PBG.202 METHODS OF PLANT BREEDING 1+1

THEORY

Reproductive and pollination systems in crop plants – Contrivances to aid self and cross pollination – Incompatibility - Sterility - Apomixis and their classification.

Centre of origin – Domestication of crop plants – germplasm, plant genetic resources – conservation and utilization – biodiversity.

Basic biometerics –nature and significance of qualitative and quantitative traits – variation – phenotypic – genotypic and environmental – heritability – genetic advance.

Selection breeding – introduction and acclimatization – self pollinated crops – genetic makeup – mass selection – pure line concept – hybridization and selection – pedigree method – bulk method – modified bulk method –mass pedigree method – single seed descent (SSD) and back cross method.

Selection breeding – cross pollinated crops – genetic makeup – mating systems – synthetics – composites – mass selection – progeny selection – recurrent selection.

Population genetics – Hardy Weinberg law – gene frequencies – random mating population - breeding asexually propagated crops – genetic makeup – clonal selection – hybridization – hybrid breeding.

Mutation breeding – types – genetic basis – mutagen and mutagenesis – handling of mutagenised population, application of mutation.

Comprehensive Plant Breeding strategy – Innovative breeding.

Special selection methods – markers – phenotypic markers – molecular markers – marker assisted selection.

PRACTICAL

Observation on reproductive and pollination systems in plants – Alternation of generation and life cycle – Description and drawing of different pollination systems – Mechanisms enforcing self and cross pollination – Morphology of pollen grains – Assessment of pollen fertility and sterility in A, B, and R line – Maintenance of A, B lines. Emasculation technique – Selfing and crossing techniques – Breeder kit – Layout of different trails – Irradiation – dosimetry – Half life period – Procedure for irradiation – Chemical mutagenesis – Molar solution – Procedure for treatment – Calculation of heterosis, PCV, GCV, heritability, genetic advance – genetic divergence – Records maintained – Wild species maintenance and utilization - screening method for specific traits – marker assisted selection.

LECTURE SCHEDULE

1. Centers of Origin – contribution of Vaviolov, Harlan and Zhukovosky – law of homologous series.
2. Conservation, evaluation and utilization - gene sanctuaries - exploration - national and international agencies engaged in conservation.
3. Incompatibility - causes of incompatibility - gametophytic and sporophytic incompatibility - significance of self - incompatibility.
4. Sterility - male sterility - genic, cytoplasmic, cytoplasmic genic male sterility and environmentally influenced male sterility - line breeding.

5. Apomixis - classification of apomixis with examples and their significance.
6. Basic biometrics –nature and significance of qualitative and quantitative traits – variation – phenotypic – genotypic and environmental – heritability – genetic advance.
7. Plant introduction - primary and secondary introduction - objectives - Acclimatization, adaptation, merits and demerits of introduction – Vilmorin principles of progeny selection - Johansen's pure line theory - Genetic structure of self-pollinated crop - Breeding methods for self pollinated crops - pure line selection - merits and demerits.
8. Mass selection and its merits and demerits - hybridization, its objectives and - types - intervarietal, interspecific - intergeneric, wide and introgressive hybridization, - choice of parents - procedures of hybridization.
9. MID SEMESTER EXAMINATION.
10. Pedigree and bulk method of selection - merits and demerits. Modified bulk method - mass pedigree method and SSD - Advantage over pedigree and bulk methods.
11. Back cross method - prerequisites and its application in transferring resistant genes - merits and demerits - Multilines, multiblends and population improvement approach in self - pollinated crops.
12. Genetic structures of a population in cross-pollinated crop Hardy - Weinberg law - Breeding methods followed in cross pollinated crops. Selection - Mass selection - Modified mass selection. Detasseling - panmixis - unit selection. Progeny testing and selection- Half sib family selection - Ear to row method, full sib family selection. Inbred or selfed family selection - Recurrent selection.
13. Heterosis, hybrid vigor and inbreeding depression. Hybrid seed production - manual emasculation and pollination - utilization of male sterility and self incompatibility.
14. Multiple Cross hybrids - advantages and disadvantages over other hybrids - Recurrent selection, types of recurrent selection and their relative advantages - synthetics and composites.
15. Genetic structure of a population of asexually reproducing crops - Clone and clonal selection - Hybridization followed by clonal selection - exploitation of heterosis to produced synthetics through poly cross technique.
16. Mutation - types of mutation - characteristics of mutation - Mutagens - Application of mutation breeding.
17. Special selection methods – markers – phenotypic markers – molecular markers – marker assisted selection.

PRACTICAL SCHEDULE

1. Pollination and Reproduction in plants - Alternation of generation and life cycle.
2. Description and drawing different pollination systems - Mechanisms enforcing self and cross pollination in crops.
3. Pollen morphology - Exine structure of different crops. Fertility and sterility in A, B, R and TGMS lines.
4. Breeder kit and its components - uses.
5. Selfing and crossing techniques in different crops.
6. Emasculation, kinds of emasculation and pollination technique.
7. Layout of different yield trials - Observing the experimental plots.
8. Irradiation - dosimetry - half life period - procedure for irradiation. Chemical mutagenesis - molar solution preparation - procedure for chemical mutagenesis.

9. Calculation of PCV, GCV, heritability, genetic advance.
10. Genetic divergence studies.
11. Maintenance of A, B and R line and TGMS lines. Hybrid seed production techniques.
12. Studies on segregating generations and maintenance of records.
13. Studies on different wild species in crop plants and wide hybridization.
14. Estimation of heterosis.
15. Screening methods – laboratory – field – for specific traits.
16. Procedure for marker assisted selection.
17. PRACTICAL EXAMINATION.

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TAMIL NADU AGRICULTURAL UNIVERSITY

PBG 202 – Methods of Plant Breeding (1+1)

Theory

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1. CENTRE OF ORGIN – CONTRIBUTION OF VAVILOV, HARLAN AND ZHUKOVOSKY – LAW OF HOMOLOGOUS SERIES.

OBJECTIVES AND SCOPE OF THE BREEDING

Plant breeding is an art and science, which tells us ways and means to change the genetic architecture of plants so as to attain a particular objective.
The objectives may be
a) Crop Improvement
b) Improved agronomic characters
c) Resistance against biotic and abiotic stress.

a) Crop Improvement:

It may be increased yield. Majority of our breeding programmes aim at increased yield. This is achieved by developing more efficient genotypes. The classical examples are utilization of Dee Gee Woo Gen in rice and Norin – 10 in wheat. Identification and utilization of male sterility is another example.

Improving the quality is another aim in crop improvement. The quality characters vary from crop to crop. For example,

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

Elimination of toxic substance is yet another aim of crop improvement.

- HCN content in Jowar plants.
- Lathyrogen content in *Lathyrus sativus* (βN oxalyamine alanine BOAA)
- Erucic acid in Brassicas
- Cucurbitacin in cucurbits.

b) Improved agronomic characters:

- Production of more tillers – E.g. Rice, Bajra.
- Elimination of season bound characters – Redgram, Sorghum
- Reducing the plant height to prevent lodging – Rice.
- Non – shattering nature – Green gram, Brassicas.
- Synchronized maturity – Pulses.
- Growth habit – From bushy to non bushy type – Pulses.
- Elimination or introduction of dormancy – Potato, Ground nut.

c) Resistance against biotic and abiotic stresses.

Biotic stress: Pests and diseases, Evolving resistant varieties reduce cost of cultivation, environmental pollution and saving beneficial insects.

Abiotic stress: It is location specific problem. Soil factors and edaphic factors some times poses severe problems. Breeding resistant varieties is the easy way to combat abiotic stress.

Scope of Plant Breeding:

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

1. Enhancing the productivity of crops

This can be done

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

2. Stabilizing the productivity achieved

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

Plant breeding, the past, present and future scopes

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

The *indica* x *japonica* cross derivative ADT 27 (GEB 24 x Norin 10) is the first high yielding rice of Tamil Nadu. The identification of Dee Gee Woo Gen and release of Wonder rice IR 8 (peta x DGWG) changed the scenario from poverty to problem of plenty.

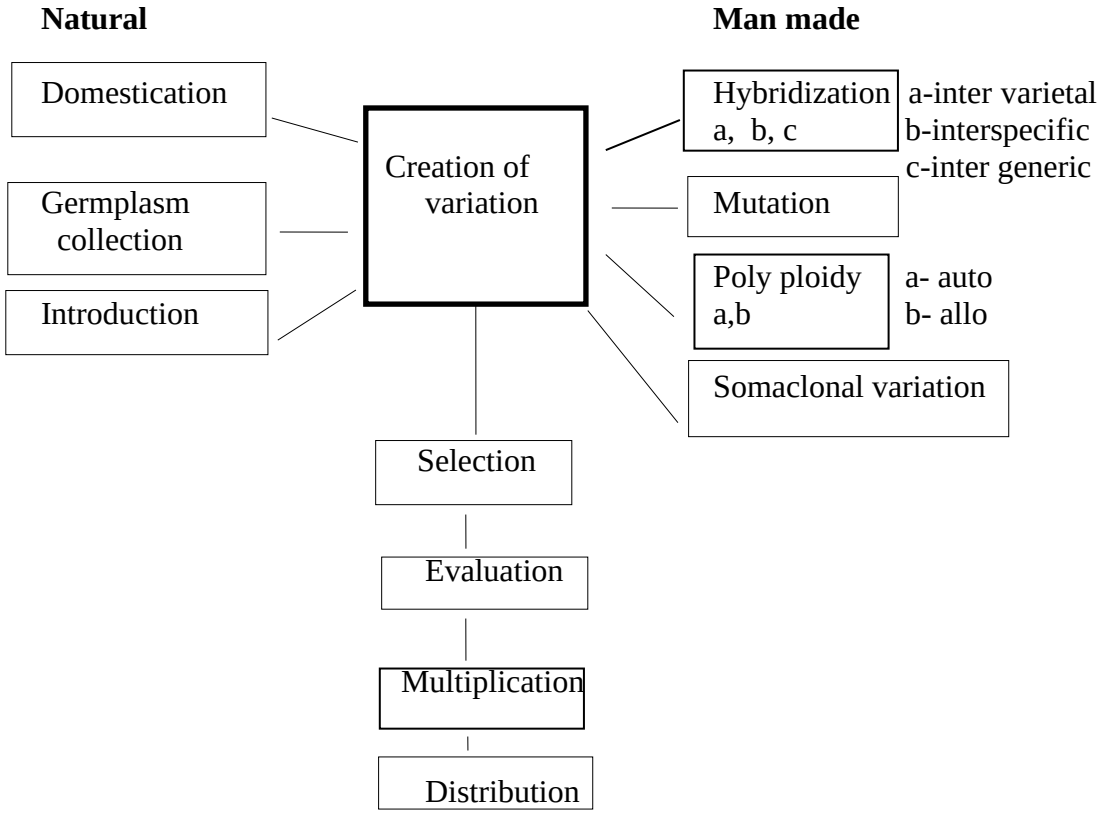
Like wise identification of dwarfing gene in Japanese wheat variety Norin – 10 by Borlaug and breeding of Mexican dwarf wheat varieties led to the release of wheat varieties life Kalyan sona in India.

In peal millet, breeding by male sterile line Tift 23A at Tifton, Georgia by Burton and his coworker and later on its introduction to India led the release of hybrid bajra HB1 to HB4 which increased bajra production many fold.

In Jowar, breeding of first male sterile line combined kafir 60A and its introduction in to India led to the release of first hybrid sorghum CSH 1 (CK 60A x IS 84) during 1970s.

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

ACTIVITIES IN PLANT BREEDING



Undesirable effects of Plant Breeding

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

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

Tift 23A	Bajra	Susceptible downy mildew
T cytoplasm	Maize	Susceptible to Helminthosporium.

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

RTV (Rice Thungro Virus)

Grey mold in Bengal gram.

4 Attainment of yield plateau - No more further increase in yield

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

- As early as 700 BC **Babylonians** and **Assyrians** artificially pollinated the date palm.
- In 1717 **Thomas Fairchild** produced the first artificial hybrid
- **Joseph Kolreuter**, a German made extensive crosses in Tobacco and *Solanum* between 1760 and 1766 and studied the progenies in detail.
- **Thomas Andrew Knight** (1759 - 1835) was the first man to produce several new fruit varieties by using artificial hybridisation.
- **Le coutier**, a farmer published his results on selection in wheat in the year 1843. He concluded that progenies from single plants were more uniform
- **Patrick Shireff** a Scotsman practiced individual plant selection in wheat and oats and developed some valuable varieties.
- **Vilmorin** (1857) proposed individual plant selection based on progeny testing. This was known as 'Vilmorins principle of progeny testing'. He proposed this progeny testing in sugar content in sugar beets (*Beta vulgaris*). But this method was in-effective in wheat. This clearly demonstrated the difference between effect of selection in cross and self pollinated crops.
- **Nilsson** and his associates at Sweedish Seed Association, Svalof Sweeden (1890) refined the single plant selection
- In 1903 **Johansen** proposed the famous 'pure line theory' which states that a pure line is progeny of a single self fertilised homozygous plant. He proposed this theory based on his studies in *Phaseolus vulgaris.*
- **G.H.Shull** work in maize is the fore runner for the present day hybrid maize programme. He described in detail about the effect of inbreeding.
- During 1960's **Norman Borlaug**, the Nobel laureate developed Mexican semi dwarf wheat varieties which paved the way for green revolution in wheat. The dwarfing gene was isolated from wheat variety **Norin 10.** Later on this Mexican dwarf were introduced in the India by Dr.M.S. Swaminathan and a number of high yielding wheat varieties like Kalyan sona, Sharbathi sonara were developed.
- In rice the identification of dwarf ***Dee Gee Woo Gen*** from a tall rice variety by a Taiwan farmer revolutionized rice breeding. Using this ***DGWG*** at IRRI during 1965 the wonder rice IR 8 was released.
- Nobilisation in sugarcane by **C.A.Barber** and **T.S.Venkatraman** is another monumental work in plant breeding.

CENTRES OF ORIGIN

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

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

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

He classified the economic plants into six classes;

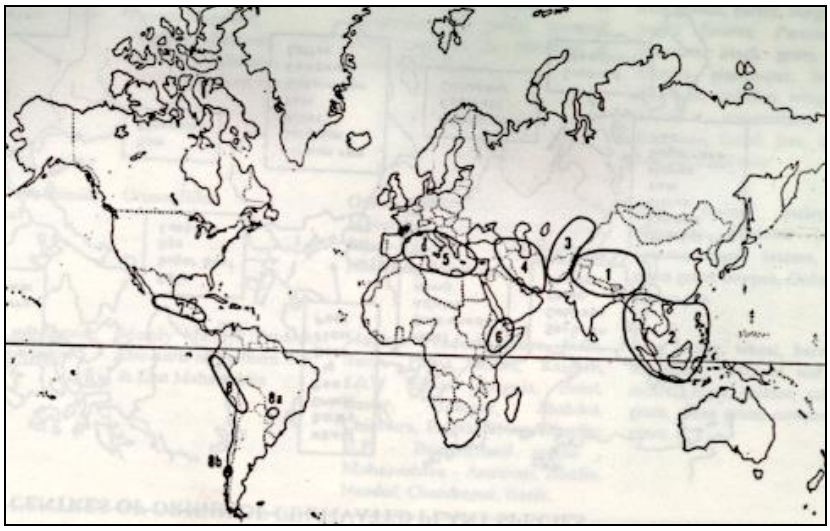
1. Plants cultivated 4000 years ago.
2. Plants cultivated 2000 years ago.
3. Plants cultivated less than 4000 years.
4. Plants cultivated 2000 to 4000 years.
5. Plants cultivated before the time of Columbus.
6. Plants cultivated after the time of Columbus.

It is **N.I.Vavilov** who proposed the concept of 'centres of origin'. He proposed the concept based on his studies of a vast collection of plants at Institute of Plant Industry, Leningrad. The concept is that crop plants evolved from wild species in the area showing great diversity and that place is termed as **primary centre of origin**. Later on from the primary centre the crops moved to other places due to the activities of man.

There are certain areas where some crops exhibit maximum diversity of forms but this may not be the centre of origin for that particular crop. Such centres are known as **Secondary centres of origin**. E.g. Sorghum

The primary centre of origin for this crop is Africa but India exhibits maximum diversity for this crop.

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



Eight main centres of origin are recognised by Vavilov, they are:
1.China
2.Hindustan
3.Central Asia
4.Asia minor
5.Mediterranian
6.Abyssinya
7.Central America
8.South America

1. The China centre: It consists of the mountainous regions of central and western China and the neighbouring low lands. It is the largest and oldest independent centre.
The crops originated in this centre are:

I .Primary centre of origin are:
Soybeans
Radish
Proso millet
Opium
Brassica
Onion

ii. Secondary centre of origin are:
Maize
Cowpea
Turnip
Sesame

2.The Hindustan Centre: This includes Burma, Assam, Malaya, Java Borneo, Sumatra and Philippines, but excludes North West India, Punjab and North Western Frontier Provinces. The crops originated in this centre are:

i. Primary centre of origin are:
Rice
Redgram
Chickpea
Cowpea
Greengram
Turmeric
Cucumber
Radish
Noble canes
Cotton *(Gossypium arboreum)*
Hemp
Coconut

3.The Central Asia Centre: It includes North West India, all of Afghanistan, the Soviet Republics of Tadjikistan and Tian Shan. It is also known as *the Afghanistan centre of origin.* The crops originated in this centre are:

i. Primary centre of origin are:	*ii. Secondary centre of origin are*:
Wheat	Rye
Pea	
Broad bean	
Green gram	
Sesame	
Safflower	
Cotton*(G.herbaceum)*	
Onion	
Garlic	

4.The Asia Minor Centre: This is also known as the *Near East or the Persian Centre* of Origin. It includes the interior of Asia Minor, the whole of Transcaucasia, Iran and Highlands of Turkmenistan. The crops originated in this centre are:

i. Primary centre of origin are:	*ii. Secondary centre of origin are*:
Triticum	Rape
Rye	Black Mustard
Alfalfa	Turnip
Cabbage	
Oats	

5.The Mediterranean Centre: The crops originated in this centre are:

i.Primary centre of origin are:

Many valuable cereals and legumes such as;

Durum Wheat	Chikpea
Emmer Wheat	Beets
Barley	Peppermint
Lentil	
Pea	
Broad bean	

6.The Abyssinian Centre: It includes Ethiopia and hill country of Eritrea. The crops originated in this centre are:

i. Primary centre of origin are:	*ii. Secondary centre of origin are:*
Barley	Broad bean
Sorghum	
Pearl millet	
Lentil	
Khesari	
Sunflower	
Castor	
Coffee	
Okra	

7.Central American Centre: This includes South Mexico and Central America.It is also referred to as the *Mexican Centre of Origin*. The crops originated in this centre are:
i.Primary centre of origin are:
Maize
Lima bean
Melons
Pumpkin
Sweet Potato
Arrowroot
Cotton *(G.hirsutum)*

8.The South American Centre: This centre includes the high mountainous regions of Peru, Bolivia, Ecuador, Colombia, parts of Chile, and Brazil and whole of Peraguay The crops originated in this centre are:

i. Primary centre of origin are:
Potato
Maize
Lima bean
Peanut
Egyptian cotton *(G.barbadense)*
Tobacco
Tapioca

Later in, 1935, Vavilov divided the Hindustan Centre of Origin into two centres, viz., *Indo Burma* and *Siam- Malaya-- Java Centre of Origin*. The South American Centre was divided into three centres, namely, *Peru, Chile* and *Brazil-Peraguay Centres of Origin*. At the same time he introduced a new centre of origin, the *U.S.A. Centre of origin*. Two plant species, Sunflower(*Helianthus annuus*) and Jerusalem Artichoke (*H.tuberosus*) originated in the U.S.A. Centre of origin.

Thus the centres of origin may be more appropriately called the centres of diversity. The centres of origin may not be the centres of origin of the species concerned, but they are the areas of maximum diversity of the species. Within the large centres of diversity, small areas may exhibit much greater diversity than the centre as a whole. These areas are known as *Microcentres* .

OBJECTIONS TO VAVILOV'S THEORY

According to **Vavilov** whenever a crop plant exhibits maximum diversity, that place is the centre of origin for that crop. But this view is no longer valid. E.g. maize and tomato.

For maize the centre of diversity is Peru but archeological evidence shows Mexico as centre of origin. For tomato, South America is considered to be primary centre of origin but it is Mexico as per archeological evidence.

Secondly Vavilov stated that primary centre is marked by a high frequency of dominant genes in the centre and recessive genes towards the periphery. But it is not so. E.g. Wheat, maize, oil palm

Vavilov's claim that centre of origin confined to mountainous regions only. But this is not the case. For E.g. Maize exhibits maximum diversity in plains

Many crops have more than one centre of origin E.g. Balsam, Sorghum. In some crops centre of domestication cannot be determined for want of suitable evidence.

To counter the objection, **Zhukovsky** student of vavilov has proposed 'mega centre' theory. He divided the world into 12 regions. Mega gene centres were the places where cultivated plant species exhibit diversity and micro gene centre is the place where wild species occur.

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

LAW OF HOMOLOGOUS SERIES:

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

2. CONSERVATION, EVALUATION AND UTILIZATION – GENE SANCTUARIES – EXPLORATION – NATIONAL AND INTERNATIONAL AGENCIES ENGAGED IN CONSERVATION.

CROP GENETIC RESOURCES AND CENTRE OF DIVERSITY

Sum total of genes in a crop species
- 'Genetic resources' or ' gene pool', 'genetic stock', 'germplasm'
- Whole library of different alleles of a species
- Basic materials for initiation of breeding programme.

Features:
- Entire genetic variability
- Land races, modern cultivars, obsolete cultivars, breeding stocks, wild forms, species
- Cultivated & wild species
- Collected from centers of diversity, gene bank, gene sanctuaries, farmers field, market and seed companies.
- Basic material

Classification of gene Pool

1) Area of collection
2) Domestication
3) Duration of conservation
4) Crossability in breeding program.

1) Area of collection: a) Indigenous b) exotic
2) Domestication: a) Cultivated b) wild
3) Duration of conservation:

a) Base collection: For long term storage. Used for regeneration. Seed viability not less than 95%
Seeds with 5 ± 1 % moisture content
$-18^{O}C$ to $-20^{O}C$

b) Active collections
Medium teem forage. (10-15 years)
Regeneration, multiplication, evaluation, distribution and documentations.
$0^{O}C$ storage moisture content around 8%.
Routine tests after every 5-10 years.
Large sample – regeneration – to reduce genetic drift.

c) Working collection: Short term (3-5 years) $5^{O}C - 10^{O}C$ seeds moisture 8 – 10%.

4) Crossability in Breeding Program

1) Primary gene pool GP_1: Intermating is easy – production of fertile hybrids. Same species or closely related.
2) Secondary gene pool GP_2: Partial fertility on crossing with GP_1 plants related species.
3) Tertiary gene pool GP_3: Sterile hybrids on crossing with primary gene pool. Needs special techniques.

Components of Genetic Resources

Various plant materials constituting gene pool.

1) Land races: Primitive cultivars.
   - evolved under sub resistance agriculture.
   - High level of genetic diversity – diseases, pests.
   - Broad genetic base
   - Less uniform
   - Low yielders.
   - N.I. Vavilov in rice.

2) Obsolete cultivars
   - Improved varieties of recent part
   - Replaced by new varieties.
   - Wheat varieties K68, K65, pb591 – Traditional Tall before Mexican wheat attractive grain color and good chapatti making.

3) Modern cultivars
   - Currently cultivated high yielding varieties.
   - High yield potential, uniformity, parents in breeding program Narrow genetic base
   - low adaptability.

4) Advanced breeding lines

Pre-released plants developed by plant breeders. Not yet ready for release.

5) Wild forms of cultivated species.

High degree of resistance.

6) Wild relatives.
   1) hybrid sterility problems in crossing
   2) hybrid invariability
   3) undisarable genes with desirable alleles.

7) Mutants

Mutant gene pool Dee-Geo-Woo-Gen in rice and Norin 10 in wheat. Valuable genetic resources. In seed propagated crops, 410 varieties have been released.

Crop Genetic Diversity

Variety of genes and genotypes found in a crop species. Genetic diversity – broad genetic base to population.

N.I. Vailov (1926, 1951) realized the importance. Proposed eight main centers of diversity and three subsidiary centers.

Vavilov not covered Africa, Australia.

Primary Centres of Diversity.

- Regions of vast genetic diversity
- Large number of dominant genes
- Mostly have wild characters
- Less crossing over
- Natural selection operates.

Secondary centers.
- lesser genetic diversity
- large number of recessive genes
- have desirable characters
- more crossing over
- both natural and artificial selections operate.

Microcenters
- Small areas – tremendous genetic diversity.
- Study of evolution of cultivated species.
- Vavilov – parallel series of variation – Law of homologous series of variation. Particular variation in a crop ⟷ another related spread.

Germplasm activities.

1) Exploration and collection.
- Collection trips tapping genetic diversity from various sources.
- assembling at one place.

i) Genetic erosion: - Reduction in genetic variability
a) Replacement of land races with improved cultivars
b) Modernization of agriculture – eliminates wild & weedy former.
c) Extension of farming into wild habits.
d) Grazing into wild habitats.
e) Growth of cities.

ii) Extraction: Permanent loss of a crop species.

Process

1) Sources of collection:	Centres of diversity gene bank gene sanctuaries seed companies farmers fields
2) Priority of collection:	Endangered areas, endangered species
3) Agencies of collection:	SAU ICAR IPGRI

4) Method of collection: Expeditions, personal visit to gene bank, correspondence, exchange of material.

