BREEDING STRATEGIES - CLONAL SELECTION

Clone

A clone is a group of plants produced exclusively from a single individual plant through asexual reproduction. Most of the fruit plants are propagated asexually which consist of large number of clones that is why these plants are known as a group of plants derived from a single plant by vegetative means. In other words all the vegetative progenies of a single plant make a clone.

Characteristics

_ Clones are stable- They retain their original traits just like pure line variety

_ Theoretically clones are immortal i.e. A clone can be maintained indefinitely by asexual reproduction. However, these are very much susceptible to diseases or insect pests depending upon the species and cultivars.

_ Homogeneous-Individual plant of a clone is a mitotic derivative of the same plant and therefore homogeneity in phenotype is the major feature of clones. A group of individual plants derived from the same tissue of the original mother plant carries the same genotype. Phenotypic variation if any in clones is due to environmental impact.

_ Continuous inbreeding of clones which are heterozygous might lead to severe loss in vigour

_ The phenotype of a clone is due to effect of gene (G), environment (E) and GxE interaction over the population mean (h). Therefore P=h+G+E+GE

_ Clones are maintained by asexual reproduction, but pure lines and inbreds are maintained by self-pollination or close inbreeding

Genetic variation within clones

Genetic variation within clones may be due to mutation, mechanical mixture and sexual reproduction.

a. Mutation

Somatic mutations are also known as bud mutations. The frequency of mutations is generally very low. A mutant allele would be homozygous only when (i) both the alleles in the cell mutate at the same time producing the same mutant allele, or (ii) the mutant allele is already in the heterozygous condition in the original clone. Dominant bud mutations express themselves more frequently than the recessive ones, as recessive mutation get expressed only under homozygous conditions. Bud mutations often produce chimeras, i.e., individuals containing cells of two or more genotypes. However, it is not a great problem because normal plants, i.e., non chimeras, may be produced from chimeras by several techniques.

b. Mechanical mixture

Mechanical mixture produces genetic variation within a clone, similar to the manner as seen in pure lines.

c. Sexual reproduction

Occasional sexual reproduction leads to segregation and recombination. The seedlings obtained from sexual reproduction are genotypically different from the asexual progeny.

Clonal degeneration

The loss in vigour and productivity of clones with the passing of time is known as clonal degeneration and it may be due to mutation and infection of virus and bacteria.

Clonal selection

The phenotypic value of a plant or a clone is due to its genotype (G), the environment (E) and the genotype x environment interaction (GE). Of these, only the G effects are heritable and stable. Therefore, a selection for quantitative characters based on single plant observation may not hold good. A selection for polygenic characters like yield on the basis of unreplicated clonal plots would also often be misleading and unreliable. The value of clone can be reliably estimated only through replicated yield trials. However, selection for highly heritable characters, such as plant height, days to flowering, colour, disease resistance, etc., is easy and effective even on the basis of single plant or plot. The various steps involved in clonal selection are briefly described below and are depicted.

First year: From a mixed variable population, a few hundred to few thousand desirable plans are selected. A rigid selection can be done for simply inherited characters with high heritability. Plants with obvious weakness are eliminated. In fruit plants, it is difficult to get large number of individual selections. In such case, few plants may be selected.

Second Year: Clones from the selected plants are grown separately, generally without replication. This is because of the limitation in propagation material in each clone, and also because of the large number of clones involved. The characteristics of clones will be clear now than in the previous generation when the observations were based on single plant. The inferior clones are eliminated at this stage. The selection is based on visual observation and on the breeder's judgment of the value of clones. Fifty to one hundred clones are selected on the basis of clonal characteristics.

Third year: Replicated preliminary yield trial is conducted. A suitable check is included for comparison. Few superior performing clones with desirable characteristics are selected for multi location trials. At this stage, selection for quality is done. If necessary, separate disease nurseries may be planted to evaluate disease resistance of the selected clones.

Fourth to Seventh years: Replicated yield trials are conducted at several locations along

with a suitable check. The yielding ability, quality and disease resistance etc. of the clones are
rigidly evaluated. The best clones that are superior to the check in one or more characteristics are
identified for release as varieties.

#ineteenth year: The superior clones are multiplied and released as varieties.

Advantages

i) Clonal selection is an easy and less time consuming method.

ii) Easy maintenance because there is no problem of out crossing and loss of seed viability. Variation occurs due to somatic mutation only which can be managed by removal of undesired plants.

iii) Heterotic clones on selection may be used as permanent hybrids. Heterosis can be exploited for longer time without production of hybrid seed every year (for vegetatively propagated vegetable crops).

iv) Clonal selection is the only method of breeding in vegetatively propagated fruit plants.

