Principles of Plant Breeding

9. Principles of Plant Breeding (HPG 101) 3 (2+1)

Plant breeding as a dynamic science, genetic basis of Plant Breeding – classical, quantitative and molecular, Plant Breeding in India – limitations, major achievements, goal setting for future. Sexual reproduction (cross and self pollination), asexual reproduction, pollination control mechanism (incompatibility and sterility and implications of reproductive systems on population structure). Genetic components of polygenic variation and breeding strategies, selection as a basis of crop breeding. Hybridization and selection – goals of hybridization, selection of plants; population developed by hybridization – simple crosses, bulk crosses and complex crosses. General and special breeding techniques. Heterosis – concepts, estimation and its genetic basis.

Practical: Breeding objectives and techniques in major field crop plants. Floral biology – its measurement, emasculation, crossing and selfing techniques in major crops. Determination of mode of reproduction in crop plants, handling of breeding material and maintenance of experimental records in self and cross pollinated crops. Demonstration of hybrid variation and production techniques.

Principles of Plant Breeding

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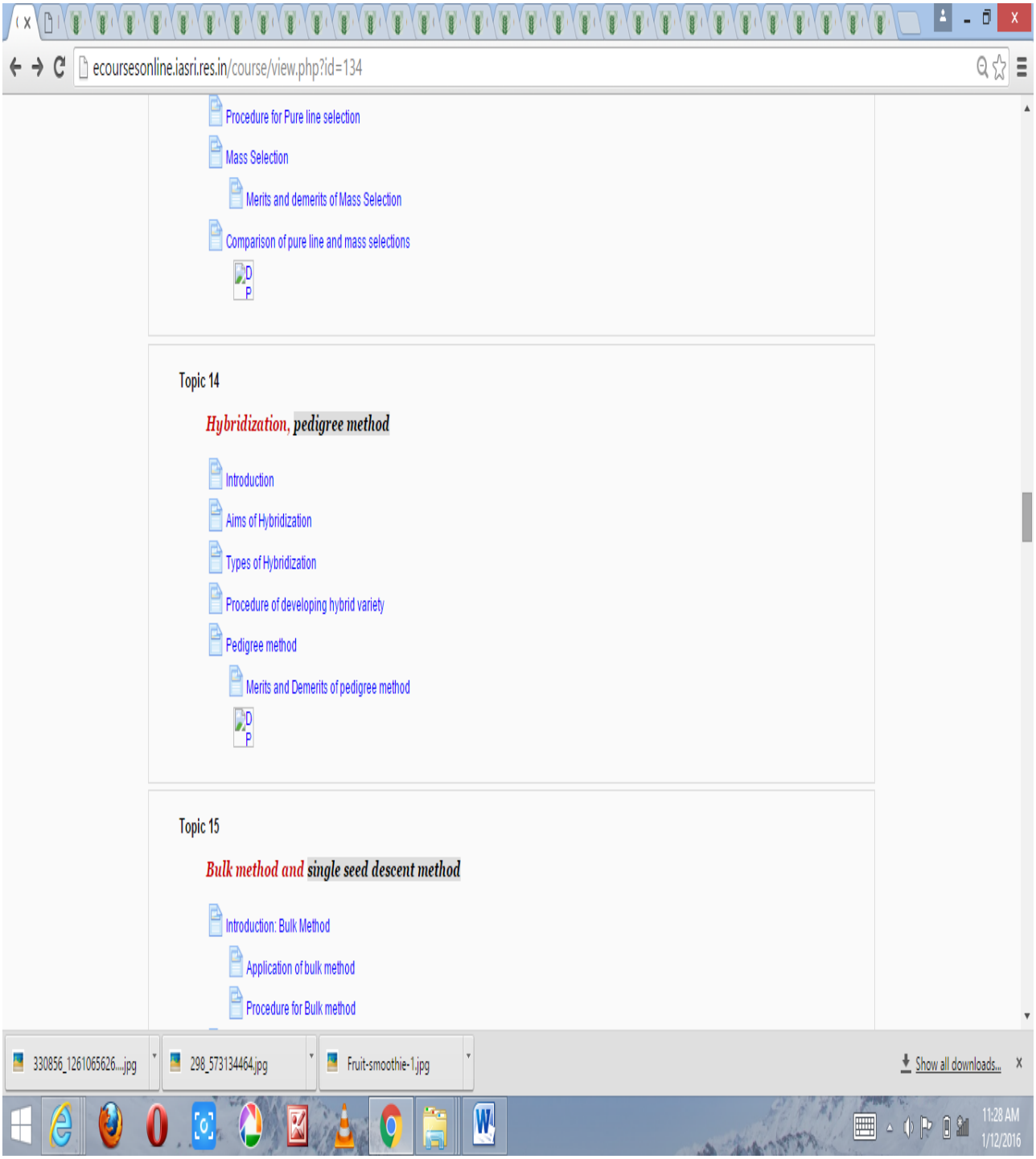
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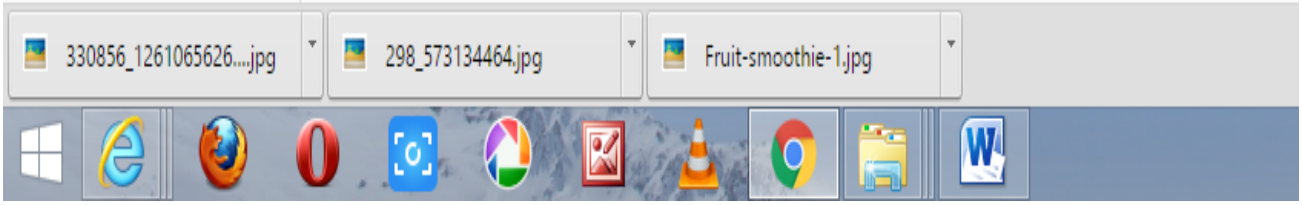

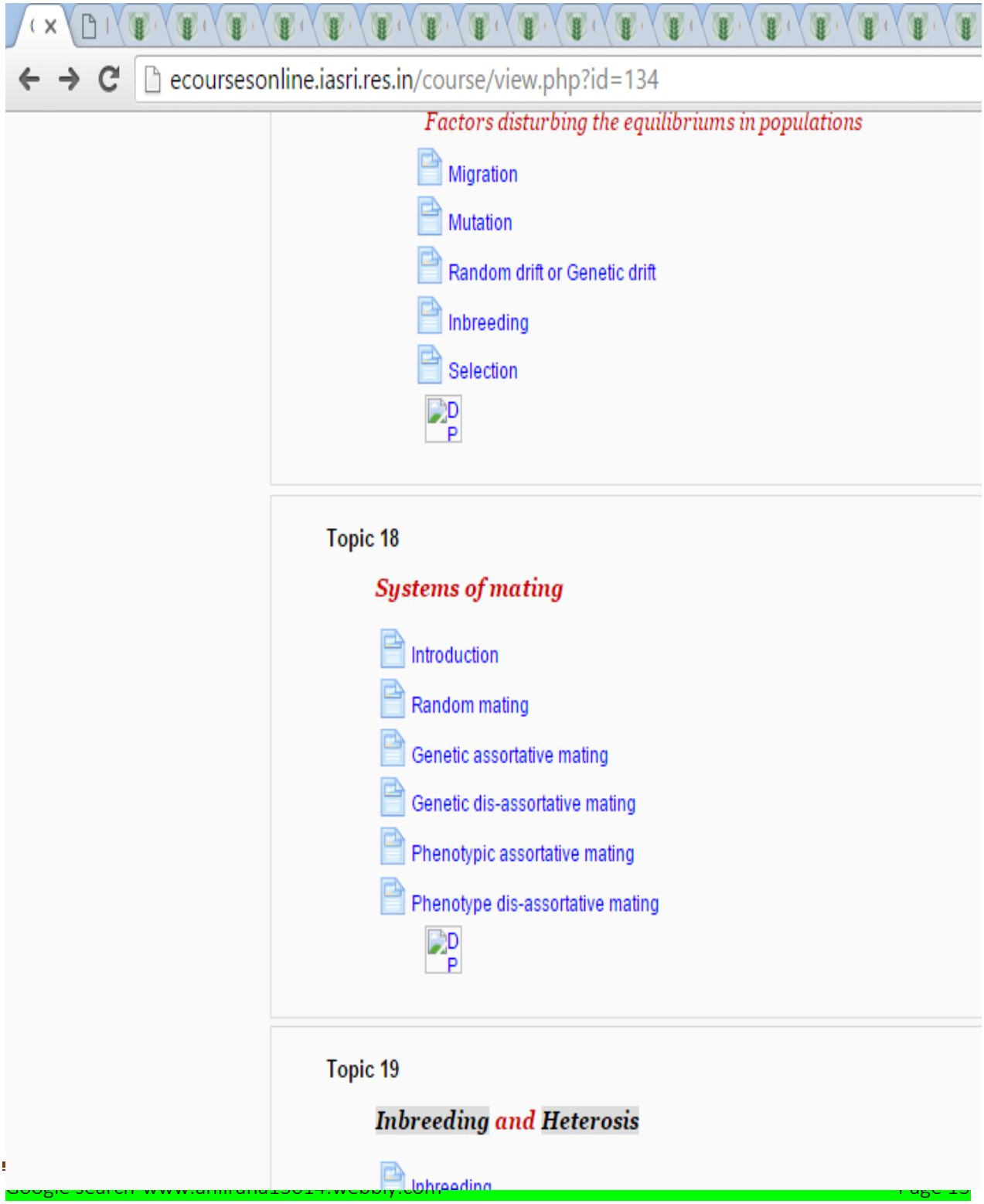
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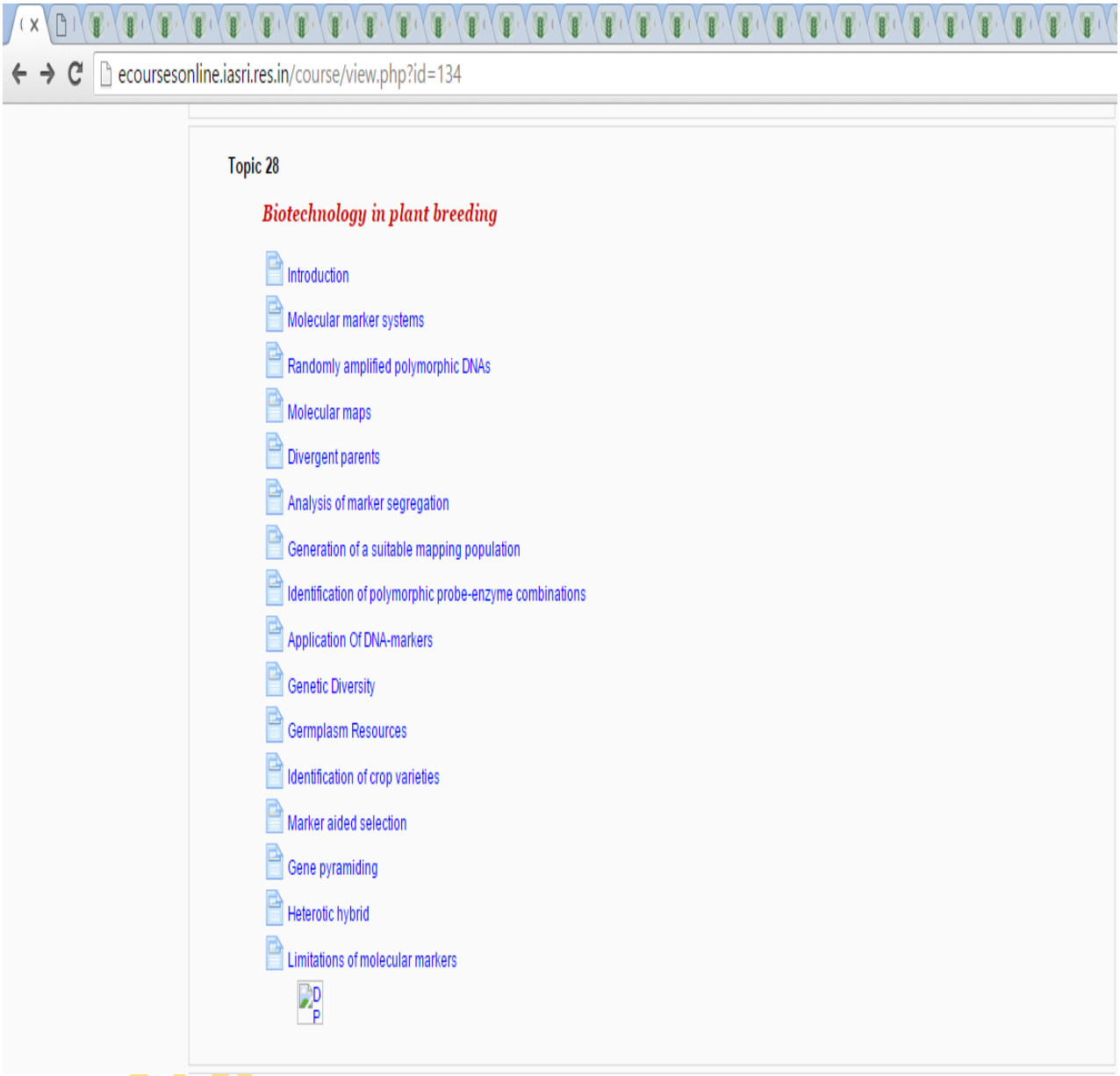
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Introduction to Plant Breeding

- Human being is almost dependent on plants for his food and all other needs. Plants are major source directly or indirectly of most food, fodder clothing, fuel, drugs etc. Considering the prime importance of plants, human being have long been concerned with developing plant types better suited to satisfying their needs.
- Art: Before the rediscovery of Mendelism plant breeding was the result of visual selection of material practiced on a trial and error basis by untrained people. Skill and proper judgment coupled with common sense formed the basis of success. Selection was the only practice of plant breeding which resulted in development of many varieties developed before 20thcentury. This art depends on breeder to assess the difference of economic values between the plants.
- Now with the growth of genetics, these practices are systematic to the point, where they can be called as a science.

Definition

- Smith defined plant breeding as the art and science of improving the genetic pattern of plants in relation to their economic use. It is a science which uses the knowledge and techniques from many basic science areas. As a continuation of natural evolution plant breeding is an ancient discipline.
- It is not only contributes to agriculture progress by information, but also by material products such as crop varieties, hybrids, composite/ synthetics, which are suited to human needs in one or more aspects. Earliest success were chance affair largely depended upon the breeder's skill.
- Thomas Robert Malthus 1798 postulated that human populations, unless checked by wars or disasters, increase under hunger. He foresaw catastrophe because he believed that population was capable of increase at a geometric rate and food supply at arithmetic rate. He predicted that Britain would be in disaster by the midnineteenth century. Fortunately his prediction was falsified. Due to tremendous increase in food supplies due to better method of production and improved varieties of plants and breeds of live stock.
- Finally we can expect plant breeding to contribute substantially to greater agriculture production. It was not only by breeding high yielding varieties, but also by stabilizing production through developing resistance to diseases, pests, drought, heat and cold etc.

Year	Area	Production	
1949-50	99.20 m ha	54.92 m tonnes	
1985-86	127.06 m ha	150.47 m tonnes	247% growth rate
2000-01		220 m tonnes	
2003-04		240 m tonnes	

So the nation became almost self sufficient in food grains but the population is growing at an alarming rate of around 2.5% per year .Food production should also increase at least at same rate or even at faster rate to improve the nutritional status.

The 25 major food crops of the world, ranked according to total tonnage produced annually

1	Wheat	10	Oat	19	Coconut
2	Rice	11	Sorghum	20	Cotton
3	Corn (Maize)	12	Sugarcane	21	Apple

4	Potato	13	Millets	22	Yam
5	Barley	14	Banana	23	Pea nut
6	Sweet potato	15	Tomato	24	Watermelon
7	Cassava	16	Sugar beet	25	Cabbage
8	Grape	17	Rye		
9	Soybean	18	Orange		

It could be possible because of

- Improved crop varieties
- Better management of inputs
- Increase net cropped area

Better environment alone cannot lead to better yields from inferior varieties beyond a certain limit. The limit is set by the genetic makeup of the variety.

Eg: Tall wheat varieties give response upto 60 kg/ ha, Dwarf wheat varieties give increase yield of120 kg/ ha

- Hence, genetic makeup of plants permits considerably endless favorable changes.
- This continuous favorable change in the genotype of crop varieties is a must for increasing yields.
- So plant breeding deals with the principles and the methods required forfavorably changing thegenetic constitution of crop plants.

Selected milestones in plant breeding

- 9000 bc First evidence of plant domestication in the hills above the Tigris river.
- 3000 bc- Domestication of all important food crops in the Old World.
- 1000 bc- Domestication of all important food crops in the New World.
- 700 bc -Assyrians and Babylonians hand pollinated date palms.
- 1694 -Camerarius demonstrated sex in plants and suggested crossing as a method to obtain new planttypes.
- 1716- Mather of USA observed natural crossing in maize.
- 1719- Fairchild created first artificial hybrid of dianthus sp. (Carnation × Sweet williams).
- 1727- Vilmorin introduced the concept of progeny testing i.e Vilmorin isolation principle.
- 1753- Linnaeus published Species plantarium. Binomial nomenclature was born.
- 1761–1766 Koelreuter of Germany demonstrated that hybrid offspring received traits from both parents and were intermediate in most traits; produced first scientific hybrid using tobacco.
- 1847- “Reid’s Yellow Dent” maize was developed.

- 1866- Mendel published his discoveries in Experiments in plant hybridization, culminating in inheritance and discovery of unit factors (genes).
- 1899- Hopkins described the ear-to-row selection method of breeding in maize.
- 1908–1909 Hardy of England and Weinberg of Germany developed the law of equilibrium of populations.
- 1908–Nilsson Ehle proposed the concept of multiple factor hypotheses.
- 1909 -Shull conducted extensive research to develop inbreds to produce hybrids of maize.
- 1917 -Jones developed first commercial hybrid maize.
- 1926 -Pioneer Hi-bred corn company established as first seed company.
- 1934- Dustin discovered colchicines.
- 1935- Vavilov published The scientific basis of plant breeding.
- 1940 -Harlan used the bulk breeding selection method in breeding.
- 1944- Avery, MacLeod, and McCarty discovered DNA is hereditary material.
- 1945- Hull proposed recurrent selection method of breeding.
- 1950 -McClintock discovered the Ac-Ds system of transposable elements.
- 1953- Watson, Crick, and Wilkins proposed a model for DNA structure.
- 1970- Borlaug received Nobel Prize for the Green Revolution.
- Berg, Cohen, and Boyer introduced the recombinant DNA technology.
- 1994 -"Flavr Savr" tomato developed as first genetically modified food produced for the market.
- 1995- Bt corn developed.

- 1996- Roundup Ready® soybean introduced.
- 2004- Roundup Ready® wheat developed.

Disciplines for Plant Breeding

Plant breeding involves several disciplines for development of improved cultivation. Knowledge of all these disciplines is essential for a plant breeder to start judicious breeding programme.

1. **Botany**: Understanding of the morphology and the reproduction of plants and also taxonomy. It helps to transfer of resistant genes.
2. **Genetics and cytogenetics**: Plant breeding is an applied branch of Genetics. It involves various genetical principles
3. **Agronomy**: A good breeder is first a good agronomist to raise good crop and to select and to evaluate varieties
4. **Plant physiology:** To develop varieties for a biotic stresses eg: drought salinity, heat and crop to develop physiological efficient genotypes
5. **Plant pathology**: Crop plants are infected by a number of fungal, bacterial, virus diseases. Plant breeder has to develop resistant varieties for various diseases and therefore should be aware of basic principles of plant pathology.

