

A TEXT BOOK ON PLANT BREEDING

GENETIC
DIVERSITY
SELECTION
HYBRIDIZATION
GENETIC GAIN
CROP IMPROVEMENT

BETTER
VARIETIES
FOR A
SUSTAINABLE
FUTURE



Y. ANBU SELVAM
R. ELANGAIMANNAN
Y. ANITHA VASLINE

Foreword

Plant breeding is certainly is a noble profession and we need to ensure the brightest minds to insure the crop yields will continue to increase to feed an ever-increasing global human population. Plant breeding in the earlier centuries has been regarded as an art and later developed into science as knowledge progressed in classical genetics as the foundation of plant breeding was based on recognition of the gene as the unit of heredity, on procedures for gene manipulation during the 19th and 20th centuries.

More recently the science of molecular genetics proposes to advance plant breeding to an even level of sophistication. This new technology becomes routinely operational; it offers an opportunity to enhance performance of crop cultivars through understanding the basic concepts of molecular biology. This book entitled 'principles of plant Breeding' aims to provide a comprehensive understanding on these basic and advanced principles.

The book will be very useful for both B.Sc (Ag) and M.Sc (Ag) students. The presentation is simple, lucid with essential photographs and illustrations which would also be useful in the preparation of junior and senior research fellowship examinations.

The author has taken commendable effort to include all the requirements necessary for a text book. I am confident that this book will adequately cater to the needs of the students of plant breeding and genetics. I congratulate him for bringing out this useful book.



(G. KUPPUSWAMY)

Dean
Faculty of Agriculture,
Annamalai University

Preface to the first edition

The goal of plant breeding is to change the plant's heredity in ways that it will improve plant performance. Improved plant performance may be manifested in many ways. Improved yield and quality are usually the primary breeding goals. Plants are the basic source of food for the human population. Higher yields of food plants contribute to a more abundant food supply. The world of plant breeding has experienced dramatic changes as understood by Changes in technology, *i.e.*, analytical methods, transgenic methods, thus enable the plant breeder to evaluate more number of germplasm in more ways and in more environment and to identify genotypes that exhibit optimal adaptation to the needs of society, to the demands of nature and the desire of the market.

It is hoped that this book will stimulate interest in plant breeding and encourage students to under stand the basic concepts of plant breeding. This book includes nature of crops and methods of breeding, origin, domestication and introduction of crop plants, breeding self-pollinated crops by introduction and selection, breeding self-pollinated crops by hybridization, methods of breeding crops-pollinated crops, breeding asexually propagated plants, heterosis breeding, mutation breeding, ploidy breeding, organization and achievements of plant breeding, The chapter on molecular breeding helps the students to understand the basic principles and tools developed by molecular biologist, such as DNA markers which are being used to map and tag important plant traits on the genomes of many of our crop plants and the utilization of marker assisted selection to improve the efficiency of conventional plant selection and in turn crop improvement.

The author acknowledges with thanks the Annamalai University authorities for granting permission to write and publish this book. Since the help of the books of various authors has been taken in preparing this book, the author does not claim my authenticity. The author always welcomes and entertains the critical and constructive suggestions whole heartedly.

Author

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CHAPTER -I

BASICS OF PLANT BREEDING

Centres of Origin of crop plants

There is considerable evidence that the cultivated plants were not distributed uniformly throughout the world. Even today, certain areas show far greater diversity than others in the forms of certain cultivated crops and their wild relatives. N.I.Vavilo proposed that *crop plants evolved from wild species in the areas showing great diversity and termed them as **primary centres of origin***. Later, crops moved to other areas primarily due to the activities of man. These latter areas generally lack the richness in variation found in the primary centres of origin. *But in some areas, certain crop species show considerable diversity of forms although they did not originate there. Such areas are known' as **secondary centres of origin** of these species.*

The concept of centres of origin was given by Vavilov based on his studies of a vast collection of plants at the Institute of Plant Industry, Leningrad. He was the director of the institute from 1916 till 1936. He also 'postulated the **Law of Homologous Series in Variation**. This law, states that characters found in one species also occur in other related, species. Thus diploid (2X), tetraploid (4x) and hexaploid (6x) wheats show a series of identical contrasting characters.

Similarly, genus *Secale* duplicates the variation found in genus *Triticum*. Thus a character absent in a species, but found in a related species, is likely to be found in the collection of that species made from the centre of its origin.

Eight main centres of origin were originally proposed by Vavilov: China, Hindustan, Central Asia; Asia Minor, Mediterranean, Abyssinia Centre and South America. Later, in 1935 Vavilov divided the Hindustan Centre of Origin into two centres, viz., *indo-Burma* and *Siam-Malaya-Java Centres of Origin*. Similarly, the South American Centre was divided into three Centres, namely, *Peru*, *Chile* and *Brazil-Paraguay Centres of Origin*. Thus the eight main centres were regrouped into 11 centres of origin. At the same time he introduced a new centre of origin the U.S.A centre, the two plant species, sunflower (*Helianthus annuus*) and Jerusalem artichoke (*Helianthus tuberosus*), are believed to have originated in the U.S.A centre of origin.

The concept that centres of diversity represent centres of origin has been seriously questioned. Plants of a species growing in different environments are likely to be different, i.e., diverse. Thus a plant species is likely to show a greater variation in a region with varied climatic and other ecological conditions. Areas

with mountains and valleys show considerable variation in the prevalent environment. Therefore, plant species would show a great variation in such areas. Interestingly, the centers of origin are situated in such mountain-valley areas. Further the centres of diversity of many species have shifted with time. This shift in diversity was brought about a shift in the area of the greatest cultivation and due to the introduction of the concerned species into an area with the greatest ecological diversity than where they existed before. These processes have given rise to the **secondary centres of diversity**. Consequently, several species have two or more centers of diversity and it is often difficult to determine which one of them is the real centre of origin,

Thus the centres of origin may be more appropriately called the centres of diversity. These centres may not be the centres of origin of the species concerned, but they are the areas of the maximum diversity of these species. These serves as an extremely useful guide to plant explorers as to where to search for variation in a given species. Within the large centres of diversity, small areas may exhibit much greater diversity than the centre as a whole; these areas are known as *micro centres*. The crop evolution appears to proceed at a more rapid rate in such microcentres. The

microcentres are important for plant collection as well as for an experimental study of the evolution of plants.

1. The Abyssinian Centre of Origin

It includes Ethiopia and hill country of Eritrea. It is the *primary centre of origin* for *H. vulgare* (barley), *Triticum durum*, *Triticum turgidum*, *Triticum dicoccum*, Jowar (*Sorghum bicolor*), bajra (*Pennisetum americanum*), gram, lentil, sem (*Dolichos lablab*), pea, khesari (*Lathyrus sativus*), linseed, saff flower, sesame, Castor (*Riccinus communis*), coffee (*Coffea arabica*), onion and okra (*Abelmoschos esculentus*). It is the *secondary centre of origin* for broad bean (*Vicia faba*).

2. The Asia Minor centre of origin

This is also known as the *Near East* or the Persian centre of origin. It includes the interior of Asia Minor, the whole of Transcaucasia, Iran and Highlands of Turkmenistan. The crop species that originated in this region (*primary centre of origin*) include nine species of *Triticum*, rye, alfalfa (*Medicago sativa*), Persian clover (*Trifolium resupinatum*), carrot, cabbage (*Brassica oleracea*), oat (*Avina sativa*), species of *Allium*, lettuce (*Lactuca sativa*), fig (*Ficus carica*), pomegranate (*Punica granatum*), apple, several species of *Pyrus*, *Prunus*, grapes, almonds,

chestnuts (*Castanea* sp.) and pistachio nut. It is the *secondary centre of origin* of rape(*Brassica campestris*), black mustard (*Brassica nigra*), leaf mustard (*Brassica japonica*), turnip (*B. napus*) and apricot.

3. The Central American Centre of Origin

This includes the region of South Mexico and Central America. It is also referred as the *Mexican Centre of Origin*. The plants that originated (*Primary centre of origin*) here are Maize, rajma (*P. vulgaris*), lima bean (*Phaseolus lunatus*), melons, pumpkin (*Cucurbita melanosperma*), sweet potato (*Ipomoea batatas*), arrowroot (*Canna edulis*), chillies (*Capsicum annuum*), cotton, (*G. hirsutum* and *G. purpureascens*), papaya (*Carica papaya*), guava (*Psidium guajava*) and avocado (*Persea americana*).

4. The Central Asia Centre of Origin

It includes North-West India (Punjab, The North-West Frontier provinces and Kashmir), all of Afghanistan, the Soviet Republics of Tadjikistan and Uzbekistan and Tian-Shan. It is also known as the *Afghanistan centre of Origin*. The crops that originated in this centre (*primary centre of origin*) are wheat (*Triticum aestivum*), club wheat (*Triticum compactum*), pea (*Pisum sativum*), broad bean (*Vicia faba*), mung, linseed (*Linum usitatissimum*),

sesame, safflower (*Carthamus tinctorius*), hemp, cotton (*Gossypium herbaceum*), radish, musk melon (*Cucurbita moschata*), carrot (*Dacus carota*), onion (*Allium cepa*), garlic (*Allium sativum*), Spinach (*Spinacea oleracea*), pistachio nut (*Pistacia v'era*), apricot, pear, almond (*Prunus amygdalus*), grape (*Vitis vinifera*) and apple (*Pyrus malus*). It is secondary centre of origin of rye (*Secale cereale*).

5. The China Centre of Origin

This centre consists of the mountainous regions of central and western China and the neighbouring lowlands. It is the largest and the oldest independent centre of origin. The crops that originated in this area (*primary centre of origin*) are: soybean (*Glycine max*), radish (*Raphanus sativus*), *Colacasia antiquorum* (bunda), *Panicum miliaceum* (proso millet) and some other species of millets, buckwheat (*Fagopyrum esculentum*), *Papaver somniferum* (opium poppy), several species of *Brassica* and *Allium*, *Solanum melongena* (brinjal), some species of *Cucumis* and *Cucurbita*, pears (*Pyrus communis*), peaches (*Prunus persica*), apricots (*Prunus armeniaca*), plums (*Prunus divaricata*), orange (*Citrus nobilis*), Chinese tea (*Camellia sinensis*) and naked oats (*Avida nuda*).

In addition, it is *secondary centre of origin* for several crop

plants, e.g., *Zea mays* (maize), *Phaseolus vulgaris* (rajma), cowpea (*Vigna unguiculata*), turnip (*Brassica rapa*) and sesame or til (*Sesamum indicum*).

6. The Hindustan Centre of Origin

This centre includes Burma, Assam, Malaya Archipelago, Java, Borneo, Sumatra and Philippines, but excludes North-West India, Punjab and North-western Frontier Provinces. It is the *primary centre of origin* of rice (*Oryza sativa*), arhar or pigeon pea (*Cajanus cajan*), gram or chickpea, (*Cicer arietinum*), cowpea, mung (*Vigna radiata*), brinjal, *Cucumis sativus* (cucumber), *Lactuca indica* (Indian lettuce), certain species of *Dioscorea* (Yams), *Raphanus indicus* (Indian radish), *Saccharum officinarum* (noble canes), several species of cotton (particularly, *Gossypium arboreum*), hemp (*Cannabis indica*), black pepper (*Piper nigrum*), indigo (*Indigofera* sp.), mango (*Mangifera indica*), orange, sour lime and some other *Citrus* species, coconut (*Cocos nucifera*), banana (*Musa sapientum*) and turmeric (*Curcuma domestica*).

7. The Mediterranean Centre of Origin

Many valuable cereals and legumes originated in this area. The species that originated in this centre (*primary centre of origin*) are: durum wheats (*Triticum durum*), emmer wheats (*Triticum*

dicoccum), and other *Triticum* species, several species of *Avena*, barley (*Hordeum vulgare*), lentil (*Lens esculenta*), several species of *Lathyrus*, pea, broad bean, lupins (*Lupinus* sp.), chickpea, clovers (*Trifolium* sp.), vetch (*Vicia sativa*), several species of *Brassica*, such as, rape, black mustard, cabbage and turnip, onion, garlic, beets, lettuce (*Lactuca sativa*), artichoke, asparagus (*Asparagus officinalis*), lavender, peppermint (*Mentha* sp.) and sage.

8. The South American Centre of Origin

This centre includes the high mountainous regions of Peru, Bolivia, Ecuador, and Columbia, parts of Chile and Brazil, and whole of Paraguay. The crops that originated in this centre (*primary centre of origin*) are many species of potatoes, maize, lima bean, peanut (*Arachis hypogaea*), pineapple (*Ananus comosa*), pumpkin (*Cucurbita maxima*), Egyptian cotton (*Gossypium barbadense*), tomatoes, guava, tobacco (*Nicotiana tabacum* and other species), quinine tree (*Cinchona calisaya*), cassava (*Manihot utilissima*), and rubber (*Hevea* sp.)(**Fig.1**)



Fig 1. Centres of origin of cultivated plant species. 1. The China Centre, 2. The Hindustan Centre, 3. The Central Asia Centre, 4. The Asia Minor Centre, 5. The Mediterranean Centre, 6. The Abyssinian Centre, 7. The Central American Centre, and 8. the South American Centre (8. Peru, 8.a. Brazil-Paraguay and 8Bb, Chile)

Domestication of Crop Plants

Domestication is the process of bringing wild species under
Human Management.

The present day Cultivated plants have been derived from wild weedy species .Therefore the first step in the development of cultivated plants was their domestication. Most of the crops were domesticated by the prehistoric man. Knowingly or unknowingly he must have selected for the characteristics that made the plants more suited to his needs. Under domestication, the crop species have changed considerably as compared to the wild species from which they originated. The changes, brought about by selection by man as well as nature, are often so great that the crops are classified as distinct species. As a result, in many cases, the parental wild species of the cultivated plants are not definitely known. The domesticated species were selected for characteristics entirely different from those for which the wild species were selected in nature. Therefore, the two groups of plants developed in two different, often opposite, directions.

Changes in Plant Species under Domestication

Almost all the characteristics of plant species have been affected under domestication. The characters that show more

distinct changes are those that have been objects of selection and are still plant breeding objectives in many cultivated species. Some of the important changes that have occurred under domestication are briefly listed below.

1. *Elimination of or reduction in shattering* of pods, spikes etc.

This change has taken place in most of the cultivated species.

2. *Elimination of dormancy* has taken place in several crop species. Lack of dormancy has become a problem in crops like barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), mung (*Vigna radiata*) etc.

3. *Decrease in toxins* or other undesirable substances has occurred in many crops. The bitter principle of cucurbitaceous plants provides an example of this type.

4. *Plant type* has been extensively modified. The cultivated plants show altered tillering, branching, leaf characters, etc.

5. In several crop species there has been a decrease in plant height, eg., cereals, millets. This is often associated with a change from indeterminate to determinate habit.

6. In some species on the other hand there has been an increase in plant height under domestication, eg., jute

- (*Corchorus* sp.), sugarcane (*Saccharum officinarum*), forage grasses etc.
7. *Life cycle has become shorter* in case of some plant species.
This is particularly so in case of crops like cotton (*Gossypium* sp.), Arhar (*Cajanus cajan*), etc.
 8. Most of the crop plants show an *increase in size of their grains or fruits*.
 9. *Increase in economic yield* is the most noticeable as well as ,desirable change under domestication. This is self-evident in every crop species.
 10. In many crop species, *asexual reproduction has been promoted under domestication*, e.g., sugarcane, potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), etc.
 11. There has been a *preference for polyploidy* under domestication. Many of the domesticated plant species are polyploids, e.g., potato, wheat, sweet potato, tobacco (*Nicotiana* sp.), etc., while diploid plants are present in nature. Thus domestication seems to have favored polyploidy in these crop species.
 12. In many species, there has been a *shift in the sex form* of the species. In many dioecious fruit trees, bisexual forms

have developed under domestication. Self-incompatibility has also been eliminated in many crop species.

13. *Variability within a variety has drastically decreased under domestication.*

The extreme case is that of pure line varieties, which are completely homogenous genotypically.

Germplasm

The genes required for crop improvement are present in different lines, varieties, strains or populations of the crop species and their relatives. Some useful genes, often critical genes, are contributed by the wild relatives of the crop. The various lines, strains, populations of a crop species and its related wild species constitute the germplasm of the crop. *Thus, the germplasm of a crop may be defined as the sum total of heredity material, i.e., all the alleles of various genes present in a crop species and its wild relatives.* Therefore germplasm consists of the following five types of materials (1) land races, (2) obsolete varieties (3) varieties in cultivation, (4) breeding lines and (5) wild forms and wild relatives.

Genetic Erosion

The gradual loss of variability from cultivated species and their wild forms and wild relatives is called genetic erosion. This variability lead arisen in nature over an extremely long period of time. Therefore, if allowed to be lost, it would be impossible to create it again during a short period. Genetic erosion is a creation of man since ironically man's success in plant breeding is the chief cause of genetic erosion.

Plant Genetic Resources-Conservation and Utilization

A centralized plant introduction agency was initiated in 1946 at the all India agricultural research institute (IARI), New Delhi. The agency began as a plant introduction scheme in the division of botany and was funded by ICAR. In 1956, during the second five year plan the scheme was expanded as the plant introduction and exploration organization. Subsequently in 1961 it was made an independent division, the division of plant introduction in IARI. The division was reorganized as National bureau of plant genetic resources (NBPGR) in 1976. The nature of activities and functions of the bureau have remained the same. The bureau is responsible for the introduction and maintenance of germplasm of agricultural and horticultural plants.

The bureau has its headquarters at IARI, New Delhi. It has four substations for the testing of introduced plant materials. These substations represent the various climatic zones of India; their locations are listed below.

1. *Simla*. It is situated in Himachal Pradesh at approximately 2.300 m above sea level. It represents the temperate zone.
2. *Jodhpur, Rajasthan*. This substation represents the arid zone
3. *Kanyakumari, Tamil Nadu*. It represents the tropical zone.
4. *Akola, Maharashtra*. It represents the mixed climatic zone, It was recently shifted from Amravati.
5. *Shillong, Meghalaya*.

Germplasm Conservation

The germplasm has to be maintained in such a state that there is minimum risk for its loss and that either it can be planted directly in the field or it can be prepared for planting with relative ease. This is called *germplasm conservation*. Germplasm can be conserved either (a) *in situ* or (b) *ex situ*.

(a) *In situ* Germplasm Conservation

Conservation of germplasm in its natural habitat or in the area where it grows naturally is known as *in situ germplasm conservation*. This is achieved by protecting this area from human

interference; such an area is often called ***natural park, biosphere reserve or gene sanctuary***. A gene sanctuary is best located within the centre of origin of crop species concerned, preferably covering the microcentre within the centre of origin. NBPGR, New Delhi, is making attempts to establish gene sanctuaries in Meghalaya for *Citrus* and in North-Eastern region for *Musa*, *Citrus*, *Oryza*, *Saccharum* and *Mangifera*.

(b) Ex situ Germplasm Conservation

Conservation of germplasm away from its natural habitat is called ex situ Conservation. It can be achieved in the following 5 ways: (1) seed banks. (2) Plant or field banks. (3) Shoot tip banks, (4) cell and organ banks, and (5) DNA banks.

(1) Seed Banks

In *seed banks* germplasm is stored as seeds of various accessions. *Virtually all gene banks are essentially seed banks*. Seeds are classified, mainly on the basis of their storability, into two major groups: (a) orthodox and (b) recalcitrant.

a. Orthodox Seeds. Seeds of this type can be dried to a moisture content of 5% or lower without lowering their viability. Most crop seeds belong to this category. Such seeds can be easily stored for long periods.

b. Recalcitrant Seeds. The viability this group of seeds drops drastically if their moisture content is reduced below 12-30%. Seeds of many forest and fruit trees and of several tropical crops like *citrus*, cocoa, coffee, rubber, oil palm, mango, jack fruit etc. belong to this group.

(2) *Plant or field banks*

A Plant or field bank is an orchard or a field in which accessions of fruit trees or vegetatively propagated crops are grown and maintained.

(3) *Shoot tip banks*

In such gene banks, germplasm is conserved as slow growth cultures of shoot-tips and node segments. Their regeneration consists of sub culturing the cultures which may be done every 6 months to 3 years.

(4) *Cell and organ banks*

A germplasm collection based on cryopreserved (at -196° in liquid nitrogen) embryogenic cultures, shoot tips and or somatic/zygotic embryos may be called cell and organ bank.

(5) *DNA banks*

In these banks DNA segments from the genomes of germplasm accessions are maintained as cosmid clones, phage

lysates or pure DNA. These DNA segments can be evaluated and the desired ones may be used to produce transgenic plants.

Germplasm Utilization

The germplasm can be used in a breeding programme in the following 3 ways.(1)direct release as a variety.(2) it may be subjected to selection for developing a variety and (3) it may be used as a parent in hybridization programmes.

(1) Direct release as a variety

Semi dwarf wheat varieties Sonora 64 and Lerma rojo were released directly for cultivation. TN 1 rice was introduced from Taiwan and directly released as a variety. Other introduced rice varieties are IR 8, IR 21 and IR 36 from IRRI, Philippines.

(2) Varieties selected from introduction

Many varieties have been developed through selection from introductions. Two varieties of wheat ,kalyan sona and sonalika were selected from introductions from CIMMYT, Mexico.

(3) Varieties developed through hybridization

All the present day semi dwarf wheat varieties are derived from crosses with Mexican semidwarf wheat varieties. All but few semidwarf rice varieties possess the dwarfing gene Dee-geo-woo-gen through usually either TN I or IR 8. Some of the other

examples of varieties developed through hybridization with introductions are pusa ruby tomato obtained from a cross between Meeruti and Sioux.

Biodiversity

Biodiversity (biological diversity) is 'the variety and variability among living organisms (plants) and the ecological complexes in which they occur'. In other words, the variety of different species and ecosystems on the planet.

Importance of biodiversity

Biodiversity benefits humans. Human activities cause biodiversity loss which threatens continuity of ecosystems and affects provision of goods and services to humans. Without biodiversity, human well-being is affected. Without biodiversity, all ecosystems suffer and the balance of life on earth is lost.

There has been a rapid decline in biodiversity over the past century. This is because economic systems don't value these essential contributions to human welfare. Climate change has gained some of the attention it needs, but biodiversity is still ignored. This is a mistake and without biodiversity no life is stable, hence our continued existence on the planet is unstable too.

CHAPTER - II

REPRODUCTION IN CROP PLANTS

The various modes of reproduction found in crop plants may be broadly grouped into the following two categories: (1) Asexual and (2) sexual.

I. Asexual Reproduction

Asexual reproduction does not involve fusion of male and female gametes. New plants may develop either from vegetative parts of the plant (*vegetative reproduction*) or they may arise from embryos that develop without fertilization (*apomixis*).

a) Vegetative Reproduction

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

1. Underground stems

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

Tuber: Potato (*Solanum tuberosum*) etc.

Bulb: Onion (*Allium cepa*), garlic, (*Allium sativum*) etc.

Rhizome: Ginger (*Zingiber officinale*), turmeric (*Curcuma domestica*) etc.

Corm: Bunda (*Colocasia ontiguorum*), arwi (*Colocasia esculenta*) etc.

2. Sub-aerial Stems

These modifications include runner, stolon, sucker etc.

Sub aerial stems are used for the propagation of mint(*Mentha* sp.), date palm(*Phoenix dactilifera*).

3. Bulbils

Bulbils are modified flowers that develop into plants directly without formation of seeds. These are vegetative bodies as their development does not involve fertilization and seed formation. The lower flowers in the inflorescence of garlic naturally develop into bulbils. Scientists are trying to induce bulbil development in plantation crops by culturing young inflorescences on tissue culture media. It has been successfully done in cardamom.

4. Artificial Vegetative Reproduction

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

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

Significance of Vegetative Reproduction

Vegetative reproduction offer unique possibilities in breeding. *A desirable may be used directly regardless of 'whether it is of homozygous or heterozygous. Further, mutant buds, branches or seedlings, if desirable can be multiplied and directly used as varieties.* However, it does not allow transfer of genes /cytoplasm from one variety into another.

b) Apomixis

In apomixes, seeds are formed but the embryos develop without fertilization. Consequently, the plants resulting from them are identical in genotype to the parental plant. In apomictic species, sexual reproduction is either suppressed or absent. When sexual reproduction also occurs, the apomixes is termed as facultative. But when sexual reproduction is absent it is referred

to as obligate. Many crop species show apomixes but it is generally facultative. Apomixis has been reported in over 300 species belonging to over 30 families.

Classification of apomixes

1. Adventive Embryony. In this case embryos develop directly from the vegetative cells of the ovule, such as nucellus, integument and chalaza. Development of embryo does not involve production of embryo sac. Adventive embryony occurs in mango (*Mangifera indica*), *Citrus. etc.*,

2. Apospory. Some vegetative cells of the ovule develop into unreduced embryo sacs after meiosis. The embryo may develop from egg cell of some other cell of such an embryo sac. Apospory occurs in some species of Hieraceum, Malus, Crepis, Ranunculus, etc.

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

3a. Parthenogenesis. In parthenogenesis the embryo develops from egg cell. Depending upon whether the embryo sac is haploid or diploid, parthenogenesis is termed as haploid or diploid

parthenogenesis. Haploid parthenogenesis occurs accidentally and has been reported in *solanum nigrum*, *nicotiana*, *crepis* and *maize*. Diploid parthenogenesis occurs in many grasses, *eg.*, *taraxacum*. In many species *eg.*, *nicotiana*, *datura*, *rice* etc pollen grains may be induced in vitro to produce haploid embryos or plantlet. This is called androgenesis. For this anthers or pollengrains are cultured on a suitable tissue culture medium. This technique offers several possibilities for crop improvement.

3b.apogamy. In apogamy, synergids or antipodal cells develop into an embryo. Like parthenogenesis apogamy may be haploid or diploid depending upon the haploid or diploid state of embryo sac. Diploid apogamy occurs in *antennaria*, *alchemilla*, *allium* and many other plant species.

Significance of Apomixes

Apomixes is a nuisance when the breeder desires to obtain sexual progeny, *ie.*, selfs or hybrids. But it is of great help when the breeder desires to maintain varieties/desirable genotypes. Thus in breeding of apomictic species the breeder has to avoid apomictic progeny when he is making crosses or producing inbred lines. But once a desirable genotype has been selected it can be multiplied and maintained through apomictic progeny. This would keep the

genotype of a variety intact. Elaborate breeding schemes are being devised to use apomixes for multiplication and maintenance of hybrid varieties and thereby fix heterosis. Asexually reproducing crop species are highly heterozygous and show severe inbreeding depression. Therefore breeding method in such species must avoid inbreeding.

II. Sexual Reproduction

Sexual reproduction involves fusion of male and female gametes to form zygote, which develops into an embryo. In crop plants, male and female gametes are produced in specialized structures known as flowers.

Flower

A flower usually consists of sepals, petals (or their modifications), stamens and/or pistil. A flower containing both stamens and pistil is a perfect or hermaphrodite flower. If it contains stamens but not pistil, it is known as staminate, while a pistillate flower contains pistil but not stamens. Staminate and pistillate flowers occur on the same plant in a monoecious species, such as maize, Colocasia, castor (*Ricinus communis*), coconut etc. But in dioecious species, staminate and pistillate flowers occur on different plants, *e.g.*, papaya, date palm (*Phoenix dactylifera*),

pistachio (*Pistacia vera*), hemp (*Cannabis indica*) etc. In crop plants, meiotic division of specific cells in stamen and pistil yields microspores and megaspores, respectively. This is followed by mitotic division of the spore nuclei to produce gametes; the male and female gametes are produced in microspores and megaspores, respectively.

Sporogenesis

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

1. Microsporogenesis

Each anther has four pollen sacs, which contain numerous pollen mother cells (PMCs). Each PMC undergoes meiosis to produce four haploid cells or microspores. This process is known as microsporogenesis **(Fig.2)**. The microspores mature into pollen grains mainly by a thickening of their walls.

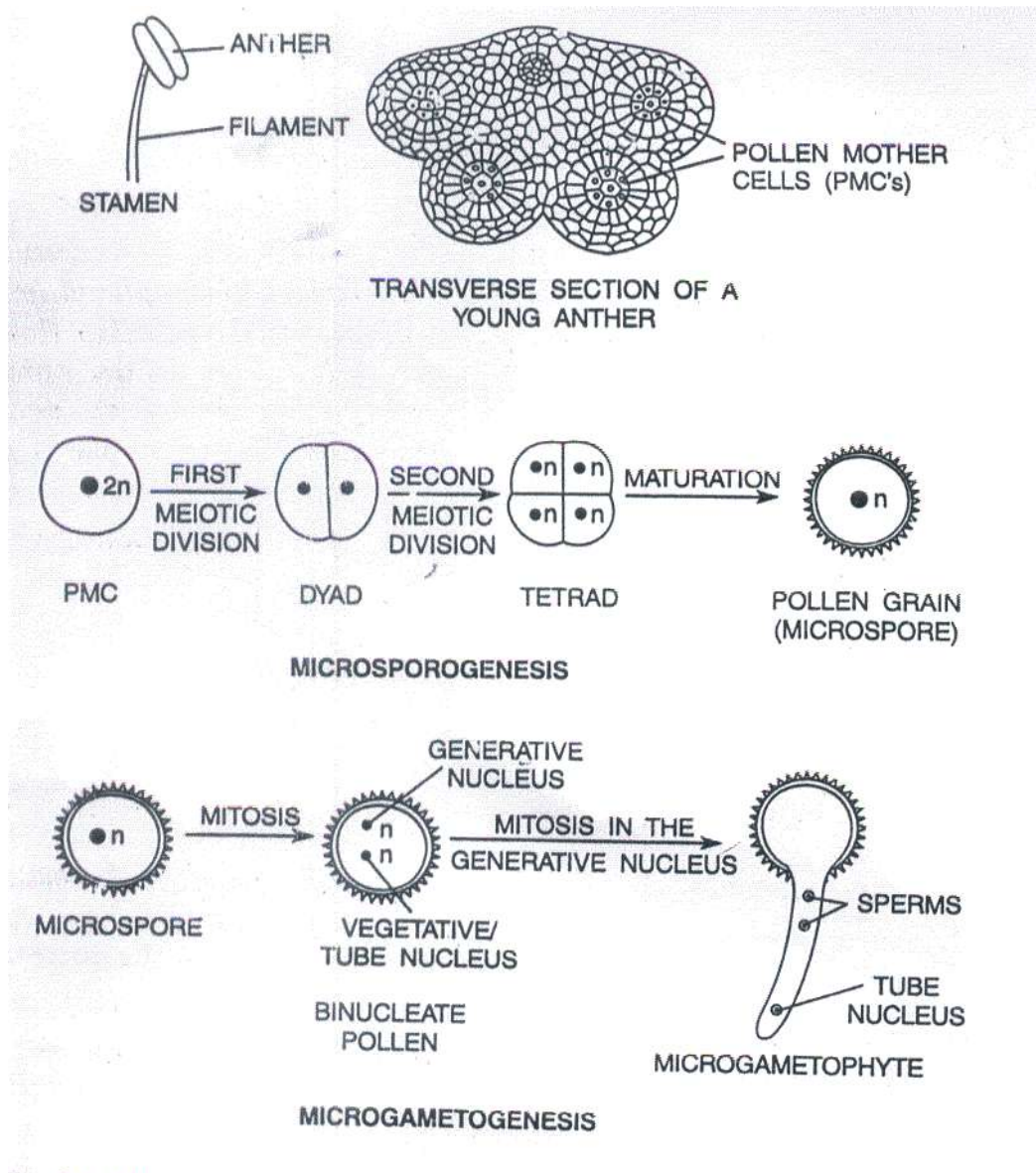


Fig 2. Microsporogenesis and microgametogenesis (a generalised scheme)

2. Megasporogenesis

Megasporogenesis occurs in ovules, which are present inside the ovary. A single cell in each ovule differentiates into a megaspore mother cell. The megaspore mother cell

undergoes meiosis to produce four haploid megaspores. Three of the megaspores degenerate leaving one functional megaspore per ovule (**Fig.3**). This completes megasporogenesis.

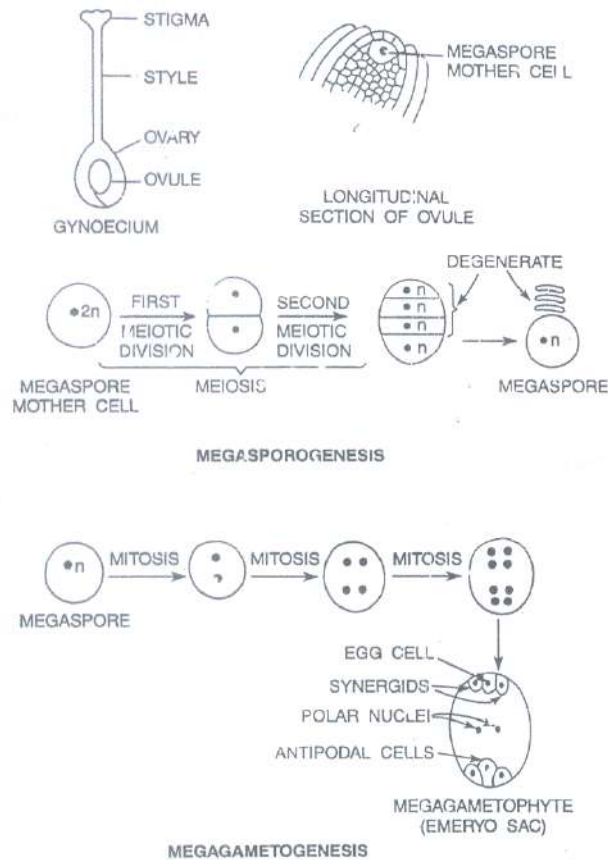


Fig 3. Megasporogenesis and megagametogenesis (a generalised scheme)

Gametogenesis

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

1. Microgametogenesis

This refers to the production of male gamete or sperm. During the maturation of pollen, the microspore nucleus divides mitotically to produce a generative and a vegetative or tube nucleus. The pollen is generally released in this binucleate stage. When the pollen lands onto the stigma of a flower, it is known as *pollination*. Shortly after pollination, the pollen germinates. The pollen tube enters the stigma and grows through the style. The generative nucleus now undergoes a mitotic division to produce two male gametes or sperms. The pollen, along with the pollen tube, is known as microgametophyte (**Fig.2**). The pollen tube finally enters the ovule through a small pore, micropyle, and discharges the two sperms into the embryo sac.

2. Megagametogenesis

The nucleus of a functional megaspore divides mitotically to produce four or more nuclei. The exact number of nuclei and their arrangement varies considerably from one species to another. In most of the crop plants, megaspore nucleus undergoes three mitotic divisions to produce eight nuclei. Three of these nuclei move to one pole and produce a central **egg cell** and two **synergid**

cells. One synergid is situated on either side of the egg cell. Another three nuclei migrate to the opposite pole to give rise to **antipodal cells.** The two nuclei remaining in the centre, the **polar nuclei**, fuse to form a secondary nucleus. The megaspore thus develops into a mature **megagametophyte** or **embryo sac.** *The development of embryo sac from a megaspore is known as megagametogenesis.* The embryo sac generally contains one egg cell, two synergids, three antipodal cells (all haploid), and one diploid secondary nucleus (**Fig. 3**).

Fertilization

The fusion of one of the two sperms producing a diploid zygote, is known as *fertilization*. The fusion of the remaining sperm with the secondary nucleus, leading to the formation of a triploid primary endosperm nucleus is termed as **triple fusion.** The zygote divides mitotically to produce a diploid embryo. The primary endosperm nucleus produces endosperm through repeated mitotic division. During seed development, endosperm produce nutrition to the developing embryo. It may be absorbed completely, *eg.*, in legumes or may form the major portion of

seeds, e.g., in monocots like maize (*Z. mays*) wheat (*Triticum sp.*) etc., and some dicots, *eg.*, castor (*R. communis*), *Brassica sp* ,*etc.*

Significance of Sexual Reproduction

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

CHAPTER -III

POLLINATION IN CROP PLANT

Anthesis

In the process of flowering, the first opening of a flower is known as anthesis. Generally, it occurs in the morning. The occurrence of anthesis vary from one species to another. They are also greatly affected by environmental factors, such as humidity and temperature. A knowledge of anthesis of a crop is desirable for making successful crosses, and also it largely determines the mode of pollination prevalent in the crop. In case of rice (*O. sativa*), a flower is enclosed by two boat shaped bracts, **lemma** and **palea**. Petals and sepals are represented by two small sac *lodicules*. It has six stamens and one ovary. The ovary has two elongated and feathery stigmas. When stigmas become receptive, the lodicules gradually swell and push the lemma and palea apart. Simultaneously, the filaments of stamens elongate and push the anthers out of the bracts. The anthers dehisce during this process and liberate most of their pollen inside the flower. The remaining pollen grain] fall outside reaching the stigma, the pollen grains

germinate within few minute. The pollen reaches the embryo sac in about 20 minutes to two hours. Sometimes the anthers may be pushed out before they dehisce, or the pollen may not be functional. In such cases, cross-pollination may take place. In rice, as high as 2-3 per cent cross pollination may occur.

Pollination systems in crop plants

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

Self-Pollination

Many cultivated plant species reproduce by self-pollination . Self-pollinated species are believed to have originated from cross-pollinated ancestors. These species, as a rule, must have hermaphrodite flowers. But in most of these species, self-pollination is not complete and cross-pollination may occur up to

5%.The degree of cross-pollination in self-pollinated species is affected by several factors, *eg.*, variety, environmental conditions like temperature and humidity and location.

Contrivances to aid Self-Pollination

There are various mechanisms that promote self-pollination, which are generally more efficient than those promoting cross-pollination. These mechanisms are briefly summarized below.

1.Cleistogamy

In this case, flowers do not open at all. This ensures complete self-pollination since foreign pollen cannot reach the stigma of a closed flower. Cleistogamy occurs in some varieties of wheat (*Triticum* sp.), oats (*Avena* sp.), barley (*H. vulgare*) and in a number of other grasses.

2. Chasmogamy

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

1.In crops like tomato (*L. esculentum*) and brinjal (*S. melongena*), the stigma are closely surrounded by anthers.

Pollination generally occurs after flowers open. But the position of anthers in relation to stigmas ensures self pollination.

2. In some species, flowers open but the stamens and the stigma are hidden by other floral organs. In several legumes, *e.g.*, pea (*P. sativum*), mung (*V. radiata*), urd (*V. mungo*), soybean (*G. max*) and gram (*C. arietinum*), the stamens and the stigma are enclosed by the two petals forming a keel.

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

Genetic Consequences of Self Pollination

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

Cross-Pollination

In cross-pollinated species, the transfer of pollen from a flower to the stigmas of the others may be brought about by wind (anemophily), water (hydrophily) or insects (entomophily). Many of the crop plants are naturally cross-pollinated . In many species, a small amount (up to 5-10 per cent) of selfing may also occur.

Contrivances to aid Cross Pollination

There are several mechanisms that facilitate cross pollination. These mechanisms are described briefly.

1.Dicliny

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

1a. Monoecy.

Staminate and pistillate flowers occur in the same plant, either in the same inflorescence, *eg.*, castor, mango (*Mangifera indica*), banana (*Musa sapientum*) and coconut, or in separate inflorescences, *e.g.*, maize. other monoecious species are cucurbits (*Cucurbita* sp.), walnut (*Juglans regia*), chestnut, strawberries (*Fragaria* sp.), rubber (*Hevea* sp.), grapes (*Vitis vinifera*) and cassava (*M. utilisissima*).

1b. Dioecy.

The male and female flowers are present on different plants, *ie.*, the plants in such species are either male or female, *eg.*, papaya (*C. papaya*), datepalm, hemp, asparagus and spinach. In general, the sex is governed by a single gene, *eg.*, asparagus and papaya. In some cases, there are hermaphrodite plants in addition to male and female plants, and a number of intermediate forms may also occur.

2.Dichogamy

Stamen and pistils of hermaphrodite flowers may mature at different times, thereby, facilitating cross-pollination.

2a. Protogyny. In crop species like bajra, pistils mature before stamens.

2b. Protandry. In crops like maize and sugarbeets, stamens mature before pistils.

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

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

promoting cross-pollination. For example, maize exhibits both monoecy and protandry.

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

4. Male Sterility. Male sterility refers to the absence of functional pollen grains in otherwise hermaphrodite flowers. Male sterility is not common in natural populations. But it is of great value in experimental populations particularly in the production of hybrid seeds. Male sterility is of two types: Genetic and cytoplasmic. Cytoplasmic male sterility is termed cytoplasmic- genetic when restorer genes are known.

Genetic Consequences of Cross-Pollination

Cross-pollination preserves and promotes heterozygosity in a population. Cross-pollinated species are highly heterozygous and show mild to severe inbreeding depression and a considerable amount of heterosis. The breeding methods in such species aim at

improving the crop species without reducing heterozygosity to an appreciable degree. Usually hybrid or synthetic varieties are the aim of breeder wherever the seed production of such varieties is economically feasible.

Often Cross-Pollinated Species

In many crop plants cross-pollination often exceeds 5 per cent and may reach 30 per cent. Such species are generally known as **often cross-pollinated species**, *eg.*, jowar, cotton, arhar (*Cajanus cajan*), safflower *etc.* The genetic architecture of such crops is intermediate between those of self-pollinated and cross-pollinated species. Consequently, in such species breeding methods suitable for both of them may be profitably applied. But often hybrid varieties are superior to others.

Mechanisms of Pollination Control in Crop Plants

There are several natural mechanisms that control the mode of pollination in crop plants. Two of these mechanisms, self-incompatibility and male sterility are of special significance because of their utilization in hybrid seed production. Therefore, these mechanisms will be considered in some detail.

Self incompatibility

Self-incompatibility (SI) was first reported by Koelreuter in the middle of eighteenth century. More than 300 species belonging to 70 families of angiosperms show self incompatibility. In case of self-incompatibility, pollen grains fail to germinate on the stigma of the flower that produced them. If some pollen grains do germinate, pollen tubes fail to enter the stigma. In many species, the pollen tubes enter the style, but they grow too slowly to effect fertilization before the flower drops. Sometimes fertilization is effected, but the embryos degenerate at a very early stage. Self incompatibility appears to be a biochemical reaction, but the precise nature of these reactions is not clearly understood. The genetic control of incompatibility reaction is relatively simpler. Lewis (1954) has suggested various classifications of self incompatibility; a relatively simpler classification is as follows: (1) heteromorphic system, (2) homomorphic system, (2a) gametophytic control, and (2b) sporophytic control.

Heteromorphic System

In this system, flowers of different incompatibility groups are different in morphology. For example, in *Primula* there are two types of flowers, ***pin and thrum***. Pin flowers have long styles and short stamens, while thrum flowers have short styles and long

stamens. This situation is referred to as ***distyly***. *Pin and thrum flowers are produce on different plants. The only compatible mating is between pin and thrum flowers.* This characteristic is governed by a single locus **s**; Ss produce thrum, while ss produce pin flowers. The incompatibility reaction of pollen is determined by the genotype of the plant producing them. Allele S is dominant over s. The incompatibility system, therefore, is **heteromorphic-sporophytic**. The pollen grains produced by pin flowers, would all be s in genotype as well as incompatibility reaction. The pollen produced by thrum flowers would be of two types genotypically, S and s, but all them would be S phonotypically. The mating between pin and thrum plants would produce Ss and ss progeny in equal frequencies **(Fig.4)**. This system is of little importance in crop plants; it occurs in sweet potato and buckwheat.

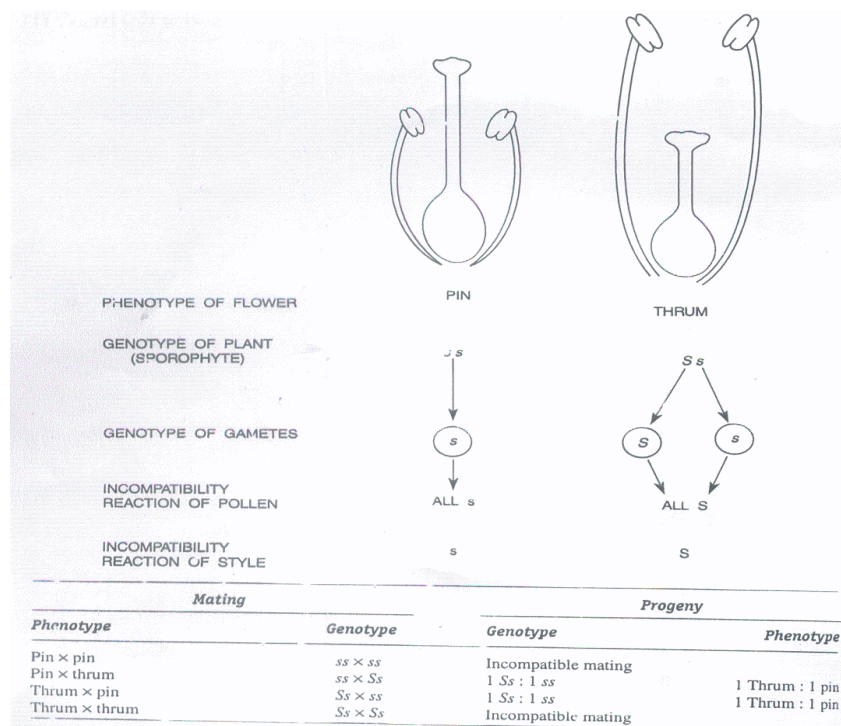


Fig 4. Hereromorphc – sporophytic system of incompatibility. The relative positions of stigma and anthers are governed by a single gene S/s. The incompatibility reaction of pollen is governed by the genotype of the saprophyte on which it is produced while that of style is controlled by it own genotype . Allele S is foraminate over S in determining incompatibility as well as the length of style. Pollen of an incompatibility group would be able to grow normally in the style of the same incompatibility group and would be unable to effect fertilization. Such\ a mating is said to be incompatible/.

Homomorphic System

In the homomorphic system, incompatibility is not associated with morphological differences among flowers. The incompatibility reaction of pollen may be controlled by the genotype of the plant

on which it is produced (**sporophytic control**) or by own genotype (**gametophytic control**).

Gametophytic System

Gametophytic incompatibility was first described by East and Mangelsdorf in 1925 in *Nicotiana sanderae*. The incompatibility reaction of pollen is determined by its own genotype, and not by the genotype of the plant on which it is produced (**Fig. 5**). This is because the biochemical substance involved in **SI** reaction of the pollen is produced after meiosis. In style, both the S alleles express themselves, *ie.*, show co-dominance. Generally, incompatibility reaction is determined by a single gene having multiple alleles, *eg.*; *Trifolium*, *Nicotiana*, *Lycopersicon.*, *Solanum*, *Petunia* etc. Sometimes, polyploidy may lead to a loss of incompatibility due to the competition between the two S alleles present in diploid pollen. Irradiation of pollen or flower buds with X-rays or gamma-rays temporally suppresses the incompatibility reaction, and thus allows the pollen tube to grow through incompatible style. In some species, *eg.*, *Phalaris*, *Physalis* etc., two loci (S and Z) govern incompatibility, while in some others, *eg.*, *Beta vulgaris* and *Papaver*, three loci are involved. The stigma surface is wet except in Graminae, so that there is no direct interaction between individual

pollen grains and stigma surface cells; all pollen grains germinate in a common stigmatic fluid. Pollen tube grows very slowly in style containing the same S allele as the pollen, and fails to effect fertilization.

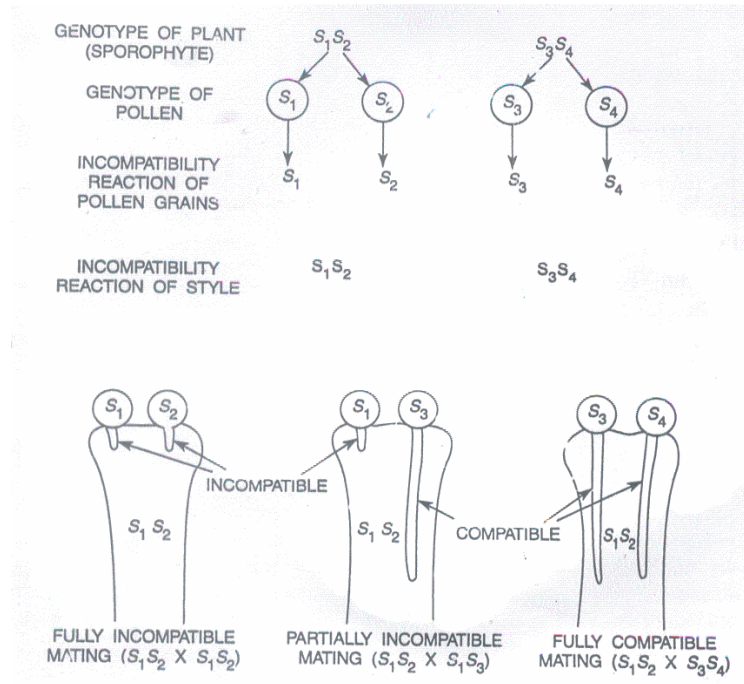


Fig 5. Gametophytic system, of self – incompatibility. The incompatibility reaction of pollen is governed by the genotype of pollen itself. The incompatibility reaction of style is determined by its own genotype and, generally, the two alleles show codominance.

Therefore all the plants are heterozygous at the S locus. In a single gene system, there are three types of matings:

- i) fully incompatible, *eg.*, $S_1S_2 \times S_1S_2$
- ii) fully compatible, *eg.*, $S_1S_2 \times S_3S_4$

iii)Partially (*ie.*,50%of the pollen) compatible ,*eg.*, $S_1S_2 \times S_2S_3$.

Sporophytic System

In the sporophytic system also, self-incompatibility is governed by a single gene, S, with multiple alleles; more than 30 alleles are known in *Brassica oleracea*. In general, the number of S alleles is considerably larger in the gametophytic than in the sporophytic system. The incompatibility reaction of pollen is governed by the genotype of the plant on which the pollen is produced, and not by the genotype of the pollen (**Fig. 6**). It was first reported by Hughes and Babcock in 1950 in *Crepis foetida*, and by Gerstel in *Parthenium argentatum*(in the same year).

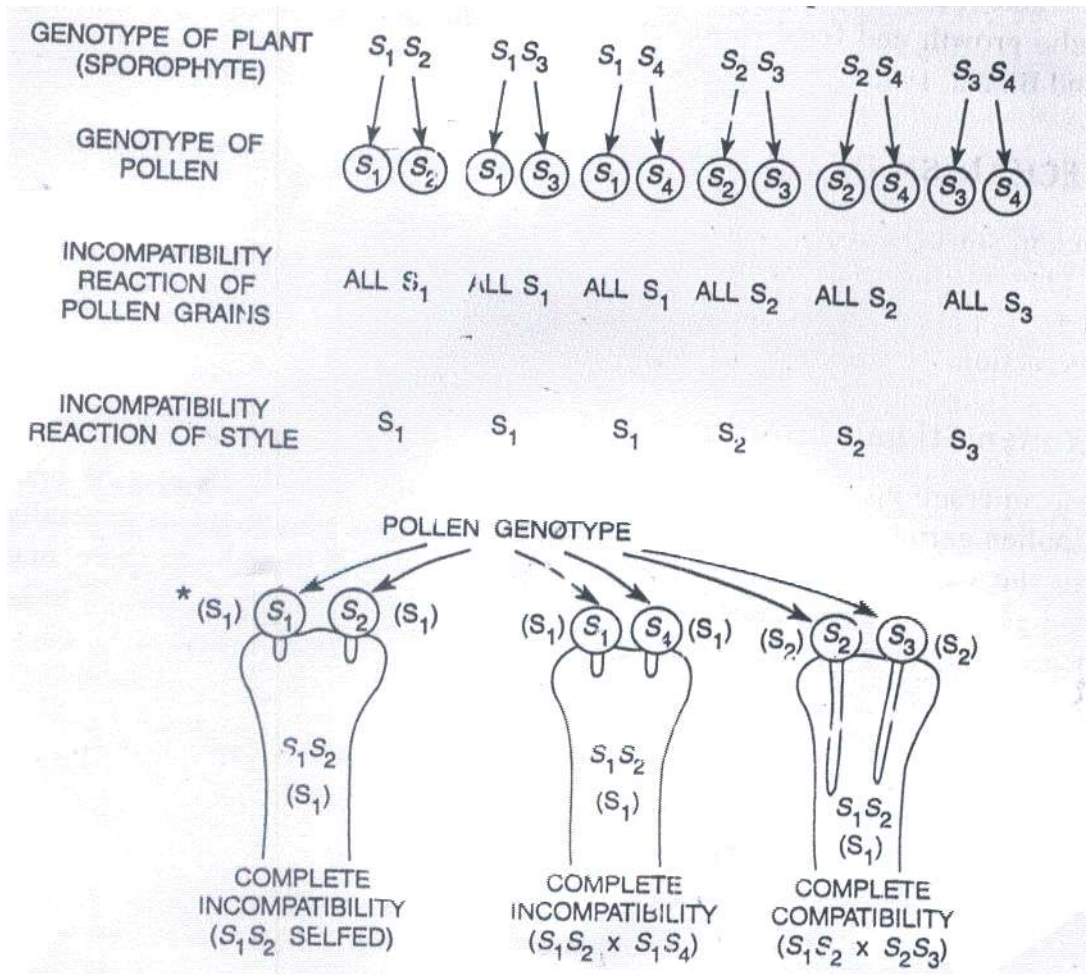


Fig. 6. Sporophytic system of self-incompatibility. The incompatibility reaction of pollen grains is controlled by the genotype of plant (sporophyte) on which they are produced. While that of style is governed by its own genotype. For simplicity it is assumed that the incompatibility alleles (S alleles) show complete dominance in the manner $S_1 > S_2 > S_3 > S_4$ etc. The actual situation is much more complicated and several different types of allelic relationships are encountered. The letters within parenthesis, e.g. (S1) and (S2) denote the incompatibility reactions of pollen grains and styles.

In the sporophytic system, the S alleles may exhibit dominance, individual action (Codominance) or competition. Consequently there may be many complex incompatibility relationships. Lewis has summarised the following characteristics of this system.

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

Sporophytic incompatibility is found in radish (*R. sativus*), diploid *Brassica* crops and *Sinapis*. In many cases, different S alleles vary in their activity leading to varying degrees of self-incompatibility, e.g., *B. oleracea*. Polygenes (modifying genes) are known to increase as well as decrease the activities of S alleles both in the gametophytic as well as sporophytic systems.

Male sterility

Male sterility is characterized by nonfunctional pollen grains, while female gametes function normally. It occurs in nature sporadically, perhaps due to mutation. Male sterility is classified into the following groups: (1) genetic (GMS), (1a.) temperature

sensitive genetic (TGMS), (1b.) photoperiod-sensitive genetic (PGMS), (1C.) Transgenic-genetic, (2) cytoplasmic (CMS), (3) cytoplasmic-genetic (CGMS), and (4) chemically induced male sterility.

Genetic Male Sterility

Genetic male sterility is of wide occurrence in plants. It is ordinarily governed by a single recessive gene, **ms**, but dominant genes governing male sterility are also known, *eg.*, in safflower. Male sterility alleles arise spontaneously or may be artificially induced. In rice, 25 *ms* are known. When a male sterile plant (*ms ms*) is crossed with a male fertile (*Ms Ms*) one, the F₁ (*Ms ms*) is male fertile. In F₂, a 3 male fertile : 1 male sterile ratio is obtained. A male sterile line may be maintained by crossing it with heterozygous male fertile (*Ms ms*) plants. Such mating produces 1 : 1 male sterile and male fertile plants (**Fig. 7**).

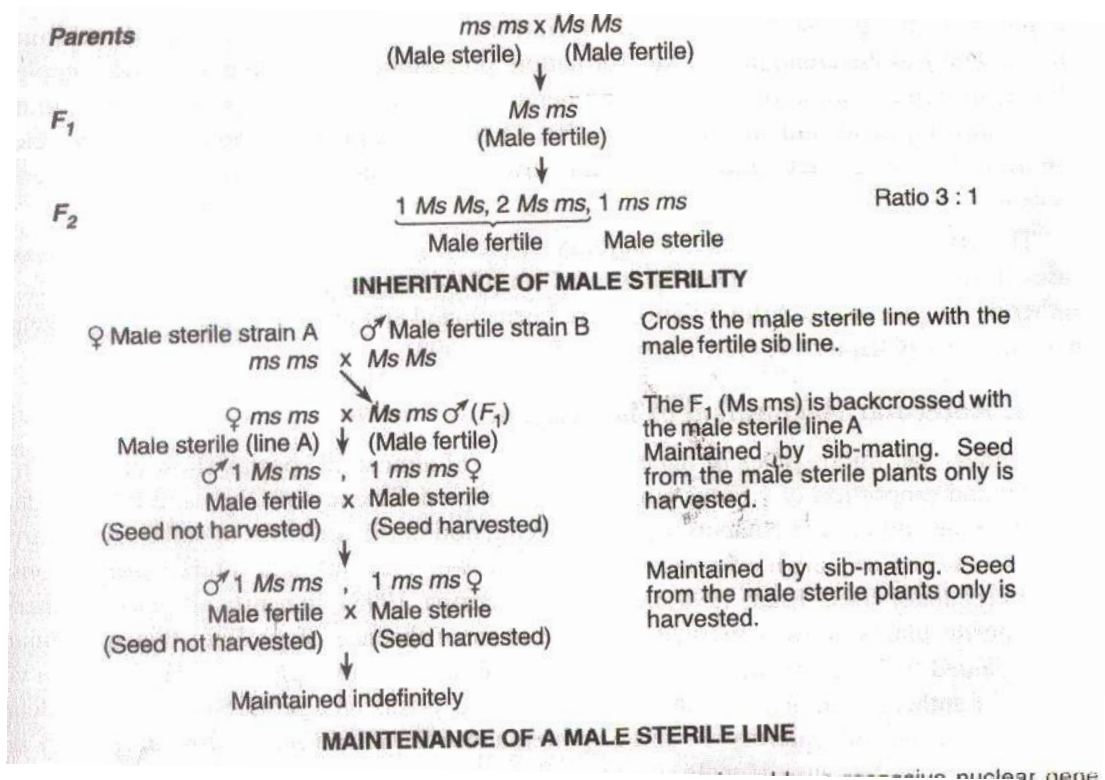


Fig. 7. Inheritance of genetic male sterility. Male sterility is produced by a recessive nuclear gene designed as ms , the dominant allele Ms produces male fertility. During the maintenance of male sterile lines, sib-mating is achieved natural pollination.

