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Plant Breeding

Breeding is an art and science, which tells us ways and means to change the genetic architecture of plants so as to attain a particular objective. Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques Plant breeding has been practiced for thousands of years, since near the beginning of human civilization. It is now practiced worldwide by individuals such as gardeners and farmers, or by professional plant breeders employed by organizations such as government institutions, universities, crop-specific industry associations or research centers. International development agencies believe that breeding new crops is important for ensuring food security by developing new varieties that are higher-yielding, resistant to pests and diseases, drought-resistant or regionally adapted to different environments and growing condition.

Mulberry Plant breeding uses principles from a variety of sciences to improve the genetic potential of plants. The process involves combining parental plants to obtain the next generation with the best characteristics. Breeders improve plants by selecting those with the greatest potential based on performance data, pedigree, and more sophisticated genetic information.

Plants are improved for food, feed, fiber, fuel, shelter, landscaping, eco-systems services and a variety of other human activities. The aim of mulberry plant breeding is to improve the characteristics of plants so that they become more desirable agronomically and economically. The specific objectives may vary greatly depending on the crop under consideration.

The followings are the objective of plant breeding

1) Higher yield
2) Improved quality
3) Diseases and pest resistance
4) Agronomic characters
5) Photo and thermo insensitivity
6) Synchronous maturity
7) Non shattering characteristics
8) Determinant growth habit
9) Dormancy

10) Varieties for a new seasons
11) Moisture stress and salt tolerance
12) Resistance against biotic and aboitic stress
13) Elimination of toxic substances
14) Wider adaptability
15) Useful for mechanical cultivation

Achievement:

Mulberry breeding and genetics units from different Seri zones of India played an important role in involving some high yielding varieties, the leaves of mulberry provide the sole food source of silkworm, (*Bombyx mori* L) (Source-CSRTI BERHAMPORE)

1	A total no. of 143 mulberry germplasm resources had been characterized and grouped into 22 clusters.
2	Flowering time and sex expression in tropical, sub-tropical and temperate genotypes have been worked out their utilization in crossing programme.
3	C1730, a new mulberry variety giving 3% higher yield under non-irrigated condition had been evolved.
4	An improved method on induction of tetraploids through *in vitro* was developed.
5	One hexaploid, 2 pentaploids, 3 tetraploids & their respective triploids and a mixoploid had been developed.
6	Somaclonal variant of $SV_{1\ has}$ been evolved with 26 % improvement in leaf yield over S1.
7	$C_{776,}$ – a salt tolerant variety can withstand salinity stress up to an Ec of 7.8 mhos/cm has been identified through in vitro screening followed by field study. The variety has shown 28.1 % superiority in leaf yield over $S_{1635.}$
8	Mulberry Genotype RG-120 identified as highest yielder (54.97 mt/ha/year) under irrigated condition, which outyielded S-1635 (43.02 mt/ha/year) by 27.8%. The genotype is ready for AICEM Trial for Phase-III.

SELECTION

It is the oldest method of breeding process. It involves picking up of better ones from the entire crop plants.

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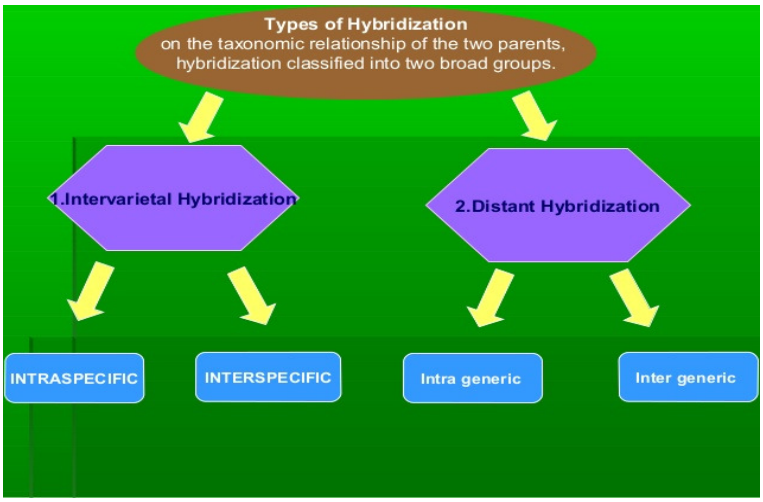
TYPES OF SELECTION:

A) MASS SELECTION: It is the simplest method followed by the farmers to improve mainly local varieties of crops. Before harvesting the crops, best looking and healthy plants are selected from the mass. Seeds of these plants are collected and sowed them to raise new generation. Mass selection is based on external character.

B) PURE LINE SELECTION: A pure line is progeny obtained from a single individual by self fertilization. A group of plants obtained from a single self fertilized homologous plant is called pure line. It involves a desirable homologous individual from the mixed population and multiplying the same.Pureline selection is the only method of improvement of local varieties of self pollinated crops. The progeny of pureline selection are similar in phenotypical and genotypical character.

C) CLONAL SELECTION: A clone is defined as a progeny of a single plant obtained by vegetative propagation.The clonal selection is concerned with the selection and propagation of best individual of clones from mixed population of vegatively propagated plant.

HYBRIDISATION -The process of crossing two or more plants together to get off springs of new desirable characters as result of genetic recombination is called Hybridization.



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TYPES OF HYBRIDISATION:

a) INTRASPECIFIC HYBRIDISATION; Crosses are made between two individuals of same species. These crosses are done to improve self pollinated crops producing homozygosity or pure offspring.

b) INTERSPECIFIC HYBRIDISATION: Crosses are made between individual of different varieties of the same species. These crosses are made to improve self pollinated and certain cross pollinated crops.

c) INTRAGENERIC HYBRIDISATION: Crosses are made between two individuals of different species belonging to same genera. It is used to produce resistance varieties from diseases, frost or drought.

d) INTERGENERIC HYBRIDISATION: Crosses are made between two individuals of different genera belonging to same family. It produces resistance varieties as well as desirable combination of all characters. Eg: Brassica X Raphanus=Raphanusbrassica

TECHNIQUES FOR HYBRIDISATION

It is necessary for the breeder to have following information: a) whether plants are self or cross pollinated. b) Whether plants are monoecious or dioecious. c) Whether the flowers are unisexual or bisexual. d) Time of Anthesis.

PROCESS OF HYBRIDISATION

The major steps involved in the process of hybridization are:

1. Selection of Parents 2. Emasculation 3. Bagging 4. Tagging 5.Pollination 6.Harvesting F 1 Seeds 7.Further handling of the plants

SELECTION OF PARENTS -The choice of the parents depends on the objective of the cross. In combination breeding, the genetic diversity of the parents is not important. In the case of transgressive breeding, genetically diverse plants are selected as parents. If the characteristics of the parents are not completely known, they are evaluated for the agronomic features.

EMASCULATION -In the case of crops with bisexual flowers, stamens of the flowers of the female parents are removed or the pollen grains are killed. This process is called emasculation. Mechanical, physiological or genetic methods of emasculation are used, depending upon the nature of the crop and the cross.

MECHANICAL METHODS OF EMASCULATION -Here, the anthers are removed from the flowers of the female parents. Hand emasculation and suction method are generally used. For hand emasculation, the flower buds are opened carefully before anthesis (First opening of the flower) and the anthers are removed with the help of forceps. Care should be taken so that the gynoecium of the flowers in not damaged. In suction method, the petals are removed from the flowers before anthesis, with the help of forceps. Then, a thin rubber or glass tube attached to a suction hose is used to suck the anthers from the flowers.

PHYSIOLOGICAL METHODS OF EMASCULATION -Here, the anthers are killed with the help of heat treatment, cold treatment or alcohol treatment.

GENETIC EMASCULATION- Genetic or cytoplasmic male sterility factors are introduced into the female parents to make them sterile.

BAGGING- The emasculated inflorescences of female plants are covered using butter paper bags or cloth bags. However, in the case of cross-pollinated crops, male plants may also be bagged if desired, so as to avoid pollen mixture. The bags are removed 2-3 days after pollination.

TAGGING- Emasculated flowers are tagged properly after bagging. Circular or rectangular tags may be used. Details of the cross, date of emasculation, date of pollination and the number of flowers emasculated must be noted on the tag. Carbon pencil or permanent ink may be used for tagging.

POLLINATION- Mature, fertile and viable pollen grains are collected from the male parent and dusted on the stigma of the female parent. Care should be taken to see that the pollen grains are dusted at the optimum stage of viability.

HARVESTING F 1 SEEDS Crossed seeds are harvested carefully and stored to raise the F 1 generation.

FURTHER HANDLING OF THE PLANTS -Further handling of the hybrids depends on the objective of the cross. In the case of hybrid seed production, the F 1 seeds are directly released to farmers. In the case of combination breeding and transgressive breeding, F 2 is raised and the most appropriate solution program is used.

Mutation Breeding

Mutation is a sudden heritable change in the genotype of an organism. The process by which the change does occur is called mutation and the individual in which it is observed is called mutant.

Mutation occurs in natural population but at a very low rate, which is called spontaneous mutation, but the mutation can be induced by chemical or physical mutagen is called induced mutation. Utilisation of characters which are the result of induced mutation in crop improvement programme is known as mutation breeding.

Objectives:

As in mutation process the ratio of beneficial and harmful character is very small, so it is very difficult to get the desirable trait within a small population. Any mutation breeding programme should have well defined and clear-cut objectives-on which the material type and also the type of mutagen to be used will depend. Furthermore it should be kept in mind that whether the character is governed by oligogene or polygene – the handling of treated material will be different.

Selection of Material:

The mutation should be attempted in the variety which is the best one in that species, as getting a desirable mutant in a poor variety is not useful for breeding programme. Though sometimes it may happen that for selection of particular character poor variety is selected, such as, for searching a ‘dwarf’ mutant some tall varieties should be considered which may not be best one.

Many plant parts, such as, seeds, pollen grains; vegetative propagules (buds or cuttings) may be used for mutagenesis. Plant parts to be used for mutagen treatment depend on the mode of reproduction of plant and the type of mutagen to be used for mutagenesis.

Selection of Mutagen:

The choice of mutagen depends on the material type used; its effectiveness and the proper dose should be considered during treatment. The mutagen may be chemical, such as, base analogues (5-Bromo-uracil), antibiotics (azasorine), alkylating agents (EMS, MMS, DES) and also miscellaneous compounds (dyes, hydroxylamine), etc. or may be physical, such as, ionising (X-ray, P-ray, y-ray) or non-ionising (UV) radiations.

An optimum dose is the one which produces the maximum frequency of mutation and causes the minimum killing. Overdose will cause death or detrimental to most whereas the under-dose will produce few mutations.

Procedure of Mutation Breeding:

A. In Self Pollinated Crops:

First Treated Generation (M_1):

The common effects of mutations are death of seedling, growth inhibition, morphological or developmental abnormalities, and the heritable changes in qualitative or quantitative characters. Among these only the heritable changes either in cytoplasm or chromosomes or genes which are observed in M_1 generation are selected as mutants and these are promoted to M_2 generation.

M_2 Generation:

In this generation a large population is essential and maximum number of mutants is recovered due to recombination and segregation. The process of recovery would depend on the efficiency of screening procedure in M_2 and also in the later generations.

Mutagen treatment of seeds and vegetative propagules produce chimeras, where some parts of plant get changed or mutated but other parts remain unchanged. Chimera may be periclinal or sectorial, the inner chimera only will be transmitted to next generation but not the outer one.

When seeds are produced in mutated M_1 plants, the mutated tissue has to pass through two sieves of selection pressure. Mutated tissue should compete with normal tissue during formation of vegetative stage and reproductive organs.

This selection is called diplontic selection. If the M_1 flower is chimeric, mutant or normal pollen will be formed and the stigma will receive two types of pollen and both will compete for fertilizing the ovule — this is called as haplontic selection.

After competing in both the selection pressure, i.e., diplontic and haplontic selection, the mutant type would appear in M_2 generation.

M_3 and later Generation:

Selected plant progenies of M_2 generation are grown and evaluated critically in M_3 generation.

Progenies of selected M_3 plants are grown as M_4 generation for further purification, evaluation and multiplication.

Most mutants get purified by M_5 generation and those are then evaluated in field trials along with parental variety as standard check variety.

B. In Cross-Pollinated Crops:

In this type of crops genetic variability occurs more naturally, so there is minimum need for mutation breeding. Also as these are opening pollinated, the detection of mutants is more difficult.

C. Asexually Propagated Crops:

Mutation breeding is more useful in this type of crops as other conventional breeding methods cannot be applied due to lack of their sexual cycle. Somatic mutant can easily be created in this type of plants, propagated and directly used.

Since such plants are more heterozygous, any mutation from dominant to recessive may be detected and used. Any phenotypic effect of chromosomal arrangements may also be utilised due to vegetative mode of reproduction.

After mutagen treatment, formation of chimera is regular occurrence. This can be avoided by irradiating the youngest possible stage of bud, i.e., less differentiated primordia. Various propagation methods like budding, grafting and prunning are used for selection of mutated parts.

Merits:

1. De Novo (New) Creation of Genetic Variability:

When the desired character does not exist, mutations are used to get the new character.

2. Breaking Undesirable Linkage:

When two undesirable characters are linked together, mutagen treatment may be used to break the linkage.

3. Production of Haploids:

Haploids may be produced using X-rayed pollen for pollination; un-fertilised egg develops into haploid which are variously used.

4. Increase or Decrease of Chiasma Frequency:

Mutation may change the chiasma frequency which is directly related with recombination and segregation of characters.

5. Production of Transitory Sexuality in Apomicts:

Apomictic plants breed like asexually propagated plants and breeding is difficult. Transitory sexuality can be induced so the better apomictic type can be selected after crossing with various sexual types.

6. Reduction of Incompatibility in Wide Crosses:

Though actual mechanism is not known but irradiation induces better pollen tube growth in few genera which enables inter-specific crosses with the irradiated pollen.

7. Variation in F_1 Hybrids:

F_1 hybrids from inter-varietal crosses may be treated with mutagens in order to increase genetic variability by inducing mutation and to facilitate recombination of linked genes.

8. Production of Distant Hybrid with Translocation:

Irradiation of inter-specific (distant) hybrid causes chromosomal segment translocation carrying some desirable genes which may help in transfer of character from one species to another.

Demerits:

1. Unpredictability:

The frequency of desirable mutants is very low, 0.1% of total mutations. So it is required to grow a large population of M_2 generation and it is laborious to screen out a few economically wanted mutated progenies.

2. Difficulty in Detection:

Mutation breeding is difficult if the detection procedure is elaborate. The disease resistance or quality characters cannot be detected easily which needs elaborate tests. Thus mutation breeding

is not easily applicable to improve this type of character in crop. Moreover most of the mutations are recessive in nature; it is very difficult to detect it in polyploid species.

3. Undesirable Expression:

Desirable mutations are sometimes associated with undesirable side effects or chromosomal aberrations. To remove these kinds of deleterious effects, back-crossing is done which requires more time and expenses.

4. Unrepeatability:

Since the process of mutation is not well understood, so there is no control on the outcome or result of mutagen treatment. Unless directed mutagenesis is applied, it is difficult to obtain the same or desired mutants every time.

Achievements:

Point mutation as well as chromosomal mutation both has been used to produce many improved varieties which represent the improvement in oligogenic as well as polygenic characteristics.