6. **Entomology**: Crop plants are attacked by large number of insect pests. And therefore plant breeder should be aware of basics of entomology.

7. **Biochemistry**: Quality tests are required to determine the quality characteristic of a crop varieties.

8. **Statistics and Biometrics**: Plant breeding has to test the performance of various breeding materials in field experiment. Knowledge of experimental designs and statistical methods are essential.

9. **Plant Biotechnology**: It is the combination of plant tissue culture and genetic engineering. It is useful tool for development of transgenic crop plants, Herbicide resistance, good quality, resistance to biotic and aboitic stress. Distant crosses possible through somatic hybridization.

Activities in plant breeding

- **Creation of variation**: Genetic variation is a prerequisite for any crop improvement.
- **Selection**: Identification and isolation of plants and growing their progeny. It is based on phenotype. The efficiency of this activity determines the success of breeding program. Various methods have been designed to develop varieties/strain.
- **Evaluation:** The newly selected lines/ strain/ population are tested for yield and other traits and compared with the existing best varieties conducted at several location 2-3 years.
- **Multiplication**: Large scale production of certified seeds of the released and notified varieties. It is produced in a step wise manner.
- **Distribution**: Seed is sold to the farmers who use it for commercial crop cultivation to reap the economic benefits.
- For an efficient crop improvement programme the above activities have to be properly co-ordinated and efficiently geared to maximize the output. A deficiency in any step will reduce the efficiency.

Achievements

- Semi dwarf wheat (Norin-10), Rice(Dee-Gee-Woo-Gen), Hybrids, Nobilisation of cane, Hybrid corn, Hybrid in Sorghum, Pearlmillet, Hybrid cotton.
- Tomato, Chilli, Cucurbits and flower crops.
- Variation occurs in nature by three sources namely mutation, hybridization (natural) and polyploidy.

- Using these three sources genetic variation can be widened in any crop plants

- **Contributions in plant breeding**

Name	Crop	Achivements
Dharm pal Singh	Rapeseed and Mustard	
C.T.Patel	Cotton Breeder	World's first interspecific cotton hybrid
V.Santhanum	Cotton Breeder	Upland and Egyptian Cotton
Bosi Sen	Maize Breeder	
D.S Athwal	Pearl millet Breeder	
Ram Dhan Singh	Wheat Breeder	C-591
Ramaiah	Rice Breeder	CRRI Cuttack
N.G.P.Rao	Sorghum Breeder	NRCS Hyderabad CSH hybrids
Push Karanth	Potato Breeder	CPRI Shimla

M.S.Swaminathan	Wheat and Rice	Green Revolution in India
B.P.Pal	Wheat Breeder	NP varieties
T.S.Venkataraman	Sugarcane Breeder	Sugarcane breeding institute, Coimbatore

Plant Breeding in India

Breeding in India

- 1871- Organized agricultural research – Establishment of department of agriculture.
- 1892- Appointment first scientist – Agricultural Chemist.
- 1905- Imperial Agricultural Research Institute at Pusa later it Shifted to Delhi.
- 1946- Establishment of Indian Agriculture Research Institute at New Delhi.
- 1901-1905 Agriculture colleges were established at Kanpur, Pune, Sabour, Llyalpur Coimbatore.
- 1946- Establishment of Indian Council of Agriculture Research at New Delhi.
- 1921- Indian Central Cotton Committee- 70 varieties of cotton were developed.
- 1956- Project for intensification of Regional Research on Cotton, Oil seeds and Millets (PIRRCOM)-17 centre in India, crops cotton, Castor, ground nut, brassica, til, toria, jowar and Bajra.

- 1957- All India Co-ordinated Maize Improvement Project to exploit heterosis. First hybrid maize was developed in 1962, looking to the success ICAR started 16 new AICRP projects.
- 1960- First Agriculture University was established at Pantnagar, now there are 43 Agriculture universities are in India

Aims and scope of plant breeding

Increased yield has been the ultimate aim of most plant breeding in any crop.

Plant breeding aims to improve the characteristic of plants so that they become more desirable agronomically and economically

1. Higher yield: Higher crops yield. Developing efficient genotypes hybrid varieties for grain fodder and fiber
2. Improved quality: It determines its suitability for various use

- Grain size, color, milling and baking qualities in wheat
- Cooking quality in rice oil content in oil seed.
- Malting quality in barley and sugar content in sugar cane
- Keeping quality in vegetables
- Protein content in cereals. Lysine in cereals methionine and tryptophan in pulse

3. Disease and insect resistant varieties are stable, safest and cheapest.

4. Change in Maturity duration- It permits crop rotation, double cropping, late planting.

5. Agronomic characteristics: Plant height, tillering, branches erect. Eg: Dwarf in cereals is associated with lodging resistance and fertilizer response

6. Photo insensitive: It promotes cultivation in new areas throughout the year.

7. Non shattering eg: Soybean, Mung.

8. Determinate growth: Eg: Mung ,Cowpea and Red Gram.

9. Dormancy Eg: Seeds germinate even before harvesting if there are rains at the time of maturity. Eg: Greengram and Ground nut.

10. Varieties for new seasons Eg: maize is grown in Kharif. Now it can be grown rabi/ summer.

11. Moisture stress and salt tolerance.

12. Varieties for rain fed areas, saline soils. In India under 7-20 m. ha area is salt effected.

Kinds Of Germplasm

- The sum total of all the hereditary material is referred as germplasm.In other words, gene pool refers to a whole library of different alleles of a species Germplasm or gene pool is the basic material with which a plant breeder has to initiate his breeding programme.

Kinds Of Germplasm

A. Land races

- Land races are nothing but primitive cultivar which were selected and cultivated by the farmers for many generations.
- Land races were not deliberately bred like modem cultivars. They evolved under subsistence agriculture.
- Land races have high level of genetic diversity which provides them high degree of resistance to biotic and a biotic stresses. Biotic stress refers to hazards of diseases and insects, whereas a biotic stress means, drought, salinity, cold, frost, etc.
- Land races have broad genetic base which again provides them wider adaptability and protection from epidemic of diseases and insects.

B. Obsolete Cultivars

- Improved varieties of recent past are known as obsolete cultivars. These are the varieties which were popular earlier and now have been replaced by new varieties.
- For example, varieties K68, K65 and Pb 591 were most popular traditional tall varieties before introduction of high yielding dwarf Mexican wheat varieties.
- These varieties are well known for their attractive grain colour and chapati making quality. Now these varieties are no more cultivated.

C. Modern Cultivars

- The currently cultivated high yielding varieties are referred to as modern cultivars. These varieties have high yield potential and uniformity as compared to obsolete varieties and land races.
- Modem cultivars constitute a major part of working collections and are extensively used as parents in the breeding programme.

D.Advanced Breeding lines

- Pre-released plants which have been developed by plant breeders for use in modem scientific plant breeding are known as advanced lines, cultures and stocks.

E.Wild forms of Cultivated Species

- Wild forms of cultivated species are available in crop plants. Such plants have generally high degree of resistance to biotic and a biotic stresses and are utilized in breeding programmes for genetic improvement of resistance to biotic and a biotic stresses.

F. Wild Relatives

- Those naturally occurring plant species which have common ancestry with crops and can cross with crop species are referred to as wild relatives or wild species.
- Wild relatives are important sources of resistance to biotic (diseases and insects) and a biotic (drought, cold, frost, salinity, etc.) stresses.

G. Mutants

- Mutation breeding is used when the desired character is not found in the genetic stocks of cultivated species and their wild relatives. Mutations do occur in nature as well as can be induced through the use of physical and chemical mutagens.

- For example, mutant genepool Dee-Geo-Woo-Gen in rice and Norin 10 in wheat proved to be valuable genetic resources in the development of high yielding and semi dwarf varieties in the respective crop species.

Gene Pool and its Type

Gene Pool

- Gene pool consists of all the genes and their alleles present in all individuals which can hybridize with each other.

Types of gene pool

Primary gene pool (GP1)

- The gene pool in which intermating (crossing) is easy and leads to production of fertile hybrids is known as primary gene pool.
- It includes plants of the same species or of closely related species which produce completely fertile offspring on intermating.

Secondary gene pool (GP2)

- The genetic material that leads to partial fertility on crossing with GPI is referred to as secondary gene pool.
- Transfer of gene from such material to primary gene pool is possible but difficult.

Tertiary gene pool (GP3)

- The genetic material which leads to production of sterile hybrids on crossing with primary gene pool is termed as tertiary gene pool. It includes material which can be crossed with GP1, but the hybrids are sterile.
- Transfer of genes from such material to primary gene pool is possible with the help of special techniques.

Vavilovian Centers of Diversity

- N. I. Vavilov (1926, 1951), a Russian geneticist and plant breeder, was the pioneer man who realized the significance of genetic diversity for crop improvement. Vavilov and his colleagues visited several countries and collected cultivated plants and their wild relatives for use in the Russian breeding programme to develop varieties for various agro climatic conditions of USSR.
- Based on his studies of global exploration and collection, Vavilov proposed eight main centres of diversity and three subsidiary centres of diversity given as follows

1. Main centres-

- Main centres of crop diversity as proposed by Vavilov are (1) China, (2) India (Hindustan), (3) Central Asia, (4) Asia Minor or Persia, (5) Mediterranean, (6) Abyssinia, (7) Central America or Mexico, and (8) South America.

2. Subsidiary Centers -

- There are three subsidiary centres of diversity. These are: (1) Indo-Malaya, (2) Chile, and (3) Brazil and Paraguay.
- All these centres are known as centres of origin or centres of diversity or Vavilovian centres of diversity.
- Vavilovian centres of diversity of crop plants

Name of centres	**Main crops for which genetic diversity is found**
A)Main centres	
I. China	Naked oat (SC), Soybean, Adzuki bean, Common bean (SC), Small Bamboo, Leaf Mustard (SC), Peach, Orai Sesame (SC), China tea, etc.
2. Hindustan	Rice, Chickpea, Moth Bean, Rice bean, Horsegram, Brinjal, Cucumber, Tree Cotton,Jute, Pepper, African Millet, Indigo, etc.
3. Central Asia	Bread wheat, Club wheat, Shot wheat, Rye (SC), Pea, Lentil, Chickpea, Sesame, Flax,Safflower, Carrot, Radish, Apple, Pear and Walnut.
4, Asia Minor or Persia	Einkorn wheat, Durum wheat, Poulard wheat, Bread wheat, Two Rowed barley, Rye, Red oat, Chickpea (SC) lentil, Pea (SC), Flax, Almond, Pomegranate, Pistachio, Apricot and Grape.

5. Mediterranean	Durum wheat, Husked oats, Olive, Broad bean and Lettuce
6. Abyssinia	Durum wheat, Poulard wheat. Emmer wheat, Barley, Chickpea, Lentil, Pea, Flax. Sesame, Castor bean, African Millet, and coffee.
7. Central America or Mexico	Maize, Common bean, Upland cotton, Pumpkin Gourd, Squash, Sisal hemp and Pepper.
8. South America	Potato Sweet potato, Lima bean, Tobacco and Sea Island cotton
B.Subsidiary entres	
9. Indo-Malaysia	Banana, Coconut, Yam, and Pomelo
10. Chile	Potato
11. Brazil and Paraguay	Peanut, Rubber, Cocoa, Pineapple.

Types of Centers of Diversity

Primary Centres of Diversity

- Primary centres are regions of vast genetic diversity of crop plants. These are original homes of the crop plants which are generally uncultivated areas like, mountains, hills, river valleys, forests, etc.

Main features of Centres of diversity

1. They have wide genetic diversity.

2. Have large number of dominant genes.

3. Mostly have wild characters.

4. Exhibit less crossing over.

5. Natural selection operates.

Secondary Centres of Diversity

- These are the areas where certain crop species show considerable diversity although they did not originate there.These are generally the cultivated areas and have following main features.

1. Have lesser genetic diversity than primary centres.

2. Have large number of recessive genes.

3. Exhibit more crossing over.

5. Both natural and artificial selections operate.

Law of Parallel Variation

- The concept of parallel variation also known as law of homologous series of variation was developed by Vavilov (1951) based on his study of crop diversity and centres of origin. Law of homologous series states that a particular variation observed in a crop species is also expected to be available in its related species. For instance, if we get dwarf collections in one species of a crop, the same may be observed in another related species also. Vavilov used principle of homologous series of variation as a clue for discovering similar characters in related species.

Law of Parallel Variation

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Gene Banks

- Gene bank refers to a place or organization where germplasm can be conserved in living state. Gene banks are also known as germplasm banks. The germplasm is stored in the form of seeds, pollen or in vitro cultures, or in the case of a field gene bank, as plants growing in the field. Gene banks are mainly of two types,

(1) Seed gene banks

(2) Field gene banks

1. Seed Gene bank

- A place where germplasm is conserved in the form of seeds is called seed gene bank. Seeds are very convenient for storage because they occupy smaller space than whole plants. However, seeds of all crops can not be stored at low

temperature in the seed banks. The germplasm of only orthodox species (whose seed can be dried to low moisture content without losing variability) can be conserved in seed banks. In the seed banks, there are three types of conservation, viz., (1) Short term, (2) Medium term, and (3) Long-term.

- Base collectionsare conserved for long term (50 years or more) at -18 or -20°C.
- Active collections are stored for medium term (10-15 years) at zero degreesCelsius.
- Working collectionis stored for short term (3-5 years) at 5-10°C.

Advantages of gene banks

1. Large number of germplasm samples or entire variability can be conserved in a very small space.

2. In seed banks, handling of germplasm is easy.

3. Germplasm is conserved under pathogen and insect free environment.

Disadvantages

1. Seeds of recalcitrant species can not be stored in seed banks.

2. Failure of power supply may lead to loss of viability and thereby loss of germplasm.

3. It requires periodical evaluation of seed viability. After some time multiplication is essential to get new or fresh seeds for storage.

2. Field Gene banks

- Field gene banks also called plant gene banks are areas of land in which germplasm collections of growing plants are assembled. This is also called ex-situ conservation of germplasm. Those plant species that have recalcitrant seeds or do not produce seeds readily are conserved in field gene banks. In field gene banks, germplasm is maintained in the form of plants as a permanent living collection. Field gene banks are often established to maintain working collections of living plants for experimental purposes. They are used as source of germplasm for species such as coconut, rubber, mango, cassava, yam and cocoa. Field gene banks have been established in many countries for different crops

Established field gene banks

Name of country	Crop species for which field gene bank is established
Malaysia	Oil palm has been conserved on 500 ha.
Indonesia	Earmarked 1000 ha. Area for coconut and other perennial crops.
Philippines	South East Asian germplasm of banana has been conserved.
India	Global collection of coconut has been conserved to Andman and Nicobar.

Advantages

1. It provides opportunities for continuous evaluation for various economic characters.

2. It can be directly utilized in the breeding programme.

Disadvantages

1. Field gene banks can not cover the entire genetic diversity of a species. It can cover only a fraction of the full range of diversity of a species.

2. The germplasm in field gene banks is exposed to pathogens and insects and sometimes is damaged by natural disasters such as bushfires, cyclones, floods, etc.

3. Maintenance of germplasm in the field gene banks is costly.

Meristem Gene Banks

- Germplasm of asexually propagated species can be conserved in the form of meristems. This method is widely used for conservation and propagation of horticultural species. In vitro method can be used in two ways.

  a. Storage of tissues under slow growth conditions.

  b. Long term conservation of germplasm by cryopreservation. In cryopreservation, the tissues are stored at a very low temperature i.e. at -1960C in liquid nitrogen.

Gene banks for various crops in India

Crop species	Location of Gene bank	Name of Research institute/Centre
Wheat	Karnal	Directorate of Wheat Research (DWR)
Rice	Cuttack	Central Rice Research Institute (CRRI)
Potato	Shimla	Central Potato Research Institute (CPRI)

Cotton	Nagpur	Central Institute for Cotton Research (CICR)
Pulses	Kanpur	Indian Institute for Pulses Research (IIPR)
Oilseed crops	Hyderabad	Directorate of Oilseed Research (DOR)
Sorghum	Hyderabad	National Research Centre for Sorghum
Soybean	Indore	National Research Centre for Soybean

- Based on status of Research Institutes, gene banks are again of two types,

  (1) National gene banks

  (2) International or global gene banks.

- National gene banks are maintained by each country and global gene banks are located in International Crop Research Institutes/Centres. In India,gene banks are maintained by concerned Crop Research Institutes of ICAR. National Bureau of Plant Genetic Resources, New Delhi is also maintaining germplasm of various field Crops.
- Germplasm can also be conserved in the form of pollen and DNA. However, these methods are not in common use. Conservation of germplasm in the form of DNA is a difficult task as it requires sophisticated laboratory and became expensive.

Evaluation of Germplasm

- Evaluation refers to screening of germplasm in respect of morphological, genetical, economic,biochemical, and physiological, pathological and entomological attributes.

Evaluation of germplasm is essential from following angles

a.To identify gene sources for resistance to biotic and abiotic stresses earliness, dwarfness, and productivity and quality characters.

b.To classify the germplasm into various groups.

Documentation

Documentation

a. Documentation refers to compilation, analysis, classification, storage and dissemination of information.

b. In plant genetic resources, documentation means dissemination of information about various activities such as collection, evaluation,conservation, storage and retrieval of data.

c. Now the term documentation is more appropriately known as information system.

Information system is useful in many ways as given below:

a. It provides information about various activities of plant genetic resources.

b. It provides latest information about characterization, conservation, distribution and utilization of genetic resources.

c. It helps explorers, evaluators and curators in the conservation of genetic resources.

Distribution

Distribution

- The distribution of germplasm is one of the important activities of genetic resources centers.

1. Distribution of germplasm is the responsibility of the gene bank centres where the germplasm is maintained and conserved.

2. The germplasm is usually supplied to the researchers who are engaged in the research work of a particular crop species.

Utilization

- Utilization refers to use of germplasm in crop improvement programmes. The germplasm can be utilized in various ways.

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Organizations Associated With Germplasm

- There are two types of organizations, viz. International and National which are associated with germplasm.
- International Plant Genetic Resources Institute (IPGRI) Rome, Italy coordinates at global level.
- In India, National Bureau of Plant Genetic Resources (NBPGR). New Delhi deals with various aspects of germplasm of agricultural and horticultural crops.
- Forest Research Institute, Dehradun deals with germplasm of forest species and Botanical Survey of India, Kolkata deals with germplasm of remaining plant species.

Quarantine

- Quarantine refers to a prophylactic means to prevent the entry of new diseases, insects and weeds from other countries. The quarantine offices are located at all seaports and international airports. Once a plant material enters to a country it will be grown in isolation to check the entry of new diseases, pest or a weed and then issue phyto sanitary certificate.

Mode of reproduction in plants

- Mode of Reproduction in Crop Plants Knowledge of processes is the first step in commencing the crop improvement work. The breeding procedures that are to be used in successful improvement of a crop are

determined by the mode of reproduction. The mode of reproduction is classified into main types Asexual and Sexual.

I. Asexual Reproduction:

- In this category plants reproduce without involvement of male and female gametes. There are three ways in which they propagate.
- **Vegetative formations:** Here specialized cells or asexual reproductive units called spores are produced by the parent body which are grown as new individuals as lower plants, e.g., Mosses.
- **Vegetative propagation:** Here various plant parts have an ability to grow as new plants. They are of two types (i) Natural vegetative propagation method and (ii) artificial vegetative propagation method.

Natural vegetative propagation methods

- Here a portion of the plant gets detached from the body of mother plant and this detached portion grow up into a new independent plant under suitable conditions. These natural methods are:

Propagation through underground modification of stem:

Plants are propagated through stems are of different type

a. Rhizome. E.g.