Genetic male sterility is subdivided into 2 broad groups: (1) Environment insensitive (ms gene expression is much less affected by the environment) and (2) environment sensitive (ms) gene expression occurs within a specified range of temperature and/or photoperiod regimes; this type of sterility is known in rice, tomato, wheat *etc.*) - The environment sensitive male sterility is further divided into two groups ; (i) temperature sensitive genetic male sterility and (ii) photoperiod sensitive genetic male sterility. In addition, genetic engineering has produced a novel type of

dominant genetic male sterility referred to, here, as transgenic male sterility.

Temperature-Sensitive Genetic Male Sterility (TGMS)

In this type of GMS, complete male sterility is produced by the *ms* gene at higher temperatures (*eg.*, 23.3°C or higher for rice TGMS line Pei-Ai645), but at temperatures below this critical point it exhibits normal fertility. The period of development sensitive to temperature lasts from the formation of PMC's to meiosis in rice. Photoperiod has a limited effect in determining the lowest temperature at which complete sterility is produced. This type of genetic male sterility is being used to develop hybrid rice in china.

Photoperiod-Sensitive Genetic Male Sterility (PGMS)

In case of PGMS, expression of *ms* gene is drastically affected by the prevailing photoperiod, provided the temperature is within a critical range (*eg.*, 23-29°C for rice PGMS). Within this temperature range, complete sterility is obtained in rice plants grown under long day conditions (day length more than 13 hr 45 min), but under short day conditions almost normal fertility is obtained. The stage of development sensitive to photoperiod lasts from differentiation of secondary rachis branches to the formation of PMC's. It is important that a temperature above the critical range

leads to male sterility under any photoperiod, while those below the critical range produce male fertility irrespective of the photoperiod. This type of male sterility is being used to develop hybrid rice in china.

Detailed studies in rice have shown that the same *ms* gene behaves as PGMS or TGMS depending on the genetic background. For example, rice male sterile lines 5047S and W6154S have the same *ms* gene from Nong-Keng 58S. Line 5047S shows PGMS, while W6154S shows TGMS. This clearly suggests that modifying genes can drastically change the expression of *ms* gene and make it either photo- or thermo sensitive.

Transgenic -Genetic Male Sterility

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

The Barnase gene of *Bacillus amyloliquefaciens* encodes an RNAase. When Barnase gene is driven by TA29 promoter, it is expressed only in tapetum cells causing their degeneration. Transgenic tobacco and *Brassica napus* plants expressing Barnase were completely male sterile. Another gene, Barstar, from the same bacterium encodes a protein, which is a highly specific inhibitor of Barnase RNAase. Therefore, transgenic plants expressing both Barstar and Barnase are fully male fertile.

The Barnase gene has been tagged with bar gene, which specifies resistance to the herbicide phosphinothricin. This male sterile line is maintained by crossing with a male fertile line. The progeny so obtained contain 1 male sterile: 1 male fertile plants; the latter are easily eliminated at seedling stage by a phosphinothricin spray. The male sterile plants are crossed with the Barstar line to obtain male fertile hybrid progeny (**Fig. 8**). This system of male sterility is yet to be commercially used.

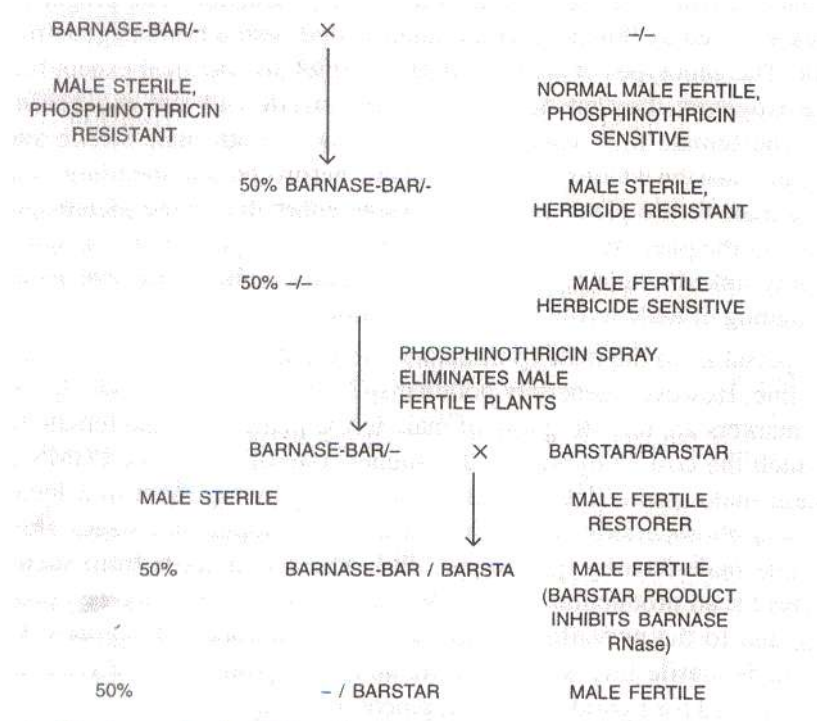


Fig 8. Male sterility due to the bacterial gene barnase and its restoration by another bacterial gene barstar. Gene barnase produces RNase, which kills the cells expressing this gene, while barstar encodes a specific inhibitor of this RNase. Herbicide resistance gene bar is linked with barnase to eliminate the male fertile segregants from the male sterile line.

Utilization in Plant Breeding

Genetic male sterility may be used in hybrid seed production. The progeny from *msms* x *Msms* crosses are used as female, and are interplanted with a homozygous male fertile (*MsMs*) pollinator. The genotypes of *msms* and *Msms* lines are identical except for the *ms* locus, i.e., they are **isogenic**; they are known as male sterile (A)

and maintainer (B) lines, respectively. The female line would, therefore, contain both male sterile and female fertile plants; the latter must be identified and removed before pollen shedding. This is done by identifying the male fertile plants in seedling stage either due to the pleiotropic effect of the *ms* gene or due to the phenotypic effect of a closely-linked gene. For example, a *ms* locus in tomato is closely linked with a recessive gene for anthocyanin production in vegetative parts; this permits roguing of male fertile plants before flowering.

Pollen dispersal from the male (pollinator) line should be good for a satisfactory seed set in the female line. However, generally pollen dispersal is poor and good, closely-linked (with the *ms* gene) markers are rare. Roguing of male fertile plants from the female line is costly as a result of which the cost of hybrid seed is higher. Use of TGMS or PGMS eliminates this problem. These male sterile lines are maintained by growing them in a locality where the temperatures and photoperiods during the sensitive developmental stages are such that they exhibit complete male fertility (phenotypically). The selfed seeds from such lines are then grown for hybrid seed production in those locations where their *ms* genes produce complete male sterility due to the prevailing temperature

and photoperiod regimes. Clearly, all the plants in the male sterile line will be sterile and no roguing will be required. TGMS and PGMS are being used for hybrid rice development in China.

Due to the difficulties narrated above, genetic male sterility has been exploited commercially only in a few countries. In USA, it is being successfully used in castor. In India, it is being used for hybrid seed production of arhar (*C.cajan*) by some private seed companies, *eg.*, Maharashtra Hybrid Seed Co. Ltd., India, produced and sold seeds of a hybrid variety of arhar. In tomato also it has been used for a limited extent to develop hybrids.

Cytoplasmic Male Sterility

This type of male sterility is determined by the cytoplasm (**Fig. 9**). Since the cytoplasm of a zygote comes primarily from egg cell, the progeny of such male sterile plants would always be male sterile. Cytoplasmic male sterility is known in many plant species, some of which are crop plants . Cytoplasmic male sterility may be transferred easily to a given strain by using that strain as a pollinator (recurrent parent) in the successive generations of a backcross programme (**Fig. 10**). After 6-7 backcrosses, the nuclear genotype of male sterile line would be almost identical to that of the recurrent pollinator strain. The male sterile line is maintained by

crossing it with the pollinator strain used as the recurrent parent in the backcross programme, since the nuclear genotype of the pollinator is identical with that of the new male sterile line. Such a male fertile line is known as the ***maintainer line*** or **B line** as it is used to maintain the male sterile line. The ***male sterile line*** is also known as the **A line**. There is considerable evidence that the gene or genes conditioning cytoplasmic male sterility, particularly in maize, reside in mitochondria, and may be located in a plasmid like element.



CYTOPLASM STERILE
NUCLEAR GENE NONRESTORER
(IT IS UNABLE TO COUNTERACT
THE EFFECT OF THE STERILE
CYTOPLASM)



CYTOPLASM FERTILE
NUCLEAR GENE NONRESTORER



Fig 9. Cytoplasmic male sterility. Male sterility is caused by the cytoplasm (depicted by S). The F cytoplasm is the normal cytoplasm. If a nuclear restorer gene(the dominant allele of r) becomes available, the male sterility will be included in the cytoplasmic – genetic group. This type of male sterility shows cytoplasmic inheritance.

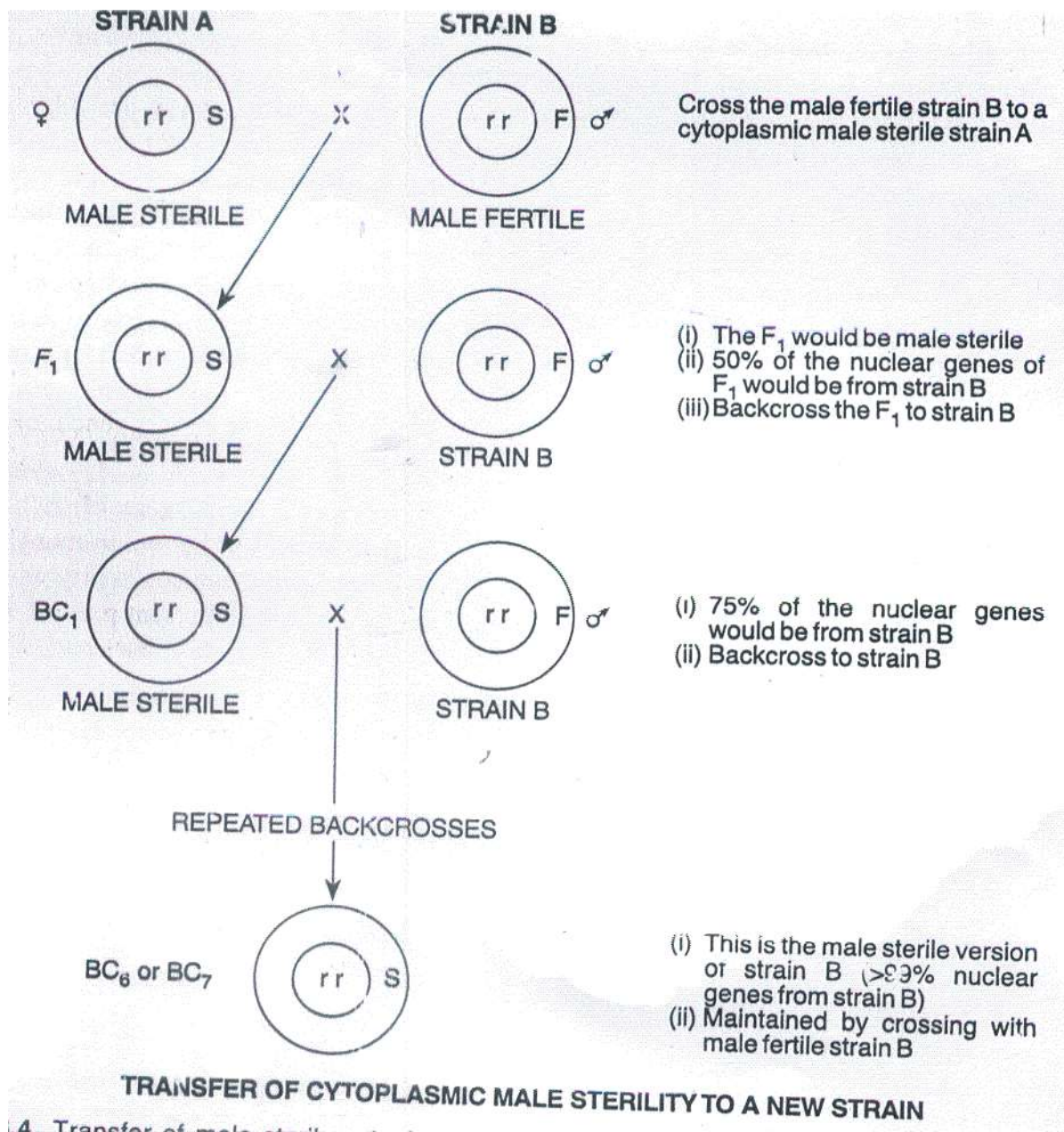


Fig 10. Transfer of male sterile cytoplasm from strain A to strain B. Strain A is used as the nonrecurrent parent in a backcross programme with strain B. After 6-7 backcrosses. The progeny will be similar to strain B in their nuclear genotype. The strain B serves as the maintainer of this new male sterile strain.

Utilization in Plant Breeding

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

Cytoplasmic-Genetic Male Sterility

This is a case of cytoplasmic male sterility where a nuclear gene for restoring fertility in the male sterile line is known. The fertility restorer gene, R, is dominant and is found in certain strains of the species, or may be transferred from a related species, *e.g.*, in wheat .This gene restores male fertility in the male sterile line, hence it is known as ***restorer gene***(Fig.11) .

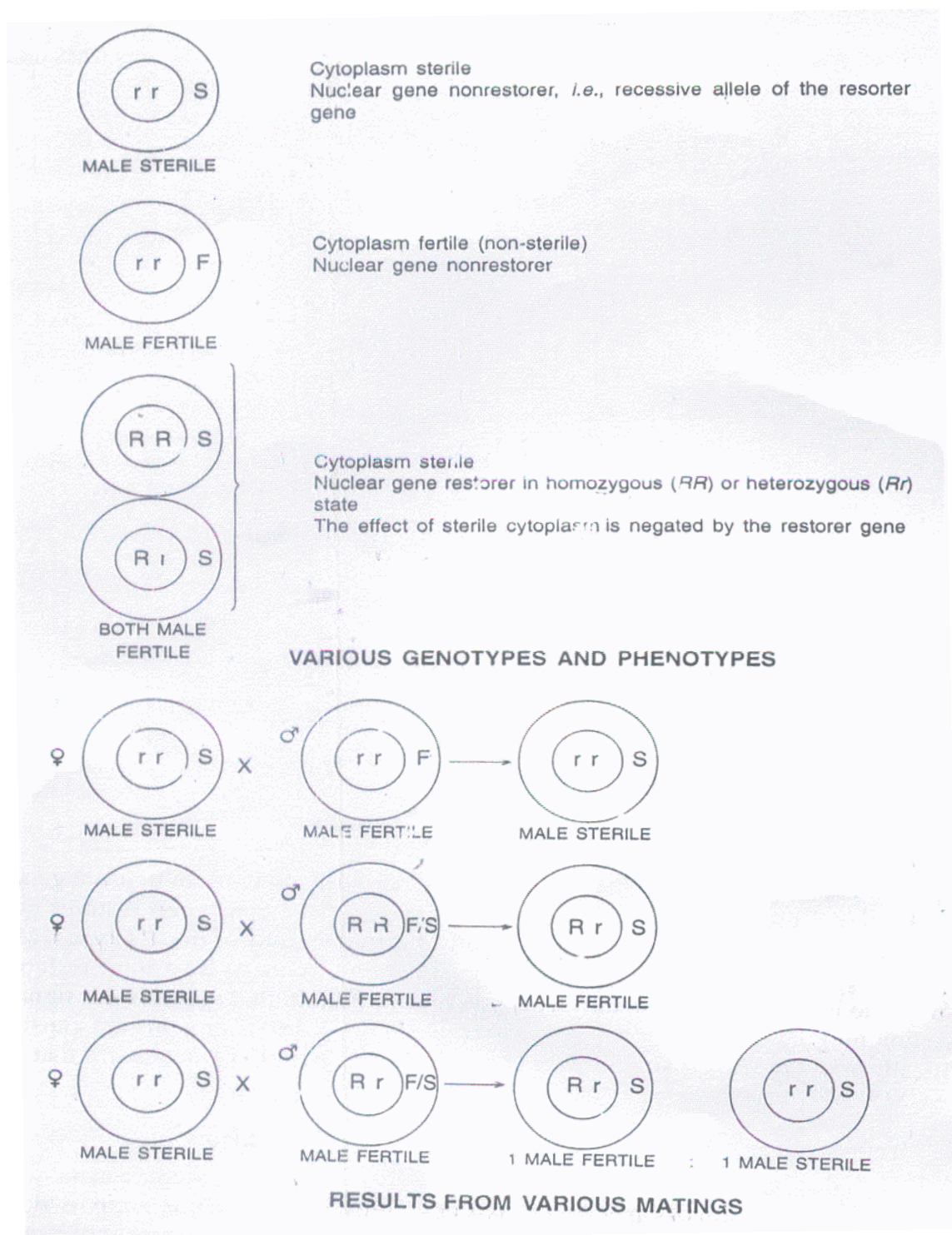


Fig.11. Cytoplasmic-genetic male sterility. Male sterility is due to a sterile cytoplasm; fertility is restored by a restorer gene, which is usually a dominant nuclear gene. F/S in the cytoplasm indicates that the cytoplasm may be either fertile (F) or sterile (S)

The cases of cytoplasmic sterility would be included in the cytoplasmic-genetic system as and when restorer genes for them would be discovered. It is likely that a restorer gene would be found for all the cases of cytoplasmic male sterility if a thorough search were made. This system is known in maize, jowar, bajra, sunflower, rice, wheat *etc.* Plants would be male sterile in the presence of male sterile cytoplasm if the nuclear genotype were **rr**, but would be male fertile if the nucleus were **Rr**, or **RR** (**Fig. 12**).

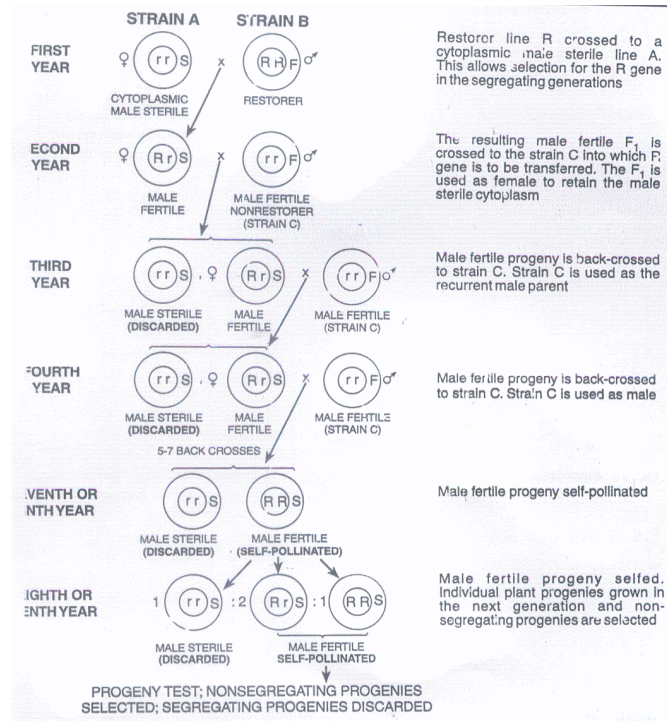


Fig 12. Transfer of a restorer gene **R** from a restorer strain (strain R) to a new strain (strain C)

Development of New Male Sterile and Restorer Lines

Sterile lines may be developed following the same procedure as in the case of cytoplasmic system (Fig.12). But the nuclear genotype of the pollinator strain used in such a transfer must be **rr**, otherwise the fertility would be restored. The development of new restorer strains is somewhat indirect (Fig. 12). First, a restorer(say **R**) is crossed with a cytoplasmic male sterile line **A**. The resulting male fertile plants are used as the female parent in repeated backcrosses with the strain **C** used as the recurrent parent, into which the transfer of restorer gene(s) is desired. In each generation, male sterile plants are discarded, and the male fertile plants are used as females for [backcrossing](#) to the strain **C**. This acts as a selection device for the restorer gene **R** during the backcross programme. At the end of backcross programme, a restorer line *isogenic* to the strain **C** would be recovered.

Utilization in Plant Breeding

The cytoplasmic-genetic male sterility is used commercially to produce hybrid seed in maize, bajra, cotton, rice, sunflower, jowar *etc.* A generalised scheme for producing a double cross in maize is presented in **Fig. 13**. Alternatively, two fertility restoring inbreds may be used to produce single cross II; in this case, one of the two inbreds will have to be detasseled for use as the female parent. In such a case all the plants in the double cross will be male fertile, since the single cross II will be homozygous for the restorer gene **R**. For producing single crosses, the scheme for production of single cross II, as presented in **Fig 13** is followed.

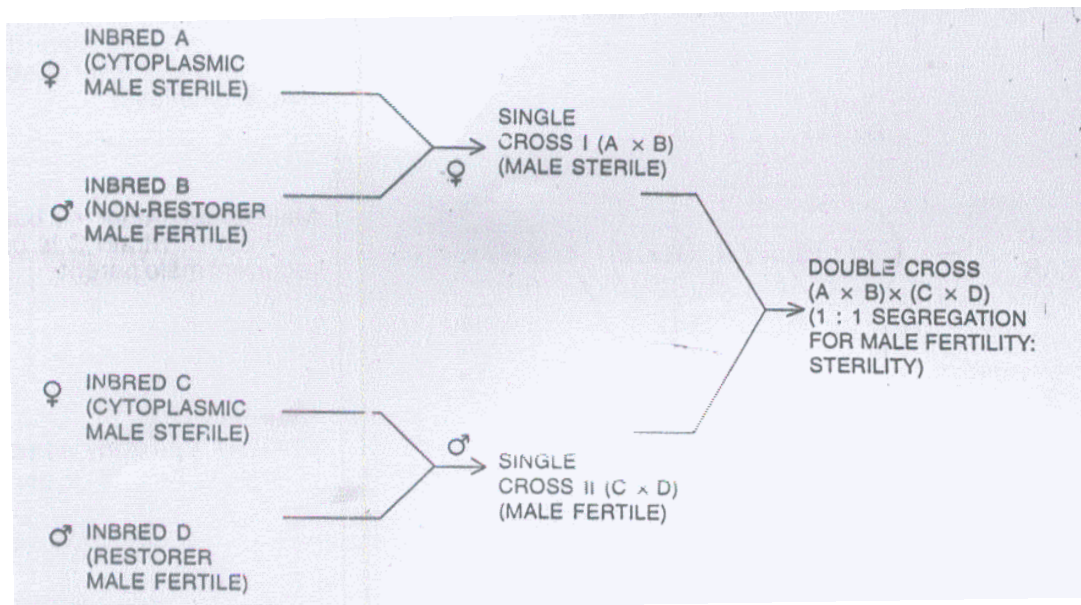


Fig 13. A generalized scheme for the production of a double cross hybrid in maize using cytoplasmic genetic male sterility.

Chemically induced male sterility

This type of male sterility is induced by application of certain chemicals like GA_3 (Gibberellic acid), NAA (Naphthalene acetic acids), **MH (Maleic Hydrazide)**, Ethrel etc. These chemicals are called **male gametocides** since they lead to pollen abortion and thereby cause male sterility; they are also called **chemical hybridizing agents**.

CHAPTER-IV

BASIC BIOMETRICS

This is special branch of mathematics, developed as a result of construction of special models and procedures to deal with the various aspects and problems of quantitative inheritance.

Nature and significance of Qualitative and Quantitative Characters

Some characters are little affected by other genes, *ie.*, the genetic background, or the environment. Such characters are generally governed by one or few genes with large, easily detectable effects; such genes are known as ***oligogenes*** or ***major genes*** or ***switch genes*** or ***qualitative genes***. The characters produced by oligogenes show-distinct classes and are known as qualitative characters or oligogenic traits.

On the other hand, the development of many characters is very much affected by the genetic background and, more particularly, by the environment. These characters are governed by several genes with small individual effects; these genes are known as ***polygenes*** or ***minor genes*** or ***quantitative genes***. The

characters produced by polygenes are referred to as quantitative characters, because they do not show clear-cut classes and have to be studied by measurement. They are also called polygenic traits since they are governed by polygenes. The inheritance of both qualitative and quantitative characters follows the laws of Mendel. But the effects of individual genes in the two cases are totally different in magnitude. Consequently, the techniques used to study the two types of characters are also different. These differences stem primarily from the ease in classifying plants into distinct phenotypic classes in one case and the inability to do so in the other. In crop improvement, both the types of characters are important. Many characters of economic value show the qualitative, while most others exhibit the quantitative mode of inheritance.

Difference between quantitative characters and qualitative characters

Qualitative character	Quantitative character
1. Governed by oligogenes.	1. Governed by poly genes.
2. The variation is discontinuous.	2. The variation is continuous.
3. The individuals can be grouped into distinct classes.	3. The individuals cannot be grouped into distinct classes.
4. The expression of the character is least influenced by the environment.	4. The expression of the character is highly influenced by the environment
5. The inheritance can be studied by grouping the individuals in to distinct mendelian ratios.	5. Since the individuals cannot be grouped into distinct ratios the inheritance can be studied by statistical quantities like mean, variance and covariance.

Phenotypic Genotypic and Environmental variability

The magnitude of variability present in a crop species is of utmost importance as it provides the basis for effective selection. The selection of plants from a population is almost always based on their appearance (Phenotype). The total variation or the phenotypic variation present in a population arises due to both heritable and nonheritable components, *i.e.*, genotype and environmental effects. *Phenotypic variation is the observable variation present in a character in a population.* It includes both genotypic and

environmental components of variation and as a result its magnitude differs under different environmental conditions.

Genotypic variation on the other hand is the heritable component and is due to the genes present in population, that is, genotype. This is due to the genotypic differences among the individuals within a population and is the main concern of the plant breeders.

Environmental variation is the nonheritable component which consists of the effects of environment. The value of progeny obtained from a selected plant, therefore," would largely depend upon the relative contributions by the heritable and the nonheritable components to its phenotype.

$$\sigma^2_P = \sigma^2_G + \sigma^2_E$$

Where, σ^2_P = phenotypic variability

σ^2_G = Genotypic variability

σ^2_E = Environmental variability

Heritability

In a general sense, heritability specifies the proportion of the total phenotypic variability that is due to genetic causes (or)

The ratio of genotypic variance to the total variance (phenotypic variance). It is a good index of the transmission of characters from parents to their offspring.

There are two types of Heritability (i) *broad sense heritability* (ii) *Real sense heritability (or) narrow sense heritability*.

(i) Broad sense heritability

It is the ratio of genotypic variance to total phenotypic variance and it is expressed in percentage.

$$H = \frac{\sigma^2_G}{\sigma^2_P} \times 100$$

Where, σ^2_G = Genotypic variance
 σ^2_P = phenotypic variance

(ii) **Narrow sense heritability (OR) Real sense heritability** is the ratio of additive or fixable genetic variance to total or phenotypic variance and it is expressed in percentage.

$$H = \frac{\sigma^2_A}{\sigma^2_P} \times 100$$

Where, σ^2_A = Additive genetic variance
 σ^2_P = phenotypic variance

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

Low: less than 30%

Moderate: 30- 60%

High: More than 60%

Genetic advance

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

$$GA = \frac{\sigma^2_G}{\sigma^2_P} \times K$$

Where, σ^2_G = Genotypic variance

σ^2_P = phenotypic variance

K= Selection differential at a particular level of selection intensity, which takes into account the mean phenotypic value of the selected families (K=2.06 at 5% intensity)

$$\text{Genetic advance as per cent of mean} = \frac{\text{Genetic advance}}{\text{Mean}} \times 100$$

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

Low: less than 10%

Moderate: 10- 20%

High: More than 20%

CHAPTER -V

BREEDING OF SELF POLLINATED CROPS

Selection Breeding

Introduction

Introduction means taking a plant variety or a species into an area where it was not grown before; even within the country.

Exchange of seed or planting material can be made directly from the Research Institutes / Centres if it is within the country. For obtaining materials from other countries, it should be done through the NBPGR (*National bureau for plant genetic resources*). Import of seeds and other propagules requires an import permit and a phytosanitary certificate for custom clearance at the entry point.

While introducing new crops, extreme care must be taken to avoid the introduction of weeds, insect pests or disease organisms along with the crops.

Importance of plant introduction

The importance and success of plant introduction can be realized when we consider the fact that the important centres of production of nearly all major crops are now far removed from their centres of origin. For example, among the groundnut growing countries of the world, India has at present the largest area under groundnut even though South America is believed to be the centre of origin of groundnut.

Utilization of plant introduction

Plant introduction can be used in three different ways.

1. *The introduced material can be used directly by increasing it en masse (multiplication). e.g.,*

'Cambodia cotton' of Tamilnadu is an example of a crop which arose from the direct increase *en masse* of an introduced plant material.

The wonder rice IR 8 released in 1966 by IRRI (Philippines) is another introduction into India. It was less susceptible to bacterial leaf blight.

Lerma Rojo 64-A and sonara 64 are some of the strains of wheat introduced from Mexico.

2. *Desirable strains can be selected from the introduced material. eg.,*

The strain TMV 1 of groundnut is a spreading type isolated from the variety, 'Saloum' introduced from Africa, by selecting elite plants, pooling their produce and multiplying them.

3. *The introduced material can be used as a parent for hybridization with local varieties. eg.,*

'Bonganhilo', a striga resistant variety of sorghum introduced from Africa, has been crossed with Co. 1, *Periamanjil cholam*, to evolve a striga resistant strain of sorghum, Co. 20.

'Jaya', a strain of rice developed by the All-India Co-ordinated Rice Improvement Project at Hyderabad, is a 'derivative of a cross between Taichung (Native) 1 and T141, a variety from Orissa. It has duration of 130 days (seed to seed).

Exploration

Explorations are trips for collection of various forms of crop plants and their related species. Therefore, cultivated forms like land races , open pollinated varieties etc., wild forms and wild relatives are all collected.

Acclimatization

The process that leads to the adaptation of a variety, line or population to a new environment is known as acclimatization.

Selection

Nature constantly selects superior types. Man also selects from mixed populations, individuals with desirable characteristics. The efficacy of selection is dependent upon the presence of genetic variability. Selection never creates variability but acts only on variability already created by spontaneous or induced mutation, or hybridization.

Genetic makeup of Self pollinated crops

Self-pollination increases homozygosity with a corresponding decrease in heterozygosity. Inbreeding also increases homozygosity and reduces heterozygosity. The term homozygosity denotes the frequency of genes in homozygous condition in the population. Segregation of the genes together or linkage between genes does not affect the percentage of homozygous genotypes in the population.

Inbreeding is mating between individuals related by descent that is having a common parent or parents in their ancestry. Self

pollination is the most intense form of inbreeding, since in this case the same individual functions as the male as well as the female parent.

The methods of selection practiced in self pollinated crops are the following: (1) Mass selection, (2) Pureline selection, (3) Clonal selection.

(1) Mass selection

In mass selection, plants are chosen on the basis of the phenotype and seeds from these selected plants are composited to form the new variety without any progeny testing.

The farmers' variety or heterogeneous local land race is likely to include plants that mature early, plants that mature late, plants that are susceptible to diseases, *etc.* These 'off-type' and undesirable plants are discarded and plants that possess the best features of the variety are selected. Seeds from these best plants are composited together for multiplication and released as an improved strain.

Mass selection is adopted as a method of crop improvement both in the self pollinated and cross pollinated crops.

Procedure of mass selection (Fig14)

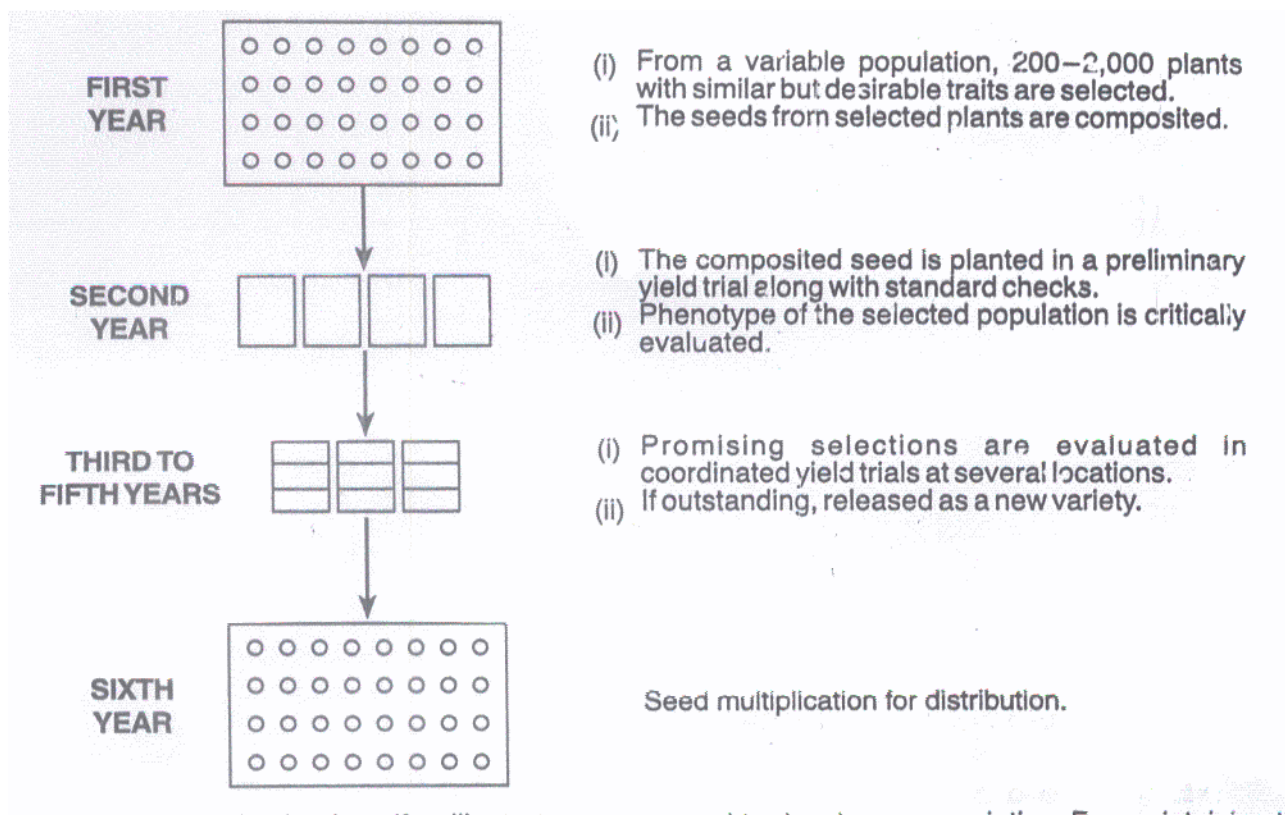


Fig. 14. Mass selection in self-pollinated crops as used to develop new varieties. For maintaining the purity of pureline varieties, operations of the first year may be repeated every year or after every few years.

First year

A large number of phenotypically similar plants are selected for their vigour, plant type, disease resistance and other desirable characteristics. The number of plants selected may vary from few hundred to few thousand. if too many plants are selected the improvement is likely to be small .but if too few plants are selected the adaptation of the variety may become poor. seeds from the selected plants are composited to raise the next generation.

Second year

The composited seeds are planted in a preliminary yield trial along with standard varieties and checks. The variety from which the selection was made should also be included as a check to determine if there has been an improvement due to selection, phenotypic characteristics of the new variety are critically observed.

Third to fifth years

The new line is evaluated in coordinated yield trials at several locations. This is done to test the performance of the new line at different locations with in an agro climatic zone and compare it with that of the popular commercial varieties used as checks. First the new line is evaluated in an initial evaluation trial for one year. If the line is promising it is promoted to uniform regional trials for two or more years. If found promising the new line will be identified for release as a new variety.

Sixth and Seventh year

The new line may be released as a new variety for cultivation if found suitable and if recommended by the competent variety

release committee.

Mass selection has the following advantages:

1) It is a rapid and less tedious method of crop improvement as compared to pureline selection, as it does not involve intensive testing and improvement can be effected within a few seasons or years.

2) Varieties evolved by mass selection are not highly homozygous and hence have wider adaptability

3) Mass selection is useful for the purification and upgradation of existing improved varieties in the production of pure seeds.

Mass selection has the following disadvantages:

1) It is not possible to know whether the selected plants are homozygous or not.

2) In mass selection, plants that look alike are selected but it is not possible to know whether the differences between the selected plants and others are due to heredity or due to environment because the performance of the progeny is not tested.

3) There is no control over pollination, as only well developed earhead is considered for phenotypic selection.

Varieties evolved by mass selection

The following varieties were evolved by mass selection:

Rice : PTB 28, PTB 29, PTB 30

Groundnut : TMV 1 and TMV 2

Sesame : TMV 1

Niger : HR 1

Greengram : ADT 1

Desi (*G. arboreum*) cotton: K 1

(2) **Pureline selection**

A pureline is a progeny of a single, homozygous, self-pollinated plant. As a result, all the individuals within a pureline have identical genotype and any variation present within a pureline is solely due to the environment. *In pureline selection, a large number of plants are selected from a self-pollinated crop and are harvested individually; individual plant progenies from them are evaluated and the best progeny is released as a pureline variety.* Therefore, pureline selection is also known as *individual plant selection*.

The Concept of Pure Line Theory

The concept of purelines was proposed by Johannsen in 1903 on the basis of his studies with French bean (*Phaseolus vulgaris*). Beans are strictly self-pollinated species. Johannsen obtained commercial seeds of the Princess variety of French bean. The commercial seed lot showed variation for seed size. He selected seeds of different sizes and grew them separately. The progenies thus obtained, *ie.*, derived from individual selected seeds, differed in seed size: progenies from larger seed generally produced larger seeds than those obtained from smaller seed. This clearly showed that the variation in seed size in the commercial seed lot of Princess had a genetic basis. As a result, selection for seed size was effective. Johannsen further studied 19 lines; each line was progeny of a

single seed from the original seed lot. He discovered that each line showed a characteristic mean seed weight, ranging from 640 mg in Line No. 1 to 350 mg in Line No. 19. The seed size within a line showed some variation, which was much smaller than that present in the original commercial seed lot. Johannsen postulated that the original seed lot was a mixture of purelines. Thus each of the 19 lines represented a pureline, and the variation in seed size within each of the purelines had no genetic basis and was entirely due to environment. Confirmatory evidence was obtained in three ways. In the first case, he classified the seeds from each pureline into 100 mg classes, and grew them separately. The mean seed weights of progenies from the different seed weight classes of a single pureline were comparable with each other, and with that of the parent pure line. 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 (**Fig. 15**).

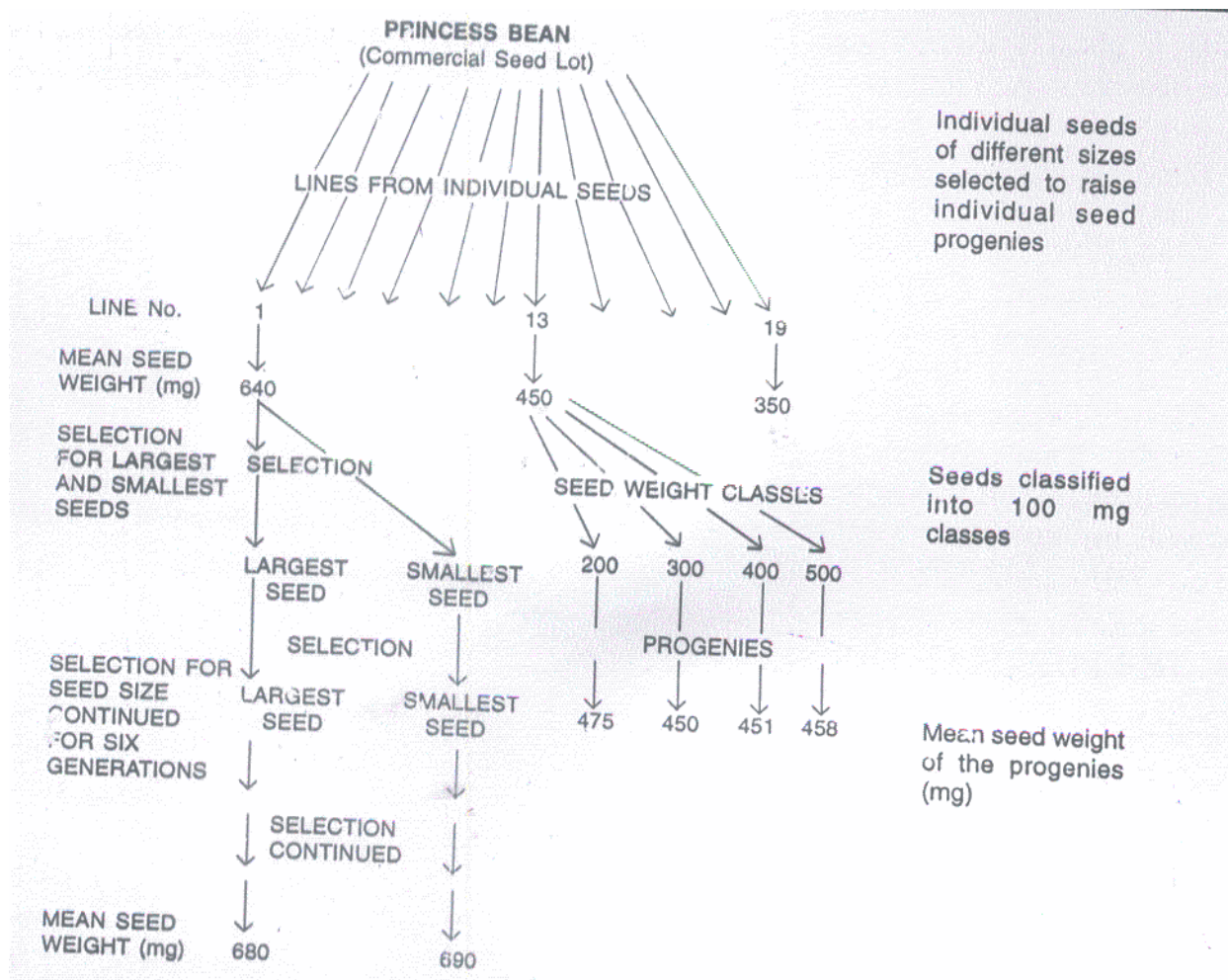


Fig 15. Selection for seed weight in a commercial seed lot of the princess variety of French bean by Johannsen (1903).

Procedure for Pureline selection (Fig.16)

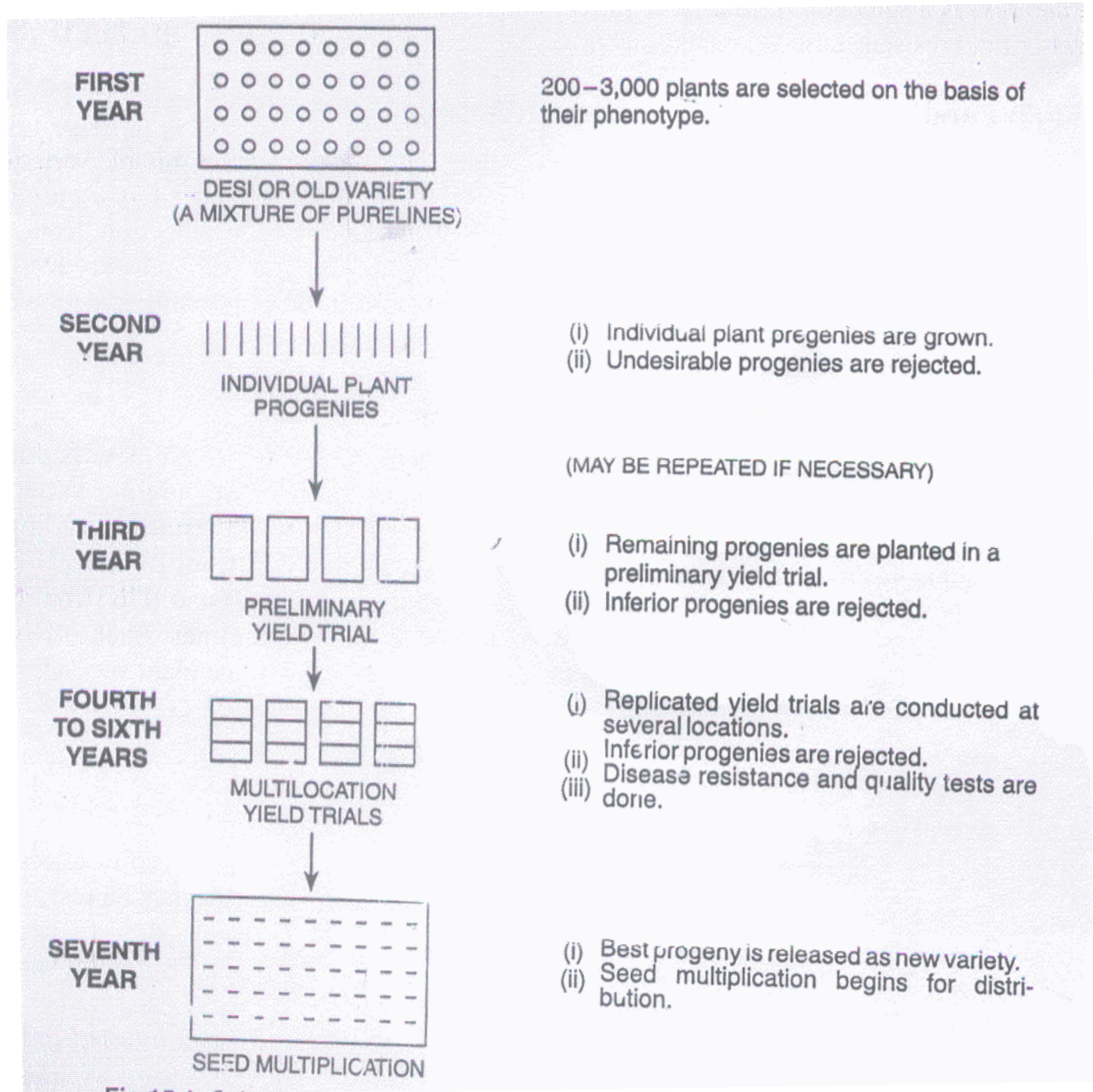


Fig. 16. Schematic representation of pureline selection in self-pollinated crops.

Step one

First year

A large number (200-3000) of plants are selected from *desi* or local variety or some other genetically variable but homozygous population and their seeds are harvested separately.

Step two

Second year

Progenies from individual plants are grown separately with proper spacing. The progenies are evaluated visually. Poor weak and defective progenies are rejected. The objective of this step is to drastically reduce the number of plant progenies, to be carried to step three.

Step three

Third year

Preliminary yield trial should be conducted and the best locally adopted variety is used as a check. Selection is made for easily observable characters and observations are recorded on disease resistance, flowering, maturity and quality characters. Yield of the progenies are used as a criterion of selection. The main objective is to further reduce the number of progenies.

Fourth year

Replicated yield trials are conducted by the breeder using the best available variety as a check. All the observations taken in the previous year is taken. Now each progeny is equivalent to a strain as it is a pureline. The promising strains are then included in coordinated yield trials for further evaluation of the strains.

Fifth to seventh year

The promising strains are evaluated at several locations along with strains from other breeders. The best released varieties are used as checks.

Eighth year

The best progeny or strain is released as a new variety and seed multiplication is initiated for distribution to the farmers.

Advantages of pureline selection

1. Pureline selection achieves the maximum possible improvement over the original variety. This is because the variety is the best pureline present in the population.
2. Pureline varieties are extremely uniform since all the plants in the variety have the same genotype. Such a uniform variety is more liked by the farmers and the consumers than a less uniform variety developed through mass selection.
3. Due to its extreme uniformity, the variety is easily identified in the seed certification programmes.

Disadvantages of pureline selection

1. The varieties developed through pureline selection generally do not have wide adaptation and stability in production possessed by the local or *desi* varieties from which they are developed.
2. The procedure of pureline selection requires more time, space and more expensive yield trials than mass selection.
3. The upper limit on improvement is set by the genetic variation present in the original population.
4. The breeder has to devote more time to pureline selection than to mass selection. This leaves less time for other breeding programmes.
5. Pureline selection has great scope as a method of crop improvement only in self pollinated species.
6. In cross pollinated species, it is not applicable as a method of crop improvement, since selfing of heterozygous plants leads to inbreeding depression and

deleterious factors that are not expressed in heterozygous state are rendered homozygous on selfing exposing certain abnormalities such as albinos, dwarfs, weaklings *etc.*

Achievements

Varieties evolved by Pureline selection

Improved varieties evolved by pureline selection in rice are the following: ADT 22, Improved White Ponni (Tamil Nadu); Rasmi (PTB 44), Suvarnamodan (ARC 11775), Vytilla - 2 (Cult. 74) (Kerala); Ambika, Improved Ambemohan, Jalgaon - 5, Terna Tuljapur - 1 (Maharashtra); GR 5, Kolhapur (Gujarat); Patel 85 (Madhya Pradesh); Janaki, Kamini, Sudha, Sugandh (Bihar); Jalamagna, Madhukar, Majhera - 3, VL 206 (Uttar Pradesh); CSR 6 (Haryana); Amulya, Jaladhi-1, Jaladhi - 2, Matangini, Nalini, Sabita (West Bengal); Khonorullu, Ngoba (Meghalaya); Giza - 14 (Jammu & Kashmir).

Hybridization and selection

Pedigree Method of Breeding (Fig. 17)

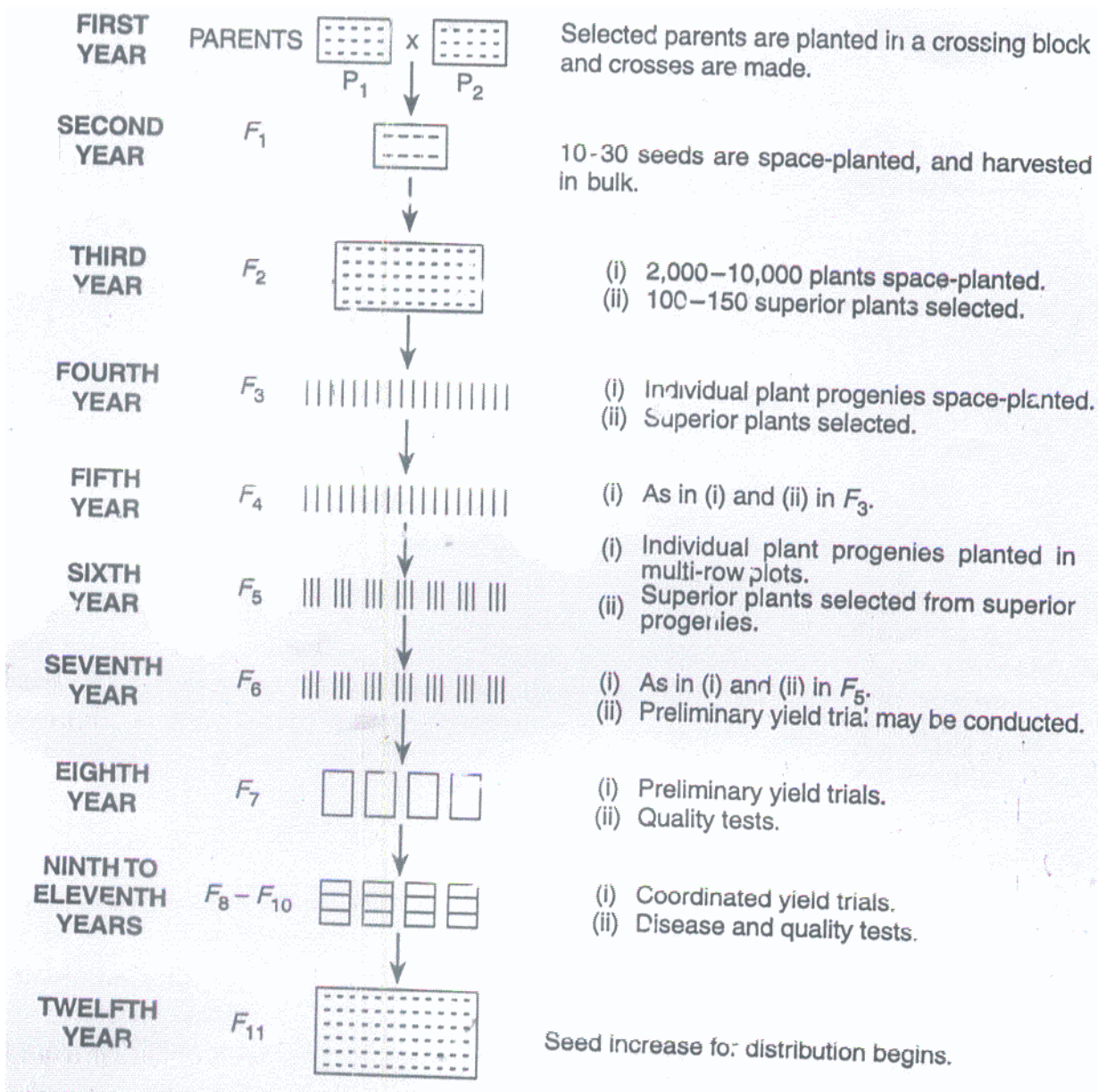


Fig 17. A Simplified schematic representation of the pedigree method of handling the segregating generations from crosses in self – pollinated crops.

Procedure: The procedure for selection in cereal crops by the pedigree method is described below:

First Year

Make crosses between two parents possessing the characters to be combined in a new variety. Collect the seeds.

Second year

Sow the hybrid seeds 20 cm apart in the row. Grow a number of F_1 plants (say, 50 to 100), sufficient to give seeds for raising a large population in the F_2 . Grow the parent varieties for comparison with the F_1 . Harvest and thresh each F_1 plant separately. Bulk the seeds from all the F_1 plants. This bulked seed constitutes the second seed generation.

Third year

Grow 2,000 to 10,000 F_2 plants. When planting, space the seeds sufficiently apart in the row so that individual plants can be examined.

Grow the parent varieties for comparison with the F_2 . One or two other standard varieties can also be grown for comparison. Take notes on important characteristics of the cross as a whole *eg.*, whether there is a large amount of plants with earliness, or of susceptibility to a given disease. All very early heading plants or plants appearing to be resistant to a disease are tagged. Select 5-20% of the population for progeny testing. *ie.*, select 200 - 1,000 plants. This would give him sufficient material for selection in further segregating generations so as eventually to have a reasonable number of promising lines to enter the preliminary yield tests.

Fourth year

Grow F_3 progeny rows from the selected F_2 plants.

Space seeds in row so that individual plants may be selected.
Grow the parent varieties for comparison.

Select the best rows and then select the best five plants in each of the selected rows. About 50 to 200 families may thus be selected.

Fifth Year

The generation is handled in much the same manner as the F_3 generation. Some lines may appear to be fairly uniform but several others may not be so. The genetic differences between the families are, however, much greater than the differences within the families. Discard undesirable families and select best plants in best rows of best families.

Sixth Year

In most crosses, most families are uniform by the F_5 generation. Discard all undesirable families. Select only the best families, say, 25 to 50. Bulk the seeds of all the plants of each family that appears uniform and conduct preliminary yield trials in the next year.

Seventh and Eighth Years

Conduct preliminary yield trials and discard mediocre cultures. Retain only the few best cultures say, 5.

Ninth, Tenth and Eleventh years

Conduct yield trials and compare the improved cultures with standard commercial varieties. Make observations on resistance to disease, resistance to drought, resistance to lodging, date of maturity and quality of grain.

Simultaneously conduct adaptive research trials (ART) and / or multilocation trials (MLT) in the farmers' holding in as many districts as possible, throughout the state or region.

Propose two or three best cultures for conducting Co-ordinated Varietal Trials under the All India Co-ordinated Research Project for the specific crop. Initial evaluation trials (IET) and advanced varietal trials (AVT) may be conducted in the co-ordinated research centres of one or more regions.

Select the best culture mainly based on ART / MLT results and supported by IET / AVT results and propose it to the State Variety Release Committee for releasing the same as a strain.

Simultaneously multiply the seed in isolation in conformity with the requirements for breeder seed production for distribution.

