SOURCES OF GENETIC VARIABILITY

What is Plant Breeding – Give or explain 2 definitions of Plant Breeding

List the sources of Genetic var. needed in Plant Breeding Explain 2

Success in Plant Breeding depends on the genetic variability in the source population as plant breeding is a selection made, possible by the existence of variability. Selection acts on the existing variability but does not creation variability sources include

(a) Gene mutations

(b) Gene recombinations

(c) Direct gene manipulation or gene transfer

(d) Somaclonal variation

(e) Plant hybridization

(f) Plant introduction

Gene mutation & recombination occur naturally; gene transfer is made possible by the use of recombinant DNA techniques while somaclonal variations involve the application of tissue and cell culture techniques.

Gene mutation:- Sudden heritable change in genes structure. This occurs at random in nature or can occur due to environment effects.

Deoxyribonucleotide

DNA Deoxyribose sugar Purines (adenine & guanine)

Nitrogen bases

Pyrimidine (thymine & cytosine)

(a) Accidental mutation may take place after a tautomeric shift of the (H) hydrogen atom which prevents the pairing of the bases in DNA

Normal pairing

A	T	C	G	A	T
I	I	I	I	I	I
T	A	G	C	T	A

A = T double

C = T triple

Abnormal

A - Adenine pairs

I - with guanine

G

As a result, different amino acids are bound resulting in the synthesis of different proteins. Chromosomal aberration (deletion, duplication inversion & translocation) also cause mutations.

In Micro organisms which have high reproductive rate, the number of mutation that occur in a single reproduction cycle is very large.

(b) Induced mutation also cause gene mutation x-rays, (irradiation) chemical mutages, fomaldehyler plenol, mustard gas, pyrimidine nitrous acid physical treatments are examples of mutagens. Note that many induced mutations are detrimental and disadvantageous to their carrier. Such mutation can be eliminated by natural or artificial selection.

(2) Gene recombination

This mans combination of genes from different parents that results in genetic variability. Assume that two parents differ in two pairs of allelic gene which are not linked, one is dominant over the other,

P_1 AAbb x aaBB P_2

F_1 AaBb

AaBb x AaBb

F_2 AABB – a new genotype

AAbb -

Parental genotype

aaBB-

aabb – a new genotype

With intra or inter-allelic gene interactions, it is possible to obtain genotypes with traits that are not possessed by either parent. However, "transgression" is another source of genetic variability leading to segregation of traits in one or both directions with regards to the parental traits. It leads to the progenies in the F_2 populations either earlier than earlier parent, shorter than the parent or superior in food content than the better parent. A level of heterosis is thereby exhibited.

Note that planned hybridization of genetically diverse individuals after selection is the richest source of new genetic variability needed for plant breeding.

(3) Gene Transfer

It is now possible, with modern technology to isolate individual genes from an organism, clone them, transfer to a vector and incorporate them into another organisms. Efforts have been made to transfer gene by recombinant. DNA from one organisms to another in order to provoke an increased production of proteins used in chemical industry and pharmacy.

At inception, viruses or plasmids were mostly used as vectors of desirable genes. However, recent studies have shown that gene transfer is possible by direct injection of genes into the cell (protoplast) or into organelles by genetic engineering.

The application of the recombinant DNA technique has helped in

(a) Successful production of human insulin

(b) Successful production of human growth hormone used in the therapy of human dwarfs.

(c) Production of interferone used in the therapy against cancer and viral infection

(d) Increased production of proteins used in pharmacy and chemical industry

(4) Somaclonal variation (S.V.)

Variability observed in single-gene traits (colour, shape, size) as well as multi-gene traits (height, branching etc) as a result of the effects of genotype and environment on a plant is called S.V. It involves the application of tissue and cell culture techniques.

Plants that are grown from in vitro culture, normally give plants that are phenotypically and genotypicaly identical to the materials from which the tissues or cells have been taken. However, the culture medium, the age of tissues or cells in culture, genotype and effects of water, light and temperature, may bring about a large variability called somaclonal variability. The new plants so formed are called somaclones. The longer the tissue culture cycle, the more is the variation found especially when undifferentiated callus (calli) or cell suspensions or protoplasts are used in culture. Meristem and shoot tip cultures do not give much culture variation.