1. Ginger (Zingiberofficinalis)

2. Turmeric (Curcuma domestica)

3. Lotus (Nelambonucifera)

b. Tuber.E.g., Potato (SolanumtuberosumL.)

c. Bulb. E.g., Onion (Allium cepa)

d. Corm.E.g.,

1.Arwi (Colocasia esculenta)

2. Gladiolus(Gladiolus communis), Saffron (Crocus sativus)

Sub-aerial modification of stem

These modified stems gives rise to new plants -3 types

a. Runners.E.g., Wood Sorrel (Oxalisacetsella),Strawbery(Frageria Sp.)Water lettuce etc.

b. Stolans.E.g., Jasmine(JasminumSp), Tecorna(Tecornagrandifalia)

c. Suckers: Like the stolans lateral branch developing from underground part of the stem.E.g., Chrysanthemum(Chrysanthemum indicum), Bamboo(Dendrocalamussp), banana (Musa Sp.)

d. Modified Roots Normal roots of wood apple are used for propagation. Some modified roots such as tuberoses, roots of sweet potato and Asparagus give rise to new individuals.

Other plants

- Many other parts like (I) Bulbils.E.g., Garlic (Allium sativum L.) and Leaf.E.g., Agave. ii. Artificial vegetative propagation methods: Here plants are propagated artificially through special methods like cuttings (E.g., Sugarcane), air layering.E.g., lemons grape wines and Jasmines, Gottee layering (E.g., Lemon and Orange), grafting (Sapota, Mango, etc.) budding (e.g., Rose) etc.

Apomixis

- Here, sexual organs or related structure form seeds, these are four important forms:

a. Apogamy: Here embryo develops from cells other than egg cells i.e., Synergids or Antipodal cells of embryo sac. E.g. Onion, Lilly. The resultant plants may be diploids or haploids.

b. Apospory: Embryo develops from cells other than embryo sac cells i.e., cells of integuments or nucleus.E.g.Citrus and Mango.

c. Diplospory: Embryo develops directly from megaspore mother cell, which may be diploid or haploid.

d. Parthenogenesis: Seedless fruits are formed from ovary without fertilization Embryo develops from either male or female gamete without fertilization. They are haploids and may be diploids depending on the ploidy level of egg cell.

Sexual reproduction

- Here specialized reproductive cells are called gametes are formed. Fusion of male and female gamete leads to the development of embryo and eventually seed which is utilized for propagation. These are two types viz., Isogamy and Heterogamy.

Isogamy: The fusion of gametes which are morphologically similar and hence termed as Isogamy.E.g., Mucor.

Heterogamy: The fusion of gametes which are morphologically dissimilar termed as heterogamy.E.g., All higher plant (Flowering plants).

Typical flower: The reproductive structure in flowers. The flowers commonly consists of four organs namely sepsis, petals, stamens (male organs) and pistil (female organ). Both stamens and pistil are known as reproductive parts.

Kinds of flower: The flower contains all four floral organs are called complete flower.E.g., Hibiscus, Soybean, etc.

Incomplete flowers: The flower lacks one or two of these floral organs. Crops belonging to grass family including corn, sorghum, and paddy lack petals and sepals. The flowers of buckwheat and sugar beet lack petals.

Classification of incomplete flowers

1. Perfect flowers:

They bear only the stamen and pistil.E.g. Cotton, Wheat, etc.

2. Imperfect flowers:

These contain either stamens or pistil but not both in the same flower. The plants which bear male and female flowers on different plants are called dioecious.E.g., Papaya .The plants which bear male and female flowers on same plant are called as monoecious.E.g., Maize, Castor, etc.

Mode of Pollination

- Pollination is transfer of pollen grains from anthers to stigma. Pollination is brought about by various agencies like air (anemophily), water (hydrophily), insects (entomophily) and animals (zoophily). The pollen grains germinate on the stigma and slender pollen tube grows through the style and enters embryo sac, where fusion of male and female gametes occurs which is called fertilization. Transfer of pollen from an anther to stigma of same flower is called “self pollination”.
- Geitenogamyis another form of self fertilization resulting from pollination between two flowers of the same plants which has same genetic consequence like autogamy.
- Transfer of pollen grains of one plant to the flower of another plant is called cross pollination. The resultants fertilization is known as cross fertilization or allogamy.E.g., Castor Niger, etc.
- The crop plant has developed various mechanisms in course of their evolution to ensure either self or cross pollination.

Mechanisms favoring self pollination:

Bisexuality:

- The male and female reproductive units are present in the same flower.E.g., Hibiscus, Wheat, Paddy, etc.

Homogamy:

- Here anther and stigma of bisexual flower mature at the same time.E.g. Green peas, etc.

Cleistogamy:

- Flowers do not open at all in case of bisexual flowers ensures self pollination.E.g., Lettuce, Legumes, etc.

Flower structure:

- In cotton the young pistil must grow through a staminal sheath, the pollen is shed on the respective stigma as it is pushed through the staminal column there by self pollination is favored.

Mechanisms favouring cross pollination

- Imperfect flowers (dicliny or unsexuality) Flowers are unisexual i.e., stamens and pistils lie on separate flowers.

a. Monoecious plants: E.g., Maize, Castor, Jack, Coconut.

b.Dioecious plants: E.g., Papaya, Hemp, Asparagus, Datepalm, etc.

1. Dichogamy:

- Unequal maturation of stamens and pistils in bisexual flowers (Anther and stigma of bisexual flowers mature at different times). They are of two types: a. Protogyny: Stigma becomes receptive earlier to stamens. E.g. Bajra. b.

Protandry: Antherdehisce and release the pollen grains before stigma of the same flower attains receptivity. E.g., Carrot, Marigold.

2. Herkogamy:

- Presence of mechanical barrier between male and female organs of a flower.E.g., Crepes.

3. Male Sterility :

- Seeds are not set due toabsence of functional pollen grains.Male sterility determined by (i) Genetic factors (E.g.Tomato, barley, Brinjal and Rice). (ii) Cytoplasmic factors (e.g., Onion) and (iii) Interaction (E.g.Chilli, etc.)

4. Self incompatibility:

- Male and female gametes are fertile but seeds are not produced when selfed because of some oppositional factors but can set seeds when there is a cross pollination.E.g., Tobacco, Mango, Tomato, Mustard, Radish, Cabbage, etc.

Classification of crops as per the pollination

I. Natural or Normally self pollinated crops

- These crops show high degree of self pollination(>95%) and cross pollination is less than five per cent.E.g., Barley, Ragi, Wheat, Beans, Grams, Groundnut, Sesamum and Tobacco.

II. Naturally or normally cross pollinated crops

- a. In these crops cross pollination occurs predominantly(>95%) with a very little (i.e., 5 %)self pollination.E.g.,a. Field Crops i.e., Alfalfa, Castor, Beans, hemp, Maize, Rye, Castor, Mustard, Sugarcane and Sunflower.
- b.Horticulture Crops.E.g., Almond, Apples, Banana, Cherries, Chestnut, Citrus, Datepalm, Grapes, Fig, Papaya, Mango, etc.)
- c. Vegetables.E.g., All Cruciferous plants.Often cross-pollinated crops.

III. Often cross- pollinated crop:

- These crops are normally self-pollinated. However, cross pollination in these crops usually exceeds five per cent due to various agencies.E.g., Sorghum, Cotton, Safflower, etc.

Determination of pollination in plants

Mode of pollination can be determined by the following methods:

a. Detailed observation of floral structure

b. Bagging:

- If the plant set seeds when the flowers or inflorescence are covered, then the mode of reproduction is self pollination. If it does not set seeds under bagged condition then it is highly cross pollinated.

c. Isolation:

- Growing single plants in isolation and observing them for seed setting is another method. If the plants set seeds in isolation then plant is said to be self pollinated, otherwise, it is cross pollinated.

Estimation cross pollination in plants

- Estimation cross pollination in plants After knowing the mode of pollination, the percentage of natural cross pollination can be determined by using certain simple inherited genetic characters determined by dominant alleles. Two varieties of the same crop having contrasting characters are to be selected. One variety may possess red flowers (dominant and another variety may possess white flowers (recessive). Theseplants with recessive trait are planted in a plot which is again surrounded by plants having dominant characters. The plants are allowed for open

pollination. Then seeds are harvested only from plants carrying recessive character and sown next year. The number of plants is counted and the percentage of natural crossing is worked out with the following formula.

$$\% \text{ of natural crossing} = \frac{\text{Number of plants showing the dominant character}}{\text{Total number of plants}}$$

Self incompatibility

- Self incompatibility refers to the inability of fertile pollen to fertilize its own egg cell resulting in failure of seed set. It differs from male sterility in that self-incompatible pollen is fertile while it is sterile in male sterile pollen.Self incompatibility has been reported in more than 3000 species of plants are covering important species such as Leguminosae, Rosacae, Solanaceae ,Compositae, Cruciferacae, Graminacae. Self incompatibility can occur at any stage between pollination and fertilization.
- Self incompatibility is of two types
    1) Homomorphic 2) Heteromorphic

Homomorphic system

- In these, there is only one type of flower. The compatible and incompatible plants cannot be differentiated on the basis of flower morphology. There are two types of incompatibility systems in these species.

- a. Gametophytic incompatibility first reported by East &Mangelsdorf (1925) in Nicotiana sanderae. Gametophyte incompatibility is governedby multiple alleles of S gene pollen. As many as 21 multiple alleles have been found to control incompatibility. These are independent in their action and do not show any dominance relationship.
- Pollen and ovule carrying dissimilar S alleles can set seed if the style does not inhibit the tube growth. The tissue is diploid and if there is any S alleles are common in pollen, it arrests the tube growth. For example, styles of S1 genotype would not allow any pollen tube either S1 or alleles to penetrate whereas it will not inhibit S3 and S3 pollen tubes. Thus, there are three situations, fully incompatibility.E.g., S1S2 x S1S3 partially incompatible.E.g., S1S2 x S2S3 and fully compatable.E.g., S1S2 x S3S4.Gametophytic incompatability has been reported in Tomato, Pear, Peach, Tobacco, Lucerne, etc.

Sporophytic incompatibility

- This was first described by Hughes and Babcock (1950) in Crepisand by Gerstel (1950) in Parthenium. This system is also governed by multiple alleles S gene. In the gametophytic system genes are not independent and show dominance relationship. The tube reaction is governed by genotype where they are produced, for example, both type of pollen produced by S1S2 plant would behave similar.
- Inhibition occurs at the stigmatic surface and the pollen does not germinate on stigma. At the incompatibility locus, there are two closely linked with genes affecting style length.

- a) Distily In primula there are two types of flower, pin type with style Short filament, large stigmatic cells and small pollen and “Thurm type” with short style, long filament small stigmatic cells and large pollen. Both types of flowers are seen incompatible but cross compatible. The thrum is governed by genes ss and pin by SS. The pollen of thrum plant, in spite their segregation into S and s types behave same.
- b) Tristyly in Lythrums there are three types of flowers; genetic constitution of the trace forms is as follows.

  Long style : mm ss

  Mid style : MM ss or MMss

  Short style : MMss, mmss, mmSS, MMSS, MMSs, MsSs
- Two independent loci S and M govern the style length. Plants with S have short style irrespective of constitutions. Plants with have medium styles if they have long styles if they have M. All the three stylar types are incompatible but cross compatible.
-

- **Uses of incompatibility**

1. Incompatibility may be used in hybrid seed production withoutEmasculation as self fertilization will not take place.E.g.,

(a) Leffel (1963) suggested production of double cross hybrid (S1S2 x S1S2) x (S3S3 x S4S4) in red clover utilizing for incompatibility alleles.

(b) Thompson (1964) Suggested the use of S gene for seed production of double cross hybrid Kale, individual lines may bemaintained by selfing either in bud or late stage.

2. Even this system is utilized by fruit breeders where so incompatible varieties are more successful in cultivation.E.g., In cherry

Pseudo-compatibility

- Incompatibility plants show some degree of self fertilization. This is known as pseudo compatibility. The avenues are used for creating homozygous S lines and maintain them by selfing. The following ways are used to maintain self incompatible species and strains by self-pollination.

a. Bud pollination:

- It is supposed that some compound which inhibits the pollen germination and tube growth is produced by stigma and style at the time of flowering. If pollination is done 24 hours before the natural flower opening, there is

compatibility. Probably the inhibitor compound is not produced by that time. This is known as bud-pollination and is widely used in maintaining lines in grapes, cabbage, cauliflower, etc.

b. End of season pollinations:

- Usually when the crop season is about to end, incompatibility mechanisms weaken, This probably due to less metabolic activity of the plant and failure of the plant to produce enough inhibitory compounds.

c. Stigma removal:

- Failure of pollen to germinate of tube growth occurs in cauliflower, cabbage and radish. In these the removal of stigma and then pollinations results in perfect seed set.

d. Grafting:

- In Trefoilparense, grafting of vegetative parts, either within a single plant (homograft) or between different plants (heterografts), have also improved the level of compatibility as reported by Evans (1969). The reason is not known. Pollination can be done after grafting.

- Making polyploidy of incompatible strains they may become compatible because the altered genic balance at the tetraploid, level. The method has been utilized in peach and potato.

Mechanism of self incompatibility

- The various phenomena observed in Self Incompatibility is grouped into 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 exinceexcudate which is either protein or glycero protein. This reacts with pellicel and induces callose formation which further prevents the growth of pollen tube.

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.

Introduction

- Apomixis, is a type of reproduction in which sexual organs or related structures take part but seeds are formed without union of gametes. It refers to the occurrence of an sexual reproductive process in the place of normal sexual processes involving reduction division and fertilization.
- 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)”.It was first reported byLeuwenhock 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.

Types of apomixis

1. Recurrent Apomixis:

- An embryo sac develops from the megaspore mother cell where meiosis is disturbed or from some adjoining cell. Consequently, the egg-cell is diploid. The embryo subsequently develops directly from the diploid egg-cell without fertilization. E.g:Crepis,Taraxacum, Poa(blue grass), and Allium (onion) without the stimulus of pollination.

Malus(apple), and Rudbeckiawhere 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.

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. Embryo within the embryo sac may also develop simultaneously, thus giving rise to poly-embryony condition, as in Citrus, Opuntia.

4. Vegetative apomixis:

- In Poabulbosaand Allium, Agave and grass species,vegetative buds or bulbils, instead of flowers are produced in the inflorescence. They can be reproduced without difficulty.

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 apospory. Since it regenerates without fertilization, it is recurrent.

Development of apomictic embryo

1. Parthenogenesis :

- 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 ina haploid embryo-sac produced by generativeapospory. It is non-recurrent in nature.

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

2. Apogamy :

- 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 thanthe 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 :

- The development of embryo neither from egg cell nor from synergidsorantipodals, 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 fromovary 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 apomixes

- 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.

Advantages of apomixis

- The two sexual processes, 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 tobreak

heterozygote superiority in such plants. On the contrary, apomicts tend to conservethe 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.

- **Uses of apomixis in plant breeding**

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 problemfor recurring seed production of F1 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 apomixes

- 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 diploidforms 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.Nonrecurrentapomixis are of academic use only.

Maintenance and transfer of apomixes

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

Self-pollination **Introduction**

- Self-pollination is considered the highest degree of inbreeding a plant can achieve. It promotes homozygosityof all gene loci and traits of the sporophyte. To be classified as self-pollinated, cross-pollination should notexceed 5%. Due to self pollination further, the progeny of a single plant ishomogeneous due to self pollination. A population of self-pollinated species, comprises a mixture of homozygous lines.
- Self-pollination restricts the creation of new gene combinations (no introgression of new genes throughhybridization). New genes may arise through mutation,but such a change is restricted to individual lines orthe progenies of the mutant plant. The proportions of different genotypes, not the presence of newly introducedtypes, define the variability in a self-pollinated species.
- Because a self-pollinated cultivar is generally one single genotype reproducing itself, breeding self-pollinated species usually entails identifying one superior genotype (or a few) and its multiplication it. Specificbreeding methods commonly used for self-pollinated species are pure-line selection, pedigree breeding, bulkpopulations, and backcross breeding Table: Examples of predominately self pollinated crops

• Genetic implications of cross-pollination

Common name	Scientific name
Alfalfa	*Medicago sativa*

Maize	*Zea mays*
Muskmelon	*Cucumismelo*
Onion	*Allium* spp.
Pepper	*Capsicum* spp.
Potato	*Solanumtuberosum*
Radish	*Raphanussativus*
Rye *Secalecereale*	
Sugar beet	*Beta vulgaris*
Sunflower *Helianthus annuus*	
Sweet potato	*Impomeabatatas*
Watermelon	*Citrulluslanatas*
Birdsfoot trefoil	*Lotus corniculatus*

Cabbage	*Brassica oleracea*
Carrot	*Dacuscarota*
Cassava	*Manihotesculentum*
Annual ryegrass	*Loliummultiflorum*
Banana	*Musa* spp.
Cucumber	*Cucumissativus*
Fescue	*Festuca*spp.
Kentucky bluegrass	*Poapratensis*

Examples of predominantly cross-pollinated species

Paraghaph 1:Soil fertility refers to the capacity of soil to supply plant nutrients. Soil fertility and fertilizers are very much closely related terms; one cannot neglect one from the other in their function. Soil fertility acts as a 'SINK' where in plants can draw nutrients for maximum yield, where as fertilizer, acts as a 'SOURCE' wherein we can draw continuously different nutrients. The importance of soil fertility and fertilizer management is being increasingly recognized in all countries recently to meet the demand for food and other agricultural raw materials.

Introduction

- *Qualitative characters:* Character under simple genetic control. i. e governed by one or few genes (Oligogenes)are known as qualitative characters.E.g. Flower colour, Fruitshape
- *Quantitative characters:* Character under complex genetic control. i. e governed by many genes (polygenes)are known as quantitative characters .E.g. Grain yield, drought tolerance, etc.

- **Economically important characters**

	Qualitative	quantitative
Nature of traits	Traits that have Mendelian inheritance and can be described according to kind.	Degree of expression of the trait.
Scale of variability	discrete or discontinuous phenotypic variation.	continuous variation.
Number of genes	single genes are readily detectable	traits are under polygenic control
Mating pattern	individual matings and their progenies.	Population of individuals that may comprise a diversity of mating kinds.
Statistical analysis	Based on counts and ratios.	Estimates of population parameters namely mean, varience, etc.
Effect of environment	Less	More

-

Environment and quantitative variation

All genes are expressed in an environment (Phenotype =Genotype X Environmental effect). However, quantitativetraits tend to be influenced to a greater degree than qualitative traits.

- In polygenic inheritance, segregation occurs at a large number of loci affecting a trait. ThePhenotypic expression of polygenic traits is dependingon variation in environmental factors to which plants in the population are subjected. Polygenic variation cannot be classified into discrete groups (i.e., variation is continuous). This is because of the large number of segregating loci, each with effects so small that it is not possible to identify of individual gene effects in the segregating population. Instead, biometrics is used to describe the population in termsof means and variances. Continuous variation is caused by environmental variation and genetic variation due tothe simultaneous segregation of many genes affecting the trait.
- In 1910, a Swedish geneticist, Nilsson-Ehle provided a classic demonstration of polygenic inheritance

Polygenic inheritance

- It may be explained by making three basic assumptions:

1.Many genes determine the quantitative trait.

2.These genes lack dominance.

3.The action of the genes are additive.

Gene action

- There are four types of gene action: additive, dominance, epistatic, and overdominance.