Limitations

_ There is limited chance of getting new and useful type of variability

_ The multiplication rate is low.

_ It is only useful for vegetatively propagated plants.

Hybridization between clones

Generally, clonal crops are cross-pollinated and they may show self incompatibility. The selected parents may be used to produce single crosses involving two parents or an equivalent of a polycross involving more than two parents (rubber).

Selection among F1 families: When the breeding value of parents is not known, and when the relative contribution of general combining ability and specific combining ability is not available, then a large number of crosses have to be made in order to ensure that at least some of the crosses would produce outstanding progenies in F1. This is particularly true in a species where crop improvement has not been done or has been done at a small scale. In such cases, it would be cumbersome to evaluate a large number of F1 progenies generally in detail. To avoid this, small samples of several F1 populations are generally grown. The general value of individual F1 families or populations is estimated noted. Inferior families are eliminated. Promising families with outstanding individuals are then grown at a much larger scale for selection. The procedure is designed to save time, space and labor by planting only small populations of a large number of crosses at the preliminary stage.

Selection within F1 families: The selection procedure within F1 families is essentially the same as that in the case of clonal selection. But in the case of fruit and plantation crops like cashew, it is difficult to follow the above steps. In these perennial crops, the steps given below may be followed:

Step I: Select two parents of desirable characters and hybridize them to produce sufficient crossed fruits.

Step II: Raise the F1 seedling populations and evaluate the individual progenies for yield and quality.

Step III: Select few superior progenies and propagate them vegetatively to produce grafts/budding on standard rootstocks.

Step IV: Evaluate the selected clonal seedling progenies (in sufficient number / clone usually minimum of 5-10) along with the parents and standard varieties.

Step V: Outstanding clones may be released as new variety.

As step I to V take at least 20-25 years, some breeders avoid step I and IV. Instead, best performing F1 progenies are assessed and the scion collected from them is multiplied as grafts / budlings for further use as next varieties.

Achievements

Clone No.51 from Dashehari, MA-1 from Alphanso, Tommy Atkin from Haden. Pusa Surya from Elden in mango, Pusa Seedless from Thompson Seedless of grape etc.

BREEDING STRATEGIES - BUD MUTATIONS AND CHIMERAS

Bud mutations

If mutation occurs in any one of the actively dividing meristematic tissues, the branch arising from them, expresses the mutant character if it is dominant and this phenomenon is known as *bud mutation* .Though mutation is most frequent at maturation divisions, it may also arise in somatic cells. If mutation occurs in cells from which buds are developed, the later are genetically different from the rest of the plant. These are termed "bud mutation" or "sports". The frequency of such mutations is very low to be of any economic importance, which is also different in different species. The bud mutation may arise through (1) gene mutation or (2) chromosomal variation. Bud variations have been noted in sugarcane. This was first noted by Lorzier in Mauritius in 1869.

Other instances of sporting are Ribbon canes of Australia, Truna canes of Mauritius and Tip canes of Hawaii which are found to throw bud variations. Barber (1906) noted bud sports in the sugarcane at Samalkota. Striped-Mauritius often sported into green canes and less often into red types. The bud sports not only varied in the colours on rind but also in some of the agricultural characters. Bud sports are frequent in ornamental plants and many new garden varieties have been established by selection of such sports. Economic types from bud sports in the case of field crops are rare. Though bud sports have been noted in crops like potato, they have not been found to be of economic type. Superior varieties in citrus have been evolved by selecting bud mutant. It is reported that in 18 years prior to 1937, about 10 million buds of varieties which originated by bud mutation have been sold in California alone. Robertson Navel orange and Dawn grape fruit are some notable examples of new varieties arising through bud mutation.

Somatic mutations

These mutations occur in tissues other than the germ track. Most mutations occur somatically, i.e., after the differentiation has set in, when a group of somatic cells is genotypically different from the other cells in the same individual, a somatic mutation may be suspected. The change occurs in the cells of the growing body. Hence the new types of cells are not only heterozygous but form a patch. In meristematic tissues of axillary buds and others a mutation often leads to a batch with new characters. Such changes occur more frequently in polyploidy and heterozygous plants and in individuals which have been grown for long as clones. If propagated vegetatively the mutated parts give rise to new types of plants. This practice is common in horticulture.

