PLANT BREEDING

- India is mainly an agricultural country. Agriculture accounts for approximately 33 per cent of India's GDP and employs nearly 62 per cent of the population. After India's independence, one of the main challenges facing the country was that of producing enough food for the increasing population. As only limited land is fit for cultivation, India has to strive to increase yields per unit area from existing farm land.
- Traditional farming can only yield a limited biomass, as food for humans and animals. Better management practices and increase in acreage can increase yield, but only to a limited extent. Plant breeding as a technology has helped increase yields to a very large extent.
- The development of several high yielding varieties of wheat and rice in the mid-1960s, as a result of various plant breeding techniques led to dramatic increase in .food production in our country. This phase is often referred to as the Green Revolution .
- Green revolution was dependent to a large extent on plant breeding techniques for development of highyielding and disease resistant varieties in wheat, rice, maize, etc .
- "Plant breeding is the purposeful manipulation of plant species in order to create desired plant types that are better suited for cultivation, give better yields and are disease resistant".
  Conventional plant breeding has been practiced for thousands of years, since the beginning of human civilization; recorded evidence of plant breeding dates back to 9,000-11.000 years ago.
- Classical plant breeding involves crossing or hybridisation of pure lines, followed by artificial selection to produce plants with desirable traits the traits or characters that the breeders have tried to incorporate into crop plants are
  (i) increased crop yield and improved quality
  (ii) Increased tolerance to environmental stresses (salinity, extreme temperatures, drought)
  (iii) resistance to pathogens (viruses, fungi and bacteria)
  (iv) increased tolerance to insect pests .
- Plant breeding programmers are carried out in a systematic way worldwide-in government institutions and commercial companies.
- The main steps in breeding a new genetic variety of a crop by selection and hybridization are -

(I) Collection of variability : Genetic variability is the root of any breeding programmer. In plant breeding collection of variability is done by collection of plant varieties.

It consists of -

(i) Cultivated improved varieties
(ii) Improved varieties that are no more cultivated
(iii) Old local or desi varieties.
(iv) Varieties produced by plant breeders (undistributed)
(v) Wild species related to the· crop species

The entire collection (of plants/seeds) having all the diverse alleles for all genes in a given crop is called germplasm collection

Significance of germplasm of wild species :-

- It is very important to conserve wild species of plant as these are highly resistant to insects, pests, disease and unfavourable growth conditions, which are necessary for survival' of plants.
- The loss of wild plants, will reduce the genetic variability and will be a great loss to gene pool.

(II) **Evaluation and selection of parents:-** The germplasm is evaluated so as to identify plants with desirable combination of characters. The selected plants are multiplied and used in the process of hybridisation. Pure lines are created wherever desirable and possible.

(III) **Cross hybridisation among the selected parents:-** The desired characters have very often to be combined from two different plants (parents). for example high protein quality of one parent may need to be combined with disease resistance from another parent. This is possible by cross hybridising the two parents to produce hybrids that genetically combine the desired characters in one plant. This is a very time-consuming and tedious process since the pollen grains from the desirable plant chosen as male parent have to be collected and placed on the stigma of the flowers selected as female parent. Also it is not necessary that the hybrids do combine the desirable characters: usually only one in few hundred to a thousand crosses shows the desirable combination.

(IV) **Selection and testing of superior recombinants:-** This step consists of selecting among the progeny of the hybrids, those plants that have the desired character combination. The selection process is crucial to the success of the breeding objective and requires careful scientific evaluation of the progeny. This step yields plants that are superior to both of the parents (very often more than one superior progeny plant may become available). These are self-pollinated for several generations till they reach a state of uniformity (homozygosity?), so that the characters will not segregate in the progeny.

(V) **Testing, release and commercialisation of new cultivars:-** The newly selected lines are evaluated for their yield and other agronomic traits of quality, disease resistance, etc. This evaluation is done by growing these in the research fields and recording their performance under ideal fertiliser application irrigation, and other crop management practices. The evaluation in research fields is followed by testing the materials in farmers' fields, for at least three growing seasons at several locations in the country, representing all the 'agroclimatic zones where the crop is usually grown. The material is evaluated in comparison to the best available local crop cultivar - a check or reference cultivar.

♦ In India, Indian Council of Agricultural Research, New Delhi (ICAR - New Delhi) carries out the evaluations.