Varieties evolved by pedigree breeding

A large number of varieties is evolved by hybridization. Some of the varieties released in Tamilnadu between 1990 and 1994 are Rice - PMK 2, TPS 3, TKM 10, ASD 18, MDU 4, CO 45, ASD 18; Sorghum - BSR 1, K 10, Paiyur 1; Blackgram - K 1, APK 1; Cowpea CO 6; Greengram - CO 5; Redgram - Vamban 1; ~b. *GD..J2*; Groundnut CO- 2, BSR 1, VRI 3; Sesame - Paiyur 1; Cotton - K 11, SVPR 1, Paiyur 1; Castor - TMV 6 and Chillies-PMK 1.

Bulk method or (Mass method or Population method) of breeding (Fig.18)

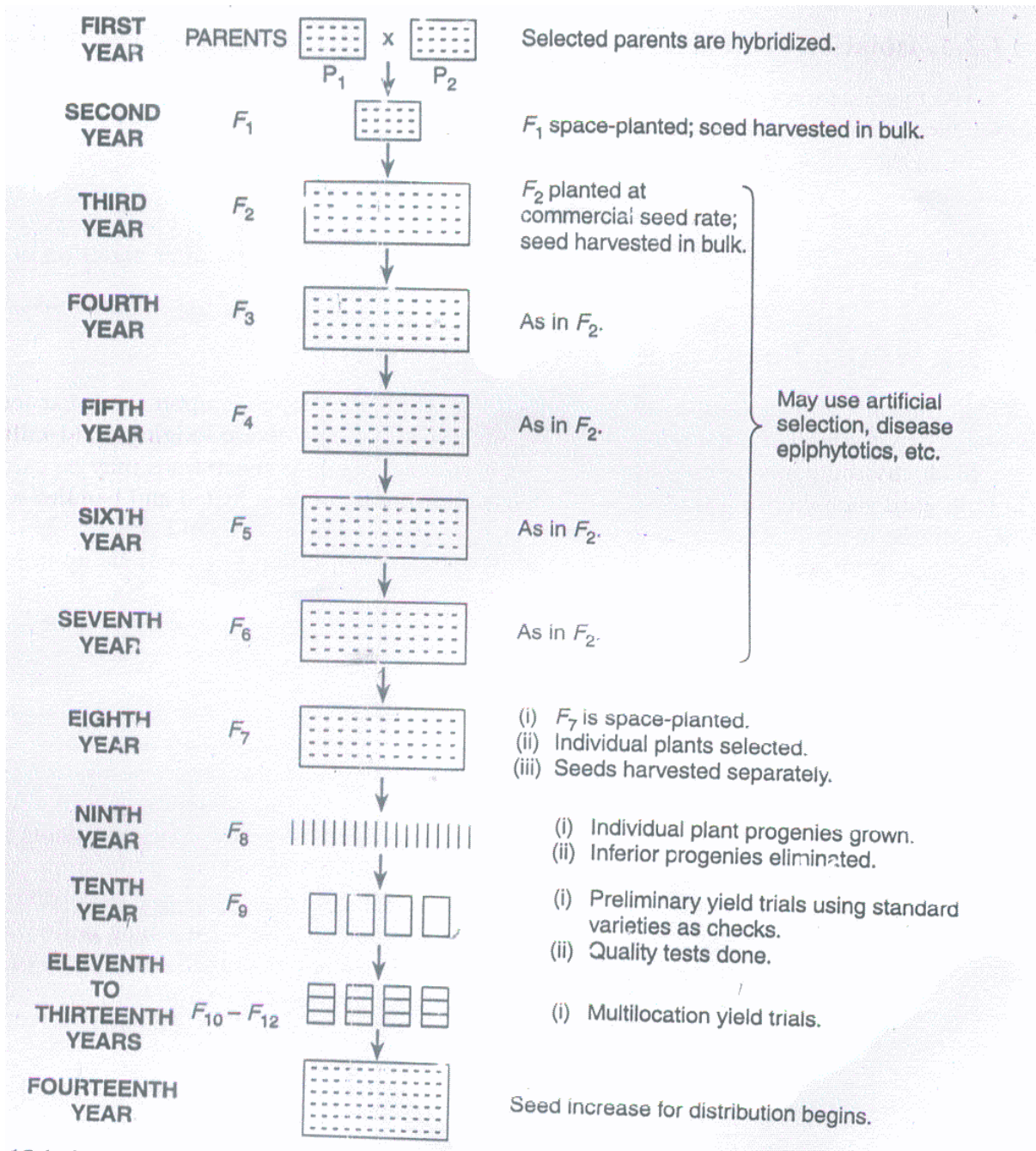


Fig. 18. A generalized scheme of bulk method for the isolation of homozygous lines of self-pollinated crops.

Procedure:

Make crosses between two parents possessing the characters to be combined in a new variety and collect the seeds. Sow the hybrid seeds 20 cm apart in the row. Grow a number of F_1 plants (say, 50 to 100), sufficient to give seeds for raising a large population in the F_2 . Grow the parent varieties for comparison with the F_1 . Harvest and thresh each F_1 plant separately. Bulk the seeds from all the F_1 plants. This bulked seed constitutes the second seed generation.

The F_2 plants are harvested in bulk and a random sample of the seed is taken for the sowing of the next generation. This procedure is repeated till the F_9 . For a cereal crop, the F_2 is sown in a plot of 1/1000 hectare, the F_3 in 1/20 ha., the F_4 in 1/2 ha., and the F_5 to F_{10} in 1 ha. each. Single plant selections are made in the F_{10} generation and progeny rows are raised in the F_{11} . Desirable rows are selected in the F_{11} and advanced to yield trials in the next year.

Merits of Bulk Method

1. The bulk method is simple, convenient and inexpensive.
2. Artificial or natural disease epiphytosis, winter killing *etc.* eliminate undesirable types and increase the frequency of desirable types. The isolation of desirable types thus becomes much easier.

3. Natural selection increases the frequency of superior types in the population. Progenies selected from long-term bulks are likely to be far superior- to those selected, from F_2 or short-term bulks.
4. Little work and attention is needed in F_2 and the subsequent generations. The breeder is free to concentrate more on other breeding projects.
5. No pedigree record is to be kept, which saves time and labour.
6. Since large populations are grown, transgressive segregates are more likely to appear and increase in frequency due to natural selection. Thus there is a greater chance of isolation or transgressive segregates than in the pedigree method.
7. Artificial selection may be practiced increase the frequency of desirable types.
8. It is suitable for studies on the survival of genes and genotypes in populations.

Demerits of Bulk Method

1. The major disadvantage of bulk method is that it takes a much longer time to develop a new variety. Natural selection becomes important only after F_8 or F_{10} and bulking may have to be done upto F_{20} or more. Thus the time required for developing a new variety is considerably longer and most breeders do not use the bulk method simply for this reason.
2. In short-term bulks, natural selection has little effect on the genetic composition of populations. But short-term bulks are useful for the isolation of homozygous lines and for specific objectives as in Harlan's mass-pedigree method.

Modified Bulk Method

A Modification of the bulk method based on artificial selection in F_2 and the subsequent generations are outlined below.

(Fig.19)

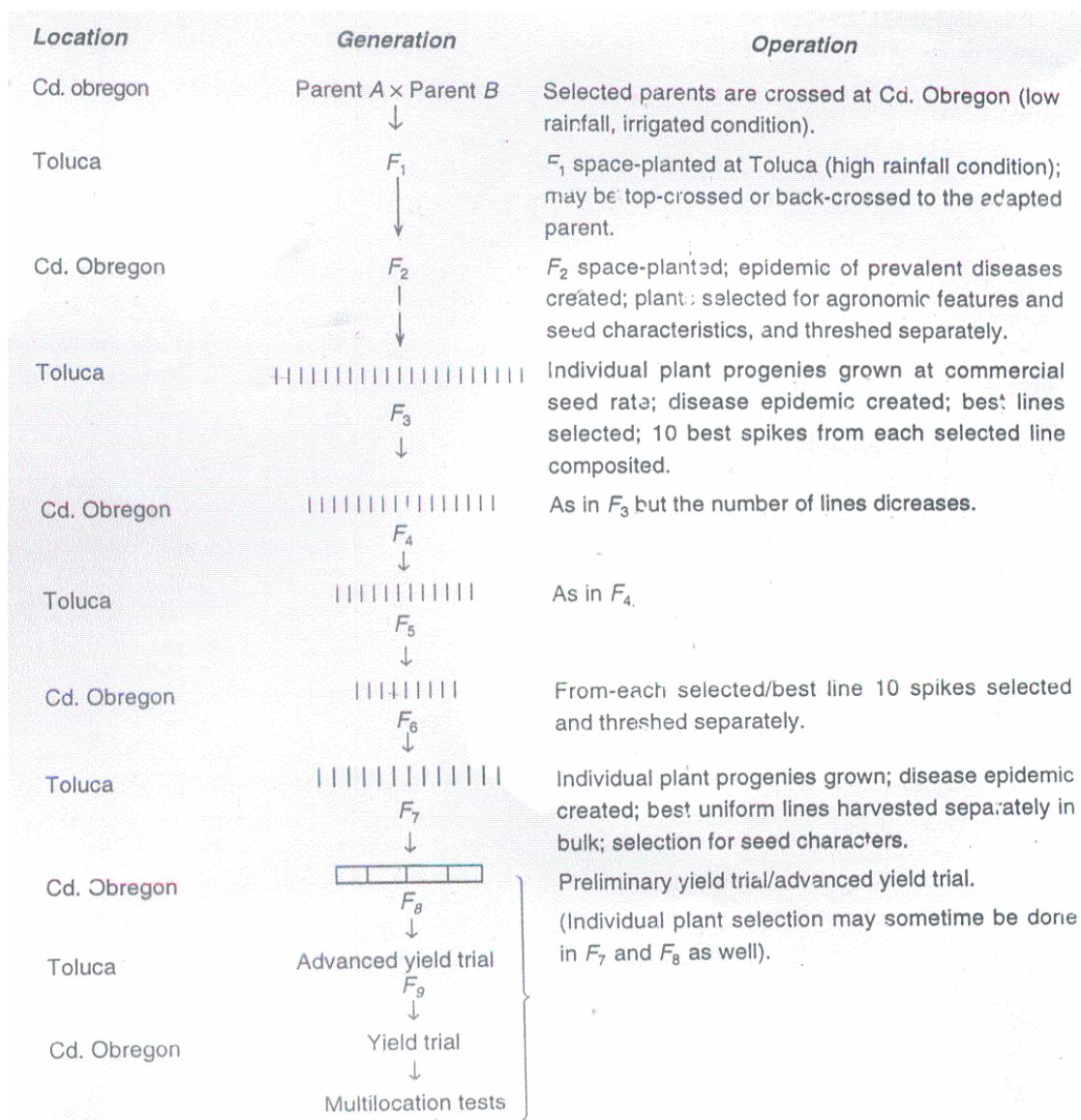


Fig 19. A schematic representation of the modified pedigree-bulk scheme of selection, which has completely replaced the pedigree selection scheme in the wheat programme at CMMYT, Mexico. The scheme shown here is an example of shuttle breeding.

1. ***F₂ and F₃ generations*** .F₂ and F₃ generations are space-planted and a large number of desirable plants are selected. Seeds from the selected plants are bulked.

2. ***F₄ generation***. F₄ is space-planted and 1000 to 5000 desirable plants are selected. Seeds from the selected plants are harvested separately.

3. ***F₅ generation***. Individual plant progenies are grown. Selection among progenies is based on plant height, disease resistance, lodging resistance, maturity date and other agronomic characteristics .Undesirable and inferior progenies are eliminated. Often only 10-30% of the progenies may be saved. Seeds from each of the selected progenies are harvested in bulk.

4. ***F₆ generation***. A preliminary yield trial is planted using the bulk seeds from the selected individual plant progenies. Observations are recorded on agronomic characteristics and yield. Quality tests may be done on superior progenies.

5. ***F₇ generation***. Superior progenies selected on the basis of yield trial or space-planted for further purification. Individual

plants are selected from the superior progenies and their seed is harvested separately.

6. ***F₈ generation.*** Individual plant progenies are grown. Inferior progenies and progenies and progenies showing segregation are ordinarily rejected. Each selected progeny is harvested in bulk.

7. ***F₉ generation.*** Preliminary yield trials are conducted to identify few superior progenies for detailed yield tests. Quality test is done to eliminate undesirable progenies.

8. ***F₁₀-F₁₃ generation.*** Replicated yield trials are conducted at several locations with standard varieties as checks. The line or lines that are found to be superior to the standard checks would be released as new varieties.

9. ***F₁₅ generation.*** Seeds of the newly released variety is multiplied for distribution among the farmers.

This modification of the bulk method provides an opportunity for the breeder to exercise his skill and judgment in selection of superior plant types in the early generations (F₂ to F₄). At the same time, it does not involve laborious record keeping as is the case with the pedigree method. Therefore, this modification has the appeal that it provides for selection in the early segregating

generations and yet it involves less time and labour than the pedigree method.

Mass pedigree method

This method was proposed by **Harlan**. selection for resistance to diseases, lodging, cold, *etc.*, depends upon the presence of suitable environmental conditions favouring disease epidemic, severe lodging, cold-killing *etc.*, such environments do not occur every year. The segregating generations may be carried in bulk until such environments occur. Individual plants are then selected and handled as in the pedigree method. Therefore, the duration of bulking in this case would depend upon the occurrence of the concerned environment; it may end in F_2 itself or may continue up to F_6 or beyond. This method is generally known as the mass-pedigree method of Harlan.

Single-Seed-Descent Method

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

plant present in the population and planted in bulk to raise the next generation. This procedure is followed till F_5 or F_6 when the plants would have become nearly homozygous. In F_5 or F_6 a large number (100 - 500) of individual plants are selected, and individual plant progenies are grown in the next generation. Selection is done mainly among the progenies, and the number of progenies is sufficiently reduced to permit replicated trial in the next generation. Individual plants may be selected only from outstanding families showing segregation. Thus preliminary yield trials and quality tests begin in F_7 or F_8 and coordinated yield trials are initialed in F_8 of F_9 generation (**Fig. 20**).

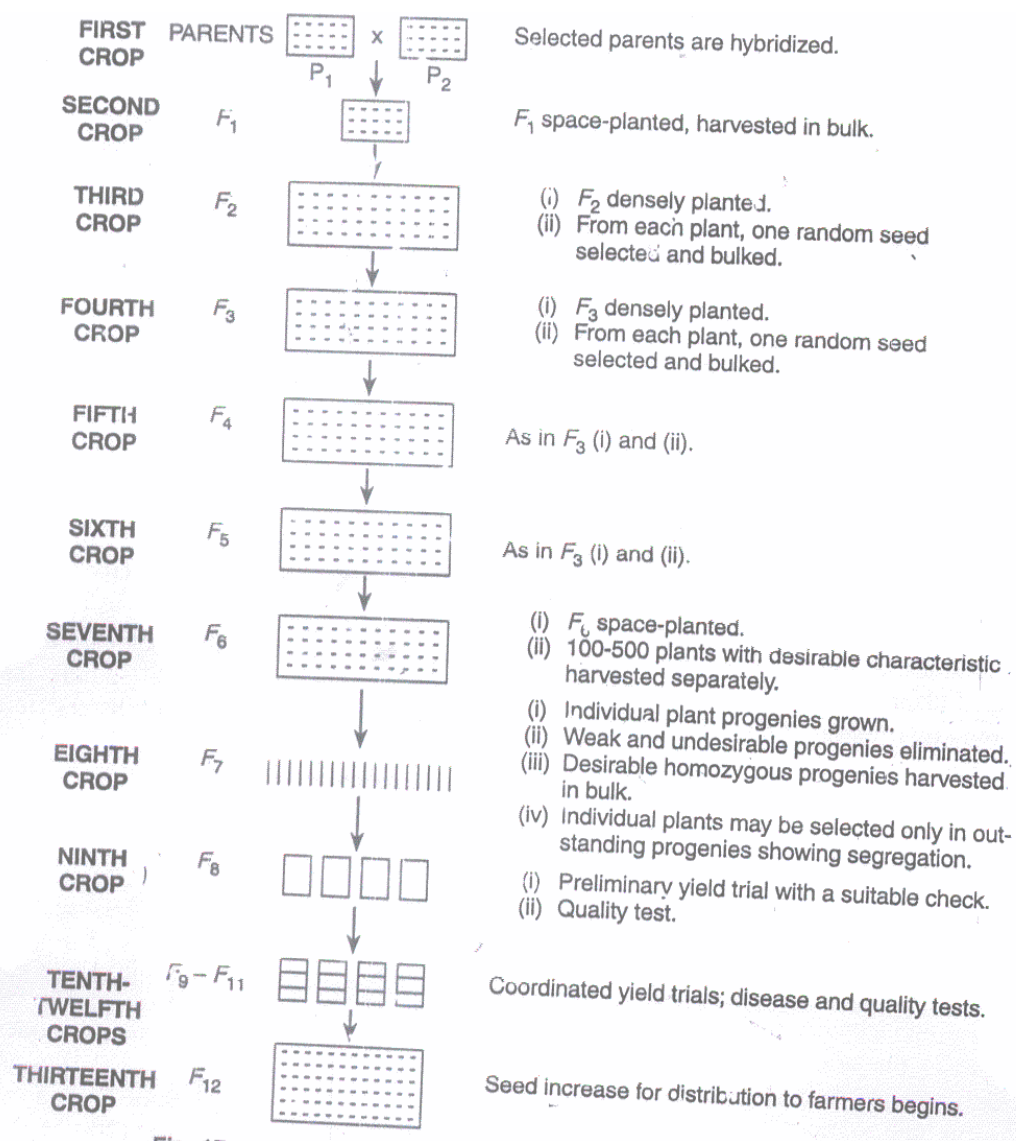


Fig. 20. Since more than one generation may be grown in each year, the number of generations does not necessarily represent the number of years.

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

The single-seed- descent scheme (1) advances the generation with the maximum possible speed in a conventional breeding method; (2) requires very little space, effort and labour; (3) makes the best use of greenhouse and off-season nursery facilities; and (4) ensures that the plants retained in the end population represent a

random sample of homozygous genotypes derived from the F_2 population. However, (1) it does not permit any form of selection (which is implied in the scheme) during the segregating generations; and (2) in each successive generation, the population size becomes progressively smaller due to poor germination and death of plants due to diseases, insect pests and accidents. In some crops, *eg.*, pulses, plant loss may be one of the most serious problems of the scheme.

Backcross method

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

Transfer of a dominant gene

Let us suppose that a high yielding and widely adapted wheat variety A is susceptible to stem rust. Further, another variety B is resistant to stem rust and the resistance to stem rust is dominant to susceptibility. A generalized scheme of the backcross programme for the transfer of rust resistance from variety B to variety A is given below (**Fig.21**).

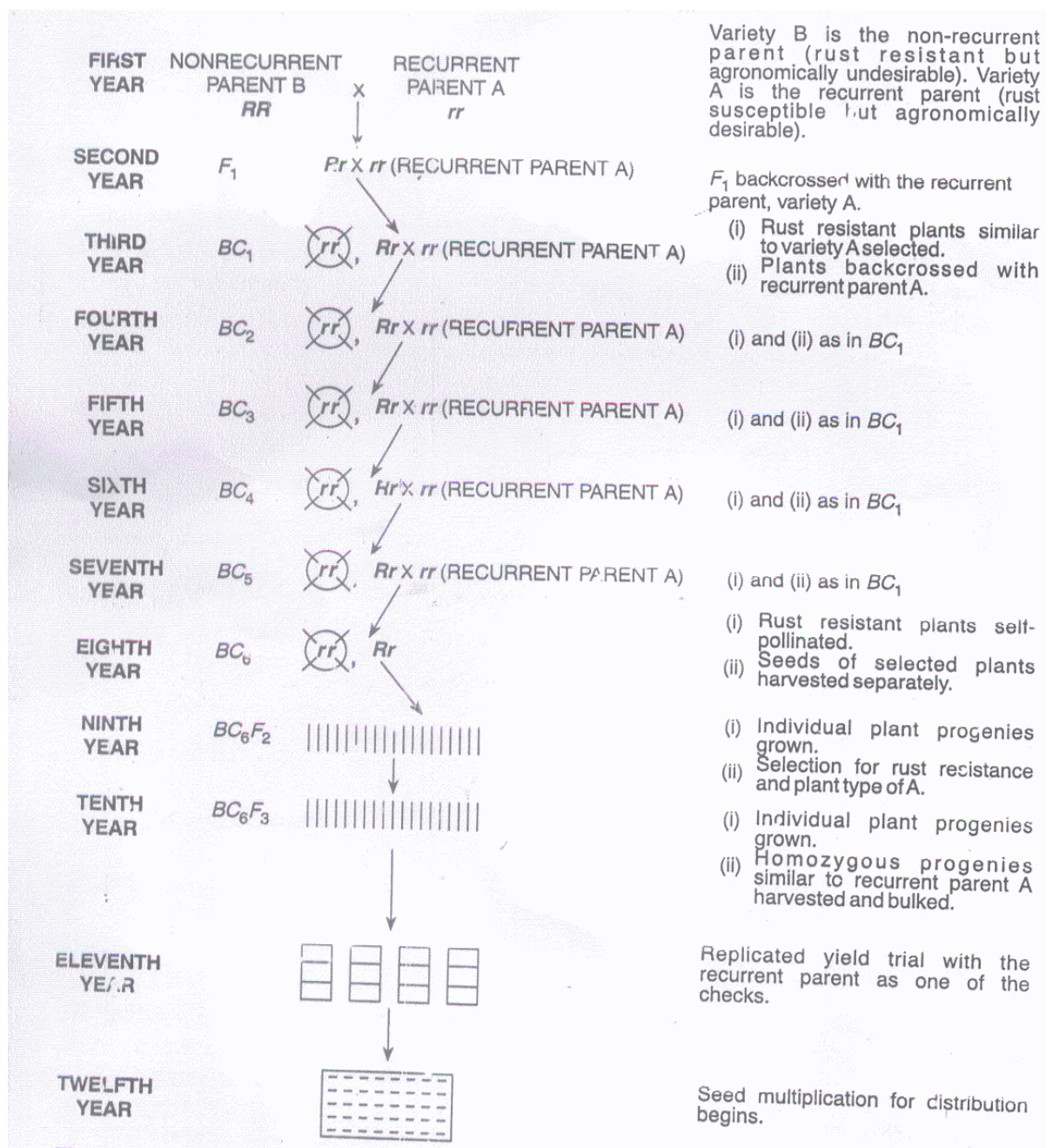


Fig 21. A generalized scheme for the transfer of a dominate gene for disease (rust) resistance through the backcross method in a self-pollinated species  denotes that plants with rr genotype are rejected.

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

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

3. **First backcross generation (BC₁).** Half of the plants in (BC₁) would be resistant and the remaining half would be susceptible to stem rust. Rust resistant plants are selected and backcrossed to variety A. BC₁ plants resistant to rust may be selected for their resemblance to variety A as well.

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

5. **BC₆ Generation.** On an average, BC₆ plants will have over 99% per cent genes from variety A. Rust resistant plant are selected and selfed; their seeds are harvested separately.

6. ***BC₆F₂ generation.*** Individual plant progenies from the selfed seeds of the selected plants are grown. Rust resistant plants similar to the plant type of variety A are selected and their selfed seeds are harvested separately.

7. ***BC₆F₃ generation:*** Individual plant progenies are grown. Progenies homozygous for rust resistance and similar to the plant type of variety A are harvested in bulk. Several similar progenies are usually mixed to constitute the new variety.

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

Transfer of a Recessive Gene

When rust (or any other disease) resistance is due to a recessive gene, all the backcrosses cannot be made one after the other. After the first backcross, and after every two subsequent backcrosses, F₂ generation must be grown to identify rust resistant

plants . The F_1 and the backcross progenies are not inoculated with rust because they would be susceptible to rust. Therefore ,Only the F_2 populations are tested for rust resistance. A generalised scheme for the transfer of a recessive gene for rust resistance is given below **(Fig. 22)**.

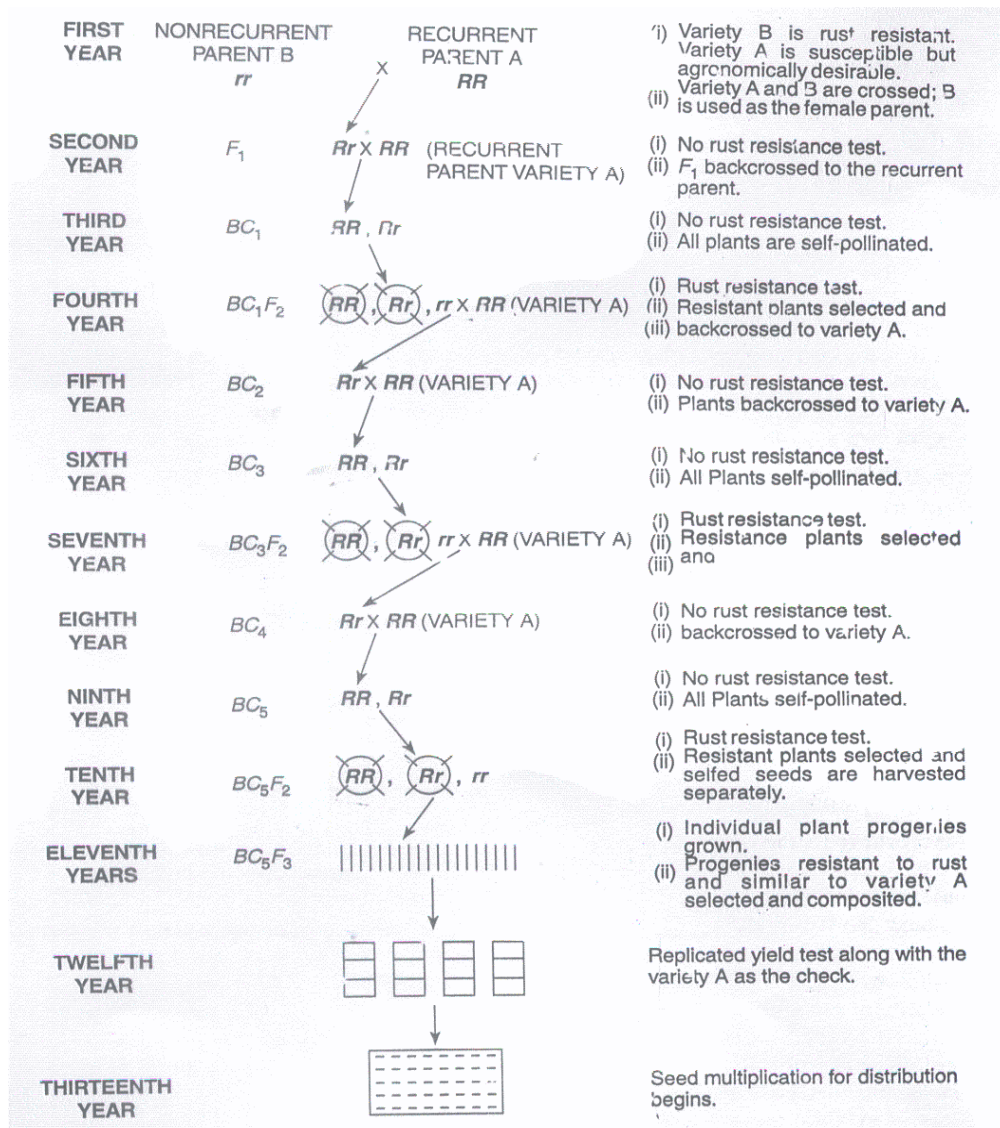


Fig. 22. A generalized scheme for the transfer of a recessive gene for disease resistance in this case (rust) resistance through the backcross method in a self –pollinated crop.

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

2.**F₁ Generation.** F₁ Plants are backcrossed to the recurrent parent.

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

4.**BC₁F₂ Generation.** Plants are inoculated with rust spores. Rust resistant plants are selected and backcrossed with the recurrent parent. Selection is done for the plant type and other characteristics of variety A the recurrent parent.

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

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

7.**BC₃F₂ Generation.** Plants are inoculated with stem rust. Rust resistant plants resembling variety A are selected and backcrossed

to variety A Selection for plant type of variety A is generally effective.

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

9.**BC₅ Generation.** There is no rust test. Plants are self-pollinated to rise F₂ generation.

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

11.**BC_sF₃ Generation.** Individual plant progenies are grown and subjected to rust epiphytotic. A rigid selection is done for resistance to stem rust and for the characteristics of variety A. Seeds from several similar rust resistant homogeneous progenies are mixed to constitute the new variety

12. **Yield Tests and seed multiplication.** It is the same as in the case of transfer of a dominant gene

Merits of Backcross Method

1. The genotype of new variety is nearly identical with that of the recurrent parent, except for the genes transferred. Thus the

outcome of a backcross programme is known beforehand, and it can be reproduced any time in the future.

2. It is not necessary to test the variety developed by the backcross method in extensive yield tests because the performance of the recurrent parent is already known. This may save up to 5 years time and a considerable expense.

3. The backcross programme is not dependent upon environment, except for that needed for the selection of the character under transfer. Therefore, off -season nurseries and green houses can be used to grow 2-3 generations each year. This would drastically reduce the time required for developing the new variety.

4. Much smaller populations are needed in the backcross method than in the case of pedigree method.

5. Defects, such as susceptibility to disease, of a well - adapted variety can be removed without affecting its performance and adaptability. Such a variety is often preferred by the farmers and the industries to an entirely new variety because they know the recurrent variety well.

6. This is the only method for inter- specific gene transfers, and for the transfer of cytoplasm.

7. It may be modified so that transgressive segregation may occur for quantitative characters.

CHAPTER -VI

BREEDING OF CROSS POLLINATED CROPS

Genetic Composition of Cross-Pollinated Populations

Cross-pollinated crops are highly heterozygous due to the free intermating among their plants. They are often referred to as random mating populations because each individual of the population has equal opportunity of mating with any other individual of that population. Such a population is also known as ***Mendelian population*** or ***panmictic population***. Mendelian population may be thought of having gene pool consisting of all the gametes produced by the population. Thus the gene pool may be defined as the sum total of all the genes present in a population. Such generation of a Mendelian population may be considered to arise from a random sample of gametes from the gene pool of previous generation. For this reason, it is not possible to follow the inheritance of a gene in a Mendelian population by using the techniques of classical genetics. To understand the genetic makeup of such populations a sophisticated field of study, population genetics.

The Hardy-Weinberg Law

“The Hardy-Weinberg law states that the gene and genotype frequencies in a Mendelian population remain constant generation after generation if there is no selection, mutation, migration or random drift”. The frequencies of the three genotypes for a locus with two alleles, say would be p^2 AA, $2pq$ Aa, and q^2 aa ; where p represents the frequency of A and q represents the frequency of a allele in the population, and the sum of p and q is one, i.e., $p + q = 1$. Such a population would be at equilibrium since the genotypic frequencies would be stable, that is, would not change, from one generation to the next .This equilibrium is known as **Hardy-Weinberg equilibrium**. A population is said to be at equilibrium when frequencies of the three genotypes, AA, Aa and aa are p^2 , $2pq$ and q^2 , respectively.

Systems of mating

(i)Random Mating

In random mating, each female gamete is equally likely to unite with any male gamete and the rate of reproduction of each genotype is equal, *ie.*, there is no selection. In such a situation, (1) gene frequencies remain constant. (2) Variance for the character is

constant and (3) the correlation between relatives or prepotency does not change.

Random mating is useful in plant breeding in several ways, *eg.*, progeny testing, production and maintenance of synthetic and composite varieties, production of polycross progeny etc.

(ii)Genetic Assortative Mating

In genetic assortative mating, the mating is between individuals that are more closely related by ancestry than in random mating. Thus in this mating system, it is not important that the genotypes of the plants selected for mating be correctly identified. This mating system is more commonly known as inbreeding. Inbreeding has the following effects on a population.

- 1.It increases homozygosity and reduces heterozygosity.

- 2.Alleles and as a result, the characters become fixed, except for the variation produced by environment. The fixation of a character is little affected by the number of genes involved and the heritability of the character, particularly under more intense forms of inbreeding, *e.g.*, self-fertilization.

- 3.Under intense inbreeding, the number of noninterbreeding groups increases rapidly. As a result, selection must be done to keep the population size manageable.

4.The total genetic variability of the population increases rapidly. The genetic variability within interbreeding groups, *ie.*, families or lines, decreases rapidly and is ultimately reduced to zero.

5.Under selection, genetic variability of the population decreases rapidly. In cases where only one line is selected, the genetic variability is very small or zero.

6.The prepotency of individuals increases under inbreeding. Prepotency is the property of an individual to produce progeny which are similar to each other and to the parent. Prepotency is affected by homozygosity, dominance, epistasis and linkage. Homozygosity is the most important factor and is under control of the breeder. As homozygosity increases, the prepotency of an individual also increases. An individual completely homozygous for all the dominant alleles will be the most prepotent. Genetic assortative mating is useful in the development of inbreds, both partial and complete.

(iii)Genetic Disassortative Mating

In genetic disassortative mating, such individuals are mated which are less closely related by ancestry than would be under random mating. Thus in this system, totally unrelated individuals

are mated. These individuals often belong to different populations. Examples of such a mating are intervarietal and interspecific crosses. The effects of genetic disassortative mating are similar to those of migration. It may be expected that this mating system would reduce homozygosity and increase heterozygosity.

(iv) Phenotypic Assortative Mating

Mating between individuals which are phenotypically more similar than would be expected under random mating is called phenotypic assortative mating. Phenotypic assortative mating leads to the following.

1. Division of the population into two extreme phenotypes. Unlike inbreeding which fixes intermediate types as well, under this system of mating the intermediate types are not fixed. Only the two extreme phenotypes, *ie.*, the lowest and the highest, remain in the population.

2. Increase in homozygosity.

3. Increase in genetic variability provided both the extreme phenotypes are kept in the population.

4. An increase in the prepotency of individuals due to an increase in their homozygosity.

These effects disappear rapidly when random mating is restored. The effects of phenotypic assortative mating become quickly obvious in case of those characters that are governed by one gene, but the effects are slower to appear in case of those governed by two genes. In the case of polygenic traits, effects are much slower to realise, and the intermediate types are seldom eliminated. Dominance and nonadditive gene actions further reduce the effects of phenotypic assortative mating. Since heritability is always less than 100 per cent for most of the quantitative traits, the effectiveness of this mating is further reduced. Therefore, the increase in homozygosity etc. is much slower than that expected with 100 per cent heritability'.

This mating system is useful in the isolation of extreme phenotypes. It is used in some breeding schemes, e.g., in recurrent selection.

(v) Phenotypic Disassortative Mating

Mating between phenotypically dissimilar individuals is referred to as phenotypic disassortative mating. The consequences of this mating are as follows.

1. Maintenance of or even some increase in heterozygosity.

2. Some reduction in population variance since it tends to produce intermediate phenotypes.
3. Reduction in correlation between relatives or in prepotency due to the increase in heterozygosity.

This mating system is very useful in making a population stable, *ie.*, in maintaining variability. Suitable parents may be selected to remove their weaknesses. The progeny from such a mating would be more desirable than the parents. It is also useful when the desirable type is an intermediate one and the available parents have the extreme phenotypes. But the most notable use of this mating system is in maintaining variability in relatively smaller populations as it reduces inbreeding.

Genetic assortative mating increases homozygosity and is useful in the development of inbred lines. Phenotypic assortative mating eliminates the intermediate types and divides the population into two extremes. This mating is desirable when the extreme types are desirable. Genetic disassortative mating is represented by inter-varietal crosses. Phenotypic disassortative mating maintains heterozygosity in the population. It is a suitable mating scheme in cases where the population is to be kept stable and heterozygous.

Synthetic Varieties

The possibility of commercial utilization of synthetic varieties in maize was first suggested by Hayes and Garber in 1919. “A *synthetic variety is produced by crossing in all combinations a number of lines that combine well with each other. Once synthesized, a synthetic is maintained by open-pollination in isolation*”. (Fig.23)

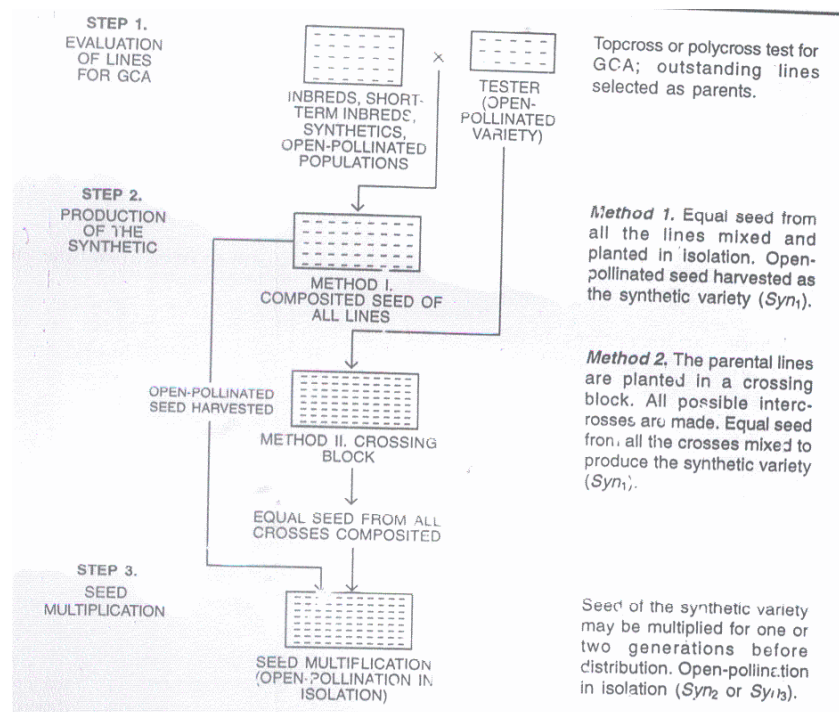


Fig. 23. Steps involved in the production of synthetic varieties.

Composites

Composite, is often used as a synonym for synthetic, which is not entirely accurate. “A composite *variety is produced by mixing the seeds of several phenotypically outstanding lines and encouraging open-pollination to produce crosses in all combinations among the mixed lines. The lines used to produce a composite variety are rarely tested for combining ability with each other*”. Consequently, the yields of composite varieties cannot be predicted in advance (in contrast to synthetics) for the obvious reason that the yields of all the F₁'s among the component lines are not available. Like synthetics, composites are commercial varieties and are maintained by open-pollination in isolation.

Germplasm complexes

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

Mass Selection In Cross Pollinated Crops

Mass selection is the oldest breeding scheme available for cross-pollinated crops. In mass selection, a number of plants are

selected on the basis of their phenotype, and the open-pollinated seed from them is bulked together to rise the next generation. The selected plants are allowed to open-pollinate, *ie.*, to mate at random including some degree of selfing (usually about 10% in maize, (*Z. mays*)). Thus mass selection is based on the maternal parent only, and there is no control on the pollen parent. Selection of plants is based on their phenotype and no progeny test is conducted mass selection, as applied to cross-pollinated crops is essentially the same as that applied to self-pollinated crops. The method is outlined in **Fig. (24)**. The selection cycle may be repeated one or more times to increase the frequency of favourable alleles; such a selection scheme is generally known as phenotypic recurrent selection. Care should be taken to select a sufficiently large number of plants in order to keep inbreeding to a minimum. The efficiency of mass selection primarily depends upon the number of genes controlling the character, gene frequencies and more importantly heritability.

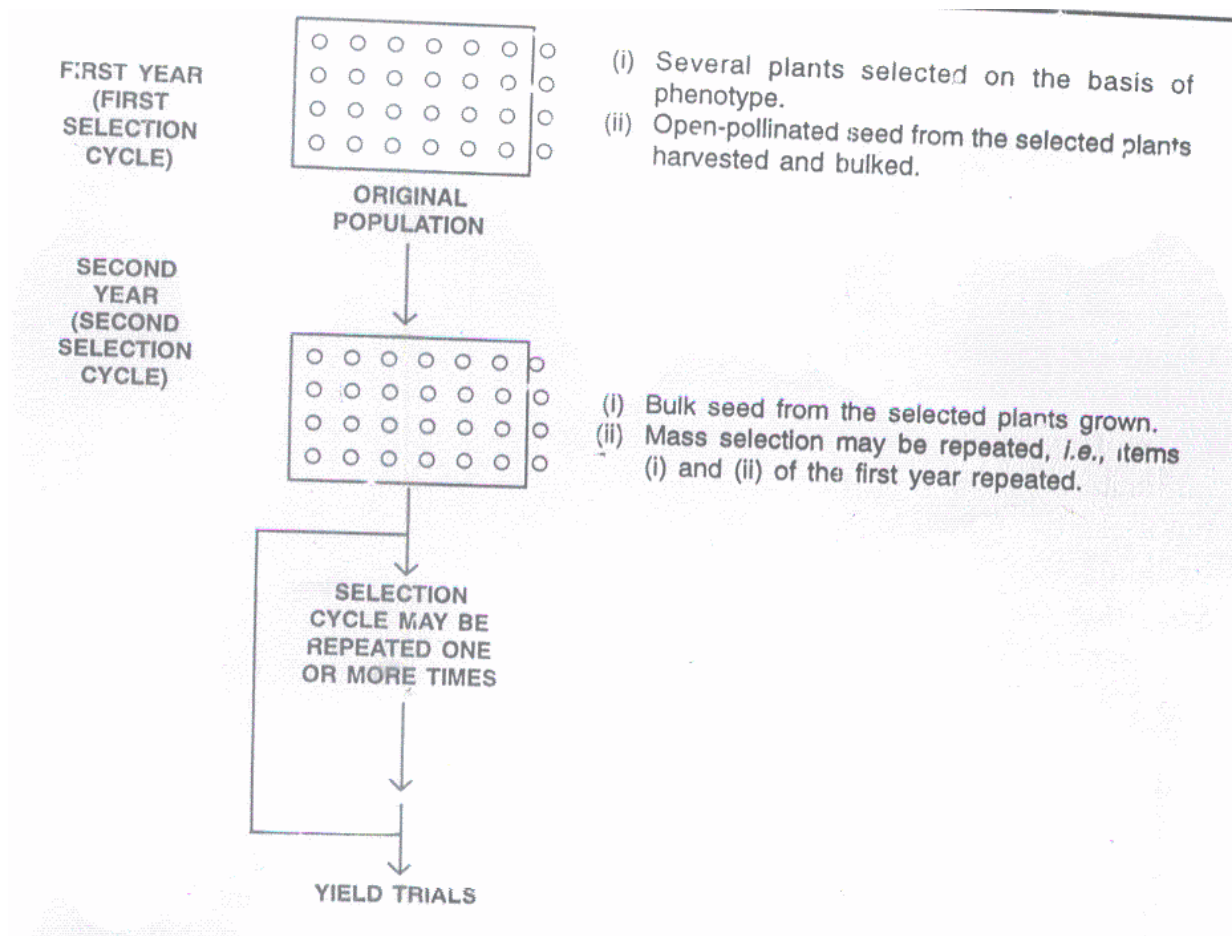


Fig. 24. Mass selection as applied to cross – pollinated crop species. When the selection is repeated one or more times, as outlined here, the scheme is known as phenotypic recurrent selection.

Merits of Mass Selection

1. Mass selection is an extremely simple breeding programme.

Work of the breeder is kept to a minimum since the selections are based on the phenotype of plants.

2. The selection cycle is very short, i.e., of only one generation. Thus in every generation, one cycle of selection is completed.

3. It is highly efficient in improving characters that are easily identified visually and have high heritability, *eg.*, plant height, size of ear, date of maturity etc.
4. If proper care is taken, mass selection is effective in improving yields in cross-pollinated crops. Most cross-pollinated crops have a high component of genetic variance which responds to selection.

Selection with Progeny Test

Progeny Selection

The simplest form of progeny selection is the ear-to-row method which has been extensively used in maize. This method was developed by Hopkins in 1908. In its simplest form, the ear-to-row method of selection is as follows (Fig.--).

1. A number (50-100) of plants are selected on the basis of their superior phenotype. They are allowed to open-pollinate, and the seeds from individual plants are harvested separately.
2. A single row of 10-50 plants, *ie.*, a progeny row, is grown from each selected plant. The progeny rows are evaluated for desirable characters and superior progenies are identified.

3. Several phenotypically superior plants are selected from the superior progenies. There is no control on pollination; the plants are permitted to open-pollinate.
4. Small progeny rows, as in item 2, are grown from the selected plants, and the process of selection is repeated.

It should be seen that this scheme is relatively simple and that the selection cycle is of one year only. However, it suffers from the defect that plants in the superior progenies are pollinated by those in both the weak and inferior progenies. This reduces the effectiveness of selection. To overcome this defect, the following modification may be used **(Fig.25)**.

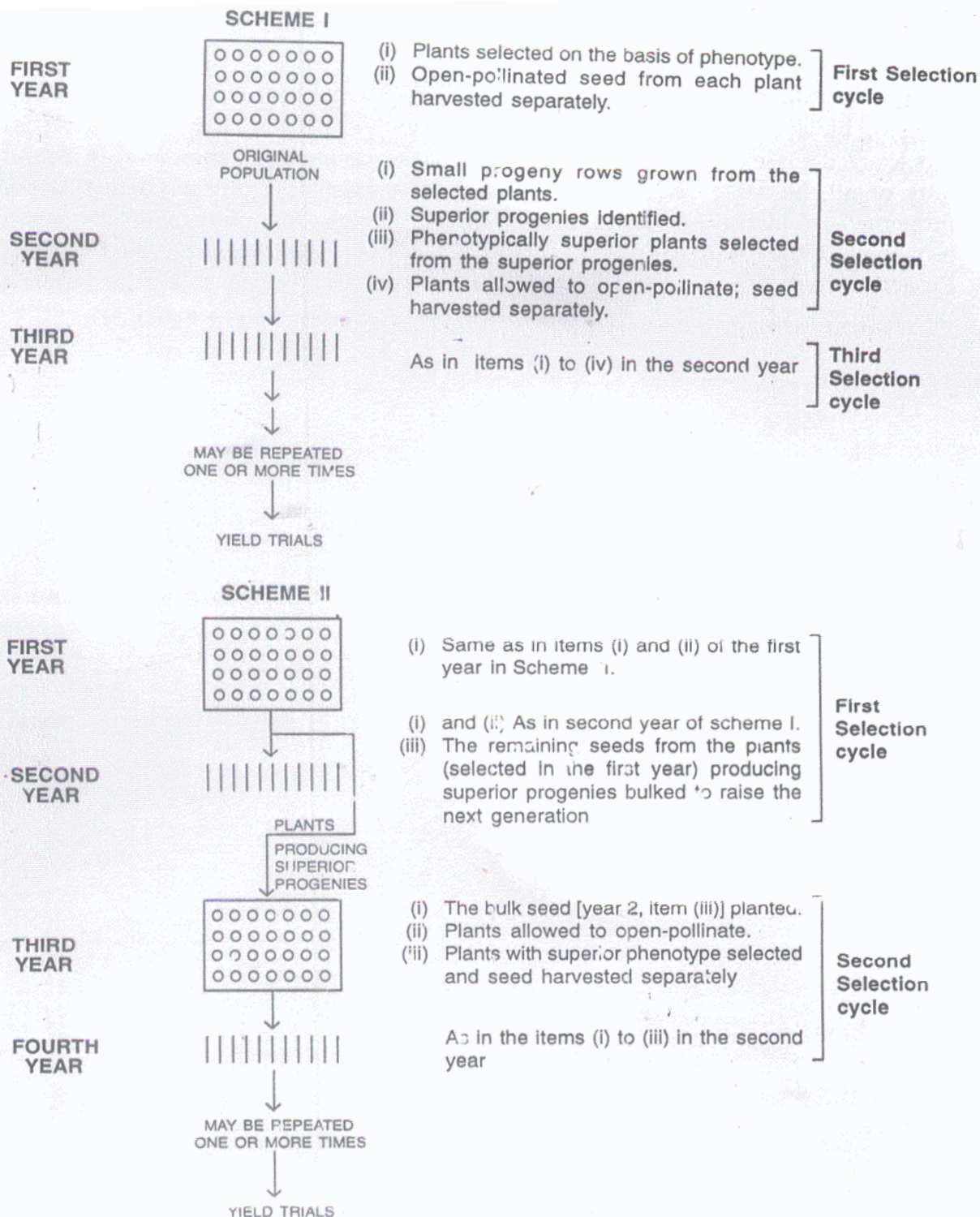


Fig. 25. Ear-to-row method of progeny selection as applied to maize. These schemes are termed as recurrent selection by many plant breeders who apply the term to any selection scheme, which has two or more selection cycles.

First Year. Several plants are selected on the basis of phenotype (as in item 1 above). Open-pollinated seed is harvested separately.

Second Year. Small progeny rows are grown (as in item 2 above) and evaluated. The remaining seed from the selected plants is kept separately. Superior progenies are identified.

Third Year. The remaining seed from the plants that produced the superior progenies (identified in the second year) is bulked to produce the next generation. Plants are allowed to open-pollinate. A number of plants are selected on the basis of phenotype and the selection cycle may be repeated one or more times.

This modification of ear-to-row method was widely used in breeding of maize in U.S.A. and was responsible for the development of several varieties. In this method, plants from superior progenies only are allowed to mate among themselves. But for each selection cycle, two years are required as compared to only one in the case of ear-to-row method.

Merits of Progeny Selection:

The progeny selection schemes, except those for recurrent selection, have the following advantages.

1. In progeny selection, the selection is based on progeny test and not on the phenotypes of individual plants. The progeny test is a far more accurate reflection of the genotype than the phenotype. Thus progeny selection is far more efficient than mass selection in the identification -of superior genotypes. This is a powerful tool for increasing the yielding ability of open-pollinated varieties of maize; the yielding ability increased at the rate of 3-8 per cent per selection cycle in the different experiments.
2. Inbreeding may be avoided if care is taken to select a sufficiently large number of plant-progenies and if the selected progenies are not closely related.
3. The selection scheme is still relatively simple and easy. But some of the modifications are more complicated and tedious.

Recurrent Selection

The idea of recurrent selection was first suggested by Hayes and Garber 1919 and independently by East and Jones in 1920. However, cohesive breeding schemes of recurrent selection were developed during 1940s, particularly after 1945 when Hull suggested that recurrent selection may be in improving specific combining ability. The recurrent selection schemes are devised in relation to heterists breeding. The idea was to ensure the oblation

of superior inbreds from the populations subjected to recurrent selection for their ultimate utilization in the production of hybrid and synthetic varieties.

Simple Recurrent Selection

In simple recurrent selection, a number of plants with the desirable phenotypes are selected and self-pollinated. Separate progeny rows are grown from the selected plants in the next generation. The progenies are intercrossed in all possible combinations by hand. Equal amount of seed from each cross is composited to produce the next generation. This completes *original selection cycle*. For recurrent selection, several desirable plants are selected from the composited population obtained from the original selection cycle; they are selected on the basis of phenotype and are self-pollinated. Progeny rows are grown and all the possible intercrosses are made by hand. Equal seeds from all the intercrosses are composited to produce the next generation. This constitutes the *first recurrent selection cycle*. The population may be subjected to one or more recurrent selection cycles **(Fig.26)**

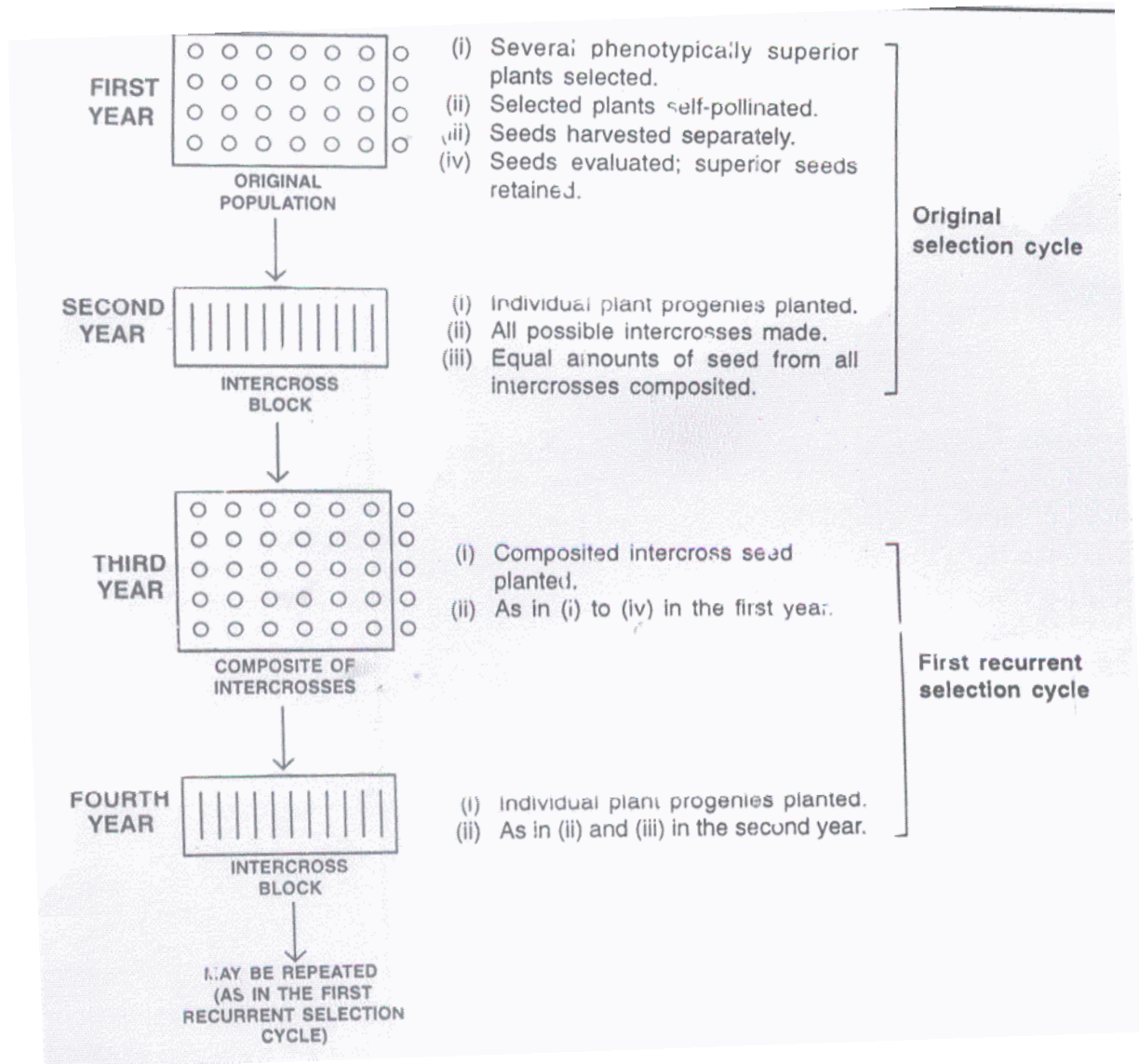


Fig.26. Simple recurrent selection when the characters under selection can be easily evaluated on the basis of phenotype of the selected or from the selfed obtained from them.

Recurrent selection is effective in increasing the frequency of desirable genes in the population. It is the most suited for characters with high heritability. The mean of selected population shifts in the direction of selection. Generally, there is no appreciable reduction in variability, and in some cases selected populations may show a relatively larger variation than the original population. Simple recurrent selection is considerably more efficient than selection with self-pollination. There is a relatively low inbreeding, and if care is taken it can be kept to a minimum. Inbreeding can be avoided in one of the following two ways: (1) The population derived from the mixture of intercrosses may be allowed to mate at random for one generation and this open-pollinated seeds should be used to establish the population for reselection. Alternatively, (2) Each intercross may be grown separately and it should be ensured that the selected plants are not related by parentage or by descent, i.e., the plants are not selected from a few of the intercross only.

Reciprocal Recurrent Selection (RRS)

Reciprocal recurrent selection was proposed by Comstock, Robinson and Harvey in 1949. The objective of RRS is to improve two different populations in their ability to combine well with each other. In this scheme, each of the two populations A and B serve as testers for the plants selected from the other population. For example, a random sample of plants from population A serves as the *tester* for the plants selected from population B. Similarly, a random sample of plants from population B serves as the *tester* for those selected from population A. It may be seen that this selection method allows for the selection for both GCA and SCA. It selects for GCA because the two testers (populations A and B) have broad genetic base since they are genetically heterogenous. Selection for SCA is accomplished because the two populations (or inbreds derived from them) would be crossed with each other to produce the commercial variety, and the plants in each of the two source populations are selected for their abilities to combine well with the gene combinations present in the other population, that is, for SCA with each other. A generalised scheme for RRS is outlined below **(Fig. 27)**.