The brown colour of the grain in sorghum in some cases is determined by the persistence of the integument in which, the colour is deposited. Often mutant patches of white occur in individual grains of panicles from homozygous brown grained line. Anatomical studies have shown the suppression of the integument in such places where

the white patch appears and genetical studies have shown that this is only affecting the somatic tissue and does not affect the germinal tissues. White grain colour is recessive to brown. In *Cosmos sulphureus,* plants with yellow petals have often been observed to appear suddenly; the usual one has orange-yellow coloured petals. Sometimes the region affected is half the head and, in such cases, in the progeny, plants with all yellow flowers have appeared. These have bred true. Somatic mutations have been recorded in vegetatively propagated plants like apples, dahlias, chrysanthemum, potato, rose, etc.

Chimeras

A chimera is an individual with one genotype in some of its parts and another genotype in the others. Somatic mutation may often lead to chimeras. When propagated asexually these chimeras may become perpetual. Certain types of *Pelargoniums* and potatoes are of such chimeras. When growth is encouraged from the concealed tissues the real nature of these chimeras is revealed. Somatic mutations either at the terminal or axillary buds in germinating seeds, seedlings or in mature plants can be produced by irradiation or chemical treatment. Artificial creations of such somatic mutations open possibilities of production of new horticultural and agricultural plants.

Treatment of seeds and vegetative propagules commonly produces chimeras..

Shoot tip meristem usually has two functional layers; the outer layer, giving rise to epidermis and a part of leaf mesophyll, and the inner layer producing the rest of the plant tissues including reproductive organs.

Chimeras are of three kinds

Periclinal chimera: When the entire outer or inner layer is affected, the chimera is known as 'periclinal chimera' (inner periclinal or outer periclinal depending upon the layer affected)

Sectorial chimera: Only a part of the inner or the outer layer is affected (inner sectorial chimera only a part of the inner or the outer layer is affected (inner sectorial and outer sectorial respectively).

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

Mutant alleles are generally recessive, but some dominant mutations may also occur. In case of sexually reproducing crops, mutation breeding utilizes both recessive and dominant mutations. In dominant mutations, the phenotype can be recognized as a somatic mutation arising from the mutated cell, for example, a colour mutation in an epidermal cell from 'aa' (colourless) to 'Aa'. How ever, recessive mutations are much more numerous than dominant ones. Recessive mutation can occur in the homozygous dominant type as AA – Aa or in the heterozygote as Aa – aa. In the former one, the selfed progeny normally segregate with 25 per cent recessive mutatnt 'aa' types.

Mutation breeding in clonally propagated crops primarily depends on dominant mutation. Recessive mutation may also be utilized provided the clone used for mutagen treatment was heterozygous; for example, if recessive mutant allele is to be useful in a clonal crop, the clone has to have the genotype Aa. Such situations are however, rare. More frequently, the mutants useful in the improvement of clonal crops are dominant mutations.

BREEDING STRATEGIES –MUTAGENESIS AND ITS APPLICATION

Mutation

Sudden heritable change in the genotype of an organism is termed as mutation. It may be spontaneous (without any treatment by man) or induced (artificially induced by a treatment with certain physical or chemical agents) in plant population. The process through which mutants get induced is called mutation and the mutated individual is called a mutant. Mutants have variously been classified as spontaneous and induced, natural and artificial based on their origin; germinal and somatic based on the tissue involved; chromosomal, genic and cytoplasmic etc.

Kind of mutations

Macro mutations are large mutations and can be recognized on a single plant basis, e.g., changes in colour, shape, etc., Micro mutations are mutations with small effects and can be recognized only when a group of 30 or more mutant plants are compared with a normal one. Micro mutants differ with normal only quantitatively; for example, mutants with larger or smaller grains or higher yield, etc., Micro mutations are more important for direct use in plant breeding.

Point mutation is another term often used to designate gene mutation but it comprises of group of changes at individual loci (point) including micro structural change, micro-deficiencies and gene mutation.

Somatic mutation refers to mutants appearing in vegetative part in M1 generation. It also refers to 'bud-sport' in the case of vegetatively propagated plants. This may occur either due to dominant mutation (aa Aa), recessive mutation in a heterozygote (Aa aa), removal of epistatic factor of chromosomal aberrations.

a. Spontaneous mutations: These are naturally occurring mutations, which arise somatically. They arise in nature continuously without any human control and create variability, which forms the basis of conventional crop breeding methods. Their frequency is extremely low (one in a million).

b. Induced mutations: Contrary to spontaneous mutations, these are induced by using various agents Physical or chemical agents, which cause mutation, are known as mutagens or mutagenic agents.

Procedure of mutation breeding

When mutations are induced for crop improvement, the entire operation of induction and isolation of mutants is termed as mutation breeding. The various steps involved in mutation breeding are as under:

_ Objectives of programme – Objective should be clear cut and well defined

_ Selection of variety for mutagen treatment – Locally accepted best variety in which improvement is needed either in polygenic or monogenic trait.