It has been suggested that the cell culture is a kind of "mutagenic treatment" because new mutations occur continuously in cell cultures.

(5) Plant Hybridization

Sexual crossing between plant parents that are genetically dissimilar. This involves making crosses within and between various plant species after careful selection merits of hybridization are that it comes out with new and desirable gene recombinations. It also allows for the vegetative use of the F_1 generation which frequently contains the best combination f genes of two parents. Hybridization results in gene recombination particularly when many parents are involved. It is the richest source of genetic diversity needed for crop improvement.

G_s = genetic advance under selection = grain from selection

depends on K, ∂_P and H

Such that

$$G_s = K\partial_P H$$

Where

Genetic advance under selection = gain from selection

(GS) = mean genetic value of source population

Mean genetic value of selected lines

Gs depends on K, ∂_p and H such that

$$G_s = K\partial_p H$$

K = selection differential taking into consideration the mean phenotypic value of selected (q) and original source (n) lines; the selection intensity and the phenotypic standard deviation (∂_P) k is expressed in standard deviation units, it is fairly stable and estimated as 2.06 for 5% selection in large samples from a normally distributed population.

For 1,2,5 and 10% selections intensity k = 2.64, 2.42, 2.06 and 1.76 respectively ∂_p = standard deviation which is the square root of phenotypic variance and H is the heritability in percentage.

Thus Gs is expressed in the unit of the character under measurement whereas GA is expressed as percentage (%) of the mean.

If phenotypic variance of seed yield in g/plot = 6355.48g

and heritability of seed yield = 39.0

$Gs = 2.06 \times \sqrt{6355.48} \times 0.39 = 64$g/plot

If mean yield per plot is 900g/plot

GA = 64/900 = 7.1%

Significant genetic variability can exist within local crop cultivars due to agricultural practices

(1) Absence of artificial selection

(2) Lack of seed quality control

(3) Use of mixed varieties for planting deliberate/accidental

(4) Natural cross-pollination and

(5) Spontaneous mutation

<u>Advantage</u>

Suitability of many local cultivars for individual and pedigree selection.

How to Evaluate Variability

Study variations in yield, yield components and other morphological characters of given local cultivars, number of branches, number of leaves, 100 seed weight, seed yield, number of pods/plant, number of seed/pod, pod length.

Observed and Expected gain per Selection Cycle

Note which of the components present the highest expected gain through selection per selection cycle.

Analyze the genetic variability of named character from chosen cultivars through individual plant selection form original population (S_0) and evaluate their progenies (S_1).

Heterosis

Heterosis, or **hybrid vigor**, or **outbreeding enhancement**, is the improved or increased function of any biological quality in a <u>hybrid</u> offspring. It is the occurrence of a genetically superior offspring from mixing the genes of its parents.

A mixed-breed dog

Heterosis (hybrid vigor): accelerated growth and increased dimensions, endurance, and fertility of various first-generation animal and plant hybrids. Heterosis is usually attenuated in the second and later generations. True heterosis, which is the ability of hybrids to leave a large number of fertile descendants, is distinguished from gigantism, which is the enlargement of the entire hybrid organism or of its individual parts. Heterosis is found in various multicellular animals and plants, including self-pollinating ones. Phenomena resembling heterosis are also observed in the sexual processes of some unicellular organisms. Heterosis often results in a considerable increase in the productivity and yield of agricultural animals and crops.

Heterosis and its converse, inbred depression, were already known to the ancient Greeks, particularly Aristotle. The first scientific studies of heterosis in plants were carried out by the German botanist J. Kölreuter (1760). Darwin generalized observations on the use of hybridizations (1876), thus greatly influencing the work of I. V. Michurin and many other breeders. The term “heterosis” was proposed in 1914 by the American geneticist G. Shull, who was the first to obtain double interlinear corn hybrids. In 1917, D. Jones developed the principles of a method of industrial cultivation of these hybrids. The application of hybridization in agriculture has increased over the years, stimulating theoretical investigation of heterosis. Species with marked heterosis have advantages in natural selection; thus, the phenomenon of heterosis increases and contributes to increased genetic variability. Often, stable genetic systems arise, ensuring predominant survival of heterozygotes by numerous genes.