5) Method of sampling: Random sampling – biotic tolerant stresses
Biosed sampling – distinct morphological characters.

6) Sample rice: 95% of total diversity should be caps there. 50 –100 individuals, 50 seeds/ plant.

Merits

1) Tapping crop genetic diversity
2) New material, prevents extraction

Demerits

entry of new disease, remote areas.

2. Conservation

Protection of genetic diversity of plants from genetic erosion.

In situ Conservation: under natural conduction.
Establishment of biosphere reserves.
- Costly method, several areas have to be conserved.

Ex situ conservation: Preservation of germplasm in gene banks.

Cheaper, easy, entire genetic diversity conserved.

Seed meristem

easy

Long term (50-100 years)
Medium term (10-15 years)
Short term (3-5 years)

Robert (1973) – Orthodox – dried to low moisture content, no loss in validity.
Eg: wheat, papaya, various beans.
- recalcitrant

Drastic loss in viability with a decrease in meristem below 12-13^{O}C
Eg: Cocoa, margo, tea, coffee, jackfruit, ruble.

Meristem

Merits – free from virus

- Vegetatively propagated crop.
- Perennial plants
- Regeneration easy

3. Evaluation:

1) to identify gene sources
2) classification of germplasm.

- by simple measures of dispersion (Range, standard deviation, SE, CV)
- by metroglyph analysis of Anderson (1957)
- D^2 statistic of Maharlanobis (1936)

4. Documentation

Compilation, analysis, classification, storage, dissemination of information.
Information system.

- provides information about various activities of plant genetic resistance.
- 7.3 million germplasm accession – 200 crop species.

IPGRI – descriptor

5. Distribution:

Specific germplasm – supplied on demand.

6) Utilization use of germplasm in crop improvement programme
cultivated germplasm - as a variety
- as parent
- as variant in gene pool.

Wild – Transfer of resistance.
IPGRI – Supervised by consultative group on international Agricultural Research (CGIAR)
CGIAR – 1972 by FAO, world bank.
UNDP to establish international research Institutes.
IPGRI established by CGIAR in 1994.

Conducting research and to promote an International Net work of plant Genetic Resources.
IBPGR Till 1993 – IBPGR 1974.

NBPGR

NBPGR – by ICAR – 1976 – New Delhi
1946 – plant introduction started at IARI, New Delhi.
1961 – separate division of Plant Introduction – Dr. H.B. Singh
1976 – NBPGR

Quarantine: 1914 – Destructive Insects and Pest Act. Photosanitary certificate.

NBPGR, FRI –

- Dehradun and Botanical survey of India. Calcutta
- Directorate of plant protection, Quarantine and storage - faredabad
- good grains & produces imported for human consumption.

GERMPLASM

The germplasm collection is a collection of large number of genotypes of a crop species and its wild relatives. In other words it is the sum total of hereditary or genes present in a species.

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.

Germplasm collections are also known as gene banks or gene pool or world collection. The term working germplasm refers to the smaller number of collections kept by a breeder for hybridization programme.

Need for Germplasm Bank :

a) The modernisation of agriculture and evolution of high yielding varieties and hybrids led to the replacement of the land races. For examples after the introduction of IR 20 rice for samba season all the local varieties like karthigai samba, Toppi sampa, Rubber samba, Thiruchengodu samba. Athur samba went out of cultivation. Along with them the beneficial genes also vanished. This is known as genetic erosion or in other words narrowing down of variability. So, to prevent the loss of variability in cultivated forms and their wild relatives (Genetic erosion) it is necessary to maintain germplasm.

b) Nature has provided enormous variability for the use of mankind. We should not destroy them and preserve them for the use of future man kind.

Germplasm conservation :

It involves short term conservation and long term conservation.

Short term conservation:

Based on the viability of the seeds the gene pool is to grown once in two years or more than two years. Each line is to be grown with proper spacing and care must be taken to ensure self pollination, so that the genetic architecture is not altered. For example in sorghum covering the panicle in boot leaf stage itself ensures selfing.

This short term conservation is a costly affair which requires much time, labour, land and cost.

Further there is every chance for mixing up of genotypes while large number is handled annually.

Long term conservation :

To over come this difficulty long term preservation in the cold storage the germplasm can be preserved. Using liquid nitrogen the germplasm can be stored for more than ten years. Complete information about the genotypes can be computerized and this is known as cataloguing and information retrieval system.

Gene Sanctuaries or Insitu conservation:

The areas of diversity are protected from trespass of human beings by fencing the area so that the plant species are preserved under natural conditions. This is known as insitu conservation.
E.g. Meghalaya for citrus, North Eastern Region for Musa, Oryza, Saccharum.

Exploratory Surveys :

NBPGR will arrange for survey and collection of germplasm. Explorations generally cover those that are likely to show great diversity of forms. Tribal areas will have more forms of diversity. Along with ICRISAT, NBPGR the TNAU has conducted exploratory surveys for collection of small millets, sorghum and pearl millet.

The palamalai hills of Coimbatore is a rich source of diversity for sorghum. *Sorghum halapense* both 2n = 20 and 2n = 40 forms are available there. The kodaikanal hills are having *S.nitidum* under natural conditions. In southern districts *S.stafii* is available. Anaikatti hills are rich source of diversity for small millets. Normally during surveys the samples collected will be of three kinds.

a) **Field Sample :** Seeds collected from field or farm areas where it is available.
b) **Market sample :** Types available in local shandies or market will be collected.
c) **Storage sample :** By visiting the houses of farmers the seeds stored for sowing will be collected.

Centres maintaining germplasm

1. Institute or plant Industry, Leningrad.
2. Royal Botanic Gardens, Kew, England.
3. USDA, Beltsville.
4. ICRI SAT. _____ Sorghum, Red gram, Ground nut, Pearl millet and Bengal gram
5. IRRI - Rice
6. AVRDC, Taiwan - Soybean (Asian Vegetable Res. Devl. centre)
7. NBPGR - Inter National Germplasm Repository.
8. NBPGR - National Germplasm Repository.

3. INCOMPATIBILITY – CAUSES OF INCOMPATIBILITY – GAMETOPHYTIC AND SPOROPHYTIC INCOMPATIBILITY – SIGNIFICANCE OF SELF - INCOMPATIBILITY

SELF INCOMPATABILITY

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

Definition

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

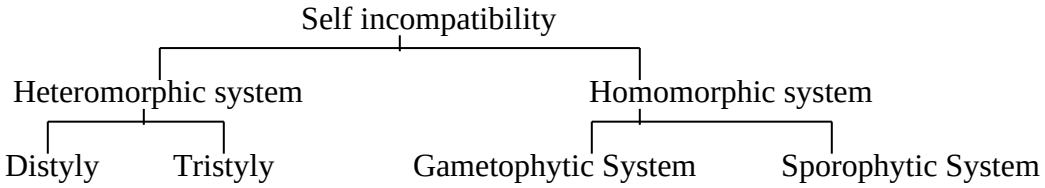
a) Self incompatible pollen grain may fail to germinate on the stigmatic surface.
b) Some may germinate but fails to penetrate the stigmatic surface
c) Some pollen grains may produce pollen tube which enters through stigmatic surface but its growth will be too slow. By the time the pollen tube enters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

Reason

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

Classification of self incompatibility

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



Heteromorphyic system

In this case there will be difference in the morphology of the flowers. For example in primula there are two types of flowers namely PIN and THRUM.

PIN flowers have long style and short stamens while THRUM flowers have short style and long stamens. This type of difference is known as Distyly

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

In case of distyly the only compatible mating is between PIN and THRUM. The relative position of anthers is determined by single gene S/s. The recessive ss produces PIN and heterozygotes Ss produces THRUM. Homozygous dominant SS is lethal and do

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

Mating		**Progeny**	
Phenotype	**Genotype**	**Genotype**	**Phenotype**
Pin x Pin	ss x ss	Incom. mating	-
Pin x Thrum	ss x Ss	1 ss : Ss	1 Thrum 1 Pin
Thrum x Pin	Ss x ss	1 Ss : ss	1 Thrum 1 Pin
Thrum x Thrum	Ss x Ss	Incom. mating	-

Homomorphic System

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

Gametophytic System

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

Genotype of Plant (Sporophyte)	$S_1\ S_2$	$S_3\ S_4$
Genotype of gametes	S_1 S_2	S_3 S_4
Incompatible reaction of pollen	S_1 S_2	S_3 S_4
[Incompatible reaction Of style	S_1 S_2	S_3 S_4
Mating	S_1S_2 x S_1S_2 -	Fullly Incompatible
	S_1S_2 x S_1S_3 -	Partially compatible
	S_1S_2 x S_3S_4 -	Fully compatible.

Sporophytic System

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

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

Lewis has summarized the characteristics of sporophytic system as follows.

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

MACHANISM OF SELF INCOMPATABILITY

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

a) Pollen - Stigma interaction
b) Pollen tube - Style interaction.
c) Pollen tube - Ovule interaction.

a) **Pollen - Stigma interaction :**

This occurs just after the pollen grains reach the stigma and generally prevents pollen from germination. Prviously it was thought that binucleate condition of pollen in gamatophytic system and trinucleate condition in sporophytic system was the reason for self incompatability. But later on it was observed that they are not the reason for S1. Under homomorphic system of incompatability there are differences in the stigmatic surface which prevents pollen germination. In gametophytic system the stigma surface is plumose having elongated receptive cells which is commonly known as wet stigma. The pollen grain germinates on reaching the stigma and incompatability reaction occurs at a later stage.

In the sporophytic system the stigma is papillate and dry and covered with hydrated layer of protein known as pellicle. This pellicle is involved in incompatability reaction. With in few minutes of reaching the stigmatic surface the pollen releases an exince excudate which is either protein or glycero protein. This reacts with pellicel and induces callose formation which further prevents the growth of pollen tube.

Pollen - Stigma interaction

Gametophytic System	Sporophytic System
Stigma surface Plumose Commonly known as wet Stigma	Stigma surface Papillate and dry. Covered with hydrated layere of protein known as pellicle which involves in incompatibility reaction.
Pollen grain germinates and incompatibility reaction occurs at a later stage.	Pollen grain releases exine exudate which is protein or Glycero-protein. This protein reaction with pellicle and induces callose formation and arrests growth of pollen type.

b) Pollen Tube - Style interaction :

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

c) **Pollen tube - Ovule interaction :**

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

Relevance of Self incompatibility in Plant Breeding :

Self incompatibility may be used for Hybrid seed production.

a) By planting two self incompatible but cross compatible varieties alternatively seeds obtained from both the lines are hybrids.
b) Alternatively by planting a self incompatible variety along with self compatible variety, the seeds obtained from self incompatible line will be a hybrid.

Hybrid seed production was made in brassicas, clover, Trifolium Solanaceous and Asteraceae crops. But there are certain difficulties in this.

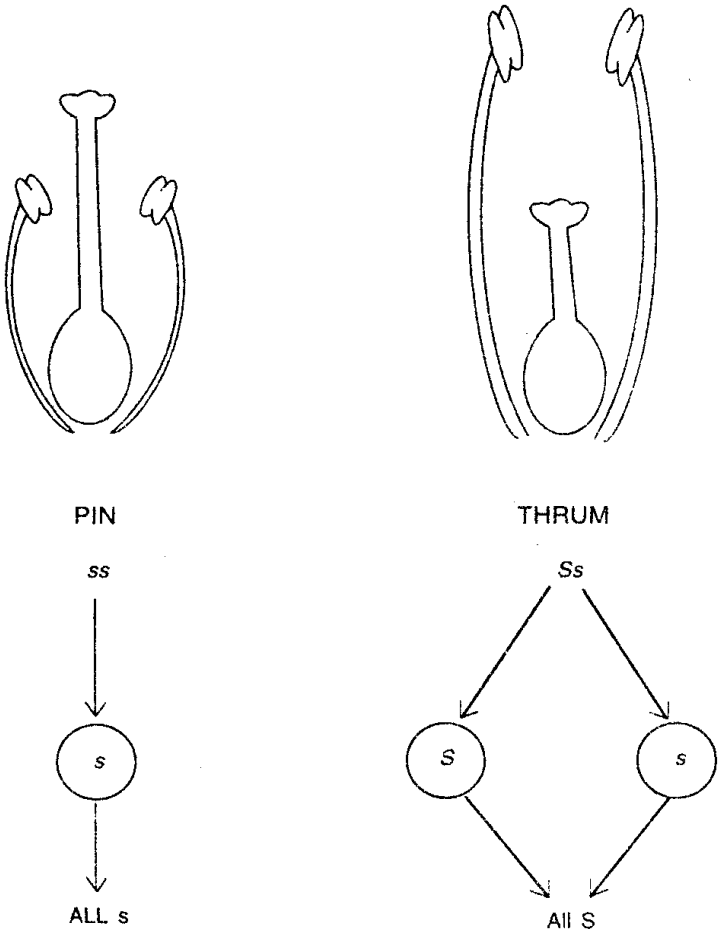
a) Production and maintenance of inbred line by hand pollination is tedious and costly.
b) Continuos selfing leads to break down of self incompatibility and self fertile lines will appear.
c) Environmental factors such as high temperature and high humidity reduce self incompatibility.
d) Bees often prefers to stay with in particular parental line which in turn increases the proportion of selfed seed.

Elimination of Self incompatibility :

1) In a single gene gametophytic system by doubling the chromosome number we can elimate self incompatibility.
2) By induced mutagenesis to produce self fertile lines.
3) By transferring self compatible alleles from related species thro' back cross breeding.

Over coming Self incompatibility

1. **By bud pollination :** Application of matured pollen to immature stigma.
2. **By surgical technique :** Removal of the stigmatic surface E.g. Brassicas or removal **of style E.g. Petunias.**
3. **End of season pollination :** In some cases self incompatibility is reduced towards the end of flowering period. Pollination at that time may be successful.
4. **Use of high temperature :** Exposure of pistil to 60^0C will induce pseudo fertility.
5. **Irradiation :**
6. **Grafting :** Grafing of a branch to another branch.
7. **Double pollination :** Application of a mixture of incompatible and compatible pollen grains.



Mating		*Progeny*	
Phenotype	*Genotype*	*Genotype*	*Phenotype*
Pin × pin	*ss* × *ss*	Incompatible mating	
Pin × thrum	*ss* × *Ss*	1 *Ss* : 1 *ss*	1 Thrum : 1 pin
Thrum × pin	*Ss* × *ss*	1 *Ss* : 1 *ss*	1 Thrum : 1 pin
Thrum × thrum	*Ss* × *Ss*	Incompatible mating	

Fig. 3.3. Heteromorphic-sporophytic system of incompatibility. The relative positions of stigma and anthers are governed by a single *S/s*. The incompatibility reaction of pollen is governed by the genotype of the sporophyte on which it is produced, while that of style is controlled by its own genotype. Allele *S* is dominant over *s* in determining incompatibility as well as the length of style. Pollen of an incompatibility group would not 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.***

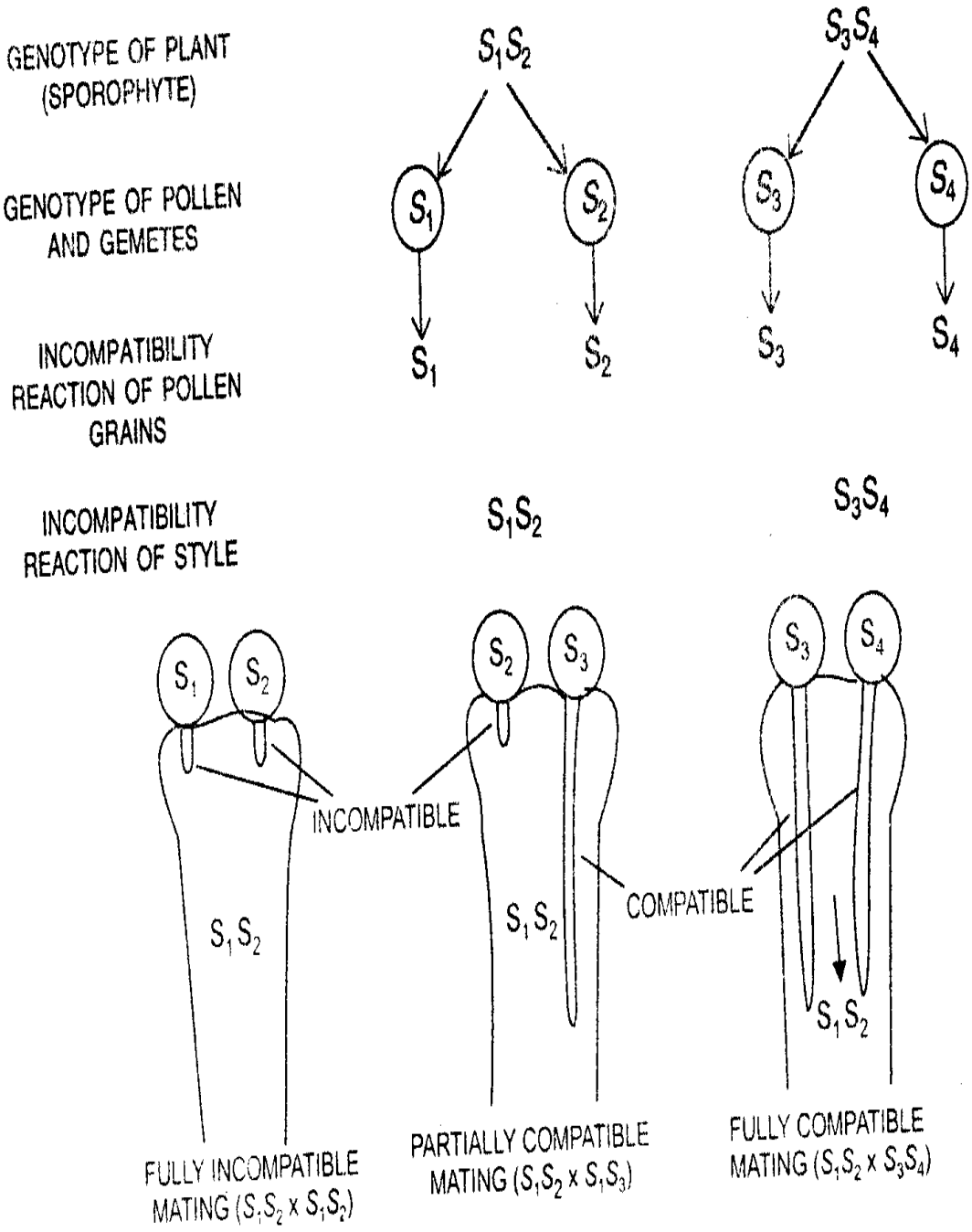
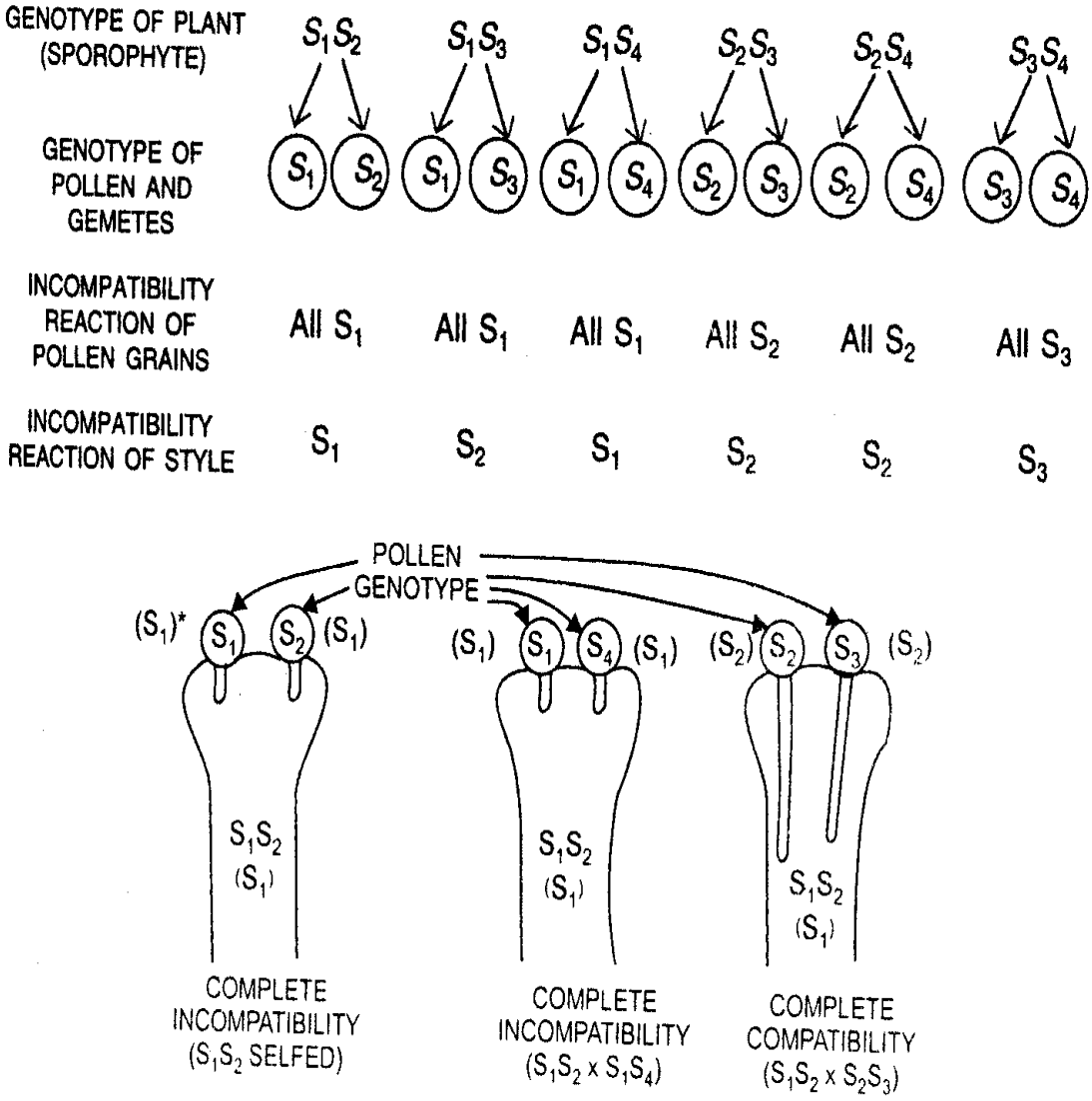


Fig. 3.4. Gametophytic system of self-incompatibility. The incompatibility reaction of pollen is governed by the genotype of pollen itself. The incompatibility reaction of style is determined by its own genotype and, generally, the two alleles show codominance.



The letters within parenthesis, *e.g.*, (S_1) and (S_2) denote the incompatibility reactions of pollen grains and styles. A complete dominance is assumed.

Fig. 3.5. 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.

4. STERILITY – MALE STERILITY – GENIC, CYTOPLAMIC, CYTOPLAMIC GENIC MALE STERILITY AND ENVIRONMENTALLY INFLUENCED MALE STERILITY – LINE BREEDING

MALE STERILITY

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

Morphological features of male sterility:

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

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

Kinds of male sterility, maintenance and uses:

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

a) Cytoplasmic male sterility (CMS)
b) Genetic male sterility (GMS)
c) Cytoplasmic-genetic male sterility (CGMS)
d) Environmental induced male sterility which is again sub divided in to
    i) TGMS (Theromosensitive)
    ii) PGMS (Photo sensitive)

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

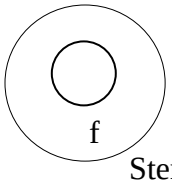
B line or maintainer line: This line is used to maintain the sterility of A line. The B line is isogenic line which is identical for all traits except for fertility status.

R line and restoration of fertility: It is other wise known as Restorer line which restores fertility in the A line. The crossing between A x R lines results in F_1 fertile hybrid seeds which is of commercial value.

1. Cytoplasmic Male Sterility (CMS):

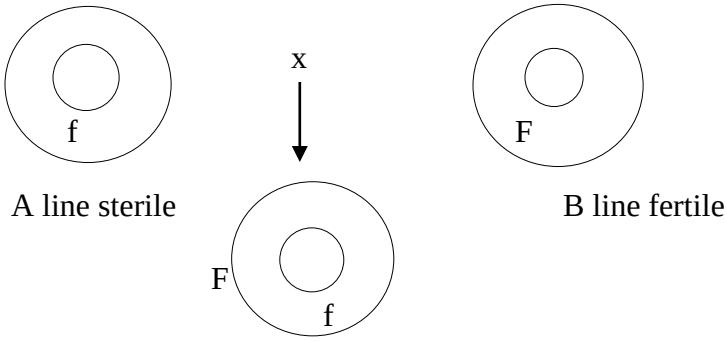
It occurs due to the mutation of mitochondria or some other cytoplasmic factors outside the nucleus. Nuclear genes are not involved here. There is considerable evidence that gene or genes conditioning cytoplasmic male sterility. Particularly in maize DNA reside in mitochondria and may be located in a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

Genetic structure



Sterile

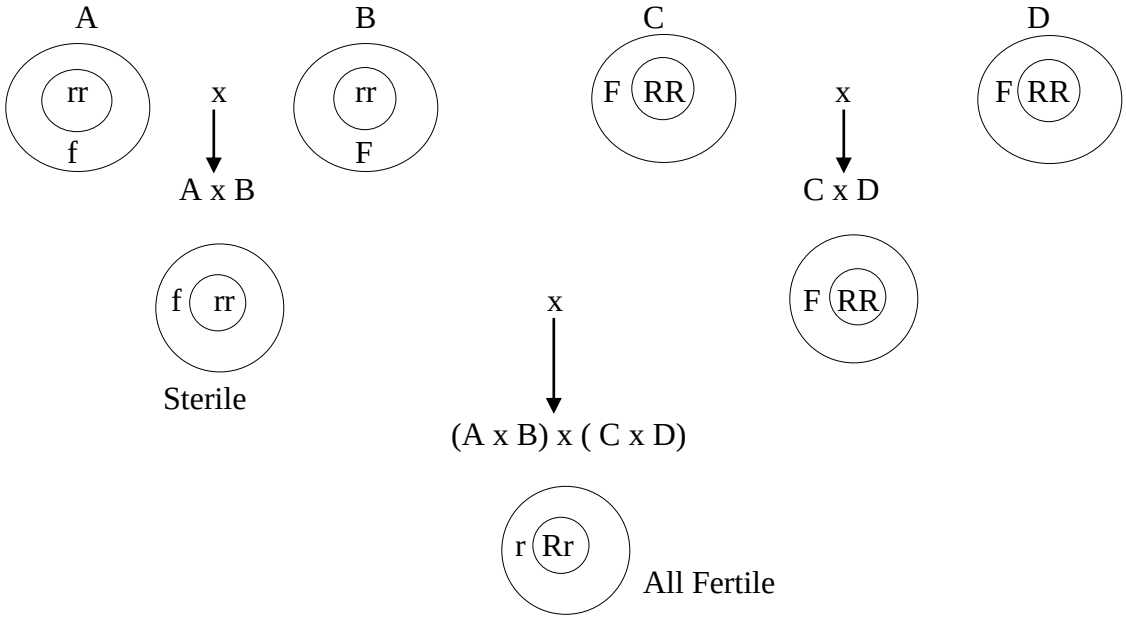
Maintenance



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

Uses:

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

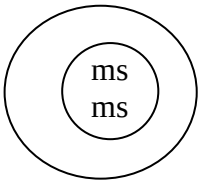


GENETIC MALE STERILITY : (GMS)

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

Genetic structure :

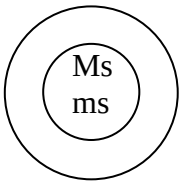
A line



In Redgram there are number of GMS lines are available. E.g. **Ms Co5, Ms T21**

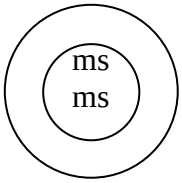
Maintenance :

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



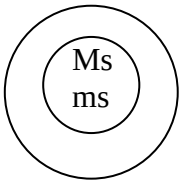
When this heterozygous line is grown in the field it will segregate in the ratio of 1 Fertile : 1 sterile.

Sterile



1 :

Fertile



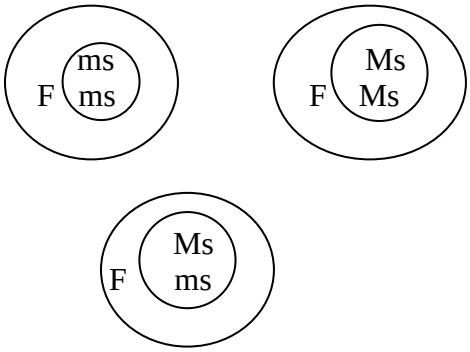
1

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

For hybrid seed production, the seeds collected from sterile plants will be grown using double the seed rate since it will segregate in the ratio of 1 fertile : 1 sterile line. At the time of flowering, the fertile line will be identified by yellow plumpy anthers and removed from the field. Only the sterile line will remain in field. These will be pollinated by the R line and the F1 obtained will be hybrid redgram.

Utilisation: Hybrid Development. Eg: Redgram

Ms T21 x ICPL 87109
A line R line



Hybrid
CoRH 1

DIFFICULTIES IN USE OF GMS

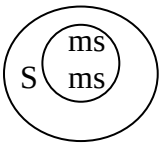
1. Maintenance of GMS requires skilled labour to identify fertile and sterile line. Labelling is time consuming and costly
2. In hybrid seed production plot identification of fertile line and removing them is costly.
3. Use of double the seed rate of GMS line is costly.
4. In crops like castor high temperature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

CYTOPLASMIC – GENEIC MALE STERILITY

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

Genetic Structure

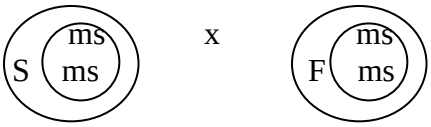
A line



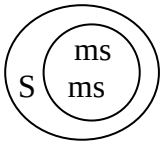
Male sterile.

Maintenance

A line x B line



sterile Fertile



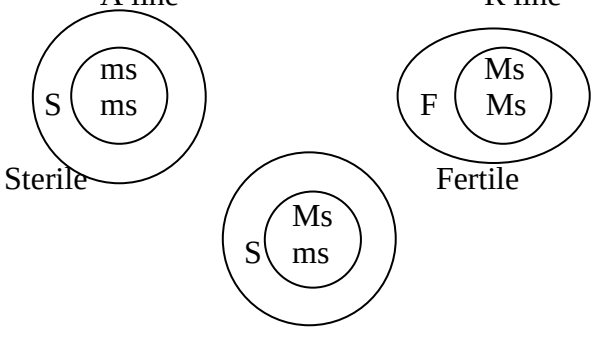
Male sterile line

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

UTILISATION :

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

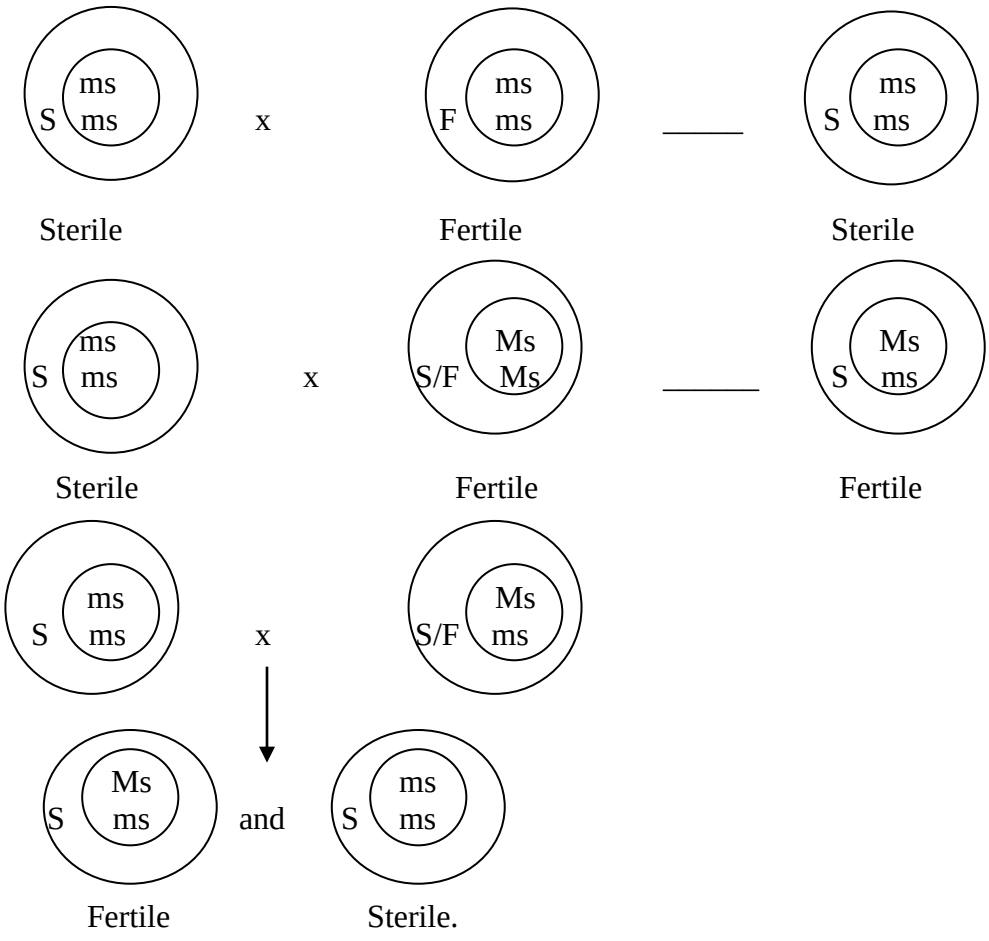
A line R line



Sterile Fertile

Hybrid
Fertile

DIFFERENT TYPES OF MATING IN CGMS LINE



Limitations of CGMS lines.

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

LINE BREEDING

The process of using different lines (genotype) and producing hybrid is known as line breeding. This terminology is used in production of rice hybrids.

The different kinds of line breeding are.

a) One line method
b) Two line method
c) Three line method

a) **One line method of rice breeding** :

Rice hybrids can be developed and propagated through the following concepts.

- Vegetative propagation. This can be done by ratooning followed by stubble planting.
- Micropropagation employing tissue culture technique.
- Anther culture hybrids. The anthers of F_1 hybrid can be cultured and plant lets developed.
- Apomictic lines.

b) **Two line method of rice breeding**.

Two line hybrids can be evolved through application of gametocides and use of environmentally induced genic male sterility.

To the selected female parent pollen suppressors can be sprayed at the time of flowering so that it will arrest the production of pollen and thus temporary male sterility is induced. The best combiner is used as a male parent and hybrid is produced.

The EGMS system is used successfully in china. Both TGMS and PGMS lines were identified. In this system male sterility is mainly controlled by one or two pairs of recessive nuclear genes and has no relation to cytoplasm. In this system only two lines viz. male sterile and Restorer lines are used. Maintainer line is not needed because by growing the male sterile line in suitable atmosphere the sterility is maintained. In this method there is no negative effect due to sterile cytoplasm.

Three line method or CGMS System

This system nowadays known as CGMS system involving three lines viz. a) Cytoplasmic genic male sterile line. b) Maintainer or B line and c) Restorer line.

TRANSFER OF MALE STERILITY OF A NEW STRAIN

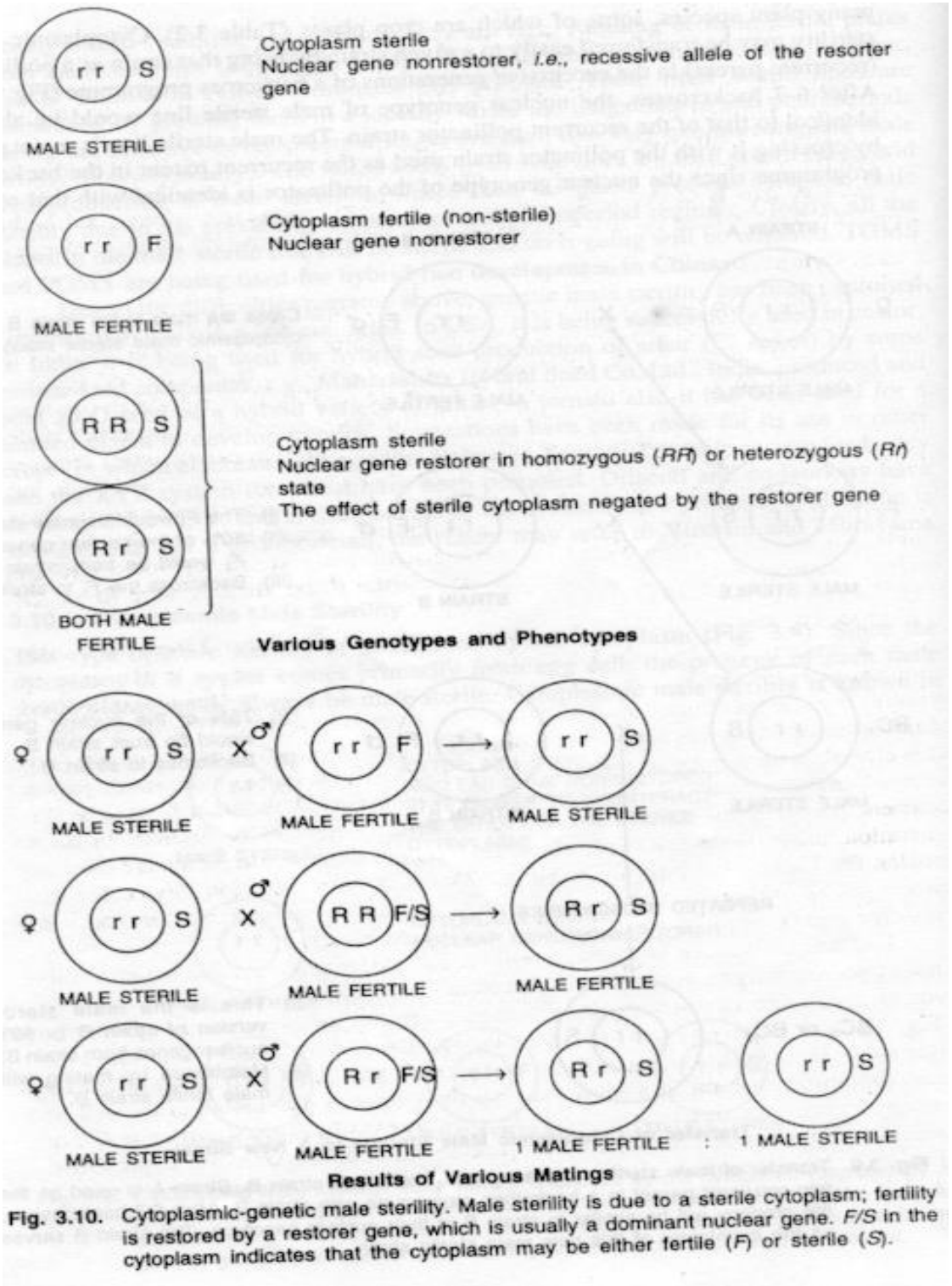
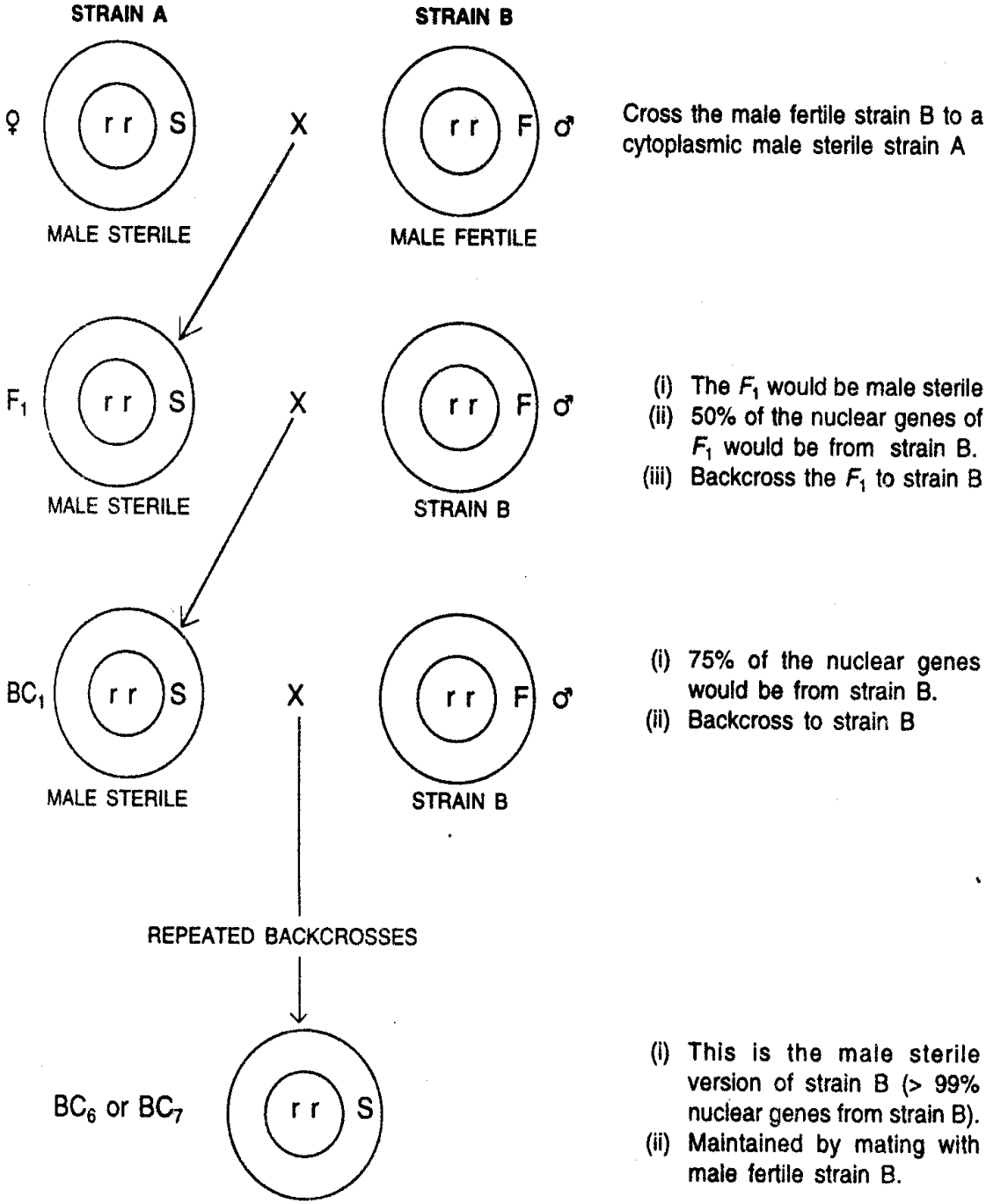


Fig. 3.10. Cytoplasmic-genetic male sterility. Male sterility is due to a sterile cytoplasm; fertility is restored by a restorer gene, which is usually a dominant nuclear gene. *F/S* in the cytoplasm indicates that the cytoplasm may be either fertile (*F*) or sterile (*S*).

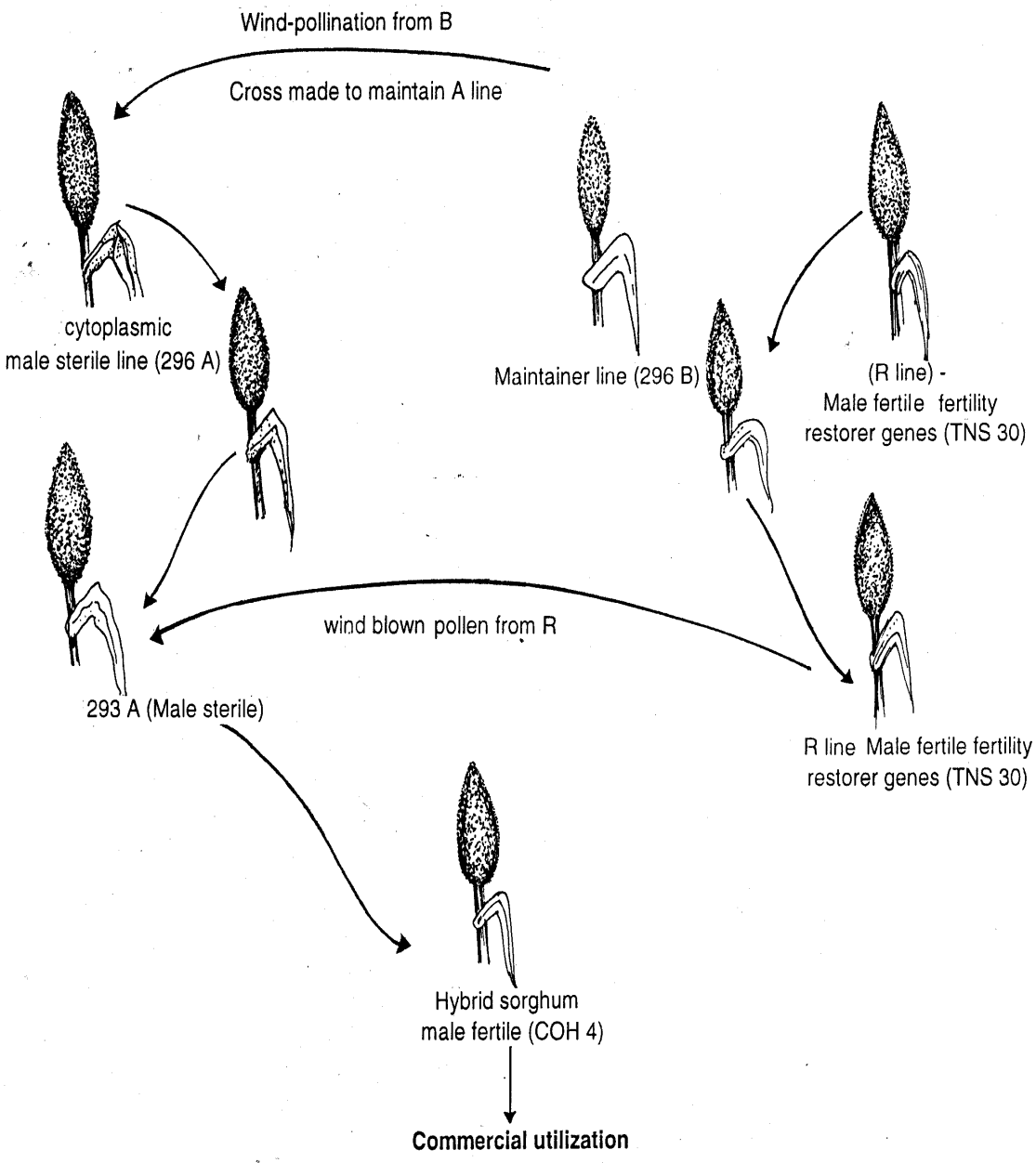


Transfer of Cytoplasmic Male Sterility to A New Strain

Fig. 3.9. Transfer of male sterile cytoplasm from strain A to strain B. Strain A is used as the non-recurrent 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.

Hybrid Seed Production using CGMS Lines

COH 4 : HYBRID SORGHUM. Seed production and Maintanance.



Scheme for producing hybrid sorghum seed, using cms and fertility restoring genes. The A line in male sterile cytoplasm is maintained by pollination from a genetically identical B line in normal cytoplasm. The hybrid seed is produced by pollinating the A line from the R line. The R line has dominant fertility restoring genes and combines with the A line to produce a high yielding hybrid.

5. APOMIXIS – CLASSIFICATION OF APOMIXES WITH EXAMPLES AND THEIR SIGNIFICANCE.

INTRODUCTION:

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

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

APOMIXIS

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

Types of apomixis :

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

1. Recurrent Apomixis :

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

2. Non -recurrent Apomixis :

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

3. **Adventive Embryony** :

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

4. **Vegetative apomixis** :

In some cases like *Poa bulbosa* and some *Allium, Agave* and grass species, vegetative buds or bulbils, instead of flowers are produced in the inflorescence. They can also be reproduced without difficulty. However, Russian workers do not group this type of vegetative reproduction with apomixis.

Now, different apomictic phenomena in each of the recurrent and non-recurrent apomicts are considered in relation to the development of the embryo sac or embryo.

Development of apomictic embryo sac

1. **Apospory** :

It involves the development of embryo sac either from the archesporial cell or from the nucellus, or from other cell. It is of two types :

(i) *Generative or haploid apospory*: If the embryo sac develops from one of the megaspores (n), the process is called generative or haploid apospory. Since it cannot regenerate, as it is haploid and fertilization fails, the process gives rise to non-recurrent apomicts.

(ii) *Somatic or diploid apospory*: When diploid embryo sac is formed from nucellus or other cells, the process is termed as somatic or diploid asopory. Since it regenerates without fertilization, it is recurrent.

Development of apomictic embryo

1. Parthenogenesis :

This refers to the development of embryo from egg-cell without fertilization, e.g. in some cases in corn, wheat, tobacco. This is also of two kinds :

(i) *Haploid parthenogenesis*: The embryo develops from egg-cell without fertilization in a haploid embryo-sac produced by generative apospory. It is non-recurrent in nature.

(ii) *Diploid parthenogenesis*: The embryo develops from egg-cell without fertilization in a diploid embryo-sac arising from somatic apospory. It is recurrent type.

2. Apogamy :

This is related to the development of embryo not from the egg-cell but from any one of the synergid or antipodal cells within the embryo sac, without fertilization. This is

haploid or diploid. In the haploid apogamy, the embryo arises from any cell other than the egg-cell without fertilization in haploid embryo -sac formed by generative apospory. By virtue of its haploid nature, it is also non-recurrent apomixis. Whereas in case of diploid apogamy, embryo develops from any cell other than the egg-cell without fertilization in a diploid embryo-sac developed by somatic apospory. It is recurrent type.

3. Androgamy :

It is the development of embryo neither from egg cell nor from synergids or antipodals, but from one of the male gametes itself, inside or outside the embryo-sac. Since it is haploid, it is non-recurrent apomixis.

In another phenomenon, i.e. **parthenocarpy**, seedless fruits are formed from ovary without fertilization. Normally, fertilization stimulates the ovary to become enlarged and form fruit. But in case of parthenocarpy, such stimulation may be received even from incompatible pollination.

Genetics of apomixis : Crosses between amphimicts and apomicts belonging to the same species, segregate for the two types of individuals in advanced generations. This suggests that apomixis is a genetically controlled phenomenon in plants. Stebbins (1958) states that, as a rule, the apomictic condition is recessive to sexuality, although polyploid apomicts show tendency towards dominance. However, this recessiveness is not usually due to a monogenic difference. Since there is frequent reversion of apomicts to normal sexuality or sterility or some abnormal genetic behaviour in crosses involving in apomict and an amphimict or involving two apomicts of diverse origins, it appears that a successful apomictic cycle is the result of an interaction of many genes which tend to break on hybridization. It is only in the relatively simple types of apomixis, like adventive embryony and vegetative reproduction that simple genetic beheaviour can be expected. Recently, Vardy *et al*. (1989) recorded three recessive genes with additive effects responsible for parthenocarpy.