♦ Ultimately a new pure line, population or hybrid that is superior to the existing varities as well as to other new material may be released as new variety.

Mutation breeding

Use of induced mutations in plant breeding to develop improved varieties. Induced mutations are useful in specific situations, when the desired alleles are absent in the germplasm.

♦ Many important varieties in different crop plants have been produced by mutation breeding

♦ **In wheat :** Sharbati sonora and pusa Ierma are two important varieties of wheat produced by gamma rays treatment of sonora-64 and Ierma rojo (Mexican dwarf wheat varieties)

♦ Sharbati-sonora is amber grain coloured variety of wheat produced by Dr. M.S. Swaminathan and is responsible for green revolution in India.

♦ **In rice :** About 45 varieties up to 1992 have been produced by mutation breeding eg Remei & Atomita-2. In mung bean, resistance to yellow mosaic virus and powdery mildew were induced by mutations.

♦ Mutation breeding has some important limitations as :

(i) Most of the mutations are recessive

(ii) Mutation rate is extremely low.

(iii) Most of the induced mutation are invaluable to the breeders and many of them are lethal.

(iv) Stability of mutant is sometimes doubtful, as some mutants have tendency to revert back to original type.

APPLICATION OF PLANT BREEDING

(I) Plant breeding for improvement-of yield:-

(a) Improvement in wheat :

- Prior to green revolution a dwarfing gene of wheat named norin-10, was noted in Japan and picked up by American plant breeder.
- Dr.N. Borlaug (Mexican wheat breeder) develop many dwarf wheat varieties like Sonora-64 and Lenna rojo-64
- N. Borlaug got Nobel prize for peace in 1970. He is also known as father of green revolution.
- In 1963 two Mexican wheat varieties viz. Sonora-64 & Lenna rojo-64 and a Japanese variety Norin-10 were introduced in India, but these varieties could not adopt to Indian conditions, they were subjected to mutations and selections at Indian Agricultural Research Institute at New Delhi under the direction of Dr. M.S Swaminathan.
- Dr. M.S. Swaminathan :- He is pioneer in mutation breeding. He has produced Sharbati sonora variety of wheat by mutation, which is responsible for green revolution in India. He is known as "Father of green revolution in India"
- In 1963 several varieties such as Sonalika and Kalyan Sona, which were high yielding and disease resistant, were introduced all over the wheat-growing belt of India.
  During the period 1960 to 2000, wheat production increased from 11 million tonnes to 75 million tones

(b) Improvement in rice :-

- Dwarfing gene "dee-geo-woo-gen" was noticed in Taiwan .
- This gene produced many improved varieties in rice .
- This gene was picked up by
  IRRI (International Rice Research Institute) Manila (Philipines) and incorporated to produce high yielding early maturing IR-8 and IR-24 varieties.
- Gurdev S. Khush and his team crossed 13 varieties of rice from six countries and wild rice Oryza nivara (from India) to develop IR-36 variety of rice.
- IR-36 variety of rice is resistant of grassy stunt virus .
- IR-36 is the high yielding variety of rice and has solved major food problem in Asia .
- Later better-yielding semidwarf varieties Jaya and Ratna were developed in India .
- During the period 1960 to 2000 rice production increased from 35 million tonnes to 89.5 million tonnes.

(c) Improvement in sugar cane :- Saccharum barberi was originally grown in north India, but had poor sugar content and yield. Tropical canes grown in south India Saccharum officinarum had thicker stems and higher sugar content but did not grow well in north India. These two species were successfully crossed to get sugar cane varieties combining the desirable qualities of high yield, thick stems, high sugar and ability to grow in the sugar cane areas of north India.

(d) Millets: Hybrid maize, jowar and bajra have been successfully developed in India. Hybrid breeding have led to the development of several high yielding varieties resistant to water stress.

(ii) Plant breeding for disease resistance :-

A wide range of fungal bacterial and viral pathogens, affect the yield of cultivated crop species, especially in tropical climates. Crop losses can often be significant, up to 20-30 per cent, or sometimes even total. In this situation, breeding and development of cultivars resistant to disease enhances food production.

Some of the diseases caused

By fungi are rusts, e.g., brown rust of wheat, red rot of sugarcane and late blight of potato;

By bacteria - black rot of crucifers

By viruses - tobacco mosaic, turnip mosaic, etc.

Some crop varieties breed by hybridisation and selection, for disease resistance to fungi, bacteria and viral diseases are released.

Table

Crop	Variety	Resistance to diseases
Wheat	*Himgiri*	*Leaf and stripe rust, hill bunt*
Brassica	Pusa swarnim (Karan rai)	White rust
Cauliflower	Pusa Shubhra, Pusa Snowball K-1	Black rot and Curl blight black rot
Cowpea	Pusa Komal	Bacterial blight
Chilli	Pusa Sadabahar	Chilly mosaic virus, Tobacco mosaic virus and Leaf curl

Several wild relatives of different cultivated species of plants have been shown to have certain resistant characters but have very low yield. Hence, there is a need to introduce the resistant genes into the high-yielding cultivated varieties. Resistance to yellow mosaic virus in bhindi (Abelmoschus esculentus) was transferred from a wild species and resulted in a new variety of A. esculentus called Parbhani kranti.

(iii) Plant Breeding for developing resistance to insect pests :-

Another major cause for large scale destruction of crop plant arid crop produce is insect and pest infestation. Insect resistance in host crop plants may be due to morphologieal, biochemical or physiological characteristics. Hairy leaves in several plants are associated with resistance to insect pests, e.g, resistance to jassids in cotton and cereal leaf beetle in wheat.

In wheat, solid stems lead to non-preference by the stem sawfly and smooth leaved and nectar-less cotton varieties do not attract bollworms.

High aspartic acid, low nitrogen and sugar content in maize leads to resistance to maize stem borers. Some released crop varieties bred by hybridisation and selection, for insect pest resistance are given in Table.

Table

Crop	Variety	Insect Pests
Brassica (rapeseed mustard)	Pusa Gaurav	Aphids
Flat bean	Pusa Sem 2, Pusa Sem 3	Jassids, aphids and fruit borer
Okra (Bhindi)	Pusa Sawani Pusa A-4	Shoot and Fruit borer

(iv) Plant breeding for improved food quality :-

More than 840 million people in the world do not have adequate food to meet their daily food and nutritional requirements. A far greater number- three billion people- suffer from micronutrient, protein and vitamin deficiencies or 'hidden hunger' because they cannot afford to buy enoughfruits, vegetables, legumes, fish and meat.

Diets lacking essential micronutrients- particularly iron, vitamin A. iodine and zinc- increase the risk for disease, reduce lifespan and reduce mental abilities.

Biofortification - breeding crops with higher levels of vitamins and minerals or higher protein and healthier fats is the most practical means to improve public health. Breeding for improved nutritional quality is undertaken with the objectives of improving -

(i) Protein content and quality;
(ii) Oil content and quality;
(iii) Vitamin content; and
(iv) Micronutrient and mineral content

In 2000, maize hybrids that had twice the amount of the amino acids, lysine and tryptophan, compared to existing maize hybrids were developed.

Wheat variety, Atlas 66, having a high protein content, has been used as a donor for improving cultivated wheat.

It has been possible to develop an iron-fortified rice variety containing over five times as much iron as in commonly consumed varieties.

The Indian Agricultural Research Institute, New Delhi has also released several vegetable crops that are rich in vitamins and minerals,

e,g,. vitamin A enriched carrots, spinach, pumpkin;
vitamin C enriched bitter gourd, bathua, mustard, tomato;
iron and calcium enriched spinach and bathua, and
protein enriched beans- broad, lablab, French and garden peas.

PLANT TISSUE CULTURE

- This is one of the latest and most promising methods of crop improvement in such plant, where all other conventional methods of breeding fail.
- Tissue culture technique is based on totipotent nature of plant cell.
- Plant tissue culture is the technique of maintaining and growing plant cells, tissues and organs in sterilized culture medium, under controlled aseptic conditions (in-vitro).

Explant - Plant part that is excised from its original location and used for initiating a culture, it may be root tip, shoot bud, anther, embryo, ovule etc. Normally undetermined cells of plant are used as explants.

Surface Sterilization - The process of treatment of explant with specific antimicrobial chemicals like sodium hypochlorite, H_2O_2, Bromine water, C_2H_5OH etc.

- ♦ The vessels, media and instruments are also sterilized by treating them with steam, dry heat or alcohol.

Culture medium or nutrient medium - Medium, which provides nutrition to explants & which is required for normal growth and development of explants.