Aside from the usual study of morphological traits, investigation of heterosis requires the application of physiological and biochemical methods, making it possible to detect fine differences between hybrids and the original forms. Heterosis has also begun to be studied at the molecular level. In particular, the structures of specific protein molecules of many hybrids (for example, enzymes and antigens) are being studied.

According to Darwin, heterosis depends on the conjunction of diverse hereditary tendencies in the fertilized egg. On the basis of this, two main hypotheses regarding the mechanism of heterosis were established. The hypothesis of heterozygosis (overdominance or single-gene heterosis) was proposed by the American researchers E. East and G. Shull. When they combine in the heterozygote, two states (two alleles) of one and the same gene reinforce each other in their action on the organism. Each gene controls the synthesis of a particular polypeptide. In heterozygotes a few different protein chains are synthesized instead of one, and often heteropolymers—that is, hybrid molecules—are formed. This process may be advantageous for the heterozygotes. The hypothesis of dominance (summation of dominant genes) was formulated by a number of American biologists, including A. V. Bruce (1910) and D. Jones (1917). Mutations (changes) of genes are, on the whole, harmful. A defense against them is increased dominance of normal genes for a population of genes (evolution of dominance). Combination in a hybrid of favorable dominant genes of two parents results in heterosis.

The two hypotheses on heterosis can be combined in the concept of genetic balance, which was developed by the American scientist D. Jones, the English scientist K. Mather, and the Russian geneticist N. V. Turbin. Obviously, heterosis is based on the interaction of both allelic and nonallelic genes; however, in all cases heterosis is associated with enhanced heterozygosity of the hybrid and with its biochemical enrichment, which also depends on an increase in the rate of metabolism.

Of particular practical and theoretical interest is the problem of the stabilization of heterosis. It can be solved by doubling sets of chromosomes, creating stable heterozygous structures, and using every possible form of apomixis, as well as by vegetative reproduction of hybrids. The effect of heterosis may also be fixed by doubling individual genes or small parts of chromosomes. The role of such duplications in evolution is very great; therefore, heterosis is considered an important stage in evolutionary progress.

Heterosis in agriculture. In the cultivation of plants, heterosis is an important way to increase productivity. Crops from heterotic hybrids are 10-30 percent greater than from ordinary varieties. In order to use heterosis in production, economically profitable means have been developed of obtaining hybrid seeds of corn, tomatoes, eggplants, peppers, onions, cucumbers, watermelons, gourds, sugar beets, sorghum, rye, lucerne, and other agricultural plants. A special position is occupied by a group of vegetatively reproducing plants—for example, a variety of potatoes and fruit and berry crops obtained from hybrid seeds—in which it is possible to fix heterosis in the descendants. To use heterosis for a practical purpose, intervarietal crossing of homozygous varieties of self-pollinating plants, intervarietal (interpopulation) crossing of self-pollinated lines of cross-pollinated plants (conjugate, trilinear, bilinear, quadrilinear, and multilinear ones), and strain-line crosses are done. The advantage of certain types of crossing for each agricultural crop is established on a basis of economic evaluation. Elimination of difficulties in obtaining hybrid seeds can be facilitated by the use of cytoplasmic male sterility, the property of incompatibility in some cross-pollinated plants, and other hereditary peculiarities in the structure of the flower and raceme, excluding large expenditures on castration. In choosing parental forms to obtain heterotic hybrids, their combination capacity is assessed. Originally, selection in this direction led to separation of the genotypes with better combination value from the population of free-pollinating varieties on the basis of inbreeding by forced self-pollination. Methods have been developed to evaluate and increase the combination capacity of lines and other groups of plants that are used for crossing.

The maximum effect in the application of heterosis is obtained with corn. The creation and introduction into production of corn hybrids made it possible to increase by 20-30 percent the total harvests of grain on the enormous areas occupied by that crop in various countries of the world. Corn hybrids have been created that combine high yield and good seed quality, drought resistance, and immunity to various diseases. Heterotic sorghum hybrids (Early 1 hybrid, Rise hybrid) and heterotic intervarietal sugar beet hybrids, of which the Ialtushkovskii hybrid has become most widespread, have been zoned. A line of sugar beet with a sterile pollen is being used increasingly to obtain heterotic forms. The phenomena of heterosis are also established with many vegetable and oil-yielding crops. First results have been obtained in studying heterosis in first-generation wheat hybrids, and sterile analogues and fertility reducers produced from sources of cytoplasmic male sterility in wheat have been discovered.