'Sonora 64', a good variety of wheat had red colour grain, ϒ-radiation produced 'Sharbati Sonora' with white colour grain. Chromosome segments of Aegilops, Secale, Agropyron carrying rust resistance genes have been trans-located to wheat by using X-rays.

High yielding groundnut variety NC_4 X modified into NC_4 by micro mutation which looks like the parent.

Point mutations have produced superior sugarcane varieties TS-1 and TS-2 which are the mutants of CO 419. In sugarcane, CO 8152 is a ϒ-ray induced mutant from CO- 527 which gives 40% higher yield, but CO 8152 has two chromosomes less than CO-527.

Duplication in a segment of chromosome 6 of barley carrying gene 'orange lemma' associated with a-amylase activity improves the matting quality of barley.

'Jagannath' rice variety is a ϒ-ray induced semi-dwarf mutant from tall cultivar T- 141, which has the improved resistance to lodging, high yield, responsive to fertiliser.

JRO-3690, a high yielding jute variety has been developed by hybridisation between two low yield mutants of jute.

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Polyploidy Breeding

Irregularities occurring during mitotic or meiotic division may lead to changes in chromosome number which might have some role in improvement of crop plants.

The type of changes in chromosome number can be broadly subdivided into two categories:

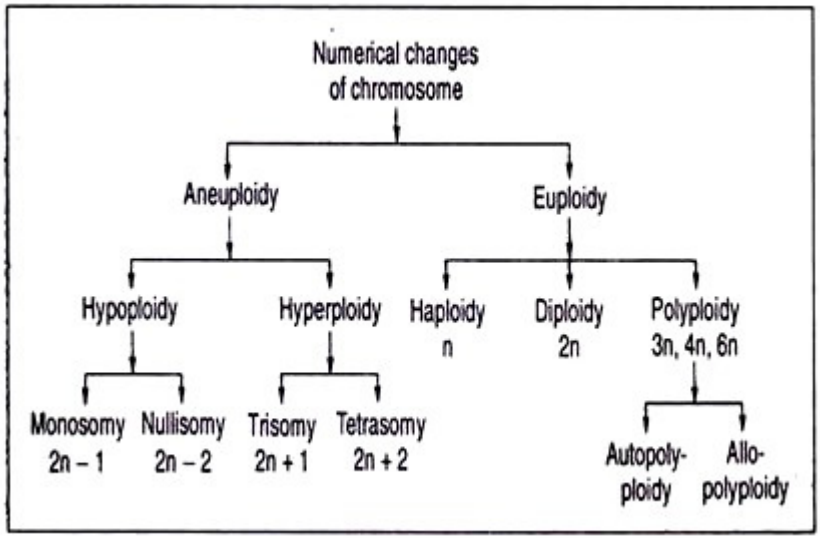
1. Aneuploidy:

Addition or loss of one or few chromosomes.

2. Euploidy:

Addition or loss of total genome set.

Aneuploids are either hypoploid (Monosomy or Nullisomy) or hyperploid (Trisomy or Tetrasomy). Euploids may be haploid, diploid, triploid, tetraploid, etc. Polyploids are euploids above the level of diploid which are either auto-polyploids or allopolyploids.



Effects of Polyploidy:

Primary Effect:

The increase in cell size in growing tissue is the primary effect of polyploidy, though the magnitude of cell elongation and increase in cell number depend on the type of plant parts. It is reflected in the plant parts where there is determinate growth habit (sepals, petals, anthers, fruits, etc.).

Secondary Effects:

Changes in Growth Rate:

Polyploidy results in slower growth rate associated with reduction in branching, etc.

Effects on Fertility and Genetic Behaviour:

Reduction in pollen viability and seed fertility occurs due to irregular chromosome distribution during meiosis.

Changes in Cell Composition:

As polyploidy is associated with cell volume expansion, so it is associated with high water content and lower somatic tension.

Effects on Segregation:

Multivalent formation causes differential behaviour in meiosis which effects the segregation pattern of genes.

Mating Barrier:

Polyploidy creates a barrier on crossing between a polyploid and the diploid progenitor.

Induction of Polyploidy:

Polyploidy is induced artificially with the help of agents like:

1. Colchicine Drug:

It is a non-toxic water soluble alkaloid obtained from Colchicum autumnale which is applied by different methods:

(a) Tube method — Treatment of shoot apex and cotyledons of rooted seedlings through a tube.

(b) Dropper method — A small drop of colchicines solution is applied on the shoot apex situated between cotyledons.

(c) Immersion method— The seeds are allowed to germinate in immersed condition in colchicine solution.

(d) Wilk method — The young seedlings are allowed to soak colchicine solution through roots.

2. Physical Agents:

Heat or cold treatment, X-ray or ϒ-ray irradiation may produce polyploids in low frequencies.

3. Decapitation:

In some plants decapitation induces polyploid cell development at cut ends and the adventitious bud formation.

4. In Vitro Culture:

Ploidy level may change during in vitro cultural condition. During protoplast culture it is a normal feature to get the auto-fused product. In another culture also, the polyploidy can be induced.

Breeding Procedure:

The procedure for polyploid breeding is mainly based on following aspects:

1. Artificial induction and collection of naturally occurring polyploids.
2. Detection of different kinds of polyploids.
3. Hybridization and selection of polyploids.

Development of Drought Tolerant Crops

Adverse environmental factors, of which water scarcity represents the most severe constraint to agriculture, account for about 70 percent of potential yield loses worldwide[1]. Agriculture is the largest consumer of water in the world, and in the drier areas of the world, which include many developing countries, the use of water for agriculture can exceed 90 percent of consumption.

Global warming is also predicted to affect most severely developing countries, where agricultural systems are most vulnerable to climatic conditions and where small increases in temperature are very detrimental to productivity. The Food and Agricultural Organization of the United Nations[2] estimates that by 2025 approximately 480 million people in Africa could be living in areas with very scarce water, and that as climatic conditions deteriorate, 600,000 square km currently classed as moderately constrained will become severely limited.

Water becomes an increasingly scarce and precious commodity. It is thus essential to improve water use efficiency in agriculture. This will require an integrated approach to water resources management to encourage an efficient and equitable use of the resource, and to ensure sustainability. The development of crop varieties with increased tolerance

to drought, both by conventional breeding methods and by genetic engineering, is also an important strategy to meet global food demands with less water.

Developing Drought Tolerant Crops

Conventional breeding requires the identification of genetic variability to drought among crop varieties, or among sexually compatible species, and introducing this tolerance into lines with suitable agronomic characteristics. Although conventional breeding for drought tolerance has and continues to have some success, it is a slow process that is limited by the availability of suitable genes for breeding. Some examples of conventional breeding programs for drought tolerance are the development of rice, wheat and Indian mustard varieties tolerant to salt and to alkali soils by the Central Soil Salinity Research Institute in Karnal, India[3]; the development of maize hybrids with increased drought tolerance; efforts to incorporate salt tolerance to wheat from wild related species; and the incorporation of drought tolerance as a selection trait in the generation of new maize and wheat germplasm by the International Maize and Wheat Improvement Center.

The development of tolerant crops by genetic engineering, on the other hand, requires the identification of key genetic determinants underlying stress tolerance in plants, and introducing these genes into crops. Drought triggers a wide array of physiological responses in plants, and affects the activity of a large number of genes: gene expression experiments have identified several hundred genes which are either induced or repressed during drought[7].

Plant Drought Tolerance Mechanisms

Plants respond to their changing environment in a complex, integrated way that allows them to react to the specific set of conditions and constraints present at a given time. Therefore, the genetic control of tolerance to abiotic stresses is not only very complex, but is also highly influenced by other environmental factors and by the developmental stage of the plant.

The physiological responses of plants to a deficit of water include leaf wilting, a reduction in leaf area, leaf abscission, and the stimulation of root growth by directing nutrients to the underground parts of the plants. Plants are more susceptible to drought during flowering and seed development (the reproductive stages), as plant's resources are deviated to support root growth. In addition, abscisic acid (ABA), a plant stress hormone, induces the closure of leaf stomata (microscopic pores involved in gas exchange), thereby reducing water loss through transpiration, and decreasing the rate of photosynthesis. These responses improve the water-use efficiency of the plant on the short term.

Plant cells are required to maintain water balance. To mantain this water balance, plants absorb water when water potential is negative Cells can decrease their water potential through the accumulation of solutes, such as sugars, amino acids, organic acids and ions – especially potassium (K+). As cellular enzymes are severely inhibited by the presence of ions, these must be removed from the cytosol (the ground fluid substance of the cell) and stored in special storage cell organelles, the vacuoles. Compatible solutes that accumulate in the cytosol and do not interfere with enzymatic reactions comprise sugar alcohols (mannitol and sorbitol), the amino acid proline, and glycine betaine. The synthesis of these compounds by the plant enhances tolerance to drought[8].

The plant's response to drought is accompanied by the activation of genes involved in the perception of drought stress and in the transmission of the stress signal. One group are genes that encode proteins that protect the cells from the effects of desiccation[9]. These genes include those that govern the: accumulation of compatible solutes; passive transport across membranes; energy-requiring water transport systems; and protection and stabilization of cell structures from desiccation and damage by reactive oxygen species[9].

A second group of genes activated by drought is comprised by regulatory proteins that further regulate the transduction of the stress signal and modulate gene expression. At least four

independent stress-responsive genetic regulatory pathways are known to exist in plants, forming a highly complex and redundant gene network[8, 9]. Two of the pathways are dependent on the hormone ABA, and two are ABA-independent. These pathways are also implicated in the perception and response to additional stress factors, including cold, high temperature and salinity.

Genetic Engineering Drought Tolerant Plants

Although not a crop plant, Arabidopsis has played a vital role in the elucidation of the basic processes underlying stress tolerance, and the knowledge obtained has been transferred to a certain degree to important food plants[10]. Many of the genes known to be involved in stress tolerance have been isolated initially in Arabidopsis. The introduction of several stress-inducible genes into plants by genetic engineering has resulted to increased tolerance of transgenics to drought, cold and salinity stresses[8, 9]. Some examples are reviewed in the following section.

Plant Breeding for Disease Resistance:

Crops are required to be disease, resistant, as a wide range of fungal, bacterial and viral pathogens that affect the yield of cultivated crop species, especially in tropical climates. Resistance of the host plant is the ability to prevent the pathogen from causing disease and is determined by the genetic constitution of host plant.

Plant breeding for disease resistance has two advantages given below:

(i) It enhances the production of food by reducing doses due to diseases.

(ii) Reduces the dependence on fungicides and bactericides.

The causative agents of diseases in plants are:

(i) Fungi Diseases caused by fungi are brown rust of wheat, red rot of sugar cane, late blight of potato, etc.

(ii) Bacteria Diseases caused by bacteria are black rot of crucifers, blight of rice, citrus canker, etc.

(iii) Virus Diseases caused by virus are tobacco mosaic, turnip mosaic, etc.

Methods of Breeding for Disease Resistance:

It is carried out by either of the following two breeding methods:

Plant Breeding for Developing Resistance to Insect Pests:

The insect resistance in host crop plants may be due to the morphological, biochemical or physiological characteristics.

The important characters that lead to pest resistance are:

(i) Hairy leaves in plants. For example, resistances to jassids in cotton and cereal leaf beetle in wheat.

(ii) Solid stem in wheat exhibits non-preference by stem sawfly.

(iii) In cotton, smooth leaf and absence of nectar repel bollworms.

(iv) In maize, high aspartic acid, low nitrogen and sugar content protect them from stem borers. Breeding methods for insect pest-resistance involve the same steps as those for any other agronomic trait.