

## HYBRIDIZATION: TECHNIQUES AND CONSEQUENCES

The mating or crossing of two plants or lines of dissimilar genotype is known as hybridization. In plants, crossing is done by placing pollen grains from one genotype, the male parent, on to the stigma of flowers of the other genotype, the female parent. It is essential to prevent self-pollination as well as chance cross-pollination in the flowers of the female parent. At the same time, it must be ensured that the pollen from desired male parent reaches the stigma of female flowers for successful fertilization. The seeds as well as the progeny resulting from the hybridization are known as hybrid or  $F_1$ .

The progeny of  $F_1$  obtained by selfing or intermating of  $F_1$  plants, and the subsequent generations are termed as segregating generations. The term cross is often used to denote the products of hybridization, i.e. the  $F_1$  as well as the segregating generations.

### OBJECTIVES OF HYBRIDIZATION

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

and the later generations, i.e. the segregating generations. The degree of variation produced in the segregating generations would, therefore, depend on the number of heterozygous genes in the  $F_1$ . This, in turn, depends upon the number of the genes for which the two parents differ. If the two parents are closely related, they are likely to differ for a few genes only. But if they are not related, or are distantly related, they may differ for several, even a few hundred, genes. However, it is not likely that the two parents will ever differ for all their genes. Therefore, when it is said that the  $F_1$  is 100 per cent heterozygous, it has reference

only to those genes for which the two parents differ.

The aim of hybridization may be the transfer of one or few qualitative characters, the improvement in one or more quantitative characters, or use the  $F_1$  as a hybrid variety. These objectives are briefly discussed below.

**Combination Breeding :** The main aim of combination breeding is the transfer of one or more characters into a single variety from other varieties. These characters may be governed by oligogenes or polygenes. The intensity of the character in the new variety is either

comparable to or, more generally, lower than in the parent variety from which it was transferred. In this approach, increase in the yield of a variety is obtained by correcting the weaknesses in the yield contributing traits, e.g., tiller number, grains per spike, test weight is that for disease resistance. The backcross method of breeding was designed for combination breeding, and often pedigree method also fulfils the same purpose. In combination breeding, the genetic divergence between parents is not the major consideration. What is important is that one of the parents must have in a sufficient intensity the character(s) under transfer, while the other parent is generally a popular variety.

**Transgressive Breeding :** Transgressive breeding aims at improving yield or its contributing characters through transgressive segregation. Transgressive segregation is the production of plants in an F<sub>2</sub> generation that are superior to both the parents for one or more characters. Such plants are produced by an accumulation of plus or favourable genes from both the parents as a must combine well with each other, and should preferably be genetically diverse, i.e., quite different. This way, each parent is expected to contribute different plus genes which when brought together by recombination give rise transgressive segregant. As a result, the intensity of character in the transgressive segregant, i.e., the new variety, is greater than that in either of the parents. The pedigree method of breeding and its modifications, particularly the population approach, are designed for the production of transgressive segregants.

**Hybrid Varieties :** In most self-pollinated crops, F<sub>1</sub> is more vigorous and higher yielding than the parents. Wherever it is commercially feasible, F<sub>1</sub> may be used directly as a variety. In such cases, it is important that the two parents should produce an outstanding F<sub>1</sub>.

### **TYPES OF HYBRIDIZATION**

The plants or lines involved in hybridization may belong to the same variety, different varieties of the same species, different species of the same genus or species from different genera. Based on the taxonomic relationship of the two parents, hybridization may be classified into two broad groups :

1. Intervarietal and
2. Distant hybridization

**Intervarietal Hybridization :** The parents involved in hybridization belong to the same species ; they may be two strains, varieties or races of the same species. It is also known as intraspecific hybridization. In crop improvement programmes, intervarietal hybridization is the most commonly used. In fact, it is so common that it may often appear to be the only form of hybridization used in crop improvement. an example would be crossing of two



Fig 7.1. Complex crosses involving 3, 4 and 8 parents.

Crop improvement progresses, the crop varieties would accumulate more and more favourable genes. This would lead to greater similarities between even unrelated varieties. In view of this, it may be expected that in future complex crosses would become more and more important. In breeding of highly improved self-pollinated crops like wheat and rice, complex crosses are a common practice today. Complex crosses would become routine in near future in the improvement of other self-pollinated crops with the progress in the level of their improvement.