In livestock breeding the phenomena of heterosis are observed in hybridization—intervarietal and intravarietal (interlinear) crossing. Heterosis causes a notable increase in the productivity of agricultural animals, and it has become most widely used in industrial crossing. In poultry farming, when egg-producing varieties of chickens are crossed—for example, leghorns with Australops or Rhode Islands—the egg production of first-generation crosses increases by 20-25 eggs per year. The crossing of meat breeds with meat-egg breeds of chickens improves meat quality. According to a complex of traits, heterosis is obtained when crossing closely related lines of fowls of one breed or by intervarietal crossing. In pig, sheep, and cattle raising, industrial crossing is used to obtain heterosis for meat productivity, which is manifested in earlier maturation, increased live eights, increased dressing percentage, and improved quality of the carcass. Meat-lard (combined) breeds of pigs are crossed with meat varieties of boars. Local breeds of small, low-productivity sheep are crossed with meat-wool sheep, and fine wool parents are crossed with early maturing meat or semifine wool breeds. To raise meat productivity, milk cows, milk-meat, and local meat breeds are crossed with specialized meat breeds of bulls

Genetic basis of heterosis

Two competing hypotheses, not necessarily mutually exclusive, have been developed to explain hybrid vigor. The **dominance hypothesis** attributes the superiority of hybrids to the suppression of undesirable (deleterious) recessive alleles from one parent by dominant alleles from the other. It attributes the poor performance of inbred strains to the loss of genetic diversity, with the strains becoming purely homozygous deleterious alleles at many loci. The **overdominance hypothesis** states that some combinations of alleles (which can be obtained by crossing two inbred strains) are especially advantageous when paired in a heterozygous individual. The concept of heterozygote advantage/overdominance is not restricted to hybrid lineages. This hypothesis is commonly invoked to explain the persistence of many alleles (most famously the erythrocyte-sickling allele) that are harmful in homozygotes; in normal circumstances, such harmful alleles would be removed from a population through the process of natural selection. Like the dominance hypothesis, it attributes the poor performance of many inbred strains to a high frequency of these harmful recessive alleles and the associated high frequency of homozygous-recessive genotypes.

Hybrid corn

Nearly all field corn (maize) grown in most developed nations exhibits heterosis. Modern corn hybrids substantially out yield conventional cultivars and respond better to fertilizer. Corn heterosis was famously demonstrated in the early 20th century by George H. Shull and Edward M. East after hybrid corn was invented by Dr. William James Beal of Michigan State University based on work begun in 1879 at the urging of Charles Darwin. Dr. Beal's work led to the first published account of a field experiment demonstrating hybrid vigor in corn, by Eugene Davenport and Perry Holden, 1881. These various pioneers of botany and related fields showed that crosses of inbred lines made from a Southern dent and a Northern flint, respectively, showed substantial heterosis and outyielded conventional cultivars of that era. However, at that time such hybrids could not be economically made on a large scale for use by farmers. Donald F. Jones at the Connecticut Agricultural Experiment Station, New Haven invented the first practical method of producing a high-yielding hybrid maize in 1914-1917. Jones' method produced a double-cross hybrid, which requires two crossing steps working from four distinct original inbred lines. Later work by corn breeders produced inbred lines with sufficient vigor for practical production of a commercial hybrid in a single step, the single-cross hybrids. Single-cross hybrids are made from just two original parent inbreds. They are generally more vigorous and also more uniform than the earlier double-cross hybrids. The process of creating these hybrids often involves detasseling.

Hybrid livestock

The concept of heterosis is also applied in the production of commercial livestock. In cattle, hybrids between Black Angus and Hereford produce a hybrid known as a "Black Baldy". In swine, "blue butts" are produced by the cross of Hampshire and Yorkshire. Other, more exotic hybrids such as "beefalo" are also used for specialty markets.