_ Part of plant to be treated- Seeds, pollen grains or vegetative propagules (buds and cuttings) may be used for mutagenesis. Selection of plant part for mutagenic treatments are based on mode of multiplication / reproduction. In sexually propagated fruit plants, seed treatment is common. Pollen grains may be used, but it has some limitations. It is difficult to collect large amount of pollen grains and pollen survival life is also short. In case of a sexually propagated fruit plant, buds or cuttings are used for mutagenic treatment.

_ Dose of mutagen – An optimum dose is that one which produces the maximum frequency of mutation and causes the minimum killing i.e. LD 50. It is that dose of mutagen which would kill 50% of the treated individual. Dose of mutagen depends upon intensity and time of treatment.

_ Mutagen treatment- Selected plant part is exposed to the desired mutagen dose. The plants are immediately planted to raise M1 plant from them. In case of seed treatment they are pre-soaked for a few hours to initiate metabolic activities and then exposed to mutagen. Treated seeds are sown immediately in field to raise M1 generation. The seeds derived from mutated pollen is considered as M1 and subsequent generations can be derived through selfing or clonal propagation.

Handling of the Mutagen – Treated Population

The following handling procedure is based on the selection for a recessive mutant allele.

i. ***M1 generation*****:** Several hundred (500 or more) seeds are treated with a mutagen and are space-planted. M1 plants will be chimeras for the mutation present in heterozygous state. About 20 seeds from each M1 plant are harvested to raise the M2 progeny rows.

ii. ***M2 generation***: About 2,000 progeny rows are grown. Careful and regular observations are made on the M2 rows. But only distinct mutations are detected in M2 because the observations are based on single plants. All the plants in M2 rows suspected of containing new mutations are harvested separately to raise individual plant progenies in M3. If the mutant is distinct, it is selected for multiplication and testing. However, most of the mutations will be useless for crop improvement. Only 1-3 per cent of M2 rows may be expected to have beneficial mutations.

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

iv. ***M4 generation***: A preliminary yield trial is conducted with a suitable check, and promising mutant lines are selected for replicated multi location trials.

v. ***M5-M8 generation***: Replicated multilocation yield trials are conducted. The

outstanding line may be released as a new variety.

It may be noted that above procedure is recommended for all horticultural crops, which are exclusively propagated by sexual means.e.g.Vegetables, *Crossandra*, *Periwinkle etc.*

A detailed method to isolate stable solid mutants in vegetatively propagated horticultural plant is presented.

Mutation breeding scheme for the improvement of horticultural tree plants

General characteristics of mutation

(i) Mutations are generally recessive but dominant mutations also occur.

(ii) Mutations are generally harmful to the organism.

(iii)Mutations are random.

(iv) Mutations are recurrent.

(v) Induced mutations commonly show pleiotrophy, often due to mutations in closely linked gene.

Mutagens

Agents used for induction of mutations, are known as mutagens. The different mutagens may be grouped as follows:

A. Physical mutagens

1. Ionizing radiations

(a) Particulate radiations – α-rays, fast neutron, thermal neutrons.

(b) Non-particulate radiations – X-rays, γ-rays.

2. Non ionizing radiation – Ultraviolet radiation.

B. Chemical mutagens

1. Alkylating agents – Sulphur mustard, mustard gas, EMS (Ethyl methane sulphonate), Ethylene Imine (EI)

2. Acridine dyes- acriflavin, proflavin, acridine orange, acridine yellow,ethedium bromide.

3. Base analogues – 5-bromouracil, 5-Chlorouracil.

4. Others – Nitric acid, hydroxyl amine.

Achievements

Mango – Rosica from Peruvian variety Rosadodelca

Papaya- Pusa Nanha from local type

Grape-Marvel Seedless from Delight

Banana- High gate from Gros Michel, Motta Poovan from Poovan

Orange-Washington Navel

Grapefruits – Marsh and Thompson

BREEDING STRATEGIES - HYBRIDIZATION AND PROBLEMS ASSOCIATED WITH HYBRIDIZATION

Hybridization

Hybridization refers to mating or crossing of two plants or lines of diverse genotypes to obtain a viable hybrid progeny. The seed as well as the progeny resulting from hybridization are known as 'hybrid' or F1.

Hybridization in self-pollinated crops

By planned hybridization between carefully selected parents, the breeder can create populations with sufficient variability from which plants combining the desirable features of the parents can be selected. Theoretically, all the plants of pure-line or a clone are of one genotype (i.e. they have identical genetic constitution). Therefore, when different pure-lines or clones are crossed, heritable variability is created by recombination. Selection in the segregating generations of a hybrid will therefore be effective.

