HYBRIDIZATION

Hybridization - producing new crop varieties - crossing two or more plants of unlike genetic constitution.

Based on the nature & relationship of plants to be crossed, hybridization can be –

Inter-varietal – Cross bet. plants of two diff. var. of same species - intra-specific hybridization. Eg. Hybrid Maize.

Intra-varietal – Cross bet. two plants of diff. genotypes but same variety.

Inter-specific – Cross bet. two species of genus - Eg. Wheat, Cotton, Tobacco.

Inter-generic – Cross bet. two diff. genera.

Eg. Sugarcane X Bamboo, Wheat X Rye, Radish X Cabbage.

OBJECTIVES

- To evolve a variety with desirable characters. Eg. high yield, good quality, disease resistance, drought tolerance, etc.
- To produce useful variations by introducing recombination of characters.
- To produce and utilize hybrid vigor.

PROCEDURE/TECHNIQUES OF HYBRIDIZATION

The following information are essential for crossing

- Details of male parent.
- Details of female parent.
- Whether plants are unisexual or bisexual.
- Time of anthesis.
- Time of harvesting of plants.
- Whether flowers are self or cross pollinated.

SELECTION OF PARENTS

- First step in hybridization.
- Desirable male & female plants are selected.
- Parent plants should be healthy & vigorous.
- Parents to be grown in isolation & self pollinated to bring homozygosity in desirable traits.
- All important characters to be combined should be kept in mind.
- Both parents should mature at the same time.

SELFING OF PARENTS

- 2nd step in hybridization.
- Make the parents pure in its characters.
- Done by artificial self fertilization.
- Bisexual flowers - covered using paper bags before anthesis to prevent natural cross pollination.
- Self pollination will occur inside the paper bags.
- In cross pollinated crops male & female flowers are bagged separately before anthesis.
- When stigma becomes receptive, pollen grains are collected & brushed on stigma – this is artificial pollination.
- Process continued till parent becomes homozygous or pure for its particular character.

EMASCULATION

- 3rd step in hybridization.
- Removal of stamens from female parent before they burst & shed pollen.
- Done to prevent self pollination.
- Done in flower buds expected to open in the following day.
- Such flower buds are recognized by their enlarged unopened corolla.
- Emasculation can be done by any of the following methods.

FORECEPS AND SCISSORS METHOD

- Done in large flowers.
- Buds are opened & anthers removed using sterile forceps & needles or scissors.
- No injury to be caused to other floral parts – calyx, corolla & especially pistil.

HOT OR COLD WATER OR ALCOHOL TREATMENT

- For small flowers like that of rice, sorghum, etc. that are difficult to be emasculated by forceps, scissors, etc.
- Emasculation is done by dipping panicles in hot water for a definite period.
- Ideal temperature is between 45^{o} C and 50^{o}C.
- Gynoecia can withstand a temperature at which anthers are killed.
- Also done by dipping inflorescence in cold water or alcohol for a definite period.

MALE STERILITY METHOD

- In self pollinated crops, emasculation is eliminated by the use of male sterile plants in which anthers are sterile.
- Male sterility can be induced by spraying 2,4-D, NAA, maleic hydrazide, etc. on immature flower buds.

BAGGING

- After emasculation flower buds are kept enclosed in bags of ideal sizes, made of plastic, cellophane or paper.
- Bags are tied by thread, wire, pins, etc.
- This process is called bagging.
- Both male & female flowers - bagged separately to prevent contamination (foreign pollen).
- Bagging is usually done before anthesis.

MALE PARENT

- Unopened flower buds of male parent whether unisexual or bisexual, are covered as like female parent to prevent contamination of foreign pollen grains.
- This is to ensure that pollen of bagged flowers are of the same plant and not of any other plant.

COLLECTION AND STORAGE OF POLLEN

- Pollen grains for crossing are collected from bagged male flowers.
- Pollen/anthers collected in petri dishes or paper bags just after dehiscence.
- Stored in vials or capsules for future use.

CROSSING OR CROSS POLLINATION

- **5^{th} step in hybridization.**
- **It is defined as artificial cross pollination between genetically unlike parents.**
- **Viable pollen is collected from desired male plant & transferred on to the stigma of the desired emasculated female parent.**
- **Pollen grains are collected in petri dishes.**
- **Bag is temporarily removed from the female parent & the collected pollen are dusted on stigma.**

- **In crops like Bajara, Jowar, etc. hand cross pollination is tedious, since flowers are small.**
- **In such crops male & female plants are grown side by side & male & female inflorescences are enclosed in one bag.**
- **Here crossing takes place inside the bag automatically.**
- **Crossing is normally done at the time of anthesis, dehiscence & stigma receptivity.**
- **Dehiscence of anther can be recognized by the yellowish powdery mass.**
- **Stigma receptivity is evident by the presence of fluid on its surface.**
- **After crossing female flowers are again bagged.**

LABELLING

- **Emasculated & crossed flowers are properly bagged, tagged & labeled.**
- **Labeling is done either on bag itself or on separate labels.**
- **Labels are tagged to bags using thread.**
- **Label should bear following details –**

  Reference number

  Date of emasculation

  Date of crossing

  Details of male and female parents

COLLECTION OF HYBRID SEEDS

- After maturity of seeds, crossed heads of desirable characters are harvested & collected along with labels in separate envelopes.
- In the next season, seeds of each head are sown separately to raise F1 generation.
- All plants of F1 are genetically similar & look exactly alike.
- Plants of F1 are progenies of crossed parents & are hybrids.
- They may exhibit hybrid vigor – increased growth, size, yield, function, etc. over the parents.