Within poultry, sex-linked genes have been used to create hybrids in which males and females can be sorted at one day old by color. Specific genes used for this are genes for barring and wing feather growth. Crosses of this sort create what are sold as Black Sex-links, Red Sex-links, and various other crosses that are known by trade names.

Commercial broilers are produced by crossing different strains of White Rocks and White Cornish, the Cornish providing a large frame and the Rocks providing the fast rate of gain. The hybrid vigor produced allows the production of uniform birds with a marketable carcass at 6–9 weeks of age.

Hybridization

The crossing of organisms differing in heredity—that is, in one or more pairs of alleles (conditions of genes) and consequently in one or more pairs of traits and properties. The crossing of individuals belonging to different species or even to less closely related taxonomic categories is called distant hybridization. The crossing of subspecies, varieties, or breeds is called intraspecific hybridization. The process of hybridization—especially natural hybridization— was observed in very ancient times. Hybrid animals (for example, mules) existed as early as the second millennium B.C.. The possibility of producing hybrids artificially was first suggested by the German scientist R. Camerarius (1694). The first to carry out artificial hybridization was the English horticulturist T. Fairchild, who crossed different species of pinks in 1717. The founder of teaching on sex and hybridization in plants is thought to be J. G. Kolreuter, who obtained hybrids of two tobacco species—*Nicotiana paniculata* and *N. rustica* (1760). G. Mendel's experiments on the hybridization of peas laid the scientific foundation of genetics, and Darwin performed an enormous number of experiments on hybridization.

The essence of hybridization is the fusion during fertilization of genotypically different sex cells and the development from the zygote of a new organism that combines the hereditary disposition of the parents. Copulation in unicellular organisms is also included among the phenomena of hybridization. The first generation of hybrids is often characterized by heterosis, which is manifested in better capacity for adaptation and greater fertility and viability of organisms. Hybridization as well as mutations are the main sources of hereditary variation, which is one of the main factors in evolution.

In natural hybridization and in artificial hybridization that is carried out by man for breeding and other purposes, flowers of the maternal form are pollinated with pollen from another species (variety) of plant, or animals of different species (subspecies, breeds) are mated. The sexual process guarantees the combining of genomes and results in the union of the nuclei of gametes—karyogamy. Therefore, it is impossible to obtain so-called vegetative hybrids. The "vegetative" hybrids described by some investigators are simply tissue chimeras.

In livestock breeding, intraspecific hybridization is a method of industrial breeding by which individuals of different breeds or strains are mated. Distant hybridization in animals is the obtaining of hybrids between varieties, species, and genera—for example, the crossing of fine-wooled sheep and Pamir argalis or cattle and zebus. This is difficult to accomplish, and the hybrids are generally sterile.

In 1935 the Soviet geneticist G. D. Karpechenko made a distinction between congruent crossings, or hybridization, and incongruent crossings in plants. Congruent crossings are intraspecific and sometimes interspecific crossings in which parental pairs with homologous chromosomes are crossed and the offspring are fertile. Incongruent crossings are generally distant crossings—that is, crossings of two individuals with structurally incompatible chromosomes and differences in the chromosome number or cytoplasm. The offspring are partly or completely sterile and the nature of the segregation is complex.

Crossings may be direct or reciprocal. For example, the hybrids ♂ A × ♀B and ♀B + ♂ A are reciprocal. If a hybridis crossed with one of the parental forms, the crossing is called a backcross. A testcross involves backcrossing a hybrid with a parent that is recessive for the trait under study. This is done to establish the hybrid's heterozygosity, linkage groups, or the frequency of crossing over between linked genes. Repeated backcrossing of a hybrid with one of the parents is called saturation. It is used to introduce into genotype A the traits of genotype B or to transfer the genome to the cytoplasm of another variety, subspecies, or species. There are also complex crossings called convergent crossings. First the parental varieties are crossed in pairs. The hybrids are then crossed with each other, and the newly produced hybrids are crossed with each other. In such cases individual hybrids often have valuable combinations of properties and traits.

Hybridization is widely used in breeding. Depending on the purpose of hybridization, there may be "combination" breeding to combine the desirable traits of the parental forms and "transgressive" breeding to obtain and select genotypes that are superior to parents in the bred trait.