Objectives of hybridization

The purpose of hybridization is to combine in a single variety, the desirable characters of two or more lines, varieties or species. Occasionally, the recombination of genetic factors leads to the production of new and desirable characters not found in either of the parents. When two parents are crossed, the resultant F1 is a homogeneous one but is heterozygous in nature, hence all plants look similar phenotypically. When they are selfed to produce F2 the population is heterogeneous and heterozygous. Hence, phenotypically many variations could be seen in this generation. Further, in this generation, a cross may frequently give rise to progenies which are beyond the range of the parents for a particular quantitative character such as height of plant, earliness, fruit size, yield etc. This phenomenon is often referred as "transgressive segregation". For example, the progenies may be taller than the taller parent or earlier than the earlier maturing parent. Such transgresive segregation may enable the breeder to attain his objective quickly.

Types of hybridization

Inter-varietal hybridization

The parents involved in hybridization belong to the same species. There may be two strains, varieties or races of the same species. It is also known as intraspecific hybridization. The intravarietal crosses may be simple or complex depending upon the number of parents involved.

a. **Simple cross:** In a simple cross, two parents are crossed to produce the F1 A x B F1

b. **Complex cross:** More than two parents are crossed to produce the hybrid.

Example

Three parent cross (A, B, C)

A x B

(A B) x C

(A B C) (Complete hybrid)

Four parents (A, B, C, D)

A x B C x D

(A B) x (C D)

(A B C D)

Eight parents (A,B,C,D,E,F,G,H)

A x B C x D E x F G x H

AB x CD EF x GH

ABCD x EFGH

ABCDEFGH (Complex hybrid)

Hybridization technique

There are seven steps involved in hybridization.

Choice of parents

It mainly depends upon the objective of breeding programme. In addition to other objectives, increased yield is always an objective for the breeder.

Evaluation of parents

If the performance of parents used for breeding is known, evaluation is not necessary. But if their performance is not known, it should be evaluated, particularly for the characters to which they are expected to contribute.

Emasculation

The removal of the stamens or anthers or the killing of pollen grains of a flower without disturbing the female reproductive organs is known as emasculation. The purpose of emasculation is to prevent self fertilization in the flowers of female parent.

Type of emasculation

1. Hand emasculation
2. Suction emasculation
3. Hot water emasculation
4. Alcohol treatment
5. Cold treatment
6. Genetic emasculation e.g.male sterility

Bagging

Immediately after emasculation, the flowers of the inflorescence are closed in suitable bags of appropriate size to prevent random cross pollination.

Tagging

Emasculated flowers are tagged just after bagging. The following information is recorded on the tags with a carbon pencil:

1. Date of emasculation
2. Date of pollination
3. Name of the female and male parents. The name of female parent written first, and then the male parent

Pollination

Pollination refers to transferring the mature and fertile pollen from the male parent to the stigma of the female parent. This is done with the help of brush delicately with out disturbing the stigma and female flower.

The pollinated flower is enclosed in a butter –paper bag or muslin cloth bag to avoid contamination from outside pollen and labeled. Few days after pollination, when the fruitset is conspicuous, the bag is removed. The seeds extracted from such crossed fruits are the F0 seeds to raise F1 or hybrid plants.

Selection procedures with hybridization

Two selection procedures are commonly followed after hybridization to isolate the desirable genotypes from the segregating progeny.

1. **The pedigree method**: This is widely followed by the plant breeders now, who maintain a detailed record of relationships between the selected plants and their progenies. It consists of the selection of individuals plants with the desired combination of characters in the F2 generation and reselection of the progenies of each selected F2 plant in succeeding generations until genetic purity is reached.
2. **The bulk method**: This method differs from the pedigree method in that the hybrids are grown in bulk till the F5 or F6 generation. Desirable individual plants are selected only in the F5 or F6 generation and these are then carried forward as families, which are evaluated in the same way as in the case of pedigree method.

Achievements

Fruit Hybrids

Mango Mallika, Amrapalli, Pusa Arunima, Arka Anmol, Arka Puneet,
Arka Aruna, Arka Neelkiran, Ratna, Sindhu, PKM-1, PKM-2.
Guava Arka Amulya, Safed Jam, Kohir Safed
Papaya CO-3, CO-2
Sapota CO-1, DHS-1, DHS-2, Hybrid 214, Hybrid-711
Banana CO-1

RESISTANCE BREEDING FOR BIOTIC ABIOTIC STRESSES

A plant is said to be healthy or normal when it carries out its physiological functions to the best of its genetic potential. These normal functions include division, differentiation, and development. Absorption of water and minerals from soil and translocation of these throughout the plants, photosynthetic product to areas of utilization or storage, the metabolism of synthesized compounds, reproduction and storage of food supplies.