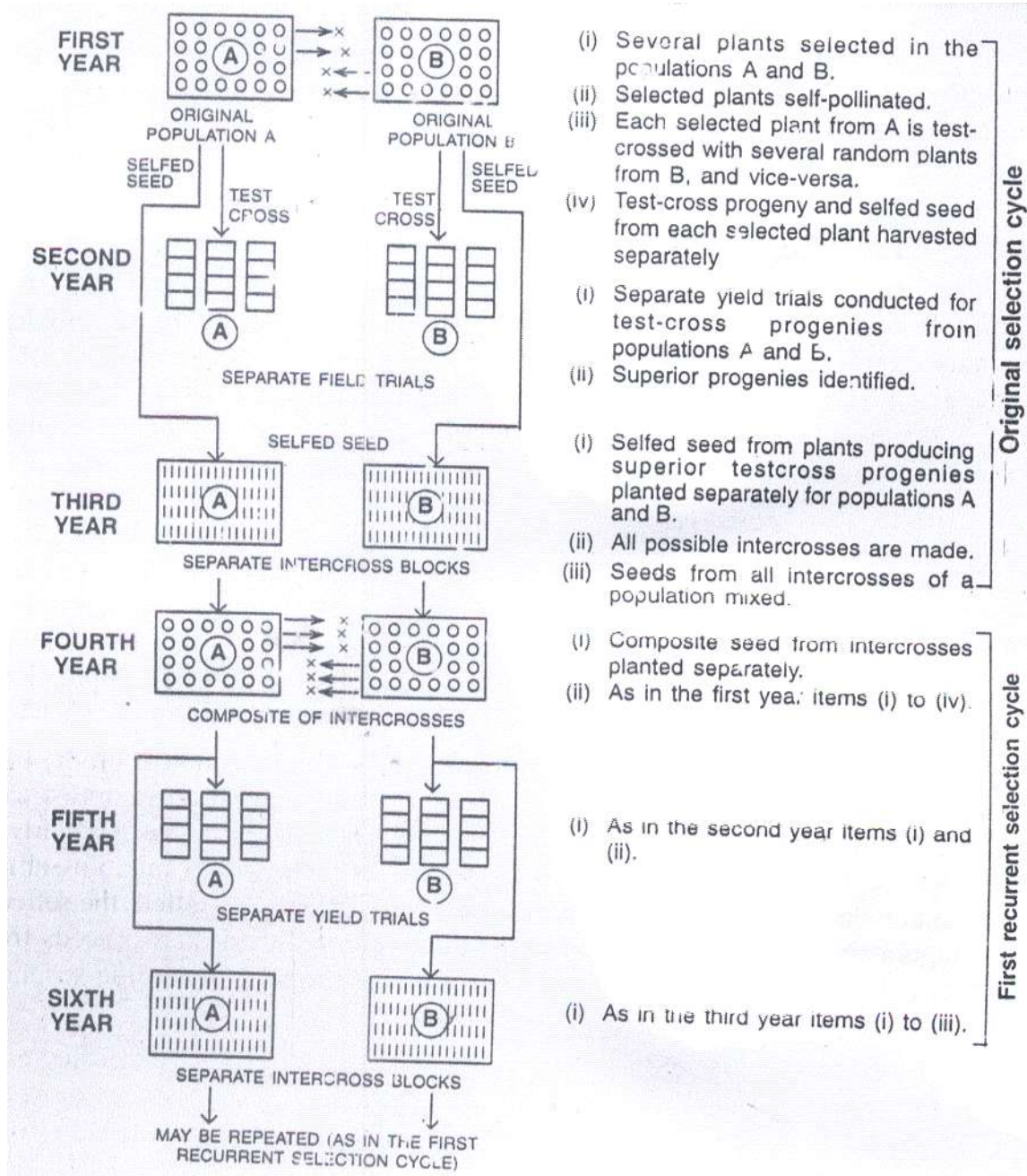


Fig. 27. Reciprocal recurrent selection. Two populations, A and B, with broad genetic base serve as testers for each other.

First Year. Several plants are selected from the populations A and B on the basis of their desirable phenotype. Each of the selected plants from population A is crossed as male with several randomly selected plants from the population B used as female. Similarly, each of the plants selected from the population B is crossed as male with a random sample of plants from the population A used as female. All the selected plants are selfed; the selfed seed is harvested separately and is stored for use in the third year. All the test cross seeds from an individual selected plant are composited and later used for progeny test in the second year.

Second Year. Two replicated yield trials are conducted: one trial is for the testcross progenies of the plants selected from population A and the other one is for those of the plants selected from population B. On the basis of these progeny tests, plants producing superior progenies are identified.

Third Year. Selfed seeds from the plants selected on the basis of progeny tests (in the second year) are planted in two separate crossing blocks in individual plant-progeny rows. In one crossing block, seeds from the plants selected from the population A are planted, while in the other crossing block seeds from plants selected from population B are sown. Within each crossing block,

all possible intercrosses among the plant progenies are made. Equal amounts of seeds from all the intercrosses in the crossing block A are mixed to raise the next generation of population A. Similarly, the next generation of population B is raised from the seeds obtained by mixing equal amounts of seeds from all the possible intercrosses in the crossing block B. This completes the original selection cycle.

Fourth Year. Populations A and B are planted from the composited seed from all the intercrosses in blocks A and B, respectively. Operations of the first year are repeated.

Fifth Year. Operations of the second year are repeated.

Sixth Year. Operations of the 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 the first recurrent selection cycle.

The two populations developed by RRS may be used in one of the following two ways.

1. They may be intermated to produce a superior population with a broad genetic base. This is similar to a varietal cross, but in this case the populations have been subjected to selection for combining ability (both GCA and SCA) with each other. In

maize (*Z. mays*), crosses between populations improved by reciprocal recurrent selection show higher yields than those between the original populations .

2. Inbreds may be isolated from the populations A and B. These inbreds may then be crossed to produce a double cross or a single cross. In this case, the inbreds should be crossed in the following order.

Single cross: ($A_1 \times B_1$)

Double cross: ($A_1 \times A_2$) $\times$ ($B_1 \times B_2$)

3. Where, A_1 and A_2 are two inbreds isolated from the population A and B_1 and B_2 are the inbreds isolated from the population B. This would permit the maximum expression of heterosis in the double cross. There is considerable evidence that reciprocal recurrent selection increases the yielding ability of hybrids produced by crossing inbreds isolated from the selected populations.

CHAPTER - VII

HETEROSIS BREEDING

Cross pollinated species and species reproducing asexually are highly heterozygous. These species usually show a severe reduction in fertility and vigour due to inbreeding (inbreeding depression). *Conversely, hybridization between unrelated strains generally leads to an increased vigour and fertility (hybrid vigour or heterosis).* These two aspects are of great significance in breeding of these species.

Inbreeding depression

Inbreeding is mating between individuals related by descent or ancestry. When the individuals are closely related, *eg.*, in brother-sister mating or ***sib mating*** the degree of inbreeding is high. The highest degree of inbreeding is achieved by selfing. The chief effect of inbreeding is an increase in homozygosity in the progeny.

Inbreeding depression may be defined as the reduction or loss in vigour and fertility as a result of inbreeding.

Heterosis

The term Heterosis was first used by Shull in 1914. Heterosis may be defined as the superiority of an F_1 hybrid over both its parents in terms of yield and some other characters. Generally,

heterosis is manifested as an increase in vigour, size, growth rate, yield or some other characteristic. Often the superiority of F_1 is estimated over the average of the two parents or the mid –parent. If the hybrid is superior to the **mid-parent** it is regarded as heterosis (**average heterosis or relative heterosis**). This practice has found some acceptance, particularly in theoretical studies. However, in practical plant breeding, the superiority of F_1 over mid-parent is of no use since it does not offer the hybrid any advantage over the better parent. Therefore, average heterosis is of little or no use to the plant breeder. More generally, heterosis is estimated over the superior parent; such an estimate is sometimes referred to as **heterobeltiosis**. The term heterobeltiosis is not commonly used since most breeders regard this to be the only case of heterosis and refer to it as such *ie.*, heterosis. However the commercial usefulness of a hybrid would primarily depend on its performance in comparison to the best commercial variety of the concerned crop species. In many cases, the superior parent of the hybrid may be inferior to the best commercial variety. In such cases, it will be desirable to estimate heterosis in relation to the best commercial variety of the crop. Such an estimate is known as **economic or useful or standard heterosis**.

Luxuriance

Luxuriance is the increased vigour and size of interspecific hybrids. The principal difference between heterosis and luxuriance lies in the reproductive ability of the hybrids. Heterosis is accompanied with an increased fertility, while luxuriance is expressed by interspecific hybrids that are generally sterile or poorly fertile.

Genetic bases of heterosis and inbreeding depression

Heterosis and inbreeding depression are closely related phenomena. In fact, they may be regarded as the two opposite sides of the same coin. The genetic theories that explain heterosis also explain inbreeding depression. There are two main theories to explain heterosis and consequently, inbreeding depression (1) dominance hypothesis and (2) over dominance hypothesis.

Dominance hypothesis

The dominance hypothesis was first proposed by Davenport in 1908. It was explained by Bruce and by Keeble and Pellew in 1910. In simpler form this hypothesis suggests that at each locus the dominant allele has favourable effects while the recessive allele has an unfavourable effect. In heterozygous state the deleterious effects of recessive alleles are masked by their dominant alleles.

Thus heterosis results from the masking of harmful effects of recessive alleles by their dominant alleles. Inbreeding depression on the other hand is produced by the harmful effects of recessive alleles which become homozygous due to inbreeding.

Overdominance hypothesis

This hypothesis was independently proposed by East and Shull in 1908. This is sometimes known as *single gene heterosis*, *super dominance*, *cumulative action of divergent alleles* and *stimulation of divergent alleles*.

According to this hypothesis , heterozygotes at atleast some of the loci are superior to both the relevant homozygotes. Thus heterozygote Aa would be superior to both homozygotes AA and aa. Consequently, heterozygosity is essential for and is the cause of heterosis,while homozygosity resulting from inbreeding produces inbreeding depression. It would be therefore,be impossible to isolate inbreds as vigour as F₁ hybrids if heterosis were the consequence of Overdominance.

Development of hybrids

Hybrids are the first generations (F_1) from crosses between the purelines, inbreds, open-pollinated varieties, clones or other populations that are genetically dissimilar. Most of the commercial hybrid varieties and F_1 'S from two or more purelines (tomato) or inbreds (maize, jowar, bajra). An **inbred** is a nearly homozygous line obtained through continuous inbreeding of a cross-pollinated species with selection accompanying inbreeding; its maintenance by close inbreeding preferably by self-pollination.

When two inbreds, say A and B are crossed to produce the hybrid ($A \times B$) it is known as single cross. When two single crosses, say ($A \times B$) and ($C \times D$), are crossed the resulting hybrid population, $(A \times B) \times (C \times D)$ is known as double cross. A three-way cross is the cross between a single cross ($A \times B$) and an inbred (C) to yield the hybrid population $(A \times B) \times C$.

When an inbred is crossed with an open pollinated variety it is known as **inbred-variety cross or topcross**. **Topcross** however denotes crosses of selected plants, lines or clones with an open pollinated variety. The purpose of top cross is to estimate GCA of the plants or lines crossed with the open-pollinated variety. When cross is made to access the combining ability, it is known as

testcross; a testcross may be made with an inbred (for SCA), hybrid, synthetic or open-pollinated variety (for GCA). The common parent used in a test cross is known as **tester**, and the progeny derived from these crosses are known as testcross progeny. **Polycross** denotes the progeny of the line produced through random pollination by a number of selected lines. When two open pollinated varieties are mated it is usually known as **varietal or population cross**.

Production of Hybrid Seed

The two requirements for commercial hybrid seed production are (1) easy emasculation of the female parent, and (2) effective pollen dispersal from the male parent to ensure a satisfactory seed set in the female parent. Both these factors are largely governed by the floral structure and the natural mode of reproduction of the crop species in question. For example, emasculation in maize, due to its peculiarly favourable floral structure, consist of a very simple operation of *detasselling* ,i.e., *removal of the entire tassel (the male inflorescence)from the plant before pollen is shed*. Thus one simple operation emasculates the whole plant. The production of hybrid maize till 1960s was essentially based on detasseling. However, male sterile and self-incompatibility offer the means for *genetic*

*emasculat*ion, that is, preventing self-fertilization by manipulating the genotype of a plant and are the bases of hybrid seed production in many crops. Pollen dispersal is often satisfactory in most cross pollinated species since it is the natural mode of reproduction. In self pollinated species however satisfactory pollen dispersal is often the limiting factor in hybrid seed production. Hybrid seeds may be produced in one of the following several ways: use of

1. Cytoplasmic –genetic male sterility
2. Cytoplasmic male sterility
3. Genetic male sterility
4. Self-incompatibility and
5. Manual emasculation and /or pollination.

These systems have been discussed in detail in chapter 3.

Production of single cross hybrid varieties

Cytoplasmic –genetic male sterility

This system is the most widely used methods in hybrid seed production .It is commercially used in maize, bajra, jowar, onions (*A.cepa*), sunflower, cotton and sugarbeets (*B.vulgaris*).This system is based on a cytoplasm that produces male sterility and on a gene that restores fertility in the presence of male sterile cytoplasm. The development of male sterile and restorer lines has been described

in chapter---and the use of this system in hybrid seed production is outlined below.

For the production of single cross ,a cytoplasmic male sterile line is used s female parent, while the male parent is the restorer. The seed set on the female parent is selfed seed. The resulting hybrid will be male fertile since it has received the restorer gene from the male parent **(Fig.28)**.Generally two rows of the male fertile inbred (the male parent) is planted after every two rows of the male sterile parent(the female parent) but when the male inbreds produces sufficient pollen,2rows of the male inbred may be planted after every 3 or 4 rows of the female parent. The present single cross hybrid varieties of maize are produced by planting male and female inbreds in the ratio of 2:4.

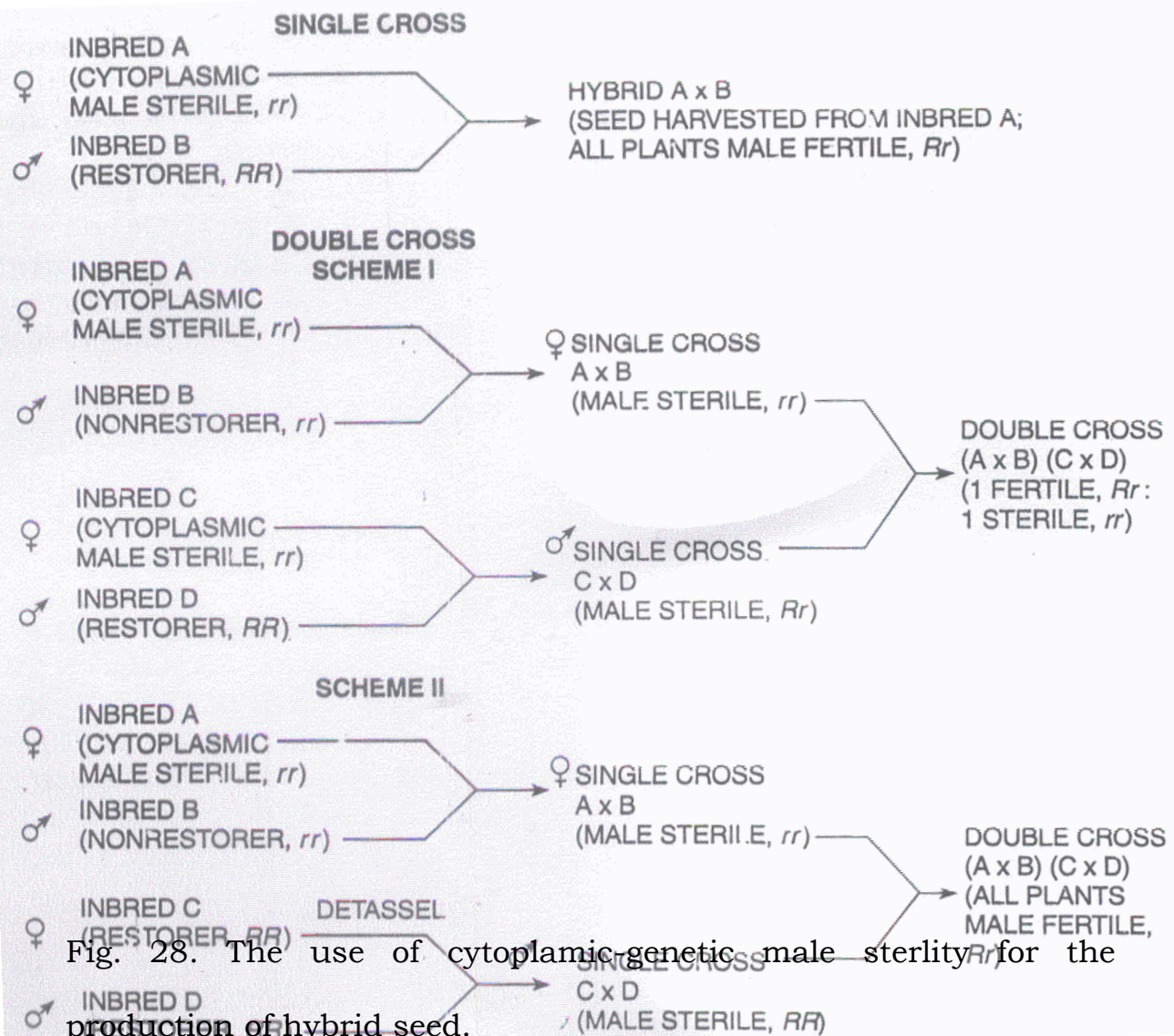


Fig. 28. The use of cytoplasmic-genetic male sterility for the production of hybrid seed.

Production of double cross hybrid varieties

Double cross hybrid varieties are produced by crossing two single crosses; One male sterile and the other male fertile. The male sterile single cross is produced by crossing a cytoplasmic male sterile line with a non-restorer male fertile line (**Fig.28**). The male fertile single cross may be produced in one of the following two ways. In the first approach, a cytoplasmic male sterile line is crossed with a restorer line(**Fig 28;scheme I**);the double cross in this case has both male sterile and male fertile plants in the ratio 1:1.in the second method ,two restorer lines are crossed together; one of the restorer line serves as female and is detasseled manually(**Fig.28;schemeII**).All the plants in the double cross ,in this case would be male fertile. Scheme II is expensive, but it may have some usefulness in crop species where pollen production is limited.

Manual emasculation and/or pollination

This method relies on manual emasculation and in many cases on manual pollination. Early hybrid maize production was based on manual emasculation *ie.*, detasseling. The scheme II (**Fig.28**) of double cross production also involves detasseling for producing the single cross(C x D).Manual emasculation and

pollination has been successfully used for hybrid seed production in tomatoes in Europe and of cotton in India. The hybrid seed produced by manual operations is costly. Consequently, this system of hybrid seed production is limited only to the crops where the returns are very high making the production of hybrid seed economically feasible.

Chemically-Induced male sterility

Several chemicals induce male sterility when applied during specific developmental stages of plants; such male sterility is known as chemically induced male sterility, and the chemical compounds are called chemical hybridizing agent(CHAs). The use of CHAs for rendering the pollen grains non functional so that the treated line is used as the female parent in hybrid seed production is termed as **Chemical emasculation**. Some of the CHAs are highly effective and are being used for commercial hybrid seed production in rice and wheat.

CHAs based hybrid seed production is being practiced in China in case of rice. Two arsenical CHAs, named MGI (based on zinc methyl arsenate) and MG2 (based on sodium methyl arsenate) have been developed for this purpose.

CHAPTER - VIII

CLONAL SELECTION AND HYBRIDIZATION

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* sp.), 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 effects of segregation and recombination, both being the inevitable consequences of sexual reproduction.

Segregation and recombination produce new gene combinations due to which the progeny differ from their parents in genotype and phenotype. Asexual reproduction, on the other hand,

produces progeny exactly identical to their parents in genotype because the progeny are derived from vegetative cells through mitosis. *“The advantage of asexual reproduction is immediately clear: it preserves the genotype of an individual indefinitely. It must be noted that this does not depend on the homozygosity of the genotype of an individual. Any genotype is preserved and maintained through asexual reproduction. In contrast, self-pollination preserves and maintains only homozygous genotypes giving rise to purelines.”*

Characteristics of Asexually Propagated Crops

Asexually propagated crops generally have the following characteristics.

1. A great majority of them are perennials, e.g., sugarcane, fruit trees, *etc.* The annual crops are mostly tuber crops, e.g., potato, cassava (*M. utilissima*), sweet potato *etc.*
2. 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.
3. They are invariably cross-pollinated.

4. These crops are highly heterozygous and show severe inbreeding depression.

5. A vast majority of asexually propagated crops are either polyploids, e.g., sugarcane, potato, sweet potato etc., or have polyploid species or varieties.

6. Many species are inter-specific hybrids, e.g., banana (*M. paradisiaca*), sugarcane, *Rubus* etc.

7. 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 plant through asexual reproduction.

Clonal selection(Fig.29)

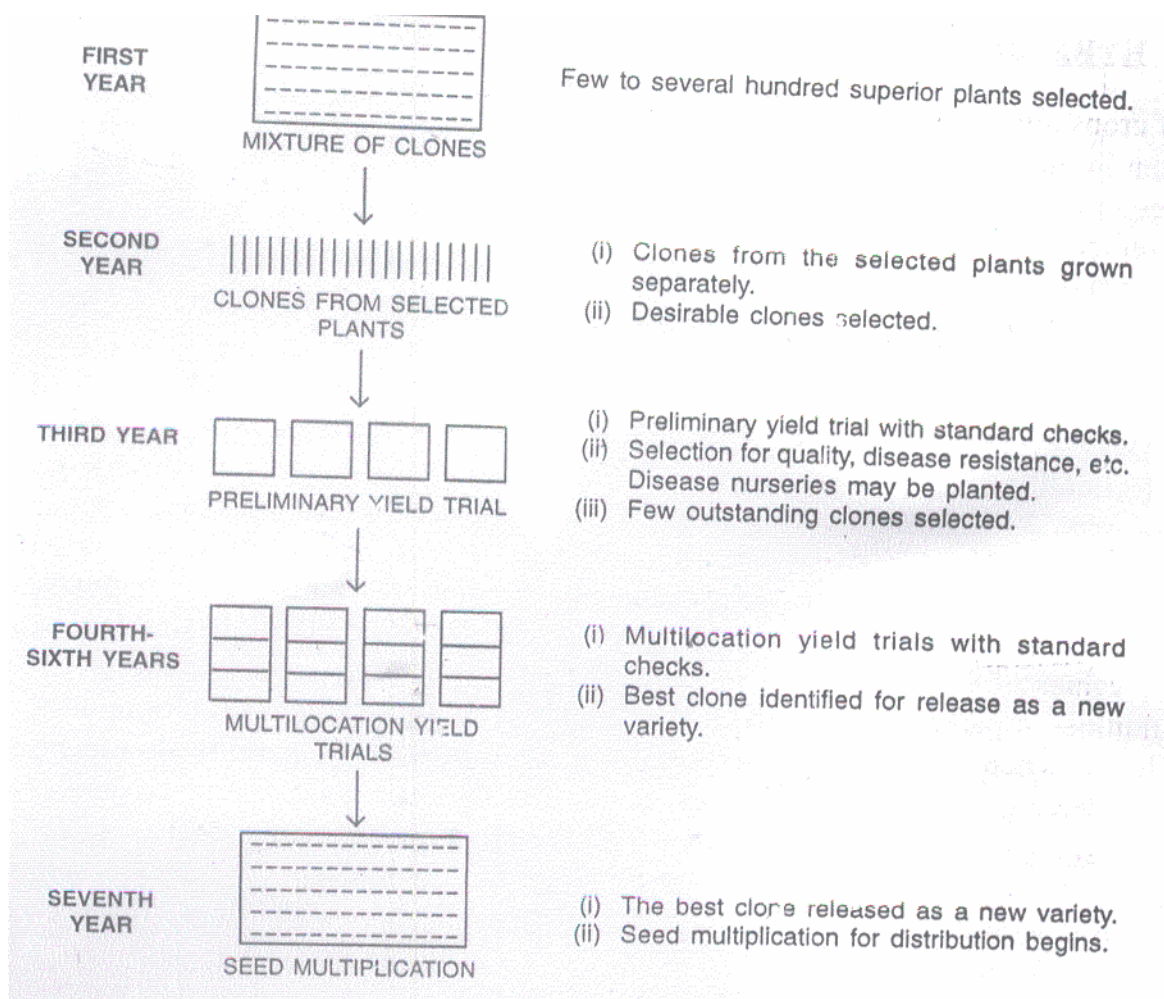


Fig. 29. A generalized scheme for clonal selection in asexually propagated species. This figure applies to a crop in which one generation does not take more than one year.

Selection procedure

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.

Second year

Clones from the selected plants are grown separately, generally without replication. This is done in view of the limited supply of propagating materials. 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 are eliminated. The selection is based on visual observations and on the breeders judgement of the value of clones.

Third year

Replicated preliminary yield trial is conducted .A suitable check is included for comparison. Few superior performing clones with desirable characteristics are selected for multilocation trials.

At this stage selection of quality is also done. If necessary, separate disease nurseries may be planted to evaluate disease resistance of the selected clones.

Fourth to sixth year

Replicated yield trials are conducted at several locations along with a suitable check. The yielding ability, quality and disease resistance of the clones are rigidly evaluated. The best clone that is superior to the check with one or more characteristics is identified for release as a new variety.

Seventh year

The superior clone is multiplied and released as a new variety.

Merits of Clonal Selection

1. It is the only method of selection applicable to clonal crops. It avoids inbreeding depression and preserves the gene combinations present in clones.
2. Clonal selection, without any substantial modification, can be combined with hybridization to generate the necessary variability for selection.
3. The selection scheme is useful in maintaining the purity of clones.

Demerits of Clonal Selection

1. This selection method utilizes the natural variability already present in the population; it is not devised to generate variability.

2. Sexual reproduction is necessary for the creation of variability through hybridization.

Clonal Selection and Hybridization (Fig.30)

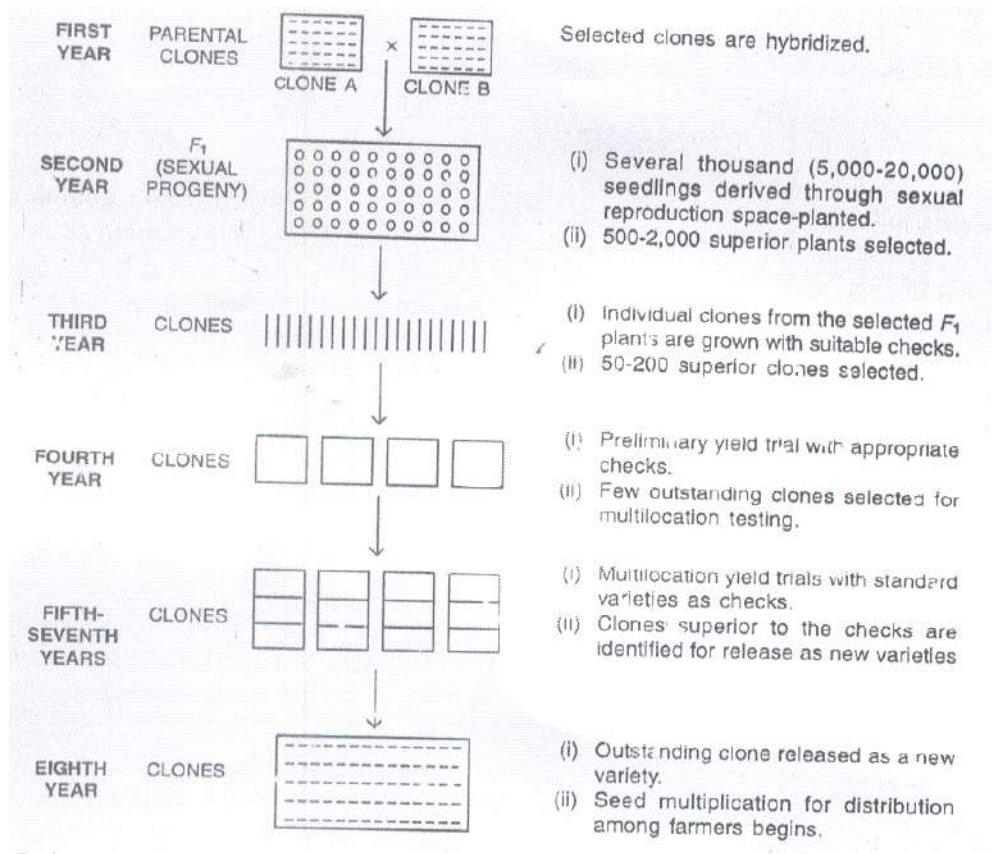


Fig. 30. A generalised scheme for breeding of annual asexually propagated species through hybridization. This figure applies to a crop in which one generation is completed in one year.

Clonal crops are generally improved by crossing two or more desirable clones, followed by selection in the F_1 progeny and in the subsequent clonal generations. Once the F_1 has been produced, the breeding procedure is essentially the same as clonal selection. The improvement through hybridization involves three steps: (1) selection of parents, (2) production of F_1 progeny, and (3) selection of superior clones.

First Year. Clones to be used as parents are grown and crosses are made to produce F_1 progeny.

Second Year. Sexual progeny from the cross, i.e., seedlings obtained from seeds, are grown. Undesirable plants are eliminated. Few hundred to few thousand desirable plants are selected.

Third Year. Clones from individual plants are grown separately. Poor and inferior clones are eliminated. Upto 200 superior clones may be selected for preliminary yield trial.

Fourth Year. A replicated preliminary yield trial is conducted. A suitable check is included for comparison. Few outstanding clones are selected for trials at several locations.

Fifth to Ninth Year. Replicated yield trials are conducted at several locations. A suitable check is included for comparison. One

or a few outstanding clones are identified and released as new varieties.

CHAPTER -IX

MUTATION BREEDING

Mutation is a sudden heritable change in a characteristic of an organism. This definition requires that the change in the characteristic be heritable, but it does not state the genetic basis of the heritable change. Clearly, a mutation (as defined above) may be the result of a change in a gene, a change in chromosome (s) that involves several genes or a change in a *plasma gene* (genes present in the cytoplasm *e.g.*, in chloroplasts, mitochondria *etc.*, which have circular naked DNA as chromosomes). Mutations produced by changes in the base sequences of genes (as a result of base pair ***transition or transversion, deletion, duplication or inversion*** etc). are known as ***gene or point mutations***. Gene mutations can be easily and clearly shown by fine genetic analysis techniques available with microorganisms. Some mutations may be produced by changes in chromosome structure or even in chromosome number; they are termed as ***chromosomal mutations***.

Characteristics 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 a small proportion (ca. 0.1 per cent) of them is beneficial.
3. Mutations are random, *i.e.*, they may occur in any gene. However, some genes show higher mutation rates than others.
4. Mutations are recurrent, that is, the same mutation may occur again and again.
5. Induced mutations commonly show pleiotropy, often due to mutations in closely linked genes.

Types of mutation

1. Spontaneous mutations
2. Induced mutations

Spontaneous Mutations

Mutations occur in natural populations (without any treatment by man) at a low rate; these are known as ***spontaneous mutations***. The frequency of spontaneous mutations are generally one in 10 lakhs, *i.e.*, 10^{-6} .

Induced mutations

Mutations may be artificially induced by the treatment with certain physical or chemical agents. Such mutations are known as induced mutations and the *agents used for producing them are termed as mutagens. The utilization of induced mutations for crop improvement is known as **mutation breeding**.*

Effects of Mutation

Generally, mutations have harmful effects on organisms. Usually they reduce viability of the individuals that carry them. Based on their effect on viability, mutations are classified into four groups: 1) lethal, 2) sublethal, 3) subvital and 4) vital.

1) **Lethal**. Lethal mutations kill each and every individual that carries them in the appropriate genotype.

Dominant lethals cannot be studied because they cannot survive even in the heterozygous state. Thus, of necessity, we have to consider only recessive lethals; that is, genes recessive in their lethal action.

Recessive lethals would kill the individuals that carry them in the homozygous state, e.g., albina chlorophyll mutations.

Sublethal and Subvital. Sublethal and subvital mutations reduce viability, but they do not kill all the individuals carrying

them in the appropriate genotype. **Sublethals** kill more than 50 per cent of the individuals, while **subvitals** kill much less than 50 per cent. A vast majority of mutations are lethals, sublethals and subvitals; these are of no value in crop improvement, although some of them may be of academic interest.

Vital. Vital mutations do not reduce viability of the individuals carrying them. Obviously, crop improvement can utilize only such mutations. Vital mutations occur in a much lower frequency (Ca 0.1% of all the mutations) as compared to the other three types.

Genetic (molecular) basis of mutation

A character of an organism is produced due to proteins (usually enzymes) produced by the genes governing that trait. The properties of the protein depends on the type and the sequence of aminoacids present in that protein molecule which, in turn ,is determined by the base sequence of the gene directing the production of their protein. An aminoacid is encoded by a set of three bases (**codon**); thus the base sequence codons determine the amino acid encoded by them. This brief consideration clearly shows that mutation in a character would generally of that trait. The change in amino acid sequence of the protein determining

expression of that trait. The change in amino acid sequence of the protein would obviously be due to a change in the base sequence of DNA molecule coding for the protein. The change in base sequence of a DNA molecule may occur in any one of the following two ways.

1. Base Substitution

2. Base Addition or Deletion

Analysis of mutations in prokaryotes has shown that both the types of changes occur spontaneously but a surprisingly large proportion of spontaneous mutations are produced by addition or deletion of single base pairs.

Base substitution

When one base in a DNA molecule is replaced by another one it is called base substitution. Base substitutions are of two types.

1. Transition

2. Transversion

Transition

When a purine is replaced by another purine (*eg.*, adenine is replaced by guanine or *vice-versa*) or a pyrimidine is replaced by another pyrimidine (*eg.*, thymine is replaced by cytosine or *vice-versa*), it is known as transition (**Fig.31**).

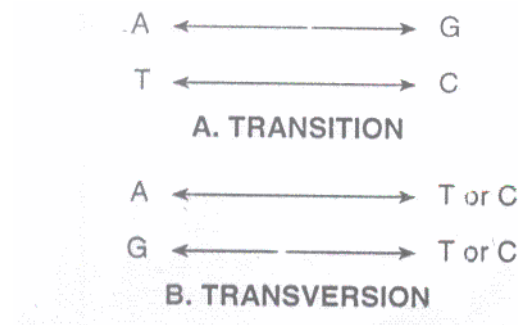


Fig 31. Types of base substitutions involved in A. transition and B. transversion.

Clearly, one base substitution affects the base sequence of only one codon; as a result only one amino acid is altered in the concerned protein.

Transversion

In transversion a pyrimidine(thymine or cytosine)is replaced by a purine(adenine or guanine)and *vice-versa*. Thus there are eight different types of possible transversion (**Fig.31**).Like transition ,one transversion event affects only one codon, and hence replaces only one amino acid in the concerned protein.

Consequently, transitions and transversions are relatively less deleterious. But sometimes ,base substitutions may generate such codons that do not code for any aminoacid(**nonsense codon**).Whenever a nonsense codon is produced, it acts as a terminator of the polypeptide chain. Consequently, the resulting polypeptide does not have the amino acids coded by all the codons

located beyond the nonsense codon. Nonsense mutations are generally more deleterious than other base substitution mutations since the polypeptides produced by such alleles are incomplete and usually totally inactive.

Base addition and deletion

Insertion of one or more bases in a DNA molecule is called base addition, while a loss of one or more bases is known as base deletion. Both addition and deletion lead to mutations. If the number of bases added or lost is a multiple of 3, one to several amino acid would be either added to or deleted from the concerned protein; such a change may or may not have a profound effect on activity of the polypeptide. But if the number of base lost or inserted is not a multiple of 3 ,the base sequences of all the codons beyond the point of insertion or deletion are altered.(Fig. 31).Consequently all the codons beyond this point will now code for a different amino acid than before. Clearly, the reading frame of the subsequent codon is shifted in such mutations; hence they are called as ***frame shift mutations(Fig.32).***

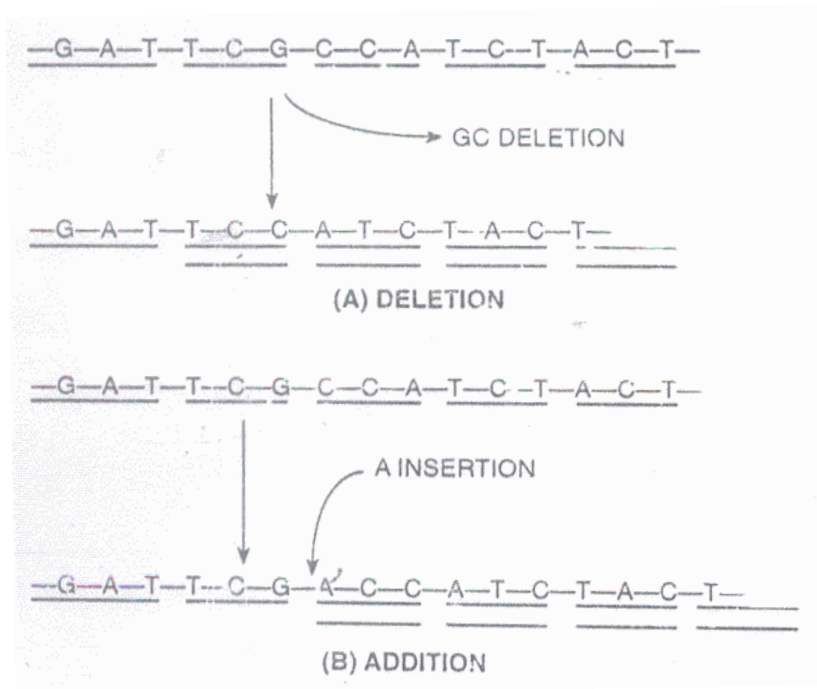


Fig.32. The production of frame-shift mutations due to the addition or deletion of bases in a number other than three or a multiple of three. Bases of codon are indicated by a single liner drawn under them, while the altered codons, due to a shift in the reading frame, are indicated by double lines under them. It may be noted that all the codons subsequent to the point of insertion or deletion are altered.

A frame shift mutation changes all the amino acids of the concerned protein, which are located beyond the point of addition-deletion of the bases. Generally proteins produced by frame shift mutations are non-functional; hence such mutations are much

more deleterious than those produced by base substitutions.
(Except for nonsense mutations).

Mutagens

Agents used for the induction of mutations are known as mutagens. Mutagens may be different kinds of radiations (*physical mutagens*) or certain chemicals (*chemical mutagens*). The different mutagens may be grouped as follows.

A. **Physical mutagens** (all of them are various kinds of radiations)

1. Ionising radiations

(a) Particulate radiations, *eg.*, α -rays (DI), β -rays (SI), fast neutrons* (DI), and thermal neutrons (DI).

(b) Non particulate radiations (electromagnetic radiations), *eg.*, X-rays* (SI), and γ -rays* (SI).

2. Nonionising radiations, *eg.*, ultraviolet radiation (UV).

B. Chemical mutagens

1. Alkylating agents, *eg.*, Sulphur mustards, nitrogen mustards, epoxides, ethylene-imines, (*eg.*, ethylene imine or EI)*, sulphates and Sulphonates (*eg.*, ethylmethane sulphonate or EMS*),

methylmethane sulphonate or MMS), diazoalkanes, nitroso compounds, *eg.*, N-methyl-N-nitro-N-nitroso-guanidine or MNNG).

2. Acridine dyes, *eg.*, acriflavine, proflavine, acridine orange, acridine yellow, ethidium bromide.

3. Base analogues, *eg.*, 5-bromouracil, 5-chlorouracil.

4. Others, *eg.*, nitrous acid, hydroxyl amine, sodium azide*.

Gamma-Garden

Gamma-garden is an area subjected to gamma-irradiation. This area is enclosed by thick-high walls to protect the plants and animals outside this area from radiation damage. The purpose of a gamma-garden is to irradiate whole plants during different stages of development and for varying durations. The source of radiation is located in the centre of gamma-garden, which is usually circular in outline. The intensity of radiation decreases as one move away from the source of radiation. The area around the radiation source is divided into a number of concentric circles representing varying intensities of radiation. Plants are placed in a suitable position in the gamma-garden, which depends upon the growth habit of the species and the intensity of radiation required. The gamma-ray source may also be used for the irradiation of seeds and other propagules.

The first gamma-garden was built in Long Island near New York, U.S.A. The first gamma-garden in India was installed in Calcutta at the Bose Research Institute in 1959. A little later, another gamma-garden was built in New Delhi at the Indian Agricultural Research Institute (IARI) in 1960. A gamma-garden has also been established at the Bhabha Atomic Research Centre, Trombay. The IARI gamma-garden has an area of 3 acres and is surrounded by a wall 12 feet high and 3 feet thick. This Brick wall is, in turn, enclosed with a barbed wire fence. The gamma-ray source consists of 6 g of ^{60}Co in the form of small pellets; ^{60}Co pellets are sealed in an aluminium capsule. The strength of ^{60}Co source is 200 Curies. The aluminium capsule is housed in a lead container, since lead is not penetrated by gamma-rays or X-rays. When irradiation is to be done, the lid of the lead container is lifted; the aluminium capsule containing ^{60}Co pellets is also raised simultaneously. After irradiation, the aluminium capsule is lowered into the lead container and the lid of the container is closed thereby enclosing the aluminium capsule in the lead container. The lead container is opened and closed by a remote control device.

Mutagenesis

Treating the biological material with a mutagen in order to induce mutations is known as ***mutagenesis***. Exposure of a biological material to a radiation like X-rays ,gamma rays etc is known as ***irradiation***. When mutations are induced for crop improvement the entire operation of the induction and isolation of mutants is termed as ***mutation breeding***. A mutation breeding programme should be clearly planned and should be large enough with sufficient facilities to permit an effective screening of large populations.

Objectives of the programme

A mutation breeding programme should have well defined and clear-cut objectives. If the experimenter starts a mutagenesis programme just with the hope that he will discover something useful, he is most likely wasting his time and resources. This is because the ratio of beneficial to useless mutations is very small (1 in 800 mutations, that is, about 0.1 per cent of the mutations) and identifying desirable mutations from among the undesirable ones is a very difficult task indeed .Further, if a character governed by oligogenes is to be improved, the procedure for the handling of

treated population would be different from that when a polygenic trait is the target for improvement.

Selection of the variety for mutagen treatment

Generally, the variety selected for mutagenesis should be the best variety available in the crop. This is particularly so when polygenic traits are to be improved. It serves no purpose to isolate desirable mutants in a less adapted inferior variety only to discover that the mutant lines have no agricultural worth or that the mutants have to be used in a hybridization programme for transferring the mutant characteristics to a superior variety. In certain situations however it may be desirable to isolate mutants in varieties other than the best ones. For example the extensive search is being made for alternative dwarfing genes in cereals particularly in wheat and rice (*O.sativa*). In this situation, dwarf and semi dwarf mutants would have to be isolated from tall varieties which obviously would not be the best varieties of these crops.

Part of the plant to be treated

Seeds , pollen or vegetative propagules (buds and cuttings) or even complete plants may be used for mutagenesis. Which plant part should be used for mutagen treatment depends primarily on whether the crop is sexually or asexually propagated and on the

mutagen to be used. In sexually propagated crops seeds are the most commonly used plant part. Mutagenic treatment of seeds is essentially a treatment of embryo meristems. Since, mutation is a single cell event the M₁ plants will carry an induced mutation only in certain parts of the shoot, *ie.*, they will be **chimeras** .

Dose of the mutagen

The usefulness of a mutagen and the type of treatment required to obtain a high efficiency are dependent upon specific properties of the mutagenic agent employed as well as on specific characteristics of the biological system to be treated. The most appropriate plant part of stage to be treated requires a thorough knowledge of the organisms and a clear definition of experimental objectives.

Mutagens generally induce a high frequency of chromosomal changes and mitotic and meiotic irregularities. Usually the damage increases with the mutagen dose, but it may not necessarily be proportional. *An optimum dose is the one, which produces the maximum frequency of mutations and causes the minimum killing.* The dose required for high mutagenic efficiency depends on the properties of the mutagenic agent of the solvent medium and of the biological system. Many workers feel that a dose close to LD₅₀

should be the optimum. ***LD₅₀ is that dose of a mutagen, which would kill 50 percent of the treated individuals.*** LD₅₀, varies with the crop species and with the mutagen used. A preliminary experiment is generally conducted to determine the suitable mutagen dose. *In general, an overdose is likely to kill too many treated individuals, while an under dose would produce too few mutations.*

Dose of the mutagen may be varied by varying the intensity of the treatment duration. In case of radiation, the intensity may be varied by changing the radiation source or by changing the distance from the radiation source of the material being irradiated. Intensity in the case of chemical mutagens may be varied by changing the concentration of mutagens.

Giving mutagen treatment

The selected plant part is exposed to the desired mutagen dose. In case of irradiation the plant parts are immediately planted to raise M₁ plants from them (pollen grains are used for pollination). In case of chemical mutagens seeds are usually presoaked for a few hours to initiate metabolic activities, exposed to the desired mutagen and then washed in running tap water to remove the mutagen present in them. The treated seeds are usually

immediately planted in the field to raise the M_1 generation. M_1 is the generation produced directly from the mutagen treated plant parts, without recourse to sexual or asexual reproduction. But when pollen grains are treated the generation resulting from the seeds that were produced by pollination with the treated pollen grains would be the M_1 generation. M_2 , M_3 , M_4 etc., are the subsequent generations derived from M_1 , M_2 , M_3 etc., plants through selfing or clonal propagation.

Handling of Mutagen-Treated Population.

Treatment of seeds and vegetative propagules commonly produces *chimeras*. A **chimera** is an individual with one genotype in some of its parts and another genotype in the others. Shoot-tip meristem usually has two functional layers: the outer layer, giving rise to epidermis and a part of leaf mesophyll and the inner layer producing the rest of the plant tissues including reproductive organs. When the entire outer or inner layer is affected, the chimera is known as *periclinal chimera* (inner periclinal or outer periclinal depending upon the layer affected), while in a *sectorial chimera* only a part of the inner or the outer layer is affected (inner sectorial and outer sectorial respectively) **(Fig.33)**.

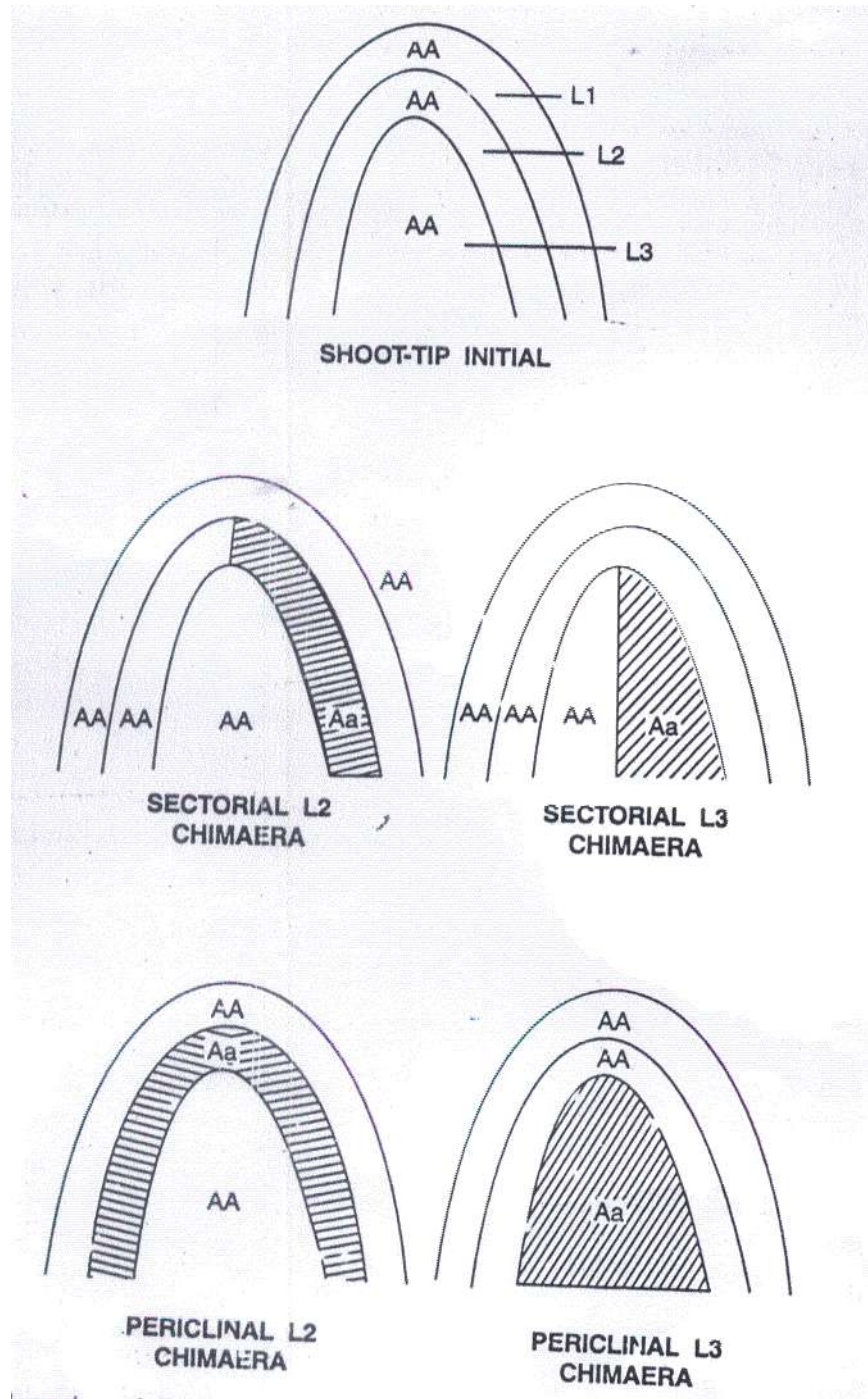


Fig. 33. Sectorial and periclinal chimaeras produced by mutagenesis.

The mutant allele (a) is recessive.

In sexually reproducing species, only the inner chimera (periclinal or sectorial) will be transmitted to the next generation; outer chimeras will not be recovered since this layer does not contribute to the production of gametes. In clonal crops, however, both outer and inner chimeras can be utilized either as periclinal chimeras (outer or inner) or by producing homogeneous individuals through sexual reproduction (only if the inner layer is affected), tissue culture or other horticultural manipulations, *e.g.*, wounding etc., which induce production of adventitious shoot buds (both inner and outer chimeras utilized). Sectorial chimeras are unstable in clonal crops and have to be made periclinal through successive clonal propagation and selection for stability.

Mutations usually occur in small sectors of the meristem and, as a result, only a part of the plant is affected. One or more sexual or clonal generations coupled with selection are necessary to obtain a stable mutant phenotype. Mutant alleles are generally recessive, but some dominant mutations may also occur. In case of sexually reproducing crops, mutation breeding utilizes both recessive and dominant mutations and in addition, excellent opportunities exist for mutation breeding for polygenic traits.

Mutations are called macro or micro mutations depending on the magnitude of phenotypic effect produced by them. A *macro mutation* produces a large phenotypic effect *ie.*, recognizable on individual plant basis; obviously ,such mutations are oligogenic in nature and can be easily selected in the M_2 generation.

In contrast, *micromutation* has a small phenotypic effect that cannot be recognized on individual plant basis; it can be detected in a group of plants and often statistical treatment of data may be necessary. Obviously , *macromutations* are polygenic in nature and selection for them is delayed till M_3 or a later generation.

The following discussions are based on sexually reproducing species, more particularly, self-pollinated species. Since *dominant mutations* are able to express themselves in heterozygous state, mutant plants are selected in M_1 and often in M_2 . In M_2 and M_3 individual plant progenies are raised and homozygous mutants are selected. Selection for *recessive mutations* however, can be taken up in M_2 only, but the mutant allele will be homozygous in the M_2 itself. Selection for polygenic traits is delayed till M_3 generation, and is based on individual plant progenies rather than on individual plants. A generalized scheme for handling the mutagen-

treated populations for oligogenic and polygenic traits is outlined below.

Mutation Breeding for Oligogenic Traits.

The handling procedure described here (**Fig.34**) is based on the selection for a recessive mutant allele.

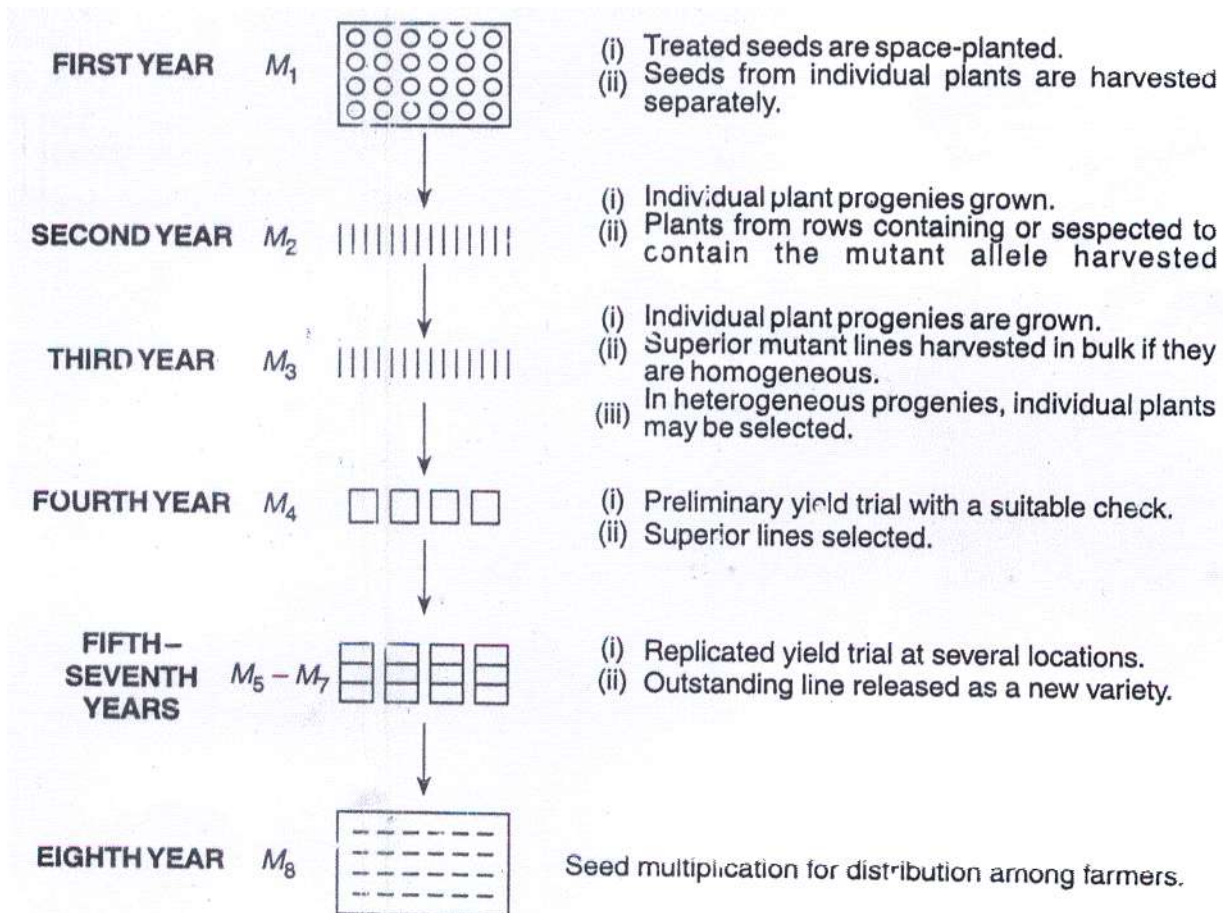


Fig. 34. A generalised scheme for mutation breeding for an oligogenic trait, the mutant allele recessive.

1. **M₁**. Several hundred (500 or more) seeds are treated with a mutagen and are space-planted. M₁ plants will be chimeras for the mutations present in heterozygous state. About 20 seeds from each M₁ spike (in case of cereals) or plant are harvested separately to raise the M₁ progeny rows.

2. **M₂**. About 2,000 progeny rows are grown. Careful and regular observations are made on the M₁ rows. But only distinct mutations are detected in M₂ because the observations are based on single plants. All the plants in M₂ rows suspected of containing new mutations are harvested separately to raise individual plant progenies in M₂. If the mutant is distinct, it is selected for multiplication and testing. However, most of the mutations will be useless for crop improvement. Only 1-3 per cent of M₂ rows may be expected to have beneficial mutations.

3. **M₃**. Progeny rows from individual selected plants are grown in M₁. Poor and inferior mutant rows are eliminated. If the mutant progenies are homogeneous, two or more M₁ progenies containing the same mutation may be bulked. Mutant M₁ rows are harvested in bulk for a preliminary yield trial in M₄.

4. **M₄** preliminary yield trial is conducted with a suitable check, and promising mutant lines are selected for replicated multilocation trials.

5. **M₅-M₈**. Replicated multi-location yield trials are conducted. The outstanding line may be released as a new variety.

Mutation Breeding for polygenic Traits(Fig.35).

Mutagenesis does produce genetic variation in polygenic traits. This variation is usually as much as 50% of that generated in F₂ generation, but sometimes it may be as much as or even greater than the latter. But often irradiation leads to a decline in the mean of M₂ populations. But some M₁ families may be superior to the unirradiated parents or the concerned F₁ populations.