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

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

1. Rapid multiplication of genetically uniform individuals can be achieved without risk of segregation.
2. Heterosis or hybrid vigour can permanently be fixed in crop plants, thus no problem for recurring seed production of F_1 hybrids.
3. Efficient exploitation of maternal effect, if present, is possible from generation to generation.
4. Homozygous inbred lines, as in corn, can be rapidly developed as they produce sectors of diploid tissues and occasional fertile gametes and seeds.

Exploitation of apomixis in crop improvement :

The use of apomixis in crops in a follow-up process, after a variety or hybrid is evolved, as reflected by the benefits it renders. Therefore, our aim in this section is to deal with only apomixis as a tool to plant breeding.

With a view to exploit apomixis in sexual crops, it needs to detect and identify an apomictic phenomenon, occurring spontaneously in any plant, or, to incorporate it artificially, perhaps through hybridization between apomicts and amphimicts.

Detection of apomixis : Positive evidence for the presence or absence of apomixis can be obtained only from an intensive screening of a large number of plants in a variety/hybrid. The screening involves a careful and systematic tracing of steps for the development of embryo-sac and embryo, through microtomy of ovule, right from megaspores to embryonic development. as such, therefore, it is a most tedious job requiring a lot of patience and persistence indeed.

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

Maintenance and transfer of apomixis : Once an apomict plant is detected its inheritance should promptly be studied by crossing a half or few flowers with the pollen obtained from normal plants and going through the segregation pattern in F_2 and onward generations. The remaining flowers may thoroughly be checked and seeds collected on maturity. The true nature of such plants would become distinct only after progeny tests. A true apomictic plant will automatically produce mother apomictic progenies which can be maintained without difficulty.

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

Lecture 6

BASIC BIOMETRICS

The phenotype may be described according to a mathematical model to facilitate statistical analysis and interpretation. The phenotypic mean i.e. *X* of a given genotype from the trial may be expressed as m,

$\bar{x} = \mu + g + e + ge$ where,

x = phenotypic mean

μ = general population mean

g = effect of genotype

e = effect of environment

ge = interaction between genotype and environment

Analysis of variance for genotypes grown in a replicated trial according to rbd

Source of Variation	d.f.	Expectation of MS
Replications	r-1	$\sigma_e^2 + g\sigma_r^2$
Genotypes	g-1	$\sigma_e^2 + r\sigma_g^2$
Error	(r-1) (g-1)	σ_e^2
Total	(rg)-1	

g and r are the number of genotypes and replications respectively; and σ_e^2, σ_r^2 and σ_g^2 denote the variances due to error, replications and genotypes respectively.

Genotypic variance (σ_g^2)=(MS due to genotypes – MS due to error)/R

Phenotypic variance (σ_p^2) = $\sigma_g^2 + \sigma_e^2$

Genotypic Co-efficient of variation

$$GCV := \frac{\sigma_g^2}{\mu} \times 100$$

Phenotypic Co-efficient of variation

$$PCV = \frac{\sigma_p^2}{\mu} \times 100$$

Heritability

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Genetic Advance

$$GA = \frac{\sigma_g^2}{\sqrt{\sigma_p^2}} \times K \text{ where,}$$

K = Selection differential which is constant for the known selection intensity (k at 5% selection intensity = 2.06).

7. PLANT INTRODUCTION – PRIMARY AND SECONDARY INTRODUCTION – OBJECTIVES – ACCLIMATIZATION, ADAPTATION, MERITS AND DEMERITS OF INTRODUCTION

PLANT INTRODUCTION

Definition

Taking a genotype or a group of genotypes in to a new place or environment where they were not grown previously.

Thus introduction may involve new varieties of a crop already grown in that area, a wild relative of the crop species or totally a new crop species for that area.

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

Primary Introduction

When the introduced crop or variety is well suited to the new environment, it is directly grown or cultivated with out any alteration in the original genotype. This is known as primary introduction. E.g. IR. 8, IR 20, IR 34, IR 50 Rice varieties. Oil palm varieties introduced from Malaysia, mashuri rice from Malaysia.

Secondary Introduction

The introduced variety may be subjected to selection to isolate a superior variety or it may be used in hybridization programme to transfer some useful traits. This is known as secondary Introduction.

E.g. In soybean EC 39821 introduced from Taiwan is subjected to selection and variety Co1 was developed.

In rice ASD 4 is crossed with IR 20 to get Co 44 which is suited for late planting.

Objectives of Plant Introduction

1. To introduce new plant species there by creating ways to build up new industries. E.g. Oil palm
2. To introduce high yielding varieties to increase food production. E.g. Rice and wheat.
3. To enrich the germplasm collection. E.g. Sorghum, Groundnut.
4. To get new sources of resistance against both biotic and abiotic stresses. E.g. NCAC accessions to have rust resistance in groundnut. Dasal rice variety for saline resistance
5. Aesthetic value - ornamentals.

Plant Introduction Agencies

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

A centralised plant introduction agency was initiated in 1946 at IARI, New Delhi. During 1976 National Bureau of Plant Genetic Resources (NBPGR) was started. The bureau is responsible for introduction and maintenance of germplasm of agricultural and horticultural plants. Similarly Forest Research Institute, Dehradun has a plant introduction organisation which looks after introduction, maintenance and testing of germplasm of forest trees. Besides NBPGR the Central Research Institutes of various crops also maintain working germplasm. All the introductions in India must be routed through NBPGR, New Delhi. The bureau functions as the central agency for export and introduction of germplasm.

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

Procedure for plant Introduction

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

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

Functions of NBPGR

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

Merits and demerits of plant introduction.

Merits.

1. It provides new crop varieties which are high yielding and can be used directly
2. It provides new plant species.
3. Provides parent materials for genetic improvement of economic crops.
4. Enriching the existing germplasm and increasing the variability.

5. Introduction may protect certain plant species in to newer area will save them from diseases. E.g. Coffee and Rubber.

Demerits

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

ACCLIMATIZATION

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

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

The success of acclimatization depends upon two factors

i) Place effect
ii) Selection of new genotypes.

8. VILMORIN PRINCIPLES OF PROGENY SELECTION – JOHANSEN'S PURE LINE THEORY – GENETIC STRUCTURE OF SELF-POLLINATED CROP – BREEDING METHODS FOR SELF POLLINATED CROPS – PURE LINE SELECTION – MERITS AND DEMERITS.

METHODS OF BREEDING AUTOGAMOUS CROPS

The following are the methods of breeding autogamous plants.

1. Introduction

2. Selection
   a) Pure line selection
   b) Mass selection

3. Hybridization and selection
   i) Inter varietal
      a) Pedigree Method
      b) Bulk Method.
      c) Single Seed Descent Method.
      d) Modified Bulk Method
      e) Mass - Pedigree Method.
   ii) Interspecific hybridization

4. Back cross method
5. Multiline varieties
6. Population approach
7. Hybrids.
8. Mutation breeding
9. Polyploidy breeding
10. Innovative techniques

SELECTION IN SELF POLLINATED CROPS

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

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

HISTORY OF SELECTION :

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

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

During 1857 Hallet in England practiced single plant selection in wheat, oats and barley and developed several commercial varieties.

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

PURE LINE THEORY

A pure line is the progeny of a single self fertilized homozygous plant.

The concept of pureline was proposed by **Johannsen** on the basis of his studies with beans *(Phaseolus vulgaris*) variety called Princess. He obtained the seeds from the market and observed that the lot consisted of a mixture of larger as well as smaller size seeds. Thus there was variation in seed size. Johannsen selected seeds of different sizes and grown them individually. Progenies of larger seeds produced larger seeds and progenies from smaller seeds produced small seeds only.

This clearly showed that there is variation in seed size in the commercial lot and it has a genetic basis. He studied nineteen lines al together. He concluded that the market lot of the beans is a mixture of pure lines.

He also concluded whatever variation observed with in a line is due to environment only. Confirmatory evidence was obtained in three ways.

In line 13 which is having 450 mg seed wt he divided the seeds on weight basis. He divided the line into seeds having 200, 300, 400 and 500 mg weights and studied the

progenies. Ultimately he got lines having weight ranging from 458 to 475. Thus the variation observed is purely due to environment.

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

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

ORIGIN OF VARIATION IN PURELINES

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

EFFECT OF SELF-POLLINATION ON GENOTYPE

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

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

This can be calculated by the formulae

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

where m = No. of generations of self-pollination.
n = No. of genes segregating.

When number of genes are segregating together, each gene would become homozygous at the same rate as Aa. Thus the number of genes segregating does not

affect the percentage of homozygosity. Similarly linkage between genes does not affect the percentage of homozygosity in the population.

Genetic advance under selection

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

$V_P = V_G + V_E$

Where P= phenotype

G = genotype

E = Environment

Genetic advance:

It is the improvement in the mean genotypic value of the selected families over the base population is known as genetic advance under selection.

Genetic advance under selection depends upon

a) Genetic variability among different plants or families in the base population.
b) The herbitability of the character under selection.
c) The intensity of selection i.e., the proportion of plants or families selected.

Genetic advance under selection may calculated as follows.

$GS = (K) (\sigma P) (H)$

Where GS = Genetic advance under selection

k = Selection differential

σP = Phenotypic standard deviation of the base population

H = Heritability of the character under selection.

PURELINE SELECTION

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

CHARACTERISTICS OF PURELINES

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

General steps for making a pureline selection

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

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

Third Season : Repeat the process of second season.

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

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

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

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

Advantage of Pureline Selection.

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

Disadvantages :

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

b) MASS SELECTION

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

Steps :

First season :

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

Second season :

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

Third to Sixth Season :

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

Merits of Mass Selection :

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

Demerits :

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

COMPARISON BETWEEN PURELINE AND MASS SELECTIONS

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

Hybrids in Self-pollinated Crops – problems and Prospects

Exploitation of heterosis through F_1 hybrids has hitherto been the prerogative of cross-pollinated crops, chiefly due to their breeding systems favouring allogamy. However, possibilities of working for such a proposition have recently been realized in self-pollinated corps also. Indeed, exploitation of hybrid vigour in autogamous crops is easy and less time-consuming in that homozygous inbreds are already available. There is practically no difference with regard to hybrid breeding between self and cross-pollinated crops. But the prospects of hybrids in selfers is dependant on three major considerations.

1. How high a heterotic effect can be gained under optimal production conditions? In fact, a breeder's main concern is the magnitude rather than the frequency of occurrence of heterosis in crops. Thus the consideration is whether or not it is possible to obtain economically viable heterosis.

2. How much of the yield surplus due to high heterosis can offset the extra seed cost? In major self-pollinated crops like wheat, barley, rice, etc., the seed rate per unit area is exorbitant and hence the hybrid seed requirement is also more.

3. How efficient and effective is the mechanism of cross-pollination in selfers? By nature, self-pollinated crops are shy pollinators with very poor pollen maneuverability (or movability to effect allogamy). Therefore, the efficiency (degree of allogamy) with which cross pollination can take place on a commercial scale is the true determinant of the success of a hybrid programme in selfers.

Among self-pollinated crops, F_1 hybrids have been graduated into the farmer's field in barely, tomato, Sorghum (often-cross-pollinated) and wheat. Briggle (1963) presented a vivid account of heterosis in wheat. Work in rice is also most encouraging (IRRI, 1972).

HYBRID WHEAT

Wheat is a self pollinated polyploid species where heterozygosity occurs due to the presence of two or more different genomes. Hence it is a permanent hybrid. By inbreeding it is not possible to remove the heterozygosity. In polyploid wheat species, part of the heterosis may already be exploited in varietal development as homozygous genomic heterosis. All these views are theoretical ones.

In wheat there occurs hybrid vigour up to 40% and it is expressed more frequently when parents involved are productive and genetically divergent. Several cytopalsmic genic male sterile liens and corresponding maintainers are available. R lines capable of fertility restoration are also available. The male sterility is derived from the species *T. timopheevi*.

However, there are three main difficulties in exploiting CGMS based on timopheevi cytoplasm.

(i) difficult fertility restoration

(ii) shriveling of F_1 kernels
(iii) dangers of genetic uniformity due to the same cytoplasm.

A chromosomal male sterility system applicable to hexaploid wheat has been already devised by Driscoli. This system is known as XYZ system. This involves use of nullisomics and irradiation. As wheat is basically a self pollinated crop a number of problems are encountered.

Hybrid rice

Heterosis in rice was reported by Jones in USA as early as 1926 and Ramiah in 1933. But the research work on hybrid rice was initiated in 1964 in China by Yuan Long Ping (Father of hybrid rice). The identification of 'Wild Abortive' or WA type ctyoplasmic male sterility in 1970 was the break through in hybrid rice breeding.

Constraints in hybrid rice programme

1. The Chinese male sterile lines have a tendency to become fertile when temperature goes up.
2. Identification of effective restorers to those Chinese CGMS lines are a failure. Chinese restorers became ineffective when used under local conditions.
3. Special techniques are required to increase the out crossing.

At present there are ways to overcome the constraints

IRRI male sterility and R lines are stable compared to Chinese lines.

A new source of male sterility using the cross TN 1 x Pankhari 203 had been identified. Similarly Assam collection of rice have larger anther size, stigma size and duration of glume opening. These characters augment the out crossing. Similarly at IRRI some accession of *O.perennis* and *O.sativa* var *O.spontanea* found to have large anthers and protruding stigma, longer duration of spikelet opening.

Hand emasculation and crossing is a success in crop where there is abundant seed. E.g. Brinjal, Tomato, Chilies, Tobacco. In a brinjal a single flower will produce about 1500 seeds after crossing.

9. MASS SELECTION AND ITS MERITS AND DEMERITS – HYBRIDIZATION, ITS OBJECTIVES AND – TYPES – INTERVARIETAL, INTERSPECIFIC – INTERGENERIC, WIDE AND INTROGRESIVE HYBRIDIZATION – CHOICE OF PARENTS – PROCEDURES OF HYBRIDIZATION.

HYBRIDIZATION AND SELECTION

Objective of hybridization

The chief objective of hybridization is to create variation. When two genotypically different plants are crossed, the genes from both the parents are brought together in F_1. Segregation and recombination produce many new gene combinations in F_2 and subsequent generations.

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

COMBINATION BREEDING

The main aim of combination breeding is the transfer of one or more characters into a single variety from other varieties. These characters may be governed by oligogenes or polygenes. In this approach, increase in yield is obtained by correcting the weaknesses in the yield contributing traits like tiller number, grains per panicle, seed weight of the concerned variety. Example for combination breeding is disease resistance achieved by backcross breeding. Pedigree method is also another example.

Transgressive breeding

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

PROCEDURE OF HYBRIDIZATION

1. Set up your objective.
2. Selection of parents.
3. Evaluation of parents.
4. Sowing plan.
5. Emasculation and dusting.
6. Labelling and bagging.
7. Harvesting and storage of seeds.

1. Objective

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

2. Selection of parents

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

3. Evaluation of parents

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

4. Sowing plan

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

5. Emasculation and dusting

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

6. Labelling and bagging

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

7. Harvesting and storage of seeds

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

DISTANT HYBRIDIZATION

When crosses are made between two different species or between two different genera, they are generally termed as - **Distant hybridization (or)**
- Wide hybridization

History :

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

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

Inter generic hybrid produced by Karpechenko, a Russian Scientist in 1928.
Raphano brassica is the amphidiploid from a cross between Radish (*Raphanus sativus*) and cabbage (*Brassica oleraceae*)

Triticale was produced by Rimpau in 1890 itself. Triticale is an amphidiploid obtained from cross between wheat and rye.

Another example is *Saccharum* nobilisation involving three species

Barriers to production of distant hybrids.

In some cases, distant hybridisation may be obtained without an appreciable difficulty. E.g. *G.hirsutum* x *G.barbadense* crosses. But in majority of cases, F_1 hybrids may be obtained with variable degrees of difficulty and in many cases, hybrids may not be obtained with currently available technique.
E.g. *Corchorus olitorius* x *C.capsularis*

The difficulties encountered in the production of interspecific hybrids may be grouped into three broad cases viz.

1. Failure of zygote formation (or) Hybrid sterility.
2. Failure of zygote development (or) Hybrid inviability.
3. Failure of F_1 seedling (or) Hybrid break down.

1. **Failure of zygote formation** :

It may be due to failure of fertilization. It may be due to

a) Slow pollen tube growth E.g. Datura.
b) Style may be longer than pollen tube - E.g. Maize and Trypsaccum crosses
c) Pollen tubes of polyploid species may be thicker than diploid species and growth of pollen tube will be slower.

2. **Failure of Zygote development** :

In many cases fertilisation takes place and zygote is produced. But the development of zygote is blocked at various stages. It may be due to

a) **Lethal genes** : These lethal genes will act only in interspecific crosses E.g. *Aegilops umbellulata* has 3 lethal genes. These will act when crosses made between *Ae.umbellulata* and diploid wheat.

b) **Genotypic disharmony between two parental genomes** :

Genetic imbalance between two species may lead to death of the embryo. E.g. Crosses with *Gossypium davidsoni* show early embryonic mortality. This may be due to lethal gene action.

c) **Chromosome elimination** :

Chromosomes are gradually eliminated from the zygote. This has been due to mitotic irregularities. Elimination is reported in *Nicotiana, Hordeum, Triticum*

d) **Incompatability Cytoplasm** :

Cytoplasm of female parent may prevent embryo development.

e) **Endosperm abortion** :

The endosperm may abort during early embryo development E.g. *Triticum* and *Secale* crosses; *Hordeum bulbosum* x *H.vulgare* crosses.

3. **Failure of hybrid seedling development**

Due to chlorophyll deficiency seedlings may die. E.g. M*elilotus* hybrids.

Techniques for the `production of distant hybrids.

1. First determine barrier to production of hybrid embryos
2. In general the species with shorter style should be used as the female parent. Where ever this is not possible the style of the parent is to be cut off.
3. In some cases autopolyploidy may be helpful in interspecific hybridisation.
   E.g. Diploid Brassicas would not cross. Making them autotetraploids leads to easy crossing.
4. Wherever embryo abortion is there, embryo rescue technique can be adopted.
5. With species having different ploidy levels, are crossed, hybridization will be difficult. In such cases higher ploidy level species is used as female for crossing. Secondly, the chromosome number of wild species of F_1 may be doubled to over come sterility.
   E.g. *Solanum tuberosum* cross with wild species.
6. When two species cannot be crossed directly, a third species can be used as bridge species. E.g. *Nicotiana*
7. Use of growth regulators like IAA, 2, 4 - D at the flowering stage increases seed set.

Role of wild species in crop improvement.

Many of our important crop species are allopolyploids. These crop evolved through distant hybridization. E.g. Groundnut, Ragi, Sugar cane, Cotton, Wheat, Brassicas, Tobacco, Potato. Many of the wild species are having resistance against both biotic and abiotic stresses which are successfully utilized for crop improvement.

Rice:

Co 31 - *O.perennis* x GEB 24 Drought resistant.

IR 34 - a derivative of complex cross and *Oryza nivara* is one of the parents having resistance against grassy stunt virus. The following wild species are being used in breeding programme.

O.barthi	BLB resistance
O.longistaminata	Drought tolerance
O.rufipogan	Source of male sterility in rice.

Sorghum : The following are the wild species available in Tamil Nadu which are used in breeding programme.

Sorghum halapense 2n : 20 form crossed with CO.11 and the fodder sorghum variety Co27 was evolved.

S.nitidum : Highly resistant to shoot fly and having high dormancy.

S.stafii : Having high dormancy occurs as weed in sorghum fields.

Sorghum sudanense is used for evolving forage sorghum.

Cumbu : *Pennilsetum purpureum* crossed with *P.glaucum* to evolve Cumbu Napier forage grass.

1. *P.glaucum* x *P.squamulatum*

   |

   Forage grass combining frost resistance.

2. *P.glaucum* x *P. orientale*

   |

   For the development of apomicts.

3. *P.glaucum* x *P.setaceum*

   |

   Development of male sterile lines.

Sugarcane : Nobilisation of sugarcane involves the wild species *S.spontaneum*.

Red gram : *Cajanus cajan* crossed with *C.lineata* and *C.scaraboides* to have resistance against wilt and also to induce male sterility.

Ground nut: *Arachis batizoccoi* crossed with *A.hypogaea* to have rust resistant lines.

Arachis villosulicarpa - for increased number of pods.

A.monticola - for thin shelled condition

Sesamum : *S.malabaricum* is crossed with *S.indicum* to have male sterile lines as well as to have resistance against powdery mildew.

S.alatum : Resistance against powdery mildew and phyllody.

Cotton : By transferring *hirsutum* genome to the cytoplasm of wild species *G.harknessi* CGMS lines were obtained.

G. tomentosum : Resistant to drought, Jassids, lint fineness and strength.

G.barbadense var. *darwinii* : jassid (Tetraploid) resistant

G.hirsutum race *punctatum* : Resistant to black arm.

A number of diploid wild species are available having resistance against pest and diseases.

Potato : Cultivated tetraploid Potato *S.tuberosum* is obtained by natural crossing of diploid wild species *S.sparsipilum* with *S.vernii* followed by natural doubling. The following diploid species are used in breeding programme.

1. *S.ajanhuii* : Frost resistant
2. *S.phureja* : Non - dormant
3. *S.ptenomum* : Having 6 month dormancy, longer in duration.

Tobacco : Cultivated tobacco which is an amphidiploid was obtained by natural crossing with wild species and doubling.

Wild species

N.debneyi - Resistant to root rot.
N. longiflora - Resistant to black shank disease.
N.glutinosa - Mosaic resistant for bridging two species.
N.digluta is used for bridging two species.

Application of wide hybridization in crop improvement :

1. **Alien - Addition lines** : An alien addition line carries one chromosome pair from a different species in addition to normal diploid chromosome complement of the parent species. When *only one chromosome* from another species is present it is known as alien addition monosome. Alien addition lines have been produced in wheat, oats, tobacco and several other species. This is generally used to transfer disease resistance from wild species.

2. **Alien - Substitution lines** : An alien-substitution line has chromosome pair from a different species in the place of one chromosome pair of the recipient species. Alien substitution monosome has been developed in wheat, Cotton, tobacco. In tobacco, mosaic resistance gene N was transferred from *N.glutinosa* to *N.tabacum*.

3. **Transfer of small chromosome segments.**
   Transfer of small chromosome segments carrying specific desirable genes have been widely used in crop improvement programmes. E.g. Transfer of black arm resistance from *G.barbadense* to *G.hirsutum*.

4. **Quality Improvement**:

   By transferring genes from wild species quality has been improved
   E.g. Genes for increased protein content in rice, soybean, oats and rye.

5. **Mode of reproduction** :

   In some cases incompatability alleles from wild species can be transferred to cultivated species for hybrid seed production.
   E.g. Brassicas.

6. **Yield** :

   Increased yield through introgression of yield gene from a related wild species into cultivated species E.g. Oats.

7. **Transfer of cytoplasm** :

   Done by repeated back crossing. Mainly used for transferring male sterility into cultivated species, E.g. *Sesamum*

8. **Development of new crop species**

   E.g. *Raphano brassica*
   Triticale.

10. Mid Semester

11. PEDIGREE AND BULK METHOD OF SELECTION – MERITS AND DEMERITS. MODIFIED BULK METHOD – MASS PEDIGREE METHOD AND SSD – ADVANTAGE OVER PEDIGREE AND BULK METHODS.

a) PEDIGREE METHOD :

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

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

Sl.No.	Cross Number	Parentage
1	X S 9801	Co2 x MS 9804
2	X S 9802	Co4 x C152
3	X S 9803	Co1 x Co4

X = Cross
S = Summer Season.
98 = Year
1 = Serial No.of Cross.

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

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

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

Merits of Pedigree Method :

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

Demerits :

1. Maintenance of pedigree record is time consuming and limits handling of larger population.

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

PEDIGREE METHOD PROCEDURE

F_1 Generation : The F_1 seeds are space planted so that full expression of F_1 can be had. It is advisable to raise the parents involved in the cross to raise as border rows so that dominance and other characters can be studied. The F_1s are harvested as single plants.

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

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

F_4 generation : Similar to F_3.

F_5 generation : Many families would have attained homozygosity and may be harvested as row bulk.

F_6 generation : The row bulk may be assessed in multi row trial. The families exhibiting segregation may be isolated and studied separately.

F_7 generation - RRYT

F_8 generation - PYT
CYT 3 seasons.

Basis of selection :

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

Early generation testing:

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

Shuttle breeding :

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

Off season nursery :

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

b) BULK METHOD

In this method F_2 and subsequent generations are harvested as bulk to grow the next generation. The duration of bulking may be 6 - 7 generations. Selection can be made in each generation but harvest is done as bulk. This is similar to mass selection . At the end of bulking period single plant selection is made and tested for yielding ability.

If bulking period is long say 20 - 30 seasons, then natural selection acts on the homozygous lines.

In this method the breeder uses his skill for selecting the plants and at the same time there is no pedigree record. This saves much time and labour.

Merits of bulk method :

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

Demerits :

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

c) SINGLE SEED - DESCENT METHOD.