Hybridization: It is the crossing of two plants differing from each other in one or more characters to get offsprings with new desirable characters, as a result of genetic recombinations. In hybridization, it is possible to combine all good characters present in different varieties in a single variety. The hybrid formed is superior over either parent in one or more characters. It is known as hybrid vigour or heterosis. The term heterosis was coined by ShuII (1914)

Based on the taxonomic relationship of the parental plants involved in a cross hybridization may be

i. Intravarietal ii. Intervarietal iii. Interspecific or iv. Intergeneric

• **Intravarietal hybridization** : This is a cross between two plants of the same variety but with different genotype. This method is helpful for the improvement of self pollinated crops.

• **Intervarietal hybridization :** It is also called intraspecific hybridization where the cross in between plants of two different varieties of same species. This is used in the improvement of self pollinated as well as some cross pollinated crops. Some improved varieties obtained through intervarietal hybridization are

i. Wheat: NP 52 x NP 165 NP 710 V '

ii. Cotton : Malvi 8 x Jarlia —» Maljari

iii. Tomato : Sioux x Meeruti —» Pusarubi planted to produce high yeilding double cross seeds.

• **Interspecific hybridization :** This is a cross between plants of different species belong to the same genus. This type of cross is mainly used to produce varieties resistant to disease, drought, pest and salinity. Genes for resistance present in wild species are brought into a hybrid by repeated crossing between the local species and wild species. Resistant varieties of wheat, potato, tomato, sugarcane etc. have been obtained by this method :

i. Cotton: *Gossypium hirsutum* x *G. arborium —» Deviraj. G. hirsutum* x *G. herbaceum —> Devitej*

ii. Potato : (*Solatium curtilobum* x *Solatium tuberosum)* x *Solatium andigena —> Kufri Kuber*

iii. Tomato: *Lycopersicum esculentum* x *L. pimpinellifolium —> Pusa red plum*

iv. Sugar cane: All the sugarcane varieties now in cultivation have been developed from complex crosses between *Saccharum officinarum, S. barberi, S. robustum* and *S. spontaneum. S. spontaneum* has been used to combine- its hardiness and disease resistance with the high sugar content and high yielding ability of *S. officinarum*.

• **Intergeneric hybridization :** This is a cross between plants of different genera belonging to the same family. It is the most difficult of all types of crosses. Hybrids produced by this method are both scientifically as well as agriculturally significant. Hybrids produced by this method are:

i. Wheat x Rye Triticale

ii. Raphanus (Radish) x Brassica (Cabbage) Raphanobrassica (Rabbage)

iii. Brinjal x Tomato —> Bromato

iv. Sugarcane x Sorghum —> Sugarcane Sorghum

Technique of Hybridization

Hybridization can be done only by skilled persons. The technique of hybridization comprises the following steps : i. selection and isolation of parents; ii. emasculation ; iii. bagging, iv. collection of pollen grains,-v. crossing, vi. Tagging, vii. Harvesting hybrid seed and raising F_1 generation plants, viii. production of F_2 generation; ix. Trials, multiplication and distribution.

For this purpose a breeder must have the following appliances in his Kit:

a. Forceps, b. Scissors, c. Needles, d. Scalpel, e. Hair brush (f) Alcohol bottle and (g.) Labels

• **Selection and isolation of parents:** Two healthy locally available plants with desirable characters are selected. Then these parent plants are grown separately on isolated plots to avoid cross pollination. These plants are self pollinated for 6 - 8 generations till the majority of plants become homozygous and true breeding. The last generations of both the parents are used for further steps of hybridization.

• **Emasculation:** It is the removal of stamens from one

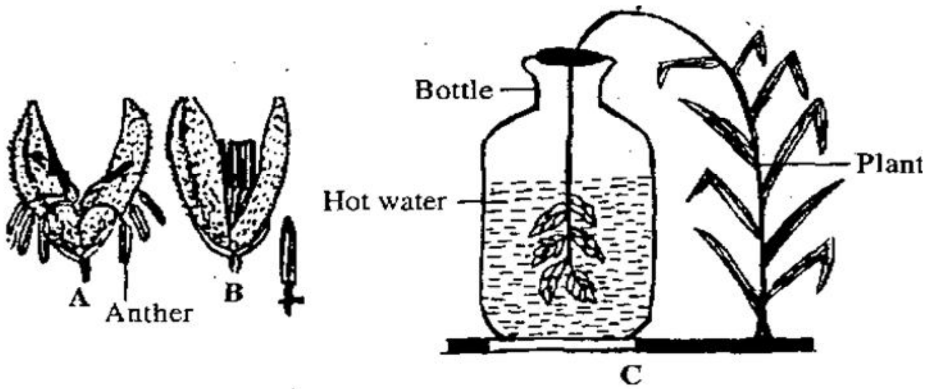

Fig. 2.5.1 : Emasculation technique. Removal of anthers by forceps in rice spikelet. **A.** Spikelet **B.** After opening the flower to removal of anthers by forceps **C.** Emasculation by hot water treatment.

of the parents (if bisexual) before they release their pollen grains. Emasculation may be done by removing anthers with the help of forceps in plants with large flowers; or by dipping the flower buds in hot water (4 - 53°C) or in ethyl alcohol for about 3-10 minutes in plants with small flowers. (Fig. 2.5.1).

Emasculation of stamens in bisexual flowers prevents the process of self pollination.

• **Bagging:** Soon after emasculation, the flowers are covered by polythene bags to prevent cross pollination by undesired pollen grains. The polythene bags are tied at the base of flower or inflorescence by threads or copper wires. (Fig. 2.5.2)

• **Crossing:** Pollen grains are collected from the flowers of

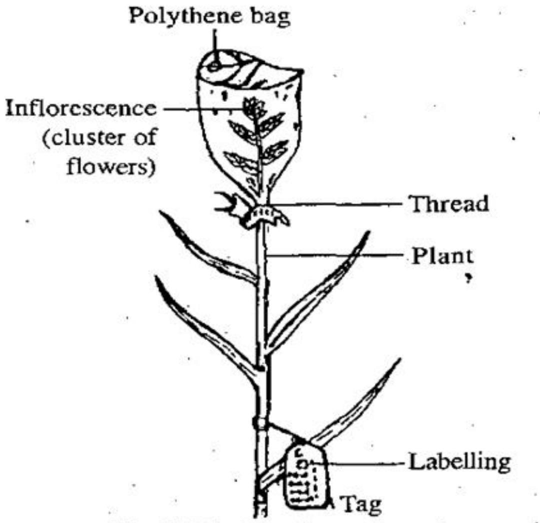

Fig. 2.5.2 : Bagging and tagging at the time of hybridization

other parent plant. When the stigma of emasculated flowers matures, the polythene bag is removed and the stigma is rusted with pollen grains collected. The flowers are again bagged immediately. It is advisable to perform crossing early in the morning as in most of the crops stigma become receptive at different times in the morning.

• **Tagging:** The flowers thus crossed are suitably labelled. A tag (label) with relevant information is attached to the plant. The tag should contain :(i.) date of crossing, (ii.) note about female plant, (iii.) note about the male plant, (iv.) serial number in the record book : and (v.) remarks, if any.

• **Harvesting hybrid seed and raising Fn generation:** When the seeds mature, the polythene bags are removed. Seeds are harvested, dried, cleaned and stored properly along with its label. In the next season, the seeds are sown in the field to raise F, generation plants. Traits in these hybrid plants are studied.

• **Production of F_2 generation plants:** F, generation plants are selfed to get F_2 generation plants. F_2 plants with desirable characters are carefully identified and developed further.

• **Trials, multiplication and distribution:** F_2 plants with desirable characters are cultivated in the separate plots to assess the characters in the subsequent generations and seeds of selected generation are distributed to the farmers. Different crop plants have been developed by the hybridization techniques. Some of them are

i. Wheat : NP 165, NP 710 etc.

ii. Rice : Jaya, Padma, Sabarmati, Krishna

iii. Maize : Deccan, Sweet maize, Ganga etc

iv. Tomato : Pusa ruby, Pusa red plum etc.

v. Cotton : Deviraj, Devitej, Laxmi etc.