A generalized scheme for the improvement of polygenic traits through mutation breeding is outlined below **(Fig.35)**

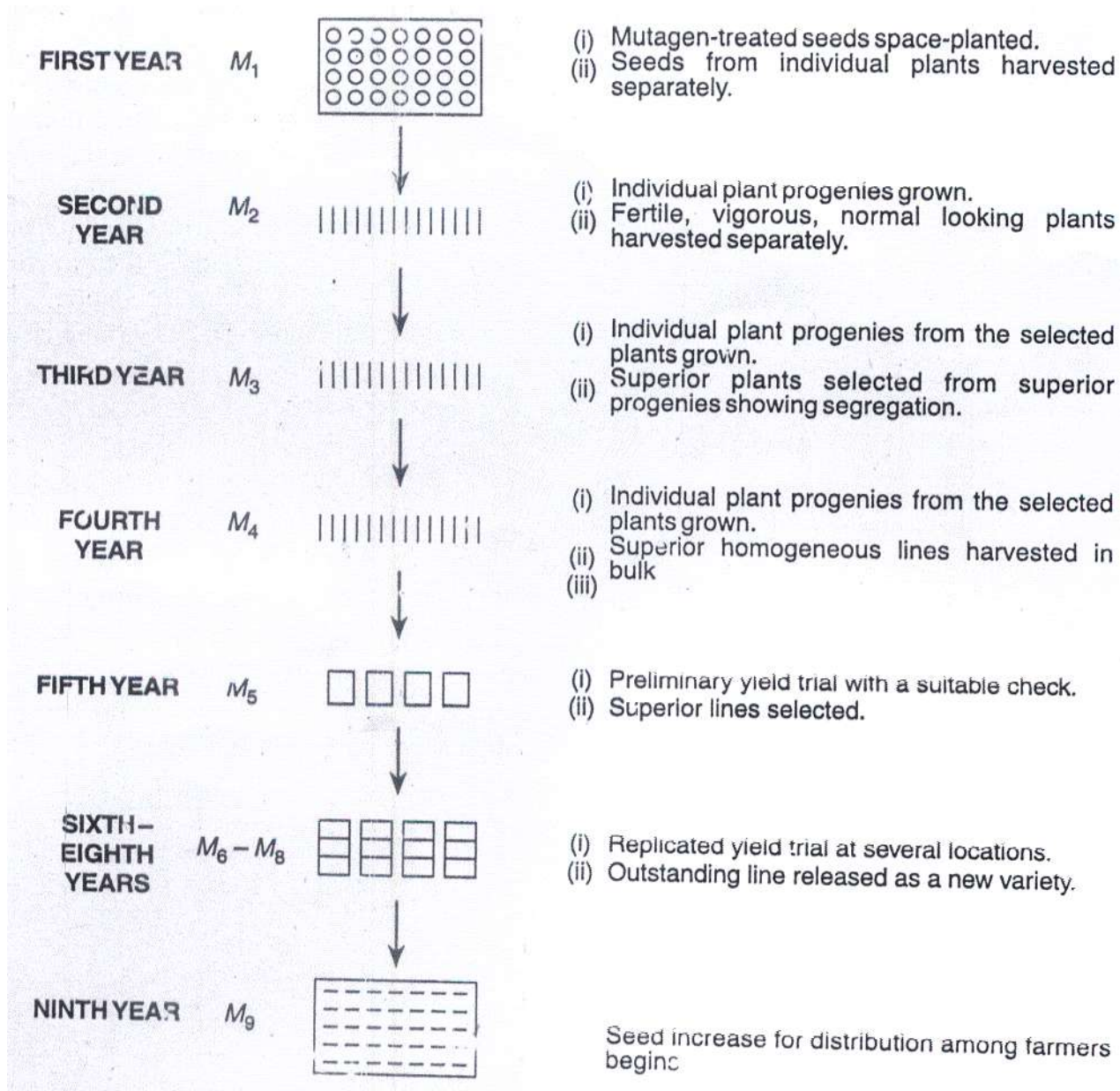


Fig. 35. A generalised scheme for mutation breeding for an oligogenic trait, the mutant allele recessive.

M_1 and M_2 . M_1 and M_2 are grown in the same way as in the case of oligogenic traits. In M_2 , vigorous, fertile and normal looking plants that do not exhibit a mutant phenotype are selected and

their seeds are harvested and separated to raise individual plant progeny rows in M_3 .

M₃.progeny rows from individual selected plants are grown.careful observations are made on M_3 rows for small deviations in phenotype from the parent variety. Inferior rows are discarded .Few rows may be homogenous and would be harvested in bulk. Selection is done in M_3 rows showing segregation; a majority of M_3 rows would show segregation. Intensive and careful evaluation of a large number (say 1000) of M_3 progeny rows allows identification of mutants with altered quantitative traits. *e.g.*, partial or horizontal disease resistance .Such mutants occur in high frequencies that approach 1% or even higher, so that their isolation becomes quite cost effective.

M₄.bulked seed from homogeneous M_3 rows may be planted in a preliminary yield trial with a suitable check; superior progenies are selected for replicated multilocation yield trials. Individual plant progenies from selected M_3 plants are critically observed. Progenies showing segregation may be subjected to selection only if they are promising. Superior homogeneous progenies are harvested in bulk for preliminary yield tests in M_5 .

M₅ to M₈.Preliminary yield trials and /or multilocation 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. It has been employed to improve morphological and physiological characters, disease resistance and quantitative characters including yielding ability. The various applications of mutation breeding may be briefly summarized as under.

i) Induction of desirable mutant alleles which may not be present in the germplasm or which may be present but may not be available to the breeder due to political or geographical reasons. To some extents mutation breeding relieves the complete dependence of breeders on the natural germplasm. But it should be remembered that mutation breeding cannot minimize the necessity of germplasm collections; it only serves as a useful supplement to the available germplasm.

ii) It is useful in improving specific characteristics of a well adapted high yielding variety. This is particularly so in the case of

clonal crops due to their highly heterozygous nature; in such a case, mutagenesis is the only method available to improve the specific characteristics of clones without changing their genetic make up.

In self-pollinated species, mutagenesis is useful in improving the specific characteristics of otherwise adapted and superior varieties. However, in such species mutagenesis may not be simpler or quicker than the standard backcross procedure, if the characteristic is available in a variety. This is more so because the desirable mutations are often associated with undesirable side effects due to other mutations, chromosomal aberrations, sterility *etc.* One or few backcrosses with the parent variety may be necessary to bring the desirable mutant allele in an acceptable genetic background

iii) Mutagenesis has been successfully used to improve various quantitative characters, including yield. Several varieties have been developed by this technique. However, there is no critical comparison available to show that the same improvement would not have been brought about by the conventional hybridization programmes.

IV) F_1 hybrids from intervarietal crosses may be treated with mutagens in order to increase genetic variability by inducing mutations and to facilitate recombination among linked genes. There is some evidence that mutagen treatment of the F_1 gives a relatively wider spread (in frequency distribution) in the F_2 . Some of the additional variability in F_2 is undoubtedly due to an enhanced recombination among linked genes. But this method has not been widely used.

V) Irradiation of interspecific (distant) hybrids has been done to produce translocations. This is done to transfer a chromosome segment carrying a desirable gene from the alien chromosome to the chromosome of a cultivated species. This illustrates another application of irradiations in crop improvement, *but this does not constitute mutation breeding*.

Achievements

More than 337 varieties have been produced as a result of mutagenesis programmes in different countries of the world. Mutant varieties have been developed in cereals, oilseeds, pulses, millets, vegetables, fruit trees etc., but wheat, barley and rice account for about 50% of the mutant varieties in all the crops. These varieties represent improvements in oligogenic as well as polygenic characteristics. These crop varieties belong to diploid and

polyploid sexually reproducing species as well as to clonal crops. The first variety developed from a mutagenesis programme was Primax White Mustard (*Brassica hirta*) released in 1950, followed by Regina II Summar Rape (*B. campestris*) in 1953. Both the varieties were developed at the Slav of station of the Swedish Seed Association. They represented a small but consistent increase in yield and oil content over the parent variety and it took 10-13 years to develop them. Further, there is some doubt that the improvements in these two varieties were due to irradiation since both the crops (mustard and rape) show a high degree of cross-pollination.

Sugarcane- variety Co 8152 is a gamma-ray induced mutant from Co 527. Co 8152 gives 40 per cent higher cane yield than the parent variety, but it has an apparently undesirable feature.

Three mutant cotton varieties have been released for commercial cultivation; two of these (MCU 7 and MCU 10) were developed through induced mutations, while one (DB-3-12) is a spontaneous mutant. MCU 7 was isolated from culture 1143 EE; it is early maturing (145 days), has medium staple length and is suitable for cultivation in rice fallow areas of Tamil Nadu. MCU 10 is a mutant from MCU 4 induced by 30 KR gamma-rays; it performs better than the parent variety (MUC 4) under rainfed conditions. DB-3-12 is a spontaneous mutant from the G.

herbaceum variety Western 1; it has a better plant type and matures 20 days earlier than the parent variety.

CHAPTER -X

POLYPLOID BREEDING

Polyploids

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

The terminology of heteroploidy is summarised in Table 2

Sl.No	Term	Type of change	Symbol*
1	Heteroploid	A change from $2x$	-
A.	Aneuploid	One of a few chromosomes extra or missing from $2n$	$2n \pm \text{few}$
	nullisomic	One chromosome pair missing	$2n-2$
	Monosomic	One chromosome missing	$2n-1$
	Double Monosomic	One chromosome from each of two different chromosome pairs missing	$2n-1-1$
	Trisomic	One chromosome extra	$2n+1$
	Double Trisomic	One chromosome from each of two different chromosome pairs extra	$2n+1+1$
	Tetrasomic	One chromosome pair extra	
B.	Euploid	Number of genomes or copies of a genome more than two	
	Monoploid	One copy of a single genome	x
	Haploid	Gametic chromosome complement	n
	polyploid	More than 2copies of one genome or 2 copies each of 2 or more genomes**	
a.	<i>Autopolyploid</i>	Genomes identical with each other	
	Autotriploid	Three copies of one genome	$3x$
	Autotetraploid	Four copies of one genome	$4x$
	Autopentaploid	Five copies of one genome	$5x$
	Autohexaploid	Six copies of one genome	$6x$
	Autooctoploid	Eight copies of one genome	$8x$
b.	<i>Allopolyploid</i>	Two or more distinct genomes(generally each genome has two copies)**	
	Allotetraploid	Two distinct genomes	$(2x_1+ 2x_2)^{**}$
	Allohexaploid	Three distinct genomes	$(2x_1+ 2x_2+2x_3)^{**}$
	Allooctoploid	Four distinct genomes	$(2x_1+ 2x_2+2x_3+2x_4)^{**}$

Table 2. $2n$ = somatic chromosome number n =gametic chromosome number x =basic chromosome number or (genome) or (chromosome complement) x_1, x_2, x_3, x_4 =distinct genomes from different species

Individuals carrying chromosome numbers other than the diploid ($2x$ and not $2n$) number are known as heteroploids, and the situation is known as heteroploidy. The change in chromosome number may involve one or a few chromosomes of the genome; this is known as **aneuploidy**. *The aneuploid changes are determined in relation to the somatic chromosome number ($2n$ and not $2x$) of the species in question.* Therefore, the terminology for aneuploid individuals arising from diploid and polyploid species is the same. Heteroploidy that involves one or more complete genomes is known as **euploidy**. By definition, therefore, the chromosome number of euploids is an exact multiple of the basic chromosome number of the species, while that of aneuploids is not.

Aneuploid individuals from which one chromosome pair is missing ($2n - 2$) are termed as *nullisomics*, while those lacking a single chromosome ($2n - 1$) are known as *monosomics*. A *double monosomic* individual has two chromosomes missing, but the two chromosomes belong to two different chromosome pairs ($2n - 1 - 1$). An individual having one extra chromosome ($2n + 1$) is known as a *trisomic*, and that having two extra chromosomes each belonging to a different chromosome pair is *double trisomic* ($2n + 1 + 1$). When an

individual has an extra pair of chromosomes, it is known as *tetrasomic*($2n+2$).

In *euploids*, the chromosome number is an exact multiple of the basic or genomic number. Euploidy is more commonly known as *polyploidy*. When all the genomes present in a *polyploid species* are identical, it is known as *autopolyploid* and the situation is termed as *autopolyploidy*. In the case of *allopolyploids*, *two or more distinct genomes are present*. Euploids may have 3 (*triploid*), 4 (*tetraploid*), 5 (*pentaploid*), 6 (*hexaploid*), 7 (*heptaploid*), 8 (*octaploid*) or more genomes making up their somatic chromosome number. In case of *autopolyploidy*, they are known as *autotriploid*, *autotetraploid*, *autopentaploid*, *autohexaploid*, *autoheptaploid*, *autooctaploid* and so on, while in the case of *allopolyploidy* they are termed as *allotriploid*, *ailotetraploid*, *allopentaploid*, *allohexaploid*, *alloheptaploid*, *allooctaploid etc.*

Amphidiploid is an *allopolyploid* that has two copies of each genome present in it, and as a consequence behaves as a *diploid* during *meiosis*. A *segmental allopolyploid* contains two or more genomes which are identical with each other, except for some minor differences.

Origin and Production of Aneuploids

Spontaneous

Aneuploids originate spontaneously at a low frequency. The earlier cases of aneuploidy were produced spontaneously in experimental populations. Meiotic irregularities lead to the formation of $n+1$ and $n-1$ gametes, *e.g.*, in *Datura* about 0.4 per cent of pollen is likely to be $n+1$.

Triploid Plants

The best sources of aneuploids are triploid plants. Distribution of chromosomes at the first meiotic anaphase is irregular leading to the production of a whole range of aneuploids in the progeny.

Asynaptic And Desynaptic Plants

In asynaptic and desynaptic plants, few to all chromosomes are present as univalents at metaphase I of meiosis. In the progeny of such plants, a relatively high frequency of aneuploids occurs.

Translocation Heterozygotes

A 3: 1 disjunction of the ring or the chain of four chromosomes in a translocation heterozygote would produce one $n + 1$ and one $n - 1$ gamete. As a result, in the progeny of

translocation heterozygotes a variable frequency of aneuploids is found.

Tetrasomic Plants

Tetrasomic ($2n + 2$) plants would produce $n + 1$ gametes in considerable frequencies. Therefore, when are crossed with normal diploid or *disomic* ($2n$) plants, they produce a high frequency of trisomics. Where possible, tetrasomics may be maintained for the production of trisomics.

Applications of Aneuploids in Crop Improvement

1. Aneuploids are useful in studies on the effects of loss or gain of an entire chromosome arm on the phenotype of an individual. Their study has clearly demonstrated that character expression is governed by a balance between a large number of genes present in the genome, that is, loss or gain of chromatin upsets the normal development.

2. Aneuploids are useful in locating a linkage group and a gene to a particular chromosome. By using a secondary or tertiary trisomic, the gene may be located to one of the two arms of a chromosome, or even to a part of the chromosome arm. The most

important application of aneuploids is in locating genes on particular chromosomes; this will be considered in some detail.

3. Study of aneuploids has shown the homoeology between A, B and D genomes of wheat (*T. aestivum*), since a chromosome of A genome would compensate for the loss of the corresponding chromosome from genome B or D. For example, tetrasomic condition of 2B compensates for the nullisomic condition of 2A or 2D so that a tetra-2A nulli-2B or 2D appears normal.

4. Aneuploids are useful in identifying the chromosomes involved in translocations.

5. They are useful in the production of substitution lines. Chromosome substitution may be desirable for studying the effects of individual chromosomes of a variety or for the transfer of the genes carried by specific chromosomes of a variety into another one.

Euploids

Autopolyploidy

In autopolyploidy are included monoploidy, triploidy, tetraploidy and higher levels of ploidy. Production of monoploids and haploids is entirely different from that of other ploidy levels and shall be considered separately. Other cases of autopolyploidy are produced directly or indirectly through chromosome doubling which is briefly considered below. It may be pointed out that, strictly speaking, monoploidy and haploidy do not constitute polyploidy. But in view of their specialized application in crop improvement, they are included here for discussion.

Origin and Production (*Induction*) of Autopolyploids

Cells/individuals having doubled chromosome numbers may originate in one of the following several ways: (1) spontaneous, (2) due to treatment with physical agents, (3) regeneration in vitro, (4) colchicine treatment, and (5) other chemical agents.

Spontaneous

Chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are also produced in low frequencies. Production of unreduced gametes is promoted by certain genes, e.g., genes causing complete asynapsis or desynapsis.

Production of Adventitious Buds

Decapitation in some plants leads to callus development at the cut end of stem. Such a callus has some polyploid cells, and some of the shoot buds regenerated from the callus may be polyploid, is of common occurrence in *Solanaceae* where 6-36 per cent of adventitious buds are reported to be tetraploid. The frequency of polyploid buds may be increased by the application of 1 per cent IAA at the cut end as it favours callus development.

Physical Agents

Heat or cold treatments, centrifugation and X-ray or gamma-ray irradiation may produce polyploids in low frequencies. Tetraploid branches were produced in *Datura* in response to cold treatment. Exposure of maize (*Z. mays*) plants or ears to a temperature of 38-45°C at the time of the first division of zygote produces 2-5 per cent tetraploid progeny. Heat treatment has been

successfully used in barley (*H. vulgare*), wheat (*T. aestivum*), rye (*S. cereale*) and some other crop species.

Regeneration in Vitro

Polyploidy is a common feature of the cells during in vitro culture. Some of the plants regenerated from callus and suspension cultures may be polyploids. Plants of various ploidy have been regenerated from callus cultures of *Nicotiana*, *Datura*, rice (*O. sativa*) and several other species.

Colchicine Treatment

Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling. It has been used with great success in a large number of crop species belonging to both dicot and monocot groups. Colchicine is a poisonous chemical isolated from seeds (0.2-0.8%) and bulbs (0.1-0.5% of autumn crocus (*Colchicum autumnale*)). It is readily soluble in alcohol, chloroform or cold water, but is relatively less soluble in hot water. Pure colchicine is $C_{22}H_{25}O_6N$. It blocks spindle formation and thus inhibits the movement of sister chromatids to the opposite poles. The resulting restitution nucleus includes all the chromatids; as a result, the chromosome number of the cell is doubled. Since

colchicine affects only dividing cells, it should be applied when the tissues are actively dividing. At any given time, only a small proportion of cells would be in division, hence repeated treatments should be given at brief intervals to double the chromosome number in a large number of cells of the shoot apex. The polyploid and diploid cells present in a shoot-tip compete with each other and diploid cells may often out compete the polyploid ones. The degree of competition varies from species to species and even among varieties within species.

The method of colchicine application varies considerably. Colchicine is usually applied as an aqueous solution, but it is relatively unstable in an aqueous solution. Therefore, it is important to use freshly prepared aqueous solutions of colchicine.

Morphological features of polyploids vary to some extent from one species to the other. Some general features are summarized below.

1. Polyploids have larger cell size than diploids. Guard cells of stomata are larger, and the number of stomata per unit area is lower in polyploids than in 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 the 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 an increase in general vigour and vegetative growth. But in some cases, polyploids are smaller and weaker.

7. Different species have different levels of optimum ploidy. For sugarbeet (*Beta vulgaris*), the optimum level is $3x$, while for timothy grass (*Phleum pratense*) it is between $8-10x$.

8. Autopolyploids generally have lower dry matter content than diploids. As a result, the increased size of many polyploids may not represent an increased dry matter production, e.g., tetraploid turnip (*Brassica rapa*) and cabbage (*B. oleracea*).

Application of Autopolyploidy in Crop Improvement

Autopolyploidy has found some valuable applications in crop improvement. These are briefly summarized below.

Monoploids and Haploids

Monoploids are weaker than diploids and are of little agricultural value directly. But they, are of great interest because they, offer certain unique opportunities in crop improvement.

(1) They are used for developing homozygous diploid lines, following chromosome doubling in two years. This greatly reduces the time and labour required for the isolation of inbreds and purelines.

(2) They may be useful in the isolation of mutants because the mutant allele (even if it is recessive) expresses itself in M_1 due to a single dose of the gene in somatic tissues. Chromosome number of mutants may be doubled to produce homozygous mutant lines in a single generation.

(3) Since desirable gametes are more frequent (p) than desirable zygotes (p^2), selection based on haploids or doubled

haploids may be expected to be more efficient than that based on diploid (zygote-derived). There is some evidence that this may be so.

(4) In autotetraploids like potato, breeding is relatively much easier at the haploid ($2x$) level than at the tetraploid level ($4x$). For comparison, consider segregation in an autotetraploid and in a diploid. There is an increasing tendency to breed potato varieties at the haploid level and then double their chromosome number to obtain tetraploid varieties.

Monoploids and haploids occur spontaneously (*e.g.*, *maize*), in low frequencies, may be induced from pollen grains through callus formation or embryo production and by chromosome elimination in certain interspecific crosses, *e.g.*, *Hordeum bulbosum* x *H. vulgare*. In the first method, the recovery of haploids is generally very low (1 in 1,000 plants or lower). But the latter two methods produce a relatively high frequency of haploids in case of those species for which appropriate techniques are available. It may be pointed out that the latter two methods are not applicable to many crop species as yet.

Triploids

Triploids are produced by hybridization between tetraploid and diploid strains. They are generally highly sterile, except in a few cases. This feature is useful in the production of seedless watermelons. In certain species, they may be more vigorous than the normal diploids, *e.g.*, in sugarbeets. These two examples are described in some detail.

Seedless watermelons are grown commercially in Japan. They are produced by crossing tetraploid ($4x$, used as female) and diploid ($2x$, used as male) lines, since the reciprocal cross ($2x \times 4x$) is not successful. The triploid plants do not produce true seeds; almost all the seeds are small, white rudimentary structures like cucumber (*Cucumis sativus*) seeds. But a few normal sized seeds may occur, which are generally empty. For good fruit setting, pollination is essential. For this purpose, diploid lines are planted in the ratio of 1 diploid: 5 triploid plants. There are several problems, *viz.*, genetic instability of $4x$ lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production (by hand-pollination).

Triploid sugarbeets (*B. vulgaris*) produce larger roots and more sugar per unit area than diploids, while tetraploids produce smaller roots and lower yields than diploids. Apparently, $3x$ is the optimum level of ploidy in sugarbeets. Triploid sugarbeet varieties have been grown commercially in Europe and Japan, but their popularity is declining rapidly. The triploid varieties are mixtures of triploid, diploid and other ploidy level plants. Seed production of triploid sugarbeet is difficult because the beet flower is small. Triploid seed may be produced in one of the following two ways: (1) using $4x$ plants as female and $2x$ as male or (2) using $4x$ as male and $2x$ as female. The first combination gives lower seed yield but a higher proportion of triploids, while the second gives a higher seed yield but a lower proportion of triploids. Commercial triploid sugarbeet seed is produced by interplanting $4x$ and $2x$ lines in the ratio of 3: 1, and seed from both $4x$ and $2x$ plants is harvested. This seed consists of about 75% triploid ($3x$) seeds. Triploid sugarbeet may give 10-15 per cent higher yields than diploids.

Tetraploids

Autotetraploids have been produced in a large number of crop species and have been extensively studied in several cases. Tetraploids may be useful in one of the following ways: in breeding, improving quality, overcoming self-incompatibility, making distant crosses and used directly as varieties.

In banana (*M. sapientum*), autotetraploids are inferior to triploids in that they have weaker leaves and increased fertility. But they offer the only available chance of adding disease resistance to commercially successful varieties. In banana, autoteraploids are produced by chance fertilization of an unreduced triploid egg (AAA) by a haploid pollen from a disease resistant diploid. A large number of such tetraploids have been produced, but they have not yet gained any commercial success. This is an unusual case where the *autotetraploidy is the only practical approach to breeding an otherwise successful triploid crop species.*

Some autotetraploids may be *superior in some quality characters* to their respective diploids, *e.g.*, tetraploid maize has 43% more carotenoid pigment and vitamin A activity than the diploid. Some teraploids may be hardier than diploids. However, it

is impossible to predict the performance of tetraploids, and a superior diploid may not necessarily produce a superior tetraploid. The tetraploids may be superior, inferior or comparable to the corresponding diploids in quality and hardness; the actual response has to be determined experimentally.

Autotetraploidy is able to overcome self-incompatibility in certain cases, e.g., some genotypes of tobacco (*Nicotiana sp.*) and white clover (*Trifolium repens*), Petunia, etc. Certain distant crosses are not successful at the diploid level, but are relatively successful at the autotetraploid level, e.g., $4x$ *Brassica oleracea* x *B. chinensis* is successful, but when *B. oleracea* is diploid it is unsuccessful. Similarly, autotetraploids of certain *Solanum* species produce hybrids with *S. tuberosum* while diploids do not.

Autotetraploids are larger in size and are more vigorous than diploids. Autotetraploid varieties of forage crops have been considerably successful. The most successful examples are, tetraploid red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). Other examples are: tetraploids of alsike cover (*Trifolium hybridum*, Variety Tetra) and berseem (*Trifolium alexandrinum*, variety Pusa Giant Berseem). Autotetraploid red clover and

ryegrass are more vigorous, digestible and palatable, and have greater resistance to nematodes as compared to the diploids. Autotetraploid turnips (*B. rapa*) and cabbages (*B. oleracea*) are larger in size, but they also have more water content than the diploids; thus they are not commercially attractive. Many ornamentals are autotetraploids. In cases of ornamentals, increased flower size, and longer flowering duration of the tetraploids are desirable.

Some autotetraploid varieties of medicinal plants have been released and adopted. Variety HMT - I of *Hyoscyamus niger* is an autotetraploid; it gives 15% more biomass and 36% greater crude drug yield than the parent. Similarly, Sugandha is an autotetraploid variety of vetiver (*Vetiveria zizanoides*); it gives 11 % more oil yield than the control.

In case of crops where seed is the commercial product, autotetraploidy has been much less successful. The chief difficulty is the high sterility and genetic instability of autotetraploids. Fertility can be improved through breeding and selection, but the progress is slow. Autotetraploids have been explored in several crop species but the most successful case is that of rye (*S. cereale*)

where tetraploid varieties have been released for cultivation (*eg.*, Double steel, Tetra Petkus). Other extensive programmes on autotetraploidy are on crops like barley (*H. vulgare*) and jowar (*S. bieolor*) where larger grains, increased protein content and higher yields are the objective. After many years of extensive breeding, some success in achieving these goals has been realised. Some generalizations may be made about autopolyploidy.

1. Autopolyploidy is more likely to succeed in species with lower chromosome numbers than in those with higher chromosome numbers.
2. Cross pollinating species are generally more responsive than self-pollinating species.
3. Crops grown for vegetative parts are more likely to succeed as polyploids than those grown for seeds.

Allopolyploidy

Allopolyploids have genomes from two or more species. Several of our crop plants are allopolyploids. Production of allopolyploids has attracted considerable attention; the aim almost always was the creation of new species. Some success has been obtained as is evident from the emergence of Triticale as a new

crop species in some areas, and the promise shown by some other allopolyploids, e.g., *Raphanobrassica* and some allopolyploids of forage grasses.

Origin and Production of Allopolyploids

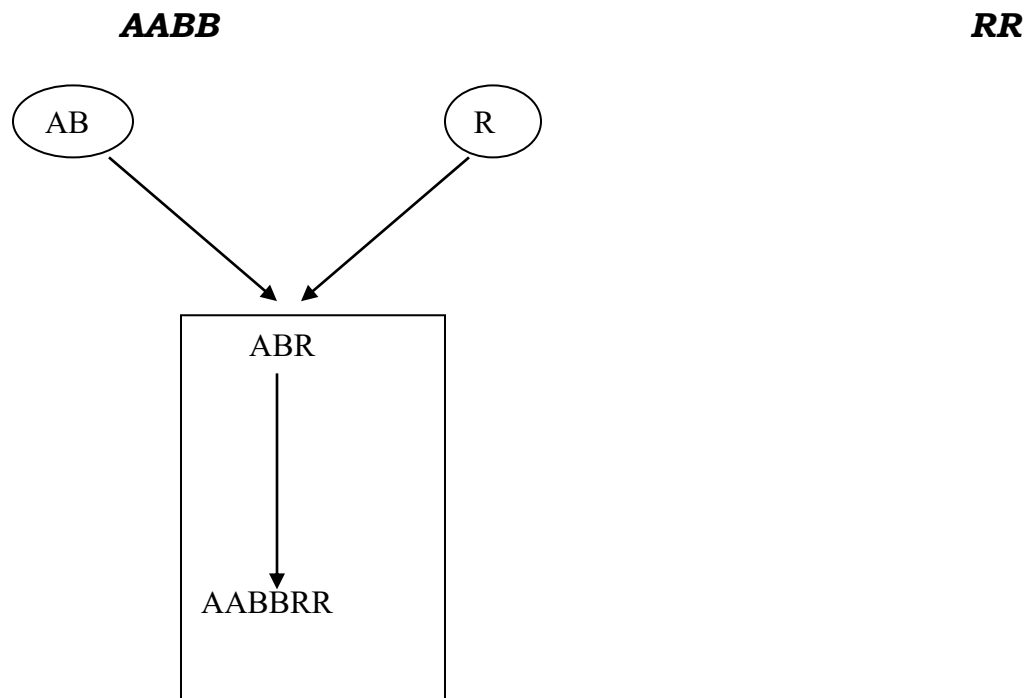
The present day allopolyploids were most likely produced by chromosome doubling in F_1 hybrids between two distinct species (distant hybrids) belonging to the same genus or to different genera. Chromosome doubling might have occurred in somatic tissues due to an irregular mitotic cell division leading to the formation of allopolyploid sectors either in the apical meristem or in the axillary buds; the latter would produce allopolyploid branches. Sexual progeny from such branches would be allopolyploids. Alternatively, irregular meiosis may lead to the production of unreduced gametes which may unite to produce allopolyploid progeny. A complete failure of pairing may be followed by the inclusion of all the chromosomes in a single nucleus at telophase I. This would lead to the production of two unreduced gametes after telophase II.

In experimentally produced distant hybrids, both the processes generating allopolyploidy have been observed. In many distant hybrids that were highly sterile, occasional branches were highly fertile and had twice as many chromosomes as those present

in the F₁ hybrid, *e.g.*, F₁ from the cross *Primula verticillata* x *P. floribunda* produced a fertile branch with twice the chromosome number. The progeny obtained from this fertile branch were allotetraploid and were named as *P. kewensis*. In many other distant hybrids, occasional unreduced gametes are produced which unite to give rise to allopolyploid progeny. The allotetraploid Raphanobrassica was obtained in this manner; the F₁ from the cross *B. oleracea* (cabbage) x *Raphanus sativus* (radish) was almost completely sterile, but produced a few seeds that gave rise to the allotetraploid Raphanobrassica.

Experimental production of allopolyploids is achieved by doubling the chromosome number of distant hybrids with the help of colchicine or some other agent. The allopolyploids produced by man are often termed as synthetic allopolyploids because they are produced (or so to say synthesized) by man from two distinct species of plants. Thus the production of an allopolyploid involves two steps: (1) production of F₁ distant hybrid, and (2) chromosome doubling (Fig. PAGE 650--). Many distant crosses not possible previously have been successfully made with the help of in vitro techniques. Chromosome doubling does not appear to be a serious problem in most of the cases since most of the plant species

respond to colchicine. Some species may respond poorly to colchicine; in such cases other agents available for chromosome doubling may be useful.



Evolutionary significance of Allopolyploids

1) ***Evolution of Nicotiana tabacum.***

N. tabacum ($n = 24$) is most likely an *amphidiploid* from the

cross *N. sylvestris* X *N. tomentosa*; both the species are diploid with $n = 12$. The Interspecific hybrids *N. tabacum* x *N. sylvestris* and *N. tabacum* x *N. tomentosa* produce 12 II and 12 I at metaphase 1. This indicates a homology between Chromosomes of *tabacum* and those of *sylvestris* and *tomentosa*. The amphidiploid from the cross *N. sylvestris* X *N. tomentosa* is similar to *N. tabacum* in many characteristics, which further supports the above conclusion. The species *N. tabacum* has undergone considerable differentiation during its evolutionary history, mostly due to the accumulation of gene mutations and, to some extent, due to the loss of some duplicated segments of the two genomes.

Evolution of *Gossypium hirsutum*

The 9 old World and the 8 New World species of *Gossypium* have $n = 13$, but the chromosomes of the New World species are smaller than those of the Old World species. The other species, *G. hirsutum*, *G. barbadense* and *G. tomentosum* (wild Hawaii cotton), have $n = 26$; in these species, 13 chromosomes are relatively larger than the remaining 13. A possible origin of *G. hirsutum* is from the cross between Asiatic cotton *G. arboreum* x *G. thurberi* (American wild cotton), followed by chromosome doubling of the interspecific

hybrid. According to a more recent scheme, *G. hirsutum* has originated from the cross *G. herbaceum* var. *africanum* x *G. raimondii*, followed by chromosome doubling of the F_1 .

Evolution of Amphidiploid *Brassica* Species

According to this scheme, *B. juncea* ($n = 18$) is an amphidiploid from *B. nigra* ($n = 8$) x *B. campestris* ($n = 10$); *B. napus* ($n = 19$) is an amphidiploid from the cross *B. oleracea* ($n = 9$) x *B. campestris* ($n = 10$), and *B. carinata* ($n = 17$) is an amphidiploid from the cross *B. nigra* ($n = 8$) x *B. oleracea* ($n = 9$). The synthetic allopolyploids produced according to the above scheme resemble the natural amphidiploids, cross easily with them, and the hybrids between the synthetic and natural amphidiploids are reasonably fertile.

Applications of Allopolyploids in Crop Improvement

Allopolyploid has three major applications in crop improvement: as bridging species in the transfer of characters from one species into another, in the production of new crop species, and in widening the genetic base of existing allopolyploid crop species.

Utilization as a Bridging Species

Amphidiploids serve as a bridge in the transfer of characters from one species to a related species, generally from wild species to cultivated species. The use of an amphidiploid as a bridging species becomes necessary when the hybrid between the cultivated species (recipient species) and the wild species (donor species) is sterile. The sterility of F_1 hybrid makes it impossible to cross the recipient species with the F_1 and this does not permit the transfer of characters from the donor to the recipient species. In such cases, chromosome number of the F_1 interspecific hybrid is doubled to produce an amphidiploid which is generally reasonably fertile and can be crossed to the recipient species. Progeny from the cross between the recipient species and the amphidiploid would have the somatic ($2n$) chromosome complement of the recipient species and one genome from the donor species. As a result, they would be sufficiently fertile to be used in backcross with the recipient species. From such a programme, alien addition and alien substitution lines are recovered, which are used in the transfer of genes, groups of genes or of small chromosome segments to the recipient species .

An example of the use of an amphidiploid as a **bridging species** is the use of synthetic *N. digluta* (allohexaploid) for the transfer of resistance to tobacco mosaic virus from *N. sylvestris* to *N. tabacum*. The F_1 hybrid from the cross *N. tabacum* x *N. sylvestris* is sterile. Chromosome doubling of the F_1 hybrid produces the synthetic allohexaploid *N. digluta*, which is reasonably fertile. *N. digluta* is backcrossed to the recipient species (*N. tabacum*) to produce a pentaploid having the complete somatic ($2n$) chromosome complement of *N. tabacum* and one genome of *N. sylvestris*. The pentaploid is sufficiently fertile to be backcrossed to *N. tabacum*. In the progeny, *N. tabacum*-like plants resistant to tobacco mosaic are selected and cytologically analysed. From the backcross progeny, both alien addition and alien substitution lines can be recovered.

Other examples of the use of an amphidiploid as a bridging species are in the cases of transfer of genes from *G. thurberi* to *G. hirsutum* and of chromosomes from *Haynaldia villosa* to *T. aestivum*. Many other examples of a similar use of an amphidiploid may be found in the literature. *For use as a bridging species, the features of the amphidiploid are not important; it has to be only sufficiently fertile to be backcrossed to the recipient species.*

Therefore, this application of synthetic amphidiploids is easy, immediate and more rewarding than the production of new crop species (in the short run).

Creation of new crop species

It was once hoped that allopolyploidy would enable man to create new species at will, and that these species would be superior to the existing crop species. This hope was based on the fact that some of the present-day important crop species are allopolyploids, and that the existing as well as new allopolyploids can be synthesized in the same manner as they would have been produced in the nature. Thus it was expected that a duplication of the nature's own methods would lead to the creation of new and superior crop species as it had occurred in the nature.

Triticale

Triticale is the most successful synthetic allopolyploid produced by crossing wheat (tetraploid or hexaploid) with rye . Triticales derived from tetraploid wheats have been the most successful, but those from hexaploid wheats may also become a successful crop species. At present, triticales are being grown

commercially in some parts of the world, *e.g.*, in Canada, and the yields of triticales are comparable to those of the best wheat varieties. The desirable features of triticales are that they combine the yielding ability and grain qualities of wheat and the hardiness (tolerance to adverse environment) of rye. But the development of such superior lines of triticales has taken 50 years of intensive research. The newly synthesized triticales were of low yielding ability due to high sterility, poor seed set and poor and variable development of grains. Triticales also show cytogenetic and genetic instability due to meiotic irregularities and produce some aneuploid progeny.

Raphanobrassica

Some other promising allopolyploids are *Raphanobrassica*, the triploid (AAC) obtained by crossing *B. napus* (AACC) with *B. campestris* (AA). In *Raphano brassica*, the breeding objectives are to combine the hardiness of *B. oleracea* with quick growth and disease resistance of fodder radish. The problems of *Raphanobrassica* are the same as those of triticales. *i.e.*, low fertility, cytogenetic and genetic instability and leafy rape-like plants that do not produce bulbs. There is evidence that

hybridization and selection at the polyploid level would be effective in improving *Raphanobrassica*.

Varalakshmi, a hybrid variety of cotton is also an interspecific hybrid between *G. hirsutum* (American cotton) and *G. barbadense* (Egyptian cotton).

Widening the Genetic Base of Existing Allopolyploids

The genetic base of some natural allopolyploids may be narrow, and it may be useful to introduce variability in such cases by producing new allopolyploids. *B. napus* is a case in point; the genetic variability of this species is narrow and the only resource available is to synthesize new allopolyploid *B. napus* to widen its genetic base. This is being done by crossing *B. campestris* ($n = 10$, AA) with *B. oleracea* ($n = 9$, CC), the parental diploid species, to produce the amphidiploid *B. napus* ($n = 19$, AACC). The two species, *B. campestris* and *B. oleracea*, have to be crossed as autotetraploids; the cross is very difficult and embryo culture has to be used.

CHAPTER -XI

MOLECULAR PLANT BREEDING

Plant breeding is a designer's activity. Conventionally, economically important traits dispersed in different parental lines are combined together for tailoring new genotypes solely through phenotype based assays. Specific selection environments are created and criteria designed to improve the efficiency of selection. However, a plant's phenotype is determined by its genetic constitution and the environment in which it grows. The effects of the environment in many cases mask those of the genotype. The phenotype, thus, provides an imperfect measure of a plant's genetic potential. In contrast, assays based on genotype would be more precise and reliable.

DNA contains an individual's genetic blue print. The sequence of nucleotides in DNA of an individual is unique and thus determines the identity. The ultimate difference between individuals lies in the nucleotide sequence of their DNA .Detection of such differences employing different molecular biology techniques has led to development of molecular markers. Molecular markers follow simple Mendelian pattern of inheritance. They are

stable and not influenced by developmental or environmental factors. Use of molecular markers to assist plant breeding has given birth to the sub-specialization of molecular breeding. This specialization is less than two decades old. In 1980, a group of researchers working in human genetics at the Whitehead Institute of Biomedical Research in USA, published a landmark paper that gave a conceptual framework for constructing saturated genome maps by employing DNA based genetic markers in humans. This was followed by rapid developments in the field concerning both theoretical and practical aspects. In plants, molecular markers were first developed in 1985-86 by two groups of researchers working independently at Native Plants Incorporated, USA and Cornell University, Ithaca, USA. Subsequently, through collaborative efforts, molecular marker systems were developed in almost all major field crops, many important vegetable crops and in some tree species including fruits.

Molecular plant breeding consist of

- 1. Developing molecular marker systems,*
- 2. Constructing high density linkage maps,*
- 3. mapping of genes,*

4. *Marker assisted selection and*

5. *map-based cloning of genes*

Which are known only by their phenotype.

BASIC TECHNIQUES

Molecular markers are based on two basic techniques: (1) *Southern blot hybridization* (Southern 1975) and (2) *polymerase chain reaction (PCR)*. The Southern blot hybridization involves several steps which include isolation of high molecular weight DNA, restriction digestion of DNA using a special class of enzymes called *restriction endonuclease*, size separation of DNA fragments by agarose gel electrophoresis, transfer of DNA fragments from gel to nitrocellulose/nylon membranes after denaturation, hybridisation of probe DNA with the membrane bound DNA and autoradiography **(Fig. 36)**.

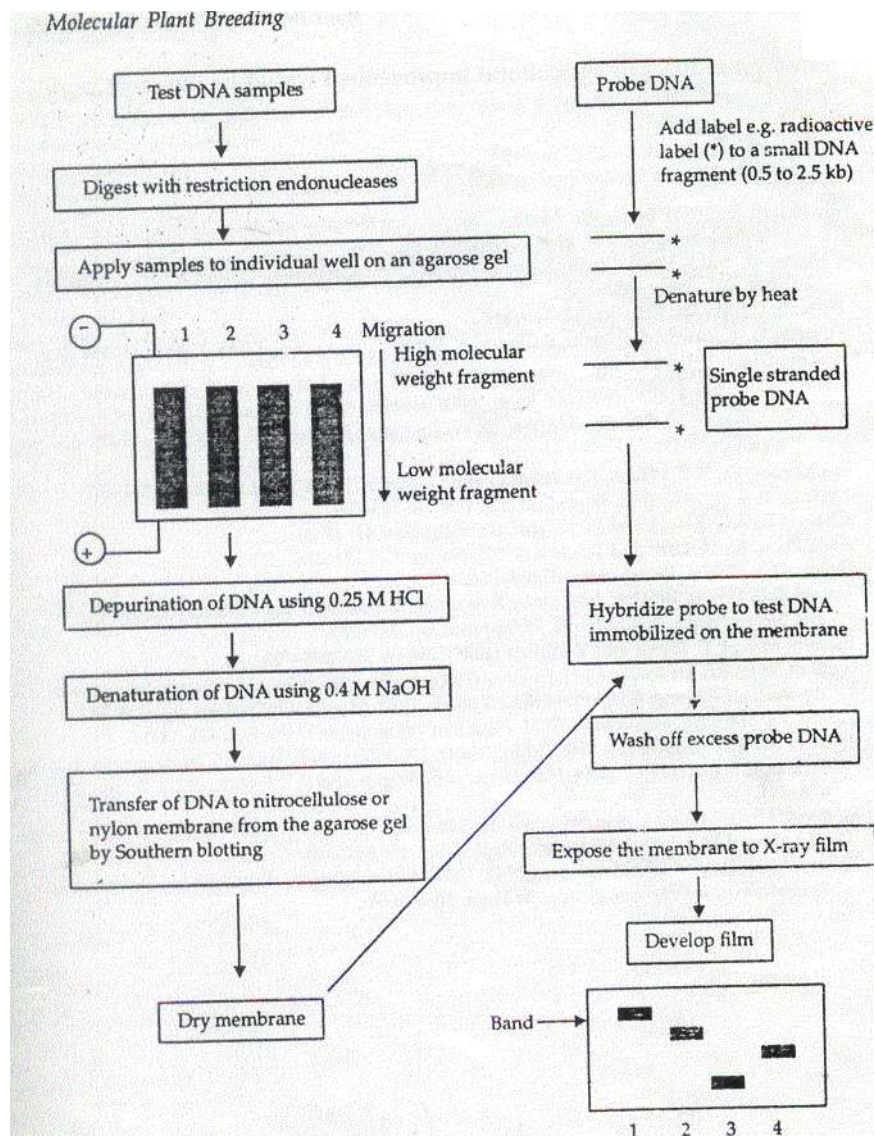


Fig. 36. Steps in southern blot hybridization. Bands in different lanes indicate position of the test DNA fragments homologous to probe DNA.

The probe DNA is prepared by incorporating radioisotopes through the use of labelled nucleotide, p^{32} PdCTP during fresh DNA synthesis. Specific DNA fragment on the membrane hybridizing with the probe DNA is detected by the presence of a dark coloured spot called band at the corresponding position on the autoradiogram.

The polymerase chain reaction is a much simpler and quicker process (**Fig. 37**) that allows automation.

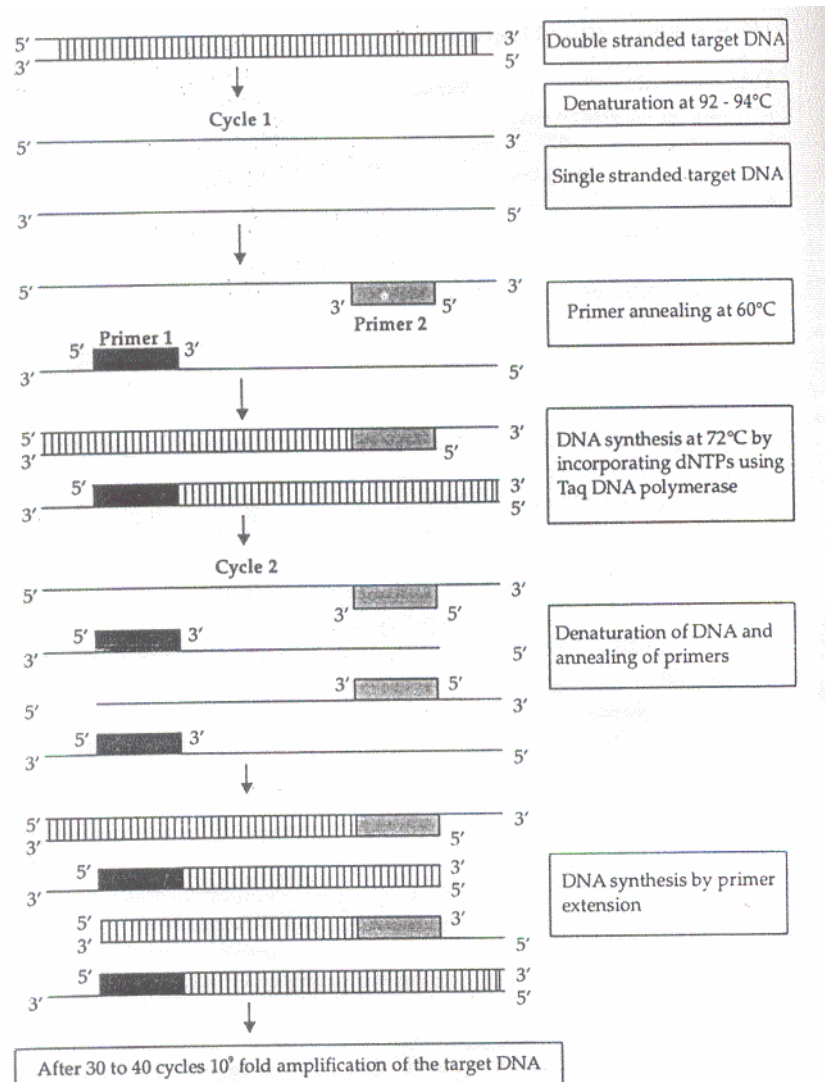


Fig. 37. Steps in polymerase chain reaction.

A PCR cycle consists of three steps: (1) *denaturation of source DNA at 92-94°C*, (2) *annealing of two 18-25 nucleotide long sequences called primers to the single stranded source (template) DNA at 55-60°C* and (3) *DNA synthesis at 72°C using a thermostable*

DNA polymerase. The target DNA flanked by two primer binding sites gets synthesized in large amounts by the end of 30-40 cycles of amplification. The amplified products is then visualized as a fluorescent band on an agarose gel under UV light after electrophoretic separation and staining with ethidium bromide. The PCR reaction requires nanogram quantities of DNA and does not involve use of radioisotopes. Since, all the steps of PCR cycle are efficiently carried out in a thermal cycler and thus are automated, it allows handling of far greater number of samples per unit time than in the Southern blot hybridization technique.

Molecular Marker Systems

A survey of literature reveals many marker systems. All these are the result of modifications of the two basic techniques. In view of their importance and the extent of use, five of the molecular marker systems are described as follow;

Restriction Fragment Length Polymorphism (RFLP)

This is the first molecular marker system that was conceived and developed by Botstein *et al.* (1980) and is based on the Southern hybridization technique. It employs restriction endonucleases which recognize four to six nucleotide long sequences—the target sites, in the DNA molecules to be cleaved. It

also utilizes single/low copy number DNA sequences as probes. The size (length) of the DNA fragments homologous to the probe DNA depends upon the location of target sites. Base changes can alter the sequences of the target site, thereby abolishing sites or creating new sites for a particular enzyme. DNA rearrangements such as deletions and insertions of sequences can also change the location of target sites. Suppose that there are two *Eco* R1 sites A and B located 1000 bases (*i.e.* 1kb) apart in the variety X (Fig. 38).

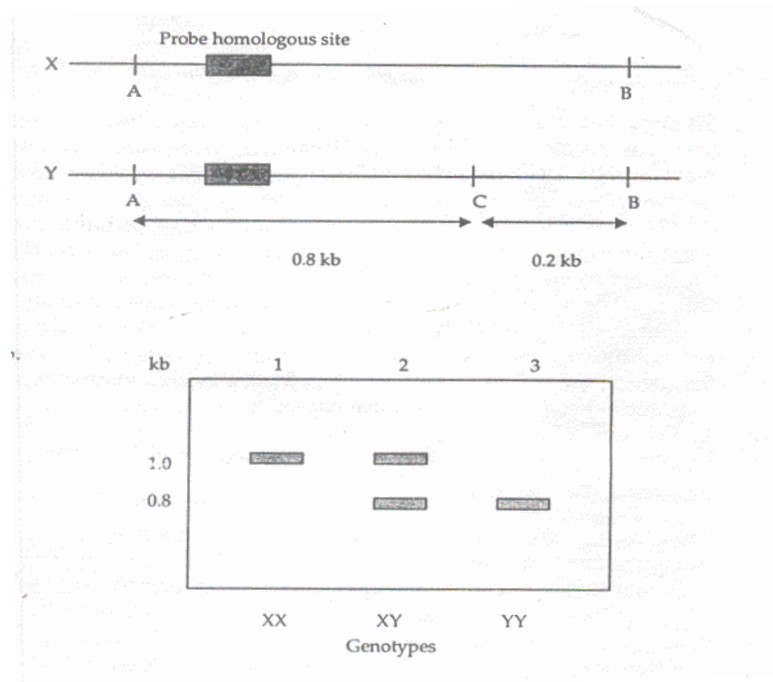


Fig. 38. Definition of RFLP.

In variety Y, however, a point mutation event has created an internal target site C which is 800 bases away from A and 200 bases away from B. Restriction digestion of DNA with enzyme Eco R1 will then generate a 1kb AB fragment in the variety X and two fragments (0.8 kb AC and 0.2 kb BC) in the variety Y. If the probe DNA is homologous to a segment of DNA between A and C target sites, on hybridization it will bind to the AB fragment in the variety X and AC fragment in the variety Y, producing 1 kb and 0.8 kb bands respectively. This difference in size of the restricted DNA fragments from homologous sites is called RFLP. In the F₁ hybrid obtained by crossing the two varieties, both 1 kb and 0.8 kb fragments will be present, thus yielding a *co-dominant marker*. RFLP profiles are highly reproducible and by employing them it is possible to determine allelic diversity and frequency in a population.

Many RFLP markers are obtained by using DNA probes from different sources. For instance, probes can be prepared from cDNA clones from libraries obtained from different plant parts like leaf, flower, root, stem and even from callus. Small genomic DNA fragments derived by restriction enzyme digestion can also be used as probes. Since a large number of DNA probes are available, there

are practically no constraints on the number of RFLP markers that can be generated. However, this is not true in all crop species. It depends on the genome structure of the species concerned. For instance, the abundance of RFLP is very high in maize and brassicas but relatively low in tomato and rice.

Random Amplified Polymorphic DNA (RAPD)

This marker system is based on polymerase chain reaction. It employs a single decamer primer of arbitrary sequence which is annealed to the template DNA typically at 37°C. The RAPD profile is obtained by resolving the amplified products on an agarose gel. The variation in RAPD profile in the form of presence or absence of band results from variation in primer binding sites. A large number of RAPD markers can be generated by the use of a variety of decamer primers. A major limitation of this marker system is non-reproducibility due to low primer annealing temperature. However, the utility of a desired RAPD marker can be enhanced by sequencing its termini and designing longer (*eg.*, 24 nt) primers for specific amplification through PCR. Such a modification that imparts the required reproducibility has been named as ***sequence characterised amplified region (SCAR)***.

Sequence Tagged Sites (STS)

This marker class was first conceived and used in human genome mapping and has been recently included in genome mapping in plants. STS markers are generated by an unmodified PCR. The primers are designed based on the sequence of cDNA, random genomic DNA and ends of large genomic DNA fragment cloned in cosmid, lambda or artificial chromosomes. Sequences flanking the microsatellites can also be used to generate PCR primers for assaying the variation in the number of tandem repeats in a given repeat motif at homologous sites. The STS marker system has all the advantages of PCR technique. A limitation of STS is that considerable effort and time are needed to generate DNA sequence data for designing suitable primers. Besides, the fragments amplified by the primers may not show length variation between individuals. This problem however, is circumvented by restriction digestion of the PCR products which detects polymorphism in the target site of a restriction enzyme internal to the two primer binding sites. This modification has led to a new marker system called ***cleaved amplified polymorphic sequence (CAPS)***.

DNA Amplification Fingerprinting (DAF)

DAF is another PCR based marker system which employs shorter (<10 nt) primers of arbitrary sequence. The primer annealing temperature is also lower (typically 30°C) as compared to RAPD. The amplified products are separated on polyacrylamide gels and detected by silver staining. This gives a better resolution essential for proper visualization and scoring of large number of bands usually obtained in DAF. The number of polymorphisms scored per run is high. Since the length of primer is short and primer annealing temperature is low, DAF suffers from the problem of non-reproducibility of some of the amplified fragments.

Amplified Fragment Length Polymorphism (AFLP)

The AFLP markers are generated by selective amplification of DNA fragments obtained by restriction enzyme digestion. High molecular weight DNA is digested by two restriction enzymes, one hexacutter (e.g. *Eco* RI) and one tetra cutter (e.g. *Msc* I). Adaptor molecules are ligated to the ends of DNA fragments.

Two primers possessing sequence complementarity to the adaptors as well as few (typically 3) extra random nucleotides at

their 3' ends are used for selective amplification of fragments employing PCR (**Fig. 39**).

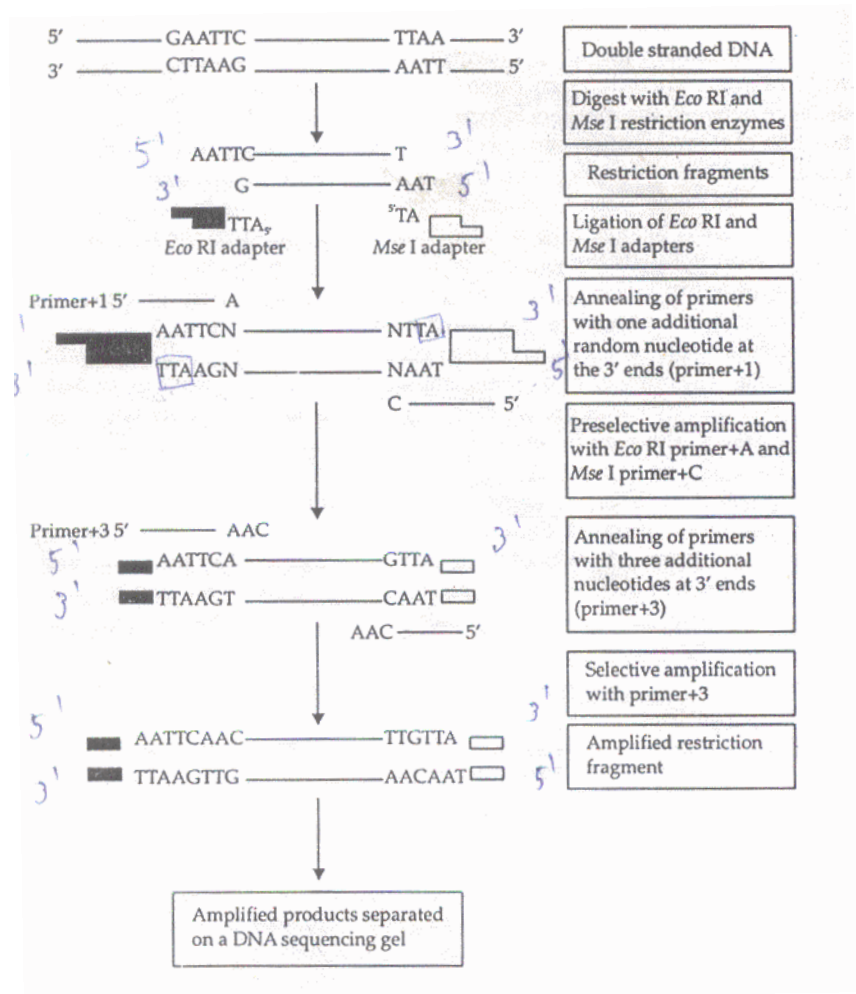


Fig. 39. Steps in the AFLP technique

The amplified products are separated on sequencing gels or even on ordinary PAGE and visualized by silver staining. Alternatively, the primers are labelled either by radioisotope or fluorescent dye so that the AFLP profile can be obtained by autoradiography or by using image analysis. The highest number of amplified products (50-100) are obtained through AFLP among all the DNA profiling systems. This increases the probability of detecting polymorphism many folds. The technique is, at present, lengthier and costlier than other PCR based techniques. It requires good quality DNA for ensuring complete digestion by enzymes because partial digestion of DNA results in non-reproducible variation in DNA profiles.