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

In this Scheme the main features are:

1. Lack of selection till F_6 or F_7 when the population becomes homozygous.
2. Each F_2 plant is represented till F_6 or F_7 generation.
3. In this method there are chances for reduction in population size due to pest, disease or poor germination.

4. Rapid generation advancement (RGA) can be made with the use of glass house or off season nursery.

d) MODIFIED BULK METHOD :

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

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

e) MASS PEDIGREE METHOD :

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

COMPARISON BETWEEN BULK AND PEDIGREE METHODS

	Pedigree method	Bulk method
1.	Individual plants are selected in F_2 and the subsequent generations and individual plant progenies are grown.	F_2 and the subsequent generations are maintained as bulks.
2.	Artificial selection, artificial disease epidemics etc., are an integral part of the method.	Artificial selection, artificial disease epiphytotics etc., may be used to assist natural selection. In certain cases, artificial selection may be essential
3.	Natural selection does not play any role in the method.	Natural selection determines the composition of the populations at the end of the bulking period.
4.	Pedigree records have to be maintained which is often time consuming and laborious	No pedigree record is maintained.
5.	It generally takes 14-15 years to develop a new variety and to release it for cultivation.	It takes much longer for the development and release of a variety. The bulk population has to be maintained for more than 10 years for natural selection to act.
6.	Most widely used breeding method.	used only to a limited extent.
7.	It demands close attention from the breeder from F_2 onwards as individual plant selections have to be made and pedigree records have to be maintained.	It is simple, convenient and inexpensive and does not require much attention from the breeder during the period of bulking.
8.	The segregating generations are space - planted to permit individual plant selection.	The bulk populations are generally planted at commercial planting rates.
9.	The size of population is usually smaller than that in the case of bulk method.	Large populations are grown. This and natural selection are expected to increase the chances of the recovery of transgressive segregants.

12. BACK CROSS METHOD – PREREQUISITES AND ITS APPLICATION IN TRANSFERRING RESISTANT GENES – MERITS AND DEMERITS – MULTILINES, MULTIBLENDS AND POPULATION IMPROVEMENT APPROACH IN SELF-POLLINATED CROPS.

BACKCROSS METHOD

In backcross method of breeding, the hybrid and the progenies in subsequent generations are repeatedly backcrossed to one of the parents. As a result, the genotype of the backcross progeny becomes increasingly similar to that of the recurrent parent.

The objective of backcross method is to improve one or two specific defects of a high yielding variety.

Pre-requisite for back cross breeding

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

Application of back cross method

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

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

E.g. Disease resistance, Seed coat colour

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

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

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

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

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

4. **Transfer of cytoplasm** :

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

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

Female parent Recurrent parent.

5. **Transgressive segregation** :

Back cross method may be modified to produce transgressive segregants. Th F_1 is back crossed to recurrent parent for 2 to 3 times for getting transgressive segregants.

6. **Production of isogenic lines.**

7. **Germplasm conversion** : E.g. Production of photo insensitive line from photo Sensitive germplasm thro' back crossing. This was done in the case of sorghum. Popularly known as conversion programme.

Procedure for backcross method

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

First Year	Non recurrent Parent B RR Resistant to rust		x	Recurrent Parent A rr Susceptible to rust	
	F_1	Rr Resistant.	x	rr	BC_1
rr		Rr	x	rr	BC_2
rr		Rr	x	rr	BC_3
rr		Rr	x	rr	BC_4
rr		Rr	x	rr	BC_5

Back cross up to
6th or 7th generation.
After 7th BC rust resistant
lines are self pollinated.
Harvest is done on single plant
basis.

8th Season
a) Individual plant progenies grown
b) Homozygous plants having resistance and resembling parent A are selected harvested and bulked.

9th Season
Yield trials.

10th Season
Seed multiplication and distribution.

Steps

First Season

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

Second Season :

Raising the F_1 and backcrossed to recurrent parent A.

Third season :

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

Fourth Season

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

Fifth Season to Eighth Season

Raising backcross F_1s and crossing resistant plants with recurrent parent is continued up to 7th $backcross_1$

Ninth season :

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

Tenth Season :

Growing the progeny rows and observing each row for resistance. Best rows are selected and harvest is done on row basis

Eleventh Season :

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

Twelfth season :

Selected plot seeds are multiplied and released as new variety.

Back Cross Method - Transfer of Recessive Gene

I Season — Non recurrent parent B — Recurrent parent A
Hybridization — Resistant — Susceptible

rr x RR

F_1 Rr

II Season — Grow the F_1

Rr

III. Season Grow F_2 ~~RR~~ : ~~Rr~~ : rr x RR BC_1

IV Season Grow BC_1 F_1 Rr

V Season Grow BC_2 F_2 ~~RR~~ : ~~Rr~~ : rr x RR BC_2

VI Season Grow BC_2 F_1 Rr

VII Season Grow BC_2 F_2 ~~RR~~ : ~~Rr~~ : rr x RR BC_3

VIII Season Raise BC_3F_1

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

X Season Do as on VIII Season

XI Season Do as in IX season.

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

Steps :

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

II Season : Grow the F_1 which will be susceptible - Harvest them.

III Season : Grow F_2 which will be segregating in the ratio of 1:2:1 i.e. 3/4 susceptible and 1/4 resistant. With the resistant lines (rr) make first backcross with parent A having dominant RR gene. Harvest BC_1 F_1

IV Season : Grow BC_1F_1

V season : Grow BC_1 F_2 which will be segregating as we saw in III season. Repeat the process of third season. This will give BC_2F_1

VI Season : Grow $BC_2 F_1$

VII Season : Grow $BC_2 F_2$ then repeat the process of V season. This will give $BC_3 F_1$.

VIII Season : Grow $BC_3 F_1$

IX Season : Grow $Bc_3 F_2$ and repeat the process of VII season. Harvest $BC_4 F_1$

X Season : Grow $BC_4 F_1$

XI Season : Grow $BC_4 F_2$ and repeat the process of IX Season. Harvest BC_5F_1

XII
XIII : Repeat the process and carry out backcross up to 7 time.
XIV

XV Season : While studying $BC_7 F_2$
Select single plants having resistance and resembling parent B.

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

XVII Season : Conduct yield trial and select best material.

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

Note: While studying Back cross F_2s they should be bombarded with artificial epiphytotic conditions.

MERITS OF BACKCROSS METHOD

1. The genotype of the new variety is nearly identical with that of the recurrent parent, except for the genes transferred. Thus the outcome of a backcross programme is known beforehand, and it can be reproduced any time in the future.
2. It is not necessary to test the variety developed by the back cross method in extensive yield tests because the performance of the recurrent parent is already known. This may save upto 5 years time and a considerable expense.
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 generation each year. This would drastically reduce the time required for developing the new variety.
4. Much smaller population 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 interspecific gene transfers.
7. It may be modified so that transgressive segregation may occur for quantitative characters.

DEMERITS OF BACKCROSS METHOD

1. The new variety generally cannot be superior to the recurrent parent, except for the character that is transferred.
2. Undesirable genes closely linked with the gene being transferred may also be transmitted to the new variety.
3. Hybridization has to be done for each backcross. This is often difficult, time taking and costly.
4. By the time the backcross is over, the recurrent parent may have been replaced by other varieties superior in yielding ability and other characteristics.

NUMBER OF PLANTS NECESSARY IN THE BACKCROSS GENERATIONS

According to the above schemes, only a few (about 10) seeds are necessary in each backcross generation for the transfer of a character governed by a single gene. This population size would almost certainly have at least one plant with the gene for rust resistance. However, if the character is governed by two or more genes, a larger number of backcross progenies would be required. A larger size of backcross population is also desirable to permit an effective selection for the plant type of the recurrent parent, and to increase the probability of recombination between the genes being transferred and the genes tightly linked with it. Therefore, more than 50, preferably 100 or more, plants should be grown in each backcross generation. In F_2 and F_3 generations, the population size should be as large as possible.

SELECTION FOR THE CHARACTER BEING TRANSFERED

A rigid selection for the character being transferred must be practiced during the backcross and the F_2 generations, otherwise the character is likely to be lost. It is, therefore, essential that the character being transferred must have a high heritability. Although monogenic characters are the easiest to transfer, the number of genes is not as important as the heritability of the character under transfer. It is desirable that the character should be easily identified either visually or through simple tests. The breeder should try to maintain the character in an intense form through selection. *Often the intensity would be lost due to modifying genes in the new genetic background. Therefore, the nonrecurrent parent should be chosen for a high intensity of the character to be transferred.*

NUMBER OF BACKCROSSES TO BE MADE

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

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

TRANSFER OF TWO OR MORE CHARACTERS TO A SINGLE RECURRENT PARENT

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

SIMULTANEOUS TRANSFER

The genes for the characteristics may be transferred simultaneously in the same backcross programme. The characters to be transferred are brought together in the hybrid by crossing each of the nonrecurrent parents to the recurrent parent and the hybrid thus produced. But in such a case, a larger backcross population would be needed than in the case of transfer of a single character. Further, the breeding programme may be delayed because the conditions necessary for the selection of all the characters may not occur every year. Sometimes, the two genes under transfer may be linked. In such a case, the transfer become very easy, and selection for only one gene may be necessary. Some examples of such a favourable linkages are between the genes Lr 24 and Sr 24, Lr 19 and Sr 25 and Lr 56 and Sr 31.

STEPWISE TRANSFER

The recurrent parent is first improved for one character. The improved recurrent parent is then used as recurrent parent in a backcross programme for the transfer of other character. If additional characters are to be transferred, they are transferred one time in a stepwise fashion. This approach takes much longer time for the transfer of two or more characters.

SIMULTANEOUS BUT SEPARATE TRANSFERS

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

MODIFICATIONS OF THE BACKCROSS METHOD

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

PRODUCTION OF F_2 AND F_3

The F_2 and F_3 generations are produced after the first and the third backcrosses. A rigid selection for the character being transferred and for the characteristics of the recurrent parent is done in the F_2 and F_3 generations. In the backcross progenies, selection need not be done either for the character being transferred or for the characteristics of the recurrent parent. The fourth, fifth and sixth backcross are made in succession. For the sixth backcross, a relatively larger number of plants from the backcross progeny is used. This method may be used for the transfer of both dominant and recessive genes. It is believed that an effective selection in F_2 and F_3 generations is equivalent to one or two additional backcrosses.

USE OF DIFFERENT RECURRENT PARENTS

Often two or more good varieties have quantitative characteristics that are desirable in the new variety. These varieties may be used as recurrent parents in the same backcross programme. Each variety is used as a recurrent parent for one or two backcrosses. *The objective of this approach is to combine in the new verity some good genes from each of the recurrent parent with the genes from the nonrecurrent parent.* Nobilisation of sugarcane is an outstanding example of this approach. Noble canes (*S.officinarum*) were first crossed with the Indian canes (*S.barberi*). The resulting hybrids were backcrossed to different varieties of noble canes to develop a large number of commercial sugarcane varieties. A similar approach was used for the transfer of scab resistance in apples and for the transfer of high vitamin C content from wild tomato (*Lycopersicon peruviamum*) to the cultivated tomato (*L.esculentum*).

BACKCROSS-PEDIGREE METHOD

In this method, the hybrid is backcrossed 1-2 times to the recurrent parent. Subsequently, the backcross progenies and handles according to the pedigree method. This approach is useful when one of the parents is superior to the other in several characteristics but the nonrecurrent parent is not desirable agronomically. The superior parent is used as the recurrent parent. The purpose of the one to two backcrosses is to make sure that the new variety would get a majority of the superior genes from the recurrent parent. It also leaves enough heterozygosity for transgresive segregants to appear. The varieties developed by this method must be put to yield trials as those developed by the pedigree method. The same holds true when two or more recurrent parents are used in the backcross programme.

APPLICATION OF THE BACKCROSS METHOD TO CROSS-POLLINATED CROPS

The backcross method is equally applicable to cross-pollinated crops. The method is essentially the same as in the case of self-pollinated crops. The only difference is that in cross-pollinated crops a large number of plants (100-300) from the recurrent parent must be used in each backcross. This is necessary so that the new variety has the same genetic constitution as the recurrent parent. For example, wilt resistance was transferred to alfalfa variety California Common from the variety Turkestan. Two hundred plants of California Common were used for each backcross. The new variety Calliverde is exactly like California Common except for its wilt resistance.

COMPARISON BETWEEN BACKCROSS AND PEDIGREE METHODS

	Pedigree method	Backcross method
1.	F_1 and the subsequent generations are allowed to self - pollinate	F_1 and the subsequent generations are backcrossed to the recurrent parent.
2.	The new variety developed by this method is different from the parents in agronomic and other characteristics.	Usually extensive testing is not necessary before release.
3.	The new variety has to be extensively tested before release	Usually extensive testing is not necessary before release.
4.	The method aims at improving the yielding ability and other characteristics of the variety.	The method aims at improving specific defects of a well adapted, popular variety.
5.	It is useful in improving both qualitative and quantitative characters.	Useful for the transfer of both quantitative and qualitative characters provided they have high heritability.
6.	It is not suitable for gene transfer from related species and for producing substitution or addition lines.	It is the only useful method for gene transfers from related species and for producing addition and substitution lines.
7.	Hybridization is limited to the production of the F_1 generation.	Hybridization with the recurrent parent is necessary for producing every backcross generation.
8.	The F_2 and the subsequent generations are much larger than those in the backcross method.	The backcross generation are small and usually consist of 20-100 plants in each generation.
9.	The procedure is the same for both dominant and recessive genes.	The procedures for the transfer of dominant and recessive genes are different.

Multiline Varieties

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

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

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

Merits of Multiline varieties

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

2. Only one or a few lines of the mixture would become susceptible of the pathogen in any one season. Therefore, the loss to the cultivator would be relatively low.

3. The susceptible line would constitute only a small proportion of the plants in the field. Therefore, only a small proportion of the plants would be infected by the pathogen. Consequently the disease would spread more slowly than when the entire population was susceptible. This would reduce the damage to the susceptible line as well.

Demerits of Multiline Varieties

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

Achievements

Multiline variety appears to be a useful approach to control diseases like rusts where new races are continuously produced. In India, three multiline varieties have been released in wheat (*T.aestivum*). Kalyan Sona, one of the most popular varieties in the late sixties, was used as the recurrent parent to produce these varieties. Variety 'KSML 3' consists of 8 lines having rust resistance genes from Robin, Ghanate, K1, Rend, Gabato, Blue Brid, Tobari etc. Multiline 'MLKS 11' is also a mixture of 8 lines; the resistance is derived from E 6254, E 6056, E 5868, Frecor, HS 19, E 4894 etc. The third variety, KML 7406 has 9 lines deriving rust resistance from different sources.

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

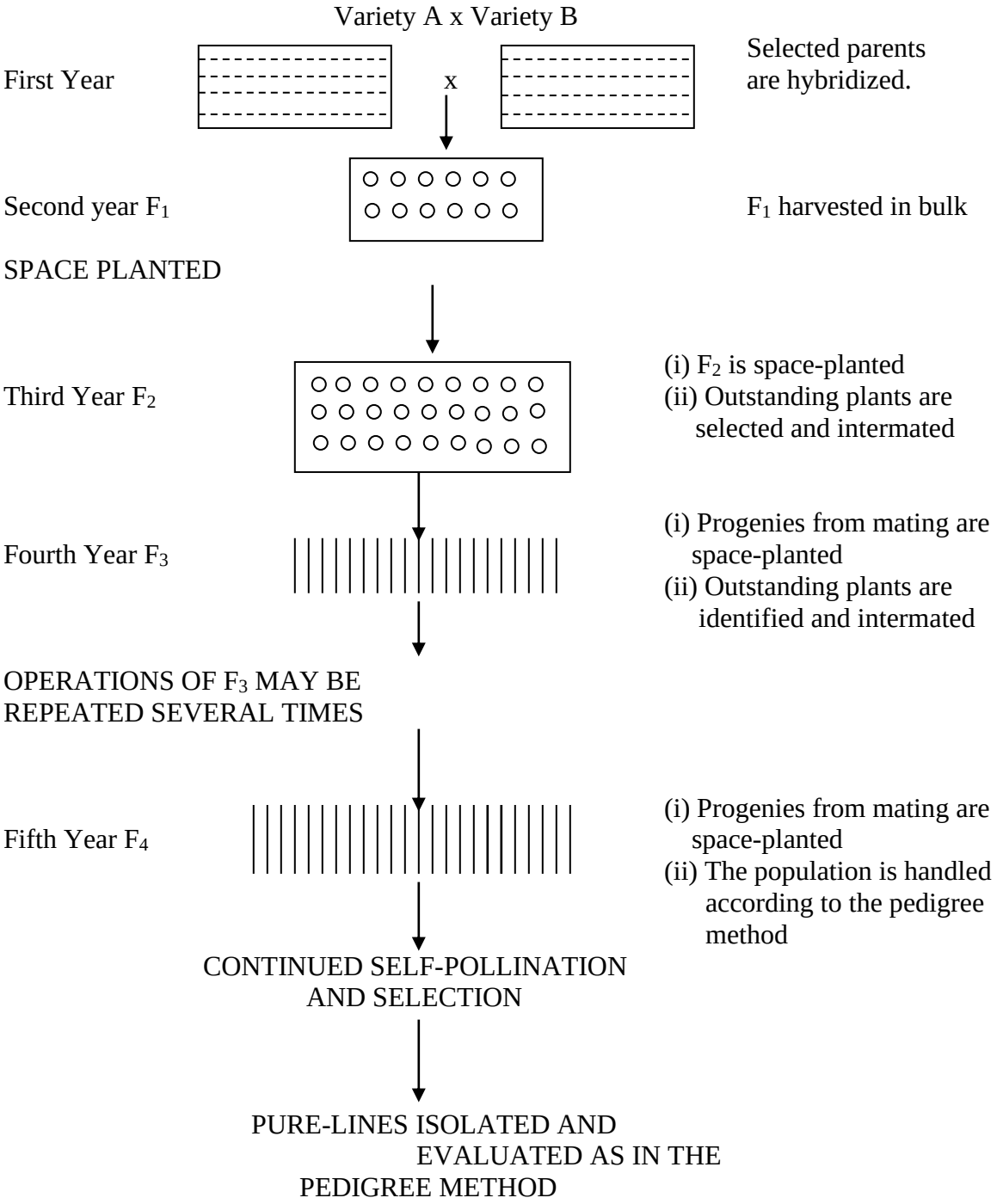
POPULATION APPROACH TO BREEDING OF SELF-POLLINATED CROPS

Self-fertilization of F_1 hybrids leads to a very rapid increase in homozygosity. After only 4 generations of self-pollination, about 94 per cent of the genes would become homozygous. Even in F_2, half of the genes are in homozygous state. Thus self fertilization quickly separates the progeny from a hybrid into a large number of purelines. As a consequence, selection in such a segregating population only picks out the genes combinations present in the population primarily as a result of recombination in F_2. This reduces the chance of recombination between linked, especially tightly linked genes and of recovery of rare transgressive segregants. There is no opportunity for changing the genotype of the plant produced by recombination in F_1, F_2 and to some extent, in F_3. Thus the two obvious limitations of breeding methods based on self-pollination of the hybrid (e.g., pedigree and bulk methods) are: first, the recombination is limited to two or, at the best, three generations, and second, there is no possibility for further changing the genotype of the segregants.

A population breeding approach has been suggested to overcome these problems. In population breeding, outstanding F_2 plants are mated among themselves in pairs or in some other fashion. The intermating of selected F_2 plants restores heterozygosity in the progeny, which provides for a greater opportunity for recombination. This also brings together the desirable genes from different F_2 plants and would help in the accumulation of favourable genes in the intermated population. Thus the chances of the recovery of transgressive segregants would increase considerably. This process may be repeated one or more times (Fig.). This procedure is similar to recurrent selection in cross-pollinated crops. A variation of this approach would be to intermate F_3 or later generation progenies. This would allow a more effective selection of desirable progenies than in the case of F_2 where individual plants have to be selected. As noted previously, selection in F_2 based on individual plants is of little value, particularly for characters like yield. Selection based on F_3 or F_4 progenies would be more desirable. Intermating of selected plants may be continued for two or more generations.

This idea of population approach was first suggested by Palmer in 1953. It is not commonly used at present, but may find a greater application in the future as improvements due to the pedigree method would become less and less marked. Evidently, the population approach is akin to recurrent selection commonly used in cross-pollinated crops and often it is referred to as such. The chief limitation of recurrent selection in self-pollinated crops is the difficulty in making the large number of required crosses by hand (emasculation and pollination). This difficulty may be overcome by using genetic or cytoplasmic male sterility. When genetic male sterility is used, selection is confined to the male sterile (ms ms) plants in each generation. Seeds from the selected male sterile plants are generally harvested in bulk. The progeny from such plants may be expected to have both male sterile (ms ms) and male fertile (Ms ms) plants in almost equal proportion. Further, the seeds produced on the male sterile plants would be produced by pollination by the male fertile plants in the population. Thus the use of male sterility effectively ensures intermating among the plants in the population and eliminates the needs for tedious and time consuming hand emasculation and pollination.

Schematic representation of population approach to breeding of self-pollinated crops



Results from recurrent selection are available in tobacco and soybean. In tobacco, Matzinger and coworkers selected the plants before flowering and intermated them. A linear response of 4.9 and 7 per cent per cycle to selection for decrease plant height and for increased leaf number, respectively, was obtained for five cylces of selection.

Further, there was no evidence for a reduction in variability as a result of the selection. Brim and coworkers carried out six cycles of recurrent selection for increased protein content in two segregating populations of soybean and three cycles of selection for yield and three cycles of selection for high oil content in another segregating population. There was an increase of 0.33 and 0.67 per cent / cycle in protein content of the two populations, of 5.3% per cycle in yield and of 0.3% per cycle in oil content. These findings amply demonstrate the effectiveness of recurrent selection in improving yield and yield traits in self-pollinated crops.

In 1970, Jensen proposed a comprehensive breeding scheme which provides for the three basic functions of a versatile breeding programme. Firstly, it allows the development of F_2, F_3 etc. (selfing series) at every stage of the breeding programme, which permits the isolation of purelines for use as commercial varieties. Secondly, it requires intermating among the selected plants/ lines in each stage; the progenies from these intermatings form the basis for the next stage of the selfing series in the breeding programme. Thus the breeding programme progresses in two different directions: (1) Vertically, through the selfing series leading to the isolation of commercial varieties, and (2) horizontally, through intermating among the selected plant / lines; this generates the recurrent selection series. Thirdly, new germplasm may be introduced at any stage of the programme by intermating it with some of the selected plants of that stage. This permits the retention and / or the creation of large amounts of variability for effective selection through several cycles, and the introduction of new genes in the breeding material, if so desired. This breeding scheme is known as Diallel Selective Mating Scheme (DSM) and is designed to serve both short-term and long-term breeding objectives. A breeder may create more than one such population for a crop, each population being developed to fulfil a specific objective. This scheme has not been widely used primarily due to the difficulties in making the large number of crosses required in this scheme. Jensen has suggested the use of male sterility to overcome this difficulty in the same way as in the recurrent selection scheme discussed earlier. Further, DSM is much more complicated than the simple pedigree method which still is the favourite breeding method for self-pollinated crops.

Merits of population Approach

2. The population approach provides for greater opportunities for recombination. This is made possible by restoring heterozygosity through intermating of selected plants.

3. This approach helps in the accumulation of desirable genes in the population. This is also brought about by the intermating of selected plants from segregating generation.

Demerits of Population Approach

1. The success of this approach depends upon the identification of desirable plants in F_2 and the subsequent segregating generations. This is very difficult, if not impossible, for complex characters like yield which show low heritability. This may be avoided to some extent by using later generation (F_3 or F_4) progenies; replicated yield data may also be used.

2. Another draw back of this approach is the intermating of selected plants. This may become a major limitation in some crops because crossing in many self-pollinated species is difficult and time consuming.

3. The time taken to develop a new variety through population approach would be always greater than that by the pedigree method.

4. There is no convincing evidence for the benefits from the population approach. It has been argued that increased recombination may be detrimental as it would break the desirable linkage. But such a criticism assumes that all or most of the new gene combinations (recombinations) will be inferior to the existing ones. Such an assumption is not entirely valid since crop improvement is based on the creation of new and desirable gene combinations.

13. GENETIC STRUCTURES OF A POPULATION IN CROSS-POLLINATED CROP & MASS SELECTION

GENETIC STRUCTURE OF CROSS POLLINATED CROPS.

Cross pollinated crops are highly heterozygous due to free intermating. These cross pollinated crops are referred as

a) Random mating population
b) Mendelian population.
c) Panmictic population.

Hardy - Weinberg Law

This law was independently developed by Hardy (1908) in England and Weinberg (1909) in Germany. According to the law that "*gene and genotypic frequencies in a random mating population remains constant generartion after generation provided there is no selection, mutation, migration or genetic drift".*

The frequencies on the three genotypes for a locus with two alleles say A and a would be p^2 AA, 2pq Aa and q^2 aa

Where p = frequency of A

q = frequency of a in the population.

The sum of p and q is one i.e. p + q =1. Such a population would be equilibrium because the genotypic frequencies would be stable from one generation to next. This equilibrium is known as Hardy- Weinberg equilibrium. A population is said to be at equilibrium when the frequencies of the three genotypes AA, Aa and aa are P^2 2pq q^2.

Whether the population is at equilibrium or not can be tested by chisquare test.

Factors disturbing the equilibrium in the population :

1. **Migration** : In plant breeding migration is represented by inter varietal crosses, poly crosses, etc., wherein a single population two or more separate populations are introduced. Migration may introduce new alleles into a population which may change the gene frequencies.

2. **Mutation** : It may produce a new allele not present in the population or it may change the frequencies of the exhibiting alleles.

3. **Random drift** : It is other wise known as genetic drift. It is a random change in gene frequency due to sampling error. In a smaller population if natural selection operates at random it will lead to sampling error. This sampling error is greater in smaller population than in a large one. Because of sampling the frequency of one of the alleles becomes zero and that of the other alleles become one. The allele having the value one is said to be fixed because there is no further change in its frequency and thus it becomes homozygous. Thus if the population is small genetic drift will occur. To over come this, one has to use larger population, which may not be possible because of limitations in space, labour and finance.

4. **Inbreeding** : In smaller populations, a certain amount of inbreeding is bound to occur and this will lead to homozygosity.

5. **Selection** : This is important because when you practice selection you allow the selected genotype to reproduce, while the undesirable genotypes are eliminated. Thus if in a random mating population if we practice selection for the allele AA alone then its frequency in the selected population will be ***one*** and the frequency of ***aa*** will be zero. This particular selection for a particular allele is known as

selection differential designated as S.

Selection differential for AA = 1

Fitness for aa = 0.

But in practice it is not possible to identify AA alone especially in case of quantitative characters. So, we will not eliminate one allele (aa) but instead the gene frequency will be changed. When the selection differential is less than ***one*** then the rate of change in gene frequency would depend on the intensity of selection and upon gene frequency.

Thus selection in a random mating population is highly effective in increasing or decreasing the frequency or alleles, but it is unable to either fix or eliminate them.

SYSTEMS OF MATING

To change the genetic composition of a population we have got different systems of mating

1. Random mating
2. Genetic assortative mating
3. Genetic disassortative mating
4. Phenotypic assortative mating
5. Phenotypic disassortative mating.

1. **Random mating** :

Here the rate of reproduction of each individual is equal i.e. there is no selection and each male or female is equally likely to combine at random. This random mating is useful in plant breeding for the production and maintenance of synthetic and composite varieties, production of polycross varieties.

2. **Genetic assortative mating**:

Here the mating will be between individuals that are closely related by ancestry ie. mating between individuals having more or less similar genotype. It is other wise known as inbreeding. The genetic assortative mating leads to

i) Increase in homozygosity
ii) Characters become fixed
iii) Lethals will be eliminated
iv) Separation of population into lines.

Genetic assortative mating is usefull for the development of inbreds.

3. **Genetic disassortative mating**

It is mating between individuals that are not closely related by ancestry.

E.g. Intervarietal and interspecific crosses.

4. **Phenotypic assortative mating**

Mating between individuals which are phenotypically more similar. This type of mating leads to increase in homozygosity and division of population into two extremes. i.e. there is highest and lowest phenotypes remain in the population and there is no intermediate types.

5. **Phenotypic disassortative meting**

Mating between phenotypically dissimilar individuals. This system leads to maintenance of or increase in heterozygosity.

Selection in cross pollinated crops

Selection in a random mating population is able to

i) Change the gene and genotypic frequency.
ii) Production of new genotypes due to changed gene frequencies.
iii) Cause a shift in the mean of population towards the direction of selection.
iv) Change in the variance of population to some extent.

The magnitude of these effects are influenced by the number of genes controlling the character, the degree of dominance, nature of gene action and to a large extent heritability.

A large number of studies on the effect of selection in random mating population has been made. The response to selection in cross pollinated crop can be divided in to five broad groups.

1. **Rapid gain followed by slow Progress**.

In some cases selection produces rapid gain for some generations. This is followed by a period of slow gain.

This type of response is seen in characters like plant height, days to flowering. These characters will be governed by a few genes with major effect and several genes with lesser effect. The major genes will give rapid gain and several genes having lesser effect gives slow effect.

2. Continued slow progress for a long period

E.g. Oil content and protein content in maize crop.

This is because, that these traits are governed by several genes, each having a small additive effect. So, progress under selection for such traits would be slow.

3. **Slow response for a shorter period only**

Here the response for selection will be for a shorter period only and afterwards there will be no response at all. This is due poly genes which may be more than 40 which control a character.

4. **Lack of response to selection**

This may be due to low heritability and additive gene action. This was seen in maize when selection is practiced for yield.

5. **Rapid gain - plateau - Rapid gain**

This is due to linked genes both positive and negative.

BREEDING METHODS FOR CROSS POLLINATED CROPS

Populations of cross pollinated crops are highly heterozygous. When inbreeding is practiced they show severe inbreeding depression. So to avoid inbreeding depression and its undesirable effects, the breeding methods in the crop is designed in such a way that there will be a minimum inbreeding. The breeding methods commonly used in cross pollinated crops may be broadly grouped into two categories.

I. Population improvement

A. Selection

a) Mass selection

b) Modified mass selection

Detasseling

Panmixis

Stratified or grid or unit selection

Contiguous control.

B. Progeny testing and selection

a) **Half sib family selection**

i) Ear to row

ii) Modified ear to row.

b) **Full sib family selection**.

c) **Inbred or selfed family selection**.

i) S_1 self family selection

ii) S_2 self family selection.

C. Recurrent selection

a) Simple recurrent selection

b) Reciprocal recurrent selection for GCA

c) Reciprocal recurrent selection SCA

d) Reciprocal recurrent selection.

D. Hybrids

E. Synthetics and Composites.

Mass selection

This is similar to the one which is practiced in self pollinated crops.

A number of plants are selected based on their phenotype and open pollinated seed from them are bulked together to raise the next generation. The selection cycle is repeated one or more times to increase the frequency of favourable alleles. Such a selection is known as *phenotypic recurrent selection.*

Merits :

i) Simple and less time consuming

ii) Highly effective for character that are easily heritable.

E.g. Plant height, duration.

iii) It will have high adaptability because the base population is locally adapted one.

Demerits :

1. Selection is based on phenotype only which is influenced by environment
2. The selected plants are pollinated both by superior and inferior pollens present in the population.
3. High intensity of selection may lead reduction in population there by leading to inbreeding.

To over come these defects modified mass selection is proposed they are

a) **Detasseling** : This is practiced in maize. The inferior plants will be detasseled there by inferior pollen from base population is eliminated.

b) **Panmixis** : From the selected plants pollen will be collected and mixed together. This will be used to pollinate the selected plants. This ensures full control on pollen source.

c) **Stratified mass selection** : It is also known as *Grid method* of *mass selection* or *unit selection.*

Here the field from which plants are to be selected will be divided into smaller units or plots having 40 to 50 plants / plot. From each plot equal number of plants will be selected.

The seeds from selected plants will be harvested and bulked to raise the next generation, by dividing the field into smaller plots, the environmental variation is minimised. This method is followed to improve maize crop.

B) PROGENY TESTING AND SELECTION

a) Half sib family selection

Half sibs are those which have one parent in common. Here only superior progenies are planted and allowed to open pollinate.

1. **Ear to row method**: It is the simplest form of progeny selection. Which is extensively used in maize. This method was developed by *Hopkins*

a) A number of plants are selected on the basis of their phenotype. They are allowed to open pollinate and seeds are harvested on single plant basis.

b) A single row of say 50 plants i.e. progeny row is raised from seeds harvested on single plant basis. The progeny rows are evaluated for desirable characters and superior progenies are identified.

c) Several phenotypically superior plants are selected from progeny rows. There is no control on pollination and plants are permitted to open pollinate.

Though this scheme in simple, there is no control over pollination of selected plants. Inferior pollen may pollinate the plants in the progeny row. To over come this defect, the following method is suggested.

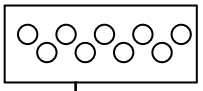
a) At the time of harvest of selected plants from base population on single plant basis, part of the seed is reserved.

b) While raising progeny rows, after reserving part of the seeds, the rest are sown in smaller progeny rows.
c) Study the performance of progenies in rows and identify the best ones.
d) After identifying the best progenies, the reserve seeds of the best progenies may be raised in progeny rows.
e) The progenies will be allowed for open pollination and best ones are selected.

There are number of other modifications made in the ear to row selection.
For example,
i) The selected progenies may be selfed instead of open pollination
ii) The selected plants may be crossed to a tester parent. The tester parent may be a open pollinated variety, or inbred.
iii) The progeny test may be conducted in replicated trial.

Ear to row method

Ist year
First selection cycle.

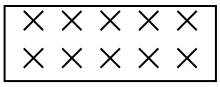


i) Plants selected on phenotype
ii) Open pollinated plants harvested on S.P.basis

Second Year

i) Raise progeny rows
ii) Superior progenies identified
iii)They are allowed to open pollinate

Third year

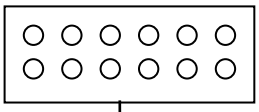


iv) Harvest on single plant basis
Repeat as in first year.

May be repeated.

Modified ear to row method

Ist year
phenotype

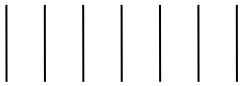


i) Selected plants based on
ii) Allow for open pollination
iii) Harvest on single plant basis

Second year

i) Reserve 50% of seeds from selected SPs.
ii) Raise progeny rows.
iii) Identify best progenies

Third year



i) Sow the seeds from reserve
ii) Allow for open pollination

Cycle repeated

b) Full sib family selection

Fullsibs are those which are produced by mating between selected plants in pairs. *Here the progenies will have a common ancestry*. The crossed progenies are tested.

A x B B x A

c) Inbred or selfed family selection

Families produced by selfing**.**

S_1 family selection :

Families produced by one generation of selfing. These are used for evaluation and superior families are intermated (Simple recurrent selection)

S_2 family selection :

Families obtained by two generations of selfing and used for evaluation. Superior families are intermated.

Merits of progeny testing and selection

1. Selection based on progeny test and not on phenotype of individual plants.
2. In breeding can be avoided if care is taken raising a larger population for selection.
3. Selection scheme is simple.

Demerits :

1. No control over pollen source. Selection is based only on maternal parent only.
2. Compared to mass selection, the cycle requires 2-3 years which is time consuming.

RECURRENT SELECTION

This is one of the breeding methods followed for the improvement of cross pollinated crop. Here single plants are selected based on their phenotype or by progeny testing. The selected single plants are selfed. In the next generation they are intermated (cross in all possible combinations) to produce population for next cycle of selection.

The recurrent selection schemes are modified forms of progeny selection programmes. The main difference between progeny selection and recurrent selection

i) The manner in which progenies are obtained for evaluation.

ii) Instead of open pollination, making all possible inter crosses among the selected lines.

14. HETEROSIS, HYBRID VIGOR AND INBREEDING DEPRESSION HYBRID SEED PRODUCTION – MANUAL EMASCULATION AND POLLINATION – UTILIZATION OF MALE STERILITY AND SELF INCOMPATIBILITY.

HETEROSIS AND INBREEDING DEPRESSION

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

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

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

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

Effects of inbreeding

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

Degrees of inbreeding depression

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

1. **High inbreeding depression** :
   E.g. Lucerne, Carrot.
   Inbreeding leads to severe depression and exhibit lethal effects. After 3 or 4 generations of selfing it is hard to maintain lines.
2. **Moderate inbreeding depression**
   Maize, Jowar, Bajra.
   Though lethal effects are there, lines can be separated and maintained.
3. **Low inbreeding depression** :
   E.g. Cucurbits, Sunflower. Only a small degree of inbreeding depression is observed.

4. **No inbreeding depression** :

The self-pollinated crops do not show inbreeding depression.

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

Types of heterosis :

1. **Average heterosis** : It is the heterosis where F_1 is superior to mid parent value. In otherwords superior to average of two parents.

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

Where $\overline{F_1}$ = Mean of hybrid

$\overline{MP}$ = Mid parental value.

$$MP = \frac{(P_1 + P_2)}{2}$$ where P_1 = Parent 1, P_2 = Parent 2

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

2. **Heterobeltiosis**: Superiority of F_1 over the better parent.

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

Where $\overline{BP}$ = Mean of Better Parent.

3. **Economic heterosis** :

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

$$\frac{\overline{F_1} - \overline{CV}}{\overline{CV}} \times 100$$

Where $\overline{CV}$ = Mean of Commercial Variety.

4. **Negative heterosis** :

Performance of F_1 inferior to better parent / mid parent value. - e.g. Duration.

Heterosis or hybrid vigour

Hybrid vigour is used as synonym of heterosis. Hybrid vigour refers to superiority of F_1 over better parent. In other words hybrid vigour is manifested effect of heterosis. Thus the term hybrid vigour is used to distinguish the F_1 superiority from negative heterosis.

Manifestation of heterosis : May be in the following form.

1. Increased yield.
2. **Increased reproductive ability.**
3. Increase in size and vigour.
4. Better quality
5. Greater adaptability.

Genetic basis of heterosis

There are two main theories of heterosis and inbreeding depression.

1. Dominant hypothesis
2. Over dominance hypothesis.

1. Dominant hypothesis : First proposed by Davenport in 1908. It was later on expanded by Bruce, Keeble and Pellow.

According to this hypothesis at each locus the dominant allele has favourable effect, while the recessive allele has unfavourable effect. In heterozygous state, the deleterious effect of recessive alleles are masked by their dominant alleles. Inbreeding depression is produced by the harmful effects of recessive alleles which become homozygous due to inbreeding.

Two objections have been raised against the dominant hypothesis.

a**) Failure of isolation of inbreds as vigorous as hybrids** :

According to dominance hypothesis it is possible to isolate inbreds with all the dominant genes

E.g. AA.

This inbreed should be as vigourous as that of hybrid. However in practice such inbreds were not isolated.

b) **Symmetrical distribution in F_2**

In F_2 dominant and recessive characters segregate in the ratio of 3:1. Quantitative characters, according to dominance hypothesis should not show symmetrical distribution. However, F_2 nearly always show symmetrical distribution.

Explanation for the two objections :

In 1917 Jones suggested that since quantitative characters are governed by many genes, they are likely to show linkage. In such a case inbreds containing all dominant genes cannot be isolated. So also the symmetrical distribution in F_2 is due to linkage.

This explanation is often known as *Dominance of Linked Genes Hypothesis*.

2. Over dominance hypothesis :

This hypothesis was independently proposed by East and Shull in 1908. It is also known as *single gene heterosis* or *super dominance theory*.

According to this hypothesis, heterozygotes or atleast some of the loci are superior to both the homozygotes. Thus heterozygote Aa would be superior to AA and aa.

In 1936 East proposed that at each locus there are several alleles a_1 a_2 a_3 a_4 etc, with increasingly different functions. Heterozygotes between more divergent alleles would be more heterotic E.g. a_1 a_4 will be superior to a_1 a_2 or a_2 a_4

Evidences for over dominance :

In maize the maturity genes in heterozygous conditions are superior i.e. Ma ma.

The heterozygote Mama is more vigorous than MaMa or mama

The human beings sickle cell anaemia is caused by ss which is lethal. But heterozygote individuals having Ss have advantage of having resistance against malaria compared to SS individuals.

Physiological basis of heterosis :

Numerous studies were made to find out the physiological basis of heterosis. Earlier studies were related to embryo size, seed size, growth rates at various stages of development, rates of reproduction

It was suggested that hybrid vigour was resulted from larger embryo and endosperm size of hybrid seeds. This was clearly demonstrated in certain cases only.

In 1952 Whaley has concluded that primary heterotic effect is due to growth regulators and enzymes in the F_1

But all these studies were highly speculative. There was no evidence to point out clearly the possible reasons for heterozygote advantage.

Recent studies about heterosis

1. **Reduced amount of single gene product :**

In certain cases the heterozygote produces an intermediate amount of a gene product which may lead to increased vigour and growth rate.

AA - more gene product
aa - Less gene product
Aa - Intermediate gene product.
This is seen in case of bread mold.

Neurospora crassa.

Gene Pab^+ Produces P. amino benzoic acid.
Gene Pab Produces Less P. amino benzoic acid.
Heterozygote Pab^+ Pab - Intermediate amount of P amino benzoic acid which leads to faster growth of the fungus.

2. **Separate gene products** :

AA - produce protein
aa - Produce protein which is slightly different.
Aa - will have both the Products.

This may have many advantages by having more adaptiveness.

Human beings : SS Resistant to sickle cell anaemia
ss - Susceptible
Ss - Resistant to Sickle cell anaemia + malaria.

3. **Combined gene product** : Otherwise hybrid product. The hybrid may produce an enzyme molecule which may be somewhat different compared to enzymes produced by homozygotes. Such heterozygote enzymes are termed as *Hybrid Substance* which may be the reason for hybrid vigour.

4. **Effect in two different tissues**.

Both homozygotes may produce high levels of an enzyme in two different tissues. But heterozygote may produce intermediate level.

E.g. Maize Adh gene for enzyme alcohol dehydrogenase in seeds.

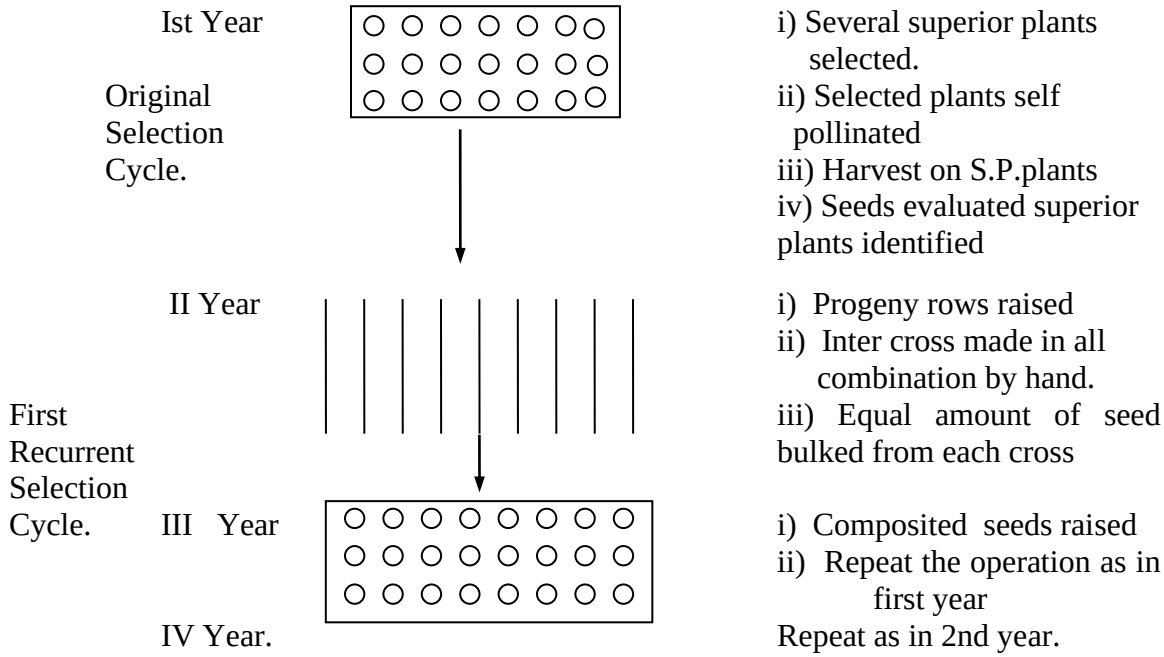
15. MULTIPLE CROSS HYBRIDS – ADVANTAGES AND DISADVANTAGES OVER OTHER HYBRIDS – RECURRENT SELECTION, TYPES OF RECURRENT SELECTION AND THEIR RELATIVE ADVANTAGES – SYNTHETIC AND COMPOSITES.

The recurrent selection schemes are of 4 different types.

1. Simple recurrent selection :

In this method a number of desirable plants are selected and self pollinated. Separate progeny rows are grown from the selected plants in next generation. The progenies are intercrossed in all possible combination by hand.

Equal amount of seed from each cross is mixed to raise next generation. This completes original selection cycle. From this, several desirable plants are selected and self pollinated. Progeny rows are grown and inter crosses made. Equal amount of seeds are composited to raise next generation. This forms the first recurrent selection cycle.



i) Recurrent selection is effective in increasing the frequency of desirable genes in the population

ii) Most suited for characters having high heritability.

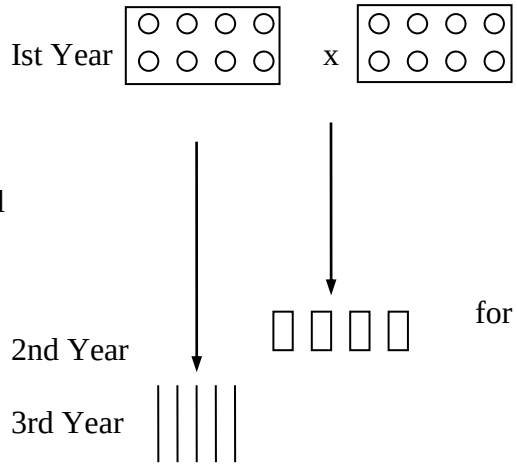
iii) Inbreeding is kept at minimum.

2) Recurrent Selection for General Combining Ability

In this case the progenies selected for progeny testing are obtained by crossing the selected plants to a tester parent with broad genetic base.

A tester parent is a common parent mated to a number of lines. Such a set of crosses is used to estimate the combining ability of the selected lines. A tester with broad

genetic base means an open pollinated variety, a synthetic variety or segregating generation of a multiple cross.



Ist Year

Original Cycle

2nd Year

3rd Year

1.Plants selected based on phenotype.
2. Selected plants selfed
3. Selected plants test crossed with tester.
4. Harvest the crossed plants on single plant basis. Reserve the seeds for III year

i) PYT conducted
ii) Superior progenies identified
1. Selfed seeds from plants producing best crosses planted
2. All possible intercrosses made
3. Equal seed from intercrosses collected bulked

First Recurrent Selection Cycle.

4th Year — 1. Sow the composited seed process of first year.

5th Year — Repeat process of 2nd year

6th Year — Repeat as 3rd year.

Recurrent selection for GCA can be used for two basically different purposes.
1. It may be used to improve the yielding ability and the agronomic characteristics of a population. In this case the end **product will be a synthetic variety**.
2. It may be used to concentrate genes for superior GCA. Here the end product will be superior inbreds. Such inbreds can be developed after a few cycles of RSGCA.

3) Recurrent Selection for Specific Combining Ability:

This is similar to RSGCA except, that in the case of Tester. Here the tester will be an *INBRED* instead of open pollinated variety.

The other operations are similar to RSGCA.

The objective of RSSCA is to isolate from population such lines that will combine well with an inbred. These lines are expected to give best hybrids in heterosis breeding.

4) Reciprocal recurrent selection

Proposed by **Comstock, Robinson** and **Harvey.**

The objective is to improve two different populations in their ability to combine well with each other. In this method we can make selection for both GCA and SCA. Basically two populations A and B are used. Each serve as a tester for the other.

1st Year — 1. Several plants selected in population A and B.

	2. Selected plants are self pollinated. 3. Selected plant from A is test crossed with plants in B and Vice versa. Harvest crossed plant on S.P.basis each
2nd Year	1. Separate yield trials conducted from test cross progenies of A and B. 2. Superior progenies identified
3rd Year	1. Selfed seed from plants producing superior test cross progenies planted. 2. All possible inter crosses made. 3. Seeds harvested and composited.
4th Year	
5th Year	
6th Year	

Use of RRS :

1. Two populations are developed by this method
2. They may be intermated to produce a superior population with broad genetic base. This is similar to a varietal cross but in this case the populations have been subjected to selection for combining ability (GCA and SCA)
3. Inbreds may be developed from populations A and B. These inbreds may be crossed to produce a single cross or double cross hybrids.

HYBRIDS:

They are the first generation from crosses between two pure lines, inbreds, open pollinated varieties of other populations that are genetically not similar.

Pure line hybrids : Tomato.

Inbred hybrids : Maize, bajra.

Kinds of hybrids :

1. **Single cross hybrids**

A x B

Crossing two inbreeds or pure lines.

2. **Three way cross hybrid**

(A x B) x C

A cross between a single cross hybrid and an inbred.

3. **Double cross hybrid**

(A x B) x (C x D)

cross between two F1s.

4. **Double Top Cross hybrid**

Double Cross hybrid crossed with open pollinated variety.

Operation in production of hybrids.

In production of hybrids inbreds are prefered rather than open pollinated varieties for the following reasons.

1. Inbreds can be maintained without a change in the genotype. Whereas open pollinated variety cannot be maintained pure. They may alter genotypically due to natural selection etc.,.
2. The hybrids derived from inbreds will be uniform where as it may not be in case of open pollinated variety.
3. The inbreds are homogenous and their performance can be predicted where as open pollinated variety are heterogenous and their prediction in performance cannot be made.

Development of inbreds :

1. By inbreeding, selfing etc.
2. Development of inbreds from haploids - rice, sorghum, maize.

Evaluation of inbreds.

a) **Phenotypic evaluation**: Based on phenotypic performance. Highly suitable for characters with high heritability.

b) **Top cross test**.

Top cross test provides a reliable estimate of GCA. The selected inbreds will be crossed to a tester parent with wide genetic base i.e. open pollinated variety. The cross progenies will be evaluated in replicated progeny rows. Based on results better inbreds can be selected.

c) **Single cross evaluation**.

The developed inbreds can be crossed and the single crosses can be estimated in replicated trial. Out standing hybrids tested over years in different locations, then released.

d) **Prediction of double cross performance**

"The predicted performance of any double cross is the average performance of the four non parental single crosses involving the four parental inbreds".