- ♦ Standard culture medium contains inorganic Salts, Vitamins, Sucrose (as a source of energy and carbon), growth regulators (2,4-D, Cytokinins, BAP-benzylaminopurine)
- ♦ Growth regulators are required for cell division and organogenesis in explant .
- ♦ Murashigice and Skoog's culture medium (Most commonly used culture medium.)

Callus - By culturing explant in culture medium an undifferentiated mass of cells is obtained which is known as 'callus'.

Types of Cultures -

(1) Callus culture & Suspension culture.
(2) Meristem culture
(3) Embryo culture
(4) Anther culture
(5) Protoplast culture

(1) Callus & Suspension culture-

Callus culture - In callus cultu;e when an explant is placed on a medium gel with agar many of the cells become meristematic and begin to divide and giving rise to callus in 2-3 weeks. The agar medium contains both type of growth regulators auxin like 2,4-D and cytokinin like BAP.

Suspension culture - In case of suspension culture a single cell or small group of cells is placed on liquid medium. The medium normally contains the auxin 2,4-D . These cells devide and form small groups of cells. The suspension cultures are continuously agitated to break the cell mass in to smaller clumps and single cells and also maintain uniform distribution of cells and cell clumps in the medium. It also allows gaseous exchange. Suspension cultures grow much faster than callus culture. Suspension culture can be maintained in either of the following two forms -

(i) **Batch culture :** are initiate as single cells in a flask and are propagated by transferring regularly small groups of suspensions to a fresh medium.
(ii) **Continuous culture :** are maintained in a steady state for long period by draining out the used medium and adding from new medium.

Formation of plantlets :-

- ♦ **Gottlied Haberlandt** was first one who grow isolated leaf cells by plant tissue culture.
- ♦ **Totipotency :-** The ability of a plant cell to regenerate into a complete plant let.
- ♦ **Regeneration** can be described as the development of specific structure like root, shoot or somatic embryo from cultured cells.

Shoot and root formation :-

- ♦ The two important components of a plant are root and shoot.

- ♦ The regeneration of root and shoot is controlled by two types of growth regulators.
- ♦ The auxin like NAA (Naphthaline Acetic Acid) promotes root regeneration whereas cytokinins like BAP promotes shoot regeneration.
- ♦ Callus cultures first kept on medium containing BAP, which initiates shoot formation from the callus.
- ♦ When shoots become 2-3 cm. long, the culture is transferred to a medium containing auxin. Roots develop from the lower ends of these shoots and develop into young plant called plantlet.

Somatic embryo regeneration :-

- ♦ Somatic embryo or embryoids are non-zygotic embryo like structures that develop in vitro cultures from somatic cells.
- ♦ The medium having ammonium nitrate and auxin (2,4-D) favour induces their differentiation.
- ♦ Mature somatic embryos germinate to yield complete plantlets.

(2) Meristem culture :-

- ♦ Use of an explant that contains pre-existing shoot meristems and produce shoot from them
- ♦ Explant (shoot tips or nodal segments) are cultured on a medium containing cytokinin (usually BAP)
- ♦ Cytokinin promotes axillary branching by overcoming apical dominance .
- ♦ When the plantlet has grown a few leaves it is transferred to soil after hardening.

Meristem culture can be used for :-

- Rapid clonal multipiication.
- Production of virus free plants.
- Conservation of germplasm.
- Production of transgenic plants.

(3) Embryo culture :-

- ♦ Application of embryo culture method to prevent the abortion of the interspecific hybrid embryo is called Embryo rescue.
- ♦ Excision of young embryos from developing seeds and their cultivation on a nutrient medium is called embryo culture.
- ♦ **Aim :-** Aim of embryo culture is to allow to young embryos to develop into complete seedlings & overcoming hybridization barriers.

Applications :-

(I) In some interspecific crosses the endosperm of developing hybrid seeds degenerate very early so young hybrid embryo which gets devoid or nutrition also dies in such cases the young hybrid embryo is excised and cultured in vitro to obtain hybrid seedling.

(II) Seeds of some plants like orchid lack stored food. In such cases embryo culture allows seedling development from the embryos. This method is also used for rapid clonal propagation in orchid.

(III) In some species seeds may remains dormant due to inhibitors present in the endosperms/seed coat.

- ♦ Embryo culture in such cases allows embryo development by eliminating the inhibitors responsible for dormancy.