Construction of Molecular Maps

Construction of genetic linkage maps using morphological markers could not be initiated in most crop plants due to lack of sufficient number of markers. Morphological marker based map making was largely confined to maize and tomato. Even in these species, map construction was highly laborious, took many years and required several mapping populations since all morphological markers could not be obtained in a single cross. These maps contained limited number of markers and, therefore, could not be

used for efficient mapping of target genes. With the availability of a large number of molecular markers, saturation mapping of plant genomes has become a reality.

Construction of molecular genome map involves five major steps: (1) identification of divergent parents,

(2) Generation of a suitable mapping population,

(3) Identification of polymorphic probe-enzyme combination and informative primers,

(4) Analysis of marker segregation in the mapping population, and (5) establishment of linkage.

Identification of Parents

A survey of the available germplasm is made for existing morphological diversity. Besides, a preliminary survey can be carried out using a few DNA markers to identify divergent parents among the available lines. It is advantageous to select parents which possess contrasting traits of agronomic importance, so that genes for those traits can also be simultaneously localized on the map.

Generation of Mapping Populations

The selected parents are crossed to obtain F_1 hybrids from which four different mapping populations can be generated: (1) F_2 (by selfing a single F_1 plant), (2) BC (by crossing the F_1 , with one of the parents), (3) Recombinant inbred lines (by selfing individual F_2 plants for 7-8 generations), and (4) doubled haploids (by *in vitro* culture of anthers/ pollens from the F_1 followed by chromosome doubling). For construction of a linkage map, 50-100 F_2 plants should be sufficient. In most of the crop plants F_2 population has been used since it can be generated in the shortest possible time with the least effort. However, for mapping genes, particularly those for quantitative traits, permanent mapping populations such as *recombinant inbred lines* and *doubled haploids* are preferred since they can be maintained over years by selfing and replicated over locations and seasons. This allows correct estimation of environmental effect which is a component of quantitative trait variation.

Identification of Informative Probes and Primers

For RFLP analysis, several DNA probes should be tried in combination with a set of restriction enzymes to find the

polymorphic probe-enzyme combinations (PEC) for the parents. Emphasis should be on finding a cheaper enzyme, since this will be required in large amounts. In crops like maize and *brassica*, one or two enzymes such as *Eco* RI and *Hind* III were sufficient to detect high level of DNA polymorphism, whereas, in rice, 11 enzymes were used to have 75% of the probes polymorphic. Therefore, depending on the species, extensive analysis is to be carried out to identify the polymorphic PECs. For PCR based markers, suitable primers are similarly identified through extensive survey.

Segregation Analysis

The individual plants of the mapping population are analyzed using the same PEC/primers which detect polymorphism between parents. The plants are categorized as parent 1 type, parent 2 type or F₁ type depending on the size of the polymorphic fragments. The observed frequency of the plants based on marker locus genotypes is compared with the expected segregation ratio of 1:2:1 in case of F₂ and 1:1 in case of other populations (**Fig. 40**).

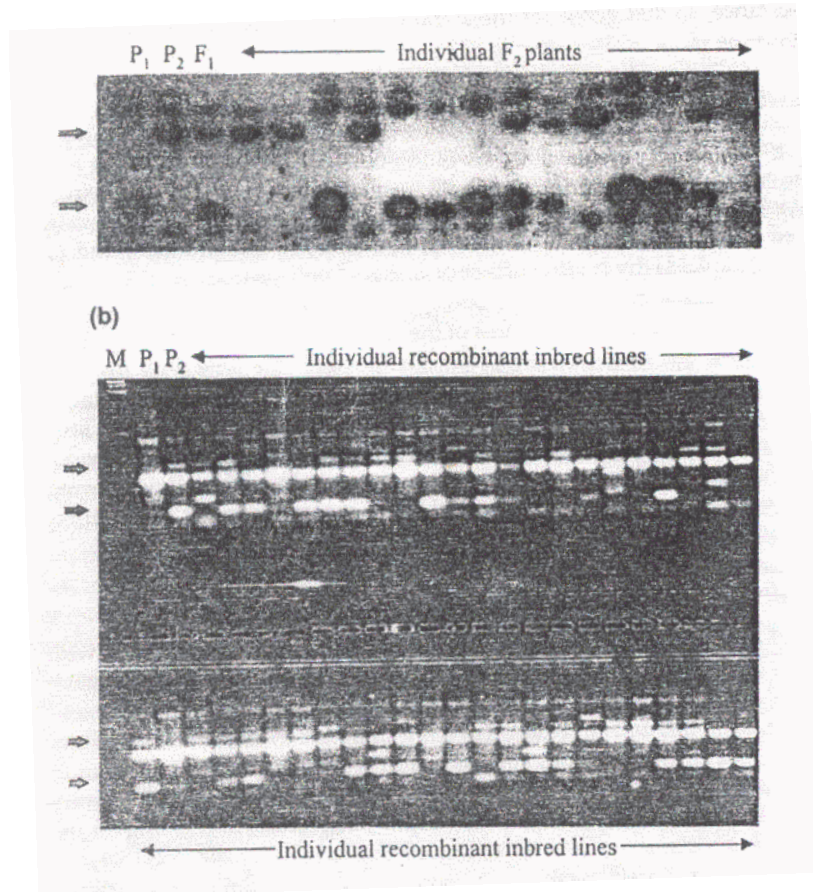


Fig. 40. Segregation pattern of RFLP (a) RAPD (b) markers in F₂ and recombinant in bred lines of mustard. M-Molecular weight standard, P₁ –cv. Varuna, P₂- cv. BEC- 144, F₁ , Hybrid of P₁ and P₂ Arrows in (a) indicted positions of two allelic fragments both of which are present in the F₁. The P₁ and P₂ and F₁ types are identifiable in F₂ based upon these two bands. Arrows in (b) indicate position of two independently segregating markers in the recombinant in bred lines.

Based on chi-square analysis, the observed segregation pattern is tested for possible distortion. Markers showing expected segregation ratio are well-suited for construction of linkage maps.

Linkage Analysis

Markers segregating in the population are tested pair-wise, *i.e.*, by a two-point test. Linkage is indicated when chi-square test reveals absence of independent assortment. Although it is possible to do linkage analysis manually, it is a stupendous task when the number of markers is more (>1000). Presently, several computer packages are being used to construct high density linkage maps. The most commonly used package is MAPMAKER developed by a group of researchers working at the Whitehead Institute of Biomedical Research, USA.

Once the linkage groups are identified by a two point test, the markers within a group are ordered by three point and multipoint linkage tests. The distance between the markers is expressed in centimorgan (cM), which is a function of recombination between them. Once the linkage groups are identified, their correspondence with the chromosomes is established by using chromosome stocks such as trisomics and monosomics or by using *in situ* hybridization. A linkage map is considered complete when the number of linkage groups equals the gametic or haploid number of chromosomes. A fair degree of saturation is assumed when the

maximum distance between any two adjacent markers is less than 10 cM.

Molecular genome maps have been constructed in almost all important crop plants. The number of markers employed to construct these maps and the marker density varies. Most of these maps are based on RFLP markers, although in few crop species such as rice, RAPD, STS, and AFLP have also being utilized. Among the crop plants, the rice genome map (Harushima *et al.* 1998) contains the maximum markers (2275) and is considered most saturated. Significantly, this map has been made using a single F₂ population. Majority of these markers are derived from cDNA and thus are ***expressed sequence tags***. Availability of such a genome map is facilitating in identification of tightly linked flanking markers for genes of agricultural importance.

MOLECULAR MAPPING OF GENES

Availability of molecular markers and saturated linkage maps has enabled mapping of genes for qualitative as well as for quantitative traits. The qualitative traits show simple Mendelian pattern of monogenic inheritance. The quantitative traits, on the other hand, are polygenically inherited. Methods employed for the

detection and mapping of loci for these two categories of traits are different.

Mapping of genes for monogenetic traits in crop plants has involved three distinct approaches:

- (1) Use of a complete linkage map,
- (2) Use of near isogenic lines and
- (3) Bulk segregant analysis of F_2 population.

The first approach essentially requires availability of a complete genetic linkage map. For the purpose of mapping a gene, markers are selected from all the chromosomes such that the maximum distance between any two adjacent markers does not exceed 20 centimorgan (cM). A gene mapping population (F_2 or BC) is created by crossing two parental lines having contrasting characteristics. The individual plants of this population are phenotyped for the target trait. Simultaneously, genotyping of these plants is carried out using markers which distinguish the two parental lines, in addition to being evenly distributed in the genome. Co-segregation of any of these markers with trait phenotype in the mapping population reveals linkage (**Fig. 41**).

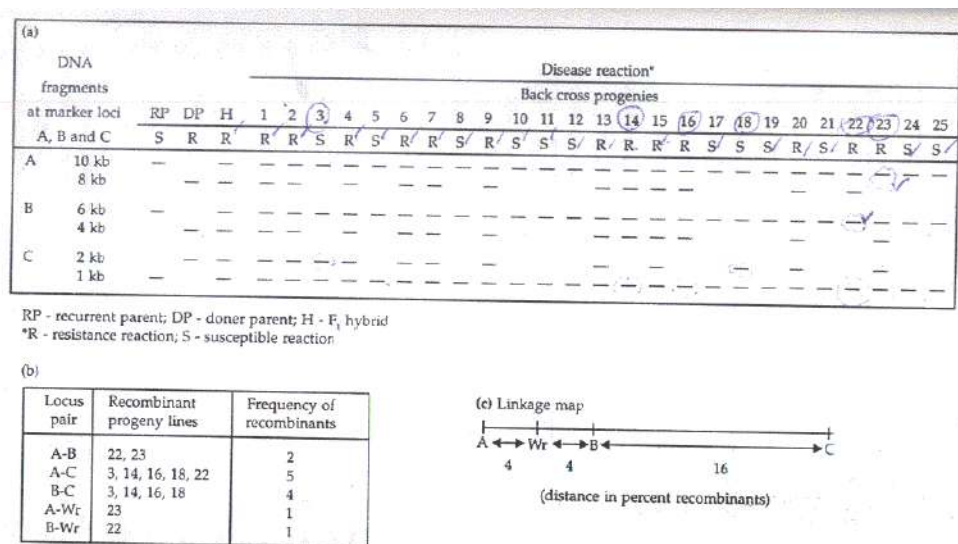


Fig. 41. Molecular mapping of a gene (W_r) for qualitative disease resistance (a) Segregation of DNA markers and the trait in back cross population (b) Frequency of recombinants in the back cross population (c) Linkage indicating the position of the disease resistance locus (W_r).

This facilitates immediate localization of the gene on the linkage map, since marker positions are already known. Although laborious, it is the most directed method of gene mapping.

The other two approaches do not require a linkage map. For instance, in case of the use of near-isogenic lines (which are developed by repeated backcrossing of the F_1 with the recipient parent and therefore, differ for a single gene), DNA markers distinguishing the two lines (e.g., resistant Vs. susceptible) are first

identified. Co-segregation of these markers with the trait is analyzed in a mapping population, often derived from the cross of the two isogenic lines. Development of near-isogenic lines, however, takes many years. This method of gene mapping therefore, is best suited to crops, in which intensive backcross breeding has been already practiced to generate isogenic lines.

Use of F_2 population in conjunction with a technique called bulked-segregant analysis, is the most rapid way of mapping genes. In this approach, DNA from ten homozygous resistant F_2 plants is bulked to constitute a resistant bulk. Similarly, a susceptible bulk is made out of DNA from ten homozygous susceptible F_2 plants. DNA markers which distinguish these two bulks are considered to have linkage with the target gene. Even though, molecular linkage map is not a pre-requisite for its success, use of markers with known map position imparts greater efficiency to gene mapping in this approach.

Many genes of interest have been located precisely on the genome maps in several crop plants following these approaches. Initially, RFLP markers were used extensively for mapping and tagging of genes. Subsequently, RAPD was preferred over RFLP markers as soon as it was developed because of its simplicity and

rapidity. There are several instances, where tightly linked (< 5 cM) markers located on either side of the target gene has been identified.

Polygenic Traits

Majority of the traits of agronomic importance are polygenically inherited. Such traits are traditionally characterized by applying the principles of quantitative genetics which enables estimation of the approximate number of loci affecting a character, average gene action, degree of interaction among polygenes and genotype-environment interaction. However, the magnitude of effect, inheritance and gene action in respect of any specific locus which is now commonly known as quantitative trait locus (QTL) and its interaction with other loci and environment remain unanswered.

The first attempt for identification of an individual QTL was made by Sax (1923). He reported a cross between two strains of *Phaseolus vulgaris*, one of which had large, coloured seeds and the other had small, white ones. The seed colour variation was due to a single gene difference, while seed size showed continuous variation. In F_2 , the plants homozygous for the allele giving coloured seed had an average seed weight greater than that of the plants homozygous

for the white colour allele. The average weight of the seed from plants heterozygous for the coloured allele was just about midway between the two. It was, thus, evident that the seed size was nearly proportional to the number of coloured alleles present in the F_2 individual. This suggested presence of linkage between the seed colour locus with one or more genes affecting seed size. Subsequently, Rasmusson (1933) provided further evidence in support of linkage of a major gene affecting flower colour with a QTL for flowering time in pea. The principle outlined in these two experiments was further extended and applied for mapping QTL in *Drosophila* (Thoday 1961).

With the advent of molecular markers and construction of high density linkage maps, it is now possible to locate most of the QTLs for a trait segregating in a cross and determine their individual as well as interaction effects on trait expression. Precise identification of QTL, however, requires permanent mapping populations such as doubled haploids or recombinant inbred lines which can be replicated over seasons and locations for estimating environmental component of the total phenotypic variance. Statistical methods being used in QTL mapping are briefly outlined.

Methods of QTL Mapping

The basic principle of determining whether a QTL is linked to a marker is to partition the mapping population into different genotypic classes based on genotypes at the marker locus. Correlative statistics is then used to determine whether the individuals of one genotype differ significantly from individuals of the other genotype with respect to the trait being measured. If the genotypes differ significantly, it is interpreted that a gene (s) affecting the trait is linked to the marker locus used for classifying the population. Three main methods of QTL mapping are:

- (1) Single marker analysis,
- (2) Interval mapping and
- (3) Composite interval mapping.

Single Marker Analysis

This is carried out by looking for associations between genotypes at individual markers and phenotypic traits of interest (Edwards *et al.* 1987). Single factor analysis of variance is done for each marker locus independent of information from other marker locus. F-tests provide evidence whether differences between marker locus genotype classes are significant or not. Although computationally simple and the first to be used to map QTL, this

method suffers from limitations: (1) As the distance between the marker and the QTL increases, the likelihood of detecting the QTL statistically decreases due to genetic recombination between the marker and the QTL and (2) the magnitude of any detected QTL will also be underestimated due to the same very reason of recombination. This method also ignores the effect of other QTLs. However, the problems due to recombination can be minimized by using a large number of markers so that most part of the genome is covered with minimum distance between a marker and the target QTL.

Interval Mapping

This method was first proposed by Lander and Botstein (1989) and it takes full advantage of a complete linkage map. In this procedure, intervals formed by pairs of adjacent markers are taken as the unit of analysis and tested for the presence of single QTL by using genetic information from the flanking markers. Presence for a putative QTL is assumed if the ***log of odds ratio (LOD)*** exceeds a critical value. This is the most widely used method easily accessible through the software package MAPMAKER/QTL. This method compensates for the recombination between marker and the QTL, thereby increasing the probability of statistically

detecting the QTL. However, when multiple QTLs are segregating in a cross, which is usually the case, it fails to eliminate the genetic variance caused by other QTL, similar to single marker method.

Composite Interval Mapping

This method was recently developed by Zeng (1994). The basic principle in this method is to combine interval mapping for a single QTL in a given interval with multiple regression analysis. It is the most powerful method of QTL analysis because of the increased precision by elimination of variance caused by other QTL. However, this method is yet to be used extensively in QTL mapping.

Prof. S.D. Tanksley and his colleagues, working at the Cornell University, Ithaca, USA were the first to use complete RFLP map of tomato and interval mapping for dissecting complex quantitative traits such as fruit mass and total soluble solid content into Mendelian factors (Paterson *et. al.* 1988). Based on information from complete molecular genome map, QTLs have been mapped for a variety of traits in many different plant species .This includes complex traits such as grain yield, drought tolerance and quantitative disease resistance. It has become possible to know the number and genomic distribution and type of gene action of QTL for the target traits. (Interestingly, the number of QTLs found to

have major effects on trait) expression are often only few (<5). This has generated optimism for a directed manipulation of complex quantitative traits for which no selection criteria are available so far.

MARKER ASSISTED BREEDING

Molecular markers with their known positions on a saturated linkage map can, in practice, aid each and every step of the plant breeding process. Specific areas of application include the following.

Estimation of Genetic Diversity

Divergent parents carrying different favourable genes are used in crossing programme for realising transgressive segregants in self-pollinated crops and for creating heterotic hybrids in cross-pollinated crops. Correct estimation of genetic diversity is the key to success in these breeding schemes. Traditionally, the extent of diversity is determined in a group of genotypes/ germplasm collections based on morphology. Two genotypes may be similar in morphology, yet the genes contributing to trait expression may be different. Besides, plant's phenotype does not precisely reflect its genotype due to its interaction with environment. It is, therefore, essential that genetic diversity estimates should be based on

genetic constitution of an organism. Molecular markers, being based on DNA differences, are best suited for this purpose.

DNA markers selected for diversity analysis should preferably belong to non-overlapping expressed part of the genome. The RFLP markers, if included in the analysis should be generated with cDNA probes. In case of PCR based markers, expressed sequence tags are most desirable. Distribution of the markers in the linkage map should be taken into account in order to cover all the genomic regions. In crop species where sufficient information on gene mapping is available, markers linked to QTLs for important economic traits will be more suitable than the unlinked ones. For genotyping of a large number of collections available in the genebank, a PCR based marker system will serve better by way of saving time and efforts.

RFLP based diversity analysis by several groups of researchers has suggested high correlation between predicted and observed yields in crop hybrids (Walton and Helentjaris 1987; Smith *et al.* 1990). A significant correlation between marker locus heterozygosity and heterosis for rice has also been reported (Saghai-Marouf *et al.* 1997). Few reports on the other hand, have indicated lack of correlation, suggesting that heterosis may not

always be predicted from the extent of genetic diversity between parental lines. The level of correlation may possibly be dependent upon the germplasm used.

Characterisation of Germplasm Resources

Indian sub-continent is very rich in crop germplasm resources. A large number of collections representing all geographic and agroclimatic regions are available in the National Active Germplasm (NAG) sites. For instance, there are 42,000 accessions of rice available at the Central Rice Research Institute, Cuttack, India and 18,000 accessions of wheat at the Directorate of Wheat Research, Karnal, India. The number is equally high in many other crops which include barley (11,030), rapeseed (8082), cotton (6896) and groundnut (6432). Keeping in view the hazards of monoculture and heavy dependence on a limited number of nuclear genes of a few cytoplasmic genomes for varietal improvement, the old and little used varieties/ landraces replaced by high yielding varieties of crops need to be preserved for genes for useful traits such as biotic and abiotic stress tolerance.

Molecular markers can aid maintenance and characterization of biodiversity in a variety of ways. Due to limitation of space, time

and effort, it may not be possible to maintain all the germplasm collections. Molecular markers can be used for identification and elimination of duplicate accessions entered in different names and thus reduces the number of accessions to be actually preserved. Simultaneously, employing markers linked to traits of agricultural importance, useful accessions in the germplasm reserve can be identified, evaluated for trait diversity and efficiently utilized in crop breeding. In rice, for instance, molecular markers have successfully been used for identification of duplicates and determination of the extent of allelic diversity both at inter and intra varietal/landrace levels.

Identification of Crop Varieties

Unambiguous differentiation and identification of crop varieties is essential for their registration, protection, utilization and quality control of seeds. Plant growth, maturity and yield related traits as well as other morphological characteristics used to establish distinctiveness, uniformity and stability (DUS) of crop varieties are not always reliable. DNA fragment profiles obtained with different marker systems should provide fingerprints highly characteristic of varieties. Among the marker systems evaluated, AFLP is turning out to be most suitable for this purpose. More than

50 scorable bands per a single run impart higher efficiency to this marker system for generating the fingerprints. It has been calculated in maize that when there are only two alleles at each locus and each allele occurs with a frequency of 0.5, the probability of distinguishing 20 of 20 inbreds is 0.99, if 20 markers are used. With many polymorphic loci and more than two alleles per locus, the resolving power of a profiling system increases many folds.

Molecular marker systems have been extensively employed for identification of crop cultivars, somatic hybrids and cybrids, genetic stocks such as somaclones and cytoplasmic male sterile lines in different crops. Another area, where molecular markers are becoming practically indispensable is identification of hybrids. Particularly, during production of F₁ hybrid seed a variety of errors and mishaps can lead to seed lots containing an unacceptable proportion of seeds produced by selfing. DNA profiling of seed samples from commercial seed lots can be used to test the purity by employing hybrid specific markers.

Marker Aided Selection (MAS)

Selection of a genotype carrying desirable gene or gene combination via linked marker(s) is called marker aided selection. Breeders practice marker aided selection when an important trait,

that is difficult to assess, is tightly linked to another Mendelian trait which can be easily scored. For example, a gene for purple coleoptile colour in some traditional rice varieties is closely linked to a gene that confers resistance to brown plant hopper (BPH). In a segregating population like F_2 , about 95% of the plants showing purple coleoptile are found resistant to BPH. In this case, coleoptile colour is a morphological marker which is used to assist selection for BPH resistance. Morphological markers are however, limited in number, are specific to particular genotypes and are dominant. Morphological markers may also show tissue and developmental stage specific expression, pleiotropy and even at times, adverse effect on plant growth, vigour and viability. Due to these reasons, morphological markers could not be of much use in MAS. Molecular markers do not suffer from these limitations and thus offer advantages over the morphological markers.

Molecular marker aided selection involves scoring for the presence or absence of a desired plant phenotype indirectly based on DNA banding pattern of linked markers on a gel or on autoradiogram depending on the marker system. The rationale is that the banding pattern revealing parental origin of the bands in segregants at a given marker locus indicates presence or absence of

a specific chromosomal segment which carries the desired allele. This increases the screening efficiency in breeding programmes in a number of ways such as:

1. The segregants can be scored at the seedling stage for traits that are expressed late in plant development. This includes traits such as grain quality, male sterility and photoperiod sensitivity.

2. It is possible to screen for traits that are extremely difficult, expensive or time consuming to score and measure such as tolerance to drought, salt, mineral deficiencies and toxicity, root morphology, resistance to nematodes or to specific races or biotypes of diseases or insects.

3. Selection can be practiced for several traits simultaneously which is difficult or even impossible by conventional means.

4. Heterozygotes are easily identified and distinguished from the either homozygotes without resorting to progeny testing. This saves time and effort.

Specific plant breeding programmes in which marker assisted selection has been already utilised are: (1) gene introgression and

elimination of linkage drag, (2) gene pyramiding and (3) development of heterotic hybrids.

Gene introgression

Wild relatives of crops constitute a rich source of genetic variation which can be efficiently utilised for improvement of both qualitative and quantitative traits. In tomato, for example, genes resistance to *Meloidogyne incognita* and tobacco mosaic virus has been introduced from the related species *L. peruvianum*. Similar potential exists in wild and exotic germplasm for most crop plants. Molecular markers can aid introgression and impart greater precision. With the use of isozyme marker it has been possible to introgress nematode resistance into the cultivated tomato from one of its wild relatives. In an interspecific cross of tomato, it has been shown that selection for the recurrent parent markers along with the introgressed gene can result in major savings in the number of generations required for completion of back crossing procedure and in the amount of field space required to test the mature individuals.

It is often observed that the desirable genes such as those for disease resistance remain linked with undesirable weedy characteristics of the alien species. During gene introgression by back crossing, the linked undesirable gene also gets transferred to

the recipient parent. This has been referred to as *linkage drag*. Even after 20 backcrosses, one expects to find a sizeable piece upto 10 cM of the donor chromosome still linked to the gene being selected. In most plant genomes, this segment is enough to contain hundreds of genes. This can, thus, result in a new variety modified for characters other than those originally targeted.

Molecular linkage maps provide a method to reduce the problems with linkage drag by allowing selection of individuals containing recombinant chromosomes which break linkage drag. It has been estimated that the use of molecular markers can reduce linkage drag at least 10 fold in a fraction of the time needed by traditional breeding (Tanksley *et al.* 1989). In tomato, molecular linkage map has been used to identify varieties carrying minimal linkage drag around several disease resistance genes transferred from wild species.

Gene pyramiding

Pyramiding of genes has been suggested as an effective way to provide durable form of disease and insect resistance in crop plants. Because of difficulty involved and the number of years it requires, development of lines carrying genes for resistance against several races of a single pathogen/ insect or against different biotic

stresses has not been very successful. Construction of such lines is much more efficient with molecular markers. Recently, successful pyramiding of four genes, *Xa4*, *xa5*, *xal3* and *Xa21* conferring resistance to four different races of bacterial leaf blight pathogen has been achieved in rice (Huang *et al.* 1997) by using molecular markers, which was otherwise impossible through conventional methods.

Construction of heterotic hybrids

Molecular marker-based genetic analysis can be utilized to identify specific genomic regions containing QTLs for yield showing dominance or over dominance gene action in the F_2 of a cross between two inbred lines. This will enable analysis of an existing cross in terms of the number of QTLs involved and the magnitude of their effect on the measured trait. Subsequently, additional inbred lines can be tested against standard lines for the presence of additional dominant or overdominant QTLs. These could then be incorporated into the standard lines by marker based introgression. In this way the existing inbreds can be improved first and then used to develop hybrids for realising higher heterosis than in the existing ones.

In maize, Prof. C. Stuber and his colleagues, working at the North Carolina State University, USA, have been successful in the above breeding scheme using molecular markers. QTLs for yield were first identified in inbred lines T x 303 and oh 43, which were then transferred through use of molecular markers to inbreds B 73 and Mo 17 respectively. By crossing the improved B 73, with the improved Mo 17, a new hybrid [B 73 (I) x Mo (I)] was constructed that out yielded the best check by 15%.

Complete integration of MAS with conventional plant breeding programmes demands consideration of two important factors: a) size of population and b) cost. Plant breeding experiment requires screening of large segregating populations routinely over generations. Genotyping of large number of samples manually is an extremely difficult task. MAS to be practicable should be amenable to automation that would allow handling of large number of samples. Development and use of PCR based markers such as STS and SCAR will be the key to success of MAS in crop improvement. As the technology develops and gets modified to analyze large number of samples, the cost will automatically go down. The investment in gene tagging, and selection based on molecular

markers should be weighted against the overall cost and time involved in traditional breeding program.

Even though, the cost of MAS is higher at the present level of estimation, its integration with traditional plant breeding is desirable because of the possibilities it offers.

Map Based Cloning Of Genes

Employing conventional methods, genes are isolated and cloned only when their products are known. For instance, the aminoacid sequence information of a purified protein can be utilized to synthesize a part of the corresponding gene that encodes the protein based on the codon dictionary. The synthesized sequence, commonly known as oligonucleotide can then be used as a probe to identify the target gene present in a library of DNA clones representing the whole genome of an organism. In plants, gene products of agronomically important characters such as disease and insect resistance are mostly unidentified. Saturated genome maps provide a way to clone a gene without prior knowledge of its molecular phenotype, *i.e.*, the product it encodes. This is a *reverse genetic* approach of gene isolation. Now it is commonly known as positional or map-based cloning of genes. The feasibility of gene cloning based on saturated RFLP maps was first

demonstrated in *Drosophila* and subsequently in human. By now, several plant genes of agricultural importance have been isolated by this approach.

The map-based gene cloning strategy is given in **Fig.42**.

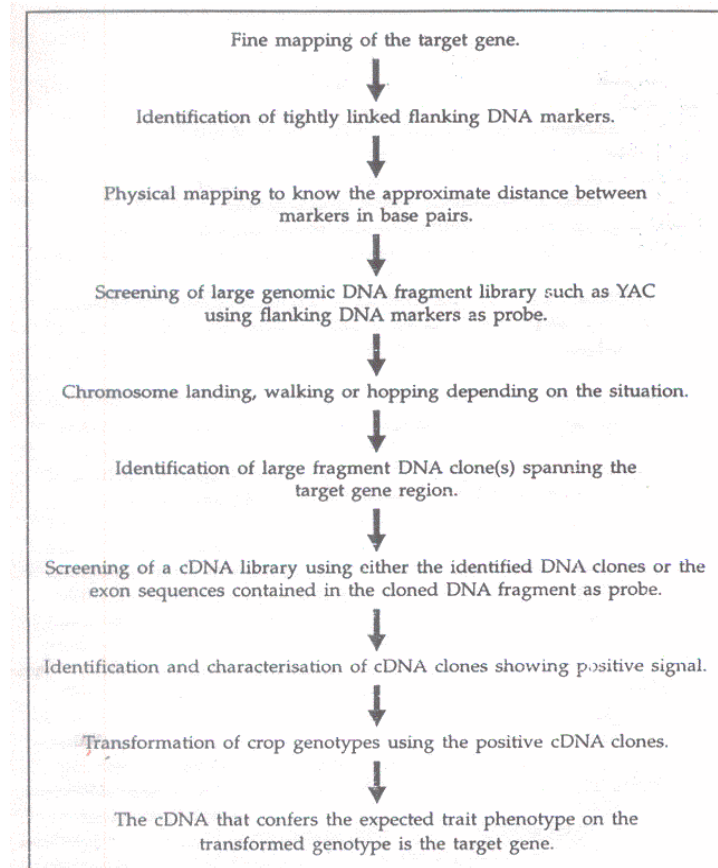


Fig. 42. A commonly used strategy for map based cloning of a gene.

The first requirement in this process is a densely populated molecular map on which the target gene has been located. The markers flanking the gene of interest should remain tightly linked even in a large mapping population so that these are physically closer to the gene. The physical distance between the linked markers can however, be estimated using pulse field gel electrophoresis (PFGE). It involves isolation of high molecular weight plant DNA (> 2 million base pairs, Mb), digestion of DNA with rare cutting restriction enzymes such as *Not* I and *Sfi* I, separation of fragments by PFGE and blotting followed by hybridization with DNA markers flanking the target gene. If both the DNA markers hybridize to the same restriction fragment then, it suggests that the maximum physical distance between the markers equals the size of the hybridizing fragment. Physical mapping helps establishing a relationship between genetic distance (expressed in percent recombination) and physical distance (expressed in base pairs). It guides the next step in the gene isolation process.

Based on the physical distance in the vicinity of the gene, an appropriate genomic DNA library is chosen. The clones in the library individually must carry large fragments such that only a few clones are needed to represent the whole genome. One such library,

often used in gene isolation is based on *yeast artificial chromosomes (YAC)* which are constructed by combining together the essential structural features of a chromosome such as telomere, centromere and origin of replication. The YACs can carry insert DNA varying in size from 100-2000 kb. In case, the flanking markers of the target gene hybridize to the same YAC, it is accepted that the gene is physically located in the same cloned DNA fragment. On the contrary, when the two flanking markers hybridize to two different YAC clones, a technique called *chromosome walking* is employed. Here, one starts walking along the chromosome from one marker and continues till the clone hybridizing to the other marker is reached. While traversing the genomic region flanked by the two DNA markers one employs DNA clones having partial overlapping at the ends.

In this process, the clone carrying the gene of interest is identified which can be directly used to transform a plant which lacks in the target trait. This way, the isolation of the target gene is confirmed. Instead of using the whole YAC, it is possible to identify DNA clones from a library of DNA sequences complementary to messenger RNA molecules and use them subsequently in genetic transformation.

Genes successfully isolated by following this approach include *Pto* that confers resistance to *Pseudomonas syringae* in tomato (Martin *et al.* 1993) and the bacterial leaf blight resistance gene *Xa 21* in rice (Song *et al.* 1995). The *Xa 21* gene has been already used to transform susceptible cultivars of rice for engineering resistance to the bacterial leaf blight pathogen. However, large genome size and presence of highly repetitive DNA sequences in majority of plant genomes are hinderances to the use of this strategy in all crop plants. Therefore, alternative strategies based on the syntenic relationship with small related genomes are being designed to deal with larger genomes like that of wheat.

**A TEXT BOOK ON
PLANT BREEDING
(ANNEXURE)**

Y. ANBU SELVAM

R. ELANGAIMANNAN

Y. ANITHA VASLINE

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CLASSIFICATION OF PLANTS

Systematic Botany or Taxonomy deals with the classification of plants. The study is based upon the structural resemblances of plants and is helpful in identifying them. The resemblances are brought out by describing, naming and arranging the plants in a methodical manner. Since for describing plants correctly, a thorough knowledge of morphology is necessary, morphology and taxonomy always go hand in hand.

Types of classification

The different types of classification proposed by earlier taxonomists can be broadly categorized into three systems– artificial, natural and phylogenetic.

Artificial system

It was based on one or at most only a few superficial characters. In 1753, **Carolus Linnaeus** of Sweden published his book “**Species Plantarum**” wherein he described 7,300 species. He divided the plants into 24 classes based on number, union, length and certain other characters of stamens. Hence, this system is also known as *sexual system of classification*.

The main defect of this system is that totally unrelated plants are brought together in a single group and those that are closely related plants are placed in widely separated groups. For example, plants belonging to Zingiberaceae of Monocotyledons and that of Anacardiaceae of Dicotyledons had been placed in one group called Monandria, as these possess only one stamen. Another defect of this system was that no importance was given to either natural or phylogenetic relationships among different groups of plants.

Natural system

In this system of classification, plants are classified based on their natural affinities. More number of characters is taken into consideration in this system. It is mainly based on all the information that were available during the time of direct observation of plants. The most important natural system of classification of seed plants was proposed by two British botanists *George Bentham and Sir Joseph Dalton Hooker*. It helps to determine the relationships between various groups of plants. However, it does not attempt to bring out evolutionary relationships among different groups of plants.

Bentham and Hooker's system of classification

Below is given a synopsis of Bentham and Hooker's system:

I. Dicotyledons

A. Polypetale (Corollas of separate petals)

Series 1- Thalamiflorae (Stamens hypogynous and usually, many, no disc present).

Cohorts: Ranales, Parietales, Polygalineae, Caryophyllineae, Guttiferales and Malvales.

Series II- Disciflorae (stamens hypogynous, disc present).

Cohorts: Geraniales, Olacales, Celastrales, , Sapindales.

Series III - Calyciflorae (Stamen perigynous or epigynous, Ovary mostly inferior).

Cohorts: Rosales, Myrtales, Passiflorales, Ficoidales and Umbellales

B. Gamopetalae (corolla of partially or completely connate petals)

Series I – Inferae (Ovary inferior).

Cohorts: Rubiales, Asterales, Campanulales.

Series II- Heteromerae (Ovary superior, androecium, 1, or 2 carpels mostly more than 2)

Cohorts: Ericales, Primulales and Ebenales.

Series III- Bicarpellatae (Ovary superior, androecium of 1 series, carpels 2).

Cohorts : Gentinales, Polemoniales, Personales, Lamiiales

C. Monochamydeae (Flowers Apetalous)

Series 1- Curvembryae (Embryo coiled, ovule mostly 1)
Nyctagineae, Chenopodiaceae, Amaranthaceae, etc.

Series II Multiovulate aquatica (Several, seeded, immersed aquatics) . Podostemaceae, etc.

Series III Multiovulatae terrestres., Nepenthaceae, Aristolochiaceae, etc.

Series IV - Micrembryae (embryo minute in endosperm)
Piperaceae. Myristicaceae, etc.

Series V- Daphnales (Ovary unicarpellate, uniovulate).
Laurinae, Proteaceae, etc.

Series VI- Achlamydosporae (Ovary usually inferior, unilocular, ovules 1-3). Santalaceae, Loranthaceae, etc .

Series VII-Unisexuales (Flowers unisexual) Euphorbiaceae, Urticaceae, etc.

Series VIII -Orders anomaly (of uncertain relationships nearer to VII than to anything else). Ceratophyllaceae, etc.

II. Gymnospermae

Gnetaceae, Coniferae, Cycadaceae

III. Monocotyledons

Series I - Microspermae.(Ovary Inferior, seeds minute).
Orchidaceae, Hydrocharitaceae, etc.

Series II -Epigynae (Ovary usually inferior; seeds generally large).
Scitamineae, Amaryllidae, Irideae, etc.

Series III-Coronarieae (Ovary superior, perianth coloured).
Liliaceae, etc.,

Series IV - Calycineae (Ovary superior, perianth greenish).
Juncaceae, Palmae, etc.

Series V - Nudiflorae (Perianth mostly none, seed albuminous).
Pandanaceae, Aroideae, etc.

Series VI - Apocarpae (Pistils more than one and distinct)
Alismaceae, Naiadaceae, etc.

Series VII- Glumaceae (Perianth reduced, Scaly bract present, conspicuous) Cyperaceae, Gramineae

Phylogenetic system

This system is based on evolutionary sequence as well as genetic relationships among different groups of plants. In addition to this, it employs as many taxonomic characters as possible. Charles Darwin's concept of Origin of Species had given enough stimuli for the creation of phylogenetic system of classification. Adolf Engler (1844-1930) and Karl Prantl (1849-1893) of Germany published a phylogenetic system in their monograph on "Die Natürlichen Pflanzen Familien". In this system, floral characters such as single whorl of perianth or no perianth and unisexual flowers pollinated by wind were considered as primitive characters when compared to perianth with two whorls, bisexual flowers pollinated by insects. According to them, members of Asteraceae of dicotyledons and Orchidaceae of monocotyledons were highly advanced.

Engler and Prantle's system of classification

Division I: Myxothallophyta - embracing the Myxomycetes.

Division II: Euthallophyta - including Algae and Fungi.

Division III : Embryophyta Zoidiogamae (Arehegoniatae) -including
Bryophytes and Pteridophytes.

Division IV: Embryophyta siphonogamia -including Gymnosperms
and Angiosperms.

I. Subdivision: Gymnospermae.

1. Class: Cycadales.

2. „ Cordaitales
3. „ Bennettitales
4. „ Coniferae
5. „ Gnetales

II. Subdivision: Angiospermae

1. Class: Chalazogamae
Series: Verticillatae
2. Class: Acrogamae

I. Sub-class-Monocotyledonae.

- | | | | |
|-----------|-------------|-----------|---------------|
| 1. Series | Pandanales. | 6. Series | Spathiflorae. |
| 2. " | Helobiae. | 7. " | Farinosae. |
| 3. " | Glumiflorae | 8. " | Liliflorae. |
| 4. " | Principes. | 9. " | Scitamineae |
| 5. " | Synanthae. | 10. " | Microspermae |

2. Sub-class-Dicotyledonae.

1. Group of Series-Archichlamydeae.

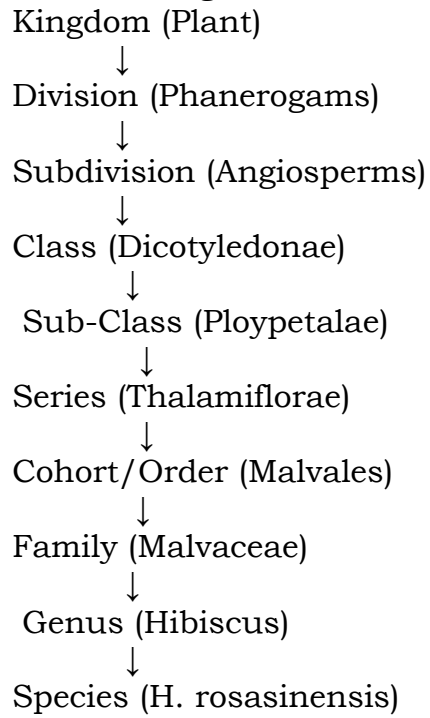
- | | | | |
|---------------|------------------|------------|---------------|
| 1. Series | Piperales | 13. Series | |
| Sarraceniales | | | |
| 2. " | Juglandales. | 14. " | Rosales. |
| 3. " | Salieales. | 15. " | Geraniales |
| 4. " | Fagales. | 16. " | Sapindales |
| 5. " | Urticales. | 17. " | Rhamnales |
| 6. " | Proteales. | 18. " | Malvales. |
| 7. " | Santalales. | 19. " | parietales |
| 8. " | Aristolochiales. | 20. " | Opuntiales. |
| 9. " | Polygoniales. | 21. " | Thymeleales |
| 10. " | Centrospermae. | 22. " | Myrtiflorae |
| 11. " | Ranales. | 23. " | Umbelliflorae |
| 12. " | Rhoeadales. | | |

2. Group of Series-Sympetalae.

- | | | | |
|-----------|-------------|-----------|----------------|
| 1. Series | Ericales. | 6. Series | Plantaginales. |
| 2. " | Primulales | 7. " | Rubiales. |
| 3. " | Ebenales. | 8. " | Aggregatae |
| 4. " | Contortae. | 9. " | Campanulatae |
| 5. " | Tubiflorae. | | |

Categories of classification

Below is given an arrangement of the various units of classification in their descending order:



BOTANICAL DESCRIPTION-FLORAL BIOLOGY

FAMILY- MALVACEAE - The cotton family

Distribution

This family includes about 82 genera and more than 1,500 species. The plants are cosmopolitan in distribution, more abundant in tropical and subtropical regions. In India, Malvaceae is represented by 22 genera and 125 species.

Habit

Plants may be annual herbs or perennial shrubs. The members of this family have mucilaginous substance. Stellate hairs occur on their young parts.

Root

Tap root system.

Stem

Aerial, erect, branched, woody and usually covered with stellate hairs.

Leaf

Petiolate, simple, entire or palmately lobed (eg. *Gossypium arboreum*), alternate, stipulate and showing reticulate venation.

Inflorescence

Solitary, terminal or solitary, axillary or terminal or axillary cyme.

Flower

Bracteate or ebracteate, bracteolate or ebracteolate, pedicellate, dichlamydeous, pentamerous, complete, actinomorphic, regular, bisexual and hypogynous.

Epicalyx

Bracteoles forming a whorl outer to calyx are called epicalyx. Bracteoles 3.

Calyx

Sepals 5, green, gamosepalous showing valvate aestivation.

Corolla

Petals 5, coloured, polypetalous but slightly fused at the base due to adhesion with staminal tube, regular and showing twisted aestivation.

Androecium

Numerous stamens, filaments are fused to form a staminal tube around the style and monadelphous. The staminal tube is fused with the petals at their bases. Anthers are monothecous, reniform, transversely attached to filaments and transversely dehiscent.

Gynoecium

Ovary superior, two to many carpels but usually 5 to 10 carpels and syncarpous. Ovary with two to many locules. Pentacarpellary.

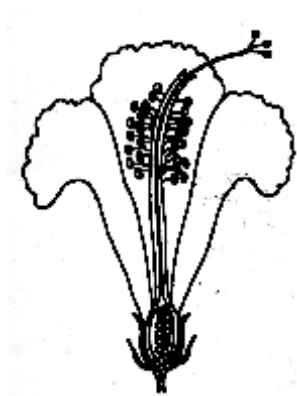
Number of locules usually corresponds to number of carpels. Each locule contains one to many ovules on axile placentation. Style long, slender and passes through the staminal tube ending in two to many distinct round stigmas.

Fruit

Boll or Loculicidal capsule

Seed

Endosperm is scanty, covered with hairs as in *Gossypium barbadense*.



L.S OF A FLOWER



Androecium



Gynoecium



Floral Diagram

Floral formula: Br., Brl. $\uparrow$ $\frac{5}{5} + \frac{5}{5} \frac{5}{5} \frac{5}{5} \frac{5}{5} \frac{5}{5}$

FAMILY: COMPOSITAE (Asteraceae) -The Sunflower Family

Commonly known as sunflower family. It is the largest family in dicots. It includes about 900 genera and 13,000 species. It has a cosmopolitan distribution. There are around 100 species in India.

Habit

Mostly annual herbs, rarely shrubs and trees

Root

Taproot system, branched

Stem

Herbaceous or woody, erect, branched.

Leaves

Simple, alternate or opposite, exstipulate, petiolate, hairy, reticulate venation.

Inflorescence

Head or capitulum, terminal or axillary. Florets surrounded by an involucre of bracts.

Flower

Bisexual, pentamerous, heterochlamydeous, epigynous.

Ray florets: Towards periphery, sessile, bracteate, pistillate or neutral, zygomorphic, ligulate.

Calyx

Absent or hairy pappus, or scaly persistent.

Corolla

Petals 5, polypetalous, ligulate.

Androecium

Absent

Gynoecium

Bicarpellary, syncarpous, unilocular, one ovule, the locule, basal placentation, style narrow, stigma branched, ovary inferior.

Disc Florets

Towards the centre, bracteate, bisexual, actinomorphic, tubular, epigynous.

Calyx

Absent or modified into pappus.

Corolla

Sepals 5, Gamosepalous, tubular.

Androecium

Stamens 2, epipetalous, syngenesious ditheous.

Gynoecium

Bicarpellary, Syncarpous, unilocular, one ovule, on basal placentation style single, stigma bifid.

Neutral florets

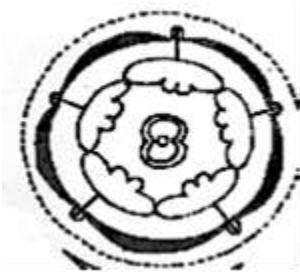
Both androecium and gynoecium are absent. Remaining characters similar.

Fruit

chene or Cypsela.

Seed

Non-endospermic.

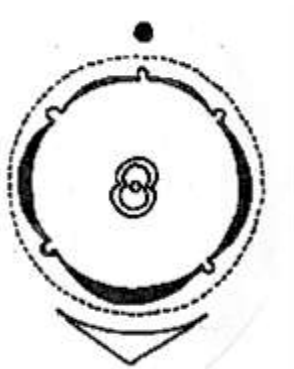


Floral Diagram of Disc Floret

Br., Eb^{rl}., $\uparrow$ ♀, K ∞, C₍₅₎, A₍₅₎, G₍₂₎



Syngenealous anthers



Br., Eb^{rl}., %, K ∞, C₍₅₎, A₀, G₍₂₎



Head or capitulum

A twig of sunflower with capitulum

FAMILY: LEGUMINOSAE (Fabaceae)-The pulse family

Root

Branched taproot, usually with nodules containing bacteria of genus *Rhizobium*.

Stem

Herbaceous or woody, branched.

Leaf

Simple or compound, alternate, stipulate, leaf base is pulvinate.

Inflorescence

Racemose, simple or compound.

Flower

Bracteate, zygomorphic, bisexual, pedicellate, heterocheilamydeous, pentamerous, hypogynous.

Calyx

Sepals 5, gamosepalous, hairy, valvate or imbricate aestivation

Corolla

Petals 5, polypetalous, papilionaceous, with a standard (vexillum) petal, two wing petals (Alae) and two keel petals (carinae). Vexillary aestivation.

Androecium

Stamens 10, monadelphous or diadelphous, anthers ditheous.

Gynoecium

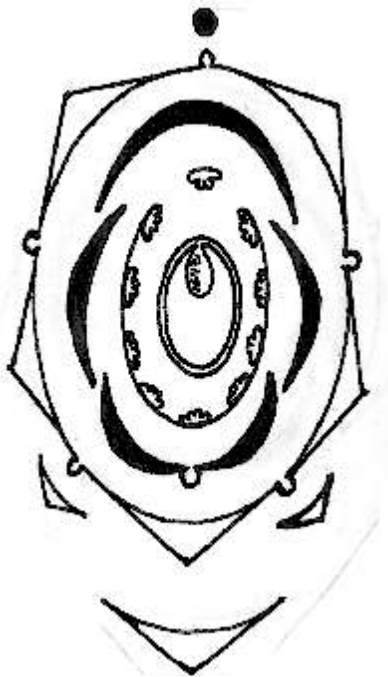
Monocarpellary, unilocular with ovules on marginal placentation. Ovary superior.

Fruit

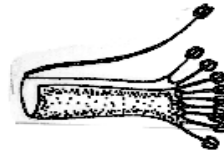
A legume or lomentum.

Seed

Large, Non-endospermic



Floral diagram



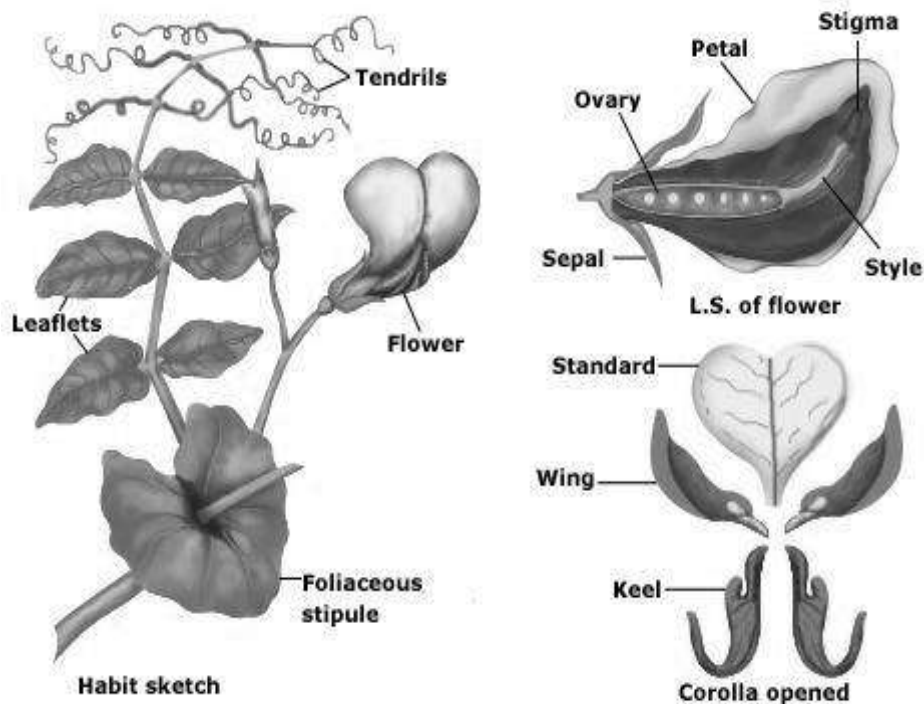
Androecium



Gynoecium

Floral formula:

Br., Brl., $\uparrow$ %, $\ominus$, K (5), C 5, A (9) + 1, G 1



FAMILY-GRAMINEAE (Poaceae) -The grass family

Habit

Mostly annual or perennial herbs. A few shrubs. Stem usually hollow in the internodal and solid at the nodal portions. In some like sugarcane and maize the stem is solid throughout. Branches arise generally in tufts from the axils of the lower leaves, while at the top there are no branches. Nodes swollen. In some cases this swelling (pulvinus) is caused by the leaf base, while in others it belongs to the stem.

Leaves

Alternate and distichous, with sheathing bases. The sheath covers the internode immediately about the node from which the leaf starts and may be either completely tubular or split longitudinally. Where the sheath joins the blade there is a ligule. Blade usually linear or linear-lanceolate, with parallel venation.

Inflorescence

Spikes or panicles. These are composed not of individual flowers, but of very small spikes known as spikelets. So the spikelets are the unit of inflorescence. Each spikelet has a central axis (rachilla) on which is arranged a number of alternate bracts. Flowers occur in the axils of these bracts. A spikelet may have 1-4 flowers. The bracts are known as glumes. Generally in each spikelet, the lower most glumes

are empty and do not have flowers in their axils. The upper ones which are fertile and have flowers in their axils are called lemmas. On the posterior side of the flower there is a bracteole known as palea. the flower is well protected between the lemma and palea.

Flowers

Usually bisexual. In some case unisexual (maize) and monoecious.

Perianth

Reduced to two very small, succulent scales known as the lodicules. They are found at the base of the flower towards the side of the lemma. The lodicules are hygroscopic and swell on absorbing moisture. They press against the bract and thus cause an opening between the lemma and palea. Through this opening, the stamens and stigma protrude.

Stamens

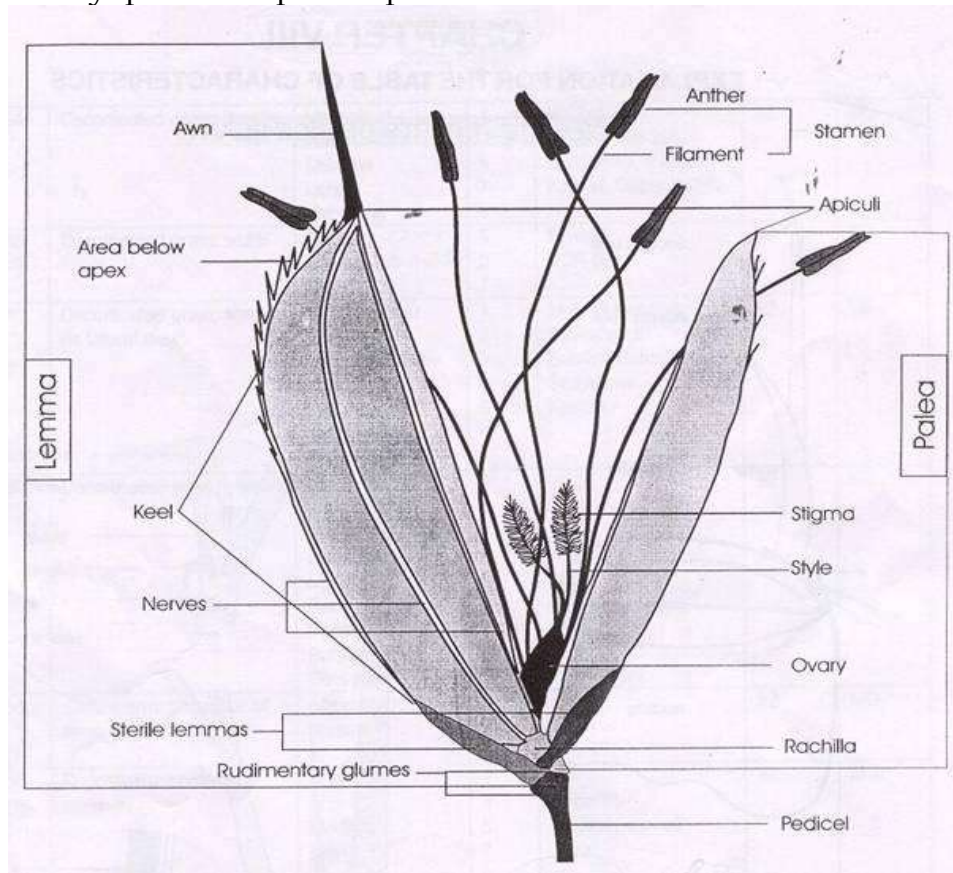
Generally 3. filaments are long and slender. Anthers versatile.

Pistil

Ovary superior, monocarpellary and single-celled with one basal, erect anatropous ovule. style 2, with feathery stigma.

Fruit

A caryopsis. The pericarp is fused with the seed coat.



FLORAL STRUCTURE OF RICE

RICE



Floral Diagram

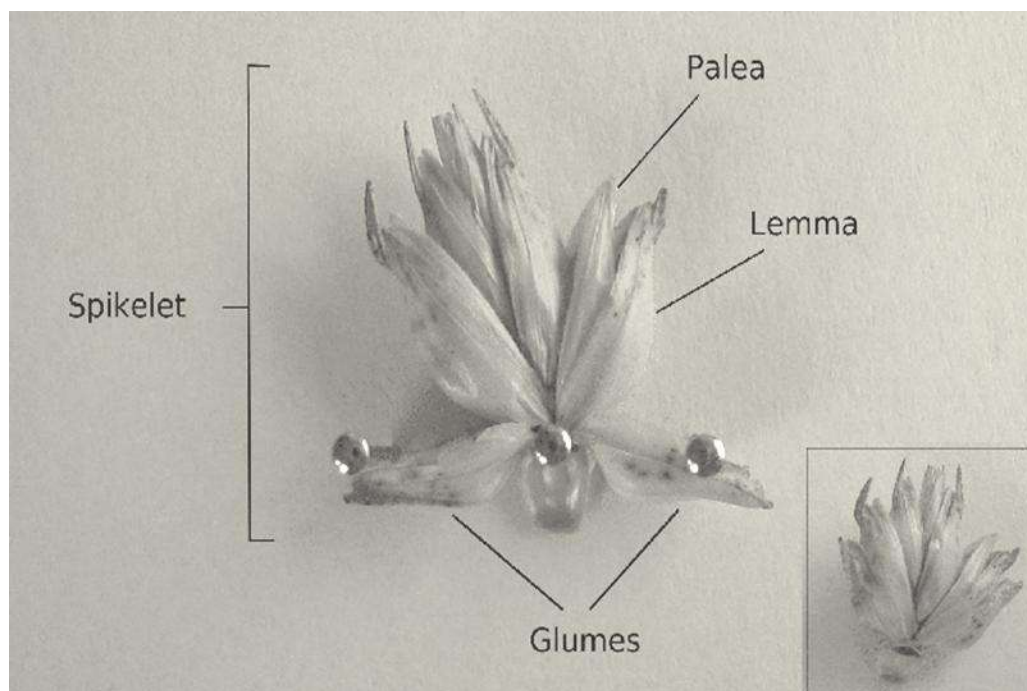
Br, Br1, %, ♂, P3(lodicules), A6, G1
+

EMASCULATION AND POLLINATION TECHNIQUE

RICE

Botany and Genetics of Rice

The genus *Oryza* is a member of the grass family, Gramineae. The rice inflorescence is a panicle which bears single-flowered spikelets. The rice flower differs from that of other cereals in having six stamens. The flower is surrounded by a lemma and palea, structures which form the hull or husk that encloses the threshed grain or paddy. The outer glumes are usually obscure, being only about one-fourth the length of the lemma and palea, although in some varieties they approach the lemma and palea in length.