METHODS OF HYBRIDIZATION

- Handling of F1 & subsequent generations is carried out by different selection methods.
- Methods of hybridization are different for self & cross pollinated crops.

HYBRIDIZATION OF SELF POLLINATED CROPS

- Selection methods of hybridization in self pollinated crops are as follows.
- Self pollinated crops include – wheat, barley, oats, rice, cotton, tobacco, potato, peas, beans, etc.

PEDIGREE METHOD

- **Desirable hybrids of F1 generation are selected on the basis of desired characters.**
- **Seeds from each selected plant are collected & grown separately in rows to raise F2.**
- **From F2 the best performers are selected & seeds from these plants are sown separately in rows to raise F3.**
- **Process repeated till F6 produced becomes homozygous & fairly uniform.**

- **Undesirable varieties are discarded during selection process.**
- **Plants uniform in desirable strains are harvested & bulked together to constitute a variety.**
- **Selected variety is further tested for yield & adaptability in different localities.**
- **Finally the variety is named, multiplied & distributed to farmers.**
- **In this method a complete ancestral record (pedigree) of each progeny is maintained.**

BULK METHOD

- F1 hybrid plants are grown in bulk.
- Their seeds are harvested & collected together – used to raise F2 generation.
- From F2 desirable plants are selected & seeds are bulked together- seeds used to raise F3 population.
- From F3 suitable plants are selected, seeds collected & bulked together.
- Process repeated till F6 or F7 generations till homozygosity is achieved.
- Plants with superior quality are named & released to farmers for cultivation.
- This is done after yield trials are done at different stations.

BACK CROSS METHOD

- Method is employed for the improvement of both self & cross pollinated crops.
- This cross is to transfer a particular quality of one parent to another lacking it.
- Disease resistance, drought tolerance, etc. are introduced into susceptible crops with other good qualities.
- This hybrid is back crossed to the desirable variety.
- Process is continued till F6.
- Plants are allowed to self pollinate so as to obtain homozygosity for the introduced characters.

- **In back cross system – desirable variety is known as recurrent or recipient variety.**
- **Undesirable variety possessing a character which is lacking in the desirable variety is known as donor parent.**

Eg. A is a good or desirable variety but it is disease susceptible.

B is an inferior variety but disease resistant.

Here A is recipient and B is donor. The desirable character ie; disease resistance of B is transferred to A by crossing A and B. Here A is female parent and B is the male parent.

- **The F1 instead of allowing to self pollinate, it is back crossed to A to transfer genes of desirable quality.**
- **This back cross generation is named as BC1.**
- **The plants of BC1 are further back crossed to A to raise the BC 2 generation.**
- **Back crossing is repeated up to BC 7 generation.**
- **Now the plants will be homozygous for all desirable characters of A parent and 50 % genes for disease resistance.**
- **At BC7 plants are allowed to self pollinate & they become homozygous for all characters including disease resistance and these plants are selected.**

A (female) X B (male with disease resistance)

Hybrid F1 X A

Hybrid BC1 X A

Hybrid BC2 X A

Hybrid BC 3 X A

Hybrid BC6 ---→ allowed to self pollinate.

(Plants with all good qualities of A + disease resistance)

Back cross method is regarded best because –

The required character of a plant can be brought easily into the desirable variety.

Fertility is established and sterility can be minimized after repeated back crossing.

MULTIPLE CROSS METHOD

- A series of bridge crosses are made.
- Desirable monogenic characters found scattered in many pure line parents are combined together in one variety.
- If there are eight varieties each with one good quality, then the qualities of these eight varieties are combined into one.

I A X B C X D E X F G X H ------→ Single crosses

II AB X CD X EF X GH ------→ Double crosses

III ABCD X EFGH -------→ Multiple cross

ABCDEFGH --→ Hybrid

- Hybrids of multiple crosses are selfed and F2 is raised.
- Further breeding is carried out either by pedigree or bulk method of selection.

HYBRIDIZATION IN CROSS POLLINATED CROPS

- Common cross pollinated crops include – maize, rye, cucurbits, fruit trees and forage crops.
- In these crops desirable characters are found scattered in different pure lines (inbreds).
- The characters can be combined in any of the following ways -

  Single cross, Three way cross, Synthetic cross, Double cross and Top cross.

SINGLE CROSS

- It is the cross between two inbreeds (pure lines) Eg. A X B or C X D.
- Hybrids are directly distributed to farmers for cultivation purpose.

DOUBLE CROSS

- **It is a cross between the F1 hybrids of two different single crosses, each involving two different inbreeds.**

1st single cross A X B

2nd single cross C X D

F1 hybrid AB X F1 Hybrid CD

ABCD Double cross hybrid

THREE WAY CROSS

- **Cross between F1 hybrid of a single cross & an inbred which is used as male parent.**

A X B Single cross

AB F1 Hybrid

AB X C Three way cross

DIFFICULTIES IN CONDUCTING HYBRIDIZATION

- **Isolation of suitable parents hybrids**

  Most difficult part of hybridization is to isolate the desirable inbreds to be used as parents and the hybrids to constitute the variety. This requires careful observation, thorough field testing, keeping records, etc.

- **Different times of maturity**

  Usually plants grown in the same season are selected for crossing but they may not flower together due to difference in their time of maturity. This can be corrected by adjusting the sowing period in such a way that both male and female plants flower simultaneously. In some cases pollen grains are preserved.

- **Incompatibility and sterility**

  This is common in both inter specific and inter generic crosses. The incompatibility may be due to lack of pollen germination, failure of fertilization, etc. Hybrid pollen sterility is the main handicap in inter generic crosses due to gene imbalance.

- **Flower damage during emasculation**

  Some times during emasculation technique the flowers can get injured or damaged leading to failure in hybridization.