1. Additive gene action

- The effect of a gene is said to be additive when each additional gene enhances the expression of the trait byEqual increments. Consequently, if one gene adds one unit to a trait, the effect of aabb = 0, Aabb = 1, AABb = 3,and AABB = 4. For a single locus (A, a) the heterozygote would be exactly intermediate between the parents(i.e., AA = 2, Aa =1, aa = 0). That is, the performance of an allele is the same irrespective of other alleles atthe same locus. This means that the phenotype reflects the genotype in additive action, assuming the absenceof environmental effect. Additive effects apply to the allelic relationship at the same locus. Furthermore, asuperior phenotype will breed true in the next generation, making selection for the trait more effective toconduct. Selection is most effective for additive variance;it can be fixed in plant breeding (i.e., develop acultivar that is homozygous).

Components of Genetic Variance

- Breeder is interested in partitioning variance into its components that are attributed to different causes or sources. The genetic properties of a population are determined by the relative magnitudes of the components of variance. By knowing the components of variance, one may estimate the relative importance of the various determinants of phenotype. phenotypic value of quantitative traits:

  P (phenotype) = G (genotype) = E (environment)
- The phenotypic value is variable because it depends on genetic differences among individuals, as well as environmental factors and the interaction between genotypes and the environment (called G = E interaction).
- Total variance of a quantitative trait may be mathematically expressed as follows:

  VP = VG +VE +VGE
- where VP = total phenotypic variance of the segregating population, VG = genetic variance, VE = environmentalvariance, and VGE = variance associated with the genetic and environmental interaction. The genetic component of variance may be further partitioned into three components as follows:

  VG = VA + VD + VI
- where VA = additive variance (variance from additive gene effects), VD = dominance variance (variance fromdominance gene action), and VI = interaction (variance from interaction between genes). Additive genetic

variance(or simply additive variance) is the variance of breeding values and is the primary cause of resemblancebetween relatives. Hence VA is the primary determinant of the observable genetic properties of the population,and of the response of the population to selection. Further, VA is the only component that the researchercan most readily estimate from observations made on the population. Consequently, it is common to partitiongenetic variance into two – additive versus all other kinds of variance. This ratio, VA/VP, gives what is calledthe heritability of a trait, an estimate that is of practicalimportance in plant breeding. The total phenotypic variance may then be rewritten as:

VP = VA + VD + VI + VE + VGE

- To obtain environmental variance, individuals from the same genotype are used. An inbred line (essentially homozygous) consists ofindividuals with the same genotype. An F1 generationfrom a cross of two inbred lines will be heterozygous butgenetically uniform. The variance from the parents andthe F1 may be used as a measure of environmental variance(VE). The procedure for obtaininggenotypic variance from F2 and backcross data. In sum,variances from additive, dominant, and environmentaleffects may be obtained as follows:

  VP1 = E; VP2 = E; VF1 = E

  VF2 = 1/2A + 1/4D + E

VB1 = 1/4A + 1/4D + E

VB2 = 1/4A + 1/4D + E

VB1 + VB2 = 1/2A + 1/2D +2E

Concept of heritability

- The concept of the reliability of the phenotypic value of a plant as a guide to the breeding value (additivegenotype variance) is called the heritability of the metric trait. As previously indicated, plant breeders are able to measurephenotypic values directly, but it is the breeding value of individuals that determines their influence on the progeny. Heritability is the proportion of the observed variation in a progeny that is inherited.
- Heritability measures this degree of correspondence. It does not measure genetic control, or trait. Heritability is, therefore, defined as a fraction: it is the ratio of genetically caused variationto total variation (including both environmental and genetic variation).

Types of heritability

- There aretwo estimates of heritability.

1 Broad sense heritability.

- Heritability estimated usingthe total genetic variance (VG) is called broad senseheritability. It is expressed mathematically as: H = VG/VP

2 Narrow sense heritability.

- Because the additivecomponent of genetic variance determines theresponse to selection, the narrow sense heritabilityestimate is more useful to plant breeders than thebroad sense estimate. It is estimated as: H2 = VA/VP
- The magnitude of narrow sense heritabilitycannot exceed, and is usually less than, the correspondingbroad sense heritability estimate

Factors affecting heritability estimates

Factors affecting heritability estimates

- The magnitude of heritability estimates depends on the genetic population used, the sample size, and the methodof estimation.

Method of computation

- Heritabilities are estimated by several methods that use different genetic populations and produce estimatesthat may vary. Common methods include the variance component method and parent–offspring regression.

Introduction

- Male sterility is a condition in which pollen is absent and non- functional. There are three types of male sterility.
- **Genetic Male sterility:** Here nuclear genes condition male sterility in recessive condition.
- **Cytoplasmic male sterility:** Male sterility is controlled by the actionof Cytoplasm. Since the cytoplasm is transmitted through the female gamete only, cytoplasmically inherited male sterility will be transferred through the female parent. This type of sterility is made use in crop plants where vegetative parts are economically important. E.g. Onion.
- **Cytoplasmic Genetic male sterility:** It is due to interaction of cytoplasimic and genetic factors. It differs from cytoplasmic male sterility in a way that, a single dominant gene coming from nucleus restores the fertility and can overcome the effect of cytoplasm responsible for sterility.
- Presence of male sterility eliminates the emasculating processes. This has been made, use of in the seed production of hybrid sorghum, bajra, sunflower and onion. The female part is either hand pollinated or allowed for free pollination.
- If the seeds do not develop after crossing the breeder will be in great loss. The failure in seed set is mainly due to the lack of fertility restoration. The term sterility in plant breeding confines only to sterility of the gametophytes, i.e., it is unable to produce viable gametes.
- Even though, the sterility is disadvantageous it could be used advantageously by inducing male sterility, which facilitates easy crossing. Hand emasculation in hermophrodite flowers which are very minute size is a very tedious job and also consumes more time. If male sterility is induced in flowers, it saves time and labour which otherwise would have been used for emasculation and reduces the cost of production of hybrid seeds.

a. There are mainly three types of male sterility

i. Genetic male sterility

ii. Cytoplasmic male sterility

iii. Cytoptasmic genetic male sterility

Genetic male sterility

- Male sterility in this case depends upon a single nuclear gene. The male sterile condition is ordinarily recessive and the sterile stock is maintained by crossing male sterile plants heterozygous plants, in which case half of the population is sterile and half are fertile plants. Fertile plants are rogued as soon they identified early by a closely linked gene as in lima beans the task producing hybrid seed is comparatively simple. The flower must be examined carefully to identify the male sterile plants.

Cytoplasmic male sterility

- This type of male sterility depends on cytoplamic genes but will produce seed if pollinators are present. The F1 seeds produce only male sterile plant, since their cytoplasm is derived entirely from the female gamete. The cytoplasmic sterility is advantageous in certain ornamental plants. It also useful in producing single or double cross

hybrids in species where vegetative part of the plant is commercial product. It is unsuitable forproduction of hybrid seed in species where in seed is the commercial product.

Cytoplasmic Genetic Male Sterility

- This type of sterility differs from cytoplasmic sterility only in that the off spring of male sterile plants can be male fertile when certain stocks are used as pollinators. Thus, cytoplasmic sterile plants are converted to fertile plants by crossing with the plants with restorer genes.
- Production of sorghum hybrids is possible through utilization of cytoplasmic genetic male sterility.
- MSJCK 60 A (Male sterile combining kafir 60)is a male sterile line of sorghum, which contains the kafir chromosomes.
- Inbred lines are converted to male sterile lines by a number of back crosses using MSJCK 60 or any male sterile as female parent and inbred line to be converted as recurrent pollen parent. The newly synthesized male sterile inbred line is designated as line A. The original male fertile line is designated as line B. There is no other difference between A and B lines except that A is male sterile and B male is fertile, which should be a pollen producing line.
- Line A is grown in an isolated field and it is wind pollinated by B. Thus male sterile line A will be maintained generation to generation. Line B is maintained by self pollination Male sterile inbred line A is grown in an isolated field and wind pollinated by unrelated inbred R which is male fertile line which possess pollen restoring genes.
- The seeds of the above single cross may be sold to farmer with the advice not to take advance generation from the year grown plants.

Production of Hybrid seed

1. Cytoplasm- genetic male sterility:

1. Single cross Hybrid:

- A cms line is used a female parent,while the male parent is a restorer. The seed set on the female parent is hybrid seed. The resulting hybrid is male fertile since it has received the restorer gene from male parent.2:2, 2:4 proportions male to female ratiocan be used

Double crosses Hybrids:

- Double cross hybrid varieties are produced by crossing two single crosses. First single cross is produced by crossing cmsline withnon restorer male fertile line. In second single cms line is crossed with a restorer line, (1:1), or two restorer line are crossed together here female line anther have been removed.

Genetic male sterility:

- The male sterile msmscms is allowed to be cross pollinated with a male fertile line. (MS MS) to yield a male fertile hybrid Msms. One of the problem, the female parent 50% of plants are male fertile (Msms), the plants Must be identified and eliminated before they shed pollen.

Chemically induced male sterility:

- Several chemicals induce male sterility when applied during specific developmental stages of plants – chemicals induce male sterility these chemicals are called chemical hybridizing agents which makes the pollen grain non functional it is also called chemical emasculation. Eg: Rice, Wheat. Commonly used chemical is rice in china are MG1= Zinc methyl arsenate MG2= Sodium methyl arsenate.

Introduction

- The Genetic constitution of plants is determined by mode of pollination.
- Self pollination leads to homozygosity and cross pollination leads to heterozygosity. So we have to exploit homozygisity in self pollinated crops and heterozygosity in cross pollinated crops. Based on genetic constitution plant breading populations are of four types.

Homogenous population:

- Genetically similar plant constitutes homogenous populations. Eg: Pure lines, inbred lines, F1between pure lines, progeny of a clone. Pure lines and inbredlines have narrow adaptation.

Heterogeneous population:

- Genetically dissimilar plants constitute heterogeneous population E.g: Land races, mass selectedpopulation,synthetics,composites, multiline. Synthetics, Composites, multiline these have wide adaptability stableperformance under different environments.

Homozygous populations:

- Individuals with like alleles at the corresponding loci are known as homozygous. Such individuals do not segregate on selfing. E.g.: Pure lines, inbred lines and mass selected population in self pollinated plants. Multiline are homozygous but heterogeneous

Heterozygous population:

- Individuals with unlike alleles the corresponding loci are heterozygous. Such individuals segregate into various types on selfing. Eg.F1, composites, Synthetics. Such populations have greater buffering capacity to environmental fluctuation

	Homozygous	**Heterozygous**
Homogenous	Genetically similar and non segregating population	Genetically similar but segregating on selfing F1, hybrids between inbred lines and progeny of a clone
Heterogenous	Genetically dissimilar but non segregating population Multiline and mass selected varieties of self pollinated crops	Genetically dissimilar but segregating population Eg. composites and synthetics

Plant breeding methods

Various approaches are used for genetic improvement of crop plants are referred to as plant breeding methods.

Choice of breeding methods mainly depends on

- Mode of pollination
- Mode of reproduction
- Gene action
- Breeding objectives of crop

- Plant breeding methods are classified based on the application in crop improvement
  - General methods
  - Special methods
  - Population improvement
  - Hybridization

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

Introduction

- 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.
- Vilmorinproposed individual plant selection based on progeny testing. This method successfully improved the sugar content in sugar beet. His method was called as “VilmorinIsolation Principle”. He emphasized that the real value of a plant can be known only by studying the progeny produced by it. This method was successful in sugar beet but not in other cross pollinated crops. 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 altogether. 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. did not change further. Thus it was proved that selection within a pure line is ineffective. In third evidence when

parent - offspring regression was worked in line thirteen found be to zero indicating that variation observed is non heritable and it is due to environment only.

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Origin of variation in purelines

1. Mechanical mixtures.

2. Natural hybridization.

3. Chromosomal aberrations.

4. Natural mutation.

5. Environmental factors.

- A Pure line is the progeny of a single, self pollinated, homozygous plant. Development of new variety through identification and isolation of single best plant progeny is known as pure line selection.
- All the individuals within a pure line have identical genotype any variation present within a pure line is solely due to environment. So Pure line is a large number of plants are selected from self pollinated crops and are harvested

individually. Individual plant progenies from them are evaluated and the best progeny is released as a pure line variety.

Characters

- All the plants within a pure line have the same genotype. Because the parent plant was homozygous and self-fertilized.
- The variation present within a pure line is environmental andnon heritable. So selection within pure line is not effective.
- Pure lines become genetically variable with time. The genetic variation is produced by mechanical mixture, Natural hybridization or mutation.
- Narrow adaptation, due to narrow genetic base.

Uses of Pure line

- A variety: A superior pure line may be used as a commercial variety. Almost all the varieties of self pollinated crops are pure line
- As a parent in a hybridization programme hybridization invariably based on pure line

- Mutation studies: Used forstudying spontaneous and induced mutation. Because genetic variation arised due to mutation.
- The concept of pure line selection was developed in the middle of the 19thcentury in Sweden Lecouteur, Sheriff, Halletand Vilmorin. The Genetic basis of pure line was explained by Johanssen in 1903.
- Pure line selection is practiced in heterogeneous population such as introduced material, land races and mass selected varieties of self pollinated species to isolate superior genotypes.
- Selection in the segregating generation from cross.

Procedure for Pure line selection

First year:

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

Second year:

- The individual progenies are grown separately with proper spacing the top 15-20 progenies are selected and they are bulked. Poor, defective, weak and segregating progenies are discarded. Selection should be based on simply

inherited character like plant type, Plant height, grain type , flowering and maturity duration disease resistance this process may be repeated

Third year:

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

Fourth year:

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

Fifth to Eighth year:

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

Advantages:

- Maximum possible improvement over original variety.Uniform and more attractive, easily identifiable.

Disadvantages:

- This method can isolate only superior genotypes, it cannotcreate new genotypes. And not applicable in cross pollinated crop. Poor adaptability due to narrow genetic base vulnarble for new diseases and pests.

Mass Selection

Mass Selection

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

Steps:

First 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. High 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 and demerits of Mass Selection

Merits

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 of pure line and mass selections

	Pure line selection	Mass selection
1.	The new variety is a pureline	The new variety is a mixture of purelines.
2.	The new variety is highly uniform. In fact, the variation within a pureline variety is purely environmental.	The variety has genetic variation of quantitative characters, although it is relatively uniform in general appearance.
3.	The selected plants are subjected to progeny test.	Progeny test is generally not carried out.
4.	The variety is generally the best pureline present in the original population. The pureline selectionbrings about the greatest improvement over the original variety.	The variety is inferior to the best pureline because most of the purelines included in it will be inferior to the best pureline.
5.	Generally, a pureline variety is expected to have narrower adaptation and lower stability in performance than a mixture of purelines.	Usually the variety has a wider adaptation and greater stability than a pureline

		variety.
6.	The plants are selected for the desirability. It is not necessary they should have a similar phenotype.	The selected plants have to be similar in phenotype since their seeds are mixed to make up the new variety.
7.	It is more demanding because careful progeny tests and yield trials have to be conducted.	If a large number of plants are selected, expensive yield trials are not necessary. Thus it is less demanding on the breeder.

Hybridization and Pedigree Method

Hybridization and Pedigree Method

Introduction

- Natural variability in self pollinatedpopulation is exhausted during selection, for further improvements new genetic variability has to be created by crossing two different pure lines. Hybridization means the mating or crossing of two plants or lines of dissimilar genotypes.
- The seeds as well as the progeny resulting from the hybridization are known as hybrid or F1. The progeny of F1 obtained by self or inter mating of F1 plants and the subsequent generations are called segregatinggenerations. Today hybridization is the most common method of crop improvement and the majority of the crops varities have originates from hybridization.
- One of the objectives of hybridization is to create genetic variation. Two genotypicallydifferent plants are crossed together to obtain F1 generation. F1 is advanced to generate F2generation. The degree of genetic variation in F2 and subsequent generation depend on number of heterozygous genes in F1.

- **Aims of Hybridization**

1. To transfer of one or few qualitative character.

2. Improvement in one or more quantitative character.

3. F1 Hybrid as variety.

I. Combination breeding:

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

II. Transgressivebreeding:

It aims at improving yield or its contributing character through transgresive segregation. It refers to theappearance of such plants in F2generation that are superior to both the parents for one or more character.It is due to accumulation of plus or favourable genes from the parents as a consequences of recombination. The parents used for crossing must combine very well and are genetically diverse. So pedigree breeding followed by population approach are designed for production of transegressivesegregants.

III. Hybrid varieties:

In self pollinated crops F1 is more vigourous and high yielding than the parents.Two parents should combine well to produce outstanding F1 hybrid.

Types of Hybridization

- Inter-varietal Hybridization / Intra specific: Parents involved in hybridization belongto the same species. They may be two strains, varietiesor races.
- Varietal crosses may be simple crosses or complex crosses

a.Simple crosses: Two parents are crossed to produce F1(A XB)

b. Complex crosses: More than two parents are crossed to produce the hybrid (AXB) X C X F1

Procedure of developing hybrid variety

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

1. Choice of parents: One of the parent involved in crosses should be a well adapted and proven variety in the area. The other variety should be having the character that are absent in this variety.Combining ability of the parents serves as useful guides in the selection of parents, which produce superior F1 and F2.

2. Evaluation of parents: Parents are evaluated for their combining ability.

3. Emasculation: The removal of stamens/anther without affecting the female reproductive organs, hand emasculation is mostly followed.

4. Bagging: Immediately after emasculation the flowers are enclosed in suitable bags to prevent cross pollination.

5. Tagging: The emasculated flowers are tiedwith a thread. The information on date of emasculation, date of pollination, name of female and male parents are recorded in the tag with pencil. The name of the female parent is written first then male parent.

6. Pollination: Mature fertile and viable pollen from the male parent should be placed on receptive stigma of emasculated flowers to bring about fertilization. Pollen grain is collected, allowed for dehiscence and pollination is carried out with camel hair brush.

7. Harvesting and storing of F1 seeds: The crossed heads/pods should be harvested and threshed. The seeds should be dried and properly stored to protect them from storage pests.

8. Rising the F1 generation: Identify the selfed seeds in the F1generation by using dominant marker gene. Larger F1 population is desirable, because both the genes are present in heterozygous condition.

9. Selfing: To avoid cross pollination andto ensureself pollination. In often cross pollinated crops they are bagged to prevent cross pollination.

Pedigree method

- In pedigree method individual plants are selected from F2 and their progenies are tested in subsequent generations. A record of the entire parent off spring relationship is maintained and known as pedigree record. The

pedigree may be defined as a description of the ancestor of an individual and it generally goes back to some distant ancestor. So each progeny inevery generation can be traced back to the F2 plant from which it isoriginated.

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

Procedure:

1. Hybridization: The selected parents are crossed to produce a simple / complex cross(F1 seed)

2. F1generation: F1 seeds are space planted to each produces maximum number of F2seed. 15-30 F1plants aresufficient to produce good F2 populations.

3. F2generation: 200-10000 plants are space planted and 100-500 plants are selected and their seeds are harvested separately. He should select as many as F2 plants as he can handle efficiently. The selection depends on skill of the breeder and his ability to judge to select F2 which produce good progeny.

4. F3 Generation: Individual plant progeny are space planted. Individual plant with desirable characters from superior progenies is selected.

5. F4 Generation: Individual plants progenies are space planted desirable pants are selected undesirable progenies are rejected. Progenies are compared visually and more plants are selected from superior progenies. Selection of desirable plants from superior progenies selection is practiced within / between family.

6. F5Generation: Many families have reached homozygous and may be harvested in bulk. The breeder has to assess the yielding potential of progenies, 25-100 progenies are advanced and tested inpreliminary yield trial.

7. F6 Generation: Multi row plots and evaluated visually progenies harvested bulk and they have become homozygous.

8. F7 Generation: Preliminary yield trail with replication to identify the superior progenies. Progenies are evaluated for other component character 2-5 outstanding lines superior to check are advanced to multi location testing.