SPIKELET OF RICE

Emasculation and Pollination in Rice

The blooming of rice normally occurs between 8 and 11 A.M. The flowers in a single panicle bloom over a period of seven to ten days. Blooming of the spikelet starts at the apex of the panicle and proceeds downwards. The rice flower is normally self-pollinated. The extent of natural crossing varies from 0 to about 3 per cent with an average of about 0.5 percent, depending upon the variety, the season, and the environment.

Crossing Technique

Before emasculation immature spikelets or those previously flowered are removed from the panicle keeping only flowers that will bloom the following day. The conventional method, still used by some rice breeders, is to separate the glumes with a pair of forceps, in the early morning about 1 to 2 hours before pollination, and remove the six stamens. Emasculation and pollination may be facilitated clipping across the anthers just above the attachment of the filament at the same time and expose the remnants of the anthers so that they can be removed with fine pointed tweezers. Emasculations are done in the afternoon and pollinations the following morning. After emasculation, flowers are covered with a butter paper bag.

Hot water may be used to open the florets of rice before crossing. In the hot water treatment the rice panicle is immersed in

water contained in a thermos flask at 42 to 44 degrees Centigrade for a period of 5 to 10 minutes. Panicles in the second or third day of blooming are chosen as female parents and spikelets that have already flowered and immature florets are removed. An hour or so before blooming normally begins; the tiller is bent over carefully to avoid breaking and inserted into the hot water. The thermos bottle may be supported on a trough like holder at an angle of about 35 degrees to prevent loss of water. This treatment causes the florets to open in a normal manner and the stamens may be removed without injury to the stigma. Pollinations must be made within a 30 minute period before the glumes close naturally.

Successful pollinations require mature and healthy anthers. Florets nearing the blooming stage are opened, and the turgid anthers are taken with forceps and broken over the stigma. Pollen may also be dusted over clipped florets by shaking a shedding panicle over them. Pollen of rice does not normally remain viable for more than few minutes. Seed set in rice is generally lower than with wheat or barley.

WHEAT

Emasculation and Pollination in Wheat

Wheat is a self-pollinating crop. Blooming normally starts several days after the wheat spike emerges. The main culm flowers bloom first and the tillers later, in the order of their formation. Flowering begins in the upper part of the spike and proceeds in both directions. Flowering continues throughout the day, with two to three days required for a spike to finish blooming. The glumes normally open during the flowering process, the anthers protrude from the glumes, and part of the pollen is shed outside the flowers. To exclude all cross-pollination in breeding or genetic studies the spike may be covered with a butter paper envelope prior to flowering.

Wheat flowers are emasculated on the day prior to their shedding pollen by clipping back the glumes and removing the anthers with fine-pointed tweezers. Pollinations are made one or two days later by breaking a ripe anther over the stigma. Crossing may be facilitated by the utilization of male sterile lines, thereby eliminating the emasculation process. Genetic male sterility and cytoplasmic male sterility are both available in common wheat.

MAIZE

Pollination in Maize

Maize bears monoecious flowers with staminate flowers produced in the tassel and pistillate flowers on the shoot. Pollination is accomplished by the transfer of pollen from the tassel to the silks (cob). About 95 per cent of the ovules on a shoot are cross-pollinated and about 5 per cent are self-pollinated. Most of the pollen that pollinates an ear of maize usually comes from stalks in the immediate

vicinity, but pollen may be carried by the wind for distances up to one kilometre.

As the tassel flowers open, the anthers are pushed out by the elongating filaments and pollen grains are emptied from the extruded anthers. It has been estimated that a single tassel from a normal plant may produce as many as 25,000,000 pollen grains, or an average of over 25,000 pollen grains for each kernel on an ear with 800 to 1,000 kernels. Pollen shedding begins one to three days before the silks have emerged from the husks of the same plant, and usually continues for a period of several days after the silks are ready to be pollinated. Hot, dry weather tends to hasten the pollen shedding.

Under favourable conditions pollen may retain its viability for 18 to 24 hours, but it may be killed in a few hours by heat or desiccation. A hot, dry wind may injure the tassel so that it does not shed pollen, or it may reduce the silk moisture so that pollen grains will not germinate. Maize propagated from seed that has been produced by uncontrolled pollination is commonly referred as *open-pollinated Maize*.

SORGHUM

Botany of Sorghum

The sorghum head varies from a compact to an open panicle. The spikelets usually occur in pairs, one being sessile and the second borne on a short pedicel, except for the terminal spikelet which is borne on a branch and is accompanied by two pediceled spikelets. The sessile spikelet contains a perfect flower. The pediceled spikelet is usually sterile.

Emasculation and Pollination in Sorghum

The amount of natural cross-pollination in sorghum averages about 6 per cent. Artificial cross-pollinations are made by emasculating the seed parent and hand-pollinating it with pollen collected from the pollen parent. Hand emasculations are made by using fine-pointed tweezers, a dissecting needle, a sharp pencil point, or a small emasculation instrument to remove the anthers. Usually, only a small branch of the panicle is emasculated. Enough of the panicle is clipped away to permit bagging the emasculated head, but too much trimming may have an adverse effect on seed set due to drying out. Pollen is collected in bags in the same manner as with maize and dusted over the exposed stigmas, or the pollen producing head may be brushed or dusted over the emasculated head. A mass method of emasculation has been devised which uses heat to kill the pollen. In this method the sorghum head is immersed in water heated to a temperature of 48 degrees Centigrade and left for 10 minutes. This temperature kills the pollen but not the pistil. Some sorghum is more sensitive to heat than others and a slightly lower temperature, 47 to 47.5 degrees Centigrade, may need to be used to avoid injury. Emasculated heads, and heads already pollinated, are enclosed in paper bags to protect them from wind blown pollen. Flowers on male sterile plants do not need to be

emasculated but must be bagged before natural cross-pollination occurs, and may then be pollinated as described above when the stigmas are receptive. Both genetic and cytoplasmic male sterility are available in sorghum.

CUMBU(Pearl millet)

Botany, Emasculation and Pollination in Cumbu

Pearl millet is robust, rapid growing, erect, tropical, annual and cross-pollinated crop due to **protogyny**.

The inflorescence is cylindrical spike like ear, with tapering tip. A rosette of bracts consisting of bristles and spikelets united at the base and known as involucre encloses a flower cluster arising from the cen'tral rachis. Each involucre may include one to nine fertile spikelets. A single spikelet is really a secondary spike having one upper and one lower floret. The lower floret is staminate or sterile. The staminate lower floret consists of a single lemma and three stamens. The upper floret is fertile and has three anthers and one pistil with two feathery stylar branches. These structures are enclosed between lemma and palea.

The seed is typically obovoid, ranging from light grey, deep grey and pearly amber to deep yellow and purple. White and brown seeds have also been reported.

Crossing

The stigmas remain receptive for one to two days. Anthers emerge in two waves. The first wave involves the perfect flowers and the second wave commences 2-3 days later involving the staminate florets. Anthesis starts from the upper third of the head and proceeds towards the base. Under field conditions pollen grains remain viable for 5-7 hours. Around 5°C, pollen grains may be viable upto four days. For self-pollination, the spike is bagged before emergence of the stigmas. Sometimes two spikes of different age may be bagged together.

For cross pollination, about four fifths of the upper portion of the spike is clipped and the left over portion is bagged before the emergence of the stigmas. Likewise the ears of the pollen parent are also bagged. As soon as the stigmas of the female parent emerge, the entire panicle is dusted by shaking a pollen shedding spike of the male parent. The bag of the female parent is cut along the top and the clipped earhead of male parent is introduced into the female bag and rotated around the earhead gently brushing the stigmas. The bag, on the female is folded down and stapled. Details of the cross, the date, and the person's name are written on the bag using waterproof marker.

When the female and male parents are closer, are of the same height and synchronous in Flowering, the earheads of both the parents are enclosed in one bag for cross-pollination. This method is particularly suitable for making crosses between maintainer male sterile and fertile lines.

RAGI(Finger millet)

Botany of Ragi

The inflorescence of ragi consists of a group of digitately arranged spikes, the fingers, with one spike, the thumb, generally below the main terminal group. The spikelets are crowded into two overlapping rows on the outer sides of the spike. Each spikelet contains from 4 to 6 flowers. The flowers are perfect except for the terminal flower which may be either staminate or sterile. The flowers overlap each other forming a compact tapering spikelet. Each perfect flower contains three stamens, a branched stigma and two lodicules. The carpel is closely covered by the palea.

Emasculation and Pollination in Ragi

The spikes may take 6 to 8 days to complete flowering. The top flower opens first with flowering proceeding downwards, but within a spikelet the lowest flower opens first and the process continues upward. The flowers open between 1 and 5 A.M. Pollen viability is very short, lasting only 10 to 15 minutes. Flowering takes place simultaneously in all Fingers. The anthers require about 45 minutes for dehiscence after emergence. This delay in opening permits emasculation after the anthers have emerged. The stigma remains receptive for about five minutes after emergence from the glumes. Since the period of anthesis is very short, self-pollination is the rule. However, a very small per cent of natural cross-pollination may occur.

Several methods have been proposed for emasculation and artificial cross-pollination. With hand emasculation the flower is selected on the evening previous to its natural opening. Other flowers in the spikelet are cut off and the three stamens of the selected flowers are removed after gently separating the lemma and palea. Hand emasculation are difficult to make due to the small size of the flowers and are usually carried out under a magnifying glass. Hot water may be used in making emasculations. Immersion of 2 minutes at 52°C centigrade is said to give good pollen killing.

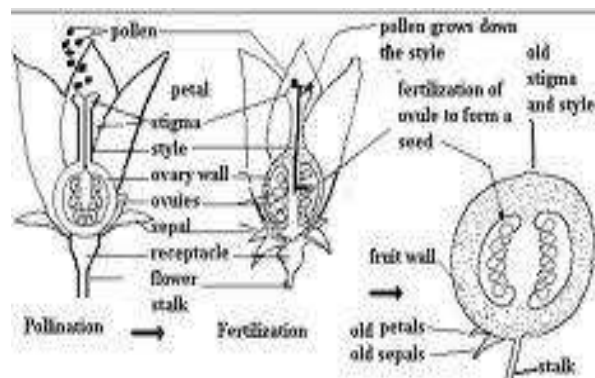
Pollinations are made by collecting ripe anthers and breaking them over the stigmas or by dusting a flowering branch of the male parent over the emasculated spikes. The contact method in which heads of the two varieties are put together under bags may also be used without emasculation.

COTTON

Emasculation and Pollination in Cotton

The base of the cotton flower is surrounded by three leaf-like triangular bracts forming what is commonly known as *squares*. Pollen is shed directly on the stigma when the anthers open, or it may be carried to the stigma by insects. Pollen is wind-borne only to a very slight extent, if at all, on account of its heavy sticky nature, but pollen carried by insects may result in considerable cross-pollination. The

amount of cross-pollination normally ranges from 5 to 25 percent although amounts in excess of 50 per cent have been reported.



A simple technique has been developed for artificially cross-pollinating cotton flowers. Crosses are made on the day preceding that in which the flowers would open normally. The corolla is cut away with small scissors or a curved scalpel and the anthers are removed. Pollen is collected from the male flower in a short length of a soda straw. The soda straw partly filled with anthers is slipped down over the exposed stigma. The bracts are pulled up around the soda straw and wired securely to hold the soda straw in place. Some breeders recommend emasculating one day and pollinating the next, but this procedure may result in unnecessary drying out of the stigma. About 75 per cent of normal seed set may be obtained after artificial crossing.

When cotton plants are to be selfed, it is necessary for the flower to be covered to prevent cross-pollination. A small paper bag is placed over the bud the afternoon before it opens.

GREENGRAM, BLACKGRAM, BENGALGRAM

Pollination and Emasculation in Greengram, Blackgram, Bengalgram

Flowers are generally emasculated in the evening and pollinated the next morning. For emasculation, flowers that will open one or two days later are selected, and the rest of the flowers and buds in a branch are removed. The stamens of the selected buds are removed with a pair of fine forceps by gently pushing the keels apart. The emasculated floral branch is then bagged. Utmost care is necessary in emasculation as the flowers in some species, as in blackgram for example, are very sensitive and may shed after emasculation or even after pollination. Magnifying glasses may be needed in emasculating very small flower buds. Ripe anthers are collected the following morning and pollination is done by gently pressing a ripe anther against the stigma. The flowers are again bagged after pollination until the pods are mature.

To ensure selfing the flowers need to be bagged, also, as insects may sometimes carry pollen to the stigma and bring about cross-pollination. In most breeding studies the amount of natural cross-pollination is so small that it may be ignored.

REDGRAM (Pigeonpea)

Emasculation and Pollination in RedGram

Selfing in Pigeonpea

Selfing is done by covering an individual inflorescence, a branch of a plant or a whole plant. When a large amount of seed is required, groups of plants are covered with bee-proof cages.

Bagging

Three types of selfing bags are used for pigeonpea

1. Glassine bag (13 cm x 8 cm) to cover an individual inflorescence.
2. A small-muslin-cloth bag (60 cm x 20 cm) to cover a flowering branch
3. A large-muslin-cloth bag (135 cm x 90 cm) to cover an entire plant.

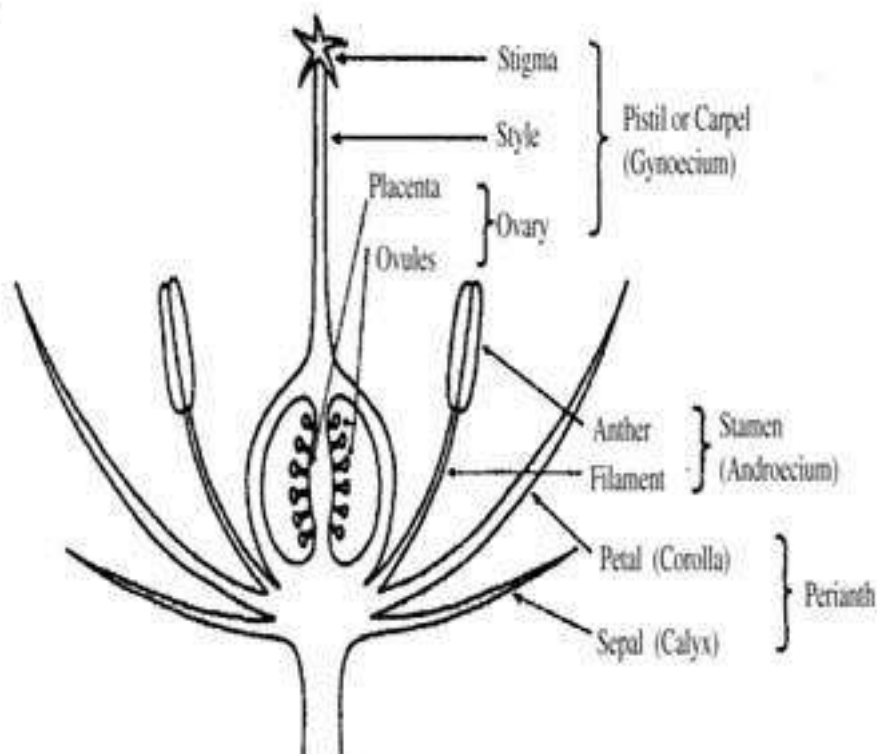
Bee-proof cages

To produce a large quantity of seed, bee-proof cages are used for handling the segregating generations. These cages are formed with nylon net on a movable angle-iron frame that covers the plot. The objective of a cage is to control the insects.

Emasculation

The bud most likely to shed pollen the next day is selected for emasculation. Hold the bud firmly between the thumb and middle finger with support of the index finger so that the keel of the standard is facing towards the emasculator. Remove or push down the sepal covering the keel, Open the corolla by inserting one tip of the forceps at the base of the keel. Move the forceps upward to the tip of the standard. Press the

bud with a slight pressure until it opens. This makes the anthers visible. With the forceps remove the anthers from the staminal column. Count the anthers or examine the bud with a magnifying glass to ensure that all the anthers or their broken parts are completely removed.



Floral structure of Redgram

Pollination

The pigeonpea stigma is receptive before anthesis, therefore, pollination can be done immediately after emasculation. Remove the corolla of the pollen-source bud so that the staminal columns and anthers are uncovered. To transfer pollen hold the flower in one hand and touch the anthers to the stigma of an emasculated bud. A single flower can be used to pollinate two or three emasculated flowers .

To identify the artificially pollinated buds small, bright-colored, thin nylon thread is tied to the peduncle of the bud. Seeds are harvested only from such pods.

BENGAL GRAM (Chickpea)

Emasculation and Pollination in Bengal Gram

Crossing in chickpea is usually difficult and time consuming as is the case with most of the legumes. For emasculation, the bud is held in the left hand and gentle pressure is applied to open the standard and wing petals. The keels are opened with forceps and the stamens removed. Pollen is collected from half-open flowers and applied on stigma. For better pod setting after crossing, the crossing should be attempted after formation of first pod on the plant and as far as possible, large bud sized cultivars should be used as female parent.

COWPEA

Emasculation and Pollination in Cowpea

Cowpea flowers are large and showy. Mostly flowers open between 7 and 9 a.m. On cloudy days the flowers may open even in the afternoon. Though the flowers open late in the morning, the dehiscence of the anthers is much earlier. It may vary from 10 p.m. to 0.45 a.m. The dehiscence is influenced by environmental factors like presence of moonlight, a clear sky and a dry warm atmosphere. During dark nights the dehiscence tends to be delayed. Due to dehiscence taking place before the opening of flowers, the cowpea is self-pollinated in nature.

Since the dehiscence of anthers is much in advance of the blooming, the emasculation needs to be carried out in mature flower buds in the preceding evening. The flower buds likely to bloom the next day (recognized by large size, the yellowish colour of the back of the standard petal) is selected for emasculation. The bud is held between the thumb and the forefinger with the keel side upper most. A needle is run along the ridge where the two edges of the standard unite. One side of the standard is brought down and secured in position with thumb. Same thing is done with one of the wings. After this the exposed keel is slit on the exposed side, about 1/16 inch from the stigma. A section of keel is also brought down and secured in position under the end of thumb. Now 10 stamens are seen. They are removed with pointed forceps. Afterwards, the disturbed parts of standard, wing and keel are brought in original position as far as possible. To prevent drying out of the emasculated bud, a leaflet may be folded and pinned around the bud. A tissue paper can be used to cover and protect the bud.

Pollination is done next morning from a freshly opened flower. The standard and wings of male flowers are removed. By slight depression of the keel, stigma covered with pollen grains protrudes out. This itself can be used as a brush for pollination. The cowpea flowers are highly sensitive and drop off easily with slight mechanical disturbance or injury. Therefore, much labour and time should be devoted to get enough crossed seed.

SOYABEAN

Emasculation and Pollination in Soyabean

In freshly Opened flower the diadelphous stamens are elevated and the anthers form a ring around the stigma. The pollen is thus directly shed on stigma resulting into a high percentage of natural self-pollination. Natural cross-pollination varies from about less than 0.5% to 1 %. The pollination may occur a day before full opening of the flower i.e., the pollination may occur within the bud.

Special care is needed while emasculating the flower buds as they are very small. Parental cultivars may be grown in pots so that the plant could be raised to the desired height for emasculation. For increased span of flowering, two-three dates of sowings of the parental cultivars are usually required. A floral bud at the appropriate stage is swollen and the corolla is visible through the calyx. Five sepals are removed with forceps to expose the corolla. Corolla is removed with forceps by jerking-one stroke operation. In this process, 10 stamens are usually removed and if a few are left, they are removed.

Pollination is carried out immediately after emasculation. Open flowers are collected from male parent. Corolla is removed and the emasculated flower-bud stigma is brushed with the anthers of the male flower whose corollas are already removed. In event of a successful pollination, a pod is visible in about seven days.

SUNFLOWER

Emasculation and Pollination in Sunflower

Sunflower is highly cross pollinated crop mainly through insects and to a limited extent by wind. The flower opening starts from outer side of the head and proceeds towards centre of the head. The heads bloom within 5-10 days depending upon size and season. Anthesis occurs between 5 to 8 AM. The pollen grain viability lasts for 12 hours. The stigma remains receptive for two-three days.

Selfing is done by bagging of the head. The bagging material could be cotton cloth, tiffany bags or paper bags or cheese cloth bags or plastic netting. Emasculation is done as follows:

Emasculation is done by removing the anther tubes with forceps early in the morning that the flowers open. Unemasculated flowers are removed. Considering hand emasculation tedious, sometimes crosses are made without emasculation. Hybrid plants are distinguished from selfed ones on the basis of vigour or the presence of marker genes.

Pollination

Pollination is carried out by collecting pollen from heads which are already bagged prior to flowering. Pollen can be collected from flowering heads into paper bags by a light tap of the hand on the back of the head. Pollination is usually done in the same morning after emasculation. Pollen can be applied by a small piece of Cotton, a camel hair brush, the corner of the cloth bag isolator, a small section or leaf, paper or other suitable material that is dipped in the pollen and gently drawn over the receptive surface of the stigmas. Freshly collected pollen is more effective in pollination. Pollen can be stored without serious loss of viability for 1-2 weeks in cork-stoppered vials at ordinary room temperature. After each cross, care must be taken to avoid contamination by wiping the hands with alcohol and cleaning or discarding the pollen applicator.

GROUNDNUT

Emasculation and Pollination in Groundnut

Anthesis

Flowering starts about 25-30 days after seedling emergence. The dehiscence of the anthers takes place from 0400 to 0500 and flowers open from 0530 to 0730.

Anthesis is affected by the temperature and humidity. Therefore, it is necessary to control these two factors in the greenhouse. During flowering time, the humidity should be above 70% and the temperature between 28° and 30°C.

Emasculation

Emasculation of groundnut can be accomplished on warm bright days from 1330 to 1630. On cloudy or rainy days, emasculation could be delayed until 2100-2200.

Steps for emasculation

- 1) Select the well-developed bud for emasculation, and remove all other buds at the node to ensure that only one flower develops at that node. Pull the leaf down gently to expose the bud.
- 2) With one hand hold the bud between your thumb and index finger.
- 3) Remove the sepal on the side of the keel and push down the sepal on the side of the standard.
- 4) Open the standard petal with the forceps and pull down the wing petals.
- 5) Hold back the standard petal with the thumb and index finger. Break open the keel with the point of the forceps. Move the keel up and pull it free of the stigma and anthers. The keel is pulled down and held out of the way with the thumb and index finger, while all anthers along with their filaments are removed.
- 6) Now return the standard petal to its original position over the stigma, usually no attempt is made to cover the emasculated flowers for protection from outside pollen.
- 7) A small thread is put on the hypanthium of the emasculated flowers for identification (Norden, 1980) or on the stem above the bud axis. Use different coloured threads on different days to identify emasculated buds for pollination. A record of the number of buds emasculated should be maintained for each parent.

Pollination

On the morning after emasculation, the standard petal is usually expanded and the stigma is exposed between 0600 and 0730. A healthy flower from the male parent (pollen source) is selected. Its corolla is removed exposing the anthers and pollen. Now directly squeeze the pollen on to the emasculated flower stigma or on to the forceps, and transfer the pollen from the forceps to the stigma of an emasculated flower. After pollination, remove all other flowers that were not hand pollinated by breaking their hypanthium near the base.

The maximum physiological development of pollen is from 0500 to 0700.

Pod development and harvest

When fertilization is successful, the tissue below the ovary, called the gynophore, elongates into a peg carrying the ovary at its tip geotropically into the soil. In the soil the ovary takes a horizontal position and a pod develops. Mature pods can be harvested usually 55-65 days after the pegs developed.

SESAMUM

Emasculation and Pollination in Sesamum

Emasculation is easily performed by pulling off the corolla from the buds on the day before opening. The flowers open at dawn; the pollen grains are shed shortly after, and remain viable for about 24 h. The stigmas remain receptive for 2 more days if not fertilized. Hand pollination is done usually. Sesame is considered a self-pollinated crop. However, if insect activity is high outcrossing can reach high levels.

OBJECTIVES AND ROLE OF PLANT BREEDING

Plant breeding aims to improve the characteristic of plants so that they become more desirable agronomically and economically. Thus the chief objective of breeding is to develop such improved varieties of crop plants that will be commercially successful. Specific objectives would vary greatly depending on the crops under consideration. Some of the main objectives of plant breeding may be summerized as follows.

Higher yields

Most of the breeding programmes aim at higher crop yields. This is achieved by developing more efficient genotypes, *eg.*, hybrid varieties of maize (*Z. mays*), sorghum (*S. bicolour*), bajra (*P. americanum*), etc

Improved Quality

The quality of plant produce determines its suitability for various uses. Therefore quality is an important aspect for plant breeders. Quality characters vary from one crop to another. *e.g.*, grain size, colour, milling and baking qualities in wheat, cooking quality in rice, malting quality in barley, size, colour and flavour of fruits, keeping quality in vegetables, protein content in cereals and legumes, lysine content in cereals, methionine and tryptophan content in pulses etc.

Disease and Insect Resistance

Resistant varieties offer the cheapest and the most convenient method of disease and insect management. In some cases, they offer the only feasible means of control, *e.g.*, rusts in wheat. Resistant varieties not only increase production but also stabilize it.

Change in Maturity Duration

It permits new crop rotations and often extends the crop area. Development of wheat varieties suitable for late planting has permitted rice-wheat rotation. Thus breeding for early maturing crop varieties or varieties suitable for different dates of planting may be an important objective in many cases.

Agronomic Characteristics

Modification of agronomic characteristics such as, plant height, tillering, branching, erect or trailing habit etc., is often desirable. For example, dwarfness in cereals is generally associated with lodging resistance and fertilizer responsiveness.

Photoinsensitivity

Development of photo insensitive and thermo insensitive wheat and photo insensitive rice (*O.sativa*) varieties has permitted their cultivation in new areas. Rice is now cultivated in Punjab, while wheat is a major rabi crop in West Bengal.

Synchronous Maturity

Synchronous maturity is highly desirable in crops like mung (*Vigna radiata*), where several pickings are necessary.

Nonshattering Characteristics

It would be of great value in a crop like mung where shattering is a major problem in case of many commercial varieties.

Determinate Growth

Development of varieties with determinate growth is desirable in crops like mung, Pigeon pea (*Cajanus cajan*), cotton (*Gossypium sp*), etc,

Dormancy

In some crops, seeds germinate even before harvesting if there are rains at the time of maturity, e.g., mung, barley, etc. A period of dormancy in such cases would check the loss due to germination. In some other cases, however, it may be desirable to remove dormancy.

Varieties for New Seasons

Traditionally, maize is a kharif crop. But scientists are now able to grow maize as rabi and zaid crops. Similarly, mung is now grown as a summer crop in addition to the main kharif crop.

Moisture Stress and Salt Tolerance

Development of varieties for rainfed areas and for saline soils would be helpful in increasing crop production in India. The major proportion (Ca. 70%) of the cropped area in the country is rainfed. The estimates of salt-affected (saline) soils in the country vary from 7 to 20 million hectares, of which about 2.8 million hectares are alkaline soils. Most of these areas are spread in the states of Uttar Pradesh, Haryana and Punjab.

Elimination of Toxic Substances

Some crops have toxic substances which must be eliminated to make them safe for consumption. For example, khesari (*Lathyrus sativus*) seeds have a neurotoxin, β - N-oxalylamine alanine (BOAA) that causes paralysis. Similarly, brassica oil has erucic acid, which is harmful to human health. Removal of such toxic substances would increase the nutritional value of these crops.

HISTORY OF PLANT BREEDING IN INDIA

- 1871 – The Government of India created the Department of Agriculture
- 1905 – The Imperial Agricultural Research Institute was established in Pusa, Bihar
- 1934 – The buildings of the institute damaged in earthquake
- 1936 – Shifted to New Delhi
- 1946 – Name was changed Indian Agricultural Research Institute

- 1901-05 – Agricultural Colleges were established at Kanpur, Pune, Sabour, Llyalpur, Coimbatore
- 1929 – Imperial council of Agricultural Research was established
- 1946 – Name was change to Indian Council Agricultural Research
- 1921 – Indian Central Cotton Committee was established – Notable researches on breeding and cultivation of cotton. Eg : 70 improved varieties of cotton
- 1956 – Project for intensification of regional research on cotton, oilseeds and millets (PIRRCOM) was initiated to intensify research on these crops – located at 17 different centres through out the country
- 1957 – All India Coordinated maize improvement project was started with objective of exploiting heterosis
- 1961 - The first hybrid maize varieties released by the project
- ICAR initiated coordinated projects for improvement of the other crops
 - 1960 – First Agricultural University established at Pantnagar, Nainital, U.P.

ACTIVITIES IN PLANT BREEDING

The desired changes in genotypes of crop species and the consequent benefits to farmers are brought about by a series of interrelated and largely interdependent activities. These activities are as follows: (1) creation of variation, (2) selection, (3) evaluation, (4) multiplication and (5) distribution.

Creation of variation

Genetic variation is a prerequisite for any improvement in a crop. Therefore, in any breeding programme, this is always the first step unless variation preexists. Genetic variation can be created by domestication, germplasm collection, plant introduction, hybridization (intervarietal, distant, somatic), mutation, polyploidy, somaclonal variation and genetic engineering.

Selection

The next step consists of identification and isolation of plants having the desirable combinations of characters, and growing their progeny; this is called selection. Selection is necessarily based on phenotype. The efficiency of this activity determines the success of a breeding program. Various breeding methods have been designed to increase the efficacy of selection. Selection finally yields an improved line/strain or population.

Evaluation

The newly selected lines/strains/populations are tested for yield and other traits and compared with the existing best varieties called **checks**. Evaluation is a stepwise process, ordinarily conducted at several locations for 3 or more years under the concerned All India

Coordinated Crop Improvement Project. If the new line/strain/population are superior to the checks, it is released and notified as a new variety and its seed can now be multiplied and, more importantly, certified by a seed certification agency for quality.

Multiplication

This step concerns with a large scale production of certified seed of the released and notified variety. Seed production is usually done by seed production agencies in a step-wise manner, and the seed is certified by a seed certification agency.

Distribution

Certified seed is ultimately sold to the farmers who use it for commercial crop cultivation. This activity alone makes it possible to reap the economic benefits from the above activities in form of (1) an enhanced and (2) stable production of (3) superior produce (4) often at a lower cost.

QUARANTINE

Quarantine means to keep materials in isolation to prevent the spread of diseases etc. present in them to the other materials.

Introduced plant propagules are thoroughly inspected for contamination with weeds diseases and insect pests. Materials that are suspected to be contaminated are fumigated or are given other treatments to get rid of the contamination. If necessary, the materials are grown in isolation for observation of diseases, Insect pests and weeds, This entire process is known as **quarantine** and the rules prescribing them are known as quarantine rules.

SIGNIFICANCE OF POLLINATION

For the breeder to understand the results of selection within a mixed population, it is necessary that he knows something about the genetic nature of the plants with which he is working. Plants that are normally self-pollinated differ in genetic make-up from plants that are normally cross-pollinated. In a crop that is self-pollinated, it is the rule that plants will be homozygous. This assumption may be made since: (a) homozygous gene pairs (AA or aa) will remain homozygous with self-pollination; (b) heterozygous gene pairs (Aa) will segregate producing homozygous and heterozygous genotypes in equal proportions. With, self-pollinations, heterozygosity are reduced by one half with each successive self-fertilization. After several successive generations of self pollination, the proportion of heterozygous plants remaining in a population is very small. Even though complete homozygosity for all characters is virtually unattainable, at least from a theoretical standpoint, a practical state of homozygosity is normally reached by the breeder after six to eight generations of selfing. For qualitative characteristics in which the dominant may be visibly distinguished

from the recessive form, complete homozygosity is desired in order to produce a variety uniform in appearance.

A mixed population of a self-pollinated crop is in reality a mixture of homozygous genotypes. If the individual homozygous genotypes are isolated and increased, each produces a pure population. Heterozygous plants may arise in a homozygous population, of a self-pollinated crop by natural cross-pollination or by mutation, but the progenies of these heterozygous plants quickly segregate again into true breeding genotypes.

In naturally cross-pollinated crops, individual plants are extremely heterozygous as a result of the mixing of the genotypes in each generation by crossing. In these species, self-pollination does not normally take place to any significant extent unless pollination is controlled. Continuous self-pollination, or inbreeding for several generations in normally cross-pollinated species is generally accompanied by a loss of vigour and productiveness. This has been well illustrated in the breeding of hybrid maize in which inbred lines are greatly reduced in size and vigour in comparison with open-pollinated varieties from which they are derived. Self-fertilization is difficult to attain in some cross-pollinated species on account of the presence of incompatibility alleles.

In a few crops such as sorghum and cotton there are varying amounts of self and cross-pollination. In these crops the amount of homozygosity or heterozygosity will vary according to the pollination since there are two opposing forces in action, self-pollination, which leads to segregation and homozygosity, and cross-pollination, which increases the heterozygosity.

VILMORIN PRINCIPLE OF PROGENY SELECTION

Evaluation of the worth of plants on the basis of performance of their progeny is known as progeny test. The progeny test was developed by Louis de Vilmorin. Therefore, it is also known as **Vilmorin Isolation Principle** or, in short vilmorin Principle. Vilmorin observed that sugarbeet plants with high sugar content could be grouped into three classes based on the sugar contents of their progenies, The first group of plants produced progenies high in sugar content, the progenies from the second group had some plants with high and some with low sugar content, while the third group produced progenies low in sugar content. Thus plants similar in phenotypes (high sugar content) produced considerably different progenies. From the observations, Vilmorin concluded that the real value of a plant can be known by studying the progeny produced by it.

Today, progeny test is the basic step in every breeding method. The progeny test serves two valuable functions. Its first function is to

determine the breeding behaviour of a plant, *i.e.*, whether it is homozygous or heterozygous, For example, consider the progeny from barley plants with the genotypes VV and Vv, both having two-rowed spikes. The second, and perhaps the more important function of progeny test is to find out whether the feature for which a plant was selected is heritable *ie.*, is due to genotype. Selections have to be based on phenotype. The relative contribution of genotype and environment to the phenotype of the selected plants can be determined through progeny test. If the phenotypic superiority of the selected plants is due their genotype, their progeny will also exhibit that superiority.

Genetic Basis of Pure Line

Number of generations of selfing	Frequency percent			Frequency percent	
	AA	Aa	aa	Homozygosity	Heterozygosity
1	0	100	0	0	100
2	25	50	25	50	50
3	37.5	25	37.5	75	25
4	43.75	12.5	43.75	87.5	12.5
5	46.875	6.25	46.875	93.73	6.25
6	48.437	3.125	48.437	96.874	3.125
7	49.219	1.562	49.219	98.436	1.562
8	49.608	0.781	49.608	99.216	0.781
9	49.803	0.39	49.803	99.606	0.39

CHOICE OF PARENTS

The choice of parents mainly depends upon the objectives of breeding programme. In addition to other objectives, increased yields are always an objective of the breeding. Therefore, *at least one of the parents involved in a cross should be a well and proven variety in the area for which the new variety is being developed.* The other variety should be having the characters that are absent in this variety. In combination breeding, the genetic diversity of parents is not important, but in transgressive breeding genetic diversity is of great importance. For transgressive breeding, it should be made sure that the parents differ for many genes affecting yield or some other character of importance. However, it would be desirable that their performance of parents is good and that they are well adapted in the areas where they are commonly grown.

COMBINING ABILITY

Some parents produce superior F₁'s and F₂'s while others do not. This property of the parents is known as combining ability and can be estimated by one of the many biometrical techniques. Combining ability

of the parents serves as a useful guide in the selection of parents for a hybridization programme.

The ability of an inbred to transmit desirable performance to its hybrid progenies is referred as its *combining ability*.

General combining Ability

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

Specific Combining Ability

The excessive performance of a cross over and above the expected performance based on GCA of the parents is known as specific combining ability.

Thus GCA is the characteristic of parents and SCA is characteristic of crosses or hybrids. The inbreds are evaluated in following way.

It is essential that all the characters sought to be improved are present in one or the other parent. If necessary, three or more parents may be included in a complex cross. One should remember that a character absent in the original parents will generally not appear in the segregating generations, except for the possibility of transgressive segregations and some gene interactions. *e.g.*, Complementary gene action. Thus the choice of parents is the basic step in a hybridization programme and often more than anything else determines its success or failure of the programme.

COMBINATION BREEDING

The main aim of combination breeding is the transfer of one or more characters into a single variety from another variety or other varieties. These characters may be governed by oligogenes or poly genes. The intensity of the character in the new variety is either comparable to or more generally lower than that in the parent variety from which it was transferred. In this approach, the increase in yields of the variety is obtained by correcting the weaknesses in the yield contributing traits. *e.g.*, tiller number, grains per spike, test weight etc., of the concerned parent variety. Examples of the methods of combination breeding are backcross breeding and pedigree breeding.

TRANSGRESSIVE BREEDING

Transgressive breeding aims at improving yield or its contributing characters through transgressive segregation.

Transgressive segregation refers to the appearance of such plants in an F_2 generation that are superior to both the parents for one or more characters. Such plants are produced by an accumulation of plus or favourable genes from both the parents as a consequence of recombination. Obviously, the parents involved in hybridization must combine well with each other, and should preferably be genetically

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

MERITS OF BULK AND SINGLE SEED DESCENT METHOD

- i) The bulk method is simple, convenient and inexpensive.
- ii) Artificial or natural disease epiphytosis, winter killing etc. eliminate undesirable types and increase the frequency of desirable types. The isolation of desirable types thus becomes much easier.
- iii) Natural selection increases the frequency of superior types in the population. Progenies selected from long-term bulks are likely to be far superior to those selected from F_2 or short-term bulks.
- iv) Little work and attention is needed in F_2 and the subsequent generations. The breeder is free to concentrate more on other breeding projects.
- v) No pedigree record is to be kept, which saves time and labour.
- vi) Since large populations are grown, transgressive segregants are more likely to appear and increase in frequency due to natural selection. Thus there is a greater chance of isolation of transgressive segregants than in the pedigree method.
- vii) Artificial selection may be practised to increase the frequency of desirable types.
- viii) It is suitable for studies on the survival of genes and genotypes in populations.

DEMERITS OF BULK AND SINGLE SEED DESCENT METHOD

- i) The major disadvantage of bulk method is that it takes a much longer time to develop a new variety. Natural selection becomes important only after F_8 or F_{10} , and bulking may have to be done upto F_{20} or more. Thus the time required for developing a new variety is considerably longer, and most breeders do not use the bulk method simply for this reason.
- ii) In short term bulks; natural selection has little effect on the genetic composition of populations.
- iii) It provides little opportunity for the breeder to exercise his skill or judgment in selection. But in the modified bulk method the breeder has ample opportunity for practicing selection in the early segregating generations.
- iv) A large number of progenies have to be selected at the end of the bulking period.

- v) Information on the inheritance of characters cannot be obtained, which is often available from the pedigree method.
- vi) In some cases, at least, natural selection may act against the agronomically desirable types.

MULTILINES AND MULTIBLENDS

One plant breeding approach to manage the 'breakdown' of disease resistance genes in crops has involved the concept of multi-lines or multi-blends.

The idea of multiline varieties was put forward by Jenson in 1952. **Multilines** are mixtures of several purelines of similar height, flowering and maturity dates, seed colour and agronomic characteristics, each of which has a different gene for resistance to the given disease. (Or)

Multi-lines consist of a mixture of inbred isogenic lines that share an identical genetic background but differ only with respect to a gene(s) for a specific trait. This concept was originally proposed as an approach to promote the durability of a cultivar by providing greater stability of production, broader adaptation to the environment, and greater protection against pathogen populations (Jenson, 1952).

In contrast, **multi-blends** involve a simple mixture of one or more cultivars that differ in a diverse range of traits. Such blends of cultivars have had a long history of use in agriculture and have often been confused with the use of multi-lines (Jenson, 1988). In modern agriculture, the use of multi-blends is more feasible in forage, forestry and perennial horticultural crops, but less appropriate in arable and vegetable crops where uniformity is essential for the mechanization of crop agronomy and harvest.

MERITS OF SYNTHETIC VARIETIES

Synthetic varieties offer several unique advantages in comparison to hybrid varieties in the exploitation of heterosis. These advantages are listed below.

1. Synthetic varieties offer a feasible means of utilizing heterosis in crop species where pollination control is difficult. In such species, the production of hybrid varieties would not be commercially viable.
2. The farmer can use the grain produced from a synthetic variety as seed to raise the next crop.
3. In variable environments, synthetics are likely to do better than hybrid varieties. This expectation is based on the wider genetic base of synthetic varieties in comparison to that of hybrid varieties.
4. The cost of seed in the case of synthetic varieties is relatively lower than that of hybrid varieties.

5. Seed production of hybrid varieties is a more skilled operation than that of synthetic varieties.
6. Synthetic varieties are good reservoirs of genetic variability. The composites and germplasm complexes also serve as gene reservoirs.
7. There is good evidence that the performance of synthetic varieties can be considerably improved through population improvement without appreciably reducing variability.

DEMERITS OF SYNTHETIC VARIETIES

1. The performance of synthetic varieties is usually lower than that of the single or double cross hybrids. This is because synthetics exploit only GCA, while the hybrid varieties exploit both GCA and SCA.
2. The performance of synthetics is adversely affected by lines with relatively poorer GCA. Such lines often have to be included to increase the number of parental lines making up the synthetic as lines with outstanding GCA are limited in number.
3. Synthetics can be produced and maintained only in cross-pollinated crop species, while hybrid varieties can be produced both in self- and cross-pollinated crops.

DIFFERENCE BETWEEN SYNTHETICS AND COMPOSITES

Synthetics	Composites
1. No. of inbredlines are less (6-8)	No. of lines are more (even upto 20)
2. GCA of parental lines is tested	No. testing
3. Performance can be predicted	Cannot be predicted
4. Broad based	More broad based
5. Synthetic can be reconstituted at a later date	Cannot be reconstituted
6. Seed replacement after 4-5 years	After 3-4 years

ACHIEVEMENTS OF SYNTHETICS AND COMPOSITES

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

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

Composite 1, has been evolved in *Brassica campestris* Var. *toria*. It was developed by compositing 10 *elite toria* strains; it matures in 100 days,

exhibits profuse branching and yields about 11 q/ha seed, which contains about 40% oil.

CHIMERAS AND ITS TYPES

Chimera is defined as a mixture of genetically diverse tissues in the same shoot. These tissues frequently form mosaic pattern. The most common and easily observed chimeras are leaf variegations observed in horticultural plants like Codium and Acalypha. The leaf variegations or chlorophyll variegations are due to plastid mutations, consisting of green and white or green and yellow patterning as also the patterning of anthocyanin distribution. These chimeras can be of three types.

- 1. Sectorial:** The tissues possess the characteristics which closely resemble the parents from which these tissues are originally derived.
- 2. Periclinal:** The core is of one plant and the epidermal region is of another plant. As a result, the leaves may be of one plant type and the flowers and fruits are of another plant.
- 3. Chromosomal chimera:** The most frequent being the ploid-chimera. It has been a very common experience to meet with cells especially in the root tips, with higher chromosomal number in an otherwise diploid tissue. Plants under experimental control have been sometimes observed to give rise suddenly to sectors or whole branches differing in chromosome number.

TREE BREEDING

OBJECTIVES

In genetic improvement of tree species major emphasis is placed on selection breeding. Some points are generally considered while choosing traits for selection purposes are as follows:

- Sufficient genetic variation for the character should be available,
- The character should be heritable and its heritability should have been worked out,
- Trials have been conducted to screen out geographic variation available in that species.

SEED ORCHARD AND CLONAL ORCHARDS

1. Seedling seed orchard
2. Clonal seed orchard

Seedling seed orchard- Seedling seed orchards are raised from the seeds collected from superior phenotypes. The seedlings planted from

this material are roughed to remove inferior trees, leaving the best trees of the best families for seed production.

Clonal seed orchard- A seed orchard composed of vegetative propagated trees established primarily from the clonal material of superior Phenotype for the production of seed of proven genetic quality.

WIDE HYBRIDIZATION OR DISTANT HYBRIDIZATION

Introduction

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

Eg. Inter specific hybridization: Eg. *Oryza sativa* x *O. perennis*

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

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

1. History : The first distant hybridization; hybrid between carnation (*Dianthus caryophyllus*) and sweet willian (*Dianthus barbatus*) by Thomas Fairchild in 1717 and the hybrid is called as ***fairchilds mule***

2. Most of the interspecific hybrids were of no agricultural value many interspecific hybrids particularly in case ornamentals, served as commercial varieties.

3. An interesting inter generic hybrid *Raphano brassica* was an amphidiploid cross between radish and cabbage but it was useless.

4. The first inter generic hybrid with a great potential was **Triticale**

Objectives:

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

eg., Many disease resistance and, insect resistance genes

Wide adaptability: (i.e. drought-resistance, cold tolerance etc.)

Quality improvement (Eg. Cotton (fibre) Tobacco (leaf)

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

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

2. Exploitation of luxuriance (heterosis) in vegetatively propagated / ornamental crops. Prolonged vegetative period, Prolonged blooming period
3. Creation of Novel genotypes : New species or F1 hybrids hitherto non-existent in nature.

BARRIERS TO THE PRODUCTION OF DISTANT HYBRIDS

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

1. Failure of zygote formation / cross incompatibility

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

1. Failure of fertilization, because the pollen may not germinate.
2. Pollen tube is unable to reach to embryo sac and hence sperms are not available for fertilization
3. Pollen tube may burst in the style of another species Eg. *Datura*.
4. The style of the female parent may be longer than the usual length of the pollen tube growth therefore the pollen does not reach the embryo sac. Eg. *Zea mays* and *Tripsacum sp.*
5. Pollen tubes of polyploidy species are usually thicker than those of diploid species.
6. When a diploid is used as female and a polyploidy as male, the polyploidy pollen tube grows at a slower rate in the diploid style than it would be in a polyploid style.

These barriers are known as pre-fertilization barriers.

Techniques to make wide crosses successful

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

2. Failure of zygote Development / Hybrid inviability

The inability of a hybrid zygote to grow into a normal embryo under the usual conditions of development is referred to as *hybrid inviability*. This may be due to :

- a. Lethal genes** : some species carry a lethal gene, which causes death of the interspecific hybrid zygote during early embryonic development. Eg. 1. *Aegilops umbellulata* carries a lethal gene with 3 alleles against diploid wheats.

- b. Genetic Disharmony between the two parental genomes.**

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

Eg. Cotton - *G. gossypoides* x other *G. sps.*

Brassica – *B. napus* x *B. oleracea*

- c. Chromosome elimination :** In some cases of distant hybridization, chromosomes are gradually eliminated from the zygote. This generally does not prevent embryo development, but the resulting embryo and the F1 plants obtained from such crosses are not true interspecific hybrids since they do not have the two parental genomes in full. Generally, chromosomes from one are successively eliminated due to mitotic irregularities.

Eg. *Hordeum bulbosum* x *H. vulgare*

Hordeum bulbosum x *Triticum aestivum*

Triticum aestivum x *Zea mays*

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

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

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

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

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

3. Failure of Hybrid seedling development / Hybrid sterility

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

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

2. Interspecific and intergeneric F1 hybrids of wheat show both chlorosis and necrosis; **Hybrid sterility :** Hybrid sterility refers to the inability of a hybrid to produce viable off spring. The main cause of hybrid sterility is lack of structural homology between the chromosomes of two species.

TECHNIQUES FOR PRODUCTION OF DISTANT HYBRIDS

1. **Choice of parents :** Genetic differences exist among parents in a species for cross compatibility. More compatible parents should be selected for use in wide crosses.

2. Pollina ting sufficiently large no. of flowers.
3. **Reciprocal crosses** : it is better to attempt reciprocal crosses when distant crosses are not successful.
Eg. : *Phaseolus aureus* and *p.mungo* are crossable only when *P. aureus* in used as female and *P. mungo* as male.
4. Determine the barrier and then take measures to overcome it:
Longer style sps - cut the style
Use more than one strain of each sps for lethal genes Autopolyploidy (*B.oleracea* x *B. compestris*)
Manipulation of ploidy – when two species of a cross differ in chromosome number, it is necessary to manipulate their ploidy
 - a. Direct crossing-use higher ploidy sp as female parent
 - b. Chromosome no. of the wild species or of the interspecies hybrid (F1) may be doubled to overcome sterility of the hybrid.
5. **Bridge crosses** : Some times, two species say 'A' and 'C' do not cross directly. In such case a third species say 'B' which can cross with both 'A' and 'C' is chosen as a bridge species. First 'B' is crosses with 'C' and then the amphidiploid is crossed with 'A'. Bridge crosses have been used in Tobacco and wheat. Eg. *Nicotiana repanda* can cross with *N. sylvestis* but not with *N. tabacum* *N. sylvestris* crosses with both *N. repanda* and *N.tabacum*. For transfer of genes from *N. repanda* to *N.tabacum* *N. sylvestis* is used as bridge species.
6. **Use of pollen mixtures**: Cross incompatibility results due to unfavourable interaction between the protein of pistil and pollen which inhibits normal germination and growth of pollen tube. This problem can be overcome by using the mixture of pollen from compatible (self) and incompatible parents.
7. **Manipulation of pistil** : In some cases, pollen tube is short and style is very long, due to species difference. Thus pollen tube cannot reach ovule to effect fertilization. In such situation either reciprocal cross should be made or the style should be cut to normal size before pollination. This technique is successful in maize – *Tripsacum* crosses, where maize style remains receptive even after cutting.
8. **Use of growth regulators** : Some times, the pollen tube growth is so slow that the egg cell dies or the flower aborts before the male gametes reach the ovary. In such cases, growth regulators should be used to accelerate the pollen tube growth or to prolong the viability of the pistil. Use of growth regulators such as IAA; NAA; 2,4-D and GA3 etc; are promising in some wide crosses.
9. **Large number of crosses** : The success of seed set is generally very low in wide crosses. Hence, large no. of crosses should be made to obtain crossed seeds.
10. **Protoplast fusion** : The wide crosses can be obtained through protoplast fusion, when it is not possible to produce such crosses through sexual fusion.

11. **Embryo culture** : This technique is being used widely to obtain viable interspecific or intergeneric hybrids. This is used when hybrid zygote is unable to develop. This technique has been successfully used in *Triticum*, *Hordeum*, *Phaseolus*, *Nicotiana*, *Gossypium*, *Lycopersicon*, *Trifolium*, *Cucurbita* etc.
12. **Grafting** : Grafting of interspecific hybrid on to the cultivated species helps in making the cross successful.

Applications of wide hybridization in crop improvement

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

Eg. A. Disease resistance:

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

B. Wider adaptation: Cold tolerance has been transferred from wild relatives to Wheat, onion, potato, tomato and grape.

C. Quality: Oil quality in oil palm was improved by genes from wild relatives.

D. Changing the mode of reproduction:

1. Self-incompatibility: S.I. genes from *B. campestris* to self compatible *B. napus* for hybrid seed production.

E. Yield:

F. Other characters:

4. Development of New crop species :

Eg. Triticale

5. Utilization as New hybrid varieties :

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

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

Sterility in Distant Hybrids

Distant Hybrids show variable sterility ranging from complete fertility to complete sterility For eg. *L. esculentum* x *L. pimpinellifolium* hybrid is completely fertile while sugarcane maize hybrid is completely sterile. Distant Hybrids are of two broad groups : The first group includes those Distant hybrids that exhibit atleast some fertility so that they can be maintained by selfing, intercrossing among themselves or backcrossing to the parental species.

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

SOMACLONAL VARIATION

The genetic variability present among the cultured cells, plants derived from such cells or progeny of such plants is called somaclonal variation. This term is introduced by Larkin and Scowcroft in 1981. However first report of morphological variation in plants regenerated from cell culture was made by Heinz and Mee in 1971 in sugarcane. Larkin and Scowcroft proposed the term Somaclonal variation to describe all those variations which occurs in plants regenerated from any form of cell culture (or) it refers to the heritable changes which accumulated in the callus from somatic explants and expression in the progeny of *invitro* regenerates obtained from callus.

The plants derived from cell and tissue cultures are termed as somaclones and the plants displaying variation as somaclonal variants. The plants regenerated from tissue culture are designated as R0 generation and their successive sexual generation as R1 and R2 and so on. The somaclonal variation may be transient (epigenetic) or genetic only the latter is transmitted to the next generation and is thus important for crop improvement. The epigenetic changes are expressed at cell culture stage but usually disappears when plants are regenerated to reproduced sexually, Somaclonal variation is a wide spread phenomenon reported in Monocots (wheat, rice, and maize) Dicots (Tomato, Tobacco, and Brassica) and asexually propagated crop plants except sugarcane.

Types of Somaclonal Variation

Based on the tissue from which variation originate Somaclonal variation can be divided into the following types

- 1) *Gametoclonal variation* : variation observed among the plants regenerated from gametic cultures
 - a) *Androclonal variation* observed among the plants regenerated from anther (or) pollen culture
 - b) *Gynoclonal variation*:- from ovule (or) ovary culture
- 2) *Protoclonal variation*:- variation observed among the plants regenerated from protoplast cultures
- 3) *Calliclonal variation*:- variation observed among the plants regenerated from callus cultures.

Origin of Somaclonal Variation

Somaclonal variation may occurs either for single trait (or) more than one trait at a time. Variation may arrive due to any one (or) a combination of following mechanisms:

1) Somaclonal variation may occur due to chromosomal abnormality in cultured cells and plants regenerated from them.

2) The chromosomal abnormality may be due to changes in chromosomal number (ploidy level) and chromosomal structure

Gene mutations:

Variation may also be due to additive effects of mutated genes change in base sequence (or) activation of some gene amplification results alternation in gene expression. changes in plasmagene which include plasmagene mutations and rearrangement in cytoplasm gene can also be altered by *in vitro* culture condition.

3) Activation of transposable elements

4) DNA methylation

5) Mitotic crossing over

The variations observed in the plants regenerated from cultured cells are derived broadly from two sources

a) Pre – existing variability-Some of the variation could be due to the inherent cellular heterogeneity of explant

b) *In vitro* induced variability -Culture conditions may bring about new genetic changes

Advantages of Somaclonal Variation

1) Use of Somaclonal variation may reduce the time required for release of new variety by two years as compared to mutation breeding

2) It can be used to isolate new genotype that retain all the favourable characters of the existing cultivars while adding one additional trait that means it may not involve a drastic change in genetic background.

3) Saves time by reducing lengthy procedures of hybridization and selection

4) In wide crosses it provides a mechanism of gene integration

5) Highly efficient as it can screen very large, number of cells rapidly with small effort time, cost labour, and space requirement.

6) Characters can be selected at the cell level which cannot be allowed at plant level

7) This is the only approach for isolation of biochemical mutants in plants

8) It is a simple and cheap form of plant biotechnology as compared with somatic hybridization and genetic transformation.

9) The *invitro* regenerates *eg.*,of tomato and potato showed increased rates of recombination. This could be useful in generating novel genetic variation by breaking undesirable close linkages and by shuffling the genes linked in the repulsion phase.

Achievements

Crop	Somaclonal variety	Parent variation	Salient features
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<i>Sugarcane</i>	Ono	Pindal	Resistant to fiji disease
<i>Sweet potato</i>	Scarlet	Selected from shoot tip culture	Contains darker and more stable Skin colour
<i>Geranium</i>	Velvet rose	Robbers lemon rose	Polyploid somaclone, sturdiness vigour and attractiveness
<i>Ciltronella java</i>	Bio-13	Not known	37 percent more oil content 39percent more citronella content
<i>Brassica juncea</i>	Pusai Jaikisan	Varuna	Bolder seeds. 17.4 percent yield advantage released by IARI NewDelhi

DOUBLED HAPLOIDS

In general the haploids are much weaker highly sterile and difficult to maintain when compare to the normal plants of concerned species. Therefore chromosome number of all haploids is doubled usually by treating with colchicine to produce doubled haploids which have the normal somatic chromosome complement (2n) of the species and are fully fertile.

The double haploid plants are completely homozygous and fully vigorous and can be used for the evaluation of performance and selection for desirable traits.

In vitro production of haploids can solve some problems in genetic studies since gene actions are readily manifested due to a single allelic dose present in chromosomes of an entire genome. Some of the applications of haploids in plant breeding include.

By releasing new varieties through F1 double haploid system. The anthers of the hybrid progeny (F1) are excellent breeding material for raising pollen derived homozygous plants (double haploids in which complementary parental characters are combined in one generation.

Variety A		Variety B
AAbb	x	aaBB
(Ab)		(aB)

F1 AaBb

Anther culture :

Haploids : AB, Ab, aB, ab.

Doubled haploids: AABB, AAbb, aaBB, aabb

Doubled haploids derived from pollen cultures expressed genetic variability to an extent; the new varieties have been synthesized in respect of Barly, Brassica, Rice, Maize Potato, Pepper and Asparagus.

Production of homozygous doubled haploid lines saves four years time when compare to conventional methods.

Advantages

Selection among double haploid lines is atleast 6-8 times as efficient as that among the segregating population. It reduces the size of breeding material. By haploid induction followed by chromosome doubling it is possible to obtain exclusively male plants in dioecious species.