(4) Anther culture & haploid production :-

- ♦ Anther culture is also known as pollen grain culture or androgenic haploid culture .

- When anthers of a plant species are cultured on a suitable medium, then haploid plants are produced, this method is called anther culture.
- This technique was first used in India to produce haploid plants of Datura innoxia by Guha and Maheshwari (1964).
- Sometime diploid plants are also formed among haploid plants. Source of these diploid plants is anther wall (which is diploid)
- **In anther culture there can be two situation :-**
  (i) Nucleus within a pollen grain may continue to divide and give rise to a pollen embryo

  or

  (ii) Continued division in a pollen produces a callus which then regenerate shoots.
- Haploid can also be produced by culturing- unfertilised ovules .
  Androgenic haploid plants have some variations which are called gametoclonal variation.
  Haploid plants are very useful in plant breeding because,
  (i) They have single set of chromosome, so even a very small change or mutation can be detected in haploids.
  (ii) These haploids are used to produce homozygous diploids (by colchicine treatment) and these homozygous diploids are used as parents in crossing.
  (iii) Use of haploids in producing pure lines has reduced the period required for developing new varieties from 10 years to 5 years.

(5) Protoplast culture:-

- **Somatic hybrid :** A hybrid produced by fusion of somatic cells of two species or varieties .
- The process of production of somatic hybrid is somatic hybridisation .
- **Protoplast :** Cell wall less plant cell is called protoplast.

Steps of somatic hybridisation :-

(A) Removal of cell wall → 2 method

(i) Mechanical method → Old method

Plant tissue → **Plasmolysing solution**
↓
Cut from sharp knife → **Deplasmolysing Solution**
↓
Protoplast

(ii) Enzymic method → New method
- Discovered.by- Cocking
- In this method cell wall is digested by using pectinase & cellulase enzyme.

(B) Fusion between protoplast →
- Protoplast of two different species are fused together.
- Substance which induces the fusion of protoplast is called fusogen or fusogenic agent.
- Fusogenic substance and condition :-
  - By treatment of $NaNO_3$
  - By treatment of Ca^{+2} ions at high pH

- By treatment of polyethylene glycol [PEG]
- By high voltage electric shock

(C) Culture of the fused protoplast :-

- Product of fused protoplast of two different species is called heterokaryon .
- Heterokaryons are mainly used in tissue culture .
- When the fused protoplasts are cultured on a suitable medium they regenerate cell walls and begin to divide to ultimately produce plantlets.

Importance of somatic hybridisation :-

(i) It allows the production of hybrids between different lines and species that can not be produced normally by sexual reproduction. eg. Rice & Carrot

♦ Pomato is a somatic hybrid between potato and tomato.

♦ Bromate - Brinjal & tomato

(ii) Use of somatic hybrid

♦ For gene transfer

♦ Transfer of cytoplasm

♦ Production of useful allopolyploids.

Sp. Points :-

♦ Somatic hybridisation is also called parasexual hybridisation .

♦ First somatic hybrids were obtained between two species of tobacco Nicotiana gluca and N. langsdorfit by Carlson et. al.

CYBRID

Cybrids possess a nuclear genome from only one parent but cytoplasmic genes from both parents. The process of protoplast fusion resulting in the development of cybrid is known as cybridisation / cytoplasmic hybrids.

APPLICATION OF PLANTLETS PRODUCED BY PLANT TISSUE CULTURE

(I) Rapid clonal propagation of superior lines :- e.g. oil palm

♦ **Clone :** Group of individuals or cells derived from a single individual or cell by asexual reproduction.

♦ Multiplication of genetically identical copies of a cultivar by asexual reproduction is called clonal propagation or cloning or micro propagation.

♦ All the cells in culture are derived from a single explant by mitotic division, so all plantlets regenerate from a culture generally, have the same genotype and constitute a clone. These plantlets can be used for rapid clonal propagation of superior lines like oil palm.

Two common types of micropropogation are :-

(a) Multiple shootlet production

(b) Somatic embryogenesis

(II) Somaclonal variation :-

♦ Genetic variation presents among plants regenerated from tissue culture have been termed as somaclonal variation .

- These variations originated by minor chromosomal aberration, by gene mutation .
- The variation which are stable and have agronomic characters like resistance to diseases and pests, stress to tolerance, early maturation, better yields are useful only.

(III) To produce transgenic plants.