Inbreds : A, B, C, D.
6 possible single crosses = A x B, A x C, A x D, B x C, B x D, C x D.
From these 3 double crosses produced = (A x B) x (C x D)
(A x C) x (B x D)
(A x D) x (B x C)

The performance of these any one double cross can be predicted from performance of the four single crosses not involved in producing that particular hybrid.

(A x B) x (C x D)
A x C
A x D
B x C
B x D

Average

PRODUCTION OF HYBRIDS

Methods

1. Hand emasculation and dusting - Cotton, Tomato, Chillies, Bhendi
2. Use of male sterile lines -

a) Cytoplasmic male sterility - ornamentals
b) Genic male sterility - Redgram, Castor.
c) Cytoplasmic - genic male sterility Jowar, Bajra, Rice

3. Use of self in compatibility

By planning cross compatible lines hybrids are produced. Here both are hybrids. E.g. Brassicas.

Success of hybrids.

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

SYNTHETIC VARIETIES

A synthetic variety is produced by crossing in all combinations a number of inbreds (4-6) that combine well with each other. The inbreds are tested for their GCA. Once synthesised, a synthetic is maintained by open pollination. The lines that make up a synthetic may be usually inbred line but open pollinated variety, or other population tested for general combining ability are also be used.

Synthetic varieties are common in grasses, clover, maize and sugar beets. The normal procedure is equal amounts of seeds from parental lines (Syno) is mixed and planted in isolation. Open pollination is allowed. The progeny obtained is Syn_1. This is distributed as synthetic variety or it may be grown in isolation for one more season and Syn_2 is distributed.

Merits :

1. Less costly compared to hybrids.
2. Farmer can maintain his synthetic variety for more seasons which is not possible in hybrids.
3. Because of wider genetic base the synthetics are more stable over years and environments.
4. Seed production is more skilled operation in hybrids where as it is not so in synthetics.

Demerits :

1. Performance is little bit lower compared to hybrids because synthetics exploit only GCA while hybrids exploit both GCA and SCA.
2. The performance may not be good when lines having low GCA are used.

COMPOSITES :

It is produced by mixing seeds of phenotypically outstanding lines and encouraging open pollination to produce crosses in all possible combinations among mixed lines. The lines used to produce a composite are rarely tested for combining ability. So the yield of composite varieties cannot be predicted easily. Like synthetics, composites are commercial varieties and are maintained by open pollination.

Composites were released in maize - Amber, Jawahar, Kissan.

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

Poly Cross Test:

This is done to estimate the GCA in crops where production of inbred is not possible. This is followed generally in grasses. Poly cross test is based on seeds obtained by random mating among the clones. Each clone is planted at different date to facilitate random mating. Polycrosses are generally not perfect since mating may not be at random.

Combining ability :

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

General combining ability:

Average performance of a strain in a series of cross combinations. The GCA is estimated from the performance of F_1s from the crosses. The tester will have a broad genetic base.

Specific combining ability:

Deviation in performance of a cross combination from that predicted on the basis of general combining ability of the parents involved in the cross. The testing will be on inbred.

16. GENETIC STRUCTURE OF A POPULATION OF ASEXUALLY REPRODUCING CROPS – CLONAL AND CLONAL SELECTION – HYBRIDIZATION FOLLOWED BY CONAL SELECTION – EXPLOITATION OF HETEROSIS TO PRODUCED SYNTHETICS THROUGH POLYCROSS TECHNIQUE.

CLONAL SELECTION AND HYBRIDISATION

Some agricultural crops and a large number of horticultural crops are vegetatively propagated.

Crops : Sugarcane, Potato, Sweet Potato, Tapioca, Ginger, Turmeric, Banana.
Trees : Mango, Citrus, Apples, Pears.

Characters of Vegetatively propagated Crops.

1. Majority of them are perennials.
   E.g. Sugarcane, Fruit trees.
   Annual Crops are mostly tubers - Potato, Sweet potato, Yams.
2. Some crops show reduced flowering and seed set.
   Many do not flower at all. Only fruit crops have shown regular flowering and seed set.
3. They are cross pollinated
4. Highly heterozygous and exhibit high inbreeding depression
5. Vegetatively propagated crops are mostly polyploids.
6. Many species are interspecific hybrids E.g. Banana, Sugarcane.
7. The variety is developed thro' clones.

The advantage of asexual reproduction is that it preserves the genotype of the individual indefinitely.

CLONE :

A clone is a group of plants produced from a single plant thro' asexual reproduction. All the members of a clone have same genotype as the parent plant.

Characteristics of clones :

1. All the individuals belonging to a single clone are identical in genotype.
2. The phenotypic variation within a clone is due to environment only.
3. Theoritically clones are immortal i.e. a clone can be maintained indefinitely thro' asexual reproduction, provided there is no disease occurrence.
4. Generally clones are highly heterozygous and exhibit severe inbreeding depression.
5. Genetic variation within a clone may occur due to natural mutation or hybridization or due to mechanical mixtures.

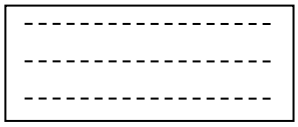
BREEDING METHODS FOR ASEXUALLY PROPAGATED CROPS.

The vegetatively propagated crops can be improved by using following techniques.

1. Clonal selection.
2. Hybridisation & Selection
a) Inter varietal
b) Inter specific
3. Polyploidy breeding.
4. Mutation breeding.
5. Tissue culture and anther culture.

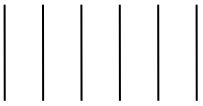
Clonal Selection : A clone is a group of plants produced through asexual reproduction from a single plant. Phenotype of a plant or a clone is due to the effects of genotype(G) environment (E) and GXE interaction. Of these only Genotype effects alone are heritable and Environmental effects and GXE effects are non-heritable.

Therefore selection for quantitative characters based on observations on single plants is highly unreliable. The value of a clone can be reliably estimated only through replicated yield trials.



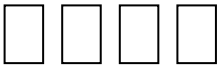
First Year — Select best plants.

Mixture of clones



Second year

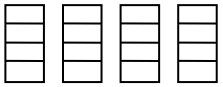
1. Clones from selected plants grown separately.
2. Desirable clones selected.



Third Year

1. Conduct PYT with local checks.
2. Select outstanding clones.

Preliminary yield trial.



Fourth to Sixth Year

Comparative yield trial. Select best clones.

MLT

ART

Multiply the best clone
Release as a variety.

Merits :

i) It is the only method applicable to clonally propagated crops. It avoids inbreeding depression and preserves the gene combination.

ii) This method can be combined with hybridization to create further variability for selection.

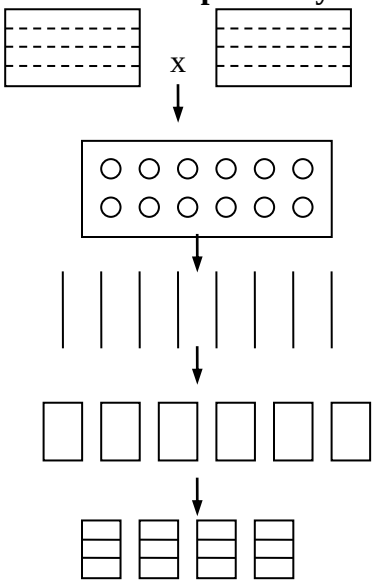
iii) The selection is used to maintain the purity of clones.

Demerits :

1. It uses the variability which is already present and it is not possible to create variability in clonal selection programme.

2. Hybridization and selection

a) Inter varietal or intra specific hybridization



Selected clones are hybridised.

Crossed plants are planted and clonal selection is made in F_1

Individual clones from F_1 are raised along with checks.
Superior clones are selected.

Conduct PYT

Conduct CYT.

b) Inter Specific hybridization

Successfully used in many crops like Rubus, Malus Strawberries.

Potato variety Kufri Kuber was developed from a complex cross.

(S. *Cuttilobum*) x (S.*tuberosum*) x S.*andigena*

Generally interspecific crosses are useful for disease resistance. For E.g. S.*demissum* has been extensively used to induce late blight resistance in potato.

Sugar cane : All varieties of sugar cane now in cultivation have been developed from complex cross between

S.*officinarum*, S.*barberi*,

S. *robustum* and S.*spontaneum*

The success of interspecific hybridization in clonal crops are due to following reasons.

i. Due to asexual: reproduction there is no segregation.

ii. Most of them are not seed crops and hence flowering and fertility are not essential for their success.

3. Mutation breeding.

By subjecting the clones, tubers or suckers to irradiation or chemical treatment, variability can be created.

4. Polyploidy breeding :

It is useful in clonally propagated crops. E.g. Bananas. Triploid bananas.

5. Tissue culture :

Disease free banana suckers can be developed by tissue culture method. High yielding suckers can be multiplied by Tissue culture.

Problems :

i. **Reduced flowering and fertility** : This acts as a barrier in making crosses. Further apomixis and parthenocarpy prevents getting sexual progeny from crosses for further selection. E.g. Mango.

ii. **Difficulties in genetic analysis**:

Estimation of GCA and SCA are very much limited in Clonal crops. Without genetic analysis successful crop improvement is very much limited.

iii) **Most of their crops are perennials**:

Because of this regular yield trials with replication are not possible.

17. MUTATION – TYPES OF MUTATION – CHARACTERISTICS OF MUTATION – MUTAGENS – APPLICATION OF MUTATION BREEDING

Mutation

Sudden heritable change in a specific character. Such a change may be large or small.

The term **mutation** was first coined by **Hugo de vries** (**1901**). He observed sudden heritable change in **Evening prime rose** (*Oenothera lamarkiana*). He called these changes as **mutation** and organism undergone mutation were termed as **Mutants** (bearing mutant gene).

The scientific study of mutation was started in 1910 by **Morgan and his workers**. In *Drosphila*. He observed white eyed male among red eyed male individuals. **The white eyed male was a mutant.**

Mutation occurs frequently in nature and has been reported in many organisms e.g. Drosphila, mice, rodents, rabbits, guinea pigs and man

Macro mutations – Large and conspicuous mutations. E.g. Change in colours, shape etc.
Micro mutants – Small and inconspicuous. E.g. yield, plant height etc.

Kind of mutations :

On the basis of occurrence, degree, origin etc., the mutations are classified as.

1. **According to types of cells in which mutation occurs.**
   a) Somatic mutations : Mutations occurring in body cells. These are not transmitted to next generation and hence termed as **non-heritable mutations**.

   b) Germinal mutations : Mutations occurring in reproductive cells and such mutations are heritable and passed on to next generation E.g. Occurrence of short legged sheep of Ancon breed in a normal one.

2. **On the basis of origin (mode of Origin)**
   a) Spontaneous mutations or Natural Mutations :
   When mutations occur naturally. Eg. Double petunia -Freaks appearing in a population

   b) Induced mutations : Produced artificially in the laboratory.
   Muller with X-rays produced mutants in *Drosophilla*.

3. **Based on the nature of their effect.**
   a) Biochemical mutations : Mutations which bring about radial changes in biochemical constitution.

   b) Spurious mutations : Mutations which remain suppressed but express in the offsprings as a result of crossing over. If crossing over does not occur they remain concealed. E.g. Pink eye colour in *Drosophila*.

4. **Mutations based on their directions**

a) Forward mutation : Development of a new mutant type from a wild type (normal type).

b) Reverse mutation - Back mutation

Mutants revert to normal type

5. **Based on type of chromosomes.**

a) Autosomal : mutation occuring in autosomes

b) Sex linked mutations - mutations occuring in sex chromosomes.

6. **Based on stages of occurrence:**

a) Gametic mutation : Mutation occurs during gamete formation.

b) Zygotic mutation : Occur during first or later mitotic divisions in a zygote. This results in the development of mutant characters only in the cells which are involved in the process. Here a mosaic organism is formed.

7. **Based on affecting factors :**

a) Endogenous mutation : Caused by certain internal factors like change in metabolism, nutrition etc.

b) Exogenous mutation : Caused by external factors like change in temperature., climate etc.

8. **Nature of mutations** : They may be

a) Gene mutation : (Point mutation) a change in the DNA molecule of an individual gene

b) Chromosomal aberration : Due to changes in the structure of chromosome.

c) Chromosomal variation : Change in number of chromosomes per cell.

Point mutation

Point mutation: DNA is a chemical molecule of heredity and has information coded in terms of four letter alphabets, A, G, C and T. (Adenine, Guanine, Cytosine, Thiamine). Its coded information are duplicated and transmitted during inheritance. Transcribed and translated during development. During replications or transcription some errors may occur in exact copying of the codes leads to change in a very small segment of DNA molecule (i.e. single nucleotide - **muton** or nucleotides). Ultimately an altered phenotype of the affected organism. **Such mutations which include very limited segment of DNA is called as point mutations.**

Muton *: The unit of mutation, the smallest unit gene (DNA) capable of undergoing mutation, represented by one nucleotide*

Normal sequence : C A T, C A T, C A T, C A T < A -------

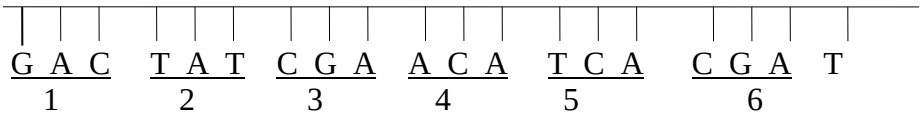
Sequence after removal of one base : C A T, T A C, A T C, A T C < A ---------

Addition of one base : C A T, C $\overset{T}{\downarrow}$ T A, T C A, T C A, T C A -

Frame shift mutations

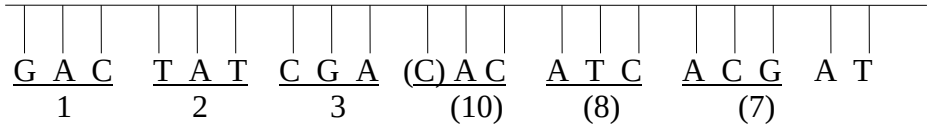
Mutations arise from the insertion (+) or deletion (-) of a nucleotide or nucleotides into or from DNA molecule. Frame shift mutations displace the starting point of genetic transcription of the genetic cell and resulting m RNA is misread by the translation process from the point of nucleotide addition or deletion. Thus once frame shift mutation introduced into a gene the reading frame is shifted. So that all codons distal to the mutations are read out of phase.

Normal sequence of nucleotide

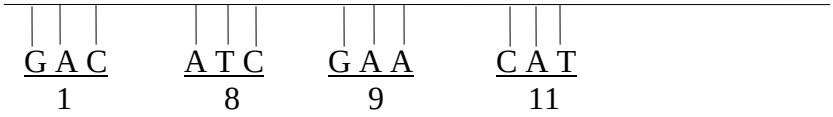


(Amino acids are symbolised 1,2,3)

Insertion of single nucleotide (C) resulting in mutant nucleotide sequence



Deletion of single nucleotide (T)



Codons which resulted often frame shift mutations cell into three categories..

1. Sense codons : Which are read or translated the same as before frame shift mutation.
2. Missense codons : Which code for a different amino acid.
3. Nonsense codons code for no amino acid.

Tautomerism

When a molecule is able to exist in more than one chemical form, it is called **tautomeric** and the phenomenon is known as **tautomerism.**

Radiation may provide the energy for the formation of tautomeric forms.

Normally

Adenine : Links with **Thyamine**

Guanine : Links with **Cytosine**.

These are the base paring found in DNA molecule, But due to tautomerism an unusual base pairing.

such as **A - C**

G – Tmay result

Such unusual base paring always, cause changes in the character of the progeny.

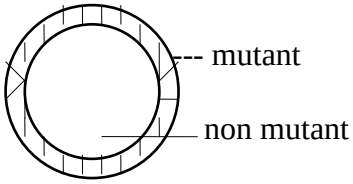
Bud mutation

If the mutation occurs in the meristematic in the early stages of bud development, all the cells of the bud will be mutant in nature, to the shoot developed from such bud will be a mutant one. This type of mutation will be called as bud sport.

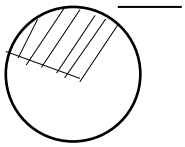
If a mutation occurs in the last stages of bud development, only some of the cells of the bud will be mutant in nature. A plant which has genetically distinct tissues lying adjacent to one another is called a **chimera**

Chimeras classified into 3 types.

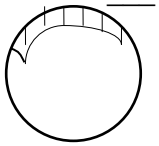
1. **Periclinal chimera**: Mutant and non-mutant tissues are in concentric layers one overlapping the other. They can be perpetuated by vegetative propagation.



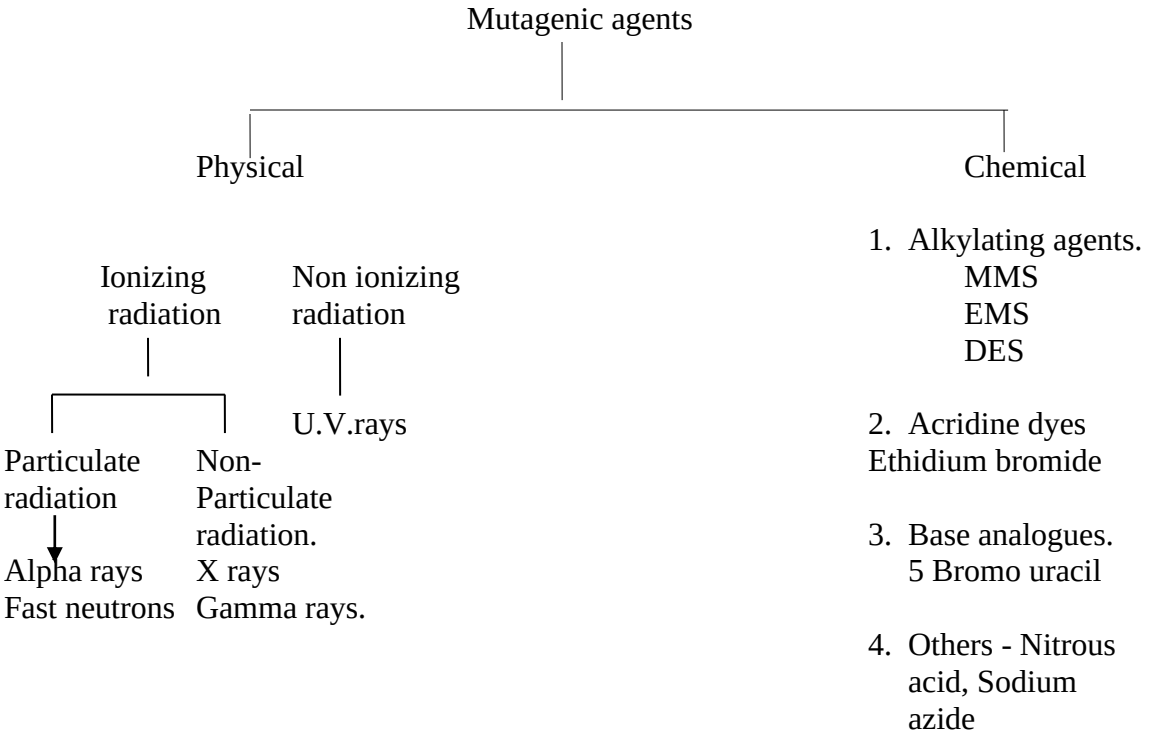
2. **Sectorial chimera**: A segment of mutant tissue extending from the epidermis towards the centre.



3. **Mericlinal** : It is incomplete periclinal chimera. The mutant tissues partly surrounds the other.



MUTATION BREEDING



Method of mutation Breeding :

1. **Objective of the programme** : Define the objective, the handling of treated population will differ. For e.g. for characters governed by oligogenes the selection can be made in M_3 generation while for traits governed by poly genes we can make selection in M_3 or M_4 generations.

2. Selection of the variety :

Usually the locally adapted best variety will be selected. For e.g. for induction of male sterility in Redgram, locally adapted short duration Co5 is to be selected. But this may not be Universal rule. For e.g. to breed alternate dwarfing gene in rice, low yielding tall *indicas* may be subjected to mutagenic treatments.

3. Part of the plant to be treated.

Depending on mode of reproduction of the crop the plant part to be treated will be decided.

I. Sexually propagated - 1. Seeds.
   a) Dry. b) Soaked
   2.. Pollen grain - limited use

II. Asexually propagated - cutting, tuber, bulbs etc.

4. Dose of the mutagen :

Beneficial mutants will be obtained around LD 50 dose. So go thro' previous literature and fix. After fixing LD 50 for a particular variety, the regular dose can be fixed.

5. Handling of M_1 generation :

The treated seeds are sown in individual plots treatment wise along with the untreated check. Seeds are sown on single seed per hill. In case of clonally propagated crops, Dominant mutants may be observed in M_1 generation. Whereas in Seed crops most of the mutant alleles will be recessive. The M_1 plants are to be harvested on single plant basis.

Observations to be recorded :

a) Germination and survival
b) Observing on chimeras if any.
c) Dominant mutants if any.
d) Biometrical observations on selected plants

6. M_2 generation :

For raising M_2 generation depending upon the availability of area we can limit the number of M_1 plants to be advanced. If the treatment is only one dose we can carry forward all the M_1 plants on single plant basis to M_2. On the other hand if the treatments are more we can limit the number of single plants to be carried forward to M_2. For e.g. 20 plants from each treatment can be selected at random from M_1 and carried forward to M_2 generation.

Immediately after germination observation on chlorophyll mutants is to be recorded to workout the mutation rate. The chlorophyll mutants may be in the form of Albino, Xantha, Viridis.

Each and every single Plant in M_2 is to be examined for detection of morphological deviants. (Macro mutants). These macro mutants are to be harvested on single plant basis and beneficial ones are to be utilized in breeding programmes and others can be included in germplasm.

The harvest of M_2 is done on single plant basis. The suspected mutant plants will be harvested on single plant basis and carried forward to M_3 generation.

7. M_3 generation :

Progenies are raised on single plant basis. For characters governed by oligo genes. Progeny rows exhibiting homozygosity may be harvested as bulk and carried forward to M_4 generation to conduct PYT.

For quantitative characters, selection can be postponed to M_4 generation if the lines are not homozygous. After selecting lines exhibiting homozygosity in M_4 those can be carried forward as bulk to M_5 for conducting preliminary yield trials.

Uses of mutation breeding.

i) New genotypes that are not present in germplasm can be created artificially.
ii) Specific characters can be improved in a variety which may be either qualitative or quantitative.
iii) F_1 can be irradiated to increase the variability further. This may be useful to break the linkage groups.

iv) Irradiation of interspecific hybrids may be done to induce beneficial translocations.
v) For induction of male sterility induced mutagenesis can be used.

Limitations :

i) Hit or miss method.
ii) Large populations are to be screened in M_2 generation. Each and every single plant is to be observed which is laborious.
iii) Desirable mutants may be associated with other undesirable traits.
iv) Most of the mutants are recessive. Recessive mutants cannot be identified in clonally propagated crops. In case of polyploids larger population is to be studied to find out recessive mutants.

Varieties released :

Castor	:	Aruna
Rice	:	Jagannath
Groundnut	:	Co2
Red gram `	:	Co5
Lablab	:	Co10
Cotton	:	MCU 10.
Black gram	:	Co 4

KINDS OF MUTATIONS

1. Gene mutation or Point mutation

Mutations produced by changes in the base sequences of genes are known as gene or point mutations. Gene mutations can be easily and clearly detected by fine genetic analysis technique available with microorganisms.

2. **Chromosomal mutations** : Mutations may cause changes in the structure of chromosome or even in chromosome number. Gross chromosomal changes. e.g. changes in chromosome number, translocations, inversions, large deletions and duplications are detectable cytologically under the microscope, but small deleterious duplications can rarely be detected.

3. **Cytoplasmic mutations** : When the mutant character shows cytoplasmic or extra nuclear inheritance, it is known as cytoplasmic mutations.

4. **Bud mutations or Somatic mutations**

Mutations occurring in buds or somatic tissues i.e. in clonal crops. Bud mutations in clonaly propagated crops depend on dominant mutations. Recessive mutations may also be utilised provided the clone used for mutagen treatment was heterozygous for the gene in question.

5. **Reverse mutation** :

Due to induced mutagensis in an organism it may revert back to original form. For example a dwarf plant would have obtained by natural mutation, when it is subjected to induced mutagenesis it may revert back to original tall plant. This is known as reverse mutation.

Lecture 18

Molecular breeding – use of marker assisted selection and utility in crop improvement

Molecular Markers

Before going to study about the molecular markers one should know about, what are conventional (or) morphological markers.

Morphological markers:

Morphological markers are based on the visually identifiable traits (eg. spikelet fertility in rice which is identified based on the apiculous colour) like wise many morphological markers are available for the identification of different traits. However, they are limited in number.

Features of morphological markers:

1. They are easy to identify, there is no need of any complex analysis. Trait of interest can be identified visually.
2. Morphological markers are inherited qualitatively, eg: presence of awns in rice or quantitatively eg: Plant height.
3. Frequency of availability of morphological markers is less (less in number). i.e morphological markers may not be available for all the traits of interest.
4. They are influenced by environment.
5. Since they are influenced by environment, they are unstable in inheritance
6. They are stage specific. (i.e) a particular marker will be usefully to identify in a particular stage of plant and not to the any stage of the plant.
7. They are influenced by genotype and developmental stages of the plant.

Besides these drawbacks, the morphological markers are useful to the plant breeders for the selection purpose in certain situation. To overcome the difficulties in the morphological markers, scientists tried to form alternate ways of identification, then comes the molecular markers. The first stage of this development leads to the identification of **'Biochemical markers'**.

Biochemical/ Protein markers:

Here the identification of trait / genotype of interest is based on the banding pattern of the proteins.