**Distant Hybridization :** Distant hybridization includes crosses between different species of the same genus or of different genera. When two species of the same genus are crossed, it is known as interspecific hybridization; but when they belong to two different genera, it is termed as intergeneric hybridization. Generally, the objective of such crosses is to transfer one or few simply inherited characters like disease resistance to a crop species. Sometimes, interspecific hybridization may be used for developing a new variety, e.g., Clintonoat variety was developed from a cross between *Avena sativa* x *A. byzantina* (both hexaploid oat species), and CO 31 rice variety was developed from the cross *Oryza sativa* var. *indica* x *O. perennis*. Almost all the present-day sugarcane varieties have been developed from complex crosses between *Saccharum officinarum* (noble canes), *S. barberi* (Indian canes) and other *Saccharum* species, e.g., *S. spontaneum* (Kans.). The improvement in fiber length of Indian Cotton (*Gossypium arboreum*) has been brought about by crossing it with American cultivated Cotton ; many improved varieties have resulted from such crosses. Intergeneric hybridization may also be used to develop a new crop species, e.g., Triticale from a cross between *Triticum* sp. and *Secale cereale* (rye). Wild species often provide genes which are not present in the cultivated species. For example, many of the genes for rust resistance in wheat are derived from related wild species. Distant hybridization is likely to become increasingly important in the correction of specific defects of crop species. In many cases, wild species may contribute valuable 'yield genes' as well to the cultivated species.

#### **Pre-requisites for hybridization**

Breeder should have clear knowledge about the following before taking up hybridization.

1. Requirements of the tract
2. Local conditions i.e. soil, climate, Agronomic practices and market requirements
3. Existing varieties of crops both local and introduced
4. Facilities like funds, land, labour and equipment

5. Plant material i.e. germ plasm
6. Objectives : Well set objectives and planning

### **Hybridization procedure or steps involved in hybridization**

Details of the following steps have to be covered in

Practical classes

1. Choice or selection of parents
2. Evaluation of parents i.e. by selfing and studying the progeny
3. Emasculation
4. Crossing or pollination
5. Bagging & Labelling
6. Harvesting of F<sub>1</sub> seed
7. Raising F<sub>1</sub> generation

From F<sub>2</sub> onwards the generations are known as segregating generations and they may be handled either by pedigree method of Bulk method or backcross method for evolving new varieties.

## **HANDLING OF SEGREGATING GENERATIONS**

### **Pedigree Method**

In the pedigree method, individual plants are selected from F<sub>2</sub> and subsequent generations, and their progenies are tested. During the entire operation a record of all parent off spring relationships is kept. This is known as pedigree record. Individual plant selection is continued till the progenies show no segregation. At this stage the selection is done among the progenies, multilocation tests are conducted and released as varieties.

The pedigree may be defined as a description of the ancestors of an individual and it generally goes back to some distant ancestors. It is useful to know the relationship of two individuals and useful for selection of parents and prediction of outcome of the cross.

Procedure of pedigree method

**1<sup>st</sup> year** : cross is made between the parents possessing desirable characters.

**2<sup>nd</sup> year** : Sow the F<sub>1</sub> seed giving wide spacing so that each F<sub>1</sub> plant produces more seeds.

Raise as many F<sub>1</sub> plants as possible to produce large number of F<sub>2</sub> seeds. Harvest in bulk.

**3<sup>rd</sup> year** : Grow 2000-10000 plants of F<sub>2</sub> giving wide spacing for full expression of the characters in F<sub>2</sub> generation plants. Grow parents for comparison. Depending upon the

facilities and objectives of the programme about 100-500 superior plants are selected. The

value of selection depend on the skill of the breeder. He has to judge which F<sub>2</sub> plant will produce superior progeny for characters under consideration. The breeder develops this skill through close study of the crop for many generations. The selection in F<sub>2</sub> is done for simply inherited characters like head type disease resistance etc. and selection for characters governed by many genes like yield will be reserved for later generations. The selected plants are harvested separately and given serial numbers and description entered in pedigree registers.

**4<sup>th</sup> year** : Progeny rows of F<sub>3</sub> i.e. seeds of one selection plant in one row are space planted along with parents and checks. From superior progeny rows, individual plants with desirable characters are selected (about 50-100 families and about 5 plants in each family and harvested separately). Diseased, lodging and undersirable progenies are discarded.

**5<sup>th</sup> year** : F<sub>4</sub> plants raised again as head to row. Desirable plants are selected from desirable rows and harvested separately.

**6<sup>th</sup> year** : F<sub>5</sub> plants raised in 3 row plots i.e. seeds of each selected plant sown in 3 rows. By this time many families might have become reasonably homozygous. For comparison check variety is grown for every 3 or 5 block. Progenies are evaluated for yield and the inferior ones are rejected. The number should be reduced to 25-50. superior plants from superior progenies are selected. Plants from each progeny are bulked.

**7<sup>th</sup> year** : F<sub>6</sub> individual plant progenies are grown in multi-row plots and evaluated. Inferior progenies are rejected and superior progenies are selected. Plants of each progeny are harvested in bulk. Diseased and inferior plants from the progenies are removed.

**8<sup>th</sup> year** : F<sub>7</sub> preliminary yield trial with 3 or more replications are conducted to identify superior lines. The progenies are evaluated for many characters including yield. Standard commercial varieties must be included as checks. Two to five outstanding lines are selected and advanced to coordinated yield trials.

**9<sup>th</sup>, 10<sup>th</sup> & 11<sup>th</sup> year** : selected lines are tested in several localities for 2 or 3 years for adaptation tests. Lines are evaluated for all characters mainly yield and disease resistance.

A line that is superior to commercial variety in yield and other characters is selected. **11<sup>th</sup> and 12<sup>th</sup> year** : Selected superior lines is named, multiplied and released as a new variety.

Number of year can be reduced if generations are advanced during off seasons either in green house or under irrigated conditions.

Several modifications for the above described pedigree method are followed by breeders depending upon the crop, time and availability of funds and facilities like labour, land etc.

**Early generation tests :**

The objective of these test is to find out superior crosses and superior progenies in early generations i.e. in F<sub>2</sub> and F<sub>3</sub>. we need not advance all the crossed and all selected progenies in each cross upto F<sub>8</sub>. much labour, time and cost would be saved by this early generation testing. A more reliable information about the potential crosses and progenies may be obtained by conducting replicated tests (preferably in more location) and evaluating them for yield and other characters in F or F itself. A desirable cross or progeny should

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have high mean yield, high genetic variance and high expected genetic advance under selection. Other crosses and progenies are rejected in the beginning i.e. F<sub>2</sub> and F<sub>3</sub> generations itself.

**F<sub>2</sub> progeny testing :** Another modification for pedigree method. In F<sub>2</sub> make as many single plants selections as possible. From F<sub>3</sub> to F<sub>6</sub> advance the progenies in bulk making selections of the progenies as a whole and discarding the inferior progenies. Thus each of the progeny is derived from the single plant selected in F<sub>2</sub> generation. In F<sub>6</sub> make single plant selections in each of the progeny. Compare the yields of the single plants with progenies from which they are selected. Select superior single plant progenies and advance to preliminary yield trials, multilocation tests etc. There are two advantages 1. No. of crosses can be handled simultaneously 2. Natural selection operates from F<sub>3</sub> to F<sub>6</sub> since they are advanced in bulk.

**Mass pedigree method :** This is another modified pedigree method. Crosses are made and further generations grown in bulk or as mass until suitable season occurs for making desirable selections against drought, insect and diseases etc. The population will be exposed to the natural conditions of vagaries. From the remaining population individual plants are selected and harvested progenies are evaluated for yield and other characters in preliminary yield trials and further generations are proceeded as in pedigree method till release of variety. The advantages of both bulk and pedigree methods can be obtained and large number of crosses can be handled at a time. The disadvantage is that it takes a bit longer time.

**Merits of pedigree method :**

1. It gives maximum opportunity for the breeder to use his skill and judgement in selection of plants
2. It is well suited for the improvement of characters which can be easily identified and are simply inherited.
3. Transgressive segregation for yield and other quantitative characters may be recovered.
4. Information about the inheritance of characters and pedigree of lines can be obtained.

5. Inferior plants and progenies are eliminated in early generations.
6. It takes less time than bulk method to develop new variety.

**Demerits of pedigree method :**

1. Valuable genotypes may be lost in early generations, if sufficient skill and knowledge are lacking in the breeder, at the time of selection.
2. No opportunity for natural selection
3. Difficult to handle many crosses
4. Maintenance of records, selections, growing progeny rows etc are time consuming and laborious.