Applications

1. Dihaploid production through bulbosum technique is successful in barley.
2. Through the induction of genetic variability (gametoclonal variation) haploids (or) plants with various ploidy levels and mutants are obtained.
3. Selection of mutants resistant to disease. Haploids provide relatively easiest system for the induction of mutations. Therefore they can be employed in rapid selection of mutants having traits for disease resistance.
 - a. Eg:- Tobacco mutants resistant to Black shank disease and wheat lines resistant to scar. *Fusarium graminearum*
4. Developing asexual lines of trees (or) perennial species.
5. Pollen derived rubber tree is taller by 6m which could be multiplied by asexual propagation
6. Transfer of desired alien genes / genetic transformation.
7. Chromosomal instability in haploids makes them potential tool for introduction of alien chromosomes (or) genes during wide crossing programme. haploids are used for the development of substitutional and additional lines.
8. Double haploids are used for QTL mapping and construction of genetic linkage maps etc.

Achievements

In china, 81 varieties and strains of rice have been developed through anther culture.

- a. Hua yu-1 possess, characters of high yield, resistant to bacterial blight and light adoptability Xin-Xiu is high yielding variation and has been released in 1lakh ha other variation
- b. Xinxu is with high yielding and blast resistance has been released in one lakh ha. The other varieties xhonghua -8 and xhonghua-9 with

high yield and blast resistance have been produced. In Europe rice variety Maria na and wheat variety Florin, Tobacco variety Biopreslavana. In china wheat variety Hua pei-1, Lung Hua-1 etc.

MARKER ASSISTED BREEDING

Morphological Markers

These markers are often detectable by eye, by simple visual inspection. Examples of this type of marker include the presence or absence of an awn, leaf sheath coloration, height, grain color, aroma of rice etc. In well-characterized crops like maize, tomato, pea, barley or wheat, tens or hundreds of genes that determine morphological traits have been mapped to specific chromosome locations.

Biochemical Markers

A protein that can be extracted and observed.*eg.*, isozymes and storage proteins.

Molecular Markers (Or) DNA Markers

A unique gene (DNA sequence), occurring in proximity to the gene or locus of interest, can be identified by a range of molecular techniques such as RFLP, RAPD, AFLP, DAF, SCAR, microsatellite or single-nucleotide polymorphism, or (SNP) detection.

Molecular marker is a DNA segment that is readily detected and whose inheritance can easily be monitored. The use of molecular markers is based on the naturally occurring DNA polymorphism.

A DNA marker is a small region of DNA showing sequence polymorphism in different individuals within a species (or) among different species.

The DNA markers are most widely used type of markers predominantly due to their abundance the first such DNA markers to be used for the fragments produced by the digestion with restriction endonucleases that means restriction fragment length polymorphism (RFLP) based genetic markers. A wide range of molecular techniques is now available to detect the polymorphism at DNA level. These have been grouped into the following.

1) Non PCR based approaches Eg: - RFLP

2) PCR based approaches Eg: - RAPD AFLP SSRS etc (plants)

Major Genes

'Major genes' that is responsible for economically important characteristics are frequent in the plant kingdom. Such characteristics include disease resistance, male sterility, self-incompatibility, and others related to shape, color, and architecture of whole plants and are often of mono- or oligogenic in nature. The marker loci that are tightly linked to major genes can be used for selection and are sometimes more efficient than direct selection for the target gene. Such advantages in efficiency may be due for example, to higher expression of the marker mRNA in such cases that the marker is actually a gene. Alternatively, in such cases that the target gene of interest differs between two alleles by a difficult-to-detect single nucleotide polymorphism.

TESTING, RELEASE AND NOTIFICATION OF VARIETIES

Botanically a variety is a sub group of a species. According to seed act (1966, sub section 16 of section 2) it is a sub division of a kind identified by its growth, yield, plant fruit, seed or other characters. Where as seed technology considers a group of plants uniform in their morphological, physiological, biochemical and other characters without any variation from generation to generation and can be differentiated from other groups of plants of the same species by some distinguishing characters as variety after its release and notification. Schematic flow chart indicating the steps involved in the development of a variety:

Plant breeding method
Development of a strain
Identification by the workshop for particular zone
Testing under AICRP
Multilocation trials in the state / zone of adoption
Release of variety by state / central Govt.
Notification of the variety by GOI
Seed production through seed multiplication chain
Use of certified seed by the farmers

In India, the release of new crop varieties consists of four major steps viz.

1. Development of new strains
2. Evaluation of performance
3. Identification of superior strains and
4. Release and notification

1. Development of new strains

The new strains are developed by ICAR crop research institutes and state agricultural universities for specific purposes. Various breeding methods are used for development of new strains in self and cross pollinated species.

2. Evaluation of performance

The performance of newly developed strains is evaluated in AICCIP, ICAR institutes, SAUs and private registered seed companies enter their improved strains / hybrids in the AICCIP of respective crop for multilocation testing. The new strains are tested at multilocations under the coordinated project for a minimum period of three years / seasons. The new variety is first tested for yield under the initial varietal trials (IVT); the in the second year. The strains that give good performance in AVT for two years are selected.

3. Identification of superior strains

The strains which show good yield performance in AVT are identified as superior strains and are considered for release in the workshop meetings. The new agro and plant protection techniques required to obtain potential yield of new strains are also worked out by that time. The workshop after considering the new promoting varieties recommend them to replace existing varieties.

4. Release and notification

The proposal for release of new varieties is put up in a prescribed proforma to variety release committee. There are two types of variety release committees viz, state variety release committee (SVRR) and central variety release committee (CVRC). In case of state variety release committee, Director of Agriculture for field crops and Director of Horticulture for vegetable and horticulture crops is the chairman. In central variety release committee, Deputy Director General (Crop Science) of ICAR is the chairman. The release proposal of varieties recommended for All India release is put up before CVRC, while for those recommended for release in a particular state is placed before the SVRC of respective state these committees consist of scientists and representatives of seed producing organizations (NSC, SSC and SSCA) and other related govt. agencies After release, the variety is notified. Seed production can be taken up only after notification of new varieties. The notification is done by the govt. of India.

SEED CERTIFICATION

Procedure for seed certification

Seed certification is voluntary and that too for the kind and variety notified by the government of India. It can be completed in six broad phases.

1. Receipt and scrutiny of the application.
2. Verification of seed source, class and other requirements.
3. Field inspection should be conducted to see that fields are up to the prescribed field standard.
4. Post harvest inspection, including processing and packing.
5. Seed sampling and testing to confirm that the seeds are up to the prescribed seed standards.
6. Grant of certificate, tagging and sealing.

Stages in seed multiplication

1. **Nucleus seed:** Nucleus seed: This is cent per cent genetic pure seed with physical purity produced under the direct supervision of the concerned plant breeder. This forms the basis for all further seed production. It has the highest genetic purity and physical purity.
2. **Breeder's seed:** This is the progeny of the nucleus seed multiplied in large area under the supervision of plant breeder and monitored by a committee. It provides cent per cent physical and genetic pure seed for production of foundation class. Golden yellow coloured certificate is issued for this category by the producing agency.
3. **Foundation seed:** Progeny of breeder's seed is handled by recognized seed producing agencies in public and private sector under the supervision of Seed Certification Agency in such a way that its quality is maintained according to the prescribed standard. Seed Certification agency issues a white colour certification for foundation class seed. Foundation seed is purchased by Seed Corporation from seed growers. Foundation seed can again be multiplied by Seed Corporation in the events of its shortage with similar seed certification standard.
4. **Certified seed:** Progeny of foundation seed produced by registered seed growers under the supervision of Seed Certification Agency by maintaining the seed quality as per minimum seed certification standards. Seed Certification Agency issues a blue colour (Shade ISI No. 104, azure blue) certificate.

TRANSGENIC CROPS

Presently, more than 50 types of genetically engineered plant species, called transgenic plants have been successfully developed. These plants were made to resist insect pests, viruses or herbicides through incorporation of foreign gene into DNA of host plant cells. Initially transgenic plants were developed more in dicotyledons, but now extended to several monocotyledons like wheat, maize, rice and

oats. Transgenic plants have also been developed and are suitable for food industries (delaying ripening in tomato).

Plants have been engineered to produce human proteins, such as hormones, in their seeds.

Transgenic dicotyledonous plants

- | | |
|-----------------------------|------------------------------|
| 1. <i>Nicotiana tabacum</i> | 4. <i>Helianthus annuus</i> |
| 2. <i>Beta vulgaris</i> | 5. <i>Solanum tuberosum</i> |
| 3. <i>Glycine max</i> | 6. <i>Gossypium hirsutum</i> |

Transgenic monocotyledonous plants

- | | |
|-------------------------|------------------------|
| 1. <i>Asparagus sp.</i> | 3. <i>Zea mays</i> |
| 2. <i>Oryza sativa</i> | 4. <i>Avena sativa</i> |

Applications of transgenics

- a) *Herbicide resistance in transgenic plants*
- b) *Improved resistance to insect pests and microbial diseases*
- c) *Practical application of genetic transformation*

VARIETAL PROTECTION, GERMPLASM REGISTRATION AND DUS

1. "*Essentially derived variety*", in respect of a variety ("the initial variety"), shall be said to be essentially derived from initial variety

(i) When the variety developed is predominantly derived from initial variety; or from a variety that is itself predominantly derived from initial variety; while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of such initial variety;

(ii) is clearly distinguishable from such initial variety; and

(iii) Conforms to such initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of such initial variety except variation in such characteristics which result in the process of derivation.

The new varieties of plants when released should protect the rights of the farmers and breeders with respect to that varieties of plants. It may include:-

(iv) The registration of extant or new plant varieties by researchers or breeders subject to terms and conditions.

(v) Developing characterization and documentation of varieties;

(vi) Compulsory cataloguing facilities for all varieties of plants, seeds and germplasm.

(vii) Ensuring that seeds of the varieties registered are available to the farmers and providing for compulsory licensing of such varieties if the breeder of such varieties or any other person are entitled to produce

such variety does not arrange for production and sale of the seed in the manner as may be prescribed.

(viii) Collecting statistics with regard to plant varieties, seeds and germplasm for compilation and publication;

(ix) Ensuring the maintenance of Register.

Registration

The Central Government shall establish for the purposes of this Act, a Registry which shall be known as the *Plant Varieties Registry*.

A register called the National Register of Plant Varieties shall be kept at the head office of the Registry, wherein shall be entered the names of all the registered plant varieties with the names and addresses of their respective breeders. The right of such breeders in respect of the registered variety, the particulars of the denomination of each registered variety, its seed or other propagating material along with specification of salient features thereof and such other matters as may be prescribed .

4. Any person specified in act may make an application to the Registrar for registration of any variety of such genera or species as specified under Act. A new variety shall be registered under this Act if it conforms to criteria of novelty, distinctiveness, uniformity and stability. However, an extant (still existing) variety shall be registered even if it does not conform to the criteria of novelty.

5. No variety shall be registered under this Act if such variety contains any gene or gene sequence involving any technology including terminator technology which is injurious to the life or health of human beings, animals or plants.

6. A new variety shall be deemed to be- (a) novel, if at the date of filing of the application for registration for protection, the propagating or harvested material of such variety has not been sold or otherwise disposed of by or with the consent of its breeder or his successor for the purposes of exploitation of such variety-

(i) In India, earlier than one year; or

(ii) outside India, in the case of trees or vines earlier than six years, or, in any other case, earlier than four years, before the date of filing such application:

Provided that a trial of a new variety which has not been sold or otherwise disposed of shall not affect the right to protection.

Provided further that the fact that on the date of filing the application for registration, the propagating or harvested material of such variety has become a matter of common knowledge other than through the aforesaid manner shall not affect the criteria of novelty of such variety.

DUS TEST

The variety should satisfy the DUS test which means

- (a) **Distinct**, if it is clearly distinguishable by at least one characteristic from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application.
- (b) **Uniform**, If it is sufficiently uniform in its essential characteristics subject to the variation that may be expected from the particular features of its propagation
- (c) **Stable**, If its characteristics remain unchanged after repeated propagation or, in the case a particular cycle of propagation, at the end of each such cycle.

BREEDING FOR BIOTIC STRESS RESISTANCE

DISEASE RESISTANCE

Stress: Constraining influence, force, pressure or adverse conditions for crop growth caused by biological or environmental factors.

Biotic (living) : Adverse effects due to pests and diseases abiotic stresses

Abiotic (non living) : Adverse effects on host due to environmental factors eg: Drought, water logging, heat, cold, salinity, alkalinity and air pollution etc.

Host : Plant effected by a disease or which can accommodate pathogen.

Pathogen : An organism that produces the disease

Disease : an abnormal conditions in the plant caused by an organism (pathogen)

Pathogenicity : The ability of a pathogen to infect a host strain

Virulence : Capacity of a pathogen to incite a disease

Avirulence : The inability of a pathogen to cause or incite a disease

Physiological race : Strains of a single pathogen species with identical or similar morphology but differ in pathogenic capabilities.

Pathotype : Strains of a pathogen classified on the basis of their virulence to known resistance genes present in the host.

Epidemic : Severe and sudden out break of disease beginning from a low level of infection.

Mechanisms of Disease Resistance

There are different ways of disease resistance *viz.*, disease escape, disease endurance or tolerance disease resistance and immunity

1. **Disease escape** : The ability of susceptible host plants to avoid attack of disease due to environmental conditions factors, early varieties, change in the date of plating, change in the site of planting; balanced application of NPK etc.

Eg. Early varieties of groundnut and potato may escape 'Tikka' and 'Late blight' diseases respectively since they mature before the disease epidemic occurs. Changing planting season in sugarcane from June to October has successfully escaped leaf-rust.

Virus free seed potato is produced by sowing the crop in October in Jullundher and other places instead of November, the normal planting time.

2. **Disease endurance or tolerance**: The ability of the plants to tolerate the invasion of the pathogen without showing much damage. This endurance is brought about by the influence of external characters. Generally, tolerance is difficult to measure since it is confounded with partial resistance and disease escape. To estimate tolerance the loss in yield and some other trait of several host varieties having the same amount of disease eg., leaf area covered by disease etc., is compared.

Eg. Wheat varieties when fertilized with potash and phosphorus are more tolerant to the rust and mildew infection. The Rice crop fertilized with silicate is resistant to blast infection in Japan.

3. **Disease Resistance** : The ability of plants to withstand, oppose or overcome the attack of pathogens. Resistance is a relative term and it generally refers to any retardation in the development of the attacking pathogen. In case of resistance, disease symptoms do not develop and the rate of reproduction is never zero but it is sufficiently lower than

1 (the rate of reproduction on the susceptible variety) to be useful. 4. **Immunity**: When the host does not show the symptoms of disease it is known as immune reaction. Immunity may result from prevention of the pathogen to reach the appropriate parts of the host e.g. exclusion of spores of ovary infecting fungi by closed flowering habit of wheat and barley. It is more generally produced by hypersensitive reaction of the host usually immediately after the infection was occurred. In immune reaction the rate of reproduction is zero i.e. $r = 0$

5. **Hypersensitivity**: Immediately after the infection several host cells surrounding the point of infection are so sensitive that they will die. This leads to the death of the pathogen because the rust mycelium cannot grow through the dead cells. This super sensitivity (hypersensitivity) behaves as a resistant response for all practical purposes. Phytoalexins are specific polyphenolic or terpenoid chemicals and are produced by the

host in response to the infection by a pathogen. More than 30 different phytoalexins have been identified. Phytoalexins are either fungicidal or fungistatic. Eg. Rust fungi and virus attack.

Genetic basis of disease resistance

The first study on genetics of disease resistance was done by Biffen in 1905. He reported the inheritance of resistance to leaf rust of wheat variety Rivet in crosses with some susceptible varieties. In F₂ there were 3 susceptible : 1 resistant plants indicating that resistance was controlled by a single recessive gene. Most of the earlier studies were conducted without taking into consideration the physiological specialization (pathotype differentiation) of the pathogen which can materially influence the conclusions drawn. It is now recognized that disease resistance may be inherited in three different ways :
Oligogenic Polygenic and Cytoplasmic inheritance

Oligogenic inheritance

The disease resistance is governed by one or few major genes and resistance is generally dominant to the susceptible reaction. The action of major resistance genes may be altered by modifying genes in many cases. Eg. Bunt resistance in Wheat. Oligogenes generally produce immune reaction. The chief characteristic of the oligogenic disease resistance is pathotypespecificity, i.e. resistant gene is effective against some pathogens, while it is ineffective against the others. In most cases, there are a number of major genes that determines resistance to a particular disease Eg. more than 20 different resistance genes are known for leaf rust of wheat, while those for stem rust resistance exceed 30. The genetics of oligogenic resistance has advanced by two events *viz.*, 1. Discovery of a resistance gene to the prevalent pathotype and 2. Evolution of a pathotype virulent to the new resistance gene. Oligogenic resistance is synonymous to *vertical resistance*.

Gene for gene hypothesis

The concept of gene for gene hypothesis was first developed by Flor in 1956 based on his studies of host pathogen interaction in flax rust caused by *Malampsora lini*. The gene for gene hypothesis states that for each gene controlling resistance in the host, there is a corresponding gene controlling pathogenicity in the pathogen. The resistance of host is governed by dominant genes and virulence of pathogen by recessive genes. The genotype of host and pathogen determine the disease reaction. When genes in host and pathogen match for all the loci, then only the host will show susceptible reaction. If some gene loci remain unmatched, the host will show resistant reaction. Now gene-for -gene relationship has been reported in several other crops like

potato, *Sorghum*, wheat etc. The gene for gene hypothesis is known as “Flor Hypothesis”.

Vertifolia Effect

Vander plank introduced the term vertifolia effect and refers to epidemic development in a variety carrying vertical resistance genes (oligogenes) leading to heavy economic losses. Total failure of vertical resistance leading to a disease epidemic is known as vertioalia effect. This failure occurs because of two reasons :

1. The level of horizontal resistance in varieties carrying oligogenes is usually low and
2. The pathogen is able to evolve new virulent pathotypes.

Polygenic inheritance

In this type the disease resistance is governed by many genes with small effects and a continuous variation for disease reaction is produced. The genes show additive and non additive effects and the environmental effect is also observed. The polygenic resistance does not show pathotype-specificity as against the oligogenic resistance. It is almost same as horizontal resistance. In some cases the polygenic inheritance may have a oligogenic component, the oligogenes acting in an additive manner eg. bacterial blight resistance in cotton.

Cytoplasmic inheritance

Resistance in some cases is determined by cytoplasmic genes or plasma gene(s). Eg. The T-male sterilizy cytoplasm (cms-T) in maize is extreamly susceptible to *Helminthosporium* leafblight, while the non-T cytoblasms are resistant to this disease.

Sources of Disease Resistance

Resistance to diseases may be obtained from four different sources

1. A known variety
2. Germplasm collection
3. Related species
4. Through mutations

Vertical and Horizontal Resistance (Van der plank)

Vertical Resistance is generally determined by major genes and is characterized by pathotype specificity. Clearly immune or susceptible response in the case of vertical resistance depends on the presence of virulent pathotype. When virulent pathotype becomes frequent, epidemics are common in the cases of vertical resistance. Thus an avirulent pathotype will produce an immune response i.e. $r=0$ or close to 0 but the virulent pathotype will lead to susceptible reaction i.e. $r=1$. It is also known as race specific, pathotype specific or simply specific resistance.

Horizontal Resistance

Race non-specific, pathotype -nonspecific and partial, general or field resistance. Horizontal resistance is generally controlled by polygenes i.e. many genes with small effects and it is pathotype nonspecific. In this case, the reproduction rate is not zero but it is less than one. Poly genes, govern horizontal resistance.

Methods of Breeding for Disease Resistance

The methods of breeding for disease resistance are essentially same as those used for other agronomic traits. They are :

1. Introduction
2. Selection
3. Hybridization
4. Budding & Grafting
5. Mutation Breeding
6. Biotechnological methods.

1. **Introduction** : Resistant varieties may be introduced for cultivation in a new area. Eg. Early varieties of groundnut introduced from USA have been resistant to leaf spot (Tikka) , Kalyanasona and Sonalika wheat varieties originated from segregating material introduced from CIMMYT, Mexico, were rust resistant. African bajra introductions have been used in developing downy mildew resistant cms lines.

2. **Selection** : Selection of resistant plants from commercial varieties is easiest method. Eg. · Kufri Red potato is selection from Darjeeling Red round · Pusa Sawani behind (yellow mosaic) selection from a collection obtained from Bihar · MCU I was selection from CO4 for black arm resistance in cotton

3. **Hybridization** : Transferring disease resistance from one variety or species to the other. a. Pedigree method is quite suitable for horizontal resistance. Artificial disease epiphytolics are produced to help in selection for disease resistance.

Eg. In wheat Kalyana Sona, Sonalaka, Malvika 12 Malvika 37, Malavika 206, Malavika 234 Laxmi in Cotton (Gadag 1 x CO2) for leaf blight resistance b. Backcross method is used to transfer resistance genes from an undersirable agronomic variety to a susceptible, widely adoptable and is agronomically highly desirable variety. If the resistant parent is a wholly unadapted variety, backcross method is a logical choice.

If resistant variety also possess some good qualities then chose pedigree method of handling segregating material.

4. **Budding & Grafting** : The disease resistance in vegetatively propagated material is transferred by adopting either by budding or grafting. By grafting or budding the resistant material, the resistance can be transferred.

5. **Mutation Breeding** : When adequate resistance is not available in the germplasm ; Mutation breeding is resorted to induce resistance.

This is also used to break the linkages between desirable resistant genes and other desirable genes.

Advantages with breeding for disease resistance

1. Helps in reducing the losses caused by pathogens
2. Reduces the high cost of disease control by chemical treatment
3. Helps to avoid the use of poisonous fungicides
4. Only method available to some specific diseases like viruses, wilt etc.

Limitations

1. Linkage of resistant genes with genes of inferior quality
2. Occurrence of physiological races of varying capacities
3. Self sterility in host plants

Utilization and achievements

1. Rice ADT 10 x Co4 (resistant to blast)
2. Potato *Solanum tuberosum* x *Solanum demissum* (susceptible to late blight) (wild resistant to late blight)
F1 backcrossed with *Sol. tuberosum*
Resistant variety

Varieties resistant to different diseases

Rice : Blast Co25, Co26,
Wheat : all three rusts : NP 809
Yellow rust : NP 785, NM86
Black rust : NP 789
Brown rust : NP 783, NP 784
Sugarcane : Red rot Co 419, Co 421, Co 527
Cotton : Wilt Vijay, Kalyan, Suyog
Groundnut : Tikka leafspot Ah 45
Chilli : Mosaic resistant Pusa red, Pusa orange

INSECT RESISTANCE

Global average loss due to insect pests is 14%. Estimated losses in individual crops vary from 5% in wheat to 26.7% in rice and still more in crops like cotton & sugarcane.

Insect Resistance

1. The ability of a plant to withstand, oppose or overcome the attack of an insect is known as insect resistance.
2. It is the property of a variety or a host crop due to which it is attacked by an insect pest to a significantly lower degree than are other varieties of the same host.

Biotypes

Strains of a species of an insect pest, differing in their ability to attack different varieties of the same host species (syn: Physiological races)

Mechanism of Insect Resistance:

Insect resistance is grouped into four categories:

1. Non preference
2. Antibiosis
3. Tolerance
4. Avoidance

1. **Non preference:** Host Varieties exhibiting this type of resistance are unattractive or unsuitable for colonization, oviposition or both by an insect pest. This type of resistance is also termed as non-acceptance and

anti-xenosis. Non preference involves various morphological and biochemical features of host plants such as colour, hairiness, leaf angle, taste etc.

2. **Antibiosis** : Antibiosis refers to an adverse effect of feeding on a resistant host plant on the development and/or reproduction of the insect pest. In severe cases, it may even lead to the death of the insect pest. Antibiosis may involve morphological, physiological or biochemical features of the host plant; some cases of insect resistance involve a combination of features. Eg. Resistance to BPT is due to antibiosis & non preference

3. **Tolerance** : An insect tolerant variety is attacked by the insect pest to the same degree as a susceptible variety. But at the same level of infestation, a tolerant variety produces a higher yield than a susceptible variety. Ability of the host plant to withstand the insect population to a certain extent which might have damaged a more susceptible host.

Tolerance is mainly a host character and it may be because of greater recovery from pest damage. Eg. Rice varieties tolerant to stem borer/gall midge produce additional tillers to compensate yield losses (as in stem borer in sorghum) or due to the ability of host to suffer less damage by the pest eg. aphid tolerance in Sugarbeet & Brassica sps. green bugs tolerance in cereals. Inheritance of tolerance is complex in many cases and is supposed to be governed by polygenes.

4. **Avoidance** : Pest avoidance is the same as disease escape , and as such it is not a case of true resistance Mostly insect avoidance result from the host plants being at a much less susceptible developmental stage when the pest population is at its peak.

Eg. 1. Early maturing cotton varieties escape pinkboll worm infestation, which occurs late in the season.

Nature of Insect Resistance / Factors for insect –resistance

Insect resistance may involve :

1. Morphological

2. Physiological (or)

3. Biochemical features of the host plant

1. **Morphological features** : Morphological factors like, hairiness, colour, thickness and toughness of tissues etc. are known to confer insect resistance.

Eg.

1. Thick leaf lamina in cotton contributes to Jassid resistance

2. Solid stem in wheat confers resistance to wheat stem sawfly

3. Thick and tough rind of cotton bolls makes it difficult for the boll worm larve to bore holes and enter the bolls. Other characters : also contribute to insect resistance.

2. **Physiological Factors** : Osmotic concentration of cell sap, various exudates etc; may be associated with insect resistance.

Eg.

1) Leaf hairs of some *solanum* sps. secrete gummy exudates. Aphids and

Colorado beetles get trapped in these exudates.

2) Exudates from secondary trichomes of *Medicago disciformis* leaves have antibiotic effects on alfalfa weevil.

3) Cotton- High osmotic concentration of cell sap is associated with Jassid resistance.

3. **Biochemical Factors** : Several biochemical factors are associated with insect resistance in many crops. It is believed that biochemical factors are more important than morphological and physiological factors in conferring non-preference and antibiosis.

Eg.1) High concentrations of gossypol is associated with resistance in several insect pests in cotton.

2) In rice – high silica content in shoots gives resistance to shoot borer

Genetics of Insect Resistance

Insect resistance is governed by -

1. Oligogenes 2. Polygenes 3. Cytoplasmic genes

1. **Oligogenic Resistance** : Insect resistance is governed by one or few major genes or oligogenes, each gene having a large and identifiable individual effect on resistance. Oligogenic resistance may be conditioned by the dominant or the recessive allele of the concerned gene. The differences between resistant and susceptible plants are generally large and clear-cut. In several cases, resistance is governed by a single gene (monogenic resistance).

Eg. In wheat to green bugs In cotton to Jassids .In apple to woolly aphids
In rice to plant & leaf hopper

2. Polygenic Resistance : It is governed by several genes, each gene producing a small and usually cumulative effect. Such cases of resistance.

- 1) Involve more than one feature of the host plant
- 2) Are much more durable than the cases of oligogenic resistance.
- 3) Difference between resistance & susceptible plants are not clear cut
- 4) Transfer of resistance is much more difficult

Examples for polygenic resistance

- 1) In wheat to cereal leaf beetle
- 2) In rice to stem borer
- 3) In maize to ear worm and leaf aphid

3. Cytoplasmic Resistance : governed by plasmagenes

- Eg. 1. Resistance to European corn borer in maize
2. Resistance to root aphid in lettuce

Sources of Insect Resistance

1. A cultivated variety
2. Germplasm collections.
3. A related wild species
4. An unrelated organisms

1. *Cultivated variety*: Resistance to many insect pests may be found among the cultivated varieties of the concerned crop.

Varieties SRT 1, Khand waz ; DNJ 286 and B 1007 of *G. hirsutum* are good sources of resistance to Jassids.

2. *Germplasm collection*:

Eg. 1) In apples for rosy apple aphid, green apple, apple maker and apple saw-fly. 2) In cotton, several strains resistant to Jassids.

3. *Related wild species* : Eg.

1) Resistance to both the species of potato nematodes has been transferred from *Solanum vernei* to potato

2) Jassid resistances is known in wild relatives of cotton *G. tomentosum*;

G. anomalum and *G. armourianum*

4. *An unrelated organism*: It is done through recombinant DNA technology

a) The 'Cry' gene of *Bacillus thuringiensis* is the most successful example. Other genes of importance are the

b) Protease inhibitor encoding genes found in many plants eg. the cowpea, trypsin inhibitor (cp TI) gene.

Breeding Methods for Insect Resistance

1. Introduction
2. Selection

3. Hybridization 4. Genetic Engineering

1. Introduction : Eg. *Phylloxera vertifoliae* resistance grape root-stocks from U.S.A. into france.

2. Selection : Eg.

1) Resistance to potato leaf hopper

2) Resistance to spotted alfalfa aphid

3. Hybridization : Pedigree oligogenic characters , Back cross Polygenic characters

4. Genetic Engineering : *B.theningiensis* (cry gene) resistance in maize, soybean, cotton etc.

Screening Techniques for determining resistance

The most crucial and perhaps, the most difficult task in breeding for insect resistance is the identification of insect resistant plant during segregation generations. There are two types of screening

1. Field Screening 2. Glass house screening

Field Screening:

The techniques designed to promote uniform infestation by an insect pest in the field are

1. Inter planting a row of known susceptible variety between two rows of testing material.

2. Screening in highly prone areas

3. in case Soil insect pests to be tested in sick plots only

4. Testing in a particular season when the infestation is very high.

Eg. Rice stem borer in off season.

5. Transferring manually equal number of eggs or larvae to each test plant.

Glass house screening

Result from glass house tests are much more reliable than those from field tests since both the environment and the initial level of infestations are more or less uniform for all the plants being tested.

Achievements

India

1. India – cotton varieties – G 27, MCU 7, LRK 516 – resistant to boll worms.

2. Rice – variety vijaya – resistant to leaf hopper

Rice – TKM 6, Ratna – Stemborer

Rice –Vajram, chaitanya, Pratibha – BPH

BREEDING FOR ABIOTIC STRESS RESISTANCE

DROUGHT RESISTANCE

Drought: Scarcity of moisture (soil moisture) which restricts the expression of full genetic yield potential of a plant.

Drought resistance: The ability of crop plants to grow, develop and reproduce normally under moisture stress.

Mechanisms of drought resistance

There are 4 mechanisms of drought resistance.

1. **Drought Escapes:** It is due to ability of a genotype to mature early, before occurrence of drought. Drought escape is most common in plants grown in arid desert region. Eg. Early maturing varieties of sorghum, maize, bajra, wheat, rice etc; give more yield than late maturing under drought.

2. **Drought Avoidance** (Dehydration avoidance): It is due to the ability of plants to maintain favourable water balance even under stress. The plants which avoid drought retain high moisture content in their tissues and lose less water. This is possible either because of:

i) Increased water uptake (due to increase in root development) plants are called water spenders.(or)

ii) Reduced water loss (due to reduction in growth of aerial parts are called water savers (i.e. to avoid transpiration)

Dehydration avoidance is interpreted as the ability of genotypes to maintain high leaf water potential when grown under soil moisture stress: Several traits contribute to dehydration avoidance Such as :

Leaf rolling, folding and reflectance narrow leaves, increased pubescence on aerial organs , presence of awns, osmotic adjustment of stomata, cuticular wax, increased water uptake ; Reduced Transpiration : Increase in concentration of Abscissic Acid (ABA), closure of stomata, ABA plays role in reduction of leaf expansion, Promotion of root growth etc.

3. **Drought Tolerance** (Dehydration tolerance) : Ability of plants to produce higher yield even under 'low water potential'. In cereals drought tolerance generally occurs during reproductive phase. Tolerant cultivars exhibit better germination, seedling growth and photosynthesis. Drought tolerance may be because of i. high proline accumulation ii. maintenance of membrane integrity

4. **Drought Resistance** : It is the sum total of avoidance and Tolerance. It refers to the genetic ability of plants to give good yield under moisture stress conditions.

Various morphological, physiological and biochemical features / parameters associated with drought resistance

a. Morphological

1. Earliness
2. Reduced tillering

3. Leaf characters: Leaf rolling, Leaf folding, Leaf shedding, Leaf reflectance
4. Reduced leaf area : Narrow leaf, Change in leaf angle
5. Hairiness (presence of hairs on leaf and other parts, lowers leaf temperature and reduce transpiration)
6. Colour of leaves
7. Wax content
8. Awns (eg. wheat and barley)
9. Root system (rooting depth and intensity)

b. Physiological

1. Photosynthesis (efficient system like C4) under stress, photosynthetic efficiency is reduced due to chloroplast damage.
2. Reduced Transpiration and reduced respiration losses
3. Stomatal behavior (closure of stomata, also change in size and number of stomata)
4. Osmotic adjustment
5. Leaf enlargement (increase in thickness)
6. Leaf cuticle wax (increases)

c. Biochemical

1. Accumulation of proline and betaine
2. Increase in Absciscic acid (barley) and Ethylene (maize & wheat)
3. Protein synthesis (increases under stress)
4. Nitrate – reductase activity

Sources of drought resistance

1. Cultivated varieties
2. Land (old or desi primitive varieties)
3. Wild relatives (reported in several crops)

For example :

S.No.	Crop	Wild sps	Resistant to
i	Wheat	<i>Aegilops. variabilis</i> <i>Aegilops speltoides</i> " <i>Aegilops umbellulata</i> " <i>Aegilops squarrosa</i> "	drought
ii	Sugarcane	<i>Sacharum. spontaneam</i>	Drought & salinity

4. Transgenes :

Eg. 'Rab' (Responsive to abscisic acid) in rice

Screening / Evaluation

1. Field Env. Highly desirable
2. Green house Env. More precisely controlled than field

Breeding Methods and Approaches

It is important that drought resistance be incorporated in material with high genetic potential for yield.

1. Yield and yield components are best evaluated under non stress / optimal environments, while
2. Drought resistance must be evaluated under water stress.

Breeding methods: Methods are same as for yield and other economic characters. Breeding for drought resistance refers to breeding for yield under moisture stress, i.e. developing varieties which can give high yields under stress. The common methods are

1. Introduction
2. Selection
3. Hybridization
4. Mutation
5. Biotechnology

Limitations

1. Generally resistant varieties have low yield
2. Do not have much wider adaptability (as abiotic resistant is location specific)
3. Drought resistant genes may have linkage with undesirable genes.
4. Transfer of resistant genes from wild types may pose problem.
5. Drought resistance is a consequence of a combination of characters and single character can be used for selection.
6. Measurement of many drought resistant traits is difficult and problematic, since virtually all the useful drought resistant traits are under polygenic control. (So pedigree method most common). But if resistant genes are from agronomically inferior race then 1-2 backcrossing with cultivated type is made. If resistance gene is from wild species-go for backcrossing breeding.
Generally selection is performed on individual plant progenies instead of individual plants (i.e. similar to line breeding)
7. Creation of controlled moisture stress Environments
8. Selection requires considerable resources

WATER LOGGING

As per Levitt (1980 b) flooding (i.e. water logging) is the presence of water in soil excess of field capacity. It leads to deficiency of O₂ and build up of CO₂, Ethylene and other toxic gases and this leads to reduction in aerobic respiration.

Effects of water logging

1. Once soil becomes water logged, air space in soil is displaced with water, the O₂ in the soil is dissolved in water. i.e. O₂ decreases; CO₂ ethylene and other toxic gases increase.
2. O₂ replacement in the soil is very inefficient. Diffusion of atmospheric O₂ into the water logged soils is very inefficient (because of the slow diffusion of atmospheric O₂ to water logged soil).
3. Root systems are suddenly plunged into an anaerobic condition. This switching from aerobic to anaerobic respiration disrupts root metabolism.
4. Carbohydrates level get depleted it is due to
 - a. Dissipation of metabolism
 - b. High water temperature
 - c. Low light

Characteristics of plants in response to water logging stress

1. Reduced growth / elongation.
2. Chlorosis, senescence and abscission of lower leaves
3. Wilting & leaf curling
4. Hypertrophy (increase in size of organ due to increase in cell size)
5. Epinasty (downward growth of petioles)

Mechanisms of tolerance

1. Adventitious root formation on lower part of stem (close to surface so that O₂ tension is quickly restored after transient water logging) eg. Tomato
2. Lenticel (i.e. raised pores in the stem of plants) formation
3. Aerenchyma formation (soft plant tissue contains air spaces found in aquatic plants) in the cortex that provide canal parallel to the axis of the root through which gases can diffuse longitudinally (eg. rice)
4. Elongation capacity (In rice – best elongation response give 100% recovery from submergence and poorest elongation gives upto 49% recovery) Scoring for elongation can be done between booting and flowering stage after flooding the crop to varying depths.

In sugarcane, *S. spontaneum* has more tolerance to flooding. Some canes gave upto 70% of their production potential when in continuous flood for 5 months (in an east at canal point Florida, USA)

Ideotype for flooded areas : The postulated ideotype for flooded areas should have the following characteristics.

1. Capacity to carry out functional activity at low O₂ concentration (i.e. High cytochrome activity)
2. Ability for photosynthesis under low light intensity
3. Capacity to synthesis food rapidly
4. Regeneration capacity of shoots when damaged by flood
5. Ability to withstand drought at later growth stage

6. Deep root system
7. Narrow, medium long and dark green leaves with high sugar and protein content.

Breeding methods : Same as in other stresses.

BREEDING FOR SALT TOLERANCE

Salt Tolerance

Salt Tolerance refers to the ability of plants to prevent, reduce or overcome injurious effects of soluble salts present in their root zone.

It is a global problem as saline and alkali soils are found in almost all the countries of the world, more in Semi Arid Tropic (SAT) of world.

Problem of salinity can be overcome by two ways:

1. Soil reclamation : costly, time consuming and short lived
2. Resistant varieties : less costly, more effective, long lasting require longer period to develop.

Behavior / characteristics of plants to salt:

1. Land races more tolerant than High yielding varieties. Tolerant plants varieties are found in salt affected areas
2. Salt tolerance capacity differs from species to species. Also genetic differences exist among cultivars for their salt tolerance capacity.
3. Different crop plants show differential response to salinity

Salinity Crops

- a. Highly tolerant crops Sugarbeet, sunflower, barley (grain), cotton, datepalm, asparagus
- b. Moderately Tolerant crops Barley (Forage), rye, sorghum, wheat, safflower, soybean
- c. moderately sensitive Rice, corn, foxtail millet, cow pea, peanut, sugarcane, tomato, potato, sweet potato, radish, alfalfa, cabbage
- d. Extremely sensitive Citrus, straw berry, melon, peas, other legumes, apple, rajmabeen, carrot, okra, onion (orange)
4. Higher ploidy level crops are more tolerant than lower ploidy level crops.

Eg. Hexaploid wheat more tolerant than tetraploid

Tetraploid *Brassica* more tolerant than diploid *Brassica*

5. In rice tall, coarse grained, late maturing varieties- more tolerant
6. In sugarcane different strains have differential tolerance Barley more tolerant than wheat.

Symptoms of plants to salt stress :

1. Retardation / cessation of growth
2. Necrosis
3. Leaf abscission
4. Loss of turgor
5. Ultimate death of plant

Mechanisms of salt tolerance

2 types of mechanisms

1. **Salt Tolerance** : Plants respond to salinity stress by accumulating salt, generally in their cells or glands and roots etc.

2. **Salt avoidance** : plants avoid salt stress by maintaining their cell salt concentration unchanged either by water absorption eg. Rice, chenopodiaceae family or by salt

exclusion eg. Tomato, soybean, citrus, wheat grass

Glycophytes (Non-halophytes) plants owe their resistance primarily to avoidance. Eg. Barley Halophytes (plants that grew in salty or alkaline soils) show tolerance by ion accumulation mechanism

Breeding methods

Breeding methods are same but breeding strategies are

1. Breeding for yield potential should have greater emphasis than breeding for salt resistance per se (As screening is done on the basis of yield reduction in stress environment as compared to non-stress Environment.).

2. Selection should be done in stresses target environments (As abiotic stress resistance is an important part of Environ. Fitness & is bound to be location specific i.e. it is related to narrow adaptation).

Screening Techniques

Common methods are

1. Sand culture by using nutrient solution in sand & irrigation with saline water

2. Solution culture by using solution culture tanks (Hydroponic culture)

3. Microplot techniques by using small microplots

Microplot Techniques: By using small microplots of size 6 x 3 x 1 m (CSSRI, Karnal, Haryana) at central soil salinity Research Institute.

Then Multilocation Trial (MLT) conducted over seasons to get more reliable results. Genotypes which survive better under salinity are considered tolerant & tested further.

Selection criteria

1. Germination (%) in saline medium

2. Dry matter accumulation (seedling / plant dry wt.) / Early vigour

3. Leaf senescence or death – Estimated by total dead leaf area or No. of dead leaves

4. Leaf necrosis

5. Leaf ion content

6. Osmoregulation (Determined as maintenance of turgor under stress)

Measured as proline or CHO accumulation or accumulation of glycine, betaine etc.

7. Yield – Economic yield

Problems

1. Creation of reliable controlled salinity Env.

2. Scoring for salinity resistance

3. Genetic control – it is complex & polygenic

4. Mechanisms of resistance poorly understood. Salinity may have interaction with other stresses.

COLD TOLERANCE

When temperatures remain above-freezing *i.e.* >0°C to <100-150°C it is called chilling. When temperature remain below freezing *i.e.* <0°C it is called Freezing.

A. Chilling Resistance

Chilling sensitive plants are typically tropical plants. Temperate plants are generally tolerant to chilling injury.

Effects of chilling stress on plants

1. Reduced germination
2. Poor seedling establishment
3. Stunted growth
4. Wilting
5. Chlorosis
6. Necrosis
7. Pollen sterility
8. Poor fruit set / seed formation
9. Reduced root growth
10. Locked open stomata
11. ABA accumulation

Chilling Tolerance

Ability of some genotypes to survive / perform better under chilling stress than other genotypes is called chilling tolerance. It is because of chilling hardening, *i.e.* an earlier exposure to a near chilling temperature for a specific period as a result of which chilling tolerance of the

concerned plants increases.

Mechanisms of chilling tolerance:

1. Membrane lipid un-saturation
2. Reduced sensitivity of photosynthesis
3. Increased chlorophyll accumulation
4. Improved germination
5. Improved fruit / seed set
6. Pollen fertility

Sources of chilling Tolerance :

1. Late adopted breeding populations *eg.* maize
2. Germplasm (*eg.* That collected from high altitude , low temperature geographic regions)
3. Induced mutants for cold tolerance
4. Cold tolerant somaclonal variants
5. Related wild species *eg.* Tomato

B. Freezing Resistance

Freezing injury / Frost injury / cryo injury

Freezing Stress : Dormant state is conducive to freezing resistance, while resistance in actively growing tissue is rare : Thus Freezing

resistance largely involves surviving freezing stress in such a manner as to enable subsequent regrowth when the temperature rises. As water in plants cools below 0°C, it may either

1. Freeze *i.e.* form ice or 2. Super cool without forming ice.

Effects of freezing stress

1. Ice formation : Two types Intercellular ice formation

Intracellular ice formation

Intercellular Ice formation: Initiation of ice formation on plant surface is sufficient to induce freezing of the internal (intercellular & xylem vessels etc.) water in most plant species.

Intracellular ice formation: It is more lethal may be due to physical disruption of subcellular structure by ice crystals. Intracellular ice formation is the major and terminal freezing stress.

Extracellular ice formation in a cases the concentrations of extracellular solutes, thereby water is withdrawn from the cells during extracellular ice formation. This creates water stress in the frozen tissue / plant.

2. Membrane disruptions :

Freezing causes disruptions and / or alter the semipermeable properties of plasma membrane · Loss of solutes from the cells occur

· Cells remain plasmolyzed even after thawing which is often called as frost plasmolysis · Cells may become highly turgid due to uptake of excess water.

3. Supercooling :

Cooling of water below 0°C without ice crystal formation is called supercooling · In plants water may cool down to -1 to -150°C in herbaceous sps and to -40 to -450°C in hardy trees.

This becomes possible apparently because internal ice-nucleators are absent in such cases. This is regarded as an important mechanism of freezing avoidance

4. Stress due to external factors : Consequent to freezing

1) Ice sheet formation below and above the ground causes reserve depletion anoxia etc. in plants.

2) Tissues killed during freeze-thaw are highly prone to pathogen attacks

3) Auto toxicity may occur

Mechanism of Freezing Resistance :

The ability of a genotype to survive freezing stress and to recover and regrow after thawing is known as freezing resistance. Freezing resistance is a complex trait involving physiological, chemical & physical processes at the tissue and cell level.

Mechanism of Freezing resistance.

1. **Freezing avoidance** : The ability of plant tissues / or genes (but the whole plants) to avoid ice formation at sub zero temperature is called freezing avoidance Supercooling is a mechanism of freezing avoidance it is controlled by

1. Lack of ice-nucleators 2. Small cell size
3. Little or no intercellular space 4. Low moisture content
5. Barriers against external nucleators 6. Presence of antinucleators
2. **Freezing Tolerance** : Ability of plants to survive the stresses generated by extra cellular ice formation and to recover and regrow after thawing is known as freezing tolerance.

The various components of freezing tolerance are as follows:

- 1) Osmotic adjustment
- 2) Amount of bound water
- 3) Plasma membrane stability
- 4) Cell wall components properties
- 5) Cold-responsive proteins Eg. ABA

Sources of freezing tolerance

1. Cultivated varieties
2. Germplasm lines
3. Induced Mutations
4. Related wild species Eg. Wheat *Agropyron* sps; rye
Barley – *H. jubatum*, *H.brachyantherum* x *H.bogdanii*, *H.jubatum* x *H.compressum*
Oats – *Avena sterilis*
5. Transgenes : Eg. chemical Synthe sized antifreeze protein gene, ala 3, in tobacco

Selection criteria

Based on

1. Field survival
2. Freezing test in laboratory
3. Cryo freezing
4. Osmoregulation

IDEOTYPE BREEDING

Crop **ideotype** refers to model plants or ideal plant type for a specific environment. In broad sense an **ideotype is a biological model which is expected to perform or behave in a predictable manner within a defined environment**. More specifically, crop ideotype is a plant model which is expected to yield **greater quantity of grains, fibre, oil or other** useful product when developed as a cultivar. The term ideotype was first proposed by **Donald in 1968 working on wheat**.

Ideotype Breeding

Ideotype breeding can be defined **as a method of crop improvement which is use to enhance genetic yield potential through genetic manipulation of individual plant character**.

Features of ideotype breeding

1. Emphasis on individual trait

In ideotype breeding, emphasis is given on individual morphological and physiological trait which enhances the yield. The value of each character is specified before initiating the breeding work.

2. Includes yield enhancing traits

Various plant characters to be included in the ideotype are identified through correlations analysis. Only those characters which exhibit positive association with yield are included in the model.

3. Exploits physiological variation

Genetic differences exist for various physiological characters such as photosynthetic efficiency, photo respiration, nutrient uptake, etc. Ideotype breeding makes use of genetically controlled physiological variation in increasing crop yields, besides various agronomic traits.

4. Slow progress

Ideotype breeding is a slow method of cultivar development, because incorporation of various desirable characters from different sources into a single genotype takes long time. Moreover, sometimes undesirable linkage affects the progress adversely.

5. Selection

In ideotype breeding selection is focused on individual plant character which enhance the yield

6. Designing of model

In ideotype breeding, the phenotype of new variety to be developed is specified in terms of morphological and physiological traits in advance.

7. Interdisciplinary approach

Ideotype breeding is in true sense an interdisciplinary approach, it involves scientist from the disciplines of genetics, breeding, physiology, pathology, entomology etc.

8. A continuous process

Ideotype breeding is a continuous process, because new ideotypes have to be developed to meet changing and increasing demands.

Features of crop ideotypes

The crop ideotype consists of several morphological and physiological traits which contribute for enhanced yield or higher yield than currently prevalent crop cultivars. The morphological and physiological features of crop ideotype differ from crop to crop and sometimes within the crop also depending upon whether the ideotype is required for irrigated cultivation or rainfed cultivation. Ideal plant types or model plants have been discussed in several crops like wheat, rice, maize, barley, cotton and beans. The important features of ideotype from some crops are

WHEAT

The term ideotype was coined by Donald in 1968 working on wheat. He proposed ideotype of wheat with following main features:

1. **A short strong stem.** It imparts lodging resistance and reduces the losses due to lodging.
2. **Erect leaves.** Such leaves provide better arrangement for proper light distribution resulting in high photosynthesis or CO₂ fixation.
3. **Few small leaves.** Leaves are the important sites of photosynthesis, respiration and transpiration. Few and small leaves reduce water loss due to transpiration.
4. **Larger ear.** It will produce more grains per ear.
5. **An erect ear.** It will get light from all sides resulting in proper grain development.
6. **Presence of awns.** Awns contribute towards photosynthesis.
7. A single culm.

RICE

The concept of plant type was introduced in rice breeding by Jennings in 1964, through the term ideotype was coined by Donald in 1968. He suggested that in rice an ideal or model plant type consists of

1. Semi dwarf stature
2. High tillering capacity and
3. Short, erect, thick and highly angled leaves
4. More panicles /m²,
5. High (55% or more) harvest index.

Now emphasis is also given on physiological traits in the development of rice ideotype.

COTTON

Ideotype for irrigated cultivation

1. Short stature (90-120 cm)
2. Compact and sympodial plant habit making pyramidal shape
3. Determinate in fruiting habit with unimodal distribution of bolling
4. Short duration (150-165 days)
5. Responsive to high fertilizer dose
6. High degree of inter plant competitive ability
7. High degree of resistance to insect pests and diseases, and
8. High physiological efficiency.

Rainfed conditions (Singh and Narayanan 1993)

1. Earliness (150-165 days)
2. Fewer small and thick leaves
3. Compact and short stature, indeterminate habit
4. Sparse hairiness,
5. Medium to big boll size
6. Synchronous bolling
7. High response to nutrients

8. Resistance to insects and diseases.

FACTORS AFFECTING IDEOTYPES

There are several factors which affect development of ideal plant type. These are briefly discussed below:

1. Crop Species

Ideotype differs from crop to crop. The ideotype of monocots significantly differs from those of dicots. In monocots, tillering is more important whereas in dicots branching is one of the important features of ideotype.

2. Cultivation

The ideotype also differs with regard to crop cultivation. The features of irrigated crops differ from that of rainfed crop. The rainfed crop needs drought resistance, fewer and smaller leaves to reduce water loss through transpiration. In dicots, indeterminate types are required for rainfed conditions, because indeterminate type can produce another flush of flowers if the first flush is affected by drought conditions.

3. Socio-economic Condition of Farmers

Socio-economic condition of farmers also determines crop ideotype. For example, dwarf *Sorghum* is ideal for mechanical harvesting in USA, but it is not suitable for the farmers of Africa where the stalks are used for fuel or hut constructions.

4. Economic Use

The ideotype also differ according to the economic use of the crop, for example, dwarf types are useful in *Sorghum* and pearl millet when the crop is grown for grain purpose. But when these crops are grown for fodder purpose, tall stature is desirable one. Moreover, less leafy types

are desirable for grain purpose and more leafy genotypes for fodder purpose. The larger leaves are also desirable in case of fodder crop.

STEPS IN IDEOTYPE BREEDING

Ideotype breeding consists of four important steps,

1. Development of Conceptual Model
2. Selection of Base Material
3. Incorporation of Desirable Traits
4. Selection of Ideal Plant Type

Practical Achievements

Ideotype breeding has significantly contributed to enhanced yields in cereals (wheat and rice) and millets (*Sorghum* and pearl millet) through the use of dwarfing genes, resulting in green revolution. Semidwarf varieties of wheat and rice are highly responsive to water use and nitrogen application and have wide adaptation. The Norin 10 in wheat and Dee-geo-Woo-gen in rice are the sources of dwarfing genes. The genic cytoplasmic male sterile systems in *Sorghum* and pearl millet

laid the foundation of green revolution in Asia (Swaminathan, 1972). Thus ideotype breeding has been more successful for yield improvement in cereals and millets than in other crops.

PROTECTION OF PLANT VARIETIES AND FARMERS RIGHTS ACT (PPV&FR, 2001)

As the existing UPOV models of plant variety protection were not suitable for our requirements, the Government of India enacted our own legislation on the In 2001 which is a unique model in the world as it provides equal rights to farmers along with breeders. For the purpose of implementation of this act, central Govt. of India established PPV&FR authority

Objectives of the Protection of Plant Varieties and Farmers Rights Act

1. Registration of plant varieties,
2. Characterization and documentation of registered varieties,
3. Documentation, indexing and cataloguing of farmer's varieties,
4. Providing compulsory cataloguing facility for all plant varieties,
5. Ensuring that seeds of all registered varieties are made available to farmers,
6. Collection of comprehensive statistics on plant varieties,
7. Maintenance of National Register of Plant variety

Plant Breeder Rights

Plant Breeder Rights are the rights granted by the Govt. to plant breeder, or owner of a variety to exclude others from producing commercially the propagating material or that variety for a period of 15-20 years.

To qualify for PBR protection a variety has to be novel, distinct from existing varieties and uniform and stable in its essential characteristics. A person holding PBR title to a variety can authorize other organizations to produce and sell the propagating material of that variety.

PBR in India

India had evolve a *sui generis* system of PBR. Which means a system of their own. The essential features of UPOV - 1978 act are being considered for adoption.

Some important features of the Indian *sui generis* system are

1. Farmers rights
2. Researchers right to use the material for research
3. Protection period of 15 years for annuals and 18 years for fruit trees
4. Compulsory deposit of the material in national gene bank

5. Establishment of National Authority for the protection of Breeders, farmers and researchers use rights.

Benefits of PBR

1. Profits obtained by breeders through PBR will act as an incentive in promoting Plant Breeder research.
2. It encourages private companies to invest in Plant Breeding Research.
3. It will enable access to varieties developed in other countries & protected by IPR laws
4. Increased competitiveness among various organizations engaged in Plant Breeding is likely to benefit both farmers and the nation

Disadvantages of PBR

1. PBR will encourage monopoly in genetic material for specific use
2. It suppress free exchange of genetic material and encourage unhealthy practices
3. The PBR holder may produce less seed and increase the price for achieving more profit.
4. Farmers privilege to resow the seed produced by him may be gradually diluted
5. PBR may result in increased cost of seed and may be burden for poor farmers

BIODIVERSITY ACT,2002

1. According to this act No person shall, without previous approval of the National Biodiversity Authority, obtain any biological resource occurring in India or knowledge associated thereto for research or for commercial utilization or for bio-survey and bio-utilisation.
2. The persons who shall be required to take the approval of the National Biodiversity Authority includes (a) a person who is not a citizen of India; (b) a citizen of India; who is a non-resident (c) a body corporate, association or organization - (i) not incorporated or registered in India; or (ii) incorporated or registered in India under any law for the time being in force which has any non- Indian participation in its share capital or management.
3. No person shall, without the previous approval of the National Biodiversity Authority, transfer the results of any research relating to any biological resources occurring in, or obtained from, India for monetary consideration or otherwise to any person who is not a citizen of India who is non-resident or a body corporate or organization which is not registered or incorporated in India or which has any non-Indian participation in its share capital or management.
Explanation-For the purposes of this section, "*transfer*" does not include publication of research papers or dissemination of knowledge in

any seminar or workshop, if such publication is as per the guidelines issued by the Central Government.

4. (i) This shall not apply to collaborative research projects involving transfer or exchange of biological resources or information relating thereto between institution's, including Government sponsored institutions of India, and such institutions in other countries, if such collaborative research projects satisfy the conditions specified in sub-section (3),

(ii) All collaborative research projects which are based on agreements concluded before the commencement of this Act and in force shall, to the extent the provisions of agreement are inconsistent with the provisions of this Act or any guidelines issued be void.

(iii) The collaborative research projects shall -

(a) Conform to the policy guidelines issued by the Central Government in this behalf; (b) be approved by the Central Government.

5. (i) No person shall apply for any intellectual property right, by whatever name called, in or outside India for any invention based on any research or information on a biological resource obtained from India without obtaining the previous approval of the National Biodiversity Authority before making such application:

Provided that if a person applies for a patent, permission of the National Biodiversity Authority may be obtained after the acceptance of the patent but before the sealing of the patent by the patent authority concerned:

Provided further that the National Biodiversity shall dispose of the application for permission made to it within a period of ninety days from the date of receipt thereof.

(ii) The National Biodiversity Authority may, while granting the approval under this section, impose benefit sharing fee or royalty or both or impose conditions including the sharing of financial benefits arising out of the commercial utilization of such rights.

(iii) The provisions of this section shall not apply to any person making an application for any right under any law relating to protection of plant varieties enacted by Parliament.

(iv) Where any right is granted under law referred to in sub-section (3), the concerned authority granting such right shall endorse a copy of such document granting the right to the National Biodiversity Authority.

6. No person, who is a citizen of India or a body corporate, association or organization which is registered in India, shall obtain any biological resource for commercial utilization, or big-survey and bio-utilisation for commercial utilization except after giving prior intimation to the State Biodiversity Board concerned:

Provided that the provisions of this section shall not apply to the local people and communities of the area, including growers and cultivators

of biodiversity, and *vaids* and *hakims* who have been practicing indigenous medicine .