9. F8 –F10 Generation: Replicated yield trial at several locations. They are tested for yield as well as for resistance.

10. F11: Seed multiplication and release.

Merits and Demerits of pedigree method

Merits:

1. Maximum opportunity for the breeder touse his skill and judgment for the selection of plants in segregating generation.

2. It provides information about the inheritance of qualitative character from the pedigree record .

3. Chances of recovering transgressivesegregants is more.

4. Weak and defect progenies are eliminated at an early stage.

Demerits:

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

2. Selection of progenies in every generation laborious, time consuming. Difficult to handle many crosses.

3. No opportunity for natural selection.

4. Possibility of losing the valuable genotype is early segregating generation.

Introduction: Bulk method

- Bulk method was first used by Nilsson Ehlein 1908. F2 and the subsequent generation are harvested as bulks to raise the next generation. At the end of bulking period individual plants are selected and evaluated in a similar manner as in the pedigree method.The duration of bulking may vary from 7-30 generation artificial selection may seldom be practiced
- Application: Cereals, small millets, grain legumes and oil seeds.

Application of bulk method

1. Isolation of homozygous lines

- It is used for the isolation of homozygous lines with a minimum of effort and expense. The population is carried to F6-F7 as Bulk, where it reaches homozygosity.Individual plants are selected and evaluated to derive pure line. So preliminary yield trails are conducted to derive homozygous lines.

2. Waiting for the opportunity for selection:

- Selection for resistance to disease, lodging and cold depends upon the presenceof suitable environmental conditions favoring epidemic.Waiting till such environment do occur so the segregating generations are carried as

bulk in such environment. Individual plants are selected and handled as inpedigree method.The duration of bulking depend upon the occurrence of the concerned environment. This is known as mass pedigree method of Harlan.

3. Opportunity for natural selection:

- Maintainance of bulk is inexpensive and without much efforts. Some bulk populations are carried up to F20 to F30 to provide an opportunity for natural selection to act on their composition. Up to F6 the population is heterozygous and after F7 natural selection to act on homozygous plants and would change the frequency of homozygous genotypes present in the population. It is assumed that natural selection would favour higher yielding genotypes and eliminate poorer genotypes.

- **Procedure for Bulk method**

1. Hybridization: Parents are selected and crossed

2. F1 generation : F1is space planted more than 200 F1 plants

3. F2-F6 Generation: Planted at commercial seed rate, spacing and harvested as bulk, during this period. Frequency of population changes due to out break of disease or pest.

4. Artificial selection is done, largepopulation is raised,30000-50000 plants in each generation.

5. F7 generation: 50000 plants are space planted about 1000-5000 plants with phenotype is selected and the seeds are harvested separately.

6. F8 generation: Individual plant progenies are single/multi row plants, since progenies are homogygous and harvested in bulk weak and inferior progenies are rejected and 100-300 individual plant progenies with desirable characters.

7. F9 Generation: Preliminary yield trial with standard check, yield and quality parameter is taken for selection.

8. F10---F12 generation: Replicated yield trails are conducted. Yield and its component characters are evaluated along with the check. Superior progenies are released as variety

9. F13 generation: Seed multiplication of the newly released variety and distribution to farmers.

Single seed descent method

- Single seed descent method This method is amodification of bulk method
- Single seed from each F2 plants is bulked to raise the F3 generation. Similarly F3, F4, F5 generation when the plants are homozygous plant progenies are advanced to next generation. Selection is done mainly among the progenies and number of progenies is sufficiently reduced to permit replicated trail. Individual plants may be selected from outstanding families showing segregation. So preliminary yield trial and quality tests beginin F7 to F8.

Objectives

1. Rapidly advance of generation of crosses.

2. F2 and subsequent generation are grown with a very high plant density.

3. F2 plant is represented equally in the end population.

4. Off season nursery/green house facilities are utilized.

5. Maximum possible speed.

6. Require very little space/effort/ labor.

7. Do not permit any form of selection during the segregating generation.

8. In each successive generation the population size become small due to poor generation and death of plants due to disease/pest.

Merits:

1. Simple, convenient and inexpensive.

2. Due to elimination of undesirable types, isolation of desirable types is easier.

3. Natural selection increase the frequency of superior types in the population.

4. No pedigree record is to be kept which save time and labor.

5. Isolation of transgressivesegregants is more.

Demerits:

1. Needs more time.

2. Little opportunity for a breeder to use selection.

3. Information on the inheritance of character cannot be obtained.

4. Naturalselection act against agronomicallydesirable types.

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Comparison of pedigree and Bulk method

Pedigree method	Bulk Method
Individuals plants are selected in F2 and Segregation generation and individual plant progenies are grown	F2 and the subsequent generation are maintained as bulk
Artificial selection, Artificial disease epidemics are an integral parts	Artificial selection, artificial disease epidemic are created to assist naturalselection.
No role of Natural section	Natural selection determines the composition of population.
Pedigree record have to be maintained which is	No pedigree records are maintained

laborious and time consuming	
It takes 12 years to develop new variety	More than 12 years bulk population > 10 years for effective natural selection
Widely used method	Limited use
Needs close attention from F2 on words	It is simple convenient.
Segregating generation are space planted to permit individual plant selection	Bulk population are planted at commercial planting rate
Population size is small	Larger population are grown and the natural selection expected to the increase the chances recovery of transgressive segregants

Backcross Breeding

Introduction

- A Crossing between a F1hybrid orits segregating generation with one of its parents is known as Back cross. The hybrid and its progenies in the subsequent generations are repeatedly back crossed to one of their parent. As a result the genotype of back cross progeny becomes increasingly similar to that parent to whom the back crosses are

made. At the end of 6-8 back crosses, the progeny would be almost identical with the parent involved in back crossing.

Objective:

1. To improve one or two specific defects of a high yielding variety and a well adapted variety with desirable character.

2. The characters lacking in this variety are transferred to it from a donor parent without changing the genotype of this variety except for the genes being transformed.

Requirement of back cross breeding

1. Suitable recurrent parent must be available which lacks in one or two characteristics.

2. A suitable donor parent must be available, the character must be highly intense.

3. Character to be transferred must have high heritability controlled by one or two genes.

4. 6-7 back crosses are required for full recovery of recurrent parent.

Applications of back cross breeding

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

Non recurrent aa	X	Recurrent parent AA
F1	Aa X AA	
BC1		50% X AA
BC2		75% X AA
BC3		81% X AA
BC4		93% X AA
BC5		98.1% X AA
BC6		98.4%

7. ***Recurrent parent:*** In back cross breeding the parent to which one or few genes from the donor parent are transferred.
8. ***Non recurrent parent:*** The donor parent from which the character is to be transferred.

Procedure for transfer of dominant gene

- E.g.Highyielding and widely adapted wheat variety A is susceptible to stem rust another variety B is resistance to stem rust. Stem rust is dominant tosusceptibility.

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

2. BC1 Generation: F1 plants are back crossed to variety A. Since all the F1 are heterozygous for rust resistance, selection for rust resistance is not necessary.

3. First BC1 generation: Half of the plants in BC1 generation are resistant and the remaining half would be susceptible to stem rust. Rust resistant plants are selected and back crossed to variety A.

4. BC2 to BC 5 generation: Segregation would occur for rust resistance. Rust resistant plants are selected and back crossed to variety A.

5. BC6 Generation: BC6 plants will have 99 percent genes from variety A Rust resistant plants are selected and selfed, their seeds are harvested separately

6. BC7Generation: Individual plants progeny from the selfed seeds of the selected plants are grown. Rust resistant plant similar to the plant type of variety A are selected and they are selfed. Seeds are harvested separately.

7. BC8 Generation: Individual plants progeny are grown. Progenies homozygous for rust resistant and similar to plant type of variety Aare harvested in bulk. Several similar progenies are usually mixed to constitute the new variety.

Yield trials:

- It is tested in replicated yield trial along with the variety A as check. All the component character are critically examined a detailed yield tests are not required and variety may be directly released for cultivation.

Transfer of a recessive gene

- When Rust resistant isrecessive, all the back crossescannot be made one after the other. For every two subsequent back crosses F2 generation must be grown to identify the rust resistant plants.

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

2. BC1 Generation: F1 plants are back crossed to the recurrent parent.

3. BC2 Generation: Since rust resistance is recessive, all the plants are rust susceptible. No test for rust resistance and all the plants are self pollinated.

4. BC3 F2 Generation: Rust resistant plantsare selectedand back crossed to recurrent parent plants which are similar to recurrent parent are selected.

5. BC4 Generation: There is no rust resistance test. Plants are selected which are resistance to recurrent parent and back crossed.

6. BC5Generation: There is no disease test. The plants are self pollinated to raise F2. Selection is usually done for the plant type of variety A.

7. BC6 Generation: Plants are inoculated with rust. Rust resistant plants are selected is back crossed to variety A.

8. BC7Generation: There is no rust resistance test plants are back crossed to variety A.

9. BC8 Generation: There is no rust test, plantsare self pollinated to rise F2 generation.

BC5 Generation:

- Plants are subjected to rest epidemic. Rigid selection is done for rust resistance. And for the characteristics of variety A. Selfed seeds of the selected plants are harvested separately. BC5

Generation:

- Individual plant progeny are grown and subjected to rust epiphytotic. A rigid selection is done for resistant to stem rust and for the characteristics of variety A. Seeds from several rust resistant homogenous progenies are mixed to constitute the new variety.
- Yield test are conducted and released as a variety
- Same procedure also to be practiced in cross pollinated crops

- **Merits and Demerits of Back cross breeding**

Merits:

1. Back cross method retains all desirable character of a popular adapted varieties and replaces undesirable allele at particular locus

2. Useful for the transfer of disease resistance and incorporation of quality traits into a variety

3. This is used for the development of isogenic lines,

4. Extensive tests are not required 2-3 generation can be raised in off season nurseries green houses, it would save time.

5. This is the only method for the inter specific gene transfer and transfer of cytoplasm.

6. Male sterility and fertility restoration genes can be transferred to various back ground.

Demerits:

1. New variety cannot be superior to recurrent parent except for the character transferred

2. It involves lot of crossing work. 6-8 back cross is often difficult and time consuming.

3. Sometime undesirable gene linked with desirable also may be transferred.

4. By the time the back cross programme the recurrent parent may have been replaced by other varieties superior in yield and other character.

Comparison of back cross and pedigree method

Pedigree	Back Cross
F1 and subsequent generation are allowed for self pollinated	F1 and subsequent generation are back crossed to recurrent parent
New variety is different from the parent	New variety is identical to recurrent parent except for the character
New variety has to be tested extensively before it is released	Extensive testing is not required
Aims at improving yielding ability and other character Not suitable inter specific gene transfer	It aims at improving specific defects of a well adopted variety Useful for the gene transfer from related species
Hybridization is limited to F1	Hybridization is required for every back cross
Handle large population	Handle Small population

Breeding procedure same for dominant and recessive gene	Different for dominant and recessive gene

Hardy-Weinberg Law

Hardy-Weinberg Law

Introduction

- Cross pollinated crops are highly heterozygous due to the free inter mating among them so these are random mating populations. Because each individual of the population has equal opportunity of mating with any other individual. It is also known as mendalian/panmictic population. A mendalian population may be thought of having a gene pool consisting of all gametes produced by the population. So gene pool may be defined as the sum total of all genes presentin the population. A population consists of all such individuals that share the same gene pool has an opportunity to inter mate with each other and contribute to the next generation of the population.
- Each generation of a mendalian population may be considered to arise from a random sample of gametes from the gene pool of previous generation. Hence, it is not easy to follow the inheritance of a gene in a mendalian population. It cannot be estimated by using the techniques of classical genetics. So tounderstand the genetic makeup of such population a population genetics has been developed.

Hardy-Weinberg law

- This law is independently developed by Hardy (1908) in England and Weinberg (1909) in Germany. The law states that the gene and genotype frequencies in a mendalian population remain constant from generation after generation if there is no selection, mutation, migration or random drift.
- The frequencies of thethese genotypes for a locus with two alleles A and a would be P^2(AA), 2pq(Aa)and q^2(aa)

  Where, p= Frequency of 'A' allele in the population.
- q= Corresponding frequency 'a' allele in the population the sum of p+q is equal=1 Such a population would be at equilibrium since the genotypic frequencies would be stable, that is, would not change from one generation to the next.This equilibrium is known as Hardy Weinberg equilibrium.

Factor effecting

Migration

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

a. Ratio of migrant individuals to those of the original population.

b. The Magnitude of difference between the values of q in the population and in the migrants.

So in plant breeding migration is by inter varietal crosses or poly crosses.

Factor effecting

Mutation

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

Random drift or Genetic drift

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

Inbreeding

- Mating between individuals sharing a common parent in their ancestry inbreeding reduces the proportion of the heterozygosity and increase the frequency of homozygosity by the rate of decrease in heterozygosity is equal to ½ N (N- Number of plants in the population) per generation in monocious or hermaphrodite species. In diocious species and monoecious species where self-pollination is prevented the decrease in heterozygosity is lower.

- In small population, even with strict random mating/ strict cross pollination the frequency of homozygotes increases while that of heterozygotes decreases due to inbreeding

Selection

- Differential reproduction rates of various genotypes are known as selection. In crop improvement selection is important because it allows the selected genotypes to reproduce. While undesirable genotypes are eliminated. So in random mating population selected genotypes would be fixed and the fitness is zero for remaining genotype. The fitness of a genotype may be defined as its reproduction rate in relation to that of other genotypes.
- It is difficult to identify genotypes for dominance and due to less than 100% heritability especially for quantitative as a result, selection is expected to change gene frequencies rather than to eliminate one or other allele.
  The selection in a random mating population is highly effective in increasing or decreasing the frequency of allele, but it is unable to either fix or eliminate them. However, in combination with a system of inbreeding, selectionis highly efficient in the fixation and elimination of an allele.
- Most of the characters of economic importance are quantitative characters which are governed by many genes. Asthese characters show a continuous distribution selection intensity is measured as the difference between mean of the population and that of the selected individuals. It is expressed in terms of K standard deviation units form

which the selection differential 'K' is calculated. The value of 'K' is related to the percent of extreme individuals saved.

- E.g: When the extreme 5% of the population is saved K=2.06. Selection of the extreme phonotype increases the frequency of desirable alleles in the population. It increasesthe frequency of desirable alleles, the frequency of desirable genotypes would also increase new genotypes would also appears producing more extreme genotypes. Therefore mean of the population would change under selection. Variance may decrease to some extent but selection would show continuous gain for several generations
- In case the quantitative character is governed by one or few genes with large effect the desirable genes may be fixed after some generation ofselection. So gain under selection would continue for several generations but in the later generations it will be small and discouraging. The progress under selection is retarded by non-additive gene action, low heritability and limit on permissible selection intensity.
- Heritability of quantitative character is less than 100 percent. Sono perfect correspondence between phonotype and genotype. So identification of desirable genotypes is difficult and reduces the progress under selection. Permissible selection intensity depends on fecundity i.e. reproductive capacity of the species. Another factor that limits selection intensity is the consideration of inbreeding. Inbreeding should be kept to a low level to maintain heterozygosity and to avoid inbreeding depression. So selected plants should be relatively large and cannot be too small.
- The breeder is interested in improvement more than one character. Selection intensity is less for one character and it is released to accommodate selective for many characters

Introduction

- The breeding has two basic tools to change the genetic composition of a population.

  a. Selection b. mating system.

Random mating

- Each female gamete is equally likely to mate with any male gamete and the rate of reproduction of each genotype is equal i.e. there is no selection.
- In such a situation.

  I. Gene frequencies remain constant

  II. Variance for the character remain constant

  III. The correlation between relatives or prepotency does not change.

- In plant breeding some form of selection is practiced on such a mating system is known as random mating with selection. Such selection changes the mean of the characters, increases the frequency of alleles for which selection is practiced. Increase the variability when selection is alone for rare allele and reduce when predominant allele. It is more observed when character is controlled by one or two gene with moderate heritability.

- Useful for progeny testing production and maintainance of synthetic and composite varieties and production of poly cross progenies.

Genetic assortative mating

- Mating between individuals that are more closely related by ancestry than in random mating. It is also known as inbreeding. So it has following effects on a population.

I. It increase homozygosity and reduces heterozygosity

II. The characters are fixed, fixation is little effected by heritability.

III. Total genetic variability of the population increases rapidly and genetic variability within inbreeding group i.e. families or lines decreases rapidly and is ultimately zero.

IV. Prepotency of individuals increases. It is the property of an individual to produce progeny which are similar to each other and to the parent. Homozygosity is most important which is under the control of breeder as homozygosity increases the prepotency of an individual increases.E. g: Useful for development of inbreds.

Genetic dis-assortative mating

- Such individuals are mated which are less closely related by ancestry than would be under random mating. So unrelated individuals are mated, which are of different population i.e inter varietal or inter specific crosses. Effects are of similar to migration. This system reduces homozygosity and increase heterozygosity.

Phenotypic assortative mating

- Mating between individuals which are phenotypically more similar than would be expected under random mating. It leads to

  1. Division of the population into two extreme phenotypes. Intermediate types are not fixed. The highest and lowest remain in the population.

  2. Increase in homozygosity and genetic variability

  3. Prepotency increases.

- These effects disappear rapidly when random mating is restored. The effects of this mating are quick in the characters governed by single gene and goes on reducing as number of genes increased. For polygenic traits intermediate types are not eliminated. The effects are further reduced due to dominance and epistatic effects and for quantitative traits which have less than 100% heritability. So homozygosity is much lower.

- The mating system is useful in the isolating extreme phenotypes. It is used in some breeding scheme. E.g: Recurrent selection.

Phenotype dis-assortative mating

- Mating between phenotypically dissimilar individuals belonging to the same population.

Consequences:

1. It leads to the maintainance or some increase in heterozygosity

2. Reduction in population variance as to produces intermediate phenotype

3. Due to increase in heterozygosity the correlation betweenrelatives or prepotency is reduced.

4. This mating system is very useful in making a population stable i.e. in maintaining variability suitable parents may be selected to remove their weaknesses. The progenyfrom such a mating would be more desirable than the parent. It is useful in maintaining variability in small population as it reduces inbreeding.

Inbreeding

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

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 were 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.

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

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

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

Heterosis

Heterosis

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

History

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

Heterosis and Hybrid Vigour

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

Heterosis is the superiority of a hybrid over its parents.

1. *Increased yield:* Heterosis is generally expressed as an increase in the yield of hybrids. The yield may be measured in terms of grain, fruit, seed, leaf, tubers or the whole plant.
2. *Increased Reproductive Ability:* More number of flowers/fruits/seeds.. Increase in Size and General Vigour: The hybrids are generally more vigorous, i.e., healthier and faster growing and larger in size than their parents. .
3. *Better Quality:* In many cases, hybrids show improved quality. For example, many hybrids in onion show better keeping quality, but not yield, than open-pollinated varieties.
4. *Earlier Flowering and Maturity:* In many cases hybrids are earlier in flowering and maturity than the parents. But earliness is highly desirable in many situations particularly in vegetables.

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

Main Difference between Inbreeding and Heterosis:

Inbreeding	**Heterosis**
1. Inbreeding results from matings between closely related individuals.	Heterosis results from crossing between unrelated strains.
2. It is decline in fitness and vigour with decreased heterozygous.	In heterosis the unfavourable recessive genes of one line (parent) are covered by favourable dominant genes of other parent.

3. It results due to fixation of unfavourable recessive genes in F2.	In this case unfavourable recessive genes of one lien (parent) are covered by favourable dominant genes of other parent.

