Practical Manual

on

Tree Improvement

FBT-211 3(2+1)

For B.Sc. Forestry students

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College of Horticulture & Forestry

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Tree Improvement FBT 211 3(2+1)

Practical: Floral biology and phonological observations in some important species. Pollen morphology. Estimation of pollen sterility and viability. Emasculation and hybridization in forest tree species. Different breeding methods – flow chart. Recording observations in provenance trial. Estimation of phenotypic and genotypic coefficient of variation. Estimation of genetic advance, heritability and GCA. Exercise in plus tree selection – recording data – design and observation in teak, eucalyptus seed orchard.

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Practical No. 1

Objective: To study floral biology

Problem: Dissect and display the parts of the given flower, identify different parts of a flower and understand their function.

Material: …….

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1. Write the name of the flower you observed……..

2. Colour of the flower……..

3. Number of calyx…….

4. Number of corolla……..……

5. Number of stamens……..

6. Number of Carpel (pistil)…….

7. Position of ovary……..……

8. