al. 1984).

Interspecific embryos between cultivated lentils and either *L. ervoides* or *L. nigricans* abort (Abbo and Ladizinsky 1991, 1994; van Oss et al. 1997; Fratini and Ruiz 2006) and embryo rescue techniques are necessary to recover hybrids (Cohen et al. 1984; Ladizinsky et al. 1985; Fratini and Ruiz 2006; Fiala et al. 2009). Nonetheless, gibberellic acid (GA 3) application after pollination aided to develop viable pods and interspecific *Lens* hybrids were obtained without the need of in vitro embryo culture (Ahmad et al. 1995).

**Mutation Breeding:** Mutation breeding can also be applied in lentil. The n- nitroso-n-methyl urea (NMU) was almost three time more effective and efficiency was 1.5 to 2 times higher than gamma rays (Sharma and Sharma, 1979).

#### Molecular markers

Lentil is a relatively minor crop compared to common bean, pea, and chickpea; as a result, genomic information regarding lentil is still limited by a relatively large genome size together with scarce information available on gene sequences, constituting a major obstacle to undertake genomic studies in lentil. So far for lentil, there exist no descriptions of a bacterial artificial chromosome (BAC) library, BAC-end sequence, or a physical map (Ford et al. 2007, 2009; Varshney et al. 2009; Pérez de la Vega et al. 2012). In comparison to other crop species, the number of *Lens* data indexed continues to be scarce, although this situation is rapidly changing, in particular for nucleotide-expressed sequence tags (ESTs) (Kaur et al. 2011; Bhadauria et al. 2013; Sharpe et al. 2013; Verma et al. 2013).

Lastly, the development of a deep and diverse transcriptome resource for lentil using next-generation sequencing technology allowed to generate data in multiple-cultivated (*L. culinaris*) and wild (*L. ervoides*) genotypes, which together with the use of a bioinformatics workflow enabled for the identification of a large collection of SNPs and SSR markers for the subsequent development of a genotyping platform that was used to establish the first comprehensive genetic map of the *L. culinaris* genome, comprising seven linkage groups corresponding to the number of chromosome pairs of lentil (Sharpe et al. 2013).

#### **International Centre**

International Centre for Agricultural Research in Dry Areas (ICARDA) is actively engaged in research on lentils. With a conservation capacity of more than one thousand accessions,

the International Centre for Agricultural Research in the Dry Areas (ICARDA; <http://www.icarda.org>), the Indian Agricultural Research Institute (<http://www.iari.res.in>), the N I Vavilov Research Institute of Plant Industry ( <http://www.vir.nw.ru>), the National Bureau of Plant Genetic Resources, India, and finally the USDA collection at the Regional Plant Introduction Station, Pullman, Washington (<http://www.ars.usda.gov>) stand out. Another national germplasm bank holding an important collection of lentils is the Spanish Plant Genetic Resource Center (<http://www.inia.es>) with more than 600 accessions. A complete picture of the genetic resources available can be obtained at the germplasm collection directory web page of Bioversity International (<http://www.bioversityinternational.org/>), which integrates information regarding most of the world germplasm collections.

### **National Programme**

All India Coordinated Pulses Improvement Project including lentil was started in 1967-68 which was upgraded to Project Directorate in 1977 and to Directorate of Pulsed Research (DPR), at Kanpur in 1984. Now DPR is known as Indian institute of Pulses Research (IIPR) w.e.f. 1993. An all India coordinated project on MULLaRP (mungbean, urdbean, lentil, lathyrus, rajmash and field pea) was started in 1993.

## MUSTARD and RAPE SEED

*Brassica spp.* ( $2n = 16, 18, 20, 22, 36, 38$  and  $48$ )

Oilseed Brassicas, rapeseed and mustard are the third most important edible oil source in world after soybean and palm and in India Brassicas rank second after groundnut. Because of their ability to germinate and grow at low temperature, the oilseed Brassicas can be grown in cooler areas and at higher elevations. Cultivated *Brassicas* can be broadly divided in to two distinct types *viz.* Vegetable type: Cabbage, Cauliflower, turnip

Oil seed type - Rape seed and mustard.

### **Origin**

Primary centre of origin of *B. campestris* is near the Himalayan region. The secondary centres of origin are located in the European -Mediterranean are and Asia (Downey and Roebbelen, 1989).

Regarding the origin if *B. juncea*, Prakash and Hinata (1980) reported that species originated in middle east where *B. campestris* and *B. nigra* might have first come into contact. However, Haminway (1976) argued that *B. juncea* has probably arisen by hybridization between different *B. campestris* and *B. nigra* genotypes at several different times and localities resulting in secondary centres of origin in China, north eastern India and Caucasus.

### **Taxonomy:**

Belongs to family Brassicaceae or Cruciferae. The genus *Brassica* contains more than 3000 species of which 40 are of economic importance. Harberd (1972) examined 85 species of *Brassica* and grouped species of the genus into cytodemes. These cytodemes are composed of different species with the same chromosome number and which are cross fertile and other having species with different chromosome number and cross infertile. According to him, most important agricultural species are four diploids, three allopolyploids, each belong to a separate cytodeme. Four diploids are:

1. *B. nigra* - Black mustard
2. *B. oleracea* - Cabbage
3. *B. campestris* - Rape seed.
4. *B. tournefortii* - Wild turnip

Three allopolyploids

1. *B. napus* - Rape seed of Europe (ghobi sarson)
2. *B. juncea* - Indian mustard
3. *B. carinata* – Ethiopian mustard (veg / oil seed)

The genetical relationship between the oilseed brassicas are diagrammatically represented as follows:

*B. napus* will cross readily with *B. campestris* but with extreme difficulty in case of *B. oleracea*.

Rape seed

1. *Brassica campestris*: Chromosome no  $2n=20$ . Also known as Indian Rape Seed. It is self-sterile in nature. Important oil seed crop of North India. three cultivated types include:

i.) *B. campestris* var. Brown sarson

ii.) *B. campestris* var. Yellow sarson

iii.) *B. campestris* var. toria

1. *B. napus*: Chromosome no. =38. Also known as European Rape Seed. It is self-fertile.

Mustard

1. *B. nigra*: Chromosome no.= 16. Known as Black mustard and native of Eurasia. 28% fixed oil. Used as medicine pungent due to glucoside sinigrin.

2. *B. alba*: Chromosome no. =24. Also known as White mustard. Young seedling used as salad, yellowish seed contains 30 % oil.

3. *B. juncea*: Chromosome no.= 36. Known as Indian mustard or Rai, seeds contain 35% oil. Leaves used as herb contains sinigrin.

### **Cytology**

Cytological analysis of chromosome pairing in the progeny of interspecific crosses has clearly established that three species with higher chromosome number are amphidiploids derived from diploid species as follows:

*B. oleracea* (CC,  $n=9$ ) x *B. campestris* (AA,  $n=10$ ) – *B. napus* (AACC,  $n=19$ )

*B. nigra* (BB,  $n=8$ ) x *B. campestris* (AA,  $n=10$ ) - *B. juncea* (AABB,  $n=18$ )

*B. nigra* (BB,  $n=8$ ) x *B. oleracea* (CC,  $n=9$ ) – *B. carinata* (BBCC,  $n=17$ )

This relationship has further been confirmed by artificially synthesizing the amphidiploid species from their diploid parents. Roebbelen (1960) carried out detailed chromosomal analysis of diploid species and concluded that only six chromosomes were distinctly different and remaining being homologous with one or another of the basic six. This suggested early evolution from a common progenitor species with basic chromosome number of  $x=6$ . The diploid Brassica species with  $n=8,9,10$  chromosome number resulted from secondary balanced polyploidy.

### **Breeding objectives**

1. Seed yield: Yield is the end product of many biological processes which are under control of complex polygenic systems. An ideal plant type is having increased branch number, pods per plant, seeds per pod and seed size. Further yield increase could result from increase in biomass and harvest index. Increased biomass can result from reduced photo respiration and increased light saturated rate of photosynthesis.
2. Early maturity: For use in various multiple cropping sequence.
3. Resistance to abiotic factors Frost resistance is needed to prevent yield losses. Winter hardiness is very important.
4. Resistance to biotic stress: Powdery mildew, Black leg, *Sclerotinia* rot, alternaria blight are major diseases affecting yield. Mustard aphid – major pest and so far no resistance source has been identified. The variety RH813 and JM1 have been developed for white rust resistance.
5. Herbicide resistance: Resistance to herbicides viz. Atrazine, simabine etc. A few sources of resistance are available.
6. Shattering resistance: Major problem in *B. napus*, highly shattering whereas *B. juncea* exhibited tolerance. Introgressive breeding done.
7. Increased oil content and quality: High oil content upto 45% in yellow seed varieties. For industrial purpose more Erucic acid varieties are required. Development of low erucic acid cultivars for edible purpose. Reduced linolenic acid content is also desirable. First “00” hybrid of *B. napus* (gobhi sarson), Hyola-401 has been developed.
8. Meal quality: Meal having less Glucosinolate content.
9. Breeding for drought tolerance: In India more than 50% of rapeseed and mustard is grown under residual moisture supply conditions. Therefore, it would be desirable to breed varieties for such situations.
10. Breeding for frost tolerance: Frost resistant variety RH781 has been commercialised.

**In Brassicas the following ideotype is suggested for moisture stress situations:**

Roots: Well developed, deep root system with thick long hairs

Seedling growth: Rapid development of hypocotyl and epicotyl

Leaf characters: Rapid build up of leaf area index at early stage, smaller, thicker, hairy, waxy, erect or moderately droopy leaves with higher photosynthetic and low dark respiration rate, rapid cell elongation and maintenance of high leaf water potential.

Plant characters: Intermediate plant height, profused primary branches, no tertiary branches, compact branches, higher siliquae density, high root shoot ratio, ability to survive internal water stress and to recover growth on hydration, long siliquae, more

number of seeds/siliquae with comparatively bold seed size, resistant to major diseases and pests

Flowering: Synchrony in flowering

Duration: Short- medium duration depending upon species and location specific rainfall

### **Breeding methods**

Breeding methods of self-pollinated crops are used in case of *B. juncea* which is self-compatible. Pureline selection, pedigree, bulk, SSD, backcross methods and doubled haploid breeding are used by Brassica breeders. Breeding methods of cross-pollinated crops are used in *B. campestris* (brown sarson, yellow sarson and toria) for improvement. Mass selection, recurrent selection, synthetic varieties and hybrid varieties are developed.

Hybrid varieties in rapeseed is dependent on cytoplasmic- genetic male sterility where three-line breeding comes in use.

Production of hybrids using sporophytic self-incompatibility has now been used in vegetable crops like cabbage, radish and other *B. campestris* vegetables. However, its application in oilseed form of self-incompatible *B. campestris* has not been successful because of high inbreeding depression by bud selfing and inbreds are difficult to maintain by continuous selfing.

1. Introduction: Regina variety introduced from Sweden
2. Simple selection: Seeta, Krishna, Kranti varieties were developed through selection.
3. Hybridization and selection
  - i.) Intervarietal ii.) Interspecific
    - a) Bulk method b) Pedigree method c) single seed descent
4. Back cross method
5. Population improvement: Recurrent selection, mass selection
6. Heterosis breeding: Heterosis can be exploited using CMS lines.
7. Mutation breeding: RLM 198 variety developed through mutation breeding at PAU Ludhiana by gamma radiation treatment of mustard cultivar RL18.
8. Tissue culture technique for production of homozygous diploids. First somaclonal variety Pusa Jai Kisan developed through tissue culture in 1993.
9. Embryo rescue technique for inter specific crosses.
10. Polyploidy breeding: Highly competitive cultivars at least for production of vegetative matter may be bred from autotetraploids of *B. campestris* and *B. oleracea*. Natural allopolyploids species *B. napus*, *B. juncea* and *B. carinata* can be resynthesized from

crosses between the parental diploid species. Amphidiploids involving leafy types as one of the parents, has a large number of primary and secondary branches and more pods. Similarly, amphidiploids from oleiferous types had more oil.

11. Interspecific somatic hybrids: Interspecific protoplast fusion has been used in Brassica to transfer disease resistance and other desirable traits from wild to cultivated species. Blackleg resistance has been transferred from *B. tournefortii* to *B. napus* through protoplast fusion. *B. napus* and *B. oleracea* protoplasts have been fused to transfer resistance to bacterium *Xanthomonas campestris* pv. *campestris* from *B. napus* to *B. oleracea*. Resultant somatic hybrids backcrossed with *B. oleracea* to develop high resistant progenies.
12. Recombinant DNA technology: In Brassicas, transgenic plants have been produced for oil quality, seed storage protein, low glucosinolates, herbicide tolerance, fungal disease resistance, virus resistance, pest resistance and other traits. Conola transgenics have been commercially released in USA, Canada and Chile.
13. Molecular markers in Brassicas: Different molecular markers have been extensively used in Brassicas for identification and characterization of transferred chromosomes from different sources of *Brassica* species.

### **National Programme**

**Directorate of Oilseeds Research (DOR), Hyderabad** is national organization with responsibility to plan, coordinate and execute the research programmes to augment the production and productivity of oilseeds.

### **National Research Centre on Rapeseed- mustard (NRC), Bharatpur**

NRC has following mandates

- National repository of rapeseed and mustard genetic resources and information
- Basic, strategic and applied research to improve the productivity and quality of oil and meal
- Development of viable agro-production and protection technologies
- To extend technical expertise and consultancies
- Establishment of linkages and promotion of cooperation with national and international agencies to achieve objectives



## **Sunflower breeding**

Sun flower (*Helianthus annuus*)

Place of origin: North America.

Classification: The genus comprises nearly 67 species - all native to America. Of these two are cultivated:

- a) *H. annuus* – diploid,  $2n = 34$ , oil seed crop.
- b) *H. tuberosus* – Hexaploid,  $2n = 102$ , known as Jerusalem artichoke and is cultivated for tuber.

Wild species: *H. hirsutus*, *H. rigidus* moderately resistant to *Alternaria*.

Putative parent: Weed sunflower gave rise to cultivated one. The weed sunflower was modified by introgression with *H. petiolaris*.

### **Cultivars of sunflower:**

- a) Giant types: 6 - 14 feet tall, late maturing, large heads 12 - 30" in diameter, seeds large, white or grey or with black stripes. Oil content is very low. E.g. Mammoth Russian.
- b) Semi dwarf varieties: Medium tall - 4 ½ to 6 feet, early maturing, heads 7 - 9" in diameter. Seeds are smaller, black, grey or striped. High oil content 35%. E.g. Jupiter, Pole star.
- c) Dwarf types: 2 to 4½ feet tall, early maturing. Head size 5½ - 6½" in diameter. Small seeds, high oil content 37%. e.g. Sunrise, Morden, Co1, Co2

### **Cytology**

The genus *Helianthus* has basic chromosome number of 17 and includes diploid, tetraploid and hexaploid species. The 17 chromosome number is divided into four groups based on position of centromere and presence of satellite or absence of satellites. First group has two pairs of satellite chromosomes with sub-median centromere, the second group has five pairs of chromosomes with median to sub-median centromere. The third group has six pairs with sub-median centromeres. The fourth group has four pairs with sub- median to sub- terminal centromeres.

### **Breeding objectives**

1. To develop short duration varieties suitable for dry land and irrigated conditions: In dryland, it is successful in black soils only. In red soil under rainfed conditions it is not successful.
2. Breeding varieties with high oil content: Ranges 38 to 48%. Complex character yield and oil content are negatively correlated. To increase oil content the shell must be thin.

3. Breeding for self-fertile lines: Protoandry and self incompatibility mechanism operates in sunflower. Hence hand pollination is necessary. To avoid this, self-fertile lines can be developed.

4. Breeding for disease resistance. Powdery mildew, rust, charcoal rot, Alternaria are major diseases of sunflower. Wild species like *H. hirsuta* are moderately resistance to Alternaria.

5. Resistant to pests: *Heliothis*, Grass hoppers, Jassids are major pests of sunflower.

#### **Breeding Methods:**

1. Introduction: Morden variety introduced from Canada.

2. Mass selection: EC 68414 from Russia. Variety Co1 was mass selection from Morden. Useful for characters which are highly heritable. For e.g. plant height, disease resistance.

3. Hybridization and selection

a) Intervarietal : Variety Co2 was derivative of multiple cross Co4 - (Dwarf x Surya)

b) Interspecific : Wild species of North American origin and best Soviet varieties were crossed and number of varieties were evolved. E.g. Progress, Novelty, Jubilee 60 which are resistant to *Verticillium* wilt also

4. Mutation: Variety Co3 (Mutant from Co2 through gamma rays)

5. Head to row and remnant seed method: Developed by Pustovoit in Russia. By this method oil content is increased. In this method the following are the steps:

a) From open pollinated type a large no (10,000 to 12,000) plants are selected based on Head size.

b) The selected lines are analysed for oil content and high oil content lines are isolated (1000 plants).

c) Part of the seed reserved and the part is sown in progeny rows along with check to estimate yield.

d) Second season testing is also done. The best lines are identified.

e) The remnant seed of elite plants which give high yield were raised in isolation and multiplied for crossing *inter se* next season.

f) The multiplied lines also tested for oil content and high yielding high oil content lines were raised in isolation and crossed *inter se*.

6. Population improvement: By mass selection, recurrent selection and use of male sterile lines population can be improved and utilised for breeding.

7. Heterosis breeding: Development of inbred lines and crossing them to harness heterosis was first done as early as 1920 in Russia. During 1970 cytoplasmic genetic male sterility was identified in wild types and obsolete cultivars. Now this system is being extensively

used for production of hybrids. First hybrid BSH 1 CMS 234 A x RHA 274 BSH 2 BSH 8. A number of CGMS lines were bred by Government as well as private seed growers and are utilised now. Male sterility can also be induced by GA 100 ppm.

Steps 1. Development of inbreds.

2. Evaluation of inbreds for combining ability.

3. Conversion of inbreds into CGMS lines and R lines.

4. Production of hybrids.

Varietal renovation: In sun flower the varieties released are renovated annually to produce super elite (Breeder seed) and Elite Seed (Foundation seed).

8. Molecular markers in sunflower breeding: Molecular markers have been successfully used in sunflower breeding to study the genetic variability of inbred lines, for identification of interspecific hybrids and MAS to identify disease and pest resistant plants during resistance breeding programmes.

### **National Programme**

Breeding research for sunflower improvement were initiated in 1972 with establishment of all India coordinated research programme on sunflower with headquarters at University of Agricultural Sciences, Bangalore. ICAR launched project to promote research and development efforts on hybrid sunflower from 1989.

## **Carrot Breeding**

### **Origin of Carrot:**

The genus *Daucus* has many wild forms that grow mostly in the Mediterranean region and south-west Asia. Afghanistan is believed to be the primary centre of genetic diversity. There are evidences that purple carrot together with a yellow variant spread from Afghanistan to the Mediterranean region as early as the tenth or eleventh century.

Wild carrot, *Daucus carota* subsp. *carota* L., (a.k.a. Queen Anne's Lace). Wild carrot is abundant in temperate regions across the globe and is widely distributed across much of the United States where it is often found along roadsides, abandoned fields, and pastures. Wild carrot is the progenitor of the cultivated carrot, *D. carota* subsp. *sativus*, and the two subspecies are sexually compatible. The cultivated carrot was likely domesticated in Central Asia roughly 1,100 yrs ago and is grown worldwide from both open pollinated and hybrid seed. The white and orange carrots are probably mutations of the yellow form. The domestic carrot readily crosses with widely adapted wild carrot known as Queen Anne's Lace.

### **Floral Biology and Pollination of Carrot:**

The inflorescence of carrot is a compound umbel. A primary umbel can have over 1000 flowers at maturity, whereas secondary, tertiary and quaternary umbels bear fewer flowers. Floral development is centripetal i.e. the flowers to dehisce first are on the outer edges of the outer umbellets. Carrot is protandrous.

After straightening of filament, the pollen is shed and stamens quickly fall. After this, the petals open fully and the style elongates. The style is divided into two parts. The petals of petaloid plants are persistent unlike those of brown-anther, male sterile plants.

Flowers are epigynous. There are five small sepals, five petals, five stamens and two carpels. Emasculation is laborious and time consuming. As soon as the first bud in an umbel opens, the whole umbel of the female parent is bagged in a muslin/cloth bag.

The flowers are removed daily until peak flowering has reached. Anthers are removed from the early opening outer flowers in the outer whorl of umbellets until sufficient flowers are emasculated.

Unopened central florets in the emasculated umbellets and all late flowering umbellets are removed. Thus, only the emasculated flowers are left on the female inflorescence inside the bag. A pollen bearing umbel from previously protected male plant is inserted into the bag of the female parent along with some house-flies to ensure pollination. Daily for a few days in the morning, the male umbel is gently rubbed against the emasculated umbel to enhance artificial cross-pollination.

Sometimes, 1-2 flowering umbels of both the parents are enclosed in the same cloth cage along with some house-flies. Seed from each parent is sown in adjacent rows. The hybrids and the parents could be identified (not always) and necessary roguing done to remove the selfed plants.

Simon has described another alternative commonly used in Europe for intercrossing male fertile plants. A single isolated umbel will not develop seeds even though pollen is present in the flowers as this is protandrous. This umbel can serve as the seed parent in a cross if, one week after anthesis, the flowers of such an isolated umbel are sprinkled with water to flush out pollen.

After it dries, pollen from the intended pollen parent can be introduced with a brush and the seed parent umbel again placed in isolation. Seeds thus produced are nearly always hybrids. Sometimes two parents to be crossed are covered by a plastic or cloth screen pollination cage. Flies are released in the pollination cage to move pollen or pollen is moved by hand or brush. In this system, selfed and crossed seeds are harvested together. The selfed and crossed progenies need to be identified by phenotypic or molecular markers or by hybrid vigour when inbreds are crossed.

### Qualitative Genes in Carrot:

As per compilation by Simon (1984) and Peterson and Simon (1986) only 20 genes (Table 22.1) have been described.

**Table 22.1. Gene listing in carrot**

Gene symbol	Characters
A	Alpha carotene synthesis
Ce	<i>Cercospora</i> leaf spot resistance
Cr	Cracking roots
Eh	Downy mildew ( <i>Erysiphe heracle</i> ) resistance
g	Green petiole
gls	Glabrous seed stalk
io	Intense orange xylem
K	Lycopene synthesis
Ms-1, Ms-2, Ms-3, Ms-4, Ms-5	Male sterility
O	Orange xylem
P-1 P-2	Purple root
Ck	Root cracking
Rs	Reducing sugar in roots
Y	Yellow xylem
Y-1, Y-2	Differential xylem/phloem carotene levels

### Breeding Goals of Carrot:

- i. High root yield
- ii. Good eating quality
- iii. Scarlet/orange colour roots
- iv. High carotene content in roots
- v. Uniformity in root shape and size

- vi Thick flesh roots
- vii. Thin and self coloured core in roots
- viii. Broad shouldered, cylindrical, uniformly tapering or stump rooted carrot with non-branching habit
- ix. Early rooting
- x. Cracking free roots (major gene Ck known for root cracking)
- xi. Smooth, shiny root
- xii. High sugar and dry matter in roots
- xiii. Slow bolting habit
- xiv. Heat tolerance
- xv. Crown or upper surface (shoulder) free from green colour, flat or slightly up-lifted rather than concave or shrunken
- xvi. Resistance to: *Alternaria* blight (*Alternaria dauci*), *Cercospora* leaf blight (*Cercospora carotae*)

#### **Varietal Groups of Carrot:**

Simon (2008) have described several varietal groups based on root colour, root length, colour intensity, core colour and size, root tip shape and days to edible root maturity. Those same distinctions are still used to help categorize carrot varietal groups even today.

#### **The major root types used today include varietal groups as:**

European – Nantes, Chantenay, Danvers, Paris Market, Flakkee, Berlicum, Amsterdam Forcing

Asian – Kuroda

North American – Emperor

South American – Brasilia

Numerous cultivars have been named for all of these root types. The typical carrot root shapes are illustrated in Fig. 22.1.

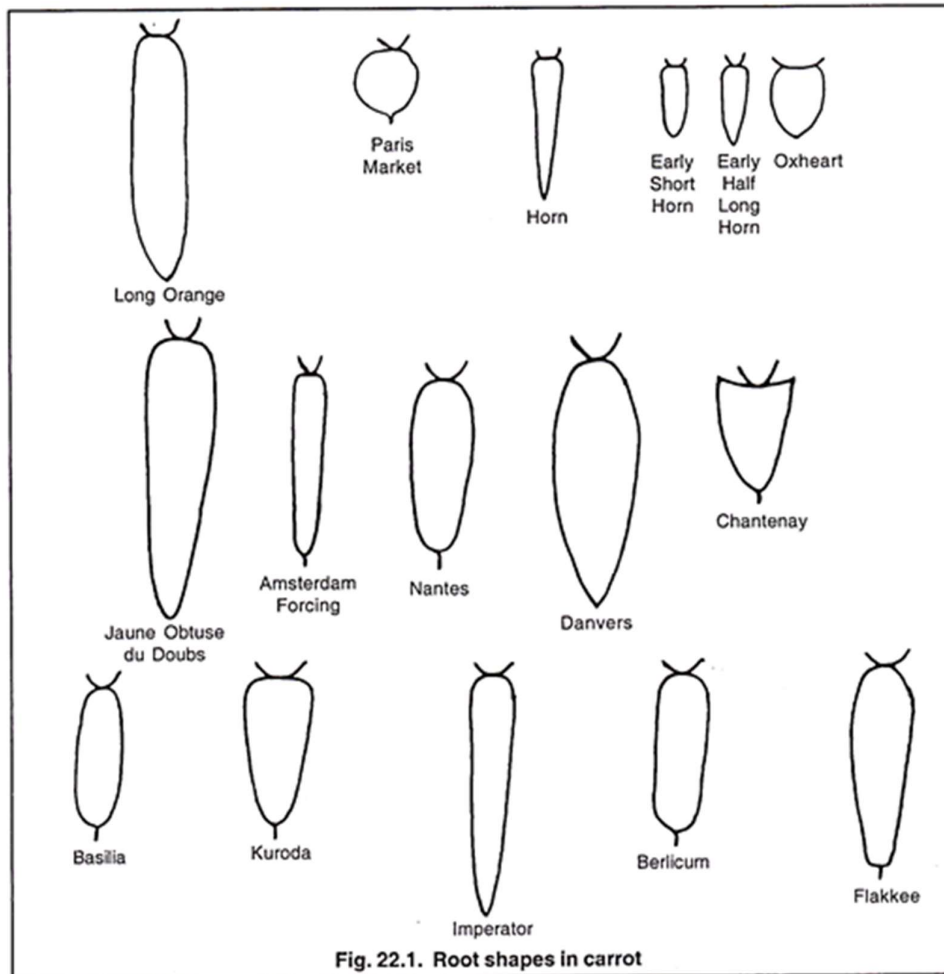


Fig. 22.1. Root shapes in carrot

European Nantes types (cylindrical roots, orange in colour) and Japanese Kuroda type (conical roots, deep orange in colour) are common in India and Asia at large. A dark orange selection from Gosun by Mr. Kuroda in 1950s resulted in the Kuroda-Gosun cultivar that has become grown widely, often simply referred to as Kuroda in India. Most of private sector seed companies are marketing Kuroda carrot on a large scale.

#### Genetic Resources of Carrot:

As for most vegetables, carrot genetic resources are in the form of open-pollinated cultivars.

#### Centres on carrot germplasm accessions are as follow:

1. USDA – ARS ([http://www\(dot\)ars](http://www(dot)ars)) = 723 accessions of *Daucus carota*.
2. European cooperative programme for Crop Genetic Resources – ECP/GR = 5037 accessions of *D. carota*
3. National Centre for Vegetable Crops research- Carrot Breeding Collection (CNPH), Eurpresa Brasileira de Pesquisa Agropecuaria-Brazil = 200 accessions.
4. BAZ – Inst, of Horticultural Crops, Germany = 5 species, 25 subspecies, 30 wild relatives.

5. National Gene Bank – Rural Development Administration, Korea = 695 accessions

**Selection Criteria of Carrot:**

**Colour and Quality:**

Visual examination of roots, cross section of roots and longitudinal section of roots is effective. Same colour should extend from crown to down tap root. The colour of xylem, phloem and vascular cambium should match as far as possible.

**Sugar and Flavour:**

A thin cross-sectional slice could be cut and tested. The roots with harsh flavour are eliminated. Total sugars which contribute to sweetness and are an important component of general preference can be estimated by a refractometer. Selection for high soluble solids and dry matter is also possible by specific gravity. High dry matter is useful for processors. Percentage dry weight is easily determined by weighing a fresh sample, drying and reweighing.

**Non-bolting:**

Bolting may cause serious losses in yield and quality, hence it is important to apply selection pressure for non-bolting.

**Disease Resistance:**

Susceptible cultivars are generally planted between rows of breeding lines and the spreader row plants are inoculated to ensure the spread of disease. This practice is applicable for both *Alternaria* leaf blight and *Cercospora* leaf spot.

**Breeding Methods of Carrot:**

**Open-pollinated Varieties and Synthetics:**

In the recent past, mass selection and pedigree selection within the different populations have been the most important breeding methods. Open-pollinated varieties are adapted to different situations. Nevertheless, all efforts to breed varieties with high level of uniformity have been limited by genetic factors originating from breeding methods used for open-pollinated varieties.

One reason for this is the heterozygosity of the open-pollinated varieties, while another is the inbreeding depression resulting from random selfing of plants within the populations. Inbreeding depression is considerable as quoted by Stein and Nothnagel (1995) in a review (Table 22.2).



**Table 22.2. Mean inbreeding depression of seven varieties in carrots after one generation of selfing**

Character	Level (%)	Range (%)
Weight of root	50.5	39.1-55.3
Weight of leaves	45.3	37.3-56.8
Length of roots	23.5	17.7-35.2
Yield of seeds	72.8	62.2-81.9

All open-pollinated varieties suffer from inbreeding depression and a limited degree of uniformity, and hybrid breeding of carrots has now been started by the carrot breeders intensively to improve uniformity.

In view of severe inbreeding depression in carrot, selection for resistance to inbreeding depression is a major consideration in carrot where self-pollination is essential to breeding progress. In this situation, it is better to develop new inbreds from intercrosses between two good inbreds.

In case where extreme uniformity seems to be unnecessary, e.g. for juice or pulp production or for regions with weakly developed agriculture, breeding of synthetics would also be worthwhile.

As an outcrossing diploid with significant inbreeding depression, adequate population size is vital to maintain population vigor in development of open-pollinated cultivars.

The development of most open-pollinated carrot cultivars begins with a pre-existing open-pollinated cultivar and selection is exercised for one or more traits. Population improvement for carrot typically starts with inter crosses among plants of open-pollinated varieties followed by phenotypic mass selection for root shape, smoothness, length and desirable root colour in a particular local production area.

Where facilities are available, selection should also be done for disease resistance (*Alternaria* leaf blight), root quality, cavity spot, nematodes, flavour, carotene content and processing quality. Plants with premature bolting, excessive root cracking and poor plant vigour are always removed.

Several of the more successful open-pollinated carrot cultivars of North America began with an intercross between two cultivars, like the breeding of 'Imperator'. Other examples include 'Waltham Hicolor' from 'Hutchinson' x 'Turkey Red Carrot' and 'Gold Pak' from 'Long Imperator' x 'Nantes'.

### **Hybrid Breeding:**

Thompson (1961) and Hanschke and Gabelman (1963) were the first scientists to detect and analyze male sterility. The first carrot hybrids were sold in early 1960s in the USA. Today

more than 100 hybrid varieties exist worldwide. The percentage of hybrids is 60-90% in Europe for early and late varieties and in USA, the value has reached to almost 100%.

Hybrid breeding in carrot is generally based on two systems of cytoplasmic male sterility (CMS) with different genetic backgrounds and origin. These are brown anther type and the petaloid type. The brown anther type (ba) is present in all cultivated orange coloured open pollinated varieties.

The phenotype is characterized by deformed, brown coloured anthers without functional pollen caused by a genetic block in meiosis. According to O. Banga and his colleagues (1964) the type ba results from an interaction of the Sa cytoplasm with two independent nuclear genes (homozygous aa or dominant B).

The two complementary genes E and D operate with their dominant alleles as restorer genes. Due to this complex inheritance, many test crosses are necessary for the development of a suitable maintainer.

