Lecture No. 18.

Backcross breeding – genetic principles – prerequisites – procedures for transferring dominant and recessive genes.

BACKCROSS METHOD

In backcross method of breeding, the hybrid and the progenies in subsequent generations are repeatedly backcrossed to one of the parents. As a result, the genotype of the backcross progeny becomes increasingly similar to that of the recurrent parent.

The objective of backcross method is to improve one or two specific defects of a high yielding variety.

Pre-requisite for back cross breeding

1. A suitable recurrent parent must be available which lacks in one or two characteristics.
2. A suitable donor parent must be available
3. The character to be transferred must have high heritability and preferably it should be determined by one or two genes.
4. A sufficient number of back crosses should be made so that the genotype of recurrent parent is recovered in full.

Application of back cross method

This method is commonly used to transfer disease resistance from one variety to another. But it is also useful for transfer of other characteristics.

1. Intervarietal transfer of simply inherited characters

E.g. Disease resistance, seed coat colour

2. Intervarietal transfer of quantitative characters.

E.g. Plant height, Seed size, Seed shape.

3. Interspecific transfer of simply inherited characters

E.g. Transfer of disease resistance from related species to cultivated species.

E.g. Resistance to black arm disease in cotton from wild tetraploid species into *G.hirsutum*

4. Transfer of cytoplasm:

This is employed to transfer male sterility. The female parent will be having the sterile cytoplasm and recurrent parent will be used as male parent.

E.g. *Sesamum malabariucum* x *S.indicum*

Female parent Recurrent parent.

5. Transgressive segregation

Back cross method may be modified to produce transgressive segregants. The F_1 is backcrossed to recurrent parent for 2 to 3 times for getting transgressive segregants.

6. Production of isogenic lines.

7. Germplasm conversion: E.g. Production of photo insensitive line from photo Sensitive germplasm through back crossing. This was done in the case of sorghum. Popularly known as conversion programme.

Procedure for backcross method

The Plan of backcross method would depend upon whether the gene being transferred is recessive or dominant. The plan for transfer of a dominant gene is simpler than that for a recessive gene.

First Year	Non-Recurrent		Recurrent
	Parent B	x	Parent A
	Resistant to rust		Susceptible to rust
	F_1 Rr	x	rr BC_1
	Resistant		
rr	Rr	x	rr BC_2
rr	Rr	x	rr BC_3
rr	Rr	x	rr BC_4
rr	Rr	x	rr BC_5

Back cross upto 6th or 7^{th} generation.
After 7^{th} BC rust resistant lines are
Self pollinated. Harvest is done on single plant basis

8^{th} Season

a) Individual plant progenies grown

b) Homozygous plants having resistance and resembling parent A are selected Harvested and bulked

9^{th} season

Yield trials.

10^{th} season

Seed multiplication and distribution

Steps

First Season

Hybridization : : Crossing between parent B donor (Female) and Susceptible parent A recipient (male)

Second Season:

Raising the F_l and backcrossed to recurrent parent A.

Third season:

Growing the BC_lF_l. It will be segregating for 1 susceptible (rr): 1 resistant (Rr). Rust resistant plants are backcrossed with recurrent parent A. This is second backcross.

Fourth Season

Raising BC_2 F_1 will again segregate in the ratio of 1: 1. Third backcross is done with resistant plants.

Fifth Season to Eighth Season

Raising backcross F_ls and crossing resistant plants with recurrent parent is continued up to 7th backcross

Ninth season:

Raising BC_7F_1 and observing resistant lines. The plants resembling parent A coupled with resistance is selected and harvested on single plant basis.

Tenth Season:

Growing the progeny row8'and observing each row for resistance. Best rows are selected and harvest is done on row basis

Eleventh Season:

The row bulk is raised in yield trial along with check and the best plots are selected.

Twelfth season:

Selected plot seeds are multiplied and released as new variety.

Back Cross Method – Transfer of Recessive Gene

I Season	Non recurrent parent B		Recurrent parent A
Hybridization	Resistant		Susceptible
	Rr	x	RR
	F_1	Rr	

II Season Grow the F_1
Rr

III Season Grow F2 RR " Rr " rr x RR BC_1

IV Season Grow BC_1F_1 Rr

V Season Grow BC_2F_2 RR : Rr : rr x RR BC_2

VI Season Grow BC_2F_1 Rr

VII Season Grow BC_2F_2 RR : Rr : rr x RR BC_3

VIII Season Raise BC_3F_1

IX Season Raise BC_3F_2 and it will segregate into 1:2:1 with resistant segregant make Backcross 4 (BC_4)

X Season Do as on VIII Season

XI Season Do as in IX season

Continue this process still 7^{th} or 8^{th} backcross. After studying 8^{th} BCF_2 select plants resembling parent B coupled with resistance. Harvest them on single plant basis. Next season raise them in progeny rows and select beast progenies. Compare them in yield trial and fix the best culture, multiply it and release it as a variety. While selecting plants artificial bombardment for disease is to be done.

Steps:

I Season : Make a cross between donor parent A and recurrent parent B and Harvest the hybrid. The donor parents A is resistant which is governed by recessive genes. The susceptibility is governed by dominant gene in parent B.

II Season : Grow the F1 which will be susceptible – Harvest them.

III Season: Grow F_2 which will be segregating in the ratio of I :2: I i.e. 3/4 susceptible and 1/4 resistant. With the resistant lines (rr) make first backcross with parent A having

dominant RR gene. Harvest $BC_I F_1$

IV Season: Grow BC_IF_1

V season: Grow $BC_1 F_2$ which will be segregating as we saw in III season. Repeat the process of third season. This will give BC_2F1_I

VI Season :Grow BC_2F_1

VII season : Grow BC_2F_2 them repeat the process of V Season. This will give BC_3F_1.

VIII Season : Grow BC_3F_1

IX Season : Grow BC_3F_2 and repeat the process of VII Season. Harvest BC_4F_1.

X season : Grow BC_4F_1

XI Season : Grow BC_4F_2 and repeat the process of IX Season. Harvest BC_5F_1.

XII, XIII & XIV : Repeat the process and carry out backcross upto 7 time.

XV Season : While studying BC_7F_2 select single plants having resistance and resembling parent B.

XVI Season : Study the progenies in progeny rows and select best progenies.

XVII Season : Conduct yield trial and select best material.

XVIII Season : Multiply the seeds and distribute it as improved variety with resistance to disease.

Note : While studying back cross F_2s they should be bombarded with artificial epiphytotic conditions.

NUMBER OF PLANTS NECESSARY IN THE BACKCROSS GENERATIONS

According to the above schemes, only a few (about 10) seeds are necessary in each backcross generation for the transfer of a character governed by a single gene. This population size would almost certainly have at least one plant with the gene for rust resistance. However, if the character is governed by two or more genes, a larger number of backcross progenies would be required. A larger size of backcross population is also desirable to permit an effective selection for the plant type of the recurrent parent, and to increase the probability of recombination between the genes being transferred and the genes tightly linked with it. Therefore, more than 50, preferably 100 or more, plants should be grown in each backcross generation. In F_2 and F_3 generations, the population size should be as large, as possible.

SELECTION FOR THE CHARACTER BEING TRANSFERRED

A rigid selection for the character being transferred must be practiced during the backcross and the F_2 generations, otherwise the character is likely to be lost. It is, therefore, essential that the character being transferred must have a high heritability. Although monogenic characters are the easiest to transfer, the number of genes is not as important as the heritability of the character under transfer. It is desirable that the character should be easily identified either visually or through simple tests. The breeder should try to maintain the character in an intense form through selection. *Often the intensity would be lost due to modifying genes in the new genetic background. Therefore, the nonrecurrent parent should be chosen for a high intensity of the character to be transferred.*

NUMBER OF BACKCROSSES TO BE MADE

In the backcross method, it is essential that the genotype of the recurrent parent should be recovered except for the gene being transferred. The recurrent parent is likely to consist of several closely similar purelines. Therefore, a sufficient number of plants from the recurrent parent should be used for the backcrosses. This would make sure that the new variety will have the same genetic composition as the recurrent parent.

Generally, six backcrosses are sufficient to recover the essential feature of the recurrent parent. Selection for the characteristics of the recurrent parent, particularly in the early backcross generations, may often have the effect of one or two additional backcrosses. *Thus six backcrosses along with selection for the recurrent parent plant type in the early backcross generations will be effective in recovering the genotype of the recurrent parent.*

TRANSFER OF TWO OR MORE CHARACTERS TO A SINGLE RECURRENT PARENT

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

SIMULTANEOUS TRANSFER

The genes for the characteristics may be, transferred simultaneously in the same backcross programme. The characters to be transferred are brought together in the hybrid

by crossing each of the nonrecurrent parents to the recurrent parent and 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 become very easy, and selection for only one gene may be necessary. Some examples of such a favourable linkages are between the genes Lr 24 and Sr 24, Lr 19 and Sr 25 and Lr 56 and Sr 31.

STEPWISE TRANSFER

The recurrent parent is first improved for one character. The improved recurrent parent is then used as recurrent parent in a backcross programme for the transfer of other character. If additional characters are to be transferred, they are transferred one 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 varieties 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 methods.

MODIFICATIONS OF THE BACKCROSS METHOD

The backcross method may be modified in various ways to suit the needs of the breeder. Following are the three common modifications of the backcross method.

PRODUCTION OF F_2 AND F_3

The F_2 and F_3 generations are produced after the first and the third backcrosses. A rigid selection for the character being transferred and for the characteristics of the recurrent parent is done in the F_2 and F_3 generations. In the backcross progenies, selection need not be done either for the character being transferred or for the characteristics of the recurrent parent. The fourth, fifth and sixth backcross are made in succession. For the

sixth backcross, a relatively larger number of plants from the backcross progeny is used. This method may be used for the transfer of both dominant and recessive genes. It is believed that an effective selection in F_2 and F_3 generations is equivalent to one or two additional backcrosses.

USE OF DIFFERENT RECURRENT PARENTS

Often two or more good varieties have quantitative characteristics that are desirable in the new variety. These varieties may be used as recurrent parents in the same backcross programme. Each variety is used as a recurrent parent for one or two backcrosses. *The objective of this approach is to combine in the new verity some good genes from each of the recurrent parent with the genes from the nonrecurrent parent.* Nobilisation of sugarcane is an outstanding example of this approach. Noble canes *(S. ofjicinarum)* were first crossed with the Indian canes *(S. barberi).* The resulting hybrids were backcrossed to different varieties of noble canes to develop a large number of commercial sugarcane varieties. A similar approach was used for the transfer of scab resistance in apples and for the transfer of high vitamin C content from wild tomato *(Lycopersicon peruviamum)* to the cultivated tomato *(L. esculentum).*

BACKCROSS-PEDIGREE METHOD

In this method, the hybrid is backcrossed 1-2 times to the recurrent parent. Subsequently, the backcross progenies and handles according to the pedigree method. This approach is useful when one of the parents is superior to the other in several characteristics but the nonrecurrent parent is not desirable agronomically. The superior parent is used as the recurrent parent. The purpose of the one to two backcrosses is to make sure that the new variety would get a majority of the superior genes from the recurrent parent. It also leaves enough heterozygosity for transgressive segregants to appear. The varieties developed by this method must be put to yield trials as those developed by the pedigree method. The same holds true when two or more recurrent parents are used in the backcross programme.

APPLICATION OF THE BACKCROSS METHOD TO CROSS-POLLINATED CROPS

The backcross method is equally applicable to cross-pollinated crops. The method is essentially the same as in the case of self-pollinated crops. The only difference is that in cross-pollinated crops a large number of plants (100-300) from the recurrent parent must be used in each backcross. This is necessary so that the new variety has the same genetic constitution as the recurrent parent. For example, wilt resistance was transferred to alfalfa variety California Common from the variety Turkestan. Two hundred plants of California Common were used for each backcross. The new variety Calliverde is exactly like California Common except for its wilt resistance.

COMPARISON BETWEEN BACKCROSS AND PEDIGREE METHODS

S. No.	Pedigree method	Bulk method
1.	F_1 and the sub sequent generations are allowed to self-pollinate	F_1 and the subsequent generations are backcrossed to the recurrent parent
2.	The new variety developed by this method is different from the parents in agronomic and other characteristics	Usually extensive testing is not necessary before release
3.	The new variety has to be extensively tested before release	Usually extensive testing is not necessary before release
4.	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
5.	It is useful in improving both qualitative and quantitative characters	Useful for the transfer of both quantitative and qualitative characters provided they have high heritability
6.	It is not suitable for gene transfer from related species and for producing substitution or addition lines	It is the only useful method for gene transfers from related species and for producing addition and substitution lines
7.	Hybridization is limited to the production of the F1 generation.	Hybridization with the recurrent parent is necessary for producing every backcross generation
8.	The F_2 and the subsequent generations are much larger than those in the backcross method	The backcross generation are small and usually consist of 20-100 plants in each generation
9.	The procedure is the same for both dominant and recessive genes	The procedures for the transfer of dominant and recessive genes are different.

Lecture No 19.

Back cross breeding – merits – demerits – multi lines and multi blends - population improvement approach in self-pollinated crops.

MERITS OF BACKCROSS METHOD

1. The genotype of the 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 back cross method in extensive yield tests because the performance of the recurrent parent is already known. This may save upto 5 years time and a considerable expense.
3. The backcross programme is not dependent upon 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 generation each year. This would drastically reduce the time required for developing the new variety.
4. Much smaller population 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 interspecific gene transfers.
7. It may be modified so that transgressive segregation may occur for quantitative' characters.

DEMERITS OF BACKCROSS METHOD

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 is often difficult, time

taking and costly.

4. By the time the backcross is over, the recurrent parent may have been replaced by other varieties superior in yielding ability and other characteristics.

MULTILINE VARIETIES

Generally, pureline varieties are highly adapted to a limited area, but poorly adapted to wider regions. Further, their performance is not stable from year to year because of changes in weather and other environmental factors. Purelines often have only one or a few major genes for disease resistance, such as, rust resistance, which make them resistant to some races of the pathogen. New races are continuously produced in many pathogens, which may overcome the resistance present in the pureline varieties. For example, Kalyan Sona wheat *(T.aestivum)* originally resistant to brown rust (leaf rust), soon became susceptible to new races of the pathogen.

To overcome these limitations, particularly the breakdown of resistance to disease, it was suggested to develop multiline varieties. *Multiline varieties are mixtures of several purelines of similar height, flowering and maturity dates, seed colour and agronomic characteristics, but having different genes for disease resistance.* The purelines constituting a multiline variety must be compatible, i.e., they should not reduce the yielding ability of each other when grown in mixture.

In 1954, Borlaug suggested that several purelines with different resistance genes should be developed through back cross programmes using one recurrent parent. This is done by transferring disease resistance genes from several donor parents carrying different resistant genes to a single recurrent parent. Each donor parent is used in a separate backcross programme so that each line has different resistant gene or genes. Five to ten of these lines may be mixed depending upon the races of the pathogen prevalent in the area. If a line or lines become susceptible, they would be replaced by resistant lines. New lines would be developed when new sources of resistance become available. The breeder should keep several resistant lines in store for future use in the replacement of susceptible lines of multiline verities.

Merits of Multiline varieties

1. All the lines are almost identical to the recurrent parent in agronomic

characteristics, quality etc. Therefore, the disadvantages of the pureline mixtures are not present in the multiline varieties.

2. Only one or a few lines of the mixture would become susceptible of the pathogen in anyone season. Therefore, the loss to the cultivator would be relatively low.
3. The susceptible line would constitute only a small proportion of the plants in the field. Therefore, only a small proportion of the plants would be infected by the pathogen. Consequently the disease would spread more slowly than when the entire population was susceptible. This would reduce the damage to the susceptible line as well.

Demerits of Multiline Varieties

I. The fanner has to change the seed of multiline varieties every few years depending upon the change in the races of the pathogen.

2. There is a possibility that a new race may attack all lines of a multiline variety.

Achievements

Multiline variety appears to be a useful approach to control diseases like rusts where new races are continuously produced. In India, three multiline varieties have been released in wheat *(T.aestivum).* Kalyan Sona, one of the most popular varieties in the late sixties, was used as the recurrent parent to produce these varieties. Variety 'KSML 3' consists of 8 lines having rust resistance genes from Robin, Ghanate, Kl, Rend, Gabato, Blue Brid, Tobari etc. Multiline 'MLKS 11' is also a mixture of 8 lines; the resistance is derived from E 6254, E 6056, E 5868, Frecor, HS 19, E 4894 etc. The third variety, KML 7406 has 9 lines deriving rust resistance from different sources.

Dirty Multiline: This term is used when a multiline is having one or two susceptible lines also. The idea of including susceptible lines is to prevent race formation

POPULATION APPROACH TO BREEDING OF SELF -POLLINA TED CROPS

Self-fertilization of F_I hybrids leads to a very rapid increase in homozygosity. After several generations of self-pollination, about 94 per cent of the genes would become homozygous. Even in F_2, half of the genes are in homozygous state. Thus self fertilization quickly separates the progeny from a hybrid into a large number of purelines. As a consequence, selection in such a segregating population only picks out the genes

combinations present in the population primarily as a result of recombination in F_2. This reduces the chance of recombination between linked, especially tightly linked genes and of recovery of rare transgressive segregants. There is no opportunity for changing the genotype of the plant produced by recombination in F_I, F_2 and to some extent, in F_3.Thus the two obvious limitations of breeding methods based on self-pollination of the hybrid (e.g., pedigree and bulk methods) are: first, the recombination is limited to two or, at the best, three generations, and second, there is no possibility for further changing the genotype of the segregants.

A population breeding approach has been suggested to overcome these problems. In population breeding, outstanding F_2 plants are mated among themselves in pairs or in some other fashion. The intermating of selected F_2 plants restores heterozygosity in the progeny, which provides for a greater opportunity for recombination. This also brings together the desirable genes from different F_2 plants and would help in the accumulation of favourable genes in the intermated population. Thus the chances of the recovery of transgressive segregants would increase considerably. This process may be repeated one or more times (Fig.). This procedure is similar to recurrent selection in cross-pollinated crops. A variation of this approach would be to intermate F_3 or later generation progenies. This would allow a more effective selection of desirable progenies than in the case of F_2 where individual plants have to be selected. As noted previously, selection in F_2 based on individual plants is of little value, particularly for characters like yield. Selection based on F_3 or F_4 progenies would be more desirable. Intermating of selected plants may be continued for two or more generations.

This idea of population approach was first suggested by Palmer in 1953. It is not commonly used at present, but may find a greater application in the future, as improvements due to the pedigree method would become less and less marked. Evidently, the population approach is akin to recurrent selection commonly used in cross-pollinated crops and often it is referred to as such. The chief limitation of recurrent selection in self-pollinated crops is the difficulty in making the large number of required crosses by hand (emasculation and pollination). This difficulty may be overcome by using genetic or cytoplasmic male sterility. When genetic male sterility is used, selection is confined to the male sterile (ms ms) plants in each generation. Seeds from the selected

male sterile plants are generally harvested in bulk. The progeny from such plants may be expected to have both male sterile (ms ms) and male fertile (Ms ms) plants in almost equal proportion. Further, the seeds produced on the male sterile plants would be produced by pollination by the male fertile plants in the population. Thus the use of male sterility effectively ensures intermating among the plants in the population and eliminates the needs for tedious and time-consuming hand emasculation and pollination.

Results from recurrent selection are available in tobacco and soybean. In tobacco, Matzinger and coworkers selected the plants before flowering and intermated them. A linear response of 4.9 and 7 per cent per cycle to selection for decrease plant height and for increased leaf number, respectively, was obtained for five cycles of selection. Further, there was no evidence for a reduction in variability as a result of the selection. Brim and coworkers carried out six cycles of recurrent selection for increased protein content in two segregating populations of soybean and three cycles of selection for yield and three cycles of selection for high oil content in another segregating population. There was an increase of 0.33 and 0.67 per cent / cycle in protein content of the two populations, of 5.3% per cycle in yield and of 0.3% per cycle in oil content. These findings amply demonstrate the effectiveness of recurrent ,selection in improving yield and yield traits in self-pollinated crops.

In 1970, Jensen proposed a comprehensive breeding scheme which provides for the three basic functions of a versatile. breeding programme. Firstly, it allows the development of F2, F3 etc. (selfing series) at every stage of the breeding programme, which permits the isolation of purelines for use as commercial varieties. Secondly, it requires intermating among the selected plants/ lines in each stage; the progenies from these intermatings form the basis for the next stage of the selfing series in the breeding programme. Thus the breeding programme progresses in two different directions: (1) Vertically, through the selfing series leading to the isolation of commercial varieties, and (2) horizontally, through intermating among the selected plant / lines; this generates the recurrent selection series. Thirdly, new germplasm may be introduced at any stage of the programme by intermating it with some of the selected plants of that stage. This permits the retention and / or the creation of large amounts of variability for effective selection through several cycles, and the introduction of new genes in the breeding material, if so

desired. This breeding scheme is known as Diallel Selective Mating Scheme (DSM) and is designed to serve both short-term and long-term breeding objectives. A breeder may create more than one such population for a crop, each population being developed to fulfill a specific objective. This scheme has not been widely used primarily due to the difficulties in making the large number of crosses required in this scheme. Jensen has suggested the use of male sterility to overcome this difficulty in the same way as in the recurrent selection scheme discussed earlier. Further, DSM is much more complicated than the simple pedigree method which still is the favourite breeding method for self-pollinated crops.

Merits of population Approach

1. The population approach provides for greater opportunities for recombination. This is made possible by restoring heterozygosity through intermating of selected plants.
2. This approach helps in the accumulation of desirable genes in the population. This is also brought about by the intermating of selected plants from segregating generation.

Demerits of Population Approach

1. The success of this approach depends upon the identification of desirable plants in F_2 and the subsequent segregating generations. This is very difficult, if not impossible, for complex characters like yield which show low heritability. This may be avoided to some extent by using later generation (F_3 or F_4) progenies; replicated yield data may also be used.
2. Another draw back of this approach is the intermating of selected plants. This may become a major limitation in some crops because crossing in many self-pollinated species is difficult and time consuming.
3. The time taken to develop a new variety through population approach would be always greater than that by the pedigree method.
4. There is no convincing evidence for the benefits from the population approach. It has been argued that increased recombination may be detrimental, as it would break the desirable linkage. But such a criticism assumes that all or most of the new gene combinations (recombinations) will be inferior to the existing ones. Such an assumption is not entirely valid since crop improvement is based on the creation of new and desirable gene combinations.

Lecture No 20.