A plant becomes diseased when it is disturbed by pathogen under certain environmental conditions which interfere with one or more of its essential functions. Diseased plant refers to any disturbance brought about by living organism under environmental factors which interfere with normal function of plant or in other words when any organ and part of plant is not doing their work properly and when either the growth or reproduction is not going forward in natural or regular manner.

Breeding varieties/hybrids resistant to biotic stresses viz., pests, diseases and nematodes and abiotic stresses viz., drought, salinity and adverse climatic conditions like frost, chilling temperature are the primary objectives in any breeding programme.

Advantages of resistant breeding

1. Farmers can use resistant varieties without incurring any extra expenditure on plant protection chemicals.
2. It is a safe measure- fungicides and other pesticides leave some residual effect.
3. It is more effective as compared to other measures of disease and pest control.
4. In case of air borne diseases, it is impossible to cover larger area with any other means of
5. disease control.

Concept of resistance breeding

Insects are usually specialized in their ability to attack the host or part of the host. An insect is capable of damaging or attacking every species of the host. The plant resistance includes those characters which enable a plant to avoid, tolerate or recover from the attack of insect under conditions that would cause greater injury to other plant of the same species.

Resistance is heritable characters possessed by the plant which influence the ultimate degree of damage done by the insect. In other words, plant resistance is defined as being the collective heritable character by which a plant species raise in groups or individually may reduce the probability of successful utilization of that plant or a host by an insect species, race, biotype or individuals. The degree of resistance is a relative term which is measured by using susceptible cultivar of same plant species as check. The degree of resistance among specific host plants may vary between two extremes i.e. immunity and high susceptibility. Any degree of host reaction less than immunity is

resistance. In case of abiotic stress, the amino acids or enzymes connected with resistance or tolerance to drought, salinity and other factors will be identified and the plants possessing the desirable traits will be used as donors in breeding programmes.

Breeding methods for biotic /abiotic stress resistance

(i) Introduction

An introduced variety resistant to the concerned insect pest and diseases or abiotic stresses may be released for cultivation if it performs well in the new environment and is agronomically desirable. Thus, it is the quickest and perhaps, the earliest method of developing a biotic stress resistant variety. e.g. introduction of *Phylloxera vertifoliae* resistant grape rootstock from USA to France. Sometimes, the introduced variety may not perform well in the new environment and it may be susceptible to the biotypes of the concerned pest prevalent in the area or to a new insect pests and/or diseases of the area.

(ii) Selection

Biotic/abiotic stress resistant variants may be found in an existing variety of a crop. In such case, selection for insect and disease resistance is practised to isolate biotic stress resistant variety. Screening large number of germplasm for resistance at field level and further confirmation through artificial testing will help in selection of a resistant line which may be directly used as variety or used as donor for developing a hybrid

(iii) Hybridization

When the desired biotic/abiotic stress resistance is present in an agronomically inferior variety of the crop or in a related wild species, hybridization is the only course of action for the breeder e.g. breeding for fruit fly resistant variety in Ber (Vashishtha et al.,1997) However breeding in ber is difficult due to incompatibility, low fruit set etc.

Backcross Method of Breeding

The backcross is a form of recurrent hybridization by which one or two desirable characteristics are added on to a superior variety, wherein the hybrids and the progenies in the subsequent generations are repeatedly back crossed to one of their parents. The object of back crossing is to transfer one or two desirable characteristics from an inferior variety to a superior variety, disturbing the genotype of the superior variety as little as possible in the process. Backcrossing is particularly well suited for the transfer of one or two simply inherited and easily recognized characters to a variety that excels in most of its characters.

In a back cross breeding programme, the genetic consequences of repeated back crossing must be understood. Repeated back crossing leads to rapid increase in homozygosity and in the frequency of homozygote's as that of selfing. The steps involved in back cross breeding depend upon the genetic nature of the gene to be transferred.

(iv) Mutation

Generally, it has not been used to produce a successful biotic stress resistant crop. The reason for this is difficulty in screening of suitable mutations, the failure of such mutagenesis to generate positive changes to the genome and large number of progeny that must be handled.

