(a) **Simple recurrent selection**: In this method a number of plants with desirable phenotypes are selected and self pollinated. In next generation the progenies from the selected plants are grown separately, and are allowed to intercross in all possible combinations. Equal amount of seeds from each plant are composited to produce the next generation. This completes the **original selection cycle**. For recurrent selection several desirable plants are selected from the composite population and are self pollinated. Progeny rows are grown and all possible intercrosses are made by hand. Equal amount of seeds from all the intercrosses are composited to produce next generation. This is the **first recurrent selection cycle**. This population may be subjected to further more recurrent selection cycle in the same way.

Recurrent selection is effective in increasing the frequency of desirable genes in the population. It is most suited for characters with high heritability. Simple recurrent selection is considerably more efficient than selection with self pollination.

4.4 CLONAL SELECTION

A **clone** may be defined as a group of plants derived from a single plant by vegetative propagation. The clone can be characterised by the following characters:

(a) Clone is homogeneous,

(b) Individuals of a clone are either homozygous or heterozygous,

(c) Clones are stable in nature,

(d) Variability can be induced through mutation,

(e) Clone is propagated vegetatively.

Normal stem, runner, sucker, stolon, tuber, rhizome, bulb and root or root cuttings can be used as clones.

The **procedure** of clonal selection is the selection of desirable clones from the mixed population of vegatatively propagated plants. The technique lies in selecting and propagating the best clone based on its performance. The steps may be summarised as follows:

1. The collection of all possible clonal variability.
2. Critical evaluation of each clone and each member of a clone for yield and quality following test for disease and pest infestation.
3. The disease free and high yielding clones are selected and evaluated and multiplied as variety.

Advantages of clonal selection are:

1. **Easy maintenance**: In this method there is no chance for outcrossing or loss of seed viability.
2. **Very quick**: As there is no time requirement for seed development, single clone can be identified very easily and can be multiplied straight way to give new variety.
3. **Permanent hybrid**: Heterotic clones can be exploited for any length of time without the need to produce hybrid seed as in seed crops.

The method of crossing or breeding of two pureline plants of two dissimilar genotypes to get the best offspring is called hybridisation

After domestication and selection, hybridization is the most potential breeding method for improvement of crop. The previous two methods utilise the existing genetic variability within the particular species or variety. Hybridization aims at to create new genetic variation of characters. It is the method of crossing of two pure line plants of two dissimilar genotypes, which will produce the F_1 hybrids and then the subsequent generations will be segregating generations.

5.1 PURPOSE

The objectives of hybridization can be categorised under 3 major criteria:

1. **Development of productive varieties**: One of the objectives is to create new variability which is further used for development of improved varieties with respect to existing varieties. This is achieved through the following methods of breeding or selection of the segregating progeny of F_1 hybrid.
   (a) Pedigree method
   (b) Mass-pedigree method
   (c) Bulk progeny
   (d) Bulk population breeding.
2. **Removal of bottle-neck genes**: The appearance of new problems (pathogen, insect) to yield can be protected through the creation of new recombinants which will help to come out from this kind of situation.
3. **Development of heterotic F_1 hybrids**: In order to exploit hybrid vigour, hybridization between two chosen inbred parents can be resorted to produce F_1 hybrids. In case of self pollinated crops two methods of breeding are used for attaining this objective.

(a) **Combination breeding**: The main objective of this method is to transfer one or more characters into a single variety from other varieties. In this breeding the genetic divergence between the two parents is not the major consideration, here among the two parents one must have the character to be transferred and another parent is the popular variety.

(b) **Transgressive breeding**: This method aims at improving yield or contributing characters through transgressive segregation. It is the production of plants in the F_2 generation which will be superior to both the parents for one or more characters. Assembly of all productive genes in transgressive progenies requires the following genetic situations:

(i) The character must be polygenic;

(ii) Parents should be completely homozygous;

(iii) Parents should be complementary to each other;

(iv) There should not be any linkage between the chosen characters.

5.2 GENERAL TECHNIQUE

Before the beginning of any hybridization programme the breeder should decide the objective of the programme. The whole hybridization programme involves the following procedures (Fig. 5.1):

(a) Choice of parents
(b) Crossing schedule
(c) Emasculation
(d) Bagging
(e) Tagging
(f) Pollination
(g) Harvesting and storage of F_1 seed.

Choice of Parents

Parents which are chosen for hybridization provide the requisite variability for isolating desirable segregants in subsequent generations. The following criteria are essential for choosing parents for hybridization.

(i) **Agronomic base**: Any well established local cultivar used as seed parent or female parent should have agronomic base for that area.

(ii) **Complementary pollen parent**: The male parent should be complementary to the female parent, i.e., it must possess the complementary attributes in their intense form.

(iii) **Homozygosity of parent**: The parent chosen for hybridization must be homozygous for the character, i.e., pureline. Though there may be cryptic variability.

(iv) **Multiple parentage**: When the desired attributes are not present in the above two parents, more than two parents can be chosen for crossing.

(v) **Combining ability**: Experimental evidences suggest that cross-combinations involving high and low combiners (H × L) or both high combiners (H × H) often provide better opportunity for superior recombinants.

(vi) **Genetic divergence**: Choice of parents based on genetic divergence will be helpful to get the divergent parents in segregating generations.

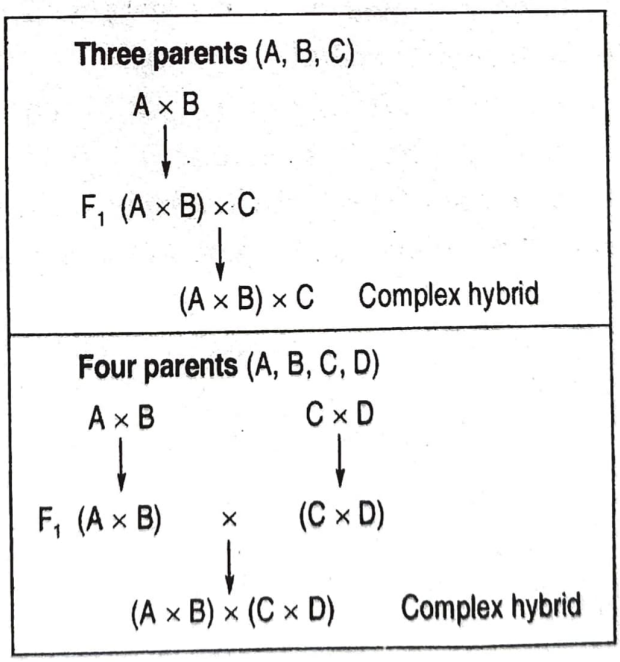
In conclusion, the breeder should have a well defined, clear cut objective, based on the present and expected future needs, in developing a new variety. Breeder should select the parents accordingly and use them in a suitable hybridisation programme.

Crossing Schedule

Crossing schedule entails development of hybrids by emasculation and pollination which helps in increasing the spectrum of variation among the hybrids, this heterogeneity is narrowed down by developing homozygous pure line.

Type of crosses: The parents involved in hybridization should belong to the same species; they may be of two strains, varieties or races of the same species. When only two parents are crossed to produce the F_1 then it is called **simple cross**; the F_1 hybrid may be selfed or back crossed with homozygous parent.

More than two parents are also crossed to produce the F_1 hybrid, which is then used to produce F_2 or is used in a back cross. Such a cross is also known as **convergent cross** because this crossing programme aims at converging, i.e., bringing together genes from several parents to a single hybrid. This is the **complex cross** where multiple convergence is attempted.



Number of crosses: How many (single, double or multiple) crosses should be developed would depend upon the objectives defined for the breeding programme, floral biology of the crop and the quality of parents chosen. Based on the recent concept of genetics, the correct choice of parents can cut short the number of crosses to develop the wanted hybrid.

Emasculation

In case of hybridization programme for self pollinated crops, it is very much essential to emasculate the plant to avoid the self pollination. Removal of male sex organs or killing that part of the flower without any damage or disturbances to female reproductive organ is known as **emasculation**. Before going to hybridization programme the efficiency of emasculation technique may be tested by bagging the emasculated flowers without pollination. The amount of seed thus set would indicate the frequency of self fertilisation occurring during emasculation.

There are various **techniques** of emasculation:

(i) **Hand emasculation**: A general procedure for hand emasculation is as follows:

(a) Emasculation is done before the anthers mature and the stigma has become receptive to minimise self pollination.

(b) The corolla of selected flowers is opened with the help of fine tip forceps and the anthers are removed.

(c) In case of epipetalous stamen the corolla lobes are removed carefully keeping the gynoecium not injured at all.

(ii) **Suction method**: This method is useful in case of small flowers where hand emasculation is not possible. The petals are generally removed with forceps exposing the anthers and stigma. A thin rubber tube or glass tube attached to a suction hose is used to suck the pollen grains from its surface. The suction may be produced by an aspirator or by a small suction pump. Care must be taken that suction should be enough to suck the stamen and pollen grains but not the gynoecium. But this method is not very efficient, as 15% of self pollination takes place. Washing the stigma with water may also reduce self pollination.

(iii) **Hot water emasculation**: Pollen grains are more sensitive to hot water than the female reproductive organs. So treatment with hot water at particular temperature and for fixed time period is helpful for killing the pollen grains without damaging the female organ. Treatment with water at 42-48°C for 10 minutes is effective in jowar, treatment at 40-44°C for 10 minutes is effective in rice; in both the cases the whole spike is immersed in thermoflask containing hot water.

(iv) **Alcohol treatment**: It is not very popular method; a particular concentration of alcohol is used for a fixed time period to kill the pollen grains. But a little bit more exposure, i.e., few seconds more than the recommended time period will reduce the female receptivity, i.e., seed set, as female organs would also be killed by this treatment.

(v) **Cold treatment**: Like hot water treatment, cold treatment can also kill pollen grains without damaging the gynoecium. Keeping rice plant at 0-6°C kills the pollen grains, and also wheat plants at 0-2°C for 15-24 hrs. kills the pollen grains. But cold treatment is less effective than hot treatment.

(vi) **Genetic emasculation**: Genetic or cytoplasmic male sterility may be used to eliminate the necessity of emasculation, i.e., male sterile plants are naturally emasculated. For self incompatible species emasculation is not necessary, but in certain genotypes male sterility can be induced by gene manipulation in cytoplasmic or nuclear genome.

Bagging

Immediately after emasculation, the flower or the inflorescence is enclosed in suitable bag to prevent random cross pollination. The bags may be made up of paper, butter paper or parchment paper, and tied at the base of the inflorescence with thread, pin or wire. As the moisture and temperature become higher within the bag so fungus may develop, which may be prevented by removing the bag after 2-3 days after pollination.

Tagging

The emasculated flower or inflorescence is tagged after bagging. The tags are made up of light weight tin-plate and are written in carbon pencil. The tag should bear the information:

(a) Date of emasculation;

(b) Date of pollination;

(c) Names of female and male parents, consecutively in the cross.

Pollination

Mature, fertile and viable pollen from donor plant (Male parent) should be placed on the receptive stigma to bring about fertilisation. During pollination pollen viability is a major factor and also is the time duration for receptivity of stigma. Both timing should match during pollination. The detail procedure for pollination vary in case of different crops.

Harvesting and Storing F_1 Seeds

The seeds are harvested from crossed heads or pods. The seeds should be dried and properly stored to protect them from storage pests. Proper care should be taken to avoid contamination of the hybrid seed with other seeds. The seeds from each cross should be kept separately and preferably, the seeds should be kept alongwith the original tags.

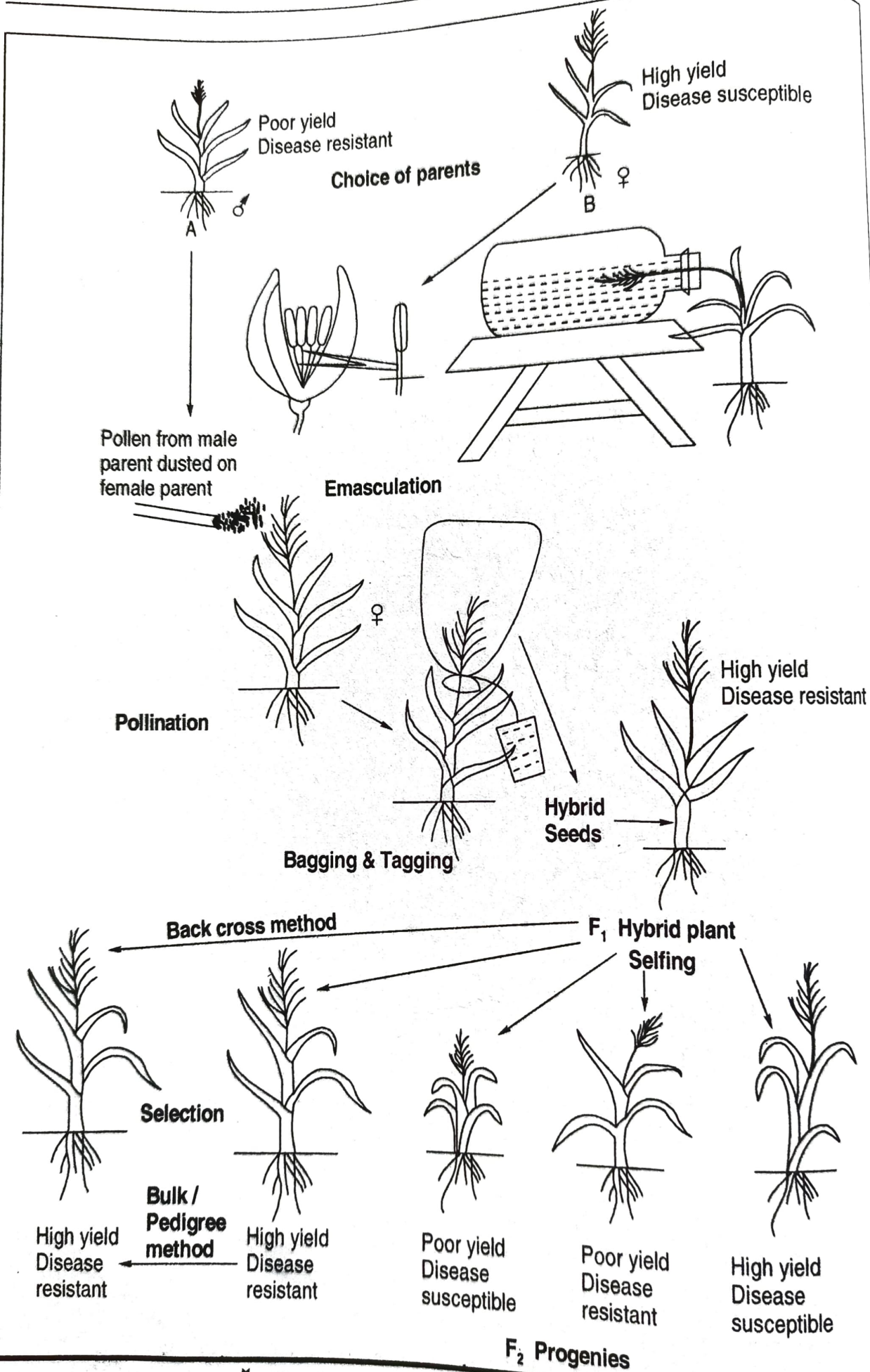


Fig. 5.1: Steps in Hybridization technique

Raising F_1 Generation

In a hybridization programme it is expected that the F_1 generation will be in heterozygous condition. The size of the F_1 generation is a crucial matter, large F_1 population is difficult to handle, but the large F_1 population has the greater opportunity of rare recombination to occur. Usually F_1 is allowed to self pollinate to get the F_2 but in back cross programme it is crossed to one of the parents.

Selfing

The technique of selfing varies from one crop to the other depending upon their mode of reproduction. In self pollinated crops selfing is not very troublesome as it is the natural mode of reproduction. But in cross pollinated crops the flowers are bagged to prevent cross pollination. If the inflorescence bears bisexual flowers then bagging is very much helpful for self pollination also.

Consequences of Hybridization

Segregation and recombination of genes would produce several new genotypes in F_2, in addition to parental types. The number of recombinant genotypes increases rapidly with the increase in number of segregating genes. The Fig. 5.2 indicates the reduction in heterozygocity by 50% in each successive generation on selfing.

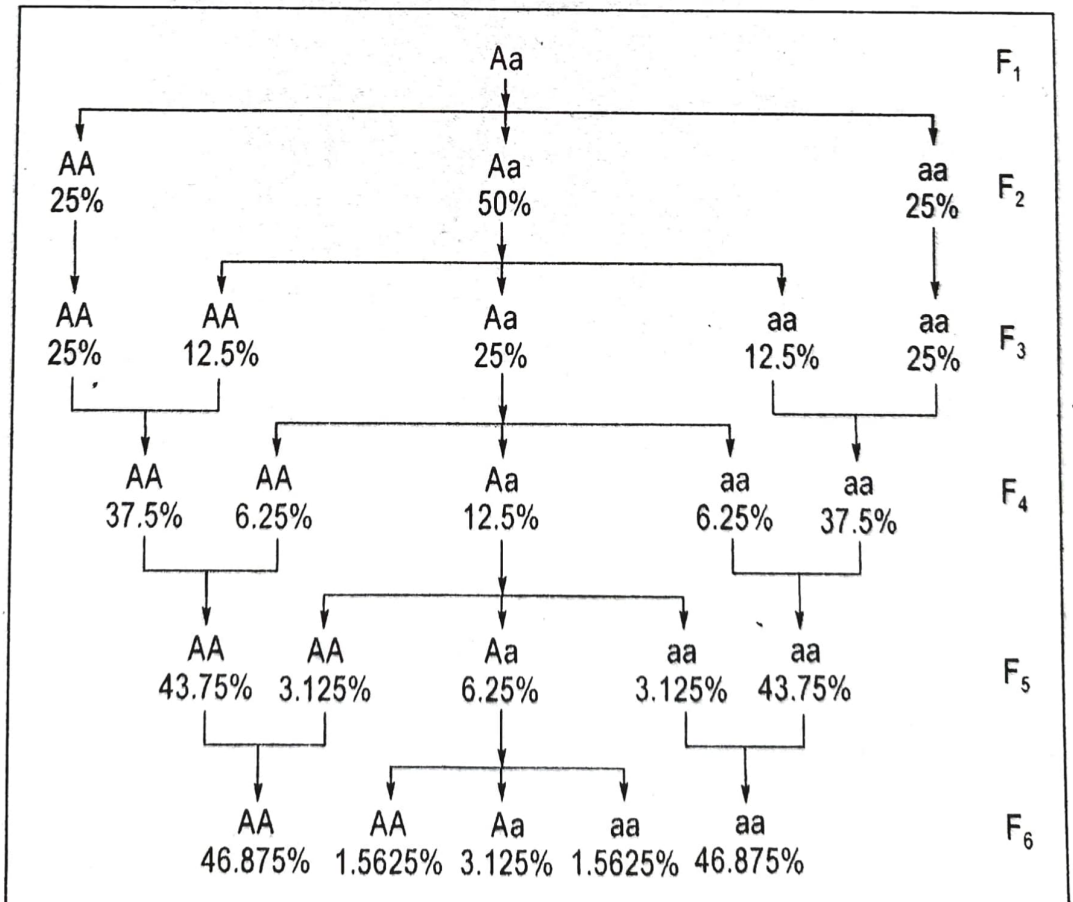


Fig. 5.2: Genetic consequences of selfing of F_1 hybrid

5.3 METHODS IN SELF POLLINATED CROP

For selection of a new variety from a segregating population after hybridization the methods followed in self pollinated crop are of three types:

(A) Pedigree method, (B) Bulk method, (C) Back cross method.

A. Pedigree Method

Individual plant progeny is selected from F_2 and subsequent generations, and their progenies are tested. During this process the record of parents as well as offsprings is kept, for which it is known as **pedigree method**. The pedigree is defined as the description of the ancestors of an individual and it is generally helpful in finding out the amount of relatedness among two individuals, i.e., whether they are related by common parent in their descent ancestry or not.

Procedure

First Year: The **hybridization** is done among two selected parents, after emasculation one become female parent and another male parent. After seed set and maturation, the F_1 seeds are harvested separately from each plant individually. On the basis of choice of parents, the type of cross will be of two types — it will be simple cross or complex cross.

Second Year: **F_1 generation** seeds are space planted and selfing is allowed, each F_1 will produce more F_2 seeds. From 15-30 selected F_1 plants, the F_2 seeds are collected to get a reasonable size of F_2 population and variation.

Third Year: In **F_2 generation,** 2000-10000 plants are space planted, 100-500 plants are selected and their seeds are harvested separately. If the parent plants are closely related varieties then the number of selected F_3 plants would be smaller whereas in case of distantly related varieties the number of F_3 progenies will be of relatively larger numbers.

Fourth Year: In **F_3 generation** also, the individual plant progenies are space planted. Each progeny should have about 30 or more plants. Individual plants with desirable characteristics are selected, disease and lodging susceptible progenies to be eliminated, and also the progenies with undesirable characters are rejected even from the selected plants. During this selection if the number of superior progenies are very small then the whole cross programme may be rejected.

Fifth Year: The selection procedure is same as previous year, only if two or more progenies coming from the same F_3 progeny are similar and comparable, then only one may be saved and others may be rejected. The emphasis is given on the selection of desirable plants from superior progenies.

Sixth Year: Individual plant progenies of **F_5 generation** are planted according to recommended commercial seed rate. Three or more rows for each progeny will help in comparison among progenies. Many progenies may have become reasonably homozygous genotype and may be harvested in bulk. If the progenies show variation then the indi-

vidual plants are selected. The number of selected progenies should be reasonable so that preliminary yield trial with 25-100 progenies can be done.

Seventh Year: Individual plant progenies of F_6 **generation** are planted in multi-row plots and evaluated visually. Progenies harvested in bulk since they become homozygous. The segregating progenies may be discarded and the preliminary yield trial may be done for the progenies which are reasonably homozygous and have enough seeds.

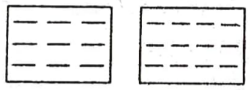
Eighth Year: **Preliminary yield trial** with three or more replications are conducted to identify few superior lines. The progenies are evaluated for plant height, lodging, disease resistance, flowering time, maturity time, etc. Quality test is done to serve as an additional basis for selection.

Ninth to Tenth or Thirteenth Year: The superior lines are tested in **replicated yield trials** at several locations. The above mentioned criteria are evaluated for these lines. The line which is superior than the best commercial variety may be released as new variety.

Eleventh or Fourteenth Year: The selected strain should get **multiplied** to release as a new variety. Breeder has the responsibility to supply the seeds to the state seeds corporation for production and marketing of the seeds.

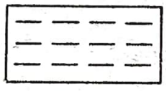
Schematic representation:

First Year



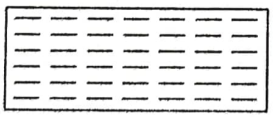
Selected plants of parental variety are hybridized to get the F_1 hybrid seeds.

Second Year



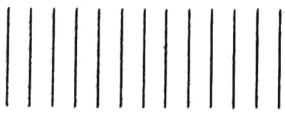
The hybrid seeds (10-30) are planted and the seeds of F_2 harvested in bulk.

Third Year



2000-1000 seeds are space planted. 100-500 superior plants are selected and seeds are harvested from those as pedigree.

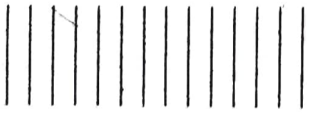
Fourth Year



Individual plant progenies are planted in row and space planted.

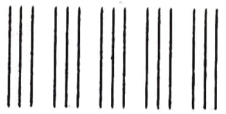
Superior plants are selected.

Fifth Year



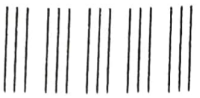
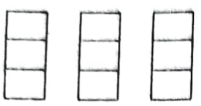
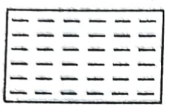
The same procedure as in fourth year.

Sixth Year



The individual selected plant progenies are in multi-row plots.

Superior plants are selected from those progenies.

Seventh Year	 F_6	The same procedure as in previous year. Enough seeds to be collected from superior plants.
Eighth Year	 F_7	The preliminary yield trial and quality test has to be done.
Ninth to Thirteenth Year	 $F_8 - F_{12}$	Co-ordinated yield trials to be done. Disease and quality tests have to be done.
Fourteenth Year	 F_{13}	Multiplication of seeds for distribution and release as a new variety.

Merits

1. This method is most useful as transgressive segregation for yield and other quantitative characters may be recovered in addition to improvement of specific characters.
2. This method is well suited for improvement of characters which can be easily identified and simply inherited.
3. Through the maintenance of pedigree record the breeder may be able to obtain the information about inheritance of characters.
4. Plants or progenies with weaker and visible defects are eliminated at an early stage in the breeding programme.
5. This method gives maximum importance on the breeder to use his/her skill and judgement about the selection of plants and progenies.
6. This method takes less time than bulk method to release a new variety.

Demerits

1. The success of the method is mainly dependent on the skill of the breeder.
2. To keep the individual pedigree record is laborious and time consuming, it may be the limiting factor for large breeding programme.
3. Selection of large number of progenies in every generation is also laborious and time consuming.
4. In F_2 and F_3, the selection for yield is not effective. If sufficient number of progenies are not retained, valuable genotypes may be lost in early segregating generations.

Achievements

Pedigree method is useful in selection of new superior recombinant types from a hybridization programme. This method is suitable for improving specific characteristics, such as disease resistance, plant height, maturity time, etc. as well as yield and quality characters.

Many improved varieties have been developed through pedigree method in many crops like wheat, rice, barley, pulses, oil seeds, cotton, tobacco, jowar, vegetables, etc.

Wheat

K 65 (tall variety) ← C 591 × NP 773

K 68 (good quality grain) ← NP 773 × K 13

WL 711 (dwarf, high yield) ← (S308 × Chris) × Kalyan Sona

Malviya 12 (good grain) ← NP 876 × Cno 66

Rice

'Jaya' and 'Padma' (short duration, finer grain) ← Taichung Native 1 × T 141

Cotton

'Laxmi' (fibre quality, early maturing, resistant to leaf blight) ← Gadag 1 × CC 2 (Combodia Coimbatore 2)

Tomato

Pusa early dwarf (more yield) ← Meeruti × Red cloud

NCBA

Dipak Kr Kar
Soma Halder

Plant Breeding Biometry Biotechnology