Heterosis and dominance in relation to parental values

Position and mean value Of the parents	Mean value of the F1 hybrid	Phenomenon
Parent A (10)	> 10	Heterosis
	10	Complete dominance
(Mid Parent) (8)	<10 but >8	Partial dominance
	8	No dominance
Parent B (6)	<8 but >6	Partial dominance
	6	Complete dominance
	<6	Heterosis

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 F2:

- In F2dominant and recessive characters segregate in the ratio of 3:1. Quantitative characters, according to dominance hypothesis should not show symmetrical distribution. However, F2nearly 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 F2is 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 heterosisor super dominance theory. According to this hypothesis, heterozygotes or at least 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 a1a2a3a4etc, with increasingly different functions. Heterozygotes between more divergent alleles would be more heterotic. E.g. a1a4will be superior to a1a2or a2a4

- **Comparison of dominance and over dominance hypothesis**

Feature	Hypothesis of heterosis	
	Dominance	**Over dominance**
Similarities		
Inbreeding leads to	Reduced vigour and fertility	Reduced vigour and fertility
Out crossing leads to	Heterosis	Heterosis
Degree of heterosis increases with	Genetic diversity in parents	Genetic diversity in parents
Differences		
Inbreeding depression is the result of	Homozygosis for deleterious recessive alleles	Homozygosity itself
Heterosis is the result of	Masking of the harmful effects of recessive alleles by their dominant alleles	Heterozygosity itself

The phenotype of heterozygote is	Comparable to that of the dominant alleles	Superior to both the homozygotes
Inbreds as vigorous as the F1 hybrids	Can be isolated	Cannot be isolated

Fixation of heterosis

- In plants, it may be achieved in the following three ways.

1. Vegetative propagation.

2. Apomixis.

3. Balanced lethal system

Estimation of Heterosis

1. Average heterosis:

- It is the heterosis where F1 is superior to mid parent value. In otherwords superior to average of two parents.

$$\frac{F1 - MP}{---------} \times 100$$

MP

Where F1 = Mean of hybrid

MP = Mid parental value.

(P1 + P2) where P1 = Parent 1

MP = ---------- P2 = 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 F1 over the better parent.

F1 - BP
--------- x 100
BP

Where BP = Mean of Better Parent.

3. Economic heterosis:

- Superiority of the F1 compared to the high yielding commercial variety in a particular crop.

F1 - CV
--------- x 100
CV

Where CV = Mean of Commercial Variety.

4. Negative heterosis:

- Performance of F1 inferior to better parent / mid parent value. - e.g. Duration.

Manifestation of heterosis

1. Increased yield.

2. Increased reproductive ability.

3. Increase in size and vigour.

4. Better quality

5. Greater adaptability.

Development and Evaluation od Inbreds

Introduction

- Inbred lines are developed from a genetically variable population through continued in breeding.

Development of inbreds:

1. By inbreeding, selfing, etc.

2. Development of inbreds from haploids - Rice, Sorghum, Maize.

Evaluation of inbreds

a) Phenotypic evaluation

- Inbreds are evaluated for their phenotypic performance which is most suited for simple inherited traits.

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 hybridprogenies will be evaluated in replicated progeny rows and based on results better inbreds are identified.

c) Single cross evaluation

- The developed inbreds can be crossed and the single crosses can be evaluated in replicated trial. Outstanding

hybrids tested over years in different locations, are released for cultivation.

d) Prediction of double cross performance

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

  Inbreds: A, B, C, D.

  Six 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 are 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 double cross can be predicted from performance of the four single crosses not involved in producing hybrid.

  (A x B) x (C x D)

  A x C

  A x D

  B x C

  B x D

Average

Production of Hybrids

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 planting cross compatible lines hybrids are produced. Here both are hybrids. – Brassicas, Fruit tree.

Success of hybrids depends on

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.

Introduction: Synthetic variety

- 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 Syn1. This is distributed as synthetic variety or it may be grown in isolation for one more season and Syn2 is distributed.

- **Merits and Demerits of Synthetic variety**

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.

- **Comparison of Synthetic and composite varieties**

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.

Mass Selection and Projeny testing

Mass selection

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

Merits:

i) Simple and less time consuming

ii) Highly effective for character that are highly 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 to reduction in population there by leading to inbreeding.

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 was followed to improve maize crop.

Progeny testing and selection

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.

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 overcome this defect, the following method is suggested.

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

b) While raising progeny rows, after reserving part of the seeds, the rest are sown in smaller progeny rows.

c) Study the performance of progenies in rows and identify the best ones.

d) After identifying the best progenies, the reserve seeds of the best progenies may be raised in progeny rows.

e) The progenies will be allowed for open pollination and best ones are selected. There are number of other modifications made in the ear to row selection.

For example, i) The selected progenies may be selfed instead of open pollination ii) The selected plants may be crossed to a tester parent. The tester parent may be a open pollinated variety, or inbred. iii) The progeny test may be conducted in replicated trial.

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
- First selection cycle. ii) Open pollinated plants harvested on Single plant basis

Inbred or selfed family selection

- Families produced by selfing.

S1family selection: Families produced by one generation of selfing. These are used for evaluation and superior families are intermated (Simple recurrent selection)

S2 family selection: Families obtained by two generations of selfing and used for evaluation. Superior families are intermated.

Merits of progeny testing and selection

Merits :

1. Selection based on progeny test and not on phenotype of individual plants.

2. In breeding can be avoided if care is taken for 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 and types

Introduction

- The recurrent selection was first suggested by Hayes and Garber in 1919. Independently byEast and Jones 1920. In 1945, Hull suggested that recurred selection will be useful in improving specific combining ability. It is

devisedinheterosisbreedingisolation of superior inbreds from the population and to utilize themin hybrid and synthetic varieties.

- **Isolation of inbred depends on two factors**

1) The proportion of superior genotypes present in the base population from which inbreds are isolated.

2) The effectives of selection during the inbreeding process increasing the frequency gene combinations.

The recurrent selection schemes are of4 different types.

Simple recurrent selection

- A number of desirable plants are selected and self pollinated. Separate progeny rows are grown from the selected plants in next generation. The progenies are intercrossed in all possible combination by hand. Equal amount of seed from each cross is mixed to raise next generation. This completes original selection cycle. From this, several desirable plants are selected and self pollinated. Progeny rows are grown and inter crosses made. Equal amount of seeds are composited to raise next generation. This forms the first recurrent selection cycle.

First Year:

i) Several superior plants are selected.

ii) Selected plants selfed.

iii) Harvest thesingle plants.

iv) Seedsare evaluated, superior plants are identified.

Second Year:

i) Progeny rows raised

ii) Inter crosses are made in all combination by hand.

iii) Equal amount of seed Recurrent bulked from each cross Selection Cycle.

Third Year:

i) Composited seeds raised

ii)Repeattheoperationasin firstyear

Fourth Year:

Repeat as in second year.

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

ii) Most suited for characters having high heritability.

iii) Inbreeding is kept at minimum.

Eg: oil content. Protein content and high heritability traits are affective for increasing the frequency of desireble genes in the selected populations

Recurrent selection for General combining ability

- Recurrent selection for general combining ability was proposed by Jenkins 1935. The progenies are crossed with tester strain with a broad genetic base. So plants are selected on the basis of superior performance of their plant X tester progenies would have superior GCA.

First Year:

- A number of phenotypically outstanding plants are selected from the source population. The source population may be open pollinated, synthetics or advanced generation of a hybrid. Selected plant areselfed as well as crossed (as male) to a number of randomly selected plants form a tester having broad genetic base. Selected seeds are harvested separately and saved for planting in the third year. The test cross progeny from each selected plant is harvested separately and used for replicated yield trial in the second year.

Second Year:

- A replicated yield trial is conducted using plant X tester progeny. Superior progeny are identified. Selected seeds form the first year from those plants that produced superior test cross progenies are planted in separate progeny row in a crossing block. These are inter crossed in all possible combination, equal amount of seeds from all the inter crosses are composited to obtain next generation. This completes the original cycle ofselection.

Fourth year:

- Seed obtained from bulking of all the inter crosses are planted as the source population for the first cycle of recurrent selection. Several plants are selected on the basis of their phenotypes. Each of them isselfed as well as crossed (as male) to a number of random plants form the tester with broad genetic base.

Fifth year:

- Operation of second year is repeated.

Sixth year:

- Operation of third year is repeated. This completes the first recurrent selection cycle.

Seventh year:

- The second recurrent selection cycle may be initiated. This method improves the GCA in the direction of selection. It increases the yielding ability of the populations to isolate inbreds with superior GCA.

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Recurrent selection for specific combining ability

- It was proposed by Hull in 1945 with an objective is to isolate lines from a population that will combine very well with a given inbred. Large part of the heterosis is due to non additive gene action i.e. Dominance and epistasis. So it depends on specific gene combination and is designed as specific combining ability. The inbred is used as tester to isolated lines with high specific combining ability.

First Year:

- Several plants are selected from the population and self pollinated. Selected plants used as males are also crossed to an outstanding inbred used as the tester.

Second Year:

- Replicated yield trial is planted using test cross progeny.

Third Year:

- Selfed seeds from the outstanding progenies are planted in separate progeny rows in a crossing block. All possible inter cross are made by hand. Equal amount of seed from the inter crosses are composited. This completes the original selection cycle.

Fourth Year:

- The composited inter cross is planted and the operation of the first year are repeated.

Fifth Year:

- Evaluation under replicated yield trial.

Sixth Year:

- Selfed seed from outstanding progenies are planted and crossed in all possible intercrosses are composited. The cycle may be repeated.

Reciprocal Recurrent selection (RRS)

- This method was proposed by Comstock, Robinson and Harvey 1949.

Objectives:

- To improve two different populations in their combining ability simultaneously whentwo populations A and B, are involved in reccurent selection. A serve as a tester for the population B, and B serves as a tester for the plants selected from A. A and B population has broad genetic base and genetically heterogenous. Selection for SCA inbreds derived from each population are crossed to derive hybrid. The method allows for the selecting both GCA and SCA.

First Year:

- Several plants are selected from the population A and B on the basis of their phenotype. Each of the selected plants form the population A is crossed as male with several randomly selected plants from the population B used as female. Similarly population B as male and population A as female. All selected plants are selfed, selfed seed is harvested separately.

Second Year:

- Two replicated trials are conducted for the test cross progeny of the plants selected from population A and population B. On the basis of progeny test plants producing superior test cross progenies are identified.

Third year:

- Selfed seed of selected plants on the basis of progeny test are planted in two separate crossing blocks as individual plant progeny rows. Progeny rows of two crossing block are planted separately. All possible inter crosses are made and equal amount of seeds from all inter cross in the crossing block A are mixed to carry to next generation of population A and B. This completes the original selection cycle.

Fourth Year:

- Population A and B are planted from the composited seeds from all intercross in block A and B separately.

Fifth Year:

- Operation of second year is repeated.

Sixth Year:

- Operation of the third year is replanted and this completes the first recurrent selection cycle.

- **Applications of reciprocal recurrent selection**

1) Production of synthetic varieties.

2) Isolation of inbred lines

3) Identification of superior single cross combination.

Breeding methods for asexual crops

Characteristics of asexual crops

- Majority of these are perennials like sugarcane and fruit trees. The annual crops are mostly tuber crops e.g. potato and sweet potato.
- These are highly heterozygous indicating the evolutionary advantage of heterozygote in these crops which leads to high degree of inbreedingdepression on account of increased homozygosity on selfing.
- Many of these crops are interspecific hybrids and are polyploids.
- These are highly cross pollinated.
- Most of these crops show reduced flowering and certain varieties do not even flower at all. The seed set through normal sexual reproduction is usually low.
- On account of their heterozygous nature the first generation of varietal crosses displays enormous variation and appearance of un-adapted and weak recessives in the progeny.

Advantages of asexual reproduction

1. It provides the quickest method to exploit the available genetic variation by selecting the out standing individual which can be quickly multiplied without any genetic changes.
2. A true to type of multiplication of an individual saves time and the resources required for progeny testing and stabilizing the genetic composition of seed Produced crops.
3. The phenomenon of apomixis is very convenient way to fix heterosis in the form of highly heterozygous hybrids.
4. Wide crosses can be easily adopted to utilize the germplasm of wild and related cultivated species. Even sterile plants can be multiplied and cultivated provided the seed is not economically important part for the crop ex. leafy vegetable
5. It provides quick method of multiplication of even sexually reproducing plants especially through micro-propagation.

Limitation of asexual reproduction

1. It leads to extreme genetic uniformity within clones which become genetically vulnerable to disease and pest or abrupt changes in environmental factors.
2. Long time storage of propagules like tubers, stem, cutting, etc. loosing vigour and viability. The long time preservation of germplasm in such crops is a practical problem. Tubers, rhizomes, stem cuttings etc. are bulky and require considerable space for conservation.
3. A large part of the produce is required for propagation.

4. The reduced fertility and seed set creates problem in making crosses on large scale. a highly photo- and thermo-sensitive nature of these crops puts restrictions on hybridisation.
5. Small mutations keep on accumulating and may ultimately alter the genetic composition of the clones.
6. The genetical analysis is difficult due difficulty to induce flowering, high sterility and perennial life cycle.

• Clone

A clone is a group of plants produced from a single plant through asexual reproduction.

Characteristic feature of a clone

1. Identical genotype.
2. Lack of genetic variation.
3. Immortality: Can be maintained indefinitely.
4. Highly heterozygous shows severe inbreeding depression.

Origin of genetic variation

Origin of genetic variation

1. **Mutation**: Dominant bud mutations are more frequent than recessive mutations.

2. **Mechanical mixture**
3. **Sexual reproduction** leads to segregation and recombination.

Breeding approaches

Breeding approaches

- The multiplication through vegetative parts in asexually propagated crops provides a unique opportunity to utilize available genetic variation in a single cycle of selection.
- Any out standing plant can be multiplied vegetatively producing unlimited number of individuals of the genetic make up.
- Since all the members of a clone are simply products of mitosis so these are genetically identical and uniform clone is thus stable over generations of multiplication and variation within clone is due to environmental or mechanical admixture.
- Before starting up of breeding programme in asexually propagated crops we have to create variation, utilization of genetic variation to isolate new population and multiplication of successful genotype for commercial cultivation.
- The identification and stabilization of desirable genetic variant from variable populations is much easier and relatively less demanding in terms of time and resource.

- Asexual reproduction provides a quick and reliable mode of multiplication of the successful clone

Introduction

The process of introduction in these crops is like any other sexually propagated crop but restricted choice of environmental condition for induction of flowering and seed set limits sexual reproduction to only few locations and thus the relevance of introductions

Selection

- The selection in these crops is restricted to the material introduce from other source from where a large number of clones are introduced and evaluated under local agro climatic conditions.
- The promising selections are tested in large scale trials which, if successful, can be released for commercial cultivation.

First year

- Grow available mixed variable population; few hundreds too few thousand plants are selected.
- Desirable plant type is selected.
- Highly heritable traits are considered for selection.

Second year

- Grow progeny of the selected clone
- Evaluate them for yield and quality trait.
- 50-100 clones are selected

Third year

- Replicated preliminary yield trial
- Few superior clones are selected.

Fourth to sixth year

- Replicated Multi location trial
- Test for yielding ability.
- Quality and Resistance are evaluated.
- A clone superior to check may be released.

Merits

1.It is the only method of selection. It avoids inbreeding depression and preserve gene combination.

2. It can be combined with hybridisation.

3. Maintain purity of clones.

Demerits

1. It utilises natural variability and cannot create variability.

2. Sexual reproduction is required to create variability through hybridisation.

Clonal selection

- Asexual reproduction covers all modes of multiplication of plants where normal gamete formation and fertilization does not take place making these distinctly different from normal seed producing crops. In the absence of sexual reproduction, the genetic composition of plant material being multiplied remains essentially the same as its source of plant.
- It was this unique characteristic of asexual reproduction that helped Thomas Knight to develop of number of cultivars of fruits and vegetables including grapes, apple, pear, and peaches during 1811 to 1838.
- In contrast to sexually producing plants, the F1 crosses in the asexually propagated plants were heterozygotes from which knight selected superior plants and propagated vegetative to develop stable varieties without any deterioration due to segregation of gene combinations.
- Asexually propagated crops are highly heterozygous but identified clone is the best amongst large number of such heterozygote present in naturally occurring or artificially created variation, there may be a presence of unfavorable alleles in homozygous.
- A process of inbreeding and selection combined with hybridization can thus lead to production of more productive hybrids. A high degree ofinbreeding depression can be expected but new clones developed by crossing of two clones from two different population of selfed ones are likely to produce highly heterozygous

and more productive clone, but crossing of inbred lines of same clone carry deleterious allele and clone in homozygous state.

Interspecific Hybridization

- Interspecific crossing has a unique place in the improvement of all the crop plants because wild and related species of cultivated plants possess several characters which are lacking in cultivated crops.
- Interspecific hybridization in sexually propagated crop faces a problem of sterility and poor seed set due to cytological difference between species.
- The asexual multiplication of plants however makes it easy to successfully adopt interspecific hybridization because any plant combining characters of two or more species can be multiplied in spite of its sterility and seed setting abnormalities. E.g: Sugarcane.

Mutation Breeding

- Induced mutations has distinct advantages and particular limitations. Any vegetative propagule can be treated with mutagens and even a single desirable mutant or a part of the mutated propagule can be multiplied as improved type of the original variety.
- The asexually propagated crops being highly heterozygous are rather difficult to be improved through sexual hybridization because of wide segregation and recombination in varietal crosses that usually loss of outstanding characteristics of the parent lines.

- The complex polyploidy nature and long juvenile period of these crops also restrict the utility of planned hybridization in these crops.
- The mutations are unicellular in event and mutagenic treatment of multicellular somatic tissues like buds, petioles, apices, tubers or bulbs leads to induction of mutation.
- Mutation breeding has successfully been used in crops commercially produced by tubers, bulbs and cuttings which include potato, sweet potato, cassava, fruit trees, and number of ornamentals maximum number of improved varieties through mutation breeding has been produced in ornamentals and fruit trees.

Breeding of apomictic crops

Apomixis is a form of asexual reproduction in which sexual organs or related structures take part but seed is formed without the union of male and female gamete.

Uses of Apomixis

- Apomixis is genetically controlled and the apomictic plants produce normally reduced male gametes which can be used to pollinate sexual plants to produce new apomictic genotypes.
- The species which can produce both sexual and apomictic seed are facultative apomictics.
- The species which can reproduce only through apomixis are obligate apomicts.
- Apomixis is usually controlled by few genes which may be either recessive or dominant.

i. Fixation of heterosis.

ii. Production of phenotypically stable populations in the form of vybrids.

iii. Production of pure homozygotes.

Mutation Breeding

Intrduction

Mutation is a sudden heritable change in a characteristic of an organism. It is the results in gene changes in chromosome or in plasma gene.

Gene or point mutations.

- Mutation produced by changes in the base sequence of genes as a result of transition, transversion, deletion, duplication or inversion etc.

Chromosomal mutation

- Mutation produced by changes in chromosome structures and chromosomal numbers.

Cytoplasm mutation is occurs in cytoplasmic genes.

History

- Term mutation was first introduced by Hugo de varies in the year 1900. The discovery of the mutagenic effects of X-rays on the fruit fly (Drosophila) by H. Muller in the 1920. And gamma rays and x-rays by L. J. Stadler, later Muller got Nobel Prize In 1946.
- Spontaneous mutation: Mutations occur in natural population at a 10 6 rate. The frequency of spontaneous mutation is one in 10 lakhs.
- Induced mutation: Mutation may be artificially induced by a treatment with certain physical or chemical agents. The chemicals used for producing them are termed as mutagens. The utilization of induced mutation for crop improvement is known as mutation breeding. Induced mutations are used more often in a supplementary role as a source of new alleles. However, it is still important in breeding vegetatively propagated species, including field crops, ornamentals, fruit and forest species. It is especially useful in ornamental plant breeding

Characteristics of mutation

1. Mutations are generally recessive, but dominant mutations do occur.
2. Mutations are generally harmful to the organism, 0.1 percent are beneficial.
3. Mutations are random that they may occur in any gene.
4. Mutation are recurrent, the same mutation may occur again and again.
5. Induced mutation commonly shows pleotropic, due to linked genes.

Types of Mutations

Transition

- Mutation by transition entails the conversion of one purine base to another purine (or a pyrimidine to another pyrimidine) When a purine is replaced by purine. E.g. Adenine is replaced by guainine or pyrimidine by pyrimidine. E.g. Thymine is replaced by cytosine.

Transversion

- A transversion involves the substitution of a purine by a pyrimidine and vice versa.
- Pyrimidine is replaced by purines. A to Z, A-C, G-C G-T Transition, Trans version are relatively also deleterious, but base substitution may generate such codon that codes for any amino acid (sense codons) whenever the nonsense codon is produced it acts on terminator of the poly peptides changes.

Base addition/ deletion

- A variety of alkylating agents (E.g., sulfur and nitrogen mustards) can act on the DNA molecule, reacting mainly with guanine (G) to alkyl ate and remove it from the DNA chain.

- The missing spot may be occupied by any one of the four bases to create mutations, usually by transition.
- Acridine is also known to express its mutagenic effect through the addition of deletion of bases.
- An insertion of one or more bases in a DNA molecule is called based addition.

Frame shift mutation

- Insertion-deletion mutations may cause significant changes in the amino acid composition of a protein and hence its function. E.g. GAG-CCG-CAA-CTT-C (corresponding to Glu-Pro-Glu-Leu) may be altered by a deletion of G that shifts the reading frame to the right by one nucleotide to produce AGC-CGC-AAC-TTC (corresponding to Ser-Arg-Asi-Phe).

Mutagens: Physical mutagens

Mutagens: Agents which induce mutation are known as mutagens.

There are two types of mutagen.

- Physical Mutagens
- Chemical Mutagens

Mutagen	Characteristics

X- rays	Electromagnetic radiation; penetrate tissues from a few millimeters to many centimeters first discovered by Roentgen 1895 wavelength varies from 10-11 to 10-7. Highly penetrating they break chromosomes and produce all types of mutation in the nucleotides like addition, deletion, inversion, transition and transversion.
Gamma rays	Electromagnetic radiation produced by radioisotopes and nuclear reactors; very penetrating into tissues; sources are Co60 and Ce137 largely used in crop plants
Neutrons	A variety exists (fast, slow, thermal); produced in nuclear reactors; uncharged particles; penetrate tissues to many centimeters; source is U235
Beta particles	Produced in particle accelerators or from radioisotopes; are electrons; ionize; shallowly penetrating; sources include P32 and C14
Alpha particles	Derived from radioisotopes; a helium nucleus capable of heavy ionization; very shallow penetrating
Protons	Produced in nuclear reactors and accelerators; derived from hydrogen nucleus penetrate tissues up to several centimeters
Non ionizing radiations	Non-ionizing radiation produced from mercury vapor lamps or tubes. They penetrate one or two cell layers eg. Pollen.

Chemical Mutagen

Chemical Mutagen

- The material is soaked in a solution of the mutagen to induce mutations.
- Chemical mutagens are generally carcinogenic and must be used with great caution.
- One of the most effective chemical mutagenic groups is the group of alkylating agents (these react with the DNA by alkylating the phosphate groups as well as the purines and pyrimidines).
- Another group is the base analogues (they are closely related to the DNA bases and can be wrongly incorporated during replication); examples are 5-bromo uracil and maleic hydrazine. (Chemical mutagens commonly used are ethyl methane sulfonate (EMS) and diethylsulfonate (DES).
- The half-life (time taken for degradation of the initial amount of alkylating agent) for EMS in water is about 3 hours at 20°C but only 10 hours at 37°C.
- Consequently, chemical mutagens are best freshly prepared for each occasion.

Mutagen group	Examples
Base analogues	5-bromouracil, 5-bromodeoxyuridine
Related compounds	Maleic hydrazide, 8-ethoxy caffeine
Antibiotics	Actinomycin D, Mitomycin C, Streptonigrin

Alkylating agents	Sulfur mustards Ethyl–2-chloroethyl sulfide Nitrogen mustards 2-chloroethyl-dimethyl amine Epoxides Ethylene oxide Ethyleneimines Ethyleneimine Sulfonates, etc. Ethyl methane sulfonate (EMS), Diethylsulfonate (DES)
Diazoalanes Nitroso compounds Azide Hydroxylamine Nitrous acid Acridines	Diazomethane *N*-ethyl-*N*-nitroso urea Sodium azide Hydroxylamine Nitrous acid Acridine orange

Situation of Mutation Breeding

1. When the desired variability is not found its cultivated varieties or in the germplasm
2. When a high yielding variety has a defect, the defect can be also being changed without much alteration in the genetic background.
3. To break the undesireble linkage
4. In crops sexual reproduction is absent and creation of variability through recombination is not possible
5. Generation cycle is very long. E.g: Plantation crops.

6. When attractive flowers and of foliage colour have to be developed in ornamental crops

Procedure for oligogenic traits

- M1- Several hundred seeds are treated with mutagen and are space planted M1 plants are selfed and harvested separately.
- M2-About 2000 progeny rows are grown. Oligo genic mutants are selected and the seed are harvested separately and advanced to M3 , M3 are grown as individual plant progeny
- M3 -Progeny rows from individual selected plants are grown. Poor and inferior mutant rows are eliminated if the mutant progenies are homogenous two or more M3 progeny may be bulked. M3 are harvested in bulk and are advanced to preliminary yield trail in M4.
- M4 – Preliminary yield trial with suitable checks, promising mutants are selected for replicated multi location trial.
- M5-M7 Replicated multi location trials are conducted. Outstanding variety may be retained for hybridization.

Procedure for Poly genie traits

Procedure for Poly genie traits

Mutagenesis does produce genetic variation in polygenic traits

1. M1 and M2 : M1 and M2 are grown, M2 vigouros fertile and normal looking plants that alone exhibit a mutant phenotype are selected and their seeds are harvested separately to raise individual plant progeny rows in M3.
2. M3 Progeny rows from individual selected plants are grown. Careful observation is made on M3 rows for small deviation in phenotype from the parent variety. Few rows which are homogenous and would be harvested in bulk. Selection is done in M3 rows showing segregation.
3. M4: Bulked seed from homogenous M3 rows may be planted in preliminary yield trial with suitable check. Superior progenies are selected for replicated multilocation yield trial superior homogenous progenies are harvested in bulk for preliminary yield trial in M4.
4. M5- M8: Preliminary yield trials and MLT are conducted depending upon the when progenies become homogenous. Outstanding progeny may be released as new variety.

Achivements in mutation breeding

Achivements

- Totally 1029 mutant varieties are released in India.
- Disease resistance: Verticilium wilt resistance in peppermint, victorial blight resistance in barley, downy mildew resistance in pearl millet.

- Modification of plant structure: E.g., bush habit in drybean, dwarf mutants in wheat and other cereals.
- Nutritional quality augmentation: E.g. Opaque and floury endosperm mutants in maize
- Chemical composition alteration: E.g., low ericic acid mutants of rape seed
- Male sterility: for use in hybrid breeding in various crops
- Horticultural variants: development of various floral mutants
- Breeding of asexually propagated species: numerous species and traits
- Development of genetic stock: various lines for breeding and research
- Development of earliness: achieved in many species

Merits

- It can be used in inducing male sterility. eg: Ethidium di bromide
- Cheap and rapid method as compared to other methods.
- More effective for oligogenic traits.

Introduction

- The change in number of chromosomes is an important source of genetic variation that resulted in the evolution of a number of crop species. The genes, which produce enzymes and finally traits are located on the chromosomes as a result of which the change in chromosome number leads to observable change in the trait of an individual.
- Each chromosome exists as a member of the pair and the number of such pairs of chromosome is specific to a particular species. As an instance the human beings have 23 pairs, maize have 10 pairs and Arabidopsis has only 5 pairs of chromosomes. A basic set of chromosomes constitute a genome containing one number of each pair of chromosomes and represents the minimum number of chromosomes that has survived in any species.
- Individuals having chromosome number more than two sets referred as polyploids. The individual having two sets of chromosomes is called as diploid while individual with only single set is called monoploid. The somatic chromosome number of an individual i.e. Sporophyte is always designated as 2n and the chromosome number of the gametes as n whereas x is basic chromosome of the genome. The basic number of any species is designated as x, which represents a complete set of non-homologous chromosomes of one genome.

- The basic number of any species is designated as x, which represents a complete set of non-homologous chromosomes of one genome. Genome of monoploid is designated by symbol x, and that of haploid with symbol n and that of sporophyte with symbol 2n. In true diploids e.g. maize x=n=10: 2n=20. But in Polyploids the monoploid and haploid chromosome number is different. For instance in common bread wheat which is polyploid, the somatic chromosome number is 42 and haploid number 21 and the monoploid basic number is 7 Thus wheat x=7, n=21,2n=42 or n=3x and 2n=6x=42. The haploids of diploids are called monohaploids, and the tetraploids are called dihaploids and those of higher polyploids are known as poly haploids. The doubling of chromosome number of haploid results into a plant called doubled haploid.

- **Types of polyploids**

Ploypoidy has played an important role in the speciation within the plant kingdom and the number of our cultivated crops is polyploids.

Classification of polyploids

Polyploids		
Euploids		Aneuploids
Autopolyploid	Allopolyploids	

Triploids Teraploids		Monosomic
Tetraploids Hexaploids		Nullisomic
Pentaploids Octaploids		Trisomic
Septaploids Hexaploids		Tetrasomics
Octaploids		

Features

1. Have larger vegetative parts
2. Slower in growth and flowering.
3. Thick and large flower and fruits than diploids.
4. Reduced fertility due to irregulaer meiosis

Features and origin of polyploids

Features

1. Have larger vegetative parts
2. Slower in growth and flowering.
3. Thick and large flower and fruits than diploids.
4. Reduced fertility due to irregulaer meiosis

Origin

1.Chromosome doubling in somatic tissues, unreduced gametes.
2. Production of adventitious buds.
3. Physical agents like heat, cold and x-ray
4. Regeneration in vitro
5. Colchicine tratment.

Aplications of polyploids

Applications

1. Low chromosome number.

2.Harvested for vegetative growth and perennial habit.

3. Cross pollinated.

4. Reproduce vegetatively.

Triploids

1. Seedless watermelon crossing tetraploid and diploid

2. Triploid sugar beet- large root, more sugar, and triploid is the optimum ploidy level.

Tetraploids

1. Breeding improved quality

2.Overcoming self incompatibility.

3. Making distant crosses

Allopolyploids

1. Hybridisation between diverse species followed by chromosome doubling.

2. Production of amphidiploids.

3.Combine complimentary characters of two species.

4. Exhibit irregular mitotic division.

5. Irregular meiosis leads to unreduced gamete

Applications of polyploids in crop improvement

Application of polyploids in crop improvement

1. Utilisation of bridging species

2. Creation of new crop species.

3. Widening of genetic base of existing allopolyploidy

Limitations

1. The effects cannot be predicted.

2. Newly synthesised allopolyploids have many defects like low fertility and genetic instability

3. Small proportion of allopolyploids are promising

Induction of polyploidy

Natural polyploids

- Polyploidy does take place in nature but at very low frequency. Some important crops like potato sweet potato and lucern are natural autopolyploids and other crops like wheat, brassica, cotton oat, tobacco sugarcane are natural allopolyploids.
- It is estimated that 70% grass species and 25% of legume species are polyploids.
- Polyploidy occurs either due to the mitotic failure or meiotic irregularities.
- During cell division the mitotic failure leads to a cell with doubled chromosome number.
- Generally such cells do not further divide and get lost but if present in the meristematic zones, these give rise to polyploid sector and occasionally the whole plant.
- The origin of polyploidy has been reviewed by Harlan and DeWet (1975) proposed that almost all polyploids arise by way of unreduced gametes and that all other.

Induction of polyploids

- Polyploids, in general, exhibit gigas characters which created interest in developing polyploids in otherwise diploid species. Polyploidy can be easily induced by using a chemical colchicine.
- The chemical prevents the spindle formation and thus, prevent the migration of the daughter chromosomes to opposite poles which leads to a polyploid cell and ultimately the plant.

- It is generally applied to the meristematic cells at the concentration ranging from 0.05-0.1 percent using cotton pads or by dipping the tissues in the colchicines solution for 2-10 hours.
- Treatment of presoaked seeds with 0.2 percent colchicines solution for 2-8 hours has also been reported to be very effective in several crops.
- In tissue culture during rapid cell divisions, endomitosis i.e. Chromosome doubling without cell division results into a high frequency of polyploid cells/plants.
- Although the poly ploids exhibit gigas characters but every species has got its optimum ploidy level at which it is more vigorous. For example corn at diploid level, banana at triploid level and potato at tetraploid level exhibit better performance and these constitute the optimum ploidy levels for these crops.
- All crops are not suitable for induction of polyploidy.

CONCLUSIONS

1. Greatest success is in diploids with low chromosome number. Increasing the chromosome level beyond hexaploid level is of little or no advantage.
2. Auto polyploidy is more useful in crops having vegetative parts of economic value.
3. Greater success lies in the annual species that must be grown for several years before they can be evaluated.
4. Vegetatively propagated crops are more suitable for induction of polyploidy as compared to seed propagated crops

Induction of aneuploids

1. The most commonly studied aneuploids in genetics and breeding are monosomics, nullisomics and trisomics.
2. These may originate spontaneously through meiotic irregularity but their frequency is very low .The aneuploids arise by fusion of unbalanced gametes, which result from meiotic irregularities.

3. Autopolyploids are characterized by increased cell size and vigorous plant growth and size of reproductive organs especially sepals, petals and fruit with few seeds in most of the cases.
4. The irregular meiosis due to multivalent formation causes high degree of pollen inviability and reduced fertility.
5. Aneuploids in general are weaker than diploids and nullisomics or even monosomics may not even survive especially in many diploid species.

6. The aneuploids show wide range of segregation distortions and variable degree of viability.
7. A very low rate of direct use in plant breeding.

Significance of polyploids

Polyploids

1. Banana and some clones of mulberry, poplar, chrysanthemum and apple are natural triploids which are commercially grown.
2. Autopolyploid rye grass, sugarbeets, turnips, and fodder beets are commercially grown.
3. Triploids sugar beet are preferred over the tetrapolids.
4. Rye grass tetraploids give higher fodder yield as compared to dipoloids.
5. Pusa Gaint Berseem, tetraploid turnips, spinach pierce grapes, tetra petkus, steel rye, and tetraploid Brassica campestris var.toria are some other example of induced autotetraploidy.
6. Autopolyploidy plays an important role in bridging ploidy levels in the interspecific crosses where hybridizing species differ in ploidy levels.
7. Haploids can be used to produce completely homozygous lines in a short period as compared to 8-10 year required in in self pollinated crops.

Allopolyploids

8. Natural allopolyploids has played important role in the evolution of our major field crops such as wheat, cotton brassica species and sugarcane.
9. Raphanobrassica was developed but unfortunately it possessed radish like leaves and cabbage like roots.
10. Triticale a man made cereal is as an artificially synthesized allopolyploid.

Aneuploids

11. The aneuploids have not been much direct use in plant breeding. Used to incorporate alien addition of complete chromosomes or preferably segments of chromosomes.
12. Monosomics has been used in genemapping.
13. Aneuploids are used in the genetic studies to identify the chromosome or arm of the chromosome on which a particular gene is located.
14. Nullisomic lines have also been used to assign genes to a particular chromosome but the nullisomics are less vigorous and less fertile than monosomics.
15. Monosomics are also very useful for intervarietal chromosome substitution.

Plant Tissue Culture

Tissue culture

- It refers to growth of living plant tissues in a suitable culture medium.
- It is defined as a technique of growing plant tissues on synthetic medium under controlled and aseptic conditions.

G. Haberlandth: German plant physiologist is considered to be the father of plant tissue culture, who conceived the idea of Totipotancy in 1902.

Totipotancy: It is the ability of an individual cell or plant part to produce whole plant. The concept of tissue culture depends on totipotency.

Explant: They are the organs excised from plants such as roots, hypocotyls, cotyledons,

Commonly used media

Commonly used media in tissue culture

- Murashige and Skoog's media (1962) - MS media.
- White media (1963)
- Gamborg et.al (1968) -B5 media
- Chu media (1978) -N6

Methods used in plant breeding: Micro propogation

Methods which are extensively used in plant breeding

Micro-propagation

Involves the production of plant from very small plant parts through tissue culture techniques.

Advantages:

- It is independent of seasonal constraints hence ensures year round rapid propagation.
- Micro propagated plants are usually true to type.
- Production of disease free plants.
- Micro-propagated field grown plants usually exhibit vigorous growth, better quality and higher yields.

Problems encountered in micro-propagation.

- Microbial contamination.
- Browning of the culture medium.
- Callusing: Extensive callusing leads to variability among the regenerated plants.
- Vitrification: Abnormal looking plants. It is because of liquid medium, it can be over come by conditioning.
- Vulnerability of micro-propagated plants to transplanting shock.
- Tissue culture induced variation.

Somaclonal variation

It is the variation among the tissues or plants derived from the In-vitro somatic cell culture i.e. Callus and suspension culture.

Variations are of two types:

1. Gamatoclonal variation
2. Protoclonal variation

Factors effecting soma clonal variation

1. Degree of departure from organized growth.
2. Genetic constitution of the donor plant
3. Culture environment.
4. Tissue source.

Significance of somaclonal variation in crop improvement.

1. It is an important alternative for creation of variation in such crops, which are extensively propagated by tissue culture.
2. This is help full in breaking linkages between certain undesirable genes.
3. New varieties are developed in tomato, sugarcane, celery, brassica and sorghum.

4. It has greater advantage for crop improvement in apomictic and vegetatively propagated crops and also in cross-pollinated crops with narrow genetic base.
5. In India somaclonal variants of medicinal plant Citronella javaI has been released as a commercial variety B-13, which gives higher yield and oil content.
6. Pusa Jai Kisan- Brassica juncea as somaclonal variant of Varuna variety.

- **Protoplast culture**

Protoplast culture or somatic hybridization

Protoplast is a nacked cell without cell wall surrounded by plasma membrane and potentially of cell wall regeneration growth and division.

It includes following steps.

1. Isolation of protoplast.
2. Fusion of the protoplast of the desired species or varieties.
3. Selection of somatic hybrid cells.
4. Culture of the hybrid cells and regeneration of hybrid plants from them.

Applications:

1. Used to produce symmetric hybrids and asymmetric hybrids.
2. Hybrids can also be produced
3. Used for anther or pollen cultures

Pollen culture

Pollen culture

- Haploids plant can be obtained from pollen grains by planting an anther or isolated pollen grains on a suitable culture medium, this is known as anther or pollen culture.

Applications of Pollen culture:

- It is useful in development of haploids.
- Haploids have been obtained by pollen culture in wheat, barley, rice. By doubling the chromosome number of haploid we get homozygous diploids.

- **Ovule culture**

Ovule culture

Regeneration of whole plant from the ovule in the nutrient medium is called ovule culture/ovary culture. Here we can use two types of ovules.

1. Fertilized
2. Unfertilized

Application of tissue culture in crop improvement

1. Generation of variability.
2. Development of haploids.
3. Embryo rescue.
4. Somatic hybridization.
5. Selection for diseases resistance.
6. Selection for salinity and metal toxicity.
7. Selection for drought resistance.
8. Micro-propagation.
9. Preservation of germplasm.