Genetic structure of a population in cross pollinated crop – Hardy Weinberg law – gene frequencies in random mating population – principles in population improvement

GENETIC STRUCTURE OF CROSS-POLLINATED CROPS.

Cross pollinated crops are highly heterozygous due to free intermating. These cross pollinated crops are referred as

a) Random mating population
b) Mendelian population.
c) Panmictic population.

Hardy - Weinberg Law

This law was independently developed by Hardy (1908) in England and Weinberg (1909) in Germany. According to the law that *"gene and genotypic frequencies in a random mating population remains constant generation after generation provided there is no selection, mutation, migration or genetic drift"*.

The frequencies on the three genotypes for a locus with two alleles say A and a would be p2 AA, 2pq Aa and q^2 aa

Where p = frequency of A

q = frequency of a in the population.

The sum of p and q is one i.e. p + q =1. Such a population would be equilibrium because the genotypic frequencies would be stable from one generation to next. This equilibrium is known as Hardy- Weinberg equilibrium. A population is said to be at equilibrium when the frequencies of the three genotypes AA, Aa and aa are p^2 2pq q^2. Whether the population is at equilibrium or not can be tested by chi-square test.

Factors disturbing the equilibrium in the population:

1. Migration: In plant breeding migration is represented by inter varietal crosses, poly crosses, etc., wherein a single population two or more separate populations are introduced. Migration may introduce new alleles into a population, which may change the gene frequencies.

2. Mutation: It may produce a new allele not present in the population or it may change the frequencies of the exhibiting alleles.

3. Random drift: It is other wise known as genetic drift. It is a random change in gene

frequency due to sampling error. In a smaller population if natural selection operates at random it will lead to sampling error. This sampling error is greater in smaller population than in a large one. Because of sampling the frequency of one of the alleles becomes zero and that of the other alleles become one. The allele having the value one is said to be fixed because there is no further change in its frequency and thus it becomes homozygous. Thus if the population is small genetic drift will occur. To over come this, one has to use larger population, which may not be possible because of limitations in space, labour and finance.

4. Inbreeding: In smaller populations, a certain amount of inbreeding is bound to occur and this will lead to homozygosity.

5. Selection: This is important because when you practice selection you allow the selected genotype to reproduce, while the undesirable genotypes are eliminated. Thus if in a random mating population if we practice selection for the allele AA alone then its frequency in the selected population will be *one* and the frequency of *aa* will be zero. This particular selection for a particular allele is known as *selection differential* designated as S.

Selection differential for AA = 1

Fitness for aa = O.

But in practice it is not possible to identify AA alone especially in case of quantitative characters. So, we will not eliminate one allele (aa) but instead the gene frequency will be changed. When the selection differential is less than *one* then the rate of change in gene frequency would depend on the intensity of selection and upon gene frequency.

Thus selection in a random mating population is highly effective in increasing or decreasing the frequency or alleles, but it is unable to either fix or eliminate them.

SYSTEMS OF MATING

To change the genetic composition of a population we have got different systems of mating

1. Random mating
2. Genetic assortative mating
3. Genetic disassortative mating
4. Phenotypic assortative mating
5. Phenotypic disassortative mating.

1. Random mating:

Here the rate of reproduction of each individual is equal i.e. there is no selection and each male or female is equally likely to combine at random. This random mating is useful in plant breeding for the production and maintenance of synthetic and composite varieties, production of polycross varieties.

2. Genetic assortative mating:

Here the mating will be between individuals that are closely related by ancestry ie. mating between individuals having more or less similar genotype. It is other wise known as inbreeding. The genetic assortative mating leads to

i) Increase in homozygosity
ii) Characters become fixed
iii) Lethals will be eliminated
iv) Separation of population into lines.

Genetic assortative mating is usefull for the development of inbreds.

3. Genetic disassortative mating

It is mating between individuals that are not closely related by ancestry.
Eg. Intervarietal and interspecific crosses.

4. Phenotypic assortative mating

Mating between individuals, which are phenotypically more similar. This type of mating leads to increase in homozygosity and division of population into two extremes. i.e. there is highest and lowest phenotypes remain in the population and there is no intermediate types.

5. Phenotypic disassortative meting

Mating between phenotypically dissimilar individuals This system leads to maintenance of or increase in heterozygosity.

Selection in cross pollinated crops

Selection in a random mating population is able to

i) Change the gene and genotypic frequency.

ii) Production of new genotypes due to changed gene frequencies.

iii) Cause a shift in the mean of population towards the direction of selection.

iv) Change in the variance of population to some extent.

The magnitude of these effects is influenced by the number of genes controlling

the character, the degree of dominance, nature of gene action and to large extent heritability.

A large number of studies on the effect of selection in random mating population have been made. The response to selection in cross pollinated crop can be divided in to five broad groups.

1. Rapid gain followed by slow Progress.

In some cases selection produces rapid gain for some generations. This is followed by a period of slow gain.

This type of response is seen in characters like plant height, days to flowering. These characters will be governed by a few genes with. major effect and several genes with lesser effect. The major genes will give rapid gain and several genes having lesser effect gives slow effect.

2. Continued slow progress for a long period

Eg. Oil content and protein content in maize crop.

This is because, that these traits are governed by several genes, each having a small additive effect. So, progress under selection for such traits would be slow.

3. Slow response for a shorter period only

Here the response for selection will be for a shorter period only and afterwards there will be no response at all. This is due poly genes which may be more than 40 which control a character.

4. Lack of response to selection

This may be due to low heritability and additive gene action. This was seen in maize when selection is practiced for yield.

5. Rapid gain - plateau - Rapid gain

This is due to linked genes both positive and negative

Lecture No. 21

Breeding methods of cross pollinated crops without involving artificial hybridization: Mass selection in cross pollinated crops – modified mass selection – unit selection – mass selection with progeny testing – half sib family selection – full sib family selection.

BREEDING METHODS FOR CROSS POLLINATED CROPS

Populations of cross pollinated crops are highly heterozygous. When inbreeding is practiced they show severe inbreeding depression. So to avoid inbreeding depression and its undesirable effects, the breeding methods in the crop is designed in such a way that there will be a minimum inbreeding. The breeding methods commonly used in cross pollinated crops may be broadly grouped into two categories.

I. Population improvement

A. Selection

a) Mass selection

b) Modified mass selection

Detasseling
Panmixis
Stratified or grid or unit selection
Contiguous control.

B. Progeny testing and selection

a) Half sib family selection

i) Ear to row
ii) Modified ear to row.

b) Full sib family selection.

c) Inbred or selfed family selection.

i) S_1 self family selection
ii) S_2 self family selection.

MASS SELECTION

Mass selection is a method of crop improvement in which individual plants are selected with similar phenotypes from a mixed population and their seeds are bulked and used to grow next generation. Phenotypic selection is done for easily observable characters like plant height, ear type, seed colour, seed size, tillering habit, shattering resistance and lodging nature etc., Sometimes yield of the single plants are taken as a criterion for selection.

This method is applicable to both self and cross pollinated crops and however more commonly used in cross pollinated crops and rarely used in vegetatively propagated crops. Generally the plants selected through this method are not subjected to progeny test. However to reject inferior progenies, Allard (1960) suggested progeny test.

In the improvement of self pollinated crops, mass selection has two major applications.

1. Improvement of local varieties
2. Purification of existing pureline varieties

Features of varieties developed through mass selection in self and cross pollinated crops.

1. Genetic constitution:

Mass selected variety of self pollinated crop is homozygous and heterogeneous, because it is a mixture of several purelines. In cross pollinated crops, mass selected variety is homo or heterozygous and heterogeneous, because it contains several homo and heterozygous genotypes.

2. Adaptation:

Mass selected varieties have broader genetic base and hence they are highly adapted. However adaptability more in cross pollinated crop than the self pollinated.

3. Variation:

Heritable variation is there in mass selected varieties besides environmental variation. This heritable variation gives more buffering capacity.

4. Selection:

In mass selected variety, selection is effective in self pollinated crops due to the presence of heritable variation. Whereas further selection leads to inbreeding depression in cross pollinated crops.

Mass selection as applied to self and cross pollinated crops

5. Quality:

Less uniform in quality than purelines because of heritable variation.

6. Resistance:

Due to genetic diversity they are less prone to pest and diseases.

Types of mass selection:

1. Positive mass selection 2. Negative mass selection

1. Positive mass selection:

In a mixed population, desired plants are selected and their seeds are mixed to grow the next generation is called positive mass selection. The base material is either land races or old varieties. It is so common method of mass selection.

2. Negative mass selection:

In this method, undesirable off type plants are only removed and the remaining plants are allowed to grow further. This is practiced for varietal purification and helps to maintain genetic purity especially in self pollinated crops.

The success of mass selection mainly depends on

1. Variability in the base population
2. Mode of inheritance of the trait under consideration
3. Heritability

Procedure for mass selection

First Year:

A large number of phenotypically similar plants are selected for their vigour, plant type, disease resistance and other desirable characteristics. The number of plants selected may vary from few 100 to few thousand. If too many plants are selected, the improvement is likely to be small. But if too few plants are selected, the adaptation of the variety may become poor. Seeds from the selected plants were composited to raise the next generation.

Second Year

The composite seed is planted in a preliminary yield trial along with the standard check. The variety from which the selection was made should also be included as a check to determine if there has been improvement due to selection. Phenotypic characters of the new variety are critically observed.

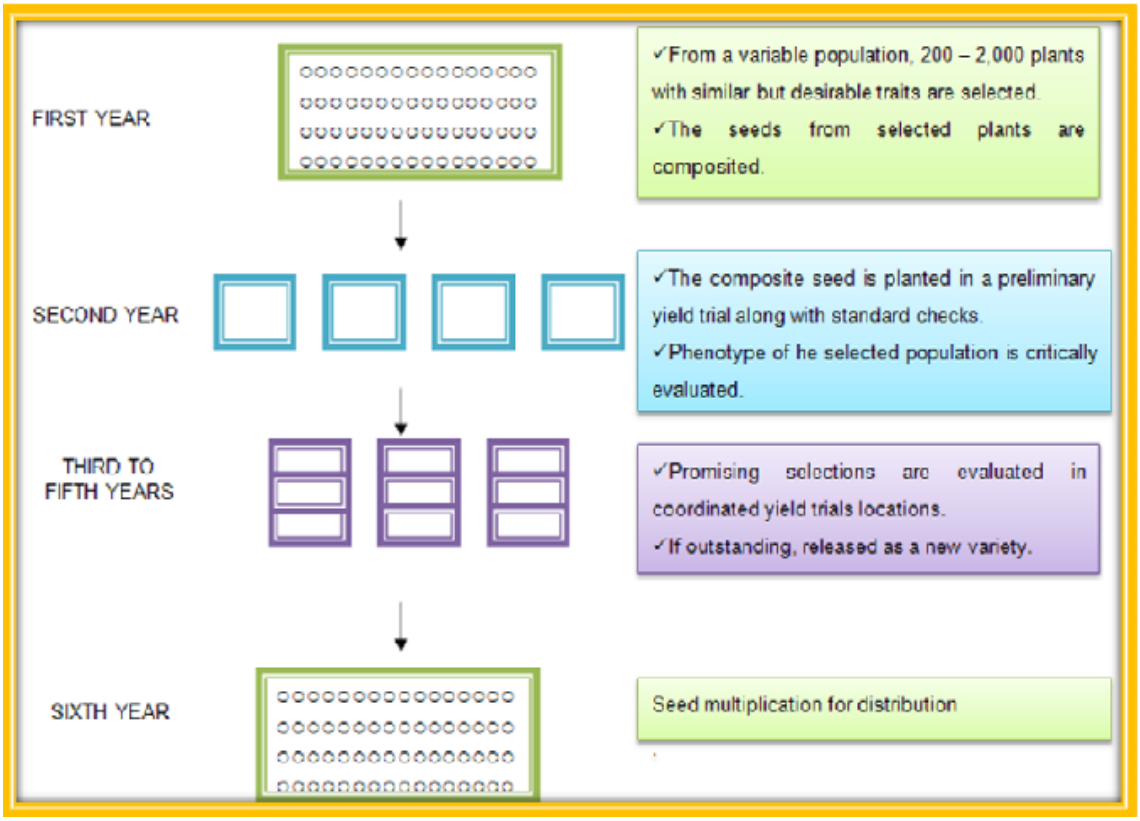
Third year to Sixth year

The new line is evaluated in coordinated yield trials at several locations. This is done to test the performance of the new line at different locations within an agroclimatic zone and compared it with that of the popular commercial varieties used as checks. First, the new line is evaluated in an initial evaluation trial for one year. If the line is promising,

it is promoted to uniform regional trials for two or more years. If found promising, the new line will be identified for release as a new variety.

Seventh Year

The new line may be released as a new variety for cultivation under guidelines of state or central variety release committee.



Merits and Demerits of mass selection

Merits of mass selection

1. Good method for the improvement of old varieties and land races and purification of improved varieties.
2. Mass selected varieties are more stable than purelines due to their buffering capacity.
3. Mass selected varieties gives good protection against diseases.
4. Mass selection is quick method and it takes fewer periods (8 years) to release a variety as compared to pureline development (10 years).
5. This method is applicable both in self and cross pollinated crops.

Demerits of mass selection

1. Progeny test is not normally carried out in mass selection. Simple phenotypic selection fails to judge the real breeding value and hence the selected plants include inferior types.
2. In cross pollinated crops, rapid deterioration of mass selected varieties occur due to no control on the pollination.
3. In cross pollinated crop large plants have to be selected for bulking because small sample lead to inbreeding depression.
4. The mass selected varieties are less uniform than purelines.
5. In self pollinated crops pureline selection is more effective than mass selection.

To over come these defects modified mass selection is proposed they are

a) Detasseling : This is practiced in maize. The inferior plants will be detasseled there by inferior pollen from base population is eliminated.

b) Panmixis: From the selected plants pollen will be collected and mixed together. This will be used to pollinate the selected plants. This ensures full control on pollen source.

c) Stratified mass **selection:**

Unit selection.

Here the field from which plants are to be selected will be divided into smaller units or plots having 40 to 50 plants / plot. From each plot equal number of plants will be selected.

The seeds from selected plants will be harvested and bulked to raise the next generation, by dividing the field into smaller plots, the environmental variation is minimized. This method is followed to improve maize crop.

It is also known as *Grid method* of *mass selection* or

B) PROGENY TESTING AND SELECTION

a) Half sib family selection

Half sibs are those, which have one parent in common. Here only superior progenies are planted and allowed to open pollinate.

1. Ear to row method: It is the simplest form of progeny selection. Which is extensively used in maize. This method was developed by *Hopkins*

a) A number of plants are selected on the basis of their phenotype. They are allowed to open pollinate and seeds are harvested on single plant basis.

b) A single row of say 50 plants i.e. progeny row is raised from seeds harvested on single plant basis. The progeny rows are evaluated for desirable characters and superior progenies are identified.

c) Several phenotypically superior plants are selected from progeny rows. There is no control on pollination and plants are permitted to open pollinate.

Though this scheme in simple, there is no control over pollination of selected plants. Inferior pollen may pollinate the plants in the progeny row. To over come this defect, the following method is suggested.

a) At the time of harvest of selected plants from base population on single plant basis, part of the seed is reserved.

b) While raising progeny rows, after reserving part of the seeds, the rest are sown in smaller progeny rows.

c) Study the performance of progenies in rows and identify the best ones.

d) After identifying the best progenies, the reserve seeds of the best progenies may be raised in progeny rows.

e) The progenies will be allowed for open pollination and best ones are selected.

There are number of other modifications made in the ear to row selection.

For example,

i. The selected progenies may be selfed instead of open pollination

ii. The selected plants may be crossed to a tester parent. The tester parent may be a open pollinated variety, or inbred

iii. The progeny test may be conducted in replicated trial.

b) Full sib family selection

Full sibs are those which are produced by mating between selected plants in pairs. Here the progenies will have a common ancestry. The crossed progenies are tested.

A x B B x A

c) Inbred or selfed family selection

Families produced by selfing.

S_1 family selection

Families produced by one generation of selfing. These are used for evaluation and superior families are intermated (Simple recurrent selection).

S_2 family selection

Families obtained by two generations of selfing and used for evaluation. Superior families are intermated.

Merits of progeny testing and selection

1. Selection based on progeny test and not on phenotype of individual plants.
2. In breeding can be avoided if care is taken raising a larger population for selection.
3. Selection scheme is simple.

Demerits

1. No control over pollen source. Selection is based only on maternal parent only.
2. Compared to mass selection, the cycle requires 2-3 years which is time consuming.

Lecture No 22.

Breeding methods of cross pollinated crops involving artificial hybridization: Recurrent selection principles – types – merits and demerits.

RECURRENT SELECTION

The idea was given by Hayes and Garber (1919) and East and Jones (1920). Jenkins 1940 described the procedure of recurrent selection. However, the word recurrent selection was coined by Hull 1945.

Definition:

Recurrent selection is defined as reselection generation after generation with inter- breeding of selects to provide genetic recombination.

It is a cyclic selection used to improve the frequency of desirable alleles for a character in a breeding population. It is also an important method of population improvement.

Main features

It is a modified form of progeny selection and however it differs from progeny selection in two ways.

1. The selected plants are selfed in recurrent selection whereas they are open pollinated in progeny selection.
2. The selected plants are inter-mated in all possible combinations in recurrent selections but they are open pollinated in progeny selection.

The characteristic features of recurrent selection are given below:

1. **Application**: This method is more commonly used in cross pollinated species than in self pollinated species.
2. **Base population**: To start recurrent selection a heterogeneous population is required. In cross pollinated species, a base population may be either of the five populations, viz., (a) an open pollinated variety, (b) a synthetic variety, (c) progeny of inter crosses among selected inbreds, (d) a double cross, and (e) a single cross.
3. **Important steps**: A simple recurrent selection scheme consists of five main steps: (1) selection of superior plants from base population, (2) selfing of selected plants (3) growing progeny of selected plants in the next season from selfed seed, (4) intermating among progeny and (5) bulking of crossed seed in equal quantity. This completes original

cycle of recurrent selection. The bulk seed is used for next cycle of selection which also involves above five steps.

4. **Use of end product**: The population developed by recurrent selection can be used in three main ways: (a) In producing homozygous inbreds by selfing, (b) in the production of hybrids varieties and (c) in the production of synthetic varieties.

5. **Basic assumption**: Recurrent selection is based on three basic assumptions, viz., absence of epistasis, absence of multiple alleles and absence of linkage disequilibrium. However, none of these assumptions is considered valid.

6. **Impact:** Recurrent selection is used to improve the frequency of desirable alleles for a character in a population. In this method the heterozygosity that is lost due to selfing is recovered by intermating of selected progeny.

TYPES OF RECURRENT SELECTION

There are four types of recurrent selection, namely, (1) simple recurrent selection, (2) recurrent selection for general combining ability, (3) recurrent selection for specific combining ability, and (4) reciprocal recurrent selection.

Each type is used under specific conditions as discussed below:

(1) Simple Recurrent Selection:

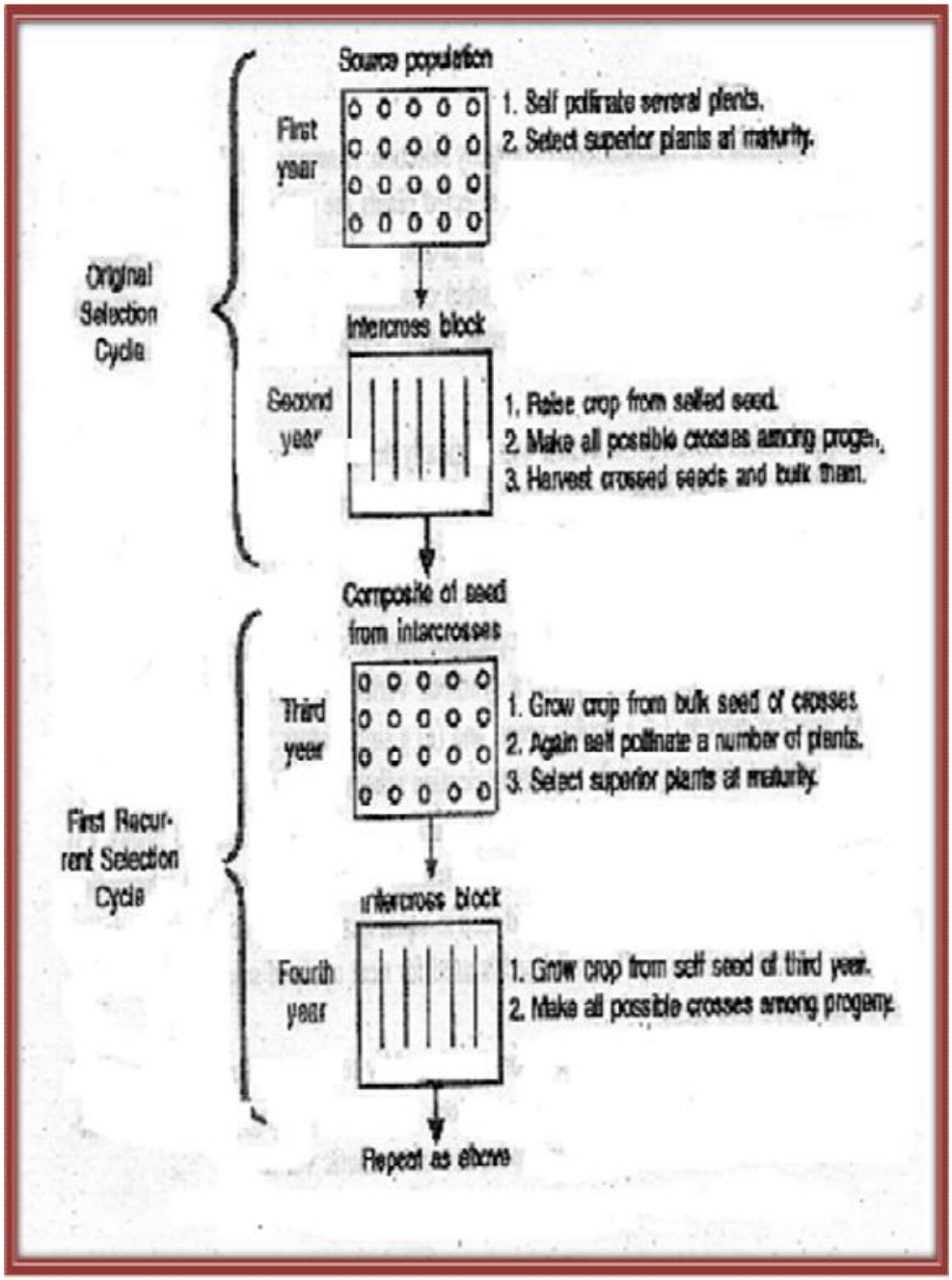
A type of recurrent selection that does not include tester is referred to as simple recurrent selection. It is also known as phenotypic recurrent selection. This method is an extension of mass selection.

Main features of simple recurrent selection are given below:

1. The tester is not used in this scheme.
2. It does not measure the combining ability.
3. The selection is based on phenotype or simple test.
4. This method is useful only for those characters which have high heritability.
5. This method requires only two seasons for the completion of one selection cycle.

In the first year, the superior plants for the character under improvement are selected from the heterozygous base population. These plants are grown from the selfed seed and intermating is done among the progeny. The crossed seed is bulked in equal quantity. This completes original cycle of selection. In the third year, bulked seed is grown and superior plants are selected and selfed like first year. In the fourth year,

progeny of selected plants are grown from selfed seed and intermating is done like first year. The crossed seed is composited in equal quantity for use in the next cycle of selection. This completes first cycle of simple recurrent selection. Thus selection cycles may be repeated till the desired improvement is achieved.



Procedure for Simple recurrent selection

Recurrent Selection for GCA

A form of recurrent selection that is used to improve the general combining ability of a population for a character and includes heterozygous tester is referred to as recurrent selection for general combining ability (RSGCA). It is also known as half sib recurrent selection with heterozygous tester. This is an extension of the Jenkin's (1940) scheme used for development of short term synthetics.

Main features of this scheme are given below:

1. This method is used for genetic improvement of quantitative characters.
2. The selection is made on the basis of test cross performance.
3. A heterozygous tester with broad genetic base is used for testing general combining ability. Generally, an open pollinated variety is used as a tester.
4. This method is used for improving general combining ability of population for a character.
5. This method is more effective with incomplete dominance and less effective with overdominance.
6. This method is used for the improvement of those characters which are governed by additive gene action.
7. This method requites three seasons or years for completion of each cycle of selection as given below:

First Year:

Superior plants for the character under improvement are selected from the base population. The selected plants are selfed and also crossed to a heterozygous tester having broad genetic base. The selfed seed is kept in cold storage.

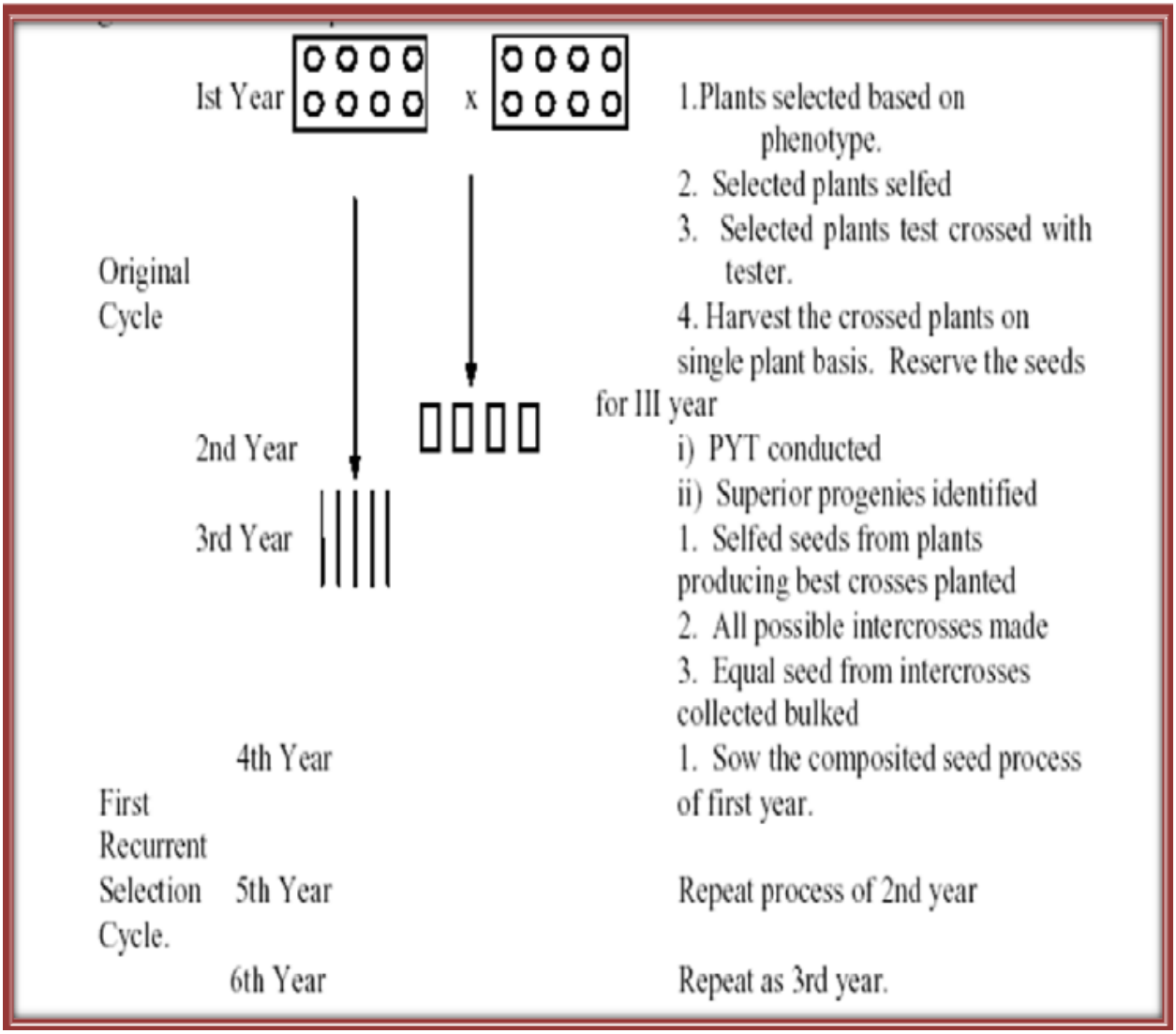
Second Year:

The crossed seed is sown and the combining ability of the selected plants is evaluated and plants with good gca are identified.

Third Year:

The progeny of selected plants with good gca are grown from their selfed seed kept in cold store. These progeny are inter- mated in all possible combinations and their crossed seed is composited to form a new source population for further selection. This completes original selection cycle. In the same way another cycle can be completed in

three years (4th to 6th year). This is called first recurrent selection cycle. Many such cycles may be made to obtain desired results.



Procedure for Recurrent Selection for GCA

Recurrent Selection for SCA

A form of recurrent selection that is used to improve the sca of a population for a character by using homozygous tester is referred to as recurrent selection for specific combining ability (RSSCA).

It is also known as half sib recurrent selection with homozygous tester. This method was originally proposed by Hull in 1945. Main features of this scheme are presented below:

1. This method is used for the genetic improvement of polygenic character.

2. Selection is made on the basis of test cross performance.

3. A homozygous tester with narrow genetic base is used for testing specific combining ability. In other words, an inbred is used as a tester.

4. This method is used for improving specific combining ability of the population for a character.

5. This method is more effective with over dominance and less effective with incomplete dominance.

6. This scheme is used when a character is governed by non additive (dominance and epistasis) gene action.

7. This method required three seasons or years for completion of each cycle of selection.

The selection procedure of this method is same as for RSGCA except that the tester is inbred lines in this case which has narrow genetic base. The differences in the performance of test crosses are due to differences in their specific combining ability.

A comparison of recurrent selection for gca and recurrent selection for sca is presented in the Table.

Comparison of recurrent selection for *gca* and recurrent selection for *sca*

Particulars	**Recurrent selection for *gca***	**Recurrent selection for *sca***
Application	Used to improve polygenic traits	Also used to improve polygenic traits
Basis of selection	Test cross performance	Test cross performance
Tester used	Heterozygous	Homozygous
Effectiveness	More effective with incomplete dominance	More effective with complete and overdominance
Condition of use	Used when additive gene action is important	Used when non-additive gene action is important
Impact	It improves *gca* of a character	It improves *sca* of a character

Reciprocal Recurrent Selection

A form of recurrent selection that is used to improve both gca and sca of a population for a character using two heterozygous testers is known as reciprocal recurrent selection (RRS). It is also termed as recurrent reciprocal half sib selection. This scheme was proposed by Comstock, *et al.* in 1949.

Main features of this method are given below:

1. This scheme is also used for the improvement of polygenic characters.

2. Selection is made on the basis of test cross performance.

3. Two heterozygous populati8ons each of which is the tester for other are used in this method. These two populations may be designated as A and B.

4. This method is used for improving a population both for gca and sca for a specific character.

5. This method is equally effective with incomplete, complete and overdominance.

6. This method is used for the improvement of those characters which are governed by both additive and non-additive gene action.

7. This method also requires three seasons or years for completion of each cycle of selection as given below:

First Year:

Several phenotypically superior plants are selected from population A and B. The pollen of some selected plants of A population is used to cross large number of randomly selected plants of population B. Similarly, pollen of some selected plants of b population is used to cross large number of plants of population A. All the plants of population A and B used as pollen patents in the crosses are selfed.

Second Year:

The progeny of test crosses made with pollen parents of A and B populations are evaluated in separate replicated trials. The superior progeny are identified.

Third Year:

The selfed seeds of those A and B plants whose progeny were found superior in replicated trials are grown in separate block. All possible crosses are made among the progeny of A plants and also among the progeny of B plants. The crossed seeds of A block are composited in equal quantity raise A1 generation. Similarly, crossed seeds of B block are bulked to raise B1 generation. This completes original cycle of selection.

Fourth Year

The A1 and B1 populations are grown from the composite crossed seeds of respective population obtained in third year.

Then operations of first year are repeated.

Fifth Year:

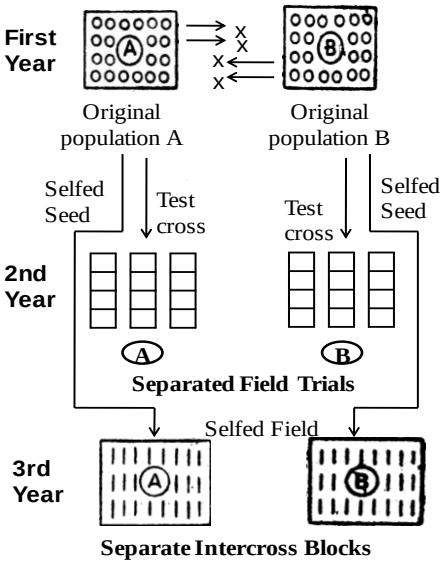
The operations of second year are repeated.

Sixth Year:

The operations of third year are repeated.

The last three years constitute first cycle of reciprocal recurrent selection. Such selection cycles may be continued till the desired improvement is achieved.

Reciprocal Recurrent Selection – Original selection cycle



1. **Several plants selected in the populations A and B**
2. **Selected plants self-pollinated**
3. **Each selected plant from A is test-crossed with several random plants from B, and vice-versa**
4. **Test-cross progeny and selfed seed from each selected plant harvested separately**

1. **Separate yield trials conducted for test-crossed progenies from populations A and B**
2. **Superior progenies identified**

1. **Selfed seed from plants producing superior test-crossed progenies planted separately for populations A and B**
2. **All possible intercrosses are made**
3. **Seeds from all intercrosses of a population mixed**

Merits and Demerits

Merits

1. Recurrent selection is an efficient breeding method for increasing the frequency of superior genes in a population for various economic characters. Thus it is an important method for population improvement.

2. Repeated intermating of heterozygous progeny provides greater opportunities for recombination to occur. Thus this method helps in breaking repulsion phase linkages.

3. This method also helps in mai9ntaining high genetic variability in a population due to repeated intermating of heterozygous plants/populations.

4. The selection is made on the basis of test cross performance (except in simple recurrent selection) and only selected plants are allowed for intermating.

Demerits

1. This method is not used directly for the development of new varieties. The new varieties are developed buy the use of end product in hybridization. This is only a method of population improvement.

2. This method involves lot of selection, crossing and selfing work.

3. This method permits selfing which leads to loss of genetic variability.

Recurrent selection has been successfully is used for the improvement of oil content in maize, fibre strength in cotton and sugar content in sugar beet and sugarcane.

Lecture No. 23

Heterosis breeding – genetic basis – hybrid vigour – estimation of heterosis – inbreeding depression – development of inbreds.

The term heterosis was coined by Shull (1914). It refers to the superiority of F1 hybrid over its parents. In other words, heterosis refers to increase of F1 in fitness and vigour over the parental values. While heterosis refers to the phenomenon (cause), hybrid vigour is the phenotypic expression (effect) of the genetical phenomenon. In current usage, heterosis and hybrid vigour are used as synonyms and interchangeable.

1. Genetic Basis of Heterosis

There are three main theories which have been advanced to explain the mechanism of heterosis. One is the dominance favaourable factor hybpothesis, the second one is over dominance hypothesis and third one is epistasis hypothesis.

(i) Dominance hypothesis

This theory was proposed by Davenport (1908), Bruce (1910) and Keeble and Pellow (1910). According to this hypothesis, heterosis is the result of superiority of dominant alleles when recessive alleles are deleterious. Here the deleterious recessive alleles of one parent are hidden by the dominant alleles of another parent and the hybrid exhibits heterosis.

P1 AAbbCC X P2 aaBBcc

F1 AaBbCc

In the hybrid, deleterious effects of all the three recessive alleles are sheltered by the respective dominant alleles. Therefore AaBbCc=AABBCC.

ii) Overdominance hypothesis

This theory was independently proposed by Shull and East (1908) and supported by East (1936) and Hull (1945). This theory is called by various names such as stimulation of heterozygosis, emulative action of divergent alleles, single gene heterosis, super dominance and over dominance. Though this theory was proposed by Shull and East (1908), the term over dominance was coined by Hull in 1945. According to this hypothesis, heterosis is the result of superiority over its both homozygous parents. Aa > AA or aa. This theory assumes a special effect for the heterozygous condition over the homozygous condition. Thus heterosis is directly proportional to the heterozygosis.

P1 AAbbCC x P2 aaBBcc

F1 AaBbCc

AaBbCc > AABBCC or aabbcc

The superiority of heterozygote over both homozygotes may arise either due to (i) production of superior hybrid substance in heterozygote which completely different from either of the homozygous products or (ii) greater buffering capacity in the heterozygote resulting from cumulative action of divergent alleles or stimulation of divergent alleles.

iii) Epistasis hypothesis

This hypothesis takes into consideration the influence of one locus on the expression of another for heterozygote adaptive superiority (Gowen, 1952). AaBbCc (with interaction). AaBbCc (without interaction). Evidence for positive interaction of non alleles as contributors to heterosis is available from many studies. There are several instances where the effects of homozygous deleterious recessive allele are epistatic to almost the entire genetic makeup of an inbred. The heterotic effect can be dramatic when such an allele is masked by its corresponding dominant allele in a hybrid (Stuber 1994). However the role of epistasis as a proportion of total genetic variance is small when compared with additive and dominance variances. Multiplicative interaction as a case of heterosis has been reported from many experiments. Multiplicative interaction as a cause of heterosis has traits must be conditioned by numerous and mutually interaction genetic factors.

2. Genetic Factors affecting Heterosis

There are four main genetic factors which affect magnitude of heterosis in crop plants. These are mode of pollination, genetic diversity of parents, their genetic base and adaptability.

i) Mode of Pollination: The magnitude of heterosis differs depending upon the mode of pollination of a species. The level of heterosis is generally higher in cross pollinated species than in self pollinated species.

ii) Genetic diversity of parents: The expression of heterosis is influenced by genetic diversity of parents. In general the level of heterosis increases with the increase in parental diversity but up to some limit and decreases with further increase in parental diversity (cross ability barriers). Thus maximum heterosis occurs at an optimal or intermediate level of parental diversity.

iii) Genetic base of parents: The manifestation of heterosis is affected by the genetic base of the parents. For example in cotton higher heterosis is associated with broad genetic base of parents.

iv) Adaptability of Parents: The magnitude of heterosis is also affected by the adaptability of the parents. In many crops, heterosis is associated with wide adaptability of the parents, because there is close association between adaptability and genetic base.

3. Estimation of Heterosis

The heterosis can be classified into three types on the basis of estimation. They are relative heterosis over mid parent, heterobeltiosis over better parent and standard heterosis over commercial hybrid/ variety.

i) **Relative heterosis:** The superiority of F1 hybrid over the mid parental value (ie., mean value or average of two parents involved in the cross) is known as mid parent heterosis, which is estimated as follows:

Relative heterosis percent = (F1-MP)/MP * 100

ii) **Heterobeltiosis;** The superiority of F1 hybrid over the better parent or superior parent out of two parents involved in the cross is referred to as heterobeltiosis, which is estimated as follows:

Heterobeltiosis=(F1-BP)/BP * 100

iii) **Standard Heterosis:** The superiority of F1 hybrid over the standard commercial variety/ hybrid is known as standard heterosis. The term useful heterosis was used by Meredith and Bridge (1972). It is also called as economic heterosis. This type of heterosis is of direct practical value in plant breeding. It is estimated as follows:

Standard heterosis= (F1-SV)/SV *100

4. Inbreeding depression:

Inbreeding depression refers to decrease in fitness and vigour due to inbreeding. The degree of inbreeding is measured by the inbreeding coefficient. Inbreeding depression results due to fixation of unfavourable recessive genes in F2. The fixation of all favourable dominant genes in one homozygous line is impossible due to linkage between some unfavourable recessive and favourable dominant genes.

Estimation: The inbreeding depressionis estimated when both F1 and F2 populations of the same cross are available.

Inbreeding depression= (F1-F2)/F1 * 100

The estimates of heterosis and inbreeding depression together provide information about the type of gene action involved in the expression of various quantitative traits. The following inferences can be drawn from the estimates of heterosis and inbreeding depression.

Ii) If high heterosis is followed by high inbreeding depression, it indicates the presence of non-additive gene action.

ii) If heterosis is followed by low inbreeding depression, it indicates presence of additive gene action

iv) The heterosis will be high when some alleles are fixed in one parent and other alleles in the other parent (dispersion of alleles).

v) The genes with lack of dominance will not exhibit heterosis in F1 but may show increase in performance in F2 (low inbreeding depression) due to fixation of genes i.e., additive gene action.

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Lecture No: 25

Synthetics and composites - steps in development of synthetics and composites – achievements – merits and demerits.

INTRODUCTION

In practical plant breeding, heterosis can be fully exploited in the form of hybrids in cross pollinated species and also in some self pollinated crops. In cross pollinated species, heterosis can also be exploited partially in the form of synthetics and composite varieties.

Synthetic cultivars have generally come to represent a specific type of synthetic that is intended for commercial (on-farm) use. As such, the parents of synthetic cultivars are also preserved for future synthesis of the cultivar and may be inbred or sibbed lines, clones, F_1 hybrids, or populations. When open-pollinated populations are intermated, the resulting population is sometimes referred to as a composite or composite variety, in contrast to synthetics or synthetic cultivars. The original concept behind the production of synthetic cultivars is attributed to Hayes and Garber and their work with maize. They described the ‘‘synthetic production of a variety’’ as involving hybridization among several inbred lines, with selection among F_1 progenies and advanced generations to produce an improved open pollinated population. In early formal definitions of synthetic cultivars, the selection of parents was necessarily based on some test of their combining ability, which could be used to differentiate synthetic cultivars from synthetics or typical open-pollinated populations. However, some plant breeders have broadened the use to the term ‘‘synthetic cultivar’’ to include any open-pollinated population produced in plant breeding that is intended for direct commercial use.

SYNTHETIC VARIETIES

A variety which is developed by intermating in all possible combinations a number of inbred lines with good general combining ability and mixing the seed of F_1 crosses in equal quantity is referred to as synthetic variety. The use of synthetic varieties for commercial cultivation was first suggested in maize (Hayes and Garber, 1919). After release, synthetic varieties are maintained by open pollination. Main features of synthetic varieties are given below:

Relevance

Synthetic varieties are relevant to cross pollinated crops. Such varieties are developed in crops like maize, pearlmillet, alfalfa and many others cross pollinated species.

Base material

A synthetic variety can be developed from inbreds, clones and open pollinated varieties. The end products of recurrent selection which are already tested for GCA are generally used to constitute synthetic variety. Generally, 5-8 good general combining inbreds are used to constitute a synthetic variety.

Genetic concept

The basic concept in the development of constitute synthetic varieties is exploitation of heterosis or hybrid vigour. Such varieties are constituted from good general combining inbreds. However, heterosis is partially utilized by synthetic varieties because some level of inbreeding takes place due to open pollination in latter generations. Synthetic exploit more of additive gene action, whereas hybrids exploit more of non additive (over-dominance and epistatic) gene action.

Genetic constitution

A synthetic variety consists of several heterozygous initially. Since subsequently the variety is maintained by open pollination, some degree of selfing occurs resulting in fixation of some genes. As a result, in later generations a synthetic variety consists of several heterozygote and homozygotes. Thus synthetic variety has heterogenous population.

Adaptation

Synthetic variety constitutes a polymorphic and stable population. Hence synthetic varieties are highly adaptable to environmental variations. In other words, synthetic varieties provide stable yield in the fluctuating environment.

Disease resistance

Synthetic varieties have better resistance to plant diseases due to their heterogenous nature and broad genetic base.

Reconstitution

A synthetic variety can safely be grown for a period of 4-5 years without reduction in the yield potential (Lonnquist and McGill, 1956). The yielding ability can be maintained in advanced generations by mass selection. Thus farmers can use their own seeds for five years. After a period of five years, it would be desirable to reconstitute the synthetic variety. The reconstitution should be based on new developments and new requirements.

Yield level

The yield of synthetic varieties is always higher than open pollinated parental variety but lower than the yield of single and double cross hybrids. The main advantage of synthetic varieties is that their seed is much cheaper than those of hybrids.

Designation

The F_1, F_2, F_3, F_4 and F_5 generations of a synthetic variety are designated as Syn_1, Syn_2, Syn_3, Syn_4 and Syn_5 respectively.

Synthetic varieties differ from mass selected varieties and lines breeding in the way of selection of component genotypes. The constituent genotypes are selected on the basis of general combining ability for a synthetic variety, whereas combining ability is not tested in mass selected varieties and line breeding. In mass selected varieties the component genotypes are selected on the basis of phenotypic performance and in the line breeding on the basis of progeny performance. Moreover, the synthetic variety is developed by crossing selected genotypes in all possible combinations and mixing the seeds of all F_1 crosses in equal quantity. Such crosses are not made to develop mass selected or line breeding varieties.

STEPS IN DEVELOPMENT OF SYNTHETIC VARIETY:

Development of synthetic varieties consists of four major steps: *viz.* (1) isolation of inbred lines, (2) evaluation of inbred lines for general combining ability, (3) intermating of good general combining inbreds in all possible combinations, and (4) mixing the seed of all F_1 crosses in equal quantity.

1. **Isolation of inbreds:**

   Various materials, *viz.* inbred lines, clones, open pollinated varieties and material developed by recurrent selection are used for development of synthetics. Jenkins (1940) suggested that inbred lines with one generation selfing can be used for development of synthetic variety. The synthetic variety developed from inbred

lines can be reconstituted exactly when the parental materials are inbreds or clones. The exact reconstitution is not possible when the parental lines are open pollinated varieties or short term inbreds because short term inbreds are also heterozygous for many gene loci.

2. **Evaluation of inbred for GCA:**

Inbred lines are evaluated for general combining ability. There are three methods for evaluating inbred lines for general combining ability (gca). These are: top cross method, polycross method and single crosses. In top cross, the inbreds are crossed with a common tester and the progeny are evaluated in replicated trials for general combining ability of yield and yield contributing characters. In polycross, selected individuals are allowed to intermate by open pollination in isolation. The topcross progeny are evaluated for gca of yield using local variety as check in replicated trial. Thus inbred lines with good general combining ability are identified and finally selected for development of synthetic variety. The first two methods are in common use for evaluation of inbreds in terms of gca, because large number of inbreds can be evaluated by this method.

3. **Intermating of good general combining inbreds:**

Inbred lines selected for superior gca are crossed in all possible combinations. The all possible single crosses would be n (n-1) / 2, where n is the number of inbred lines. The seed of each cross can be obtained in adequate quantity to produce a synthetic variety.

4. **Mixing the F_1 seeds**

The seed of all possible F_1 crosses made between the selected inbred lines is mixed together in equal quantity or equal number to constitute synthetic variety. The variety thus developed is called as Syn_1. The seed of such variety is generally multiplied by open pollination in isolation for one or two generations (Syn_1 and Syn_2) and then distributed to farmers for commercial cultivation (Syn_3).

Procedure for developing synthetic cultivar from already available inbred lines involves the following steps.

First year:

Selection of inbred lines, crossing with a common tester, harvesting top crossed seed separately.

Second year:

Evaluation of top crosses in replicated trails using standard hybrid or open pollinated variety as check. Identification and selection of good general combining inbred lines based on top cross performance.

Third year:

Making all possible single crosses among selected inbreds and harvesting seeds of single crosses separately.

Fourth year and fifth year:

Mixing seeds of single crosses in equal quantity seed multiplication by open pollination in isolation for one or two generations.

Sixth year:

Release as a new synthetic variety and distribution of seed to the farmers for commercial cultivation.

FACTORS AFFECTING PERFORMANCE OF SYNTHETIC VARIETIES:

There are three main factors which affect the performance of synthetic varieties in advanced generations. These factors are: (1) the number of parental lines included, (2) the mean performance of these parental lines, (3) the mean performance of all possible crosses among the n lines.

1. **Number of inbred lines;**

   The performance of synthetic varieties depends on number of inbred lines which constitute such variety. In maize, it was observed that the yield of synthetics increased by increasing the number of inbred lines upto 5 and thereafter the yield decreased (Kinman and Spague, 1945). The decrease in yield by inclusion of inbreds beyond 5 resulted due to decrease in prepotency. The prepotency is compensated upto 5 inbred lines due to increase in variability and thereby in heterozygosity. The highest yielding synthetic variety was obtained when only 5 or 6 best combining inbreds were included.

2. **Mean performance of inbreds:**

   The mean performance of parental inbreds also affects the yield potential of synthetic varieties. A positive association is found between the yield of a synthetic variety and the yield of its component lines. High yielding and vigorous inbreds give rise to high yielding synthetics. On the other hand low yielding synthetic varieties are obtained when low yielding and less vigorous lines are used as constituent lines. The performance of inbred lines can be evaluated while

testing for their combining ability. The high combining inbreds can be identified on the basis of top cross performance. The yields of synthetic varieties are the highest when the yields of parental lines are high. Moreover, smaller number of parental lines is required for the development of high yielding synthetics when the yields of parental lines are high.

3. **Mean performance of F_1 crosses**

The third factor which influences the yield potential of synthetic varieties is the mean performance of F_1 crosses among selected inbred lines. High performing F_1 crosses are expected to give rise to high yielding synthetic varieties. Thus improvement in the mean yield of F_1 will enhance the yield of synthetic variety.

ACHEIVEMENTS:

Synthetic varieties have been developed in cross pollinated crops like maize, pearlmillet, sumflower, sugarbeet, alfalafa, Lucerne and several other crops in USA. In India, synthetic varieties have been developed in pearl millet at ICRISAT and in sugarbeet at Pantnagar University. In sugarbeet Pant synthetic 3 is worth mentioning. In cauliflower synthetic 3 has been developed.

COMPOSITE VARIETIES

In cross pollinated crops, the mixture of genotypes from several sources that is maintained in bulk from one generation to the next is referred to as composite variety. Composites are constituted by seed mixture of advanced generation material of Intervarietal or inter racial cross. Main features of composite are given below.

Base material

Composite varieties are developed by mixing the seed of various genotypes which are similar in maturity, height, seed size, colour, *etc.* The variety is maintained by open pollination. Farmers can use their own seed for 3-4 years.

Genetic concept

Composite varieties are developed to make use of heterosis in cross pollinated crops

Adaptation

Composite are more stable and highly adaptable to environmental fluctuations.

Reconstitution

Since composites are developed from heterozygous genotypes, it is not possible to reconstitute the composite varieties, because in segregating populations gene frequency changes with time.

Genetic constitution

Composite varieties consist of several homozygotes and hetrozygotes constituting a heterogenous population.

STEPS IN DEVELOPMENT OF COMPOSITE VARIETY

There are four major steps in development of composite cultivars *viz.* (1) selection of base or parental material, (2) intermating of selected genotypes, (3) evaluation of crosses, and (4) mixing of parental material of superior crosses in equal quantity.

1. Selection of base material

In developing composite cultivars, open pollinated varieties or other heterozygous sources are used as the base material. Several open pollinated varieties with similar maturity duration, height, seed size and seed colour are selected for this purpose.

2. Intermating selected genotypes

Selected genotypes are crossed in all possible combinations. Total crosses to be made equal to $n\ (n\text{-}1)\ /\ 2$, where n is the number of varieties selected for intermating. If there are 10 varieties, there would be $10(10\text{-}1)/2 = 45$ single crosses. The seed of each cross is harvested separately.

3. Evaluation of crosses

All the single crosses are evaluated in replicated trails for yield performance in F_1, F_2, F_3 and F_4 generations using standard hybrid or open pollinated variety as check. Crosses exhibiting little or no inbreeding depression in F_2, F_3, and F_4 generations are identified and selected for the development of composite cultivar.

4. Mixing parental seed of superior crosses

Parental seed of the superior crosses is mixed in equal quantity to develop a composite variety. The seed multiplication is carried out by open pollination in isolation for one or two generations. In this way a composite cultivar is released. Composite cultivars also exploit more of additive gene action, because composites are constituted

from the parents of only those crosses which exhibit little or no inbreeding depression in F_2, F_3 and F_4 generations.

Year wise procedure of developing composite cultivars

First year

Selection of base material, intermating in all possible combinations and harvesting crossed seed separately

Second year

Evaluation of F_1 crosses in replicated trail using hybrid or open pollinated variety as check

Third and fifth year

Evaluation of F_2, F_3 and F_4 generation in replicated trails using standard check, identification of crosses exhibiting little or no inbreeding depression.

Sixth and seventh year

Mixing parental seed of superior crosses in equal quantity to constitute composite variety

Eighth year

Release of new composite variety, distribution of seed to the farmers for commercial cultivation.

ACHIEVEMENTS

Several composite varieties have been developed in maize in India. The important varieties are Jawahar, Vijay, Kisan, Sona, Vikram, Protina, Rattan, Shakti, Vikas, Tarun, Renuka, Kanchana, Co 1, etc., In *Brassica campestris var* toria an early maturing variety known as composite 1 has been released.

MERITS AND DEMERITS:

Merits:

1. Use of synthetic varieties permits appreciable exploitation of heterosis in those cross pollinated species where hybrid seed production is difficult.
2. The seed of synthetic varieties is much cheaper than seeds of single or double cross hybrids. Moreover, the seeds can be afforded even by small farmers.
3. Synthetic varieties are more adaptable to environmental changes than hybrids due to greater variability and broad genetic base.

4. Synthetic varieties have vast genetic variability which provides better protection for the new races of diseases.
5. There is no need to purchase new seed every year. Farmers can use their own seed for 4-5 years.

Demerits:

1. The produce of synthetic hybrid are less uniform and less attractive than hybrid due to higher variability and heterogeneity.
2. The yield of synthetic varieties is poorer than single or double cross hybrids due to partial exploitation of heterosis. They exploit GCA only.
3. Synthetic varieties are utilized only in cross pollinated species, whereas the hybrids can be developed in both self and cross pollinated species.

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Lecture No: 26

Genetic characters of asexual reproduction – breeding methods – clonal selection – hybridization and clonal selection – merits and demerits – achievements; Chimeras and its types; Tree breeding – clonal orchards.

INTRODUCTION

Progeny of a single plant obtained by asexual reproduction is known as **Clone**. A procedure of selecting superior clones from the mixed population of asexually propagating crops is referred to as **Clonal selection**. Crops which are propagated asexually or by vegetative means are known as asexually propagated or vegetatively propagated or clonal crops. There are some agriculture crops like sugarcane, potato, sweet potato etc, and horticulture crops like banana, citrus, mango, apple, pears, litchi etc, crops that are propagated by asexual means. The main reasons of asexual reproduction are:

(1) Reduced flowering and seed set: sugarcane, potato, sweet potato etc,

(2) Non flower in many cases: garlic, ginger, betel, several yams.

(3) To avoid inbreeding depression in certain crops: citrus, mango, apple, pears, litchi and many ornamental plants. These are highly heterozygous and vegetative propagation is essential to maintain the heterozygous balance, and

(4) Apomixies in some species: seed develop asexually in such species and many fruit crops exhibit apomixes.

CLONAL SELECTION AND HYBRIDISATION

Some agricultural crops and a large number of horticultural crops are vegetatively propagated.

Crops : Sugarcane, Potato, Sweet Potato, Tapioca, Ginger, Turmeric, Banana.

Trees : Mango, Citrus, Apples, Pears.

Characters of Vegetatively propagated Crops.

1. Majority of them are perennials. E.g. Sugarcane, Fruit trees.

Annual Crops are mostly tubers - Potato, Sweet potato, Yams.

2. Some crops show reduced flowering and seed set.

Many do not flower at all. Only fruit crops have shown regular flowering and seed set.

3. They are cross pollinated

4. Highly heterozygous and exhibit high inbreeding depression.

The advantage of asexual reproduction is that it preserves the genotype of the individual indefinitely.

Characteristics of clones :

1. All the individuals belonging to a single clone are identical in genotype.

2. The phenotypic variation within a clone is due to environment only.

3. Theoritically clones are immortal i.e. a clone can be maintained indefinitely through asexual reproduction, provided there is no disease occurrence.

4. Generally clones are highly heterozygous and exhibit severe inbreeding depression.

5. Genetic variation within a clone may occur due to natural mutation or hybridization or due to mechanical mixtures.

6. Vegetatively propagated crops are mostly polyploids.

7. Many species are interspecific hybrids E.g. Banana, Sugarcane.

8. The variety is developed thro' clones.

MAIN FEATURES OF CLONES

Clones have several important features such as:

(1) Homogeneous constitution,

(2) Heterozygosity,

(3) Vigorous growth

(4) Wider adaptation etc.

These are briefly discussed below:

Homogeneous constitution:

The progeny of a clone is genetically identical. In other words, all the plants of a clone have similar genetic constitution. Thus clones are homogeneous. There is no genetic variation within a clone. The variation is only environmental hence selection is not effective within clones.

Heterozygosity:

The asexual propagated crops are heterozygous and hence clone is also heterozygous. Progeny of a clone looks similar phenotypically but is heterozygous. If a

clone subjected to inbreeding, it will produce various types of segregations and exhibit inbreeding depression.

Vigorous growth:

Clones have hybrid vigour which is conserved due to asexual reproduction. Most of the varieties of sugarcane and potato are hybrids. In other words, clonal selection is useful in conserving the heterosis for long period, because clones are stable and are not prone segregation.

Wider adaptation:

Generally, clones are more adaptable to environmental variation due to high level of heterozygosity than pure lines. A deliberate mixture of genetically different but phenotypically similar clones give better yield in variable environments than a single clone and also provides better protection from the infestation of diseases.

Source of variation:

There are three sources of variation in a clone, *viz.* bud mutations, mechanical mixtures, and occasional sexual reproduction. The frequency of bud mutation is very low. But once bud mutation occurs it will lead to deterioration of a clone by adding new variation in the population. Viral and bacterial diseases also lead to a clonal variety.

Segregation in F_1:

When hybridization is done between different clones, segregation occurs in F_1 generation. Each F_1 plant is potentially a new variety; therefore, selection is practiced in F_1.

BREEDING METHODS FOR ASEXUALLY PROPAGATED CROPS.

The vegetatively propagated crops can be improved by using following techniques.

1. Clonal selection.
2. Hybridisation & Selection

a) Inter varietal

b) Inter specific

3. Polyploidy breeding.
4. Mutation breeding.
5. Tissue culture and anther culture.

BREEDING PROCEDURE OF CLONAL SELECTION

Improvement of asexually propagated crops by selecting superior clones is known as clonal selection. Superior clones can be isolated from three types of material, viz. (1) Local variety, (2) Introduced variety, and (3) Intercrossed populations. In other words, colnal selection is outlined as follows:

1. In the first year, superior plants are selected from a mixed population of vegetatively propagated crops. Superior plants are selected on the basis of yield, maturity, disease resistance, etc.
2. In second year, the progeny of each selected plants is asexually propagated and grown separately for evaluation without replication. Clones superior to check variety are selected and evaluated in replicated preliminary trials in the third year.
3. In the third year, Preliminary yield trail is conducted and suitable checks are included for comparison. Few superior performing clones with desirable characteristics are selected for multilocation trails.
4. In fourth to sixth year, replicated trails were conducted at several locations along with suitable checks. Clones which are superior to check variety in yield, disease resistance and quality are identified for release.
5. In seventh year, the superior clone is multiplied and released as a new variety.

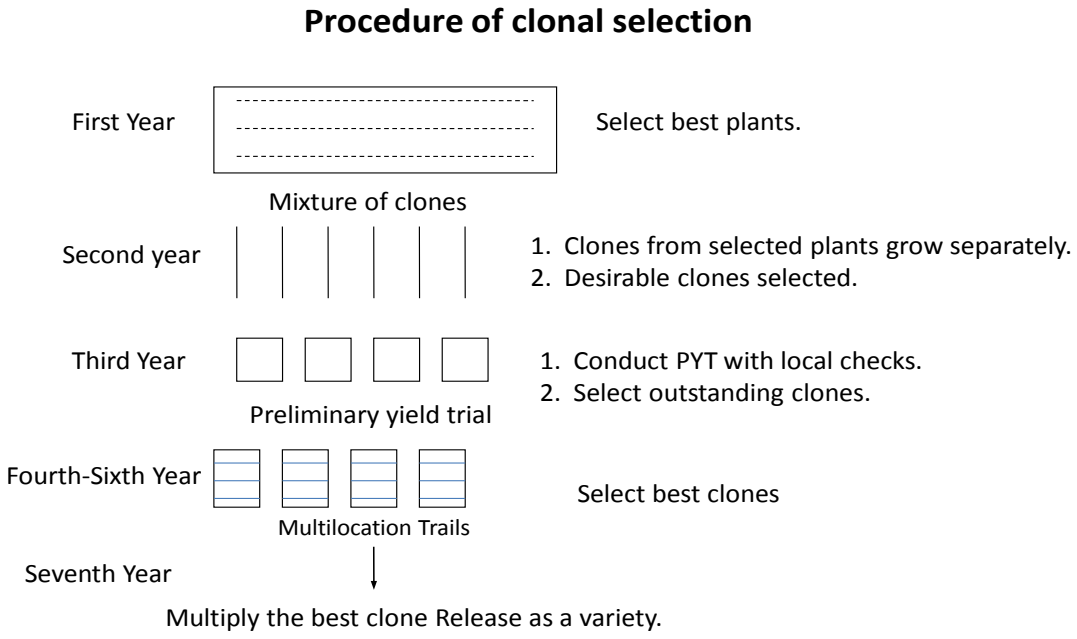


Fig1. A generalized scheme for clonal selection in asexually propagated crops

Besides clonal selection, interspecific hybridization and mutation breeding are also used for the improvement of asexually propagated crops. These methods have been successfully used in sugarcane and potato. Interspecific hybridization has been extensively used in the breeding of sugarcane. Many modern cultivars have been derived from crosses of ***saccharam officinarum*** with ***S.spontaneum or S.barberi***. These crosses are useful in combining high sugar content of the first species with diseases resistance, cold tolerance and vigour of last two species. Backcross method is used for transfer of desirable gene to the cultivated one in sugarcane and potato.

MERITS

- Only method of selection applicable to clonal crops.
- Avoids inbreeding depression and preserves the gene combination present in the clones.
- Maintaining the purity of clones.

- Combined with hybridization to create variability.
- Highly uniform, highly stable (because no risk of detoriation due to segregation and recombination).
- Effective method for genetic improvement of asexually propagated plants.

DEMERITS

- Varieties developed by clonal selection are highly prone to new races of a disease.
- Sexual reproduction is a prerequisite for creation of variability through hybridization.

ACHIEVEMENTS

- In India, colnal selection has been successfully used for new varieties in potato, sugarcane, citrus, grapes.

  Horticultural crops

  - ✓ Potato – Kufri red, Kufri safed,
  - ✓ Tomato – CO1, CO2
  - ✓ Mango – Neelam, PKM1 and Paiyur 1.
  - ✓ Banana – Bombay green (bud selection)
  - ✓ Sapota – CO2 and PKM1
  - ✓ Papaya – CO5 and CO6
  - ✓ *Jasminum grandiflorum* - CO1
  - ✓ *Jasminum auriculatum*- Parimullai, CO1,CO2
  - ✓ Marigold – MDU1
  - ✓ Chrysanthemum - CO1,CO2 and MDU1
  - ✓ Gerbera - YCD 2
  - ✓ Gladiolus - KKL 1
  - ✓ Barleria – CO1

- Interspecific hybridization followed by clonal selection.
  - ✓ Sugarcane – Co 541, Co 510.
  - ✓ Potato – Kufri sinduri, Kufri kuber.
  - ✓

Lecture No 27.

Polyploidy breeding – classification – induction of polyploidy – diploid x tetraploid and diploid x hexaploid crosses - achievements – limitations

Living organisms vary in their chromosome number. The chromosome number in a particular organism is constant and maintained for generation. Diploid organisms exist more commonly and have two sets of chromosomes. Polyploidy has more than 2 sets of chromsomes has been regarded as a major force in evolution and speciation. It is estimated that between 47% and 70% of angiosperm species are **polyploidy.** One of the early examples of a natural polyploid was one of **De Vries's** original mutations of *Oenothera lamarckiana* (mutant. *gigas*). The first example of an artificial polyploid was by **Winkler** (1916) who in fact introduced the term polyploidy. The number of chromosomes in single set is referred to n and thus diploid has 2n number of chromosomes. Number chromosomes within the set vary from crop to crops. Maize is diploid has 20 (2n) chromosmes and the n = 10. In pearl millet 2n = 14 an n = 7. A single set of chromosome is designated by the capital letter. For example A denotes the single set of chromosome in maize then AA is given to designate diploid. In wheat the species Triticum has three different gemome viz., AA, BB and DD which are genetically as well as structurally different. Differences in genome as well as differences in chromosme number exists in both cultivated as well as wild types.

Chracteistics of polyploids

The following characteristics of polyploidy provided adaptive and evolutionary advantages

- Polyloids are more heterozygous then their diploid counter parts. Allopolyploids exhibit more heterozygosity by having genomes from different species which contribute to heterosis or hybrid vigour
- The heterozygosity is stable and maintained in allopolyploidy because of preferential pairing with similar homologus chromosomes and segregation during meiosis as in the case of dipoids which preserves the genomes of both the parental species as well as maintain the diploid ratios.

- Since all polyploids have certain amount of genetic redundancy, the extra copies of gene can mutate and diverge resulting in new traits
- Polyloid populations often demonstrate extensive genomic rearrangement including the novel regions of DNA.
- Polyloids are Self fertile and apomictic which favours its propagation
- Inbreeding is less deleterious for allopolyploids due to their greater heterozygous nature
- Enzyme multiplicity provides greater biochemical flexibility and hence adaptability to different environments
- Other changes in gene expression, altered regulatory interaction and rapid genetic and epigenetic changes could further contribute to increased variation and new phenotypes.

Classification of polyploidy

An individual carrying chromosome numbers other than true monoploid or diploid numbers is called heteroploid (Sharp, 1934). Heteroploidy is divided into euploidy and aneuploidy. In the euploid, an individual carries an exact multiple of the basic chromosome number, while in the aneuploid, the chromosome number is some number other than an exact multiple of the basic set. In polyploids, x is the basic (monoploid) chromosome number and n is the gametic chromosome number, and 2n is the zygotic or somatic chromosome number. For example, the genomic formula of *Triticum aestivum* is 2 n = 6 x = 42 and *Hordeum vulgare* is 2 n = 2 x =14. Inboth cases, the basic chromosome number x is seven. The basic set of chromosomes in a diploidis called a genome

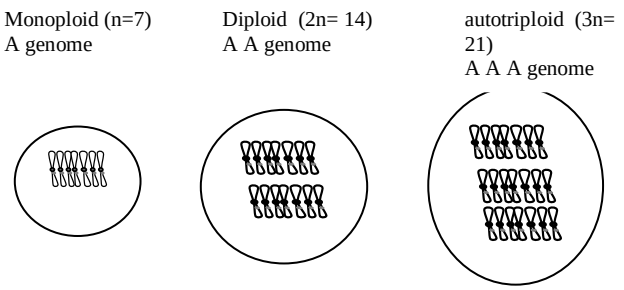
Euploidy

Euploidy is the condition in which changes in the number of chromosomes in the living cells occur in multiples of odd numbers. The change in number could occur as a single set (monoploidy) or in many multiples of the basic set of chromosomes. Euploidy is divided in auto and allopolyploidy. In the autopolyploids, the genomes are alike, because one basic genome is multiplied (x, monoploid; 2 x, diploid; 3 x, triploid; 4 x, tetraploid; 5 x, pentaploid; 6 x , hexaploid; 7 x , heptaploid; 8 x, octoploid). In allopolyploids, two or more genomes derived from different genomically unlike, distinct

species are present. Stebbins (1950) recognized two additional euploid categories, namely, segmental allopolyploids and autoallopolyploid combinations. Segmental allopolyploids carry genomes intermediate in degree of similarity and generally exhibit preferential pairing. For example, a segmental allotetra-ploid with genomes B1B1B2B2usually forms bivalents and occasionally quadrivalents. Autoallopolyploidy is confined to hexaploidy and higher levels of polyploidy (Stebbins, 1950). The term amphiploidy or amphidiploidy denotes polyploids derived after hybridization between two or more genomically dissimilar species separated by chromosomal sterility

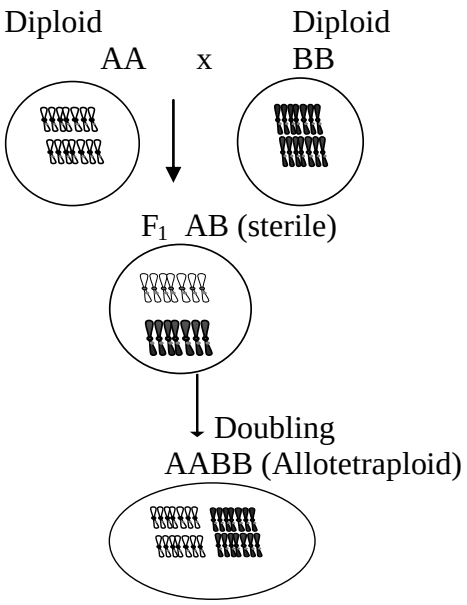
Autopolyploidy

Autopolyploidy are polyploids with multiple chromosome sets derived from a single species. Autopolyploids are also called polysomicpolyploids. Autopolyploidy includes triploidy (AAA), tetraploidy (AAAA) etc. Potato, alfalfa (4n), sugarcane (8-18n), sugar beet (3n), ryegrass (4n), bermuda grass (3-4x), cassava (4n), red clover (4n), Gros Michel banana (3n), apple cultivars (3n), and many ornamentals (3n) are autopolyploids. Most of autopolyploids are **biomass** crops, grown for vegetative parts other than seeds. Autopolyploidy may be a *strict /true autoployploid* (AAAA) or an *interracial autopolyploid* (AA**A"A"**). The ploidy series may consist of individuals with even or odd multiples of the basic chromosome number (eg: *Chrysanhemum* (x=9); series 2x, 4x, 6x, 8x,10x) or odd multiples of the basic chromosome number (eg: *Crepis occidentalis* (x=11); series 2x, 3x, 4x, 5x, 7x and 8x forms)(2). In mode oforigin,autopolyploids arise within single populations or between ecotypes of a singlespecies (M¨untzing, 1936; Darlington, 1937; Burnham, 1962; Gottschalk, 1978) and incytological criteria, autopolyploids will exhibit multivalent configurations,nonpreferential pairing at metaphase, and multisomic inheritance (Stebbins,1980).



Allopolyploidy

Allopolyploid have genome from two or more species. Several of our cultivated crop plants are allopolyploids. Most allopolyploid species are tetraploids. Hexaploids are less common. It is difficult to obtain ploidy level higher than octoploid experimentally. In nature allopolyploids are produced by chromosome doubling in F_1 hybrids. Experimental production of allopolyploid is achieved by doubling the chromosome number of distant hybrids with the help of colchicine or some other agent. The allopolyploids produced by man often termed as synthetic allopolyploids. Allopolyploids are produced by crossing two or genitically distant species and doubling the F1 hybrid because the F1 are mostly sterile because chromosomal dissimilarity both in size and genetic content (fig.1).



Aneuploid

Aneuploid is the condition in which an individual is either lacking one or two chromosomes (hypoploidy) or having additional one or two chromosomes (hyperploidy) from their diploid complement. Aneuploidy arises due to chromosomal non disjunction during meiosis results in production of gametes with abnormal number chromosomes. Aneuploids are usually lethal in animal but can be tolerated in plants. The various aneuploids are monosome, nullisome, trisome and tetrasome. Aneuploids are for the most part less vigorous than normal plants, because of physiological disturbances that are associated with unbalanced and reduced number of chromosomes and genes. Aneuploids

also tend to be irregular meiotically, and as a result they are likely to be partly or even highly sterile. This characteristics of aneulpoids is very antithesis of progress in plant breeding and as a result aneuploids have found little use as varieties. But it is highly useful in genetic studies like detection of linked genes on a chromosome and transfer of chromosome from one species to another.

Monosomes

Monosome is an individual lacking a chromosome from normal diploid complement. The term monosome (2n– 1), first coined by Blakeslee (1921), designates a primary monosome (monosomic) where one of the chromosomes is missing from the normal diploid complement.

Nullisomes

Nullisome is lacking a pair of chromosomes(2n-2). Nullisomics are not viable in diploid and tetraploid species but are tolerated in some hexaploid species, such as wheat and oats. Nullisomics are usually not found in natural populations. They can be obtained for example by selfing a monosomic to produce disomic, monosomic, and nullisomic progeny.

Trisome (2n+1) Trisome is an individual carrying a extra chromosme (2n+1) in addition to their normal comement.

Role of Autopolyploidy in Evolution

- Autopolyploid has contributed to a limited extent in evolution of plant species.
- Potato (4x), peanut (4x), coffee (4x), alfalfa (4x), banana (3x) and sweet potato (6x) are autopolyploids.
- Autotetraploids appear to have been more successful as crops than other forms of autopolyploidy.
- Forage grasses sevaral ornamentals are autopolyploids.
- Recent studies using genomic *in situ* hybridization (GISH) revealed peanut (*Arachis hypogaea*) and coffe (*Coffea arabica*) to be autopolyploids.
- GISH is a powerful tool for investigation of genome organization and evolutionary relationships.

- GISH has revealed that the parental species of *A. hypogaea* are most likely the diploid wild species *A. villosa* and *A. ipaensis.*
- Similarly *C. congensis and C. eugenioides* are the diploid progenitors of *C. arabica.*

Applications of Autopolyploidy in Crop Improvement

- Autopolyploidy is more likely to succeed in species with lower chromosome numbers than in those with higher chromosome numbers.
- Cross-pollinating species are generally more responsive than self pollinating species.
- Crops grown for vegetative parts are more likely to succeed as polyploids than those grown for seeds.

Triploids

- Triploids are produced by hybridization between tetraploid and diploid strains.
- Seedless watermelons are grown commercially in Japan. The triploid plants do not produce true seeds; almost all the seeds are small, white rudimentary structures.
- Triploid sugarbeets produce larger roots and more sugar per unit area than do diploids.
- The triploid cultivar of tea, TV 29, produces larger shoots and thereby, biomass, yields more cured leaf per unit area and is more tolerant to drought than the available diploid cultivars.

Tetraploids

- Autotetraploids are larger in size and are more vigorous than diploids. Autotetraploid varieties of forage crops have been considerably successful. The most successful examples are tetraploid red clover and rye grass.
- In rye (*S. cereale)* where tetraploid varieties have been released for cultivation (e.*g.*, Double Steel, Tetra Petkus)

- Autotetraploidy is able to overcome self- incompatibility in certain cases e.*g.*, some genotypes of tobacco, white clover, petunia, etc. Autotetraploids of of certain *Solanum* species produce hybrids with *S.tuberosum*, while others do not.

- Pusa Giant Berseem is the first autotetraploid variety released for general cultivation in India. It yielded 20 – 30 per cent more green fodder than the diploid berseem varieties.

- Other examples on autotetraploidy are on crops like barley *(H. vulgare)* and jowar (*S. bicolor)* where larger grains, increased protein content and higher yields are the objective.

Limitations :

1. Autopolyploids are successful in species with lower chromosome number.
2. Cross pollinated species are more responsive than self pollinating species.
3. Larger size of autopolyploids is generally accompanied with a higher water content. This is not a desirable character in cabbage and turnip.
4. In crops grown for seed exhibit high amount of sterility though seed size is increased.
5. Triploids cannot be maintained except through clonal propagation.
6. New autopolyploids cannot be used directly as crops because they will have some undesirable characters e.g. poor strength of stem in grapes, irregular fruit size in water melon.
7. Effects of autopolyploidy cannot be predicted.

Induction of autopolyploids

Plant breeders were initially attracted to induce polyploidy primarily because of the gigas effects, which increased cell size (but also reduced fertility). These pros and cons of the gigas effects make the induction of autopolyploids more suited to crops whose economic part is vegetative. The primary technique for inducing autoploids is the use of colchicine ($C_{22}H_{25}O_6$), an alkaloid from the autumn crocus (*Colchicum autumnale*).This chemical compound works by disrupting the spindle mechanism in mitosis, thereby preventing the migration of duplicate chromosomes to opposite poles at anaphase. Consequently, the nucleus is reconstituted with twice the normal number of chromosomes, without any nuclear or cell division.

Ryegrass is one of the species that has been successfully improved by the induction of autoploidy. Rye (*Secale cereale*) is perhaps the only grain-producing cropfor which synthetic autoploids have been developed. Meristematic tissue is most susceptible to colchicine treatment. Hence a germinating seed, a young seedling, or a developing bud, are the commonly used plant material for autoploid induction. The chemical may be applied in aqueous solution or through various media (e.g., agar lanolin paste). Seeds may be soaked in aqueous colchicine at a concentration of 0.05–0.4% for 30 minutes to 3 hours. Buds are treated differently, for example, by intermittently exposing the selected plant material for 2–6 days at concentrations of 0.2–0.5%. The breeder should determine the best treatment condition by experimentation. The material treated should be thoroughly washed after application to remove excess chemicals.

Breeding autopolyploids

In developing and using autopolyploids in plant breeding, certain general guidelines may be observed.

1. Generally, species tend to have an optimum chromosome number (optimum ploidy number) at which they perform best. Because chromosome doubling instantly and drastically increases chromosome number, selecting parents with a low chromosome number for autoploid breeding would reduce the risk of meiotic complications that are often associated with large chromosome numbers. This would increase the chance of obtaining fertile autoploids.

2. Autopolyploids tend to have gigas features and a high rate of infertility. Consequently, autoploidy is more useful for breeding species in which the economic product is not seed or grain (e.g., forage crops, vegetables, ornamental flowers).

3. Producing autopolyploids from cross-fertilizing species promotes gene recombination among the polyploids, with a better chance of obtaining a balanced genotype.

D. R. Dewey summarized the properties of a species suited for the induction of polyploidy as follows:

1. The species has low chromosome number.

2. The economic part of the plant is the vegetative material

(e.g., forage grasses).

3. The plant is cross-pollinated (allogamous).

4. The plant is perennial in habit.

5. The plant has the ability to reproduce vegetatively.

Limitations of Autopolyploidy

- The larger size of autopolyploid is generally accompanied with a higher water content
- Autopolyploids of the crop species grown for vegetative parts do not always produce more dry matter than the respective diploids.
- e.g., tetraploid turnip (*B. rapa*) and cabbage (*B. oleracea*) out yield the diploids in fresh weight, but are comparable, or even inferior, to them in terms of dry matter production.
- Autopolyploids show high sterility accompanied with poor seed set
- Larger seed size of autotetraploids does not generally lead to an increased seed yield per unit area.
- Due to the complex segregation in autotetraploids, progress under selection is very low
- New polyploids are always characterized by a few or more undesirable features e.g., poor strength of stem in grapes

Role of Allopolyploid in evolution

i. About 1/3 of angiosperms are allopolyploids. These suggest that allopolyploids have significant role in the evolution of crop species.

ii. Allopolyploids have contributed great extent in the evolution of plants than auto polyploids.

iii. The identification of diploid parental species is primarily based on pairing between the chromosome of diploid and the allotetraploid species.

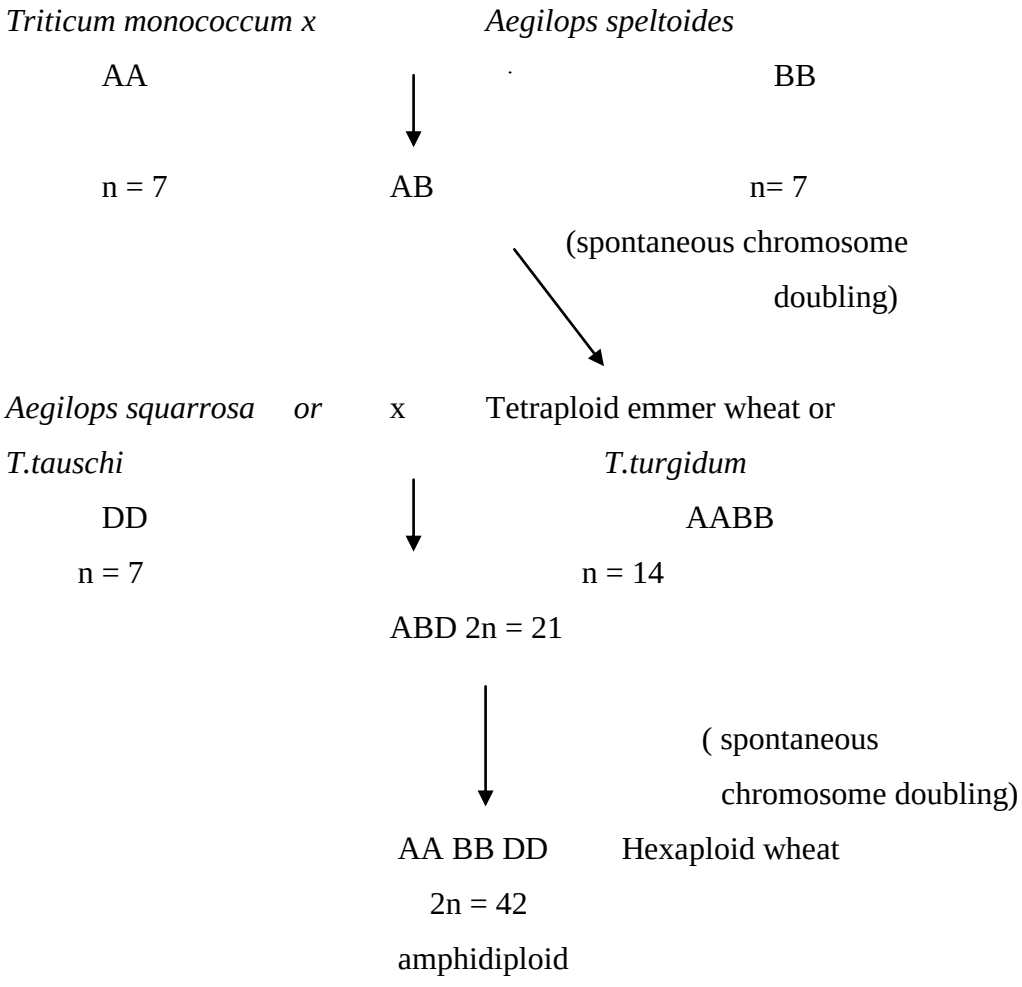
iv. Allopolyploids combine the genome of different species, hence the resulting individuals differ from progenitor.

v. Evolution is a slow process; but due to allopolyploids new species orignate very quickly.

vi. Polyploids have wider adaptation to different environmental condition than diploids.

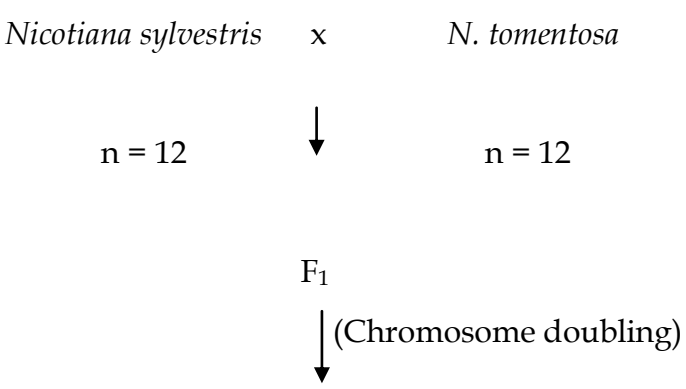
1.Evolution of hexaploid wheat

In cereal crops, wheat is a widely studied alloploid that comprises genomes from three species.



2. Evolution of cultivated tobacco

- N. tabacum (n = 24) is most likely an amphidiploid from the cross *N. sylvestris X N. tomentosa*; both the species are diploid with n = 12.
- The interspecific hybrids *N. tabacum x N. sylvestris* and *N. tabacum* x *N. tomentosa* produce 12 II + 12 I at metaphase I. This indicates a homology between chromosomes of *N. tabacum* and those of *N. sylvestris* and *N. tomentosa.*
- The amphidiploid from the cross *N. sylvestris* x *N. tomentosa* is similar to *N. tabacum* in many characteristics, which further supports the above conclusion.
- The species *N. tabacum* has undergone considerable differentiation during its evolutionary history, mostly due to the accumulation of gene mutations and to some extent, due to the loss of some duplicated segments of the two genomes.

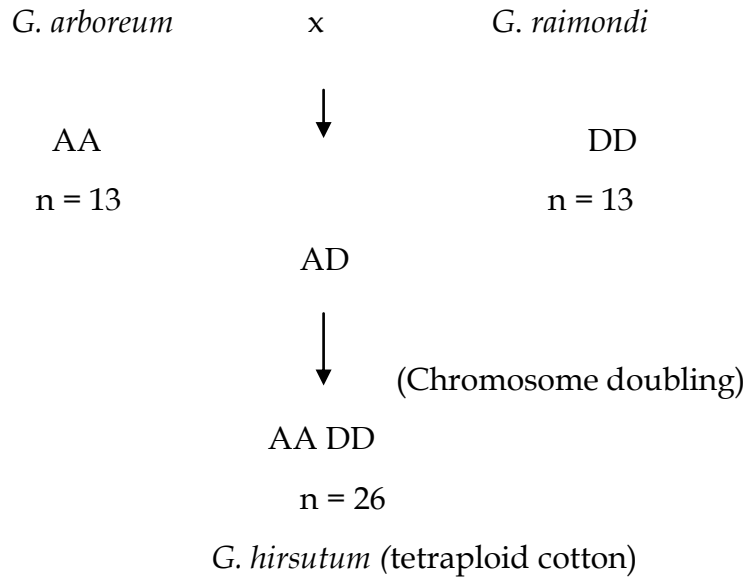


3. Evolution of tetraploid cotton

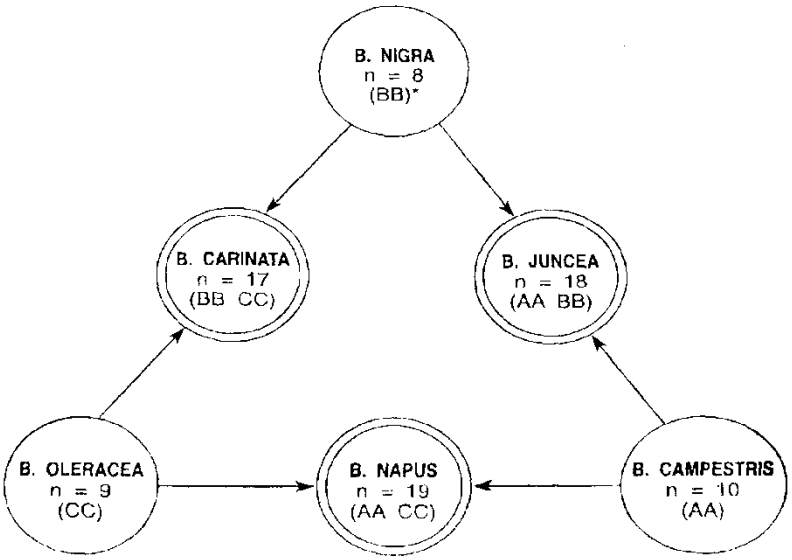
- The 9 old World and the 8 New World species of *Gossypium* have n = 13, but the chromosomes of the New World species are smaller than those of the Old World species.
- Three other species, *G. hirsutum*, *G. barbadense* and *G. tomentosum* (wild Hawaii cotton), have n = 26; in these species, 13 chromosomes are relatively larger than the remaining 13.

- A possible origin of *G. hirsutum* is from the cross between Asiatic cotton *G. arboreum* X *G. thurberi* (American wild cotton), followed by chromosome doubling of the interspecific hybrid.
- According to a more recent scheme, *G. hirsutum* has originated from the cross *G. herbaceum* var. *africanum* x *G. raimondii*, followed by chromosome doubling of the F1.

Evolution of tetraploid cotton



4. Evolution of Brassica



- U's triangle showing the relationships between diploid and naturally occuring amphidiploid species of *Brassica*. The three diploid species are represented at the three tips of the triangle; their amphidiploids are presented midway between the parental species (and are encircled by two concentric circles)

- *B. juncea* (n = 18) is an amphidiploid from the interspecific cross *B. nigra* (n = 8) x *B. campestris* (n = 10), *B. napus* (n =19) is an amphidiploid from the cross *B. oleracea* (n = 9) x *B. campestris* (n = 10), and *B. cartnata* (n = 17) is an amphidiploid from the cross *B. nigra* (n = 8) x *B. oleracea* (n = 9).

- The synthetic allopolyploids produced according to the above scheme resemble the natural amphidiploids, cross easily with them, and the hybrids between the synthetic and natural amphidiploids are reasonably fertile.

Applications of Allopolyploidy in Crop Improvement

- Bridging species in the transfer of characters from one species into another. An example of the use of an amphidiploid as a bridging species is the use of

synthetic *Nicotiana digluta* for the transfer of resistance to tobacco masaic virus from *N.sylvestris* to *N.tabacum*

- Production of new crop species. The most noted success with induced alloploidy is the commercially grown amphiploid, **triticale** derived from a cross between wheat (*Triticum*) and rye (*Secale*).

- Widening the genetic base of existing allopolyploid crop species. Genetic variability of *Brassica napus* is narrow and the only source available is to synthesize new allopolyploid *B.napus* to widen its genetic base. This is being done by crossing *B.campestris* with *B.oleracea* to produce the amphidiploid *B.napus*.

Genetics of allopolyploids

As previously indicated, alloploids arise from the combination and subsequent doubling of different genomes, a cytological event called **allopolyploidy**. The genomes that are combined differ in degrees of homology, some being close enough to pair with each other, whereas others are too divergent to pair. Sometimes, only segments of the chromosomes of the component genomes are different, a condition that is called **segmental allopolyploidy**. Some of the chromosomes of one genome may share a function in common with some chromosomes in a different genome. Chromosomes from two genomes are said to be **homeologous** when they are similar but not **homologous** (identical).

Most allopolyploids have evolved certain genetic systems that ensure that pairing occurs between chromosomes of the same genome. A classic example occurs in wheat ($2n = 6x = 42$) in which a gene on chromosome 5B, designated *Ph*, enforces this diploid-like paring within genomes of the allopolyploid. When this gene is absent, pairing between homeologous chromosomes, as well as corresponding chromosomes of the three genomes occurs, resulting in the formation of multivalents at meiosis I.

Allopolyploids exhibit a variety of meiotic features. Sometimes chromosomes pair as bivalents and thereby produce disomic ratios. Where the component genomes have genes in common, duplicate factor ratios will emerge from meiosis, an event that sometimes is an indication of allopolyploid origin of the species. Whereas significant duplications of genetic material have been found in wheat, the genomes of upland cotton have little duplication. Tetrasomic ratios are expected for some loci where multivalent associations are found in allotetraploids.

Breeding allopolyploids

Allopolyploids may be induced by crossing two species with different genomes, followed by chromosome doubling of the hybrid. Compared to autopolyploids, inducing allopolyploids is not commonly done by plant breeders. If successful, the newly induced amphiploid instantly becomes a new species (unable to cross to either parent). It also becomes reproductively isolated from its parents. Success of induced allopolyploids is enhanced by the proper choice of parents. In particular, using parents with low ploidy levels increases the chance of high fertility and seed set in the amphiploid. Commercially successful induced alloploids are few. The most noted success with induced alloploidy is the commercially grown amphiploid, **triticale** derived from a cross between wheat (*Triticum*) and rye (*Secale*).

The objective of developing triticale is to obtain a product that combines the qualities of wheat with the hardiness of rye. In lieu of doubling the F_1 to produce the desired synthetic product, a wheat × rye cross may be undertaken. The F_1 plant possesses 28 chromosomes and exhibits intermediate traits that favor rye (hairy neck, spike length). All F1s are sterile because of the formation of univalent and irregular gametogenesis. F1s are backcrossed to wheat to produce progenies containing 42 chromosomes (seven from rye and the rest from wheat). The wheat chromosomes form bivalents at meiosis, while the rye chromosomes form univalents. The bivalent wheat chromosomes are irregularly arranged. Fertilization of an ovule with 21 + 7 chromosomes by pollen with the same genomic constitution will contain the full complement of chromosomes for wheat and rye

(56 chromosomes). This product is the synthetic alloploid called triticale. Hexaploid triticale (*AABBRR*, $2n = 6x = 42$) is superior agronomically to octoploid triticale (*AABBDDRR*, $2n = 8x = 56$), but it requires embryo culturing to obtain F1s between durum wheat and rye.

All amphiploid breeding is a long-term project because it takes several cycles of crossing and selection to obtain a genotype with acceptable yield and product quality. Common undesirable features encountered in triticale breeding include low fertility, shriveled seeds, and weak straw. Even though tetraploid ($2n = 2x = 28$), hexaploid ($2n = 6x = 42$), and octoploid ($2n = 8x = 56$) forms of triticale have been developed, the hexaploid forms have more desirable agronomic traits and hence are preferred. Allopolyploids have been used to study the genetic origins of species. Sometimes, amphiploidy is used by breeders as bridge crosses in wide crosses.

Limitations of allopolyploidy

- The effects of allopolyploidy cannot be predicted.
- The allopolyploidy have some features from both the parental species, but these features may be the undesirable ones, e.g., *Raphanobrassica*, or the desirable ones, e.g., *Triticale*
- Newly synthesized allopolyploids have many defects, e.g., low fertility, cytogenetic and genetic instability.
- The synthetic allopolyploids have to be improved through extensive breeding at the polyploid level. This involves considerable time, labour and other resources.

Only a small proportion of allopolyploids are promising; a vast majority of them are valueless for agricultural purposes

Lecture No 28.

Wide hybridization-history-importance-barriers and techniques for overcoming barriers-utilization

Earlier crop improvement programmes were made based on the variability present in the local land races by means of selection method. On exploitation of these variability , hybridization among different cultivated varieties becomes the method to create new variability. The variability in the cultivated species was found to be limited and genes conferring resistance to pest and diseases were introducted in the cultivars from wild/related species which were genetically different. The first authentic record of distant hybridization for crop improvement is the production a hybrid between Carnation *(Dianthus caryophyllus*) and sweet William (*Dianthus barbatus*) by Thomas Fairchild in 1717. A new species hybrid Raphanobrassica was produced by a Russian scientist Karpechenko in 1928 by crossing Cabbage and radish. Triticale an intergeneric hybrid was produced by Rimbau in about 1890. Improvement through wide hybridization takes longer than intraspecific breeding programmes, because of the problems associated in combining two genomes in one species, and breaking linkages with unwanted wild type genes and recovering genotype associated with commercial quality. Because of genetic disimilarity, chromosomal disharmony, the inter specific or intergeneric hybridization often results in failure of fertilization. The failure to hybridize occur either before fertilization or after fertilzation.

Pre fertilization barriers

Self incompatability is the major pre fertilization barrier in many of the crop plants. The failure to fertilization is due incompatibility between pollen and style. This may be due to a.) mismatch in the length of style and the pollen tube b.) thicker pollen tube and slender style c.) non synchronization in stigma receptivity and pollen maturity and d.) immbolization of nutrients from stigma for conducive pollen germination. These barriers could be overcome by

a. Manipulating style

In the case longer style length than the penetrating pollen tube, style length is reduced by amputation and pollinating the cut stump to enable seed set. Maize x Trispacum cross and Nicotiana hybrids were produced by this way. Thick pollen tubes

of polyploidy species some times have difficulty in growing in the slender style of lower polyploids. This can be over come by making reciprocal cross.

b. Effective pollination

In experimental hybridization success could be obtained by effective pollination, i.e., application of pollen at correct time and correct position of the stigma, since the stigma receptivity varies from species to species and the area of stigma receptivity may be very small. The stigma has to be rubbed to release the stigmatic exudates, which is necessary for hydration of pollen grains. Since the humid conditions are more favourable for pollen germination, stigma may be sprayed with water or pollen culture solution.

d. Mentor pollination

In mentor pollination, compatible killed pollen (mentor pollen) grain is mixed with incompatible pollen grain and applied on the intended parent. This techniques have been used successfully in Populus and Cucumis. Mentor pollen facilitate the style to mobilize nutrients for pollen tube growth.

e. Bud pollination

Bud pollination is the application of pollen on immature style and the immature style may not have developed the ability to inhibit incompatible pollen tubes. This was successful in Lycopersicum peruvianum x Tomato which has unilateral incompatibility

f. Grafting

Grafting a pollinated style and stigma on to allien style cut below the zone in which incompatible pollen tubes would be inhibited. This technique has been successful in Datura and Oenothera.

g. Ovule pollination

Application of pollen directly to the ovules either by injection of pollen nutrient solution or by pollinating the surface of the ovules.

g. Excess pollen application

Successful fruit set may depend on ample pollination of stigma. In apple about 50 pollen grains are required per flower for fruit set.

h. Growth regulators

Spraying of growth regulators on or near flowers or applied to pedicel or ovary at or after pollination found to improve fruit set.

Post fertilization barriers

After successful pollination and fertilization, failure of zygote development may takes place due physiological barriers and genetic barriers viz., lethal genes, genotypic disharmony, genetic disharmony chromosome elimination, endosperm imbalance and cytoplsmic incompatability.

Physiological barriers

1. Removal of competing sinks: In interspecific cross has a greater chance of success if the cross is made using the first flowers to open on the maternal parent, or if all immature fruits already set are removed before the cross is made.

2. Seeds containing hybrid tissues develop slowly and may not be able to compete with vegetative sinks. So pruning all active growing points may therefore help to channel resources to the feeble sinks represented by the hybrid embryos

Genetic barriers

a. Lethal genes

The lethal genes will be effective only in hybrids. e.g., *Aegilopes umbellulata* has lethal genes. This lethal gene is active against the zygote produced by crossing *A. umbellulata* with diploid wheats.

b. Genetic imbalances.

Cause death of the embryo., e.g, Gossypium gossypoides is involved in cotton crosses.

c. Endosperm imbalance

Endosperm development depends upon the balance between the ploidy level of the different tissues and/or between maternal and paternal genomes of different tisues. Normal endosperm development requires a balance between two maternally derived genomes and one parental genomes. If this balance is disrupted, the seed abort. This could beover come by a. Doubling the chromosome of F1 of inter specific crosses b. Using female parent having higher loidy levelE.g. ***Solanum acaulae*** (synthetic octaploid) x ***S. tuberosum*** (tetraploid) produce viable seeds if acaule is used as female parent. A

cross between diploid ***S. chacoense*** and synthetic autotetraploid ***S. chacoense*** seldom or never produces viable seed because seed contains two maternal genomes from polar nuclei and two parental genomes from the sperm of autotetraploid c.doubling chromosome number of the wild species: Many wild species of potato were doubled before it is crossed with cultivated variety d. doubling the chrmosome number of polyploidy species. 8x ***Nicotiana tabacum*** x ***N. otophora*** **and** e. Reducing chromosome number of the higher ploidy level.

3. Chromosome elimination

The cytoplasmic incompatability between the cytoplasm of the species used as female and the genome of the species used as male. Such an interaction leads to hybrid weakness. In the hybrid progeny of the cross *Hordeum bulbosum* x *Triticum aestivem*, the genome of *Hordeum. bulbosum* was eliminated when *bulbosum* was used as female parent. The embryo obtained from such crosses were haploids. Endosperm abortion occurs when endosperm development is poor. e.g., the hybrids of Triticum x Secale. Interspecific transfer of genes can be overcome by bridge species where ever there is incompatibility between cytoplasmic and chromosomal imbalance. Bridge species is the one which is compatable with both the parents used in hybridization. Bridge crosses were used in wheat and tobacco.

Barriers to hybrid seedling development

Some distant hybrids die during seedling development or even after initiation of flowering. e.g., Melilotus hybrids. Such plants can be grown to maturity by grafting them on to normal plants or by growing them invitro. The hybrid weakness result from the interaction of hybrid genotypes and cytoplasm of the female parent. The reciprocal cross is resorted to overcome this, e.g. Epilopium, Oenothera etc.

Hybrid inviability

If genetic unbalance affects metabolism in early or later stages of development, the result is hybrid inviability.

Hybrid sterility

If, however the unbalance is not expressed until gametogenesis, the result is hybrid sterility. Due to lack of structural homology between genomes of parental species, many hybrids shows reduced pairing, presence of ring and chain configurations, bridge

fragment configurations and other abnormalities which indicates heterozygosity for translocations, inversions and differences in the position of the centromere. This behaviour result in unorganised disjunction of chromosomes result in non functional gametes. Chromosome pairing is regular in some hybrids, but they show variable sterility due to genes rather than chromosomal changes. Sterility associated with completely normal pairing is usually called geneic sterility. The sterility associated with small structural differences is known as cryptic structural hybridity. e.g., The F1 of hybrid between foxtail millet, Setaria itallica x Setaria viridis and in rice between Indica x Japonica hybrids fails to develop due to this reason.

Applications and achievements

The distant hybrids would be highly heterozygous and the segregating progenies deviate from mendelian ratio. Some transgressive variant could be obtained.

a. Pest and disease resistance

Addition and substitution lines:

Addition and substitutution lines are developed to incorporate diseases resitance. In addition lines either a pair of chromosome is line carries one chromosome pair from a different species in addition to the normal diploid chromosome complement of the parent species. Alien addition monosome has one chromosome instead of one pair. Allien substitution lines has one chromosome pair from a different species in the place of one chromosome pair of the recipient species. Alien substitution monosome has single chromosome. Addition and substitution lines have been produced in tobacco for transfer mosaic resistance from Nicotiana glutinosa to N. Tabacum. Substitution and addition lines are produced through backcrosses.

Nicotiana. tabacum (4x=48) x N. glutinosa(2x =24)

F_1 (3x) doubling using colchicines

F_1 (6x) x N. tabacum Back crossed for 6 generation

After six generation alien addition monosome carrying resistant gene could be obtained. Alien addition monosomes are selfed to isolate alien addition resistant plants and alien substitution lines.

Transfer of chromosomes segemets: is possible through recombination. The recombination in allopolyploidy can be promoted by genetic manipulation and using x rays. Many characters were transferred using this technique. A glaring example is the potato famine in Europe during 1849's caused by late blight; about one million Irish died. *Solanum demissum* provided genes for late blight resistance. A recent example is found in bhendi. The variety Parbhani Kranti bhindi is derived from *Abelmoschus esculentus* cv. Pus Sawani x Abelmoschus manihot and is completely resistant to yellow vein mosaic virus.

b. Abiotic stress resistance

Cold tolerance had been transferred from wild relatives to wheat, onion, potato, tomato, grape, rye and pepper mint. Earliness had been introduced to cultivated species of Brassica. Salt tolerance to tomato, tolerance to calcareous soils in grape and lack of photosensitivity in pennisetum.

c. Quality improvement

Protein content in rice, soybean, oats and rye, oil quality in oil palm, Increased soluble solids (from green fruited species) and carotenoid content (B gene from L. hirsutm) in tomato, improved leaf quality in tobacco, improved fiber strenth in *Gossypium. hirsutum* from lintless *G. thurberri*.

Genes for apomixis have been transferd to maize from tripsacum and sugarbeet from wild Beta sp.. Cleistogamy and self sterilty from wild Secale sp. to Secale cereale.

25-30% improvement in yield was achieved in Avena sativa by crossing with A. sterilize.

Cytoplasmic male sterile lines:

Male sterile lines were produced in many crops like tobacco, wheat and rice.

Interspecifc hybrids

The hybrid cotton variety Varalakshmi is a cross between *G. hirsutum* x Sugarcane hybrids, Brassica napus x B. campestries, hybrids, rice variety Co 31 (*Oryza*

sativa x *O. perennis*), ADT 27 from japonica x India are Interspecific crosses. A high proportion of the most popular perennial herbs and shrubs are hybrid in origin.

New crop species

The new crops species like Raphanobrassica and tritikale were produced.

Lecture No 29.

Mutation breeding: mutation – types – mutagens – breeding procedure – applications – achievements – limitations.

Sudden heritable change in a specific character is called mutation such a change may be large or small. The term **mutation** was first coined by **Hugo de vries** (**1901**). He observed sudden heritable change in **Evening prime rose** (*Oenothera lamarkiana*). He called these changes as **mutation** and organism undergone mutation were termed as **Mutants** (bearing mutant gene). The scientific study of mutation was started in 1910 by **Morgan and his workers** in *Drosphila*.

He observed white eyed male among red eyed male individuals. The white eyed male was a mutant.

Mutation occurs frequently in nature and has been reported in many organisms *e.g.* Drosphila, mice, rodents, rabbits, guinea pigs and man

Macro mutation – Large and noticeable mutations. *E.g.* Change in colours, shape *etc*.

Micro mutation – Small and inconspicuous. *E.g*. yield, plant height *etc*.

Kind of mutations:

On the basis of occurrence, degree, origin *etc*., the mutations are classified as.

1. According to types of cells in which mutation occurs.

a) Somatic mutations: Mutations occurring in body cells. These are not transmitted to next generation and hence termed as **non-heritable mutations**.

b) Germinal mutations: Mutations occurring in reproductive cells and such mutations are heritable and passed on to next generation *E.g*. Occurrence of short legged sheep of Ancon breed in a normal one.

2. On the basis of origin (mode of Origin)

a) Spontaneous mutations or Natural Mutations:

The mutations occur naturally. *Eg*. Double petunia -Freaks appearing in a population

b) Induced mutations: Produced artificially in the laboratory.

Muller with X-rays produced mutants in *Drosophilla*.

3. Based on the nature of their effect.

a) Biochemical mutations: Mutations which bring about radial changes in biochemical constitution.

b) Spurious mutations: Mutations which remain suppressed but express in the offsprings as a result of crossing over. If crossing over does not occur they remain concealed. *E.g*. Pink eye colour in *Drosophila*.

4. Mutations based on their directions

a) Forward mutation: Development of a new mutant type from a wild type (normal type).

b) Reverse mutation - Back mutation: Mutants revert to normal type

5. Based on type of chromosomes.

a) Autosomal: mutation occuring in autosomes

b) Sex linked mutations - mutations occuring in sex chromosomes.

6. Based on stages of occurrence:

a) Gametic mutation: Mutation occurs during gamete formation.

b) Zygotic mutation: Occur during first or later mitotic divisions in a zygote. This results in the development of mutant characters only in the cells which are involved in the process. Here a mosaic organism is formed.

7. Based on affecting factors:

a) Endogenous mutation: Caused by certain internal factors like change in metabolism, nutrition *etc*.

b) Exogenous mutation: Caused by external factors like change in temperature, climate etc.

8. **Nature of mutations**: They may be

a) Gene mutation: (Point mutation) a change in the DNA molecule of an individual gene

b) Chromosomal aberration: Due to changes in the structure of chromosome.

c) Chromosomal variation: Change in number of chromosomes per cell.

Point mutation

Point mutation: DNA is a chemical molecule of heredity and has information coded in terms of four letter alphabets, A, G, C and T. (Adenine, Guanine, Cytosine, Thiamine). Its coded information are duplicated and transmitted during inheritance.

Transcribed and translated during development. During replications or transcription some errors may occur in exact copying of the codes leads to change in a very small segment of DNA molecule (*i.e*. single nucleotide - **muton** or nucleotides). Ultimately it produced an altered phenotype of the affected organism. Such mutations which include very limited segment of DNA are called as point mutations.

Muton*: The unit of mutation, the smallest unit gene (DNA) capable of undergoing mutation, represented by one nucleotide*

Normal sequence: C A T, C A T, C A T, C A T < A -------

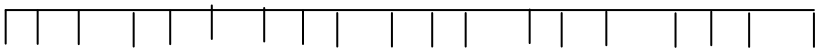
Sequence after removal of one base: C A T, T A C, A T C, A T C < A ---------

T

Addition of one base : C A T, C↓T A, T C A, T C A, T C A –

Frame shift mutations

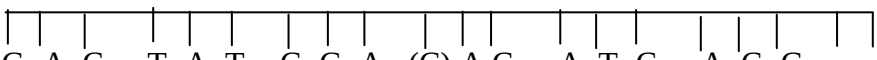
Mutations arise from the insertion (+) or deletion (-) of a nucleotide or nucleotides into or from DNA molecule. Frame shift mutations displace the starting point of genetic transcription of the genetic cell and resulting mRNA is misread by the translation process from the point of nucleotide addition or deletion. Thus once frame shift mutation introduced into a gene the reading frame is shifted. So that all codons distal to the mutations are read out of phase.



Normal sequence of nucleotide G A C T A T C G A A C A T C A C G A T

1 2 3 4 5 6

(Amino acids are symbolised 1,2,3)



Insertion of single nucleotide A T (C) resulting in mutant nucleotide sequence

G A C T A T C G A (C) A C A T C A C G

1 2 3 (10) (8) (7)

Deletion of single	G A C	A T C	G A A	C A T
nucleotide (T)	1	8	9	11

Codons which resulted often frame shift mutations cell into three categories..

1. Sense codons: Which are read or translated the same as before frame shift mutation.
2. Missense codons: Which code for a different amino acid.
3. Nonsense codons code for no amino acid.

Tautomerism

When a molecule is able to exist in more than one chemical form, it is called **tautomeric** and the phenomenon is known as **tautomerism.** Radiation may provide the energy for the formation of tautomeric forms.

Normally

Adenine : Links with **Thyamine**

Guanine : Links with **Cytosine**.

These are the base paring found in DNA molecule, But due to tautomerism an unusual base pairing.

such as **A - C**

G – T may result

Such unusual base paring always, cause changes in the character of the progeny.

Bud mutation

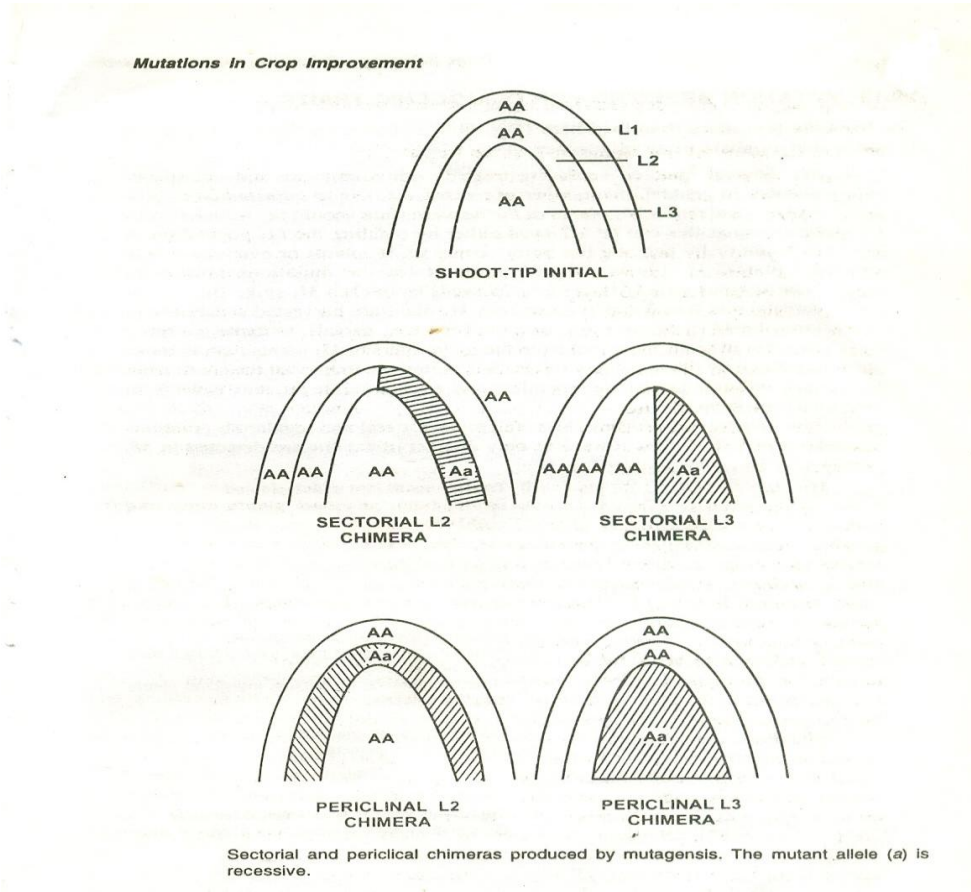
If the mutation occurs in the meristematic in the early stages of bud development, all the cells of the bud will be mutant in nature, to the shoot developed from such bud will be a mutant one. This type of mutation will be called as bud sport.

If a mutation occurs in the last stages of bud development, only some of the cells of the bud will be mutant in nature. A plant which has genetically distinct tissues lying adjacent to one another is called a **chimera**

Chimeras classified into 3 types.

1. **Periclinal chimera**: Mutant and non-mutant tissues are in concentric layers one overlapping the other. They can be perpetuated by vegetative propagation.
2. **Sectorial chimera**: A segment of mutant tissue extending from the epidermis towards the centre.

3. **Mericlinal**: It is incomplete periclinal chimera. The mutant tissues partly surrounds the other.



Sectorial and periclical chimeras produced by mutagensis. The mutant allele (*a*) is recessive.

MUTATION BREEDING

Mutagenic agents

Physical — Chemical

Ionizing radiation — Non ionizing radiation

Particulate — Non- Particulate — (U.V.rays)

Alkylating

Radiation — Radiation — MMS

EMS

Alpha rays — X rays — DES

1.

Fast neutrons Gamma rays. 2. Acridine dyes

Ethidium bromide

3. Base analogues

Bromo uracil

4. Others - Nitrous acid, Sodium azide

Method of mutation Breeding :

1. **Objective of the programme** : Define the objective, the handling of treated population will differ. For e.g. for characters governed by oligogenes the selection can be made in M_3 generation while for traits governed by poly genes we can make selection in M_3 or M_4 generations.

2. Selection of the variety :

Usually the locally adapted best variety will be selected. For e.g. for induction of male sterility in Redgram, locally adapted short duration Co5 is to be selected. But this may not be Universal rule. For e.g. to breed alternate dwarfing gene in rice, low yielding tall *indicas* may be subjected to mutagenic treatments.

3. Part of the plant to be treated.

Depending on mode of reproduction of the crop the plant part to be treated will be decided.

I. Sexually propagated - 1. Seeds.

a) Dry. b) Soaked

2. Pollen grain - limited use

II. Asexually propagated - cutting, tuber, bulbs etc.

4. Dose of the mutagen :

Beneficial mutants will be obtained around LD_{50} dose. So go thro' previous literature and fix. After fixing LD 50 for a particular variety, the regular dose can be fixed.

5. Handling of M_1 generation :

The treated seeds are sown in individual plots treatment wise along with the untreated check. Seeds are sown on single seed per hill. In case of clonally propagated crops, Dominant mutants may be observed in M_1 generation. Whereas in Seed crops most of the mutant alleles will be recessive. The M_1 plants are to be harvested on single plant basis.

Observations to be recorded :

a) Germination and survival

b) Observing on chimeras if any.

c) Dominant mutants if any.

d) Biometrical observations on selected plants

6. M_2 generation :

For raising M_2 generation depending upon the availability of area we can limit the number of M_3 plants to be advanced. If the treatment is only one dose we can carry forward all the M_1 plants on single plant basis to M_2. On the other hand if the treatments are more we can limit the number of single plants to be carried forward to M_2. For e.g. 20 plants from each treatment can be selected at random from M_1 and carried forward to M_2 generation.

Immediately after germination observation on chlorophyll mutants is to be recorded to workout the mutation rate. The chlorophyll mutants may be in the form of Albino, Xantha, Viridis.

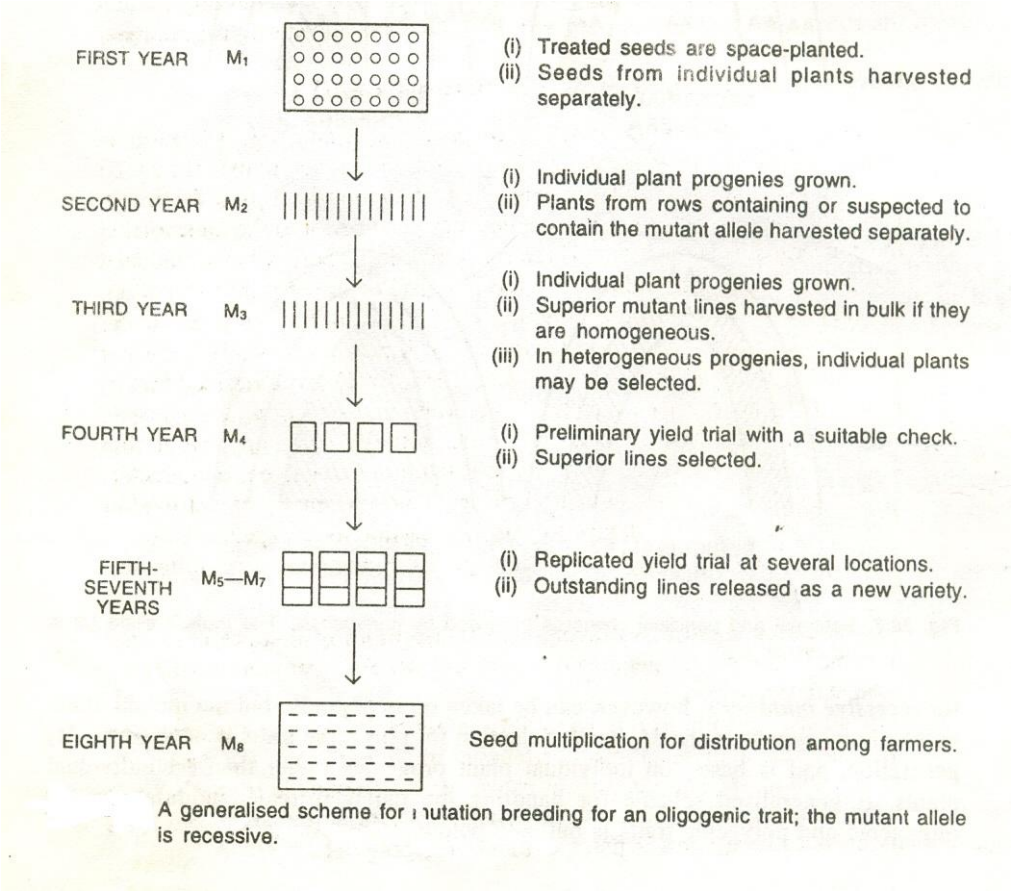
Each and every single Plant in M_2 is to be examined for detection of morphological deviants. (Macro mutants). These macro mutants are to be harvested on single plant basis and beneficial ones are to be utilized in breeding programmes and others can be included in germplasm.

The harvest of M_2 is done on single plant basis. The suspected mutant plants will be harvested on single plant basis and carried forward to M_3 generation.

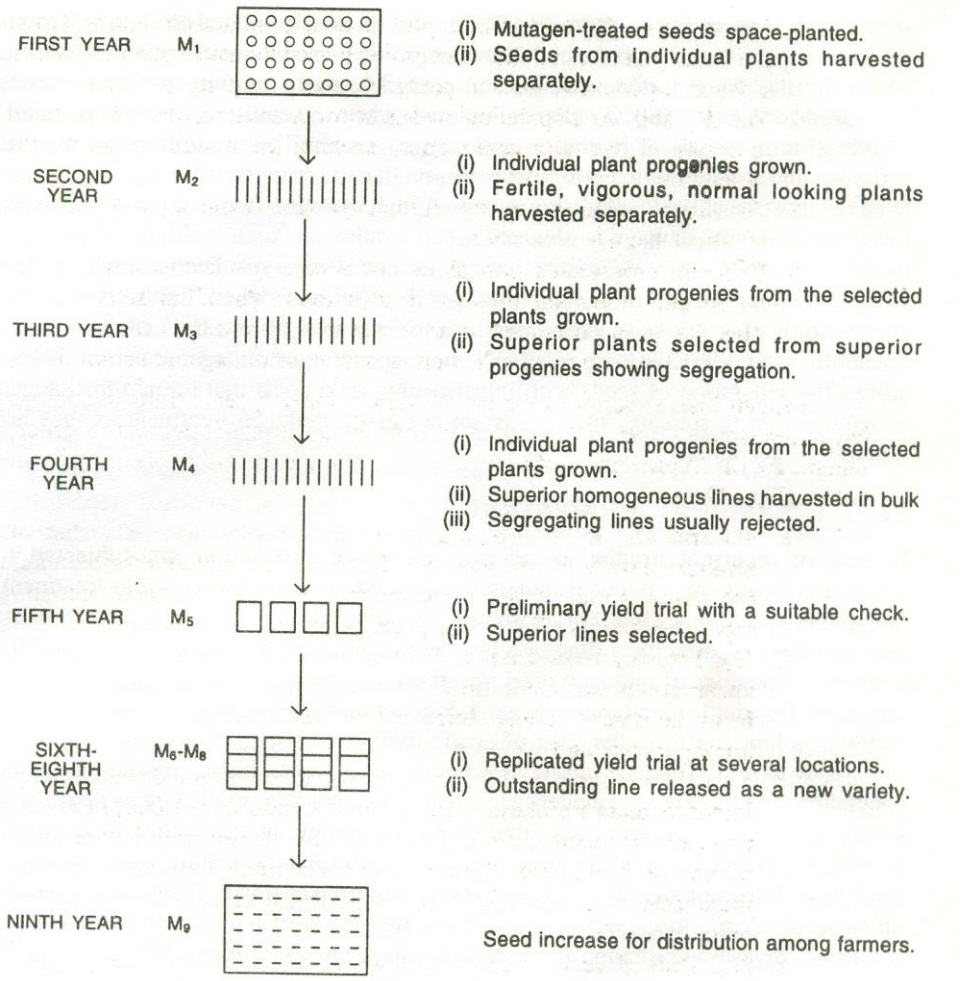
M_3 generation :

Progenies are raised on single plant basis. For characters governed by oligogenes. Progeny rows exhibiting homozygosity may be harvested as bulk and carried forward to M_4 generation to conduct PYT.

For quantitative characters, selection can be postponed to M_4 generation if the lines are not homozygous. After selecting lines exhibiting homozygosity in M_4 those can be carried forward as bulk to M_5 for conducting preliminary yield trials.



A generalised scheme for mutation breeding for an oligogenic trait; the mutant allele is recessive.



A generalised scheme for mutation breeding for polygenic traits.

Uses of mutation breeding.

i) New genotypes that are not present in germplasm can be created artificially.

ii) Specific characters can be improved in a variety which may be either qualitative or quantitative.

iii) F_1 can be irradiated to increase the variability further. This may be useful to break the linkage groups.

iv) Irradiation of interspecific hybrids may be done to induce beneficial translocations.

v) For induction of male sterility induced mutagenesis can be used.

Limitations :

i) Hit or miss method.

ii) Large populations are to be screened in M_2 generation. Each and every single plant is to be observed which is laborious.

iii) Desirable mutants may be associated with other undesirable traits.

iv) Most of the mutants are recessive. Recessive mutants cannot be identified in clonally propagated crops. In case of polyploids larger population is to be studied to find out recessive mutants.

Varieties released :

Castor : Aruna

Rice : Jagannath

Groundnut : Co2

Red gram : Co5

Lablab : Co10

Cotton : MCU 10.

Black gram : Co 4

KINDS OF MUTATIONS

1. Gene mutation or Point mutation

Mutations produced by changes in the base sequences of genes are known as gene or point mutations. Gene mutations can be easily and clearly detected by fine genetic analysis technique available with microorganisms.

2. **Chromosomal mutations** :

Mutations may cause changes in the structure of chromosome or even in chromosome number. Gross chromosomal changes. e.g. changes in chromosome number, translocations, inversions, large deletions and duplications are detectable cytologically under the microscope, but small deleterious duplications can rarely be detected.

3. **Cytoplasmic mutations** : When the mutant character shows cytoplasmic or extra nuclear inheritance, it is known as cytoplasmic mutations.

4. **Bud mutations or Somatic mutations**

Mutations occurring in buds or somatic tissues *i.e.* in clonal crops. Bud mutations in clonaly propagated crops depend on dominant mutations. Recessive mutations may also be utilised provided the clone used for mutagen treatment was heterozygous for the gene in question.

5. **Reverse mutation**:

Due to induced mutagensis in an organism it may revert back to original form. For example a dwarf plant would have obtained by natural mutation, when it is subjected to induced mutagenesis it may revert back to original tall plant. This is known as reverse mutation.

Lecture No 30.

Somaclonal variation - utilization in crop improvement; *In vitro* selection techniques — Use of doubled haploids in crop improvement.

Somaclonal Variation and Crop Improvement

Larkin and Scowcroft (1981) proposed the term somaclone to describe the plants originating from any type of tissue culture. Genetic variation (Genotypic and Phenotypic Variability) found to occur between somaclones in plant tissue cultures was then called somaclonal variation. This variation includes aneuploids, sterile plants and morphological variants, sometimes involving traits of economic importance in case of crop plants. The usefulness of variation was first demonsrated through the recovery of disease resistant plants in potatao (resistance against late blight and early blight) and sugarcane (resistance against eye-spot disease, Fiji disease and downy mildew)

Genetic variation - mutations or other changes in the DNA of the tissue those are heritable .This is only transmitted to the next generation and is thus important for crop improvement. Therefore it is necessary to study the transmission of variation to sexual progeny to facilitate the estimation of its utility for improvement of a sexually propagated crop. In several crops R_0, R_1 and R_2 progeneies were analysed for genetic analyses and 3:1 segregation leading to the isolation of true breeding variants was observed.

Epigenetic variation- non-heritable phenotypic variation. Epigenetic changes can be temporary and are ultimately reversible. However, they may also persist through the life of the regenerated plant.

Physiological variation- emporary in response to stimulus and disappear when it is removed.

Causes for variation

Changes of mother plant origin

Chimeral rearrangement of tissue layers Many horticultural plants are periclinal chimeras, that is, the genetic composition of each concentric cell layer (LI, LII, LIII) in the tunica of the meristematic tissues is different. These layers can be rearranged during rapid cellular proliferation. Therefore, regenerated plants may contain a different chimeral composition or may no longer be chimera at all. Cell variation also occurs if

callus is initiated from explants containing differentiated and matured tissue s that have specialized function.

Explant derived variation

The most stable cultures are obtained from meristematic tissue of a mature plant or tissues of a very young organ of meristematic nature.Polyploid cells can give more variability than diploids

Genetic changes arising in culture

Ploidy Changes: Three phenomena that occur during mitosis lead to most changes in ploidy:

endomitosis (sister chromatids separate within the nuclear membrane, but there is no spindle formation for cytoplasmic division)

endoreduplication (chromosomes at interphase undergo extra duplications)

spindle fusion (giving binucleate or multinucleate cells).

Gross structural rearrangements appear to be a major cause of somaclonal variation. These involve large segments of chromosomes and so may affect several genes at a time. Deletions (genes missing, for example 1,2,3,4 now 1,2,4)

Inversions (gene order altered, for example 1,2,3,4 now 1,3,2,4)

Duplications (1,2,3,4 now 1,2,2,3,4)

Translocations (whole chromosomal segments moved to a new location, for example 1,2,3,4 now 1,2,3,4,A,B,C).

Transposable elements are segments of DNA that are mobile and can insert into coding regions of genes, typically resulting in a lack of expression of the gene. The culture environment may make the transposable elements more likely to excise and move.

Point mutations (the change of a single DNA base), if they take place within a coding region of a gene and result in the alteration of an amino acid, can lead to somaclonal variation. Point mutations are often spontaneous and are more difficult to detect. Note that they result in single gene changes

Structural changes in the DNA sequence

Chromosomal rearrangements, point mutations, or transposition of transposable elements can occur during culture. These changes can occur spontaneously or can be induced with chemicals or radiation

DNA methylation: Most of the mutational events occasioned by tissue culture are directly or indirectly related to alterations in the state of DNA methylation. A decrease in methylation correlates with increased gene activity

Lack of nucleic acid precursors: Shortage of the precursor necessary for rapid nucleic acid biosynthesis, which occurs in many tissue cultures

Growth regulators: One of the triggers of polyploidy *in vitro* is growth regulators; both kinetin and 2,4-D have been implicated.

Composition of culture medium: The level of KNO3 influence the albino plants from wheat cultures. Level of organic N2, chelating agents and other micro nutrients are other factors.

Culture conditions: Temperature, Method of culture

Effect of the genotype

Effects of the culture process itself (lengthy culture periods, growth and other aspects of the culture medium may also affect the ploidy of the cultured cells. Medium that places cells under nutrient limitation will favor the development of "abnormal" cells. Chromosomal alterations, like ploidy changes, increase with increased lengths of culture. In mixed populations of cells with different ploidys, diploid cells retain their organogenic potential better than polyploid and aneuploid cells (probably due to an enhanced ability to form meristems).

One common alteration seen in plants produced through tissue culture is *rejuvenation*, especially in woody species. Rejuvenation may lead to changes in morphology, earlier flowering, improved adventitious root formation, and/or increased vigor.

Selection of somaclonal variants on subjecting the cells to selection pressure

Selection	**Selection of cells in the presence of**
Resistance to herbicide	- Herbicide
Resistance to salt	- Sodium chloride / Aluminium
Resistance to drought	- PEG / Mannitol
Resistance to frost	- Hydroxy proline resistant lines
Resistance to pathogens	- Pathotoxin / Culture filtrat

Lecture No 31

Introduction to markers – morphological – biochemical- DNA markers – uses of marker assisted selection - major genes – merits – demerits – achievements

Molecular markers consist of specific detectable molecules which show easily difference among different species. A readily detectable sequence of DNA or protein whose inheritance can be monitored.

There are several DNA markers which are used plant breeding. The commonly used markers are

- Restriction Fragment Length Polymorphism (RFLP)
- Randomly Amplified Polymorphic DNA (RAPD)
- Sequenced Tagged Sites (STS)
- Sequence Characterised Amplified Regions (SCAR)
- Variable Number Tandem Repeats (VNTR)
- *Minisatellites*
- *Microsatellites or Simple Sequence Repeats (SSR)*
- Inter Simple Sequence Repeats (ISSR)
- Amplified Fragment Length Polymorphism (AFLP)

Constraints in using morphological markers

- Less in number
- Confer indistinguishable phenotypes
- Influenced by the environment
- Influenced by the genetic background
- Influenced by the ontogeny
- No stable inheritance

Properties of DNA markers

- Abundant
- Ubiquitous

- Highly polymorphic
- Stable inheritance
- No environmental influence
- No influence of ontogeny of individual
- Codominant or dominant

RFLP

Restriction Fragment Length Polymorphism analysis refers to variation found with in a species in the length of DNA fragment generated by a specific endonuclease. Polymorphism is detected based on the differential hybridization of cloned DNA to DNA fragments in a sample of restriction enzyme digested DNAs. A typical RFLP assay has the following procedure: (i) Digestion of plant genomic DNA with restriction endonucleases (s) ; (ii) Separation of DNA fragments via electrophoresis and transfer to membranes using Southern blotting; (iii) Membranes exposed to probes labeled with radioactive isotopes via southern hybridization; and (iv) Detection of polymorphism using x-ray autoradiography or chemiluminescent technique. Polymorphisms in this assay occur because the sequence of the probe may be homologous to restriction fragments of different sizes in different genotypes.

Strengths

- RFLPs have good repeatability
- Particularly useful in comparative genome mapping

Constraints

- The assay is tedious and time-consuming
- Requires large quantities of DNA
- Extremely limited utility in marker-assisted selection due to very low assay-efficiency.

RAPD

The Random Amplified Polymorphic DNA markers are based on the differential

PCR amplification of a sample of DNAs from short oligonucleotide sequences (Williams *et al*. 1990; Welsh and McClelland, 1990), and are genetically dominant in nature. Polymorphisms found within a species in the randomly amplified fragments of DNA generated by restriction endonuclease. For amplification products to occur, the binding must be to inverted repeats sequences generally 150-400 base pairs apart. Number of amplification products is directly related to the number and orientation of the sequences that are complementary to the primer in the genome.

Strengths

- Requires small quantities of DNA
- Needs limited investment in time and training
- Sets of several hundred primers are commercially available

Constraints

- Lack of reproducibility in marker patterns across labs and across experiments, as the assay is sensitive to variation in DNA concentration, optimal primer concentration, and thermal cycling conditions.
- Inability to discern differences in sequence homology among similarly-sized fragments.

SCAR & STS

These PCR based markers are derived by sequencing the termini of RFLP, RAPD or AFLP fragments or known genes. Sequence Chararcterized Amplified Region (SCAR) primers are usually 18-25 nucleotides in length. Reproducibility and utility of SCARs is much greater than RAPDs. Although SCARs are genetically dominant, they can be converted as codominant markers by digestion with restriction enzymes. The Sequence Tagged Site (STS) markers are generally mapped, co dominant, and show stable amplification and god repeatability. STS assay is easy to adopt and amenable for automation. The major constraint is that not many polymorphic STSs are currently available in crop plants.

SSR

Microsatellite markers, also known as simple sequence repeats (SSRs),

short tandem repeats (STRs) or simple sequence length polymorphisms (SSLPs), are tandem repeats of mono, di, tri, tetra, or penta-nucleotide units dispersed throughout the genomes of most eukaryotic organisms. SSRs have been recently characterized in many crop species including maize, rice, grapevine, soybean, Brassica, barley and tomato (Gupta *et al*., 1996; Powell *et al*., 1996a,b). SSR primer pairs (forward and reverse) are based on conserved flanking regions. The primers are usually18-25 bp in length, and the SSR polymorphisms are usually based on variation in the number of specific repeat units at a locus.

Strengths:

- Abundant and uniformly distributed in the genome.
- Hypervariable (large number of alleles per locus)
- Codominant markers with known genomic locations
- Highly reliable and reproducible assay.
- Powerful tools in genotype differentiation, seed purity evaluation, making, marker-assisted selection, population genetic studies, and genetic diversity analysis.

Constraints

- Expensive and time-consuming to detect SSR loci and design primers (in many crop plants, such as maize, rice and wheat, a large number of SSR primers are available in public domain)
- Not available for all plant species; Primers usually species-specific.

AFLP

AFLP is the one of the more recent molecular marker systems (Vos *et al*., 1995). The AFLP loci are generated using a procedure that combines restriction digestion and PCR amplification. The basic procedure is as follows : (i) Digestion of genomic DNA with a combination of two restriction enzymes-a rare cutter and a frequent cutter (for example, EcoRI/Msel, EcoRI/Pstl); (ii) Ligation of double-stranded adapters to cut ends of DNA fragments by ligation; (iii) Preselective amplification using primers with a single selective nucleotide; (iv) use of amplified products as templates for

selective amplification using labeled primers having longer selective extensions; (v) Separation of the amplified fragments via electrophoresis; and (vi) visualization using autoradiography or silver staining.

Strengths

- Stable amplification and high repeatability.
- Hypervariability, coupled with generation of a larger number of mappable loci with a single amplification (high assay efficiency) – facilitates saturation of a region of the genome rather quickly**.**
- Provides raw materials for STS derivation
- Can generate fingerprints of any DNA regardless of their origin or complexity
- Can act as bridge between genetic and physical maps

Constraints

- Time consuming procedure
- Requires significant technical skills and financial resources

EST

Expressed sequence tags (EST) are subsets of STSs derived from cDNA clones. ESTs can serve the same purpose as the random STSs, with the advantage that ESTs, are derived from expressed genes, that is, from spliced mRNA which is usually free of introns as well as repetitive DNA. A large number of ESTs have already been detected in plants, such as rice and maize, with the availability of a large amount of cDNA sequence data in a relatively short time. Polymorphic ESTs will be increasingly available and used more widely than at present.

Strengths

- Represent real functional genes; therefore, more useful as genetic markers than anonymous nonfunctional sequences.
- Advantages for comparative genome analysis and gain of information on genome structure.

Constraints

- High development/start-up costs
- Available at present in a very limited number of crop plants.

SNPs

Single nucleotide polymorphisms (SNPs) can be considered as 'third generation markers'. These are point mutations in which one nucleotide is substituted for another at a particular locus. SNPs are the most common type of sequence differences between alleles, are codominant in nature, and represent an inexhaustible source of polymorphic markers for use in high resolution genetic mapping of traits. Detection of the codominant SNPs is based on DNA amplification using primers based on known sequence information for specific genes. SNP assays can be carried out in plants, such as rice and maize, where genomics is either well advanced or is progressing at a rapid pace.

Use of EST sequences has proved to be useful for discovery of SNPs in plants such as maize because of the high rate of SNP polymorphism found in it. Thus, for some species, pre-screening of amplicons may be necessary to determine whether sufficient polymorphism exists to justify further screening for SNPs. Denaturing high-pressure liquid chromatography (dHPLC), single strand conformational polymorphism (SSCP), or various chemical or enzymatic cleavage methods may be used for pre-screening. There are many commercially available assays for SNP genotyping; but none has yet emerged as a dominant leader for this application.

Strengths

- Easier to work with than SSRs or AFLPs
- Most useful when several SNP loci are closely positioned and allow haplotype definition, and development of haplotype tags.
- Serve to integrate physical and genetic maps.

Constraints

- Requirement for sequence information for the genes
- High development / start-up costs

Detection of DNA polymorphisms

Gel electrophoresis is most widely adapted technique for detecting polymorphism. For RFLP and RAPD procedures, agarose is the polymer of choose. Because microsatellites and AFLPs procedures generate smaller fragments for comparison. PAGE is typically used, followed by detection of bands through silver staining procedure (for SSRs) or by autoradiography if radiolabeled isotypes are used, as is often the case in AFLP assay.

Laser technology has also been applied to the microsatellite marker system. When using this approach, the primer is labeled with a fluorescent dye. Samples are then separated in a polyacrylamide gel. As the samples flow through the bottom of the gel, fragments are detected by a laser that detects the presence of the fluorescent dye. Computer programmes output data in a form that can be analyzed. With the advent of the genomic era, techniques that rapidly screen large numbers of samples have also emerged.

Choice of a Molecular Marker System

Potential users should recognize that development and applications of molecular markers is a rapidly evolving field in which technology is advancing quite fast. It is important, therefore, to select suitable technique (s) based on the objectives of the experiment, resolution required and operational constraints, if any. The main criteria are degree of polymorphism, and reproducibility and repeatability of the marker data.

As highlighted earlier, various marker systems have different strengths and constraints. However, SSRs and AFLPs offer distinct advantages over RAPDs and RFLPs, and are widely preferred currently for fingerprinting, genetic diversity analysis and mapping experiments in various crop species.

General Applications of DNA markers

- Diversity analysis at molecular level to characterize the germplasm entries
- Markers aided selection for pest resistance in crop improvements
- DNA finger printing of crop species from different geographical regions
- To establish phylogenetic and taxonomic relationship among individuals
- Tagging of major and minor QTLs

- Physical mapping and map based cloning of genes for producing transgenic organisam

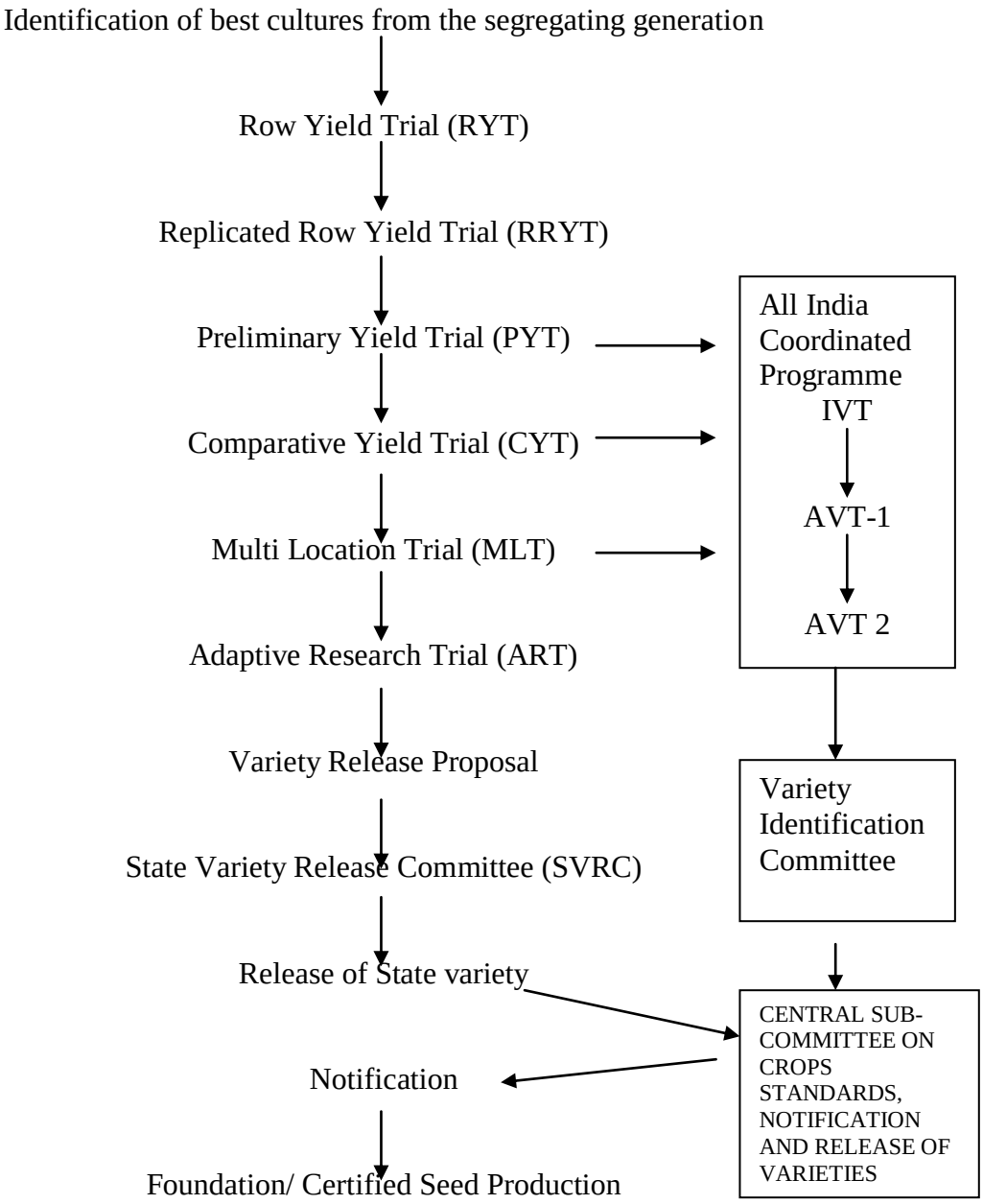
DNA markers are used for evaluation of germplasm in four main ways

- Identification of germplasm
- Screening of duplicates
- Assessment of genetic diversity
- Monitoring the genetic stability of conserved germplasm.

Lecture No 32.

Types of cultivars – procedure for release of new varieties – stages in seed multiplication – seed certification and TC plants certification.

PROCEDURE FOR RELEASE OF A VARIETY



After identification of the best cultures from the segregating generation or any other source it has to undergo the following trials.

1. Row yield trial (RYT)

For every 10 row, there will be a check entry and the trial will be non replicated.

2. Replicated row yield trail (RRYT)

From the row yield trial, the best cultures will be tested in RRYT along with appropriate check. The best entries from RRYT will be carried forward to preliminary yield trial.

3. Preliminary yield trial (PYT)

Replicated trial conducted with appropriate checks. PYT will be conducted normally for two seasons. While conducting, PYT, the best entries will be nominated to All India trials also. Screening for biotic and abiotic stresses will be done during PYT stage. The best entry will be carried to comparative yield trial. The entries entered into All India trial will be given project number. For *E*.g. sorghum entry will be given SPV (Sorghum Project Variety). Rice - IET (Initial Evaluation Trial), *etc*.

4. Comparative Yield trial (CYT)

CYT is replicated one conducted with more than one check. The trial will be repeated for 3 seasons. The entry proved to be superior in all the 3 seasons will be proposed for multilocation trial (MLT).

5. Multilocation trial (MLT)

The entries for MLT will be decided at Crop scientists meet held once in a year. Each station will propose its own entry. Based on discussion of merits and demerits of each culture, the entries will be nominated. The MLT will be conducted at Research Stations of TNAU spread over the State. The best entries will be proposed for Adaptive Research Trial (ART).

6. Adaptive Research Trial (ART)

ART will be conducted at farmers' field by the Agricultural Department Staff. The entries for ART will be decided during Scientific Workers Conference (SWC) which will be held once in a year at TNAU. Both scientists of TNAU and Agri. Dept. Staff will participate. At SWC, the entries will be fixed and each Joint Director of Agriculture will fix number of trials for his district. The entries performing well in ART will be proposed for release as a variety. Each culture has to be tested at least 40 trials, if the variety is to be considered for location specific. Other wise 120 trials are needed. If a culture is non season bound, it will be tested in all the three seasons. If it is not so, one or two seasons result is enough. If sufficient data is not available, the same entry will be tested for more than one year based on the recommendations of the Crop Scientist Meet of the University.

7. Variety Release Proposal

The scientist incharge of the culture will propose the culture for release as a variety. There is a proforma for variety release. This proforma will contain all the information about the culture *viz*., parentage, parent's morphology, cultures morphology,

key characters of the culture for identification, agronomic practices, pest and disease resistance, quality characters and yield trial results.

The variety release proposal will be screened at University Variety Identification Committee. The committee consist of various technical directors under the chairmanship of Director of Research. After approval, the proposal will be presented before Variety Release Committee at the state level.

8. Variety release committee

It will be headed by Commissioner and Secretary, Agrl. Dept. members will be Director of Agriculture Joint Directors of Agriculture and TNAU scientists. Besides these, two leading farmers of the state will also be the members. After discussion, based on merit the VRC will approve it for release. Then the culture will be released for general cultivation.

9. Notification of the variety

For certified seed production, the variety is to be notified by the central variety release committee, Delhi. After release of the variety for notification purpose the information will be furnished in the prescribed proforma. At that time details about All India trial will also be furnished. The culture or parents of the hybrid need to be registered with NBPGR. NBPGR will allot a specific identification number for each culture. This will help to protect the rights of the breeder and the institution. After notification only, a variety can be multiplied under certified seed production.

Procedure for Notification of Varieties

Steps

1. Evaluation
2. Identification
3. Release and Notification

Evaluation

Consists of various trials and tests to determine its superiority over the best existing variety in yield, agronomic traits and its suitability for consumption

Identification

Outstanding cultures are identified for release as new varieties at the Annual workshops of the coordinated projects on the respective crops. Proposals for the identification of cultures may be prepared by the respective breeder in a prescribed format.

Proposals should consist of information on the results of the various centres of AICRP for at least two years, pest and disease reactions and quality parameters. The proposals will be examined by an Identification committee. The criteria for identification as variety may vary form one crop the other. Culture after consideration called as Identified variety

Release and Notification

After identification, the variety is to be tested for at least one year for disease and quality tests. The breeder should submit a proposal for release as a new variety for approval by the Central Sub-Committee on Crop Standards, Notification and release of varieties. After a variety has been released for a zone by the Central Sub-Committee, the Director, HYV, Ministry of Agriculture and Irrigation, GOI notifies the concerned authorities of the states within that zone for seed multiplication and distribution of variety. This is known as notification of variety.

Release of a variety by a State Variety Release Committee

The breeder concerned should submit Variety Release Proposal (12 copies) in the prescribed format to the Director of Research, TNAU, Coimbatore. They will initially scrutinize the proposals. After scrutinization, it will be examined by State Variety Release Committee consists of the following members

S.No.	Officials	Position
1	Secretary to Government of Tamil Nadu, Agrl. Department	Chairman
2	Vice-Chancellor, TNAU, Coimbatore	Member
3	Director of Agriculture	Member
4	Chief Engineer (Agrl.Engg.)	Member
5	Director of Seed Certification, Coimbatore	Member
6	Professor and Head, Dept. of SS&T, TNAU, Coimbatore	Member
7	Joint Director of Horticulture	Member
8	Dean, Faculty of Agriculture, Annamalai University, Chidambaram	Member
Non-official members		
1	Leading farmers – 2 Nos.	
2	President, Tamil Nadu Seed Association, Coimbatore	

The official release of the new varieties will be made by the Hon'ble Minister for Agriculture in Farmers' Day celebration

Notification

The breeder concerned should submit 70 copies of the notification proposal in the proforma to the State Seed Sub-Committee for onward transmission to Central Sub-Committee. Deposition of seed material to Gene Bank, NBPGR, New Delhi is a pre requisite. The State Seed Sub-Committee is to be constituted by the Central Committee, Ministry of Agriculture, GOI, New Delhi which consists of the following members

S.No.	Officials	Position
1	Secretary to Government of Tamil Nadu, Agrl. Department	Chairman
2	Director of Agriculture	Member
3	Director, CPBG, TNAU, Coimbatore	Member
4	Director of Seed Certification, Cbe	Member
5	Addl. Director of Agriculture (Input)	Member
6	Seed Testing Officer, Cbe.	Member

7	Regl. Manager, NSC, Ambattur	Member
8	Director, SFC, Chengam	Member
9	E.I.D. Parry Ltd., Chennai	Member
10	TUCAS, Coimbatore	Member
11	Leading Farmers – 2 Nos.	Member
12	Secretary, TN Seed Assn. Cbe.	Member
13	JDA, (SSF), DA, Chennai	Co-convener
14	Deputy Commissioner (QC), New Delhi	Co-convener

Lecture No: 33

Maintenance Breeding: General seed production techniques – steps in nucleus and breeder seed production – varietal rundown and renovation.

General principles of Nucleus seed production

Genetic Principles: Genetic purity of a variety can deteriorate due to several factors during production cycles such as developmental variation, mechanical mixture, mutations, natural crossing, minor genetic variations selective influence of diseases and the techniques of plant breeder. The importance safeguards for maintaining the genetic purity during nucleus seed produciton. The steps involved in maintaining the genetic purity during seed production comes under genetic principles.

The important safeguards for maintaining genetic purity during seed production are

1. Control of seed source: The use of seed of an appropriate class and from an approved source is necessary for raising a seed crop. Four classes of seed namely breeders, foundation, registered and certified seed are generally recognized in seed certification. The classes are given below as defined by the Association of Official Seed Certifying Agencies (AOSCA)

Nucleus seed: Two types

Basic Nucleus seed / Primordial seed: The seed of the notified variety collected from the evolving institute with all the pass port information is considered as Basic Nucleus seed. This is cent per cent genetic pure seed with physical purity produced under the direct supervision of the concerned plant breeder.

Nucleus seed for seed multiplication chain: This is also cent percent genetic pure seed with physical purity produced by a plant breeder form basic nucleus seed stock or produce of the progenies of the plants selected from breeder seed production plot.

Breeder's Seed: Breeder seed is the seed or vegetatively propogated material directly controlled by the originating or sponsoring plant breeder of the breeding program or institution. The breeder seed production is personally supervised by a qualified breeder. The BS is the source for the initial and recurring increase of foundation seed. The BSP plot is subjected for inspection by monitoring team consists of a nominee from the Nodal Officer for breeder seed production, Deputy Director of Seed Certification, Area Manager, National Seed Corporation and the Producing breeder. The team will inspect

the crop during flowering and maturity stages. The breeder seed after passing the field and seed standards should be bagged with breeder seed tag containg all details about crop, variety, lot number, date of test, physical and genetic plurity, germination percentage. Grow out test to determine the genetic purity of the seed lot should be conducted for all the breeder seed produced by the breeder.

Foundation seed: Foundation seed is obtained from breeder seed by direct increase. FS is genetically pure and is the source of registered and or certified seed. Production of FS is the responsibility of NSC. FS is produced on government farms, at experiment stations, by agricultural universities or competent seed growers under strict supervision of experts from NSC. These class of seed should be produced in the area of adaptation of the concern variety

Registered Seed : RS is produced from foundation seed or from registered seed. It is generically pure and is used to produce certified seed or registered seed. It is usually produced by progressive farmers according to technical advice and supervision provided by NSC. Often registered seed is omitted and certified seed is produced directly from foundation seed, this is the general practice in India.

Certified Seed: CS is produced form FS. This is so known because it is certified by the State Seed Certification Agency. The certified seeds is usually produced by progressive farmers according to standard seed production practice. To be certified the seed must meet the prescribed requirements regarding purity and quality. These standards vary from one crop to another. Certified seed is available from general distribution to farmers for commercial crop production. Its production is generally supervised by State Seeds Corporation but NSC also undertakes the supervision of certified seed production if required.

ISOLATION: The crop raised for seed production should be separated from other fields of the same crop species by a minimum distance is called isolation distance. Isolation is essential to prevent pollination from unwanted pollen in the case of cross pollinated and often cross pollinated species to avoid mechanical mixture and chance cross pollination in self pollinated crops. The isolation distance varies from 3m in self pollinated crops like rice, wheat to 200m in maize etc. It also varies with the type of seeds. In some cases like

hybrid maize, the minimum isolation distance may be considerably reduced by planting border rows of pollinator parent and by choosing a large field for seed production.

Grow out tests:

The tests are much useful in self pollinated than in CSP. The authentic sample is planted after every 10 test samples for close comparison. Observations are made both on qualitative and quantitative traits of the test and authentic sample plots during the entire growing period. The frequency of various off types is recorded. This test is used to observe the genetic purity of the crop. Though it is more precise it is rarely used as it requires a much longer time, an excellent green house facility.

Agronomic Principles

1. Land requirement

a) Selection of suitable agro climatic region: The variety to be grown for seed production must be adopted to photoperiod and temperature, conditions prevailing in that area. Regions of moderate rainfall and humidity are more suited for seed production. Most crops require a dry sunny period and moderate temperature for flowering and pollination. Excessive dew & rainfall lead to poor seed set, pest & disease incidence, heavy seed loss & lodging at harvest. High temperature results in pollen desiccation at flowering, premature flowering and poor quality seeds.

b) Selection of seed plot: plot must has the following characters

-Good soil texture & fertility

-It should be free from volunteer plants & weed plants

-Free from soil borne diseases & insect pests.

-The plot must not have the same crop in the previous season also

-Well leveled field with isolation.

c) Preparation of land: It must be prepared well to suit the requirement of the crop improved germination, good land establishment and destruction of potential weeds.

Cultural practices

a) Time of planting

b) Lower seed rate than for raising commercial crops

c) Method of sowing - line sowing for varieties, for hybrids male and female rows in definite proportion

d) Seed treatment, depth of sowing

e) Recommended doe of fertilizers and irrigation must be given to get higher yield

Plant protection:

Insect pests and diseases may cause considerable damage to the crop reducing the yield and quality of seed. Incidence of some diseases may lead to the rejection of seed by the certifying agency as unfit for use. Ex. Maximum permissible limit for Head smut in sorghum is 1/1000. So plant protection should be given

Weed control:

Effective weed control is a must for good seed production. Weeds reduce crop yield and their seeds contaminate the crop seed. It acts as host for number of pathogens. Some weeds are classified as noxious or object ional weeds . Their seeds are similar to and very difficult to separate from those of the concerned crops. They are difficult to eradicate once they infest a field and act as host of disease or pest.

Roguing

Special operations : Seed production may require some special operation ex. Hybrid maize seed production requires detassaling of plants of the female parent before they shed pollen male parents are harvested first. Supplementary pollination by keeping beehives

Harvesting

Considerable care should be taken to prevent mechanical mixture from other crops or weed seeds. Threshing floor should be clean, while threshing - damage to seeds.

Seed processing:

Drying: appropriate moisture level to facilitate processing to prevent losses in germination and to reduce the chances of insect attack during storage

i) Natural drying ii) artificial drying

iii. Heated or unheated air

Cleaning and grading: separation of inert matters weed seeds and seeds of other crops from seed is known as cleaning

1. Specific gravity separators are used based on weight and size of seeds
2. Pneumatic separators : resistance to airflow
3. Spiral separators : Seed shape
4. Velvet roll: surface smoothness
5. Electronic separators: electrical properties
6. Electronic colour separators: Seed colour

Grading: Removal of smaller and shriveled seeds from the well filled healthy seeds . Air and screen machine is used for cleaning and grading

Testing: After cleaning and grading the seed lots are tested for percentage of pure seed, weed seeds, seeds of other crops , inert matter and germination - Seed testing labs.

Treating: Seeds are treated with suitable fungicides

a) helpful in controlling seed borne diseases
b) protects against damage by storage pest

Bagging: Seeds are distributed in bags of appropriate size.

Labelling: It should contain the following details

1) Kind of seed 2) name of variety
3) Purity 4) germination percentage
5) Date of germination test 6) percentage of weed seeds
7) Percentage of inert matter 8) name and address of the seller
9) Period of validity of certification 10) other information pertained to the seed
11) Treated with poison

Breeder seed - Yellow colour tag Foundation seed – White colour tag
Certified seed - Blue colour tag