Production of disease resistant plant by non-conventional breeding

Basic technique in plant cell culture

a. Callus and suspension culture
b. Haploid culture from pollen
c. Protoplast isolation and culture
d. Embryogenesis in cell culture
e. Selection of mutation from pathotoxin resistant cells and clones
f. Regeneration within heterogeneous materials
g. Regeneration of plants from somaclonal/protoclonal variation
h. Resistant plant through fusion of protoplast
i. Disease resistance through uptake of foreign genetic material

Genetic engineering or Recombinant D3A technology

There is scope of genetic engineering in fruit crops for the development of transgenic varieties resistant to biotic/abiotic stresses. This technology involves the isolation of gene of desired character. Insertion of this isolated gene in a suitable vector (making it a recombinant vector). Insertion of the recombinant vector into a suitable host (organism/cell) known astransformation. Selection of the transformed host and multiplication followed by expression of the introduced gene into the host is the normal procedure adopted.

Role of genetic engineering and biotechnology in improvement of fruit crops

Biotechnological tools are appropriate for accelerating the productivity.

Application of biotechnological tool in plant improvement has been found effective in three ways (i) rapid multiplication of existing allied clones and varieties (ii) speeding up the process of conventional breeding and (iii) conservation of germplasm and evolving novel genotypes through genetic engineering technology. Realizing the importance of biotechnology in National development, the Government of India set up a full-fledged Department of Biotechnology (DBT) in 1986 to coordinate and oversee priority areas. DBT has initiated a number of programmes to promote fruit industries. As a result of this, biotechnological revolution has taken place in horticulture.

Biotechnological application

a. Micro propagation

Superior selections and hybrids developed at various research centers failed to reach the orchardists due to lack of sufficient planting material. It leads to non-realization of the potential of improved cultivars, thus making the efforts of fruit improvement programme unfruitful. In this case, micropropogation can be a powerful tool for large scale propagation of fruit crops. This is also an ideal system for production of disease free plants. Among the fruits, micro propagation has been most successful in banana, papaya and date palm multiplication. Long term micro propagation of passion fruit by formation of multiple shoot primordial initiated from leaf explants has been reported (*Kawate et al*., 1995). *In vitro* propagation of grape vine is also possible (Heloir et al., 1997. Gray and Fisher, 1985)

b. Conservation of germplasm

The potential importance of natural gene pool to meet the future need of crop improvement by introducing specific characters such as abiotic stress resistance can not be under estimated. However, the number of wild species and their natural habitats are disappearing rapidly, as a result of introduction of highly bred modern varieties, growing urbanization and an increased pressure on land for cultivation. This leads to the erosion of the natural germplasm to such extent that there is a general fear that potentially valuable germplasm is being lost irretrievable. In plant improvement, it is necessary to facilitate the assimilation of germplasm collection in working for the use of the breeders. The process of genetic erosion necessitates measure that germplasm must be conserved in such a manner that there should be minimal losses of genetic variability of a population. The conventional methods of germplasm conservation may be vulnerable to losses due to (i) Attack by pest and pathogens (ii) Climatic disorders (iii) Natural disasters and (iv) Political and economic causes. Besides this, the seeds of many important fruit plants such as mango, litchi etc, may loose their viability in a short time under conventional storage system.

National Bureau of Plant Genetic Resources, New Delhi is maintaining large *invitro* germplasm collection of banana, phalsa, bael, jackfruit, pomegranate etc. There are two basic approaches followed to maintain the germplasm *in-vitro.*

Conservation of germplasm through biotechnology is a more efficient tool for short and medium term storage. It can be achieved by reduced temperature and light, incorporation of sub lethal levels of growth retardants, induction of osmotic stress and maintenance of culture of a reduced nutritional status particularly reduced carbon and reduction of gas pressure over the culture. Advantage of this approach is that culture can be readily brought back to normal culture conditions to produce plants on demand. But the disadvantage is that culture may be subjected to contamination and somaclonal

variation.

Cryopreservation at ultra low temperature of liquid nitrogen at -190ºC offers the possibility for long term storage with maximum phenotypic and genotypic stability.

c. Anther culture

In-vitro androgenesis holds a myriad of possibilities for improvement of horticultural crops. This technology has been extended for a number of horticultural crops. The purpose of anther and pollen culture is to produce haploid plants by the induction of embryogenesis from repeated divisions of monoploid spores, either microspore or immature pollen grains.

The major interest in haploids is based upon the production of homozygous plants as an alternative for repeated cycles of inbreeding in self pollinated crops. In cross pollinated species, double haploids are more to be used as parents in the production of single or double cross hybrids which are as follows.

_ As a result of haploid induction, chromosome homozygosity is attained in a very short time. This is particularly useful in heterozygous and self incompatible crops like mango, etc.

_ With the use of homozygous parents in crossing programme, the production of pure F1 hybrids become possible.

_ Haploid cell lines have great advantages in studies on mutant selection *in-vitro*.

d. Overcoming crossing barriers (embryo culture)

This technique pertains to the cultivation of excised plant embryo in artificial medium. Embryo culture technique has found its application both in the applied and basic research. In the conventional plant breeding programme, breeder often faces problem in transferring resistance from wild species to the cultivated species and getting the desirable interspecific hybrids (Yeung *et al.,* 1981). Application of embryo rescue can overcome some of the pre and post-fertilization barriers in fruit crops. Further, most useful and popular application of zygotic embryo culture has been used in raising hybrids. Embryo culture technique has important role in haploid production, shortening of breeding cycle (Lammerts, 1942) rapid seed viability test and propagation of rare plants.

e. Somaclonal variation

Somaclonal variation explores the naturally occurring or *in-vitro* induced variability of somatic cells following plant regeneration. Somaclonal variation is an excellent method for shortening breeding programmes. Somaclonal variation may be due to variation in chromosome number, structural variation of chromosomes due to deletions, duplication, translocation, genetic and cytoplasmic mutation etc.

Hwang and Ko (1987) identified Somaclonal variation in the cultivars Giant Cavendish with putative field resistance to Fusarium wilt (race 4) but inferior in

agronomic characters. A somaclonal variant of Cavendish banana expressing resistance to Yellow Sigatoka Leaf Spot disease with satisfactory yield has been reported (Chandha and Sahiram, 2000).

f. Somatic hybridization

It is an approach of immense value in the area of fruit breeding. Somatic hybridization provides a method where sexual incompatibility in the plants can be bypassed.

Protoplast culture includes a series of operation such as isolation of the protoplasts from cells, culturing them in a suitable medium, inducing them to divide and then regenerating plantlets from them. Fusion of protoplasts may occur spontaneously or they may be induced to fuse in the presence of fusigenic agents. The polyethylene glycol (PEG) is the most widely used fusigenic agent (Chandha *et al*., 2000)

Important fruit plants in which protoplast fusion is practised are as under:

$ame	**Method of fusion**
Citrus (Tangelo)+*Murrya paniculata*	Electrofusion
(*Citrus reticulate x Citrus paradisi)+ Citrus jambhiri*	Electrofusion
Citrus sinensis+*Citrus reticulata*	Peg mediated

g. Molecular approaches

Morphological characters, chemical composition and cytological information have been used over the years for the classification of plants. However, these techniques have certain limitation as they could be influenced by environmental and developmental effects. The molecular markers are now being increasingly used in the areas of plant classification and breeding. Polygenic characters which are very difficult to analyse using traditional plant breeding methods can be easily analysed using molecular markers.

h. Genetic engineering

The advent of recombinant DNA technology has opened tremendous possibilities for transforming almost any plant by transferring any gene from any organism across, taxonomic barriers. The recombinant DNA technology involves the following major steps.

_ Isolation of gene of desired characters.

_ Insertion of the isolated gene in a suitable vector (making it a recombinant vector).

_ Transformation – Insertion of the recombinant vector into a suitable host (organism /cell).

_ Selection of the transformed host.

_ Multiplication followed by expression of the introduced gene into the host.

Gene transfer technology

Important gene transfer methods used for production of transgenic plants are as under:

_ Agrobacterium-mediated transformation (Hohn et al., 1989)
_ Microprojectile bombardment-mediated transformation (Sanford, 1990)
_ Propoplast-mediated transformation (Paszkowski et al., 1989)
_ In-planta electroporation (Chowrira et al., 1996)
_ Intact tissue electroporation (D'Halluin et al., 1992)
_ Silicon carbide fibres (Songstad et al.,1995)
_ Electrophoresis (Songstad et al.,1995)
_ Microinjection (Neuhaus and Spangenburg,1990)
_ Sonication (Joerbo and Brunstedi,1992)
_ Laser-mediated gene transfer (Guo et al.,1995)

i. Biotechnology for biotic/abiotic stress management

Fruit crops suffer from a variety of insect pests. It is possible to implement biotechnological approaches to manage insect pests in a rational, durable and eco friendly manner. Therefore, novel insecticidal proteins and their respective genes need to be identified and used in conjunction with Bt to prevent development of resistant insect. In addition, Integrated Pest Management will have to play a central role in sustainable horticulture. Disease resistance, herbicides resistance, abiotic resistance etc. are the areas where genetic engineering can play an important role in imparting resistance in fruit crops.

Eg: In apple gene attacin (from *Hyalophora cecropia*) *lysozyme* (farm chicken) and cercropin B (from *H.cecropia*) can be used for disease resistance against *Eriwinia amylovora*.