Biotechnology in Plant Breeding

Introduction

- The new tools of molecular biology have broadened the scope of gene manipulations at the level of specific DNA segments to produce novel genomes with enhanced levels of resistance to biotic and abiotic stresses, physiological efficiency, quality of product and yield potential.
- The current plant breeding programme are being complimented by biotechnology which involves the utilization of biological agents such as micro organisms, cells of higher plants and animals for the isolation of useful products.
- The molecular biology is applied in the plant breeding for the identification, location, isolation, characterization, cloning and transfer of specific genes in crops plants.

- **Molecular marker systems**

Molecular marker systems:

1. Restriction fragment length polymorphism (RFLP)
2. Randomly amplified polymorphic DNAs (RAPDs)
3. Sequence tagged sites (STS)

4. DNA Amplification Fingerprinting (DAF)

5. Amplified fragment length polymorphism (AFLP)

Randomly amplified polymorphic DNAs

Randomly amplified polymorphic DNAs (RAPDs)

- This is a PCR (Polymerase chain reaction) based technique where a single short oligonucleotide primer which binds to many different loci, is used to amplify random sequences from a complex DNA template such as plant genome.
- For most plants the primers that are 9-10 nucleotide long are expected to generate 2-10 amplification products.
- The primers are generally of random sequence, biased to contain at least 50% GC content and to lack internal repeats.
- The products are easily separated by standard electrophoretic technique and visualized by UV illumination of ethidium bromide stained gels.

- **Molecular maps**

Construction of molecular maps

It involves 5 major steps:

1. Identification of divergent parents
2. Generation of a suitable mapping population
3. Identification of polymorphic probe-enzyme combination and informative primers
4. Analysis of marker segregation in the mapping population
5. Establishment of linkage.

Divergent parents

Identification of divergent parents:

- A survey of the available germplasm is made for existing morphological diversity.
- A preliminary survey can be carried out using a few DNA markers to identify divergent parents among the available lines.

- It is advantageous to select parents which posses constricting traits of agronomic importance, so that genes for those traits can also be simultaneously localized on map

Analysis of marker segregation

Analysis of marker segregation in the mapping population

- The individual plants of the mapping population are analyzed using the same primers which detect polymorphism between parents.
- The plants are categorized as parent 1 type, parent 2 type or F1 depending on the size of the polymorphic fragments.
- The observe frequency of the plants based on the marker locus genotypes is compared with the expected segregation ratio of 1:2:1 in case of F2 and 1:1 in case other populations. Based on the chi-square analysis, the observed segregation pattern is tested for possible distortion.

Generation of a suitable mapping population

- Selected plants are crossed to obtained f1 hybrids from which four different mapping population can be generated: F2 (by Selfing a single F1 plant) , BC (by crossing F1 with either of parents) ,Recombinant Inbredlines (by Selfing individual F2 plants

for 7-8 generations), and doubled haploids (by invitro culture of anthers or pollens from the F1 followed by chromosomes doubling).50-100 F2 plants are required for construction of linkage map.

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Identification of polymorphic probe-enzyme combination and informative primers

- For RFLP analysis, several DNA probes should be tried in combination with set of restriction enzymes to find the polymorphic probe-enzyme combinations for the parents.
- One or two enzymes such as Eco RI and Hind III were sufficient to detect high level of DNA polymorphism

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Application Of DNA-markers

Application Of DNA-markers In Plant Breeding:

- Estimation of Genetic Diversity
- Characterization of Germplasm Resources
- Identification of crop varieties
- Marker aided selection

Estimation of Genetic Diversity

1. Divergent parents carrying different favorable genes are used in crossing programme for realizing trsangressive segregants in self-pollinated crops and for creating heterotic hybrids in cross pollinated crops.
2. DNA selected for diversity analysis should preferable belongs to non-overlapping expressed part of the genome. The RFLP markers, if included in the analysis should be generated with cDNA probes.
3. For genotyping large number of collections available in the gene bank, a PCR based marker system will serve better by way of saving time and efforts.

Germplasm Resources

Characterization of Germplasm Resources

1. Molecular markers can aid maintenance and characterizations of bio diversity in a variety of ways. Due to limitation of space, time and effort, it may not be possible to maintained all the germplasm collections.
2. Molecular markers can be used for identification and elimination of duplicate accessions entered in different names and thus reduce the number of accession to be actually reserved. Simultaneously, employing markers link to traits of agricultural importance, useful accession in the germplasm reserve can be identified, evaluated for trait diversity and efficiently utilized in crop breeding.
3. Molecular markers have successfully been used for identification of duplicates and determination of the extent of allelic diversity both at inter and intra varietals or land race levels.

Identification of crop varieties

Identification of crop varieties:

1. Unambiguous differentiation and identification of crop varieties is essential for their registration, protection,

utilization and quality control of seeds.

2. DNA fragment profiles obtained with different marker systems should provide fingerprints highly characteristic of varieties.
3. Molecular markers systems have been extensively used for identification of crops cultivars, somatic hybrids and cybrids. Genetic stocks such as somaclones and cytoplasmic male sterile lines in different crops.

Marker aided selection

Marker aided selection:

1. Selection of a genotype carrying desirable gene or gene combination via linked marker is called MAS.
2. Breeders practice MAS when an important trait, that is difficult to assess, is tightly linked to another Mendelian trait which can easily scored.
3. MAS involve scoring for the presence or absence of a desired plant phenotype indirectly based on DNA banding pattern of linked markers on gel or on autoradiogram depending on the marker system.
4. The rationale is that the banding pattern revealing parental origin of the bands in segregants at a given marker locus indicates presence or absence of a specific chromosomal segment which carries the desired allele. This increases the screening efficiency in the breeding programmes in a number of ways such as.

5. Gene introgression and elimination of linkage drag
6. Molecular markers can aid introgression and impart greater precision. With the use of isozyme marker it has been possible to introgress nematode resistance in to the cultivated tomato from one of its wild relatives.
7. It is often observed that the desirable genes such as those for disease resistance remain linked with undesirable weedy characteristic of the alien species. During gene introgression by back crossing, the linked undesirable genes are also get transferred to the recipient parent. This has been referred as linkage.

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Gene pyramiding

Gene pyramiding:

1. Pyramiding of genes has been suggested as an effective way to provide durable form of disease and insect resistance in crop plants. Because of difficulty involved and number of years it requires, development of lines carrying genes for resistance against several races of a single pathogen/insect or different biotic stresses has not been very successful.
2. Recently successful pyramiding of four genes, Xa4, Xa5, Xa13 and Xa21 conferring resistance to four different races of bacterial blight has been achieved in rice by using molecular markers.

Development of heterotic hybrid:

- Molecular markers based analysis can be utilized to identify specific genomic regions containing QTLS for yield showing dominance or over-dominance gene action in f2 of a cross between two inbred lines.
- This will enable analysis of an existing cross in terms of the number of QTLS involved and the magnitude of there effect on the measured trait. Subsequently additional Inbredlines can be tested against standard lines for the presence of additional dominant or over-dominant QTLS. These could then be incorporated into the standard lines by marker based introgression. In this way existing inbred can be improved first and then used to develop hybrids for realizing higher heterosis than in the existing ones.

Limitations of molecular markers

Limitations in the use of molecular markers

- The cost and time required to undertake genetic analysis, besides lack of detectable polymorphism in certain crops also restricts the use of such markers.
- High density maps have been developed using very wide crosses but the extent of polymorphism is much less in routine breeding populations particularly in self pollinated crops.

Varietal release

Before a variety is released and reaches to the farmer, the All India Co-ordinated Crop Improvement Project identifies the variety for release in its workshop as per the established norms for testing for its value for cultivation. Presently, All India Co-ordinated Crop Improvement (AICCPs) Projects have been created for almost for all the crops or groups of crops (Paroda, 1992).

AICCIPs follow a three tier system of multilocation evaluation spread over a minimum of three years involving the following stages:

- First year - (IET, Initial evaluation trial)

- Second year — Advance varietal trial — I (AVT - 1)
- Third year — Advance varietal trial — II (AVT-II)

Evaluation of test entries

The initial evaluation trial includes the following entries:

- The entries to be nominated must have undergone critical evaluation/screening in the station/ station trials conducted by the sponsoring breeder. Secondly, the entries to be Nominated must have high degree of phenotypic uniformity and genotypic stability.
- The IET is conducted across the zones of all over the country along with check varieties. A minimum of three check varieties comprising of the following is used. i. National check, ii. Zonal check, iii. Location check, IV. Qualifying check
- All the trials are monitored by a team of scientists deputed by project coordinator to record the observations on the uniformity of crop stand, disease and insect-pest, resistance, bird damage etc.
- The main composition of the monitoring team is:

a) Project Director/Project Co-coordinator/PI/Zonal Co-ordinator Team leader

b) Breeder Member

c) Agronomist Member

d) Pathologist/Entomologist Member

e) Scientist from any other specified discipline Member

- The observations recorded according to guidelines on the data books for further supply to the co-ordinators
- The data received at the co-coordinator's cell is critically examined for the inclusion in the Annual Report.
- Outstanding performance for yield of a variety by a margin of 10% over the best performing check is promoted to advance varietal trial.

Advance varietal trials

Advance varietal trials (AVT-1)

- Advance varietal trial is constituted by the entries promoted from IVT on the criteria specified above.
- Limited number of entries in AVT-1 (not exceeding 16) is tested along with a minimum of three checks comprising of national check, zonal check and local check.
- All these entries are evaluated in a randomized block design with 3-4 replications at the different locations.
- The monitoring is done by the same team as given for IVT.

- Besides the agronomic and morphological observations, the additional data may be generated by the co-operators on disease and insect-pest resistance, and quality.
- Again, if a variety gives significant superior performance by a margin of 10% over the, best performing check in combination with other attributes is promoted to next stage

Advance varietal trials (AVT-II)

- Same steps are followed as mentioned under AVT-1. However, the additional data to be generated at this stage.
- Response to agronomic variables such date of sowing, population densities and weedicide may be recorded.
- Data on diseases, insect-pests, quality parameters and abiotic stresses may also be recorded and discussed during the workshop.
- If the variety gives outstanding performance over the check (by a margin of 10%) besides having all the favourable attributes, then the proposal for identification of a given variety is submitted by the concerned breeder on a variety identification proforma specified by the ICAR

Variety Identification System

- The proposal containing all available data for the variety is considered by the variety identification committee constituted by the ICAR which meet during each AICCIP workshop.
- Recommendations are made for country-wide release or for a specific zone or states.
- Afterwards, the sponsoring agricultural university/research institute then submits the proposal for its release and notification to central sub-committee with the support of the Project.
- Once the Central sub-committee accepts the proposal, the variety/hybrid is released for the specific state or zone. The release proposal also ensures the availability of enough seed stock.

State Varietal Identification System

- The State Seed Sub-Committee (SSSCs) is constituted by Central Seed Committee and these SSSCs have been given responsibility to set up a State, Seed Certification Agency (SSCA), a State Seed Laboratory, and to appoint a seed analyst and seed inspectors.
- The SSCC is responsible for the release of a variety in its own state on the basis of data generated by State Agriculture University.

- The concerned breeder along with agronomist, pathologist, entomologist and biochemist generate sufficient data (usually more than three years).
- Secondly, sample variety must be evaluated in All India Co-ordinated crop improvement projects trials.
- Thirdly, on-farm trial data for a year or two are collected by extension agencies of State Department of Agriculture.
- After having all the above information, the State Agriculture University deliberates on the release proposal of a variety in a series of meetings before recommending to the SSC.
- Once approved by the SSSC for release in a state, the variety is requested to be notified for seed production purpose by the Central Sub-committee.

Intellectual Property Rights

- Intellectual property is a legal field that refers to creations of mind such as musical literacy, artistic work, inventions symbols, names, images, and designs used in commerce, including copyright, trademarks, patents & related rights. Under Intellectual Property Law the holders of one of these abstract properties have certain exclusive rights to the creative works, commercial symbols or inventions which is covered by it.

- Depending on the nature of innovation, the subject matter involved and the manner in which it is used for gaining economic benefit, the intellectual property is divided into two basic groups; the industrial property and copyrights.
- Copyright automatically assigns the exclusive right to the creative owner of a literary, all performing art like dramatic, musical or artistic work, paintings, photographs, sculptures advertisements, maps, technical drawings, cinematograph film, photography, sound or video recording, broadcast/telecast, phonograms, a computer programme, etc to reproduce the work, to issue copies to or perform or communicate in public, make any translation or an adaptation of the work, offer on sale or rental and to authorize others for doing so.
- The industrial property includes patents, trademarks, industrial designs, trade secrets and geographical indications. Among these, patent assumes highest importance in view of its impact on technological and economic aspects of peoples and nations. The earliest industrial property act was enacted in Europe.
- This was followed by legislation of US Patent Act in 1793. In 1883, eleven western countries joined together to establish the Paris Convention for the Protection of Industrial Property, which was aimed to harmonize the IPR law and practices across member countries. Currently 169 countries are its members. Pre-Colonial India had an IPR regime that was practiced by the Britain.
- India is a signatory to the TRIPS Agreement hence it modified its patents law in conformity with TRIPS Agreement. We should also consider the use of Information and Communication Technology (ICT) for an effective enforcement of the Intellectual Property Rights in India. It is desirable that more initiatives on the

lines of ICT HELPDESK must be undertaken so that contemporary International Standards can be adopted in India too.

The protective umbrella of TRIPS covers;

7. Copyright and Related Rights
8. Trademarks
9. Geographical Indications
10. Industrial Designs
11. Patents
12. Layout designs of Integrated Circuits and
13. Protection of Undisclosed Information.

- Intellectual property rights give creators exclusive rights to their creations, thereby providing an incentive for the author or inventor to develop and share the information rather than keep it secret. The legal protections granted by Intellectual property laws are credited with significance contributions toward economic growth.
- Economists estimate that two third of the value of large businesses in the USA can be traced to intangible assets, Likewise industries which rely on intellectual property protections are estimated to produce 72% more value per added employee than non intellectual property industries.
- Intellectual Property (IP) has emerged as a strong tool for building and sustaining competitive advantage in the contemporary knowledge economy. Both domestic and international competition is boosted by the

scientific and technological progress in a sustainable manner by utilizing the Intellectual Property Rights (IPRs).

- There is a direct rationale for providing a healthy and global system for protection of IPR, due to their role in wealth creation. Moreover, the technology, investment and trade, which are key drivers of economic growth, are imbedded in IPR protection.
- The Indian Intellectual Property Office is dedicated to mobilize the use of intellectual property for economic and social development by creating an IP (Intellectual Property) culture and enhancing knowledge & competencies is synchronizing with the global environment.

Orthodox or conventional IPRs

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- These are the conventional IPRs used by individuals, companies, association of persons etc.
- They are further segregated in different types like Copyright, Patent and Trademark.

Copyright

Copyright

- Copyright is an IPR deals with expression of idea not an idea itself. Copyright comes into existence immediately after expression and requires no formal registration.
- Copyright is an expression and manner of expression.
- It protects original literary works, ie books, novels, lyrics, songs, computer programmes, etc.
- Whenever these are created, they become the property of the creator.

Copy right law is used in creative and artistic works (For example: books, movies, music, paintings, photographs and software) and gives the holder the exclusive right to control reproduction or adaptation of such works for a certain period of time (historically, a period of between 10-30 years depending upon jurisdiction, but more recently the life of the author, plus several decades)

Patents

- Patent is a right exclusively used for ideas but it is of commercial use. Patents are generally of three types viz. Novelty, non-obviousness and novel art.
- A patent is the right of an individual or of a company/ organization to gain profit from a particular invention or unique manufacturing process.
- A patent is an intellectual property relating to scientific and technological inventions.
- It is granted by the government of the country to the applicant and gives the inventor the right for a limited period to prevent others from using that invention in any form without permission.
- Like any other form of property, a patent can be transacted purchased, sold or even mortgaged.

- Patent law protects inventions it gives the patent holder a right to prevent others from participating and practicing the inventions without a license from the inventor for a certain period of time.
- Patents can protect the functional features of a process, machine, manufactured item, asexually reproduced plant or composition of matter.
- The main Amendments to Indian Patent Act are; new chemical entity will not be treated as patent, they must pass the test to complete patent and registration in any member country is applicable to all members of WTO.

Trademark

- Association of traders name with the commodity traded gives rise to trade mark.
- A trademark is a distinctive sign, which is used to distinguish the products or services of different businesses.
- Trade mark law protects words, phrases, logos, symbols, sound or even smell or combinations used to distinguish one product from another, which gives a distinct identity to a good.
- In circumstances where a competitor uses a protected trademark can go to court and obtain an injunction to stop the use.
- Trade secret protection helps protect the secrets of a product or work.

Unorthodox or Innovative IPRs

- These are the new entrants in the field of IPRs.

- All due to globalization and whole world has become a single platform, so do these gained importances.
- They include Industrial Design, Geographical Indications, Plant Varieties, Semi conductor layout design (SCLD).
- Out of these four semi conductor layout design is more vulnerable to copying. SCLD law was brought in reference to agreements of TRIPs.
- A design is the presentation of a product resulting from features of colour, size, shape, texture or materials of a product or packaging.
- An industrial design right protects the form of appearance, style or design of an industrial object for example: Spare parts, Furniture, textiles etc.

Cyber Law

- It is a term used to describe the legal issues related to use of communications technology, particularly cyberspace i.e. the internet.
- It is an interaction of many legal fields, including intellectual property, privacy, freedom of expression and jurisdiction.
- In essence cyber laws are designed for the physical world to human activity on the internet.

Geographical Indications of goods

- In recent years, the Indian government has become aware of the importance of GI, following the debacles related to Basmati and Turmeric GI is a notice of a definite product have been produced in a particular place.
- The producer can use this sign only for products from the specified region.
- Unlike a trademark however the GI is not an individual property for use by the geographical indication of that region which any producer may use.

Organizations involved in IPR

Organizations involved in IPR in India

- The Ministry of Commerce and Industry, Ministry of Human Resource development, Ministry of Information and Broadcasting, CII – Confederation of Indian Industries, FICCI- Federation of Indian Chambers of Commerce and Industries, ASSOCHAM- Associated Chambers of Commerce and Industries.
- Department of Science and Technology (TIFAC-Technical Information Forecasting and Assessment Council)

World Intellectual Property Organization (WIPO) of the UN, based in Geneva is the apex intergovernmental body dealing with IPRs.

Plant Varieties Protection and Farmers Rights Act (2001)(PPVFR)

Criteria for registration of varieties UPOV1991

- Registration of the variety will not be done in case the variety does not fulfill the criteria.
- **Novelty**: A variety should not have been commercially exploited more than one year.
- **Distinctiveness**: Distinguishable for characters.
- **Uniformity**: uniform in appearance in specified environment.
- **Stability**. Stable in appearance over successive generations in specified environment.

Rights of breeders and farmers

Rights of Breeder or his Successor

- A certificate of registration for a variety shall confer an exclusive right on the breeder or his successor, his agent or license to Produce, Sell, market, Distribute and Import or export the variety

Farmers' Right

- Farmer who has bred or developed a new variety is entitled for protection as a breeder of a variety at free of cost.
- Farmer, who is engaged in conservation of genetic resources of land races and wild species entitled for recognition and reward from the National Gene Fund.
- Farmers will be entitled to save, use, sow, re-sow, exchange, share, store and sell his farm produced seed of a variety, protected under this act.
- However the farmer is not entitled to sell as branded seed of a protected variety.
- The pivotal importance of the farmer having the right to sell seed has to be seen in the context of seed production in India, where the farming community is the largest seed producer providing about 80 per cent of the country's annual requirement

********Thanks********

All the best