Isozymes:

These are the enzymes, which are having varying molecular weight and doing the same function.

They are influenced by a) Environment.
b) Genotype and
c) Ontogeny.

These are products of gene expression. Just based on the banding pattern of the proteins, one cannot identify a particular character/ trait of interest. (To carry out the isozyme analysis one should know the protein structures thoroughly). During gell running there is a chance of degradation of proteins and which will lead to multiple banding instead of single banding.

eg: *'Peroxidase'*, (just a hypothetical example) while banding it should give only 2 bands. But in most of the cases up to 10 bands are obtained due to the degradation of protein.

The isozyme markers can be useful, provided if we know the genetics of these markers.

GENETIC CAUSES OF ISOZYME VARIATION

1. Gene duplication with subsequent mutation at gene loci.
2. More than one gene contribute to the structure of protein.
3. Other possible variation are : Binding of high polypeptides to varying number of coenzymes or other prosthetic groups.

Note: It is always advisable to use genetically well defined isozymes as marker. In other words, it would be advisable for the investigators to follow the ontogeny of isozymes, which are known to be regulated by specific genes in order to minimize the risks.

To overcome these problems DNA markers are now used by the breeders.

Ref: Annual review of plant physiology (1974). Author : Scandalios Isozymes in Plant Development.

DNA markers:

While cutting the DNA into segments several million beads/ fragments can be obtained. Therefore millions of individual fragments of DNA can be used, as the markers, so called DNA markers or molecular markers.

Advantages:

1. Genotypic specificity.
2. They are not stage specific (i.e.) can be used in any stage (starting from seed to maturity).
3. Not influenced by environment and ontogeny.
4. Highly polymorphic
5. Stable inheritance
6. Abundant in availability.

The DNA markers are off two types viz.,

a) Dominant markers
b) Codominant markers

a) Dominant Markers:

If we obtain the bands of any one of the parent in the F_1 progeny then they are called dominant markers.

P_1 x P_2

eg: __ } bands.

↓

F_1 __ band of P_1 (dominant marker).

b) Codominant markers:

If the bands of both the parents appeared in the progeny means, than the marker is said to be codominant marker.

P_1 x P_2

eg: __ } bands.

↓

F_1 __

__ band of both P_1 & P_2 (Codominant marker).

Unlike morphological and isozyme markers the DNA markers are not influenced by the environment. They are stabily inherited. They are not influenced by environment, genotype and ontogeny.

DNA markers are stabily inherited

Eg:	Resistant genotype		Susceptible genotype
	P_1	x	P_2
	RR		rr
	8kb		6kb
		↓	
		F_1	
		Rr	
		8kb	
		6kb	
		↓	
	RR	Rr	rr
F_2:	8kb	8kb	
		6kb	6kb

genotypic 1 : 2 : 1
phenotypic 3 : 1

Properties of DNA markers:

1. Abundant
2. Ubiquitous
3. Highly polymorphic
4. Stable inheritance
5. No environmental influence
6. No ontogeny effect
7. Codominent or dominant

Markers of 'DNA' level:

1. Restriction fragment length polymorphism (RFLP)
2. Random amplified Polymorphic DNA (RAPD)
3. Sequence Tagged Sites (STS)
4. Amplicon length polymorphism (ALP)

   or

   Specific Amplicon length polymorphism
5. Variable Number Tandom Repeats (VNTR).

These markers can be grouped into tow groups viz.,

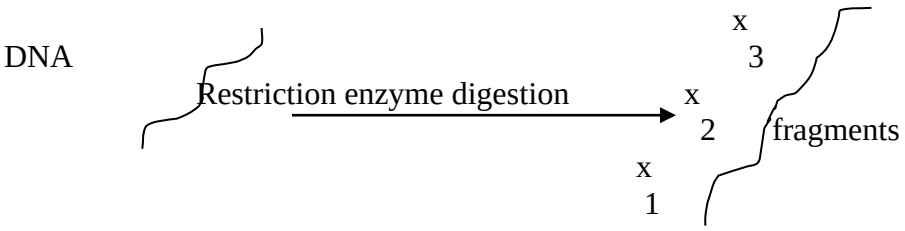
1. Hybridization based markers.
2. 'PCR' based markers.

I. Hybridization Based markers:

RFLP: Here, DNA is subjected to Restriction enzyme, after digestion by the restriction enzyme. We can get DNA fragments of variable size (DNA fragment size variation is observable after restriction enzyme digestion)

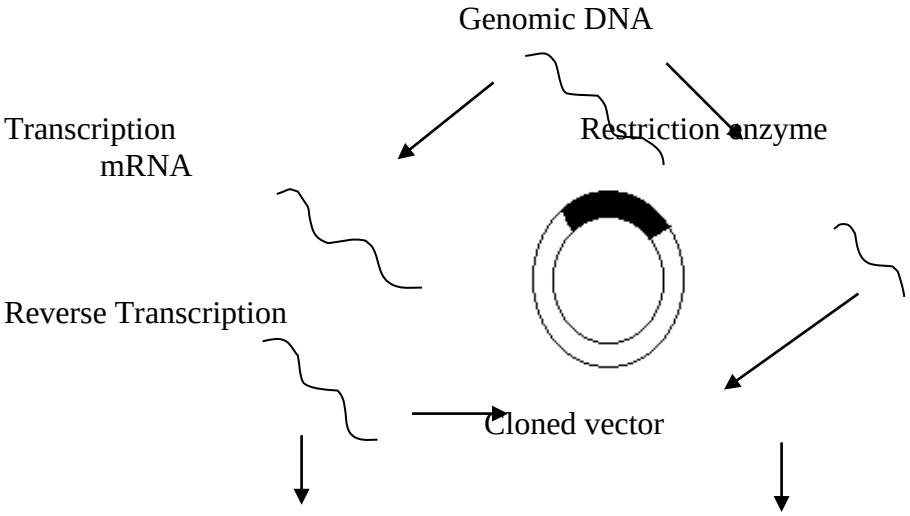
DNA —**Restriction enzyme digestion**→ **Fragments.**

To visualize these polymorphism in the fragment lengths (RFLPs), libraries of cloned probes are required. It may be genomic clones or cDNA clones.



DNA libraries :

DNA fragments after digestion can be stored in *in vitro* condition.



cDNA libraries

Gene to express-reverse transcription – cDNA library

More advantageous but, more difficult to construct (or) synthesize.

genomic DNA libraries

clone the DNA in the vector & maintain as individual fragment – genomic library – had both coding and non-coding regions.

Gel Electrophoresis & Southern blotting:

After fractionazation of DNA, the restriction products are then transferred to gel electrophoresis and subsequently transferred to Nitrocellulose filter paper followed by Southern hybridization and Autoradiography.

Fragmented DNA (by restriction enzyme)

↓

Gel electorphoresis

↓

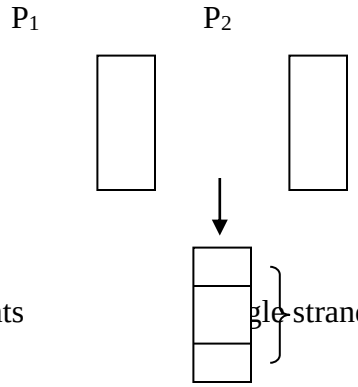
DNA fragments in nitrocellulose
Filter paper

↓

Southern hybridization

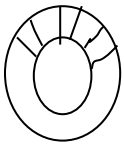
↓

Autoradiography



DNA fragments gle strands.

In Nitrocellulose filter. They are single stranded form.



Labelling cDNA

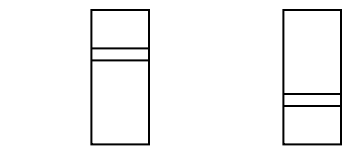
Specific piece of cDNA is labelled using 32_P

Southern hybridization at appropriate temperature & Buffer.

The labelled cDNA & the DNA fragments in the filter paper get hybridized. Hence both are single strands.

Unhybridized label is then washed off

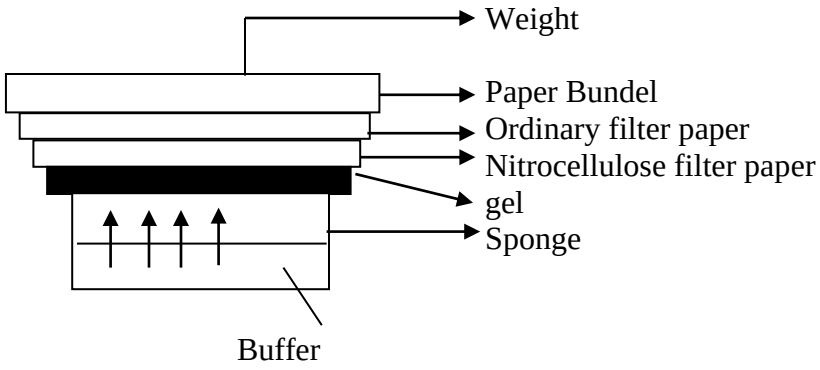
Subjected to Autoradiography



Hybridized strands / brands

The exposed film (or) autoradiogram reveals a band, where the labelled probe has bound to a region homologous to the sequence on the restricted DNA. When the size (molecular weight) of the hybridized fragments are different in different individuals, this represents a polymorphism and is referred to as RFLP. RFLPs are caused by variation in the distribution of restriction sites and not due to the restriction enzymes.

These variation in banding in different individuals is due to addition or deletion of the DNA fragments. (i.e) point mutation results in either disappearance of restriction site or appearance of restriction site.



Southern blotting.

II. PCR-Based markers : -

PCR based marker technique is conceptually a very simple method for amplifying selected nucleic acid (DNA or RNA) *in vitro*.

Invented by, Kary Mullis in (1987). The method consists of repeated cycles of DNA denaturation, primer annealing and extension by Taq DNA polymerse.

Step I: DNA is heated to high temperature of about 92-94^{0}C this step is to convert all the double stranded DNAs into single stranded one.

Step II: In this step, two oligonucleotide primers (these flank the DNA segment to be amplified ; the two primers hybridize to opposite strands of the target sequence) are allowed to anneal at temperatures ranging from 30^{0}C to 65^{0}C.

Step III: This is the extension step, where the DNA synthesis proceeds, mediated by Taq polymerase, across the region between the two primers there by replicating the DNA. The optimum temperature for this step is 70^{0}C to 75^{0}C.

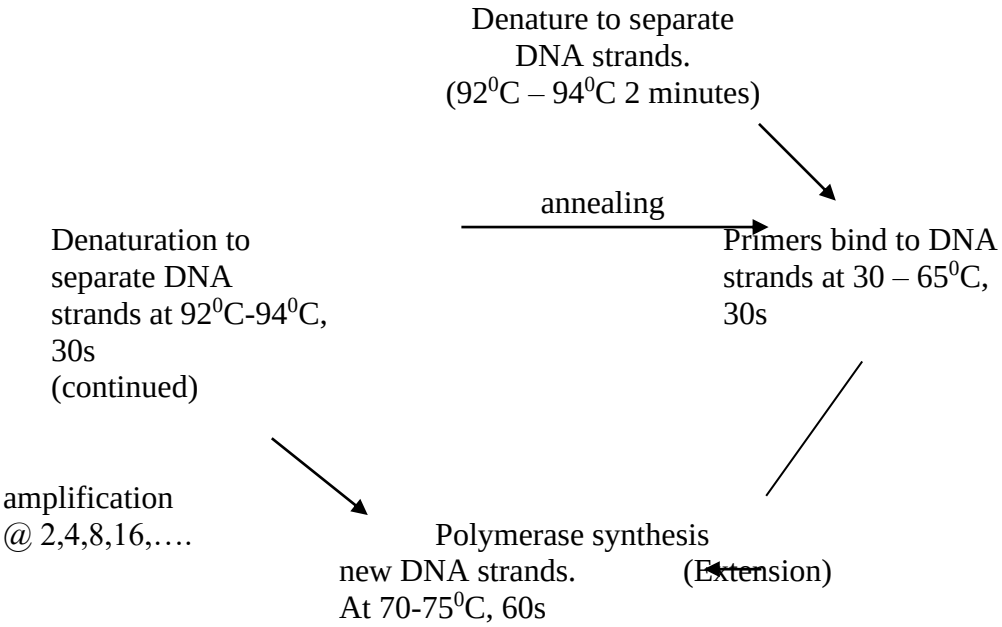
The above mentioned three steps are repeated many times (25-40 cycles) and as a result the target DNA is doubled in each successive cycle.

Primers:

They are oligonucleotide segments. They are off two types viz.,

a) Random primers $\Rightarrow$ Random amplification means they get amplified at random sites.
b) Specific primers $\Rightarrow$ specific amplification (i.e) these primers get amplified at specific sites.

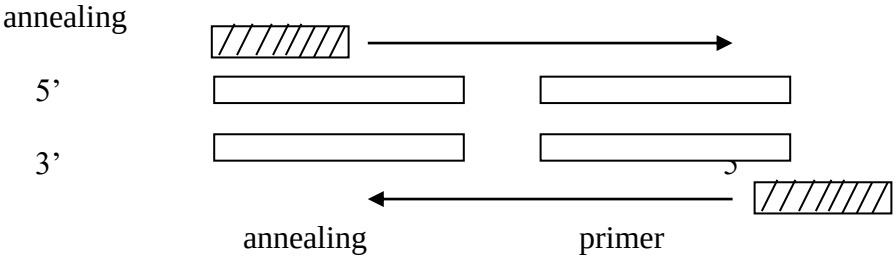
Steps involved in 'PCR' reaction



Random Amplified polymorphic DNA (RAPD)

This is a polymorphic chain reaction based on DNA amplification. But, the DNA amplified along the genome based on the binding sites to single oligonucleotide primer (10-decamer). The amplification products of RAPDs when run out an agarose gel reveals, usually several bands. The presence of a DNA fragment in one individual and the absence of the same molecular weight fragments in the another gives rise to polymorphism the unique fragment constitutes as a genetic marker.

The primers used in RAPDs random primers, with no self-complementary ends. The distance between two primes should not be less than 100 bases and the optimum is 100-2000 bases. It should not be smaller than the lower detection limit of the separation medium used to analysis.

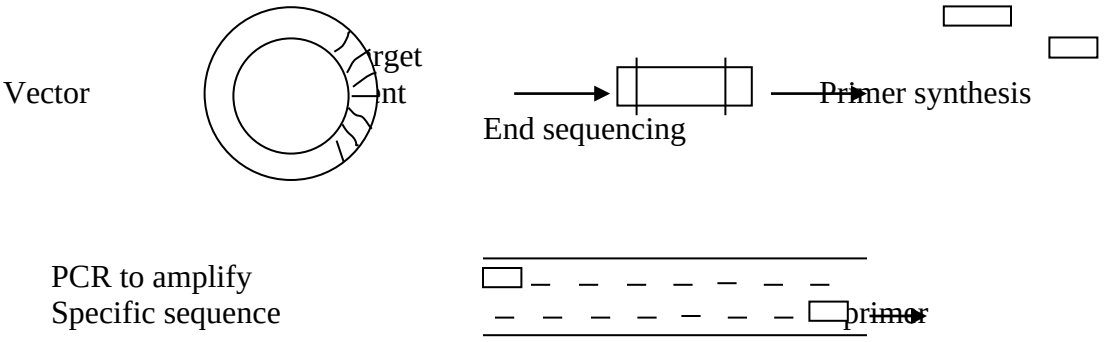


Sequence Tagged sites (STS):

In this first we have to identify the targeted fragment in the vector and the region is isolated from the vector, then sequencing the ends of the fragment called end sequencing,

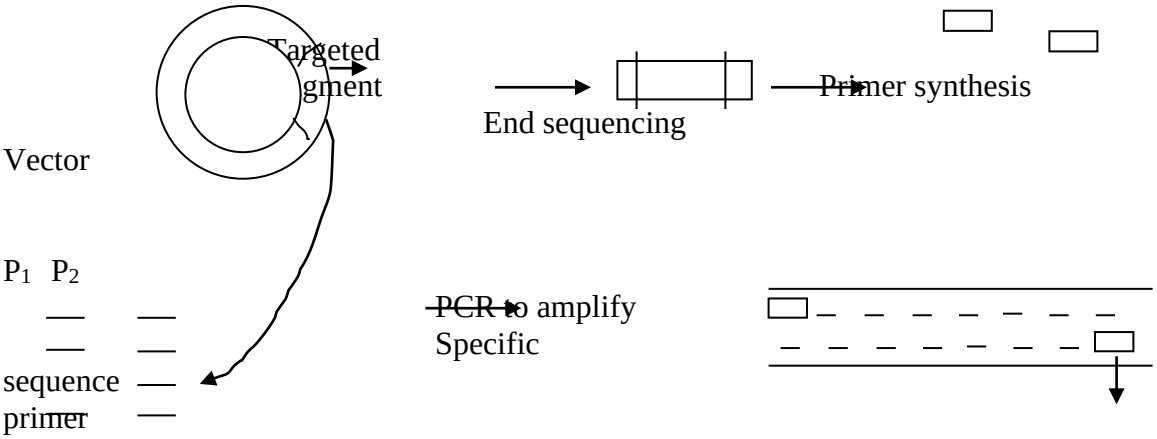
followed by primer synthesis based on the sequences. Lastly run the PCR to amplify the specific sequence. Here we are supposed to be use two primers.

Forward – is one sequence & Reverse – is the another sequence.



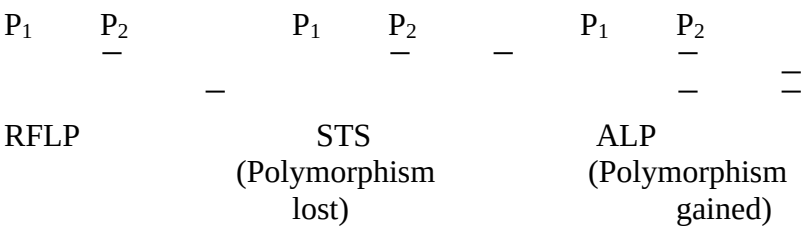
Sequence characterized amplified region (SCAR) :

The difference between the fragments of P_1 & P_2 is observed because of the third fragment (if it is single). Cull out and cloned in a vector and incorporated in the library. Then follow the same steps in STS that is, cut the ends, primer synthesis and sequencing.



Amplicon length Polymorphism (or) Specific amplicon length polymorphism:

Actually, while converting the RFLPs into PCR based markers, the polymorphism is lost. To regain the polymorphism ALP is employed.



Repeat sequences:

1) Simple sequence repeats

Try bases AT AT AT AT . . . . . AT (7 times)

Tetra bases AT AT AT AT . . . . . AT (9 times)

The primers will be specific. These differences can not be visualized in gel electrophoresis, for this we have to go for polyacralamide gel electrophoration.

2) Inter simple sequence repeats:

This is to find differences in molecular weight in the fragments, which are in inbetween repeated sequences. Design the primers based on the repeats. We can use any kind of difference in sequence repeat as markers.

Microsatellites:

Microsatellites are tandem repeats of simple sequences (1 to 6bp) that occur at random throughout the genome. They are usually less than 100bp long and are interspersed in the DNA within a region of unique sequence. Thus it is possible to amplify these sequences using a pair of unique flanking oligonucleotide primers in a polymerse chain reaction. Microsatellites are highly polymorphic due to variation in the number of repeat units. This polymorphism can be used to construct genetic maps.

Minisatellites (or) Variable Number of Tandem Repeats (VNTRs):

Minisatellites or VNTRs exhibits polymorphism due to variable number of tandom repeats of simple sequences say 9-64bp. Polymorphism results from allelic differences in the number of repeats. The resulting length variation can be detected using restriction enzymes which do not cleave the repeats and thus provides a genetically stable marker for this loci. The variation in length can be detected using Southern blotting (or) PCR can be used to detect allelic variation within the minisatellite loci.

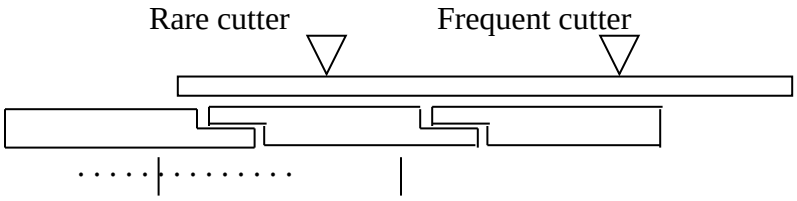
Amplified fragment length polymorphism (AFLPs):

Steps involved:

1. Restriction enzyme digestion of DNA
2. Ligation of adapters
3. Pre amplification
4. Selective amplification
5. Detection.

Restriction Digestion:

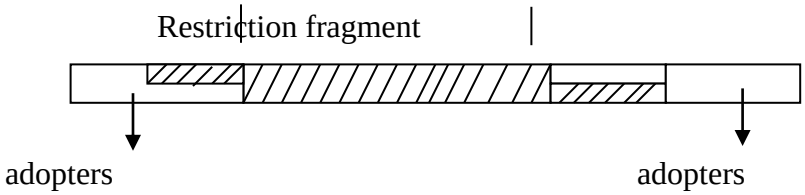
This is carried out by using rare cutters and frequent cutters. The rare cutters will have six cutters (Restriction site having 6 bases) it produce less no. of fragments and the frequent cutters will have four cutter (Restriction site having 4 bases), it produce more no. of fragments.



Here, Pst_1 (CTGCAG) is used as the rare cutter and Msc_1 is used s frequent cutter

Ligation of adaptors:

After cutting the fragments by using cutter then the cut fragment should be ligated with adaptors to get three kind of fragments.



The three possible fragments are

1) Fragments with Pst_1 site & Pst_1 site
2) Fragments with Pst_1 stie & Msc_1 site
3) Fragments with Msc_1 site & Msc_1 site

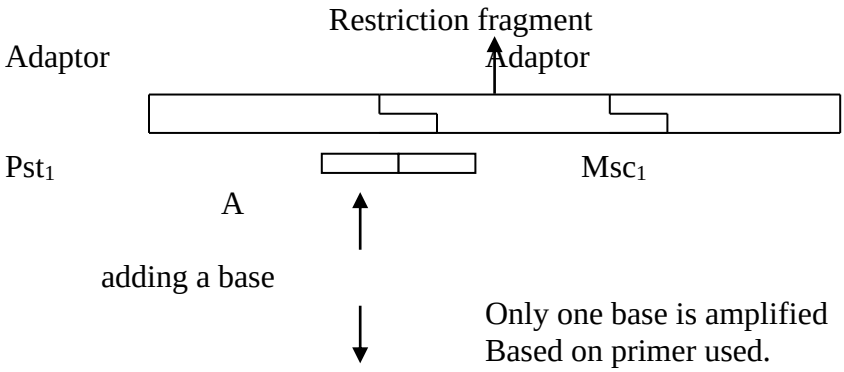
Then follows, the synthesis of primers. Synthesis of primers is based on adapters and restriction sites. Primers should be in such a way that it will amplify only the suspected adapters & restriction sites.

Pre amplification of TRF:

All the fragments can be amplified at any number of time using two primers (by using PCR-using universal fragment) called preamplifier. This will prevent the repeatation of restriction fragment sites, provide template for several PCR reactions.

Selective amplification:

This by adding one base 'A' to Pst_1 site, it will amplify the T. Here, we can use any no. of primers but it will amplify the only one base called selective amplification.



Like this by having 16 primer combinations the variation will be created. The optimum level is +3 base & don't use +1 and +2.

addition of 1 base G 4 primers 16 primer combinations.
addition of 2 bases GG 16 primers 256 primer combinations.
addition of 3 bases GGG 64 primers 4096 primer combinations.

Depends on No. of selective nucleotides the marker characters are:

Markers	Poly information content (PIC)	Map resolution	Marker type
RFLP	1-2	1,000	Codominant
AFLP	4-6	10,000	Dominant
RAPD	3-16	10,000	Dominant
SSR	10-15	>10,000	Codominant

Application of markers:

1) Genome mapping (to construct genetic maps)
2) Gene mapping & gene tagging (olegogenic)
3) QTL mapping (polygenic)
4) Diversity analysis
5) Marker aided selection.
6) Genetic purity analysis.

TAMIL NADU AGRICULTURAL UNIVERSITY
Centre for Plant Breeding and Genetics
PBG 202 Methods of Plant Breeding (1 + 1)
Model Mid Semester

Time : 1 hr. Max. Marks: 20

PART – A

I. Answer any **EIGHT** questions only **8 x 0.5 = 4**

Define

1. Centres of origin
2. Introduction
3. Inbred
4. Gene erosion
5. Apomixis
6. Self incompatibility
7. Progeny selection
8. Population
9. Law of homologous series
10. Pure line

PART – B

II. Distinguish the following (any **SIX** only) **6 x 1 = 6**

1. Distinguish Primary introduction and secondary introduction.
2. List out merits of pure line selection
3. Distinguish heteromorphic and homomorphic system incompatability
4. List out different methods to create variation in plant breeding experiments.
5. Write the important of Plant Genetic Resources
6. What are the application aspects of self incompatibility in plant breeding?
7. What is heterosis? List out different types.

PART – C

III. Answer any **FIVE** questions only **5 x 2 = 10**

1. Explain the mass selection method with a schematic diagram.
2. Give the classification of Apomixis as suggested by Nogler.
3. Compare the salient features of GMS, CMS and CGMS systems.
4. What were all the objectives made to the Concept of Vavilov's theory?
5. Explain the major activities involved in Plant Genetic Resources Management.
6. List out the methods for overcoming self incompatibility in crops.