The cytoplasm of the petaloid type (pt) is derived from a wild form of *Daucus carota* L. and has been introduced into many open-pollinated varieties of cultivated carrot. The 'pt' type is characterized by a transformation of the anthers into petals or petal like structures which are unable to produce functional pollen.

For the inheritance of this type, an interaction between Sp-cytoplasm and two independent dominant genes ( $M_1$ ,  $M_2$ ) was postulated by T.S. Morelock in 1974. A maintainer for this type cannot be detected in the F) because of the dominant state of the M genes. Backcrosses should be performed by use of the male-fertile genotype (Sp)  $m_1m_1$ ,  $m_2m_2$  as a tester in order to find dominant alleles.

As a matter of fact, petaloid male sterility was discovered in North American wild carrot by Munger in 1953. It has also been found in other North American wild carrots. Petaloidy is a homeostatic mutation where a second whorl of petals exists in place of anthers.

Petaloid CMS is the most widely used form of male sterility for production of commercial carrot hybrids in North America today. It is stable over a wide range of environments throughout flowering and seed production, although in some genetic backgrounds petaloidy breaks down and late season umbels can be fertile.

The incorporation of CMS into carrot inbreds for production of hybrid cultivars is similar to that for other crops. Generally, this process begins at the F<sub>2</sub> or F<sub>3</sub> generation with the intercrossing of individual selected fertile plants to a male sterile plant. In the next generation progeny from the male sterile are examined for male fertility in the process of backcrossing for sterile line development.

Presence of male fertile plants indicates the presence of restorer alleles from the original fertile parent. It has been easy to identify and select male sterility maintaining carrot inbreds from a wide range of germplasm backgrounds but the incidence of restorers varies widely.

A third CMS system has been detected in an alloplasmic form of orange-coloured carrot originating from a cross between the wild carrot *D. carota gummifer* Hook. fil. and the cultivated carrot *D. C. sativus* Foffm by T. Nothangel in 1992.

This type of male sterility called 'gum' type, is characterized by a total reduction of anthers and petals. Recent results on the genetic mechanisms suggest that an interaction of the 'gummifer' cytoplasm with a recessive allele (gugu) in the nucleus is responsible for the expression of this type of male sterility.

The two CMS systems 'ba' and 'pt' generally suffer from instability of male sterility under specific conditions. The instability is mainly influenced by high temperature. Nevertheless, observations over many years have revealed that other factors such as dry conditions, growing time or long day conditions operate provocatively.

A strict selection scheme is therefore necessary because carrot is partially andromonoecial, i.e. in umbels of higher order, male flowers can be produced. Umbels of the 5—7th order must be examined carefully.

The development of the male sterile (ms) and maintainer lines is very laborious process due to the dominant state of male sterility. Crossing, backcrossing, selfing and testing of the progenies in the following two generations, including isolation of the positive progenies, are characteristic steps in breeding processes.

The breeder is forced to eliminate male fertile plants or phenotypes with partial male fertility within the ms lines developed, and to develop new lines with low inbreeding depression. Such lines can be found but they rarely have good combining ability.

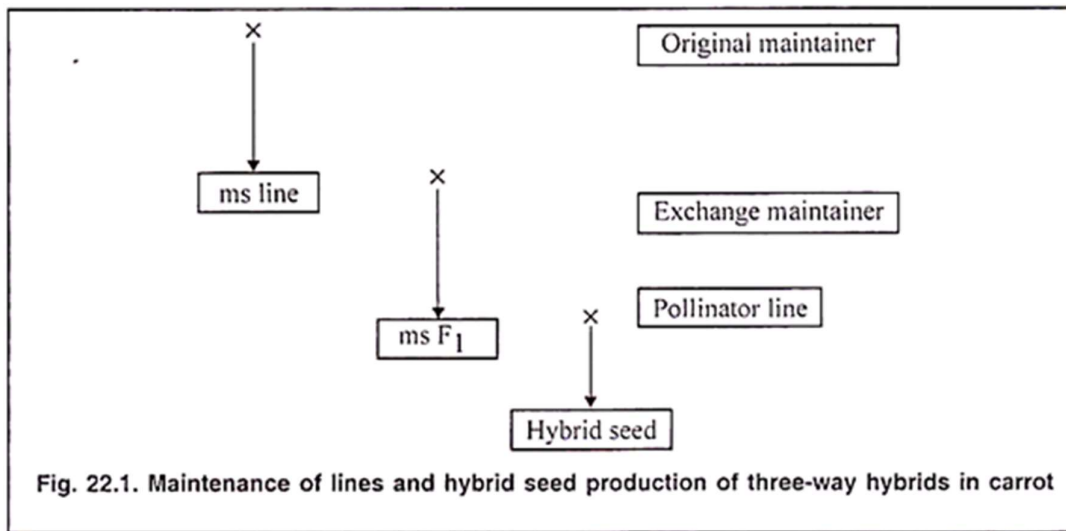
An excellent uniformity of the hybrid varieties demands a high degree of homozygosity in the ms and maintainer lines but selection of lines with low inbreeding depression reduces the probability of finding good parents for hybrid varieties. To overcome this problem, the development of three-way hybrids has been recommended. In this way, the production of hybrid seed is realized on ms F<sub>1</sub> lines (Fig. 22.1)

For this method, an original maintainer and a second line (exchange maintainer) are necessary. The universal maintainers which can maintain all ms lines due to complete homozygous state of the maintainer genes is desirable for such a system.

A universal maintainer must also possess the specific characters of the ms line and a good combining ability. Exchange maintainers have therefore been propagated which were selected following a diallel with all lines of one type.

These lines must have the same genetic state as the specific ms line, a state which can be found relatively frequently. Thus, the best line for performance and uniformity can be selected.

Recently, exchange maintainer lines for the most important ms lines have become available and can be used for seed production of new lines. Pollinator lines which are necessary for hybrid seed production are derived from op-varieties or special breeding lines by using a top-cross system for the testing of general combining ability.



### Applications of Biotechnology of Carrot:

Genome size is relatively small. Variation in molecular genetic markers is quite extensive, and both genetic transformation and regeneration of carrot are readily achieved. Therefore, carrot is a good candidate for biotechnological applications.

The carrot genetic linkage map includes around 1000 isozymes, RFLPs, AFLPs, RAPDs, and other molecular markers. From 10-25% of genomic clones tested are estimated to be in high copy numbers, while 20-30% of the RFLP probes and RAPD primers, and 40% of AFLP bands were polymorphic. The incidence of molecular marker polymorphism and map size to date makes marker-assisted selection a viable option.

Reports of success have not yet been published but markers linked to nematode resistance, CMS restorers, carotene content, and sugar content are being sought. One significant limitation to the application of molecular markers to carrot improvement is the need for radioactive labels, which cannot to be used in many laboratories. Because of this, efforts are underway to convert AFLPs to PCR-based co-dominant markers and to develop microsatellite markers.

One of the most intensively studied traits of carrots today continues to be CMS with a particular emphasis on molecular evaluation. Mitochondrial restriction fragment pattern and protein product differences between fertile, petaloid, and brown anther cytoplasm's have been compared and analysis of variation for a few specific genes, such as *atpA* have been reported. Seven monogenic traits have been mapped for carrot. These are *yel*, *cola*, *Rs*, *Mj-1*, *Y*, *Y<sub>2</sub>* and *P<sub>1</sub>*. QTLs have been mapped for carrot total carotenoids and five component carotenoids namely, phytoene, alpha-carotene, beta-carotene, zeta-carotene and lycopene and majority of the structural genes of the carotenoid pathway is placed into this map (Just et. al., 2007). MAS has been reported for two genes: *Mj-1* and *Rs*.

With the pioneering work of F. C. Steward and co-workers in 1964, totipotency of plant cells was first demonstrated using carrot. Carrot has, in fact, proven to be a model organism for plant tissue culture, transformation, and regeneration.

Taking advantage of carrot's facile manipulation in cell culture, carrot- *Daucus capillifolius* and carrot-bishop's weed (*Aegopodium podagraria*) protoplast fusions were among the first examples of successfully regenerated somatic hybrids.

Maize transposable elements have been successfully introduced into carrot cell cultures and found to be mobile. Two interesting experiments with carrot cell cultures demonstrate evidence for easy regeneration of carrot callus after one year of being air-dried and stored at room temperature.

Transgenics with genes for disease resistance and enhanced root color have been field-tested. With more genes of potential agricultural application cloned and improved, public perception of transgenics, generation, and testing of carrot transgenics will increase.

Now there are reports that carrot transformation is quite efficient. Carrot transformants have been developed for hepatitis B vaccine and other unique plant products but like most other vegetables, commercial release of the carrot transgenics has not materialized. However, it is to be recognized that herbicide and fungal resistance could have lot of advantages in crop production.

### **Research and Trends in Carrot Seed Production:**

The individual carrot flowers, in common with most other species in Umbelliferae, are borne on terminal branches in compound umbels. There is a distinct order of flowering which relates to umbel position. The first umbel to flower is the primary (sometimes referred to as the 'king' umbel) which is terminal to the main stalk.

Branches from the main stalk form secondary umbels, and subsequent branches from these form tertiary umbels. Quaternary branches and umbels may also be formed.

The modern methods of carrot root crop production for the fresh market and processing require high quality carrots with the minimum of variation between individual roots derived from the same seed lot. The relationships between seed weight and endosperm characteristics and effect of seed position on seed quality have been reviewed by George (1999).

The variation in weight of individual seeds and weight of their embryo can contribute to subsequent variability in seedling size. Seeds from primary umbels are of better quality in terms of uniformity and producing heavy seedlings. Therefore, it is better to have high plant densities as there will be less branching and therefore a high proportion of primary to secondary umbels than at lower plant densities.

Further overall seed quality is improved if manual harvesting is restricted to primary umbels only so that seeds of shorter maturity span are harvested. This is usually needed to propagate basic seeds.

For quality seed production in early stages, root-to-seed production is followed where fully evaluated and selected roots are planted for seed production. Root to seed production is used for nucleus/breeder/foundation seed production. It is also quite common for commercial carrot seed production as this method provides an opportunity to the breeder and the seed producer for selection of roots with desired quality and phenotype.

The production location should be free of wild carrots. Limited size plot can be covered by pollinating honey-bee hives. There could be geographical isolation by at least a few kilometers and pollination be accomplished by honeybees or naturally occurring insect pollinators. Seed to seed production though possible should be avoided.

### **Cultivar Description of Carrot:**

There are open-pollinated and hybrid cultivars. The following outline is based on UPOV (1990) and as described by George (1999).

#### **1. Leaf:**

Length (including petiole): very short, short, medium, long or very long

Division: very fine, fine, medium, coarse or very coarse

Intensity of green colour: light, medium or dark

Anthocyanin coloration of petiole: absent or present

#### **2. Carrot roots:**

Length: very short, short, medium, long or very long

Width: narrow, medium or broad

Ratio width/length: very small, small, medium, large or very large

Shape of longitudinal section: circular, obovate, obtriangular or narrowly oblong

Shape of shoulder: flat, flat to rounded, rounded, rounded to conical or conical

Tip: blunt, slightly pointed or pointed

External colour: white, yellow, orange or red

Intensity of external colour: light, medium or dark

Extent of green colour of skin of shoulder: absent or very small, small, medium, large or very large

Diameter of core relative to total diameter: very small, small, medium, large or very large

Colour of core: white, yellow, orange or red

Intensity of colour of core: light, medium or dark

Colour of cortex: white, yellow, orange or red

Intensity of colour of cortex: light, medium or dark

Colour of core compared with colour of cortex: lighter, same or darker

Green coloration of interior of top (longitudinal section): absent or very weak, weak, medium, strong or very strong

Time of maturity: very early, early, medium, late or very late

**Isolation Distance:**

Breeder/foundation seed – 1600 m

Certified seed – 1000 m

**Seed Production Methods:**

1. Seed – to – seed
2. Root – to – seed

**Seed Yield:**

1. 600-1000 kg/ha
2. 1000 seed weight – Approximately 0.8 g
3. SMR – 50

**Varieties of Carrot:**

**Pusa Kesar:**

This has been bred at Indian Agricultural Research Institute, New Delhi and is an old release of 1963. It was evolved from a cross of Local Red and Nantes. The roots are scarlet in colour. It is rich in carotene (38 mg/100 g edible portion). Roots stay longer in field without bolting. Seed production in north Indian plains is successful.

**Pusa Meghali:**

This is a tropical or Asiatic type cultivar. It has been developed at Indian Agricultural Research Institute, New Delhi from a cross between Pusa Kesar and Nantes and was released by the Institute in 1985.

It has short top, smooth roots with orange flesh and self-coloured core. It is richer in carotene content (11571 IU/100 g) than Pusa Kesar (7753 IU/100 g) and produces seeds in plains. It is suitable for early sowing and takes 110-120 days to attain harvest maturity and yields 260-280 q/ha.

**Nantes:**

This variety belongs to European or temperate types. Its seed production is possible only in the hilly areas. It is an introduction by IARI Regional Station, Katrain. The roots are half long (12-15 cm), slim, well-shaped, cylindrical with stumped end forming a small thin tail.

The cortex and core are deep orange. It ranks good in quality, but the top is brittle making pulling difficult. Keeping quality is poor. It is suitable for cultivation in cooler months. It takes 110-120 days for root formation.

**Pusa Yamdagni (Selection 5):**

This has been developed at IARI, Regional Station, Katrain from an inter-varietal cross between EC 9981 and Nantes to combine earliness and size of root of the former and self-coloured core character of the latter. It has performed well both in the hills and plains. It is early in maturity. It has been released by the central variety release committee.

**Kuroda:**

It is an old variety developed in Japan by Mr. Kuroda but is very popular in Indian seed companies for large scale sales. It has long, sweet, tender orange colour roots with wide shoulders. Roots are tapering to a blunt point. Roots are smooth, uniform, and conical in shape. Roots have better storability.



## Pea breeding

Peas (*Pisum sativum* L.) are consumed as fresh vegetable or dry seeds in most of the countries. In India it is grown as winter vegetable in plains and summer vegetable in the hills.

## Origin of Pea

The geographical region comprising of Central Asia, the Near East, Abyssinia, and the Mediterranean is considered as centre of origin based on genetic diversity. According to Blixt (1970), the Mediterranean is the primary centre of diversity with secondary centres in Ethiopia and the Near East.

The genus *Pisum* comprises of only a small number of taxa. All taxa within *Pisum* are diploid ( $2n = 2x = 14$ ) and the majority are fully inter-crossable with a few being more difficult, but possible. *Pisum humile* syn. *syriacum* is considered a possible candidate as progenitor, as it resembles closely to the cultivated form. There has been introgression to cultivated types from *P. humile* and *P. elatius*.

**The characters associated with domestication are as follows:**

Trait	Wild type	Cultivated	Gene basis
Testa surface	Gritty/rough	Smooth	<i>Gty</i>
Testa thickness	Thick	Thin	
Pod dehiscence	Strongly dehiscent	Non-dehiscent	<i>Dpo</i>
Seed size (mg)	60-120	80-550	

## Botany of Pea

Pea is an annual herbaceous plant. It has a tap root system. Stems are slender, usually single, and upright in growth. Leaves are pinnately compound with two to several leaflets. The rachis terminates in a simple or branched tendril. There are large stipules at the base of leaf.

The plant may be single stemmed or many axillary stems may originate at the cotyledonary node or any superior node, especially if the apical growing point is destroyed, leaflets of a pair are opposite or slightly alternate. The lower leaflets are larger than the upper leaflets. The margins of leaflets and stipules may be entire or serrated.

The inflorescence is raceme arising from the axil of a leaf. The lowest node at which flower initiation occurs, is normally constant under a given set of conditions and is used in classifying the varieties into early and late types.

Most early cultivars produce the first flower from nodes 5 to 11 and the late cultivars start flowering at about nodes 13 to 15. Early cultivars are often single flowered or bear some single and some double flowers. Late cultivars are usually double/triple flowered.

The flowers are typical papilionaceous with green calyx comprising of five united sepals, five petals (one standard, two wings and two keels). The stamens are in diadelphous (9+1) condition. Nine filaments are fused to form a staminal tube, while the tenth is free throughout its length.

The gynoecium is monocarpellary, with ovules (up to 13) alternately attached to the two placentas. Style normally bends at right angle to the ovary. Stigma is sticky. Pea is strictly self-pollinated in nature. Stigma is receptive to pollen from several days prior to anthesis until 1 day or more after the flower wilts.

Pollen is viable from the time anthers dehisce till several days thereafter. For emasculation, the flower bud chosen should have developed to the stage just before anther dehiscence, indicated by extension of petals beyond sepals. Flowers can be emasculated at any time.

The first step in emasculation is to tear away with the forceps the tip of the sepal from in front of the keel. The forefinger is positioned behind the flower and thumb in front and a light pressure is applied. This spreads the standard and wings to expose the keel. The exposed keel is slit-open by tips of forceps. Pressure can be applied by the thumb and finger on keel for increased exposure of the pistil and stamens. The 10 stamens are pulled out.

Pollen can be obtained throughout the day, preferably from a freshly opened flower. For pollen collection, it is more convenient to pick the male flowers, remove the standard and wings, pull back the keel so that the style protrudes and use the pollen covered stylar brush as an applicator to transfer the pollen to the stigma of the emasculated bud.

Older flowers and other flower buds not used in crossing are removed from the peduncle to increase the pod set after crossing. Normally emasculation is done in afternoon followed by pollination next forenoon/morning.

### **Cytology**

The somatic chromosome number of peas is 14. The translocations and other chromosome arrangements are common. The seven characters studied by Mendel have been mapped as indicated below:

- (i) The shape of mature seeds, smooth/wrinkled (*R/r*), in chromosome 7
- (ii) Seed colour, yellow/green (*I/i*), in chromosome 1
- (iii) Flower colour, purple/violet or white (*A/a*), in chromosome 1
- (iv) Mature pods, smooth and expanded/wrinkled and indented (*V/v*) in chromosome 4
- (v) Colour of unripe pods, green/yellow (*Gp/gp*), in chromosome 5
- (vi) Inflorescence, axillary/terminal (*Fa/fa*), in chromosome 4
- (vii) Plant height, tall/dwarf (*Le/le*) in chromosome 4

Thus, Mendel probably dealt with the genes *a* and *i* in chromosome 1, *le*, *fa* and *v* in chromosome 4, *gp* in chromosome 5, and *r* in chromosome 7. One may ask as to why he did not run into the complication of linkage while formulating the law of independent assortment. The answer is that with respect to *a* and *i* in chromosome 1 and *fa* in relation to *le* and *v* in chromosome 4, these genes are so distant, that linkage is not realized. The only two genes which could have shown linkage were *le* and *v*. As far as is known, Mendel did not study the simultaneous segregation in these two.

### **Breeding Goals of Pea**

1. High green pod yield
2. Long, attractive green pods with more seeds/pod (9-12 seeds)
3. Sweetness
4. High shelling percentage
5. Specific maturity (early, mid)
6. Suitable for freezing and canning
7. Resistant/tolerant to frost
8. **Resistant to diseases, namely:**
  - (i) Downy mildew (*Peronospora viciae* (Berk.) de Bary)
  - (ii) Powdery mildew (*Erysiphe polygoni* DC)
  - (iii) Rust (*Uromyces viciae – fabes* (Pers). Schroet, and *U. pisi* (Pers.) Wint.)
  - (iv) Wilt (*Fusarium oxysporum* Schl. f. sp. *pisi* (van Hall) Synd. & Hans.)
9. **Resistance to insects, namely:**
  - (i) Leaf miner
  - (ii) Aphids
  - (iii) Pod-borer
  - (iv) Pea stem fly

### **Varietal Groups:**

The primary characters used for grouping of varieties include traits related to seed and pod types, maturity groups and plant height.

**A useful point of reference list of characters and descriptors used in the UPOV guidelines is given in Table 15.2:**

**Table 15.2. List of UPOV characters, phenotypes and related loci used for varietal grouping in vegetable pea**

Character	Descriptor state	Loci
<b>Seed</b>		
1. Shape of starch grain (cotyledonary character)	Round, wrinkled, dimpled	<i>R, Rb-</i>
2. Cotyledon colour	Yellow, green, mixed	<i>I</i>
3. Testa marbling	Orange	<i>Ore</i>
4. Testa anthocyanin	Brown patterning	<i>M</i>
	Violet or pink spots, stripes	<i>F, Fs</i>
5. Hilum colour	Cream, black	<i>F, F<sub>s</sub></i> <i>PI</i>
<b>Plant</b>		
6. Anthocyanin colouration	Purple, red to pink	<i>A, B, Am</i>
7. Leaf	Leaflets	<i>Af</i>
8. Stipule	Small, rudimentary	<i>St</i>
9. Stipule	Rounded apex, pointed	' <i>Rogue syndrome</i> '
10. Stipule	Flecked, non-flecked	<i>Fl</i>
<b>Pod</b>		
11. Pod well parchment		<i>P, P'</i>
12. Thickened pod wall		<i>N</i>
13. Shape at distal end	Blunt, pointed	<i>Bt</i>
14. Colour	Yellow	<i>Gp</i>
	Blue-green	<i>Dp</i>
	Purple	<i>Pu, Pur</i>
15. Intensity of green		<i>Pa, Vim</i>

### Genetic Resources of Pea

A large number of ex-situ germplasm collections have been reported around the world in public domain as compiled by Ambrose (2008) and given in Table 15.3.

**Table 15.3. Germplasm resources of peas at major centres**

FAO Inst. Code	Country	No. of accessions
AFTC	Australia	6567
SAD	Bulgaria	2787
ICAR-CAAS	China	3837
GAT	Germany	5336
BAR	Italy	4297
CGN	Netherlands	1008
WTD	Poland	2899
VIR	Russia	6790
ICARDA	Syria	6105
NGB	Sweden	2724
JTC	UK	3194
USDA	USA	3710

These centers have been actively collaborating with each other in PGR management of peas. In absence of CGIAR funded international agricultural research center with global mandate on peas, an international consortium for pea genetic resources (Pea GRIC) has been formed that links key collections in Europe, USA, ICARDA and Australia.

In India, about 2000 pea germplasm accessions are conserved at NBGPGR, New Delhi, IIVR, Varanasi and IIPR, Kanpur, Besides, a few state agricultural universities rich in vegetable pea germplasm are G.B. Pant University of Agric. and Technology, Pantnagar, Punjab Agricultural University, Ludhiana, Haryana Agriculture University, Hisar, JNKVV, Jabalpur and Indian Agric.Res. Inst., New Delhi.

### **Important Donors for Pea Breeding Programme**

Singh (1991, 1995) has compiled extensive information on genetics and breeding of peas including listing of superior lines with multiple disease resistance in pulse crops. Kalloo (1993) and Narsinghani and Tewari (1993) have also given detailed accounts of pea breeding.

#### **A few examples of donors on peas are as follows:**

Earliness: Asauji, Lucknow, Bonia, Hans, EC 3

More pods/plant: PL P 26, 50, 69, 179, 279, 496

Long pods: EC 109171, 109176, 109190, 109195

Bold pods: EC 4103, 6185, 95924

Powdery mildew: EC 326, 42959, 109190, 109196, T 10, P 185, P 288, PC 6578, B 4048, P 6587, P 6588, BHU 159, EC 42959, IC 4604, JP 501, VP 7906

Wilt: Early Perfection, Bonneville, PL 43, 124, 6101, Glacier

Rust: PJ 207508, 222117, EC 109188, EC 42959, IC 4604, PJ 207508, JP Batri Brown 3, JP Batri Brown 4

Pea mosaic: American Wonder, Perfection Canner's Gem, Dwarf White Sugar, Little Marvel

Leaf miner: EC 16704, 21711, 25173

Pea stem fly (tolerant): Bonneville, Asauji, Boach Sel., GC 141, IP 3 (Pant Uphar), Dwarf Gray Sugar, T 10, T 163

### **Breeding Procedures of Pea**

Peas are self-pollinated due to cleistogamy and accordingly, the common breeding procedures applicable to self-pollinated crops viz. pedigree, bulk, single seed descent (SSD), back-cross and mutation breeding are used in pea breeding.

Single seed descent method is now becoming common in peas. This is particularly, useful in those situations where selected better lines are intercrossed. F<sub>1</sub> plants are grown to produce 500 or more F<sub>2</sub> seeds.

One seed is harvested from each F<sub>2</sub> plant and the harvested seeds are bulked to plant F<sub>3</sub>. This procedure continues till F<sub>5</sub> in which phenotypically, superior individual plants are selected for future plant to progeny planting and evaluation. A major advantage of this method is, that, it

can be carried out with less resources and the rapid advancement of generations is possible in field and glass-house/off season-nursery.

While advancing the generations, selection for highly heritable traits is practiced frequently in early generations, before lines are grown out as small plots in F<sub>4</sub>/F<sub>5</sub> generation. Shuttle breeding is also practised in peas where alternate generations (like F<sub>3</sub> and F<sub>5</sub>) are grown at off-site locations. For example, in India alternate generations can be grown in late kharif in Pune and Nasik in Maharashtra and followed by winter season in northern plains.

In this way, 2 generations can be grown in a year. There is widespread use of SSD utilizing glasshouses or plant growth chambers to speed-up early generations while also maintaining a wider level of variability between lines before growing plant to progeny rows for field evaluation and selection. Bulk selection is also used by some breeders.

In garden pea number of green pods/plant, green pod weight, pod length and number of seeds/pod have been shown to be the major yield components affecting the green pod yield. These yield components usually do not show component compensation effect and therefore, simultaneous improvement for these characters should be possible.

Prospects of mutation breeding in peas have been discussed by Jaranowski and Micke (1985). A dose of 10-15 krad of gamma rays is appropriate for seeds. A good criterion of effectiveness of any mutagen is germination reduction, not exceeding 50% for gamma radiation, better only 30% for neutrons and certain chemical mutagens.

Stronger germination reduction may result for a high number of chromosomal aberrations and this in turn will lead to high sterility. Among chemicals, EMS, NEU, EI, NMU and sodium azide seem to be the most efficient mutagens for peas.

Chemical compounds are applied as water solutions and seeds are usually presoaked for 12-16 hours. Presoaking facilitates the penetration of the mutagen into the tissues. The optimum temperature and duration are 21-24°C and 2-4 hours, respectively.

**Recommended concentrations of certain chemical mutagens for pea seeds are:**

EMS: 0.05 – 0.3%

NEU: 0.20 – 0.40 millimole

EI: 0.05 – 0.15%

NMH: 0.01 – 0.03%

DES: 0.03%

The solutions should be fresh prepared and buffered to pH 5-6. All the mutagenic compounds are toxic and some are very carcinogenic and should be handled with extreme care. The

probability of obtaining a favourable variant/mutant (as in other breeding approaches) is strongly related to the size of the plant population screened.

Treating only a few hundred seeds can hardly be expected to give positive results. Approximately, 1000 surviving and fertile plants in M<sub>1</sub> generation are certainly the minimum, considering that even the effective mutagen treatments may lead to only one mutation per locus in 100000 cells.

M<sub>1</sub> generation should be grown under optimum conditions. Each M<sub>1</sub> plant should be harvested individually for growing M<sub>2</sub> progenies. If M<sub>1</sub> generation is large, a single pod or even a single seed/M<sub>1</sub> plant may be harvested.

Starting with the M<sub>2</sub> generation, the optimum methods are very similar to those used for hybridization programme from F<sub>2</sub> onwards. Most reliable is pedigree selection. Bulk handling followed by pedigree method is also recommended.

However, it should be recognized that in spite of lot of efforts on mutation breeding in peas, in Sweden, Italy, Germany, Poland and Bulgaria, hardly a few mutations have been released as commercial cultivars. Thus, it is clear that mutants have provided a significant range of variation, but that is widely represented in modern day pea breeding, materials.

As a matter of fact, applied mutation breeding is certainly less charming and is on decline, although one can find numerous mutagenesis programmes in public sector research. In India, this facility is easily available in Nuclear Agric. and Biotech Division of Bhabha Atomic Research Centre, Trombay, Mumbai.

### **Idotype breeding**

A major step in producing a plant model more suited to the crop environment was made by B. Snoad who introduced the '*st*' gene for reduced stipule size and the '*af*' gene, which substitutes tendrils for leaflets. A detailed review on this has been written by Hedley and Ambrose (1981). Plants with the genetic constitution '*afafstst*' have acquired the descriptive name of 'leafless'. The main advantage of the 'leafless' pea is its improved standing ability due to its greater number of tendrils. The risk of lodging is reduced and the crop can be more easily harvested. The improved canopy structure may also allow the crop to dry more rapidly with a reduced risk of disease.

Comparisons between near-isogenic leafed and leafless lines grown as spaced pot plants, however, have shown that the yield per plant of the mutant is reduced relative to that of the leafed plant.

However, there are also reports where no significant difference in yield per plant between comparable near-isogenic lines has been observed when grown in randomized plots. More

research is still needed regarding these plant types but it seems that they do not offer yield advantage as such.

### Resistance to diseases

It is convenient to plant spreader rows of a highly susceptible cultivar for field screening against powdery mildew and rust. Plants can also be inoculated by dusting the powdery mildew spores from freshly infected leaves. Similarly, for rust, spore suspensions prepared from infected plants can be sprayed. Screening for wilt will be more effective in the 'sick' plots.

Considering the fact that powdery mildew is a serious disease of pea and for which new good resistant donors are available, a typical backcross breeding approach as applicable to a character governed by a single recessive gene (powdery mildew in pea is under a single recessive gene) as outlined by Gritton (1986) has been shown in Fig. 15.4.

		Powdery mildew resistant cultivar A (T - 10) r r	×	Powdery mildew susceptible cultivar B (Arkel) RR
F <sub>1</sub>		Rr (50% B) susceptible		B (RR)
BC <sub>1</sub>	simultaneously, test cross to select only those backcrosses that have been made with Rr*	RR : Rr* (75% B) × Suscep : Suscep.		B (RR)
BC <sub>2</sub>	- do-	RR : Rr* (87.5% B) × Suscep : Suscep.		B (RR)
BC <sub>3</sub>	- do-	RR : Rr* (93.75% B) × Suscep : Suscep.		B (RR)
BC <sub>4</sub>	- do-	RR : Rr* (96.375% B) × Suscep : Suscep.		B (RR)
BC <sub>5</sub>	- do-	RR : Rr* (98.4375% B) × Suscep : Suscep.		B (RR)
Self all plants	- do-	All, RR, 1 RR : 2Rr : 1rr Suscep : Suscep : Resistant		New resistant line

**Fig. 15.4. Backcross breeding procedure to develop powdery mildew resistant line in pea**

### Molecular Markers in Pea

Since the development of the polymerase chain reaction (PCR) in generating random amplified polymorphic DNA in 1990, this technique has been found valuable in the construction of genetic maps in several species and in production of genetic markers linked to specific phenotypic traits in particular using bulked segregant pools. RAPD technique became popular because of its simplicity and ease of use.

Laucou et al. (1998) constructed a genetic linkage map of *Pisum sativum* L. based primarily on RAPD markers that were carefully selected for their reproducibility and scored in a



population of 139 recombinant inbred lines (RILs). The mapping population was derived from a cross between a protein-rich dry-seed cultivar ‘Terese’ and an increased branching mutant (K 586) obtained from the pea cultivar ‘Torsdag’.

The map currently comprises nine linkage groups with two groups comprising only 6 markers (n = 7 in pea) and covers 1139 cM. This RAPD-based map has been aligned with the map based on the (J1281 x J1399) RILs population that includes 355 markers in seven linkage groups covering 1881 cM.

For this alignment 7 RFLPs, 23 RAPD markers, the morphological marker *le* and the PCR marker corresponding to the gene *Uni* were used as common markers and scored in both populations. Genes for which linked markers have been reported in the pea are listed in Table 15.4.

**Table 15.4. Molecular markers linked with certain genes in pea**

Trait	Gene	Marker
Cotyledon shape	<i>r<sub>b</sub></i>	Vc-5 (RFLP)
Pea seedborne mosaic	<i>sbm-1</i>	GS 185 (RFLP)
Virus resistance		
Pea enation mosaic	<i>en</i>	Adh-1 (isozyme)
virus resistance		
Bean yellow mosaic	<i>mo</i>	Pgm-p (isozyme), P 252 (RFLP)
virus resistance		
Fusarium wilt resistance	<i>Fw</i>	H19, Y14, Y15 (RAPD) : p254, p248, p277, p105 (RFLP)
Powdery mildew resistance	<i>er (-1 ? )</i>	p236 (RFLP) PD10 <sub>650</sub> , (RAPD converted to SCAR)
	<i>er-1</i>	Sc-OPO-18 <sub>1200</sub> , Sc-OPE-16 <sub>1600</sub> (SCAR)
	<i>er-2</i>	3 AFLP primers
Ascochyta blight resistance	Several QTL	af & 1 (linkage group I); p227, p105 (RFLP, linkage group IV ; p236 RFLP, linkage group VI)

McClendon et al. (2002) identified DNA markers linked to fusarium wilt race 1 resistance in pea. Eighty recombinant inbred lines (RILs) from the cross of Green Arrow (resistant) and PI 179449 (susceptible) were developed through single-seed descent, and screened for disease reaction in race 1 infested field soil and the greenhouse using single-isolate inoculum.

The RILs segregated 38 resistant and 42 susceptible fitting the expected 1: 1 segregation ratio for a single dominant gene ( $\chi^2 = 0.200$ ). Bulk segregant analysis (BSA) was used to screen 64 amplified fragment length polymorphism (AFLP) primer pairs and previously mapped random amplified’ polymorphic DNA (RAPD) primers to identify candidate markers. Eight AFLP primer pairs and 15 RAPD primers were used to screen the RIL mapping population and generate a linkage map.

One AFLP marker, ACG: CAT\_222, was within 1.4 cM of the *Fw* gene. Two other markers, AFLP marker ACC: CTG-159 at 2.6 cM linked to the susceptible allele, and RAPD marker

Y15 1050 at 4.6 cM linked to the resistant allele, were also identified. The probability of correctly identifying resistant lines to fusarium wilt race 1, with DNA marker ACG: CAT\_222, is 96%. These markers will be useful for marker assisted breeding in applied pea breeding programs.

Marker assisted selection (MAS) is now being integrated in on-going conventional pea breeding programmes. MAS is particularly, useful to speed-up selection for those traits that express late in plant development. Such target traits include resistance for diseases and even lodging and seed characters.

Isozyme marker alcohol dehydrogenase (Adh 1) has been shown to be linked with resistance to pea enation virus (En.). Two recent examples related to disease resistance are development of PCR markers designed from cDNA-AFLP fragments providing tight linkage to genes (*subm-1* and *mo*) conferring resistance to pea seed borne mosaic virus and an SSR marker suitable for resistance to powdery mildew of peas as mentioned by Ambrose (2008). QTLs for lodging resistance have been reported.

### **Integration of Biotechnology in Conventional Pea Breeding:**

Transformation and regeneration protocols are now available in peas. The most common method involves *Agrobacterium tumefaciens* mediated transformation. The major difficulty lies in the fact that this transformation is genotype specific and only a small portion of cultivars have responded to this technique.

Somaclonal variation arising from the regeneration of plants from callus, led to the use of cotyledonary meristem from freshly imbibed seed as a source of tissue for successful transformation. The use of this technology in the pea breeding is limited to proof of concept.

Partial resistance to alfalfa mosaic virus (AMV) has been reported as a consequence of transformation with chimeric virus coat protein gene,  $\alpha$ -amylase inhibitor ( $\alpha$ -A 1) and the promoter phytohemagglutinin, both found in French-bean when transferred to pea, have shown constitutive expression and resistance to pea weevil. The expression of inhibitor ( $\alpha$ -amylase) served to block the development of the larvae at an early stage and this resulted in less seed damage and better seed quality.

This transgenic pea product could not reach to large scale field testing due to legal issues. Transfer of herbicide resistance both as a reportable marker and a trait have also been reported, but not carried through to commercial release.

While GM crops are on increase in many parts of world with global acreage of 134 million hectares in 2009, the adverse reaction to GM crops in Europe and low rates of transfer have all

contributed to the pea breeding industry not engaging in the development and release of GM peas till date.

#### **Disease Resistance Breeding and Markers:**

Pea powdery mildew, caused by *Erysiphe pisi*, is an air-borne disease with a worldwide distribution, being particularly important in climates with warm dry days and cool nights. Although varying levels of resistance to *E. pisi* have been observed in pea only three genes for resistance named *er1*, *er2* and *Er3* have been described so far. Gene *er1* is widely used in pea breeding programmes and provides complete or incomplete resistance depending on the locations.

Resistance conferred by this gene has been proved to be stable and is caused by a barrier to the pathogen penetration. RFLP, RAPD/SCAR and SSR markers linked to the *er1* have been identified. Gene *er2* is not used commercially. The gene confers a high level of resistance in some locations, but is ineffective in others. The expression of *er2* is influenced by temperature and leaf age.

Gene *er2* governed resistance is based mainly on post-penetration cell death complemented by a reduction of percentage penetration success in mature leaves. AFLP, RAPD and SCAR markers linked to *er2* are available. Gene *Er3* was recently identified in *P. fulvum* and has successfully been introduced into the adapted *P. sativum* material by sexual crossing.

Resistance conferred by the gene *Er3* is due to a high frequency of cell death that occurs both as a rapid response to attempted infection and a delayed response that follows the colony establishment. RAPD markers tightly linked to *Er3* have been identified and converted into SCARs.

Pea rust has become an important pathogen of pea particularly in regions with warm, humid weather. Pea rust has been reported to be caused either by the fungus *Uromyces viciae-fabae* (Syn. *U. fabae*) or *U. pisi*. *U. viciae-fabae* is the principal causal agent of pea rust in tropical and subtropical regions such as India and China, where warm humid weather is suitable for the appearance of both the uredial and the aecidial stage.

Several sources of incomplete resistance against *U. viciae-fabae* have been reported. A single major gene (*Ruf*) has been reported as responsible for this partial resistance. Two RAPD markers have been detected flanking the gene *Ruf*, but both markers were not close enough to the gene to allow a dependable marker-assisted selection for rust resistance.

Only recently, pea germplasm collections have been screened to identify sources of resistance to *U. pisi* both under field and growth chamber conditions. No complete resistance has been identified so far. However, incomplete resistance has been observed in the collections.

All the identified accessions have displayed a compatible interaction (high infection type) both in adult plants under field conditions and in seedlings under growth chamber conditions, but with varying levels of disease reduction.

This resistance was not associated with host cell death. Preliminary results performed on F2:3 revealed two QTLs for resistance to *U. pisi* in the field and controlled conditions, respectively, which seems to be the reason for high percentage of the phenotypic variance.

Aschochyta blight, caused by *Mycosphaerella pinodes*, the teleomorph of *Ascochyta pinodes* (Berk & Blox) Jones, is a widespread pea disease. Nineteen QTLs associated with resistance have been reported. More recently, 6 QTLs (mp1-mp6) have been associated with resistance to *M. pinodes* in a cross of the cultivar Messire with *P. sativum subsp. syriacum*.

Resistance to fusarium wilt in peas caused by *Fusarium oxysporum* Schlecht. f. sp. *pisii* race 1 (van Hall) Snyd. & Hans, is conferred by a single dominant gene *Fw*. The gene has been located in the pea genome by analyzing progenies from crosses involving genetic markers across all pea linkage groups.

*Fw* has been shown to be located on linkage group III, about 13 map units from *Lap-1* and *b* and 14 map units from *Td*. The relatively large distances between these markers and *Fw* precludes the use of the linked markers in marker-assisted selection for wilt resistance.

DNA markers linked to recessive gene *sbm-1* for resistance to pea seed-borne mosaic virus (PSbMV) pathotype P-1 have been identified. Markers linked to the dominantly inherited gene *En* for resistance to pea enation mosaic virus (PEMV) have also been reported.

### **Snap Pea**

The snap pea is a type of edible-podded pea that is conspecific to field and garden peas (*P. sativum* L.). Edible-podded peas lack pod parchment or fibre. Most snap pea cultivars have wrinkled seeds with green cotyledons, white flowers and short internodes. Snap pea cultivars released by public and private breeders and seedsmen in 20th century in USA are as follows: Sugar Stick, Round Podded Sugar, Sugar Snap, Sugar Bon, Sweet Snap, Early Snap, Honey Pod, String-less Sugar Snap, Sugar Daddy, Sugar Gem. Sugar Pop, Sugar Boys. Mega. Super Sugar Snap, Crystal. Sugar Lady, Sugar Star and Jessy, etc.

Among edible podded types. Oregon Sugar Podded (Mithi Phali) has been made popular at PAU, Ludhiana in 1996 through introduction. Pods are light green and devoid of parchment layer. Its average yield is about 100-110 q/ha and it is tolerant to powdery mildew under field condition.

### **Vegetable Pea Varieties:**

In vegetable peas, early introductions from Europe and USA were found quite successful and popular in India. These included Arkel (early maturing, dwarf type, introduction from England in 1970s) and Bonneville (main season, late maturing, tall type, introduction from USA in 1970s). These introductions were obtained at IARI, New Delhi and were released for commercial cultivation after preliminary evaluation.

**Arkel** is still a very popular variety grown throughout the country. Arkel has 45-60 cm plant height, long well filled, sickle shape green pods with 7-9 seeds/pod. The seeds are sweet and become wrinkled at maturity. First picking is done in 60 days. It is highly susceptible to powdery mildew and rust. It has double flowers at lower nodes and single onwards. Shelling percentage is about 40%. The yield potential is 75-80 q/ha.

**VL Ageti Matar** (VL-7) is an early maturing variety developed at VPKAS, Almora in 1995. Plants are dwarf with dark green foliage. Pods are light green, completely filled, two pods in a bunch with 6-7 seeds/pod and 42% shelling. Its average yield is about 80-90 q/ha. Seeds are wrinkled.

**Kashi Nandini** (VRP-5) is an early maturing variety developed at IIVR, Varanasi in 2006 through hybridization (P-1542 x VT-2-1) followed by pedigree selection. Plants are dwarf, erect and come to flowering in 34 days after sowing. Pods are 8-9 cm long, well filled with 8-9 seeds having 48% shelling. Its average yield is 110-120 q/ha. Seeds are wrinkled.

**Matar Ageta-6** is an extra early maturing variety (45-50 days) developed at PAU, Ludhiana in 1996 through hybridization (Massey Gem x Harabona) followed by pedigree selection. Plants are dwarf, erect and bear 12-15 pods with 6 seeds/pod and 45% shelling.

First picking is done in 45-50 days after sowing and about 50% of green pod yield is obtained in the very first picking. Plants are dwarf (40 cm), erect and green. Dry seeds are light green, smooth with slight dimples. It is tolerant to high temperature and its yield potential is about 60-65 q/ha. This variety is suitable for single harvest. Seeds are wrinkled.

**Pant Sabji Matar 3** (Arkel x GC 141) developed at Pantnagar is similar to Arkel. however, the pods are slightly longer and attractive. Pusa Pragati developed at IARI is an early maturing cultivar with slightly straight green pods having 8-10 seeds/pod. First green pods are harvested in 60 days. It has become popular due to longer pods and higher yield potential (100 q/ha).

Kashi Udai (VRP 6) is an early maturing cultivar developed through pedigree method of breeding from the cross of Arkel x FC 1 at IIVR, Varanasi. Plant height is 58-62 cm and 50% plants bear flowers at 35-37 days after sowing. Plants have dark green foliage and short internodes with 8-10 pods/plant. Green pods are attractive, 9-10 cm long, filled with 8-9 bold seeds. Shelling percentage is 48% and green pod yield potential is 100-110 q/ha.

Among main season/late varieties requiring 100 days for first green pod harvest, the popular cultivars are Bonneville, Perfection New Line, Lincoln, Jawahar Matar-1, Jawahar Matar-4, Arka Ajeet, Azad Pea 2, Azad Pea 5, etc. These varieties are not very popular and are on decline from cultivation and the market.

**Bonneville** is an introduction from USA and made popular by IARI. Plants are medium tall (60-70 cm) come to flowering in about 60 days having two pods per peduncle. Pods are light green, straight having 6-7 seeds/ pod with 45% shelling and seeds are green, bold and wrinkled. Its average yield is 100 q/ha and it is susceptible to powdery mildew. Green pods are harvested in 100 days.

**Arka Ajit** (FC-I) is a variety of medium maturity group developed at IIHR, Bangalore in 2006 through multiple cross involving Bonneville, IIHR 209 and Freezer 656. Pods are 8-9 cm long with green bold seeds having 55% shelling. It takes 90 days from sowing to first picking. It has resistance to powdery mildew and rust. Its average yield is about 95-100 q/ha.

**Azad P-5** (KS-225) is late maturing variety developed at CSAUAT, Kanpur in 2006. Plant growth is medium with straight pods full of grains and bearing may be extended up to March. Its average yield potential is 95-105 q/ha and has resistance to powdery mildew.

**Pant Sabji Matar-4** (Arkel x HFP 4) developed at Pantnagar is late maturing and is classified as leafless type as leaflets are converted into tendrils.

## **Sugarcane breeding**

Sugarcane is a large grass of the genus *Saccharum*, tribe Andropogoneae, family Poaceae. Modern sugarcane (*Saccharum spp.*) cultivars are interspecific hybrids derived from a hybridization process involving *Saccharum officinarum* (or “noble cane”) and *Saccharum spontaneum* (wild cane), followed by a series of backcrosses to the noble parent (Daniels and Roach, 1987). The earliest known historical record of sugarcane and sugar is from Indian writings from 3000 to 3400 years ago. The generic name for sugarcane, *Saccharum*, originated from the Indian Sanskrit term “sharkara” for the crude sugary product obtained from the honey reeds. Dispersal of Indian sugarcane westward seems to have occurred during the first millennium BC. Soldiers of Alexander the Great are known to have carried it to Europe from India about 325 BC. Later, Greek and Roman writers were familiar with the concept of the Indian honey reed and its “honey” (sugar) product. The early history of sugarcane is covered by a number of authors, including Deer (1949) and Barnes (1964).

## **Origin**

The origin of sugarcane is a complex question that is best discussed in relation to its taxonomy and distribution in Southeast Asia, the Indonesian Archipelago, and New Guinea. Different species likely originated in various locations with *S. officinarum* and *Saccharum robustum* in New Guinea, *Saccharum barberi* in India, and *Saccharum sinense* in China. Dispersal of *S. officinarum* over a period of thousands of years is believed to have occurred both into the Pacific Ocean area, and along the island chain into Asia, whilst the thinner Indian canes were developed and cultivated in the North India/South China region. The wild progenitor of *S. officinarum* is *S. robustum*.

Sugarcane technologists consider six species to be important as progenitor species in the origin of modern commercial hybrids (*Saccharum spp.*). These six species (listed below) are distinguishable on the basis of sugar content, thickness of stalk, floral characteristics, chromosome number and epidermal hairs. The first four in the list below are in cultivation, while the last two (*S. spontaneum* and *S. robustum*) are wild species growing in southern Asia and New Guinea.

*S. officinarum* L.: sweet, juicy, thick stalk garden cane, initially in New Guinea

*S. barberi* Jesw.: sweet, thin stalk Indian canes

*S. sinense* Roxb.: sweet, thin stalk Chinese canes

*S. edule* Hassk.: edible inflorescence garden cane, New Guinea, Melanesia

*S. spontaneum* L.: very thin, hardy wild canes, low sugar, New Guinea and southern Asia

*S. robustum* Brandes & Jeswiet ex Grassl: tall, harder, thick stalk wild canes, a little juice and sugar, New Guinea and eastern Indonesia

### **Cytology**

*Saccharum spp.* having basic chromosome number  $x=6,8,10$ . *S. officinarum* has diploid chromosome number of  $2n=80$  with minor deviation in chromosome number (aneuploids) are also found. Triploids are rarely found in among selfed progenies of *S. officinarum* and are indistinguishable from diploid progenitors. Diploid chromosome number in *S. barberi* and *S. sinense* has been reported to vary in the five morphologically distinct groups as:

- i. Sunnabile- $2n=82$  and  $116$
- ii. Mungo-  $2n=82$
- iii. Nargori-  $2n=107$  and  $124$
- iv. Suretha-  $2n=90$  and  $92$
- v. Pansahi- $2n=118$

First four groups are considered as *S. barberi* and the Pansahi group is equivalent to *S. sinense*. Bremer (1966) suggested that a subgenus in *Saccharum* with  $2n=68,102$  and  $136$  chromosomes and having secondary basic chromosome number of  $x=17$ .

### **Breeding objectives**

- i. High cane yield
- ii. Moderate to high sucrose content
- iii. Early to full season maturity
- iv. Resistance to diseases
  - A) Red rot
  - B) Smut
  - C) Wilt
  - D) Mosaic
  - E) Ratoon stunting disease
  - F) Grassy shoot disease
- v. Resistance/tolerance to insect pests
  - a) Shoot borer
  - b) Cane borer
  - c) Pyrilla
  - d) Mealy bugs
  - f) Whiteflies
  - g) Termites



- h) White grub
- vi. Tolerance to abiotic stresses
  - a) Drought
  - b) Salinity
  - c) High temperature

Breeding methods of sugarcane are based on following consideration:

- The sugarcane plant is complex polyploid and heterozygous
- The flowering occurs under specific environmental conditions
- Self-incompatibility and male sterility are present
- Sugarcane propagated vegetatively by stem cuttings or sets

### **Breeding methods**

Breeding methods used for developing improved varieties of sugarcane are similar to the methods used in vegetatively propagated crops. Clonal selection and clonal hybridization methods are used to develop improved varieties in sugarcane. In hybridization three types of crosses are made:

**Biparental crosses:** Crosses between two known parental clones. This is easily achieved by bringing together two parents in an isolated area or under lanterns.

**Area crosses:** In this system several male sterile female clones are pollinated by one male parent in isolated area.

**Melting pot crosses:** or polycrosses are made by bringing together groups of large number of superior/ potential parental cultivars in an isolated area. Natural cross pollination allowed. This procedure allows the evaluation of breeding behaviour of large number of clones with minimum expense.

**Mutation breeding:** Presently, mutation breeding in sugarcane aims at creating economic mutants for higher cane yield, non-flowering and resistance to various diseases like red rot, smut, downy mildew and various borers. Difficulty with mutation breeding in sugarcane is that at least three time consuming vegetative generations are needed to get rid of chimerism and to test for stable mutants.

### **National Programmes**

The research on sugarcane in India is being carried out :

- i. Under all India coordinated research project

- ii. At two central institutes, namely, Indian Institute of Sugarcane Research, Lucknow and Suagrcane breeding Institute, Coimbatore

Limitations of Conventional Breeding and Rationale for Transgenic Approaches Conventional breeding in sugarcane has several limitations that can be overcome by transgenic approaches. Listed below are a few such examples.

1. Commercial sugarcane cultivars possess different proportions of chromosomes, complex recombinational events, and varying chromosome sets (aneuploidy) (Sreenivasan et al., 1987). This genomic complexity brings difficulties in applying conventional plant breeding for cultivar improvement. In addition, conventional breeding is a multistage, laborious, and time-consuming process requiring 10–14 years to develop a new cultivar. A single fault, such as disease susceptibility in an otherwise elite cultivar, could cause the cultivar to be abandoned. Conventional breeding approaches to correct such faults in an existing cultivar are impractical in sugarcane, due to the genetic complexity of cultivars (Birch and Maretzki, 1993). The capacity to introduce specific genes by transgenic approach, without major genetic reassortment following crossing, could be used to rescue flawed cultivars (Birch and Maretzki, 1993). For example, the successful production of sugarcane plants resistant to leaf scald disease was achieved by transgenic approach (Zhang et al., 1999).

2. Although breeding efforts in sugarcane have been successful in increasing cane production, only limited success has been achieved recently in increasing sugar content. For example, there has been no increase in sugar content over the last 40 years in Australian sugarcane (Bonnett et al., 2004b). In the USA, Legendre (1995) reported that the average sucrose content of new candidate varieties decreased 3.5% on the fifth cycle of recurrent selection, as compared to the previous cycle, indicating that a limit has been reached for this trait. The QTL analysis of interspecific F<sub>1</sub> populations also indicated that modern sugarcane cultivars have a limited (biased subset) population of genes controlling sugar content (Ming et al., 2001b). In contrast, metabolic engineering of sugarcane through transgenic approaches could improve sugar content. For example, transgenic sugarcane with doubled sugar content was achieved when attempting to produce isomaltulose in sugarcane (Wu and Birch, 2007).

3. Production of novel products in sugarcane is not possible by conventional breeding. In contrast, metabolic engineering through transgenic approaches could produce new products, such as alternative sugars, biopolymers, pharmaceuticals, and high-value proteins. For example, successful production of sorbitol (Chong et al., 2007), isomaltulose (Wu and Birch, 2007), p-hydroxybenzoic acid (pHBA) and biodegradable polymer (McQualter et al., 2005;

Petrasovits et al., 2007) has been achieved in transgenic sugarcane, which cannot be achieved through conventional breeding.

4. Conventional breeding allows transfer of traits and genes only between sexually compatible species. Hence, transfer of traits from noncompatible species is impossible. In contrast, transgenic approaches allow insertion of novel genes from sexually noncompatible plants/organisms, enable expression of native genes at different levels in specific tissues or under novel developmental patterns of expression.

5. The number of traits to be considered when selecting for variety development is determined by the degree of genetic linkage among those traits. If linkages are rare, several traits can be selected simultaneously. In the case of sugarcane the extent of those linkages is still uncertain (Ming et al., 2006). Recent advances in molecular marker-assisted selection and transformation technologies can alleviate the problem. Thus, genetic transformation by modern molecular techniques has the potential to enhance a host of traits including sugar, pest and disease resistance, tolerance to drought and cold, vigor, plant architecture and fiber, and to produce alternative products such as biopolymers and isomaltulose in sugarcane.