**Achievements :** Large number of varieties have been developed by pedigree method in many crops.

A few examples are

Wheat – NP -52, 120,125, 700 and 800 series

Rice – ADT – 25, Jaya, Padma

Cotton – Lakshmi, Digvijay, Sorghum – Co 18, RS 610 etc., Tobacco – NP

222 Sorghum – Co 18, Rs 610, Tobacco – NP 222

## **BULK METHOD**

The bulk method was first proposed by Nilsson Ehle in 1908 at Svalof. This method is also known as mass method 'or' Population method of breeding

- ❖ Isolation of Homozygus lines
- ❖ Waiting for the opportunity for selection
- ❖ Opportunity for natural selection.
- ❖ F<sub>2</sub> and subsequent generations are harvested in mass as bulk to raise the next generation.
- ❖ At the end of the bulking period (after attaining homozygosity) individual plants are selected and evaluated similar manner as pedigree method of breeding.

### **THE PROCEDURE FOR BULK METHOD**

The exact procedure for the bulk method would vary depending upon the objective of breeder. The following procedure is described for the isolation of homozygous lines. The breeder may introduce various modifications in the scheme to suit his needs.

**Hybridization :** Parents are selected according to the objective of the breeding programme. A simple or a complex cross is then made depending upon the number of parents involved.

**F1 Generation :** F<sub>1</sub> is space-planted and harvested in bulk. The number of F<sub>1</sub> plants should be as large as possible ; usually more than 20 plants should be grown.

**F2-F6 Generations :** F<sub>2</sub> to F<sub>6</sub> generations are planted at commercial seed rates and spacings. These generations are harvested in bulk. During this period, environmental factors, disease and pest outbreaks would change, the frequencies of different genotypes in the population. Artificial selection is generally not done. The population size should be as large as possible, preferably 30,000-50,000 plants in each generation.

**F7 Generations :** About 30-50 thousand plants are space-planted. 1000 to 5000 plants with superior phenotypes are selected and their seeds harvested separately. Selection is based on the phenotype of plants, grain characteristics, disease reaction, etc.

**F8 Generation :** Individual plant progenies are grown in single or multi-row plots. Most of the progenies would be reasonably homozygous and are harvested in bulk. Weak and inferior progenies are rejected on the basis of visual evaluation. Only 100-300 plant progenies with desirable characteristics are saved.

Some progenies which show segregation are generally rejected unless they are of great promise. In promising pr ogenies, individual plants may be selected ; preliminary yield trial will be delayed for one year in such cases.

**F9 Generation :** Preliminary yield trial is conducted by using standard commercial varieties as checks. The progenies which are superior than the check are advanced. Quality test may be conducted to further reject undesirable progenies. The progenies are evaluated for height, lodging resistance, maturity date, disease resistance and other important characteristics of the crop species.

**F10-F13 Generations :** Replicated yield trials are conducted over several locations using standard commercial varieties as checks. The lines are evaluated for important characteristics in addition to yield, disease resistance and quality. If a line is superior to the standard varieties in yield trials, it would be released as a new variety.

**F14 Generation :** Seed of the released variety is increased for distribution to the cultivators.

### ***MERITS OF BULK METHOD***

1. The bulk method is simple, convenient and less expensive.
2. Since, each F<sub>2</sub> plant is equally represented till F<sub>6</sub>, no chance of elimination of good genotypes in early generations.

3. Artificial or natural disease epiphytics, winter killing high temperature etc. eliminates undesirable types and increases the frequency of desirable type. Thus isolation of desirable types becomes easier.
4. Progenies select from long term bulks are superior than the selection from F<sub>2</sub> or short term bulk.
5. Since, little work and attention is needed in F<sub>2</sub> and subsequent generation more no. of crosses can be handled.
6. No pedigree records which saves time
7. Since large population are grown, transgressive segregants are more likely to appear and increase due to natural selection. Hence, there is a greater chance to isolate good segregants than pedigree method.

#### **DEMERITS OF BULK METHOD**

1. The major disadvantage of bulk method is that it takes a much longer time to develop a new variety. Natural selection becomes important only after F<sub>8</sub> or F<sub>10</sub>, and bulking may have to be done upto F<sub>20</sub> or more. Thus the time required is considerably longer, and most breeders do not use the bulk method simply for this reason.
2. In short-term bulks, natural selection has little effect on the genetic composition of populations. But short -term bulks are useful for the isolation of homozygous lines and for specific objectives as in Harlan's mass -pedigree method.
3. It provides little opportunity for the breeder to exercise his skill or judgement in selection. But in the modified bulk method, the breeder has ample opportunity for practicing selection in the early segregating generations.
4. A large number of progenies have to be selected at the end of the bulking period.
5. Information on the inheritance of characters cannot be obtained which is often available from the pedigree method.
6. In some cases, at least, natural selection may act against the agronomically desirable types.

#### **Comparison between bulk and pedigree method.**

<b>S. No.</b>	<b>PEDIGREE METHOD</b>	<b>S. No.</b>	<b>BULK METHOD</b>
1	Most widely used Breeding method	1	Used only to a limited extent
2	Individual plants are selected in F <sub>2</sub> and subsequent generations and individual plant progenies are grown	2	F <sub>2</sub> and subsequent generations are grown in bulk
3	Artificial selection ; artificial disease epidemics etc. are an integral part of the method	2	Mainly natural selection. In certain cases artificial selection may be essential

3	Natural selection does not play any role	3	N.S. determines the composition of the pop n at the end of the bulking period
4	Pedigree Records have to be maintained which is often time consuming and laborious	4	No pedigree records are maintained
5	Generally its taken 12-13 years to release a new variety	5	Takes more than 15 years.
6	Requires close attention of breeder from F <sub>2</sub> onwards	6	It is quite simple and does not require much attention
7	Planting (spacing) the segregating generations are space planted to permits effective individual plant selection	7	The bulk populations are generally planted at commercial planting rates
8	Population size is small in comparison to bulk	8	The population size is large

Much improvement in crop plants could not be done through this method reason being.

1. Long time required for Natural Selector
2. Lack of opportunity for the breeder to use his skills
3. Lack of facilities to raise large population

**Achievements of bulk method:**

The method has been used to a limited extent is Barley breeding in U.S.A. and more than 50 varieties were developed. They are : ARIVAL, BEECHER, GLACIER, and GEM. Originated from a cross : Atlas x Vaughn. The bulk was maintained for 7 to 8 months.

**SINGLE-SEED-DESCENT METHOD**

Another modification of the bulk method is the single-seed-descent method, which is becoming increasingly popular. In this method, a single seed from each of the one to two thousand F<sub>2</sub> plants is bulked to raise the F<sub>3</sub> generation. Similarly, in F<sub>3</sub> and the subsequent generations one random seed is selected from every plant present in the population and planted in bulk to raise the next generation. This procedure is followed till F<sub>5</sub> or F<sub>6</sub> when the plants would have become nearly homozygous. In F<sub>5</sub> or F<sub>6</sub>, a large number (1 to 5 hundred) of individual plants are selected and individual plant progenies are grown in the next generation. Selection is done mainly among the progenies, and the number of progenies is sufficiently reduced to permit replicated trial in the next generation. Individual plants may be selected only from outstanding families not showing segregation. Thus preliminary yield trials and quality tests begin in F<sub>7</sub> or F<sub>8</sub> and coordinated yield trials in F<sub>8</sub> or F<sub>9</sub>.

The objective of single-seed-descent method is to rapidly advance the generations of crosses ; at the end of the scheme, a random sample of homozygous or near homozygous genotypes/lines is obtained. F<sub>2</sub> and the subsequent generations are grown at very high plant densities as vigour of individual plants is not important. In each year, 2-3 generations may be raised using off-season nurseries and greenhouse facilities. The important features of this scheme are : (1) lack of selection, natural or artificial, till F<sub>5</sub> or F<sub>6</sub> till the population is reasonably homozygous , and (2) raising of F<sub>3</sub> and later generations from a bulk of one seed from each F<sub>2</sub> and the subsequent generation plant in order to ensure that each F<sub>2</sub> plant is represented in the population. As a result of the speed and economy, the single-seed-descent scheme is becoming increasingly popular with the breeders.

The single-seed-descent scheme (1) advances the generation with the maximum possible speed in a conventional breeding method; (2) requires very little space, effort and labour ; (3) Makes the best use of greenhouse and off -season nursery facilities; and (4) ensures that the plants retained in the end population are random sample from the F<sub>2</sub> population. However, (1) it does not permit any form of selection (which is implied in the scheme) during the segregating generations; and (2) in each successive generation, the population size becomes progressively smaller due to poor germination and death of plants due to diseases, insect pests and accidents. In some crops, e.g., pulses, plant loss may be one of the most serious problems of the scheme.

## BACK CROSS

Breeders of early 20<sup>th</sup> century engaged in the development of disease resistant varieties observed that pureline selections with genes for resistance from intra-or inter-specific hybridization were inferior to the generally acceptance superior parent in yield or quality characteristic. To overcome this problem, (Harlan and Pope (1922) suggested the back cross method by which an undesirable allele at a particular locus is replaced by the desirable allele in otherwise elite variety. In other words, B.C. procedure conserves all good characteristics of a popular adapted variety and incorporates a desirable character from another variety.

**Back cross :** A cross between a hybrid (F<sub>1</sub> or a segregating generation) and one of its parents is known as backcross.

**Back cross method :** In the B.C. method, the hybrid and the progenies in the subsequent generations are repeatedly back crossed to one of their parents.

**Objective :** To improve or correct one or two specific defects of a high yielding variety, which is well adapted to the area and has other desirable characteristics.

**Recipient parent :** Well adapted, high yielding variety, lacking one or two characters and hence receives these genes from other variety.

**Donor parent :** The variety which donates one or two useful genes.

**Recurrent parent :** Since the recipient parent is repeatedly used in the backcross programme, it is also known as the recurrent parent.

**Non-recurrent parent :** The donor parent, on the other hand, is known as the non-recurrent parent because it is used only once in the breeding programme (for producing the F<sub>1</sub> hybrid).

### **REQUIREMENTS OF A BACK CROSS PROGRAMME**

1. Existence of a good recurrent parent variety which requires improvement is some qualitatively inherited character or a quantitative character with high heritability.
2. A suitable donor parent must be available possessing the character or characters to be transferred in a highly in tense form.
3. High expressivity of the character under transfer through several back crosses in the genetic back ground of the recurrent parent.
4. The character to be transferred must have high heritability-preferably determined by one or few genes.
5. Simple testing technique for detecting the presence of the character under transfer.
6. Recovery of the recurrent genotype in a reasonable number of back cross generations.

### **Applications of Back Cross method**

B.C. method in applicable to both S.P. & C.P. crops.

1. Inter varietal transfer of simply inherited characters : characters governed by one or two major genes – Eg. disease resistance, used color.

Linkage drag : Failure of transfer of simply inherited characters like disease resistance by B.C. method due to a tight linkage between the gene being transferred and some other undesirable gene.

2. Inter varietal transfer of Quantitative characters : Quantitative characters with high heritability can be transferred.

Eg. Early ness, Pl. height seed size, seed shape.

3. Inter specific transfer of simply inherited characters : Mostly disease resistance from related species into a cultivated species.

Eg. 1. Leaf and stem rust resistance from *Triticum timopheevii*

*T. monococcum, Aegilops speltoides and rye (S. cereale ) to T. aestivum*

2. Black arm resistance from several *Gossypium* species to *G. hirsutum*

4. Transfer of cytoplasm : Back Cross method used to transfer cytoplasm from one variety or species to another. This is especial desirable in cases of Cytoplasmic or Cytoplasmic-genetic male sterility.

E. Transfer of *T. timopheevii* cytoplasm to *T. aestivum*

5. Transgressive segregation : Back cross method may be modified to obtain transgressive segregants. It may be modified in one of the following two ways.

I. The  $F_1$  may be Back crossed only 1 or at most 2 times to the recurrent parent

leaving much heterozygosity for transgressive segregants to appear.

II. Two or more recurrent parents may be used in the back cross programme to accumulate genes from them in to the back cross progeny. Such a modification of the back cross would produce a new variety that would not be exactly like any one of the recurrent parents.

6. Production of Isogenic lines : Isogenic lines are identical in their genotype, except for one gene. Such lines are useful in studying the effects of individual genes on yield and other characteristics. Isogenic lines are easily produced using the back cross method.

7. Germplasm conversion : Conversion of photosensitive germplasm lines (using as recurrent parent) to photo insensitive line (using a photo insensitive line as a donor or non-recurrent parent).

### Transfer of a Dominant Gene

Let us suppose that a high yielding and widely adapted variety A is susceptible to stem rust. Another variety B is resistant to stem rust, and that resistance to stem rust is dominant to susceptibility. A generalized scheme of the backcross programme for the transfer of rust resistance from variety B to variety A is given below.

**Hybridization :** Variety A is crossed to variety B. Generally, variety A should be used as the female parent. This would facilitate the identification of selfed plants, if any.

$F_1$  Generation :  $F_1$  plants are backcrossed to variety A. Since all the  $F_1$  plants will be heterozygous for rust resistance, selection for rust resistance is not necessary.

**First Backcross Generation (BC<sub>1</sub>):** half of the plants would be resistant and the remaining half would be susceptible to stem rust. Rust resistant plants are selected and backcrossed to variety A. BC<sub>1</sub> plants resistant to rust may be selected for their resemblance to variety A as well.

**BC<sub>2</sub>-BC<sub>5</sub> Generations:** In each backcross generation, segregation would occur for rust resistance. Rust resistant plants are selected and backcrossed to the recurrent parent A. Selection for the plant type of variety A may be practiced, particularly in BC<sub>2</sub> and BC<sub>3</sub>.

**BC<sub>6</sub>- Generation:** On an average, the plants will have 98.4 per cent genes from variety A. Rust resistant plants are selected and selfed; their seeds are harvested separately.

**BC<sub>6</sub> F<sub>2</sub> Generation:** Individual plant progenies are grown. Progenies homozygous for rust resistance and similar to the plant type of variety A are harvested in bulk. Several similar progenies are mixed to constitute the new variety.

**Yield Tests :** The new variety is tested in a replicated yield trial along with the variety A as a check. Plant type, date of flowering, date of maturity, quality etc. are critically evaluated. Ordinarily, the new variety would be identical to the variety A in performance. Detailed yield tests are, therefore, generally not required and the variety may directly be released for cultivation.

#### **Transfer of a Recessive Gene**

When rust resistance is due to a recessive gene, all the backcrosses cannot be made one after the other. After the first backcross, and after every two backcrosses, F<sub>2</sub> must be grown to identify rust resistant plants. The F<sub>1</sub> and the backcross progenies are not inoculated with rust because they would be susceptible to rust. Only the F<sub>2</sub> is tested for rust resistance. A generalized scheme for the transfer of a recessive gene for rust resistance is given below.

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

**F<sub>1</sub> Generation :** F<sub>1</sub> plants are backcrossed to the recurrent parent.

**BC<sub>1</sub> Generation :** Since rust resistance is recessive, all the plants will be rust susceptible. Therefore, there is no test for rust resistance. All the plants are self-pollinated.

**BC<sub>1</sub> F<sub>2</sub> Generation :** Plants are inoculated with rust spores. Rust resistant plants are selected and backcrossed with the recurrent parent. Selection is done for the plant type and other characteristics of the variety A.

**BC<sub>2</sub> Generation :** There is no rust resistance test. Plants are selected for their resemblance to the recurrent parent A, and backcrossed with the recurrent parent.

**BC<sub>3</sub> Generation :** There is no disease test. The plants are self-pollinated to raise F<sub>2</sub>. selection is usually done for the plant type of variety A.

**BC<sub>3</sub>F<sub>2</sub> Generation :** Plants are inoculated with stem rust. Rust resistant plants resembling variety A are selected and backcrossed to variety A. Selection for plant type of A is generally effective.

**BC<sub>4</sub> Generation :** There is no rust resistance test. Plants are back-crossed to variety A.

**BC<sub>5</sub> Generation :** There is no rust test. Plants are self -pollinated to raise F<sub>2</sub> generation.

**BC<sub>5</sub>F<sub>2</sub> Generation :** Plants are subjected to rust epidemic. A rigid selection is done for rust resistance and for the characteristics of variety A. Selfed seeds from the selected plants are harvested separately.

**BC<sub>5</sub>F<sub>3</sub> Generation :** individual plant progenies are grown and subjected to rust epiphytotic. A rigid selection is done for resistance to stem rust and for the characteristics of variety A. Seeds from several similar rust resistant homogeneous progenies are mixed to constitute the new variety.

**Yield Tests :** It is the same as in the case of transfer of a dominant gene.

## BACK CROSS

### Transfer of Two or More Characters into a Single Recurrent Parent

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

**Simultaneous Transfer:** Genes for the different characteristics may be transferred simultaneously in the same backcross programme. The characters to be transferred are brought together into the hybrid by successively crossing each of the non-current parents to the recurrent parent or the hybrid thus produced. But in such a case, a larger backcross population would be needed than in the case of transfer of a single character. Further, the breeding programme may be delayed because the conditions necessary for the selection of all the characters may not occur every year. Sometimes, the two genes under transfer may be linked. In such a case, the transfer becomes very easy, and selection for only one gene may be necessary. some examples of such a favourable linkage are; between the genes Lr 24 and Sr 24, Lr 19 and Sr 25, and Lr 26 and Sr 31.

**Stepwise Transfer :** The recurrent parent is first improved for one character. The improved recurrent parent is then used as the recurrent parent in a backcross programme for the transfer of the second character. If additional characters are to be transferred, they are transferred one

at a time in a stepwise fashion. This approach takes much longer time for the transfer of two or more characters.

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

#### **Merits**

1. The genotype of new variety is nearly identical with that of the recurrent parent, except for the genes transferred. Thus the outcome of a backcross programme is known beforehand and it can be reproduced any time in the future.
2. It is not necessary to test the variety developed by the backcross method in extensive yield tests because the performance of the recurrent parent is already known. This may save up to 5 years' time and a considerable expense.
3. The backcross programme is not dependent upon the environment, except for that needed for the selection of the character under transfer. Therefore, off-season nurseries and green-houses can be used to grow 2-3 generations each year. This would drastically reduce the time required for developing the new variety.
4. Much smaller populations are needed in the backcross method than in the case of pedigree method.
5. Defects, such as susceptibility to disease, of a well-adapted variety can be removed without affecting its performance and adaptability. Such a variety is often preferred by the farmers and the industries to an entirely new variety because they know the recurrent variety well.
6. This is the only method for inter-specific gene transfers, and for the transfer of cytoplasm.
7. It may be modified so that transgressive segregation may occur for quantitative characters.

#### **Demerits**

1. The new variety generally cannot be superior to the recurrent parent, except for the character that is transferred.
2. Undesirable genes closely linked with the gene being transferred may also be transmitted to the new variety.

3. Hybridization has to be done for each backcross. This often difficult, time taking and costly.
4. By the time the backcross programme improves it, the recurrent parent may have been replaced by other varieties superior in yielding ability and other characteristics.

### **Achievements**

1. Two cotton varieties 170-C o-2 and 134 – Co 2m were developed
2. Kalyana sona susceptible to leaf rust. Resistant has been transferred from several diverse sources *i.e.*, Robin, K1, Blue bird, Tobari, Frecor and HS-19
3. Tift 23A is susceptible to downy mildew. The line backcrossed with MS-521A, MS-541 A, MS-570A resistant hybrids were produced.

## **Comparison between backcross and pedigree methods**

<b>Pedigree method</b>	<b>Backcross method</b>
F <sub>1</sub> and the subsequent generations are allowed to self-pollinate	F <sub>1</sub> and the subsequent generations are backcrossed to the recurrent parent
The new variety developed by this method is different from the parents in agronomic and other characteristics	The new variety is identical with the recurrent parent, except for the character under transfer
The new variety has to be extensively tested before release	Usually extensive testing is not necessary before release
The method aims at improving the yielding ability and other characteristics of the variety	The method aims at improving specific defects of a well adapted, popular variety
It is useful in improving both qualitative and quantitative characters	It is useful for the transfer of both quantitative and qualitative characters provided they have high heritability
It is not suitable for genes transfer from related species and for producing substitution of addition lines	It is the only useful method for gene transfers from related species and for producing addition and substitution lines
Hybridization is limited to the production of the F <sub>1</sub> generations	Hybridization with the recurrent parent is necessary for producing every backcross generation
The F <sub>1</sub> and the subsequent generations are and	The backcross generations are small

much larger than those in the backcross method usually consist of 20-100 plants in each generation

The procedure is the same for both dominant and recessive genes

The procedures for the transfer of dominant and recessive genes are different

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Multiline varieties are mixtures of several pure lines of similar height, flowering and maturity dates, seed colour and agronomic characters of each of which has a different gene for resistance to the given disease.

#### **Characteristics of a good Multiline**

1. Its genetic diversity for vertical resistance genes for the concerned disease
2. The vertical resistance genes should be strong enough
3. It should have normal resistance to other diseases
4. Components of multiline should be uniform for agronomic and other features.
5. It should have yield advantage

#### **Development of multiline varieties**

A multiline variety is usually created by mixing the seeds of several lines that are similar in appearance but have different genes for resistance to a given disease. There are two main steps in the development of multilines:

1. Development of component lines
2. Evaluation and grouping of the components.

#### **Development of component lines**

The resistance genes are incorporated in an elite variety or line to produce as many near-isogenic lines as there are distinct R genes. This is done through a conventional backcross programme (5-6 backcrosses), a limited backcrossing (2-3 backcrosses, followed by pedigree selection) or by making double or multiple crosses. The lines obtained from the last two approaches are likely to differ for agronomic and other features as well; therefore, a detailed evaluation of such lines is essential.

#### **Evaluation and grouping of the components**

The number of component lines should be large, 15-20 according to Borlaug (1959), if durability of resistance is desired. But if a reduced level of disease is the objective, a rather small number of component lines would be adequate.

#### **Achievements**

Multiline varieties appear to be a useful approach to control disease like rusts where new races are continuously produced. In India, four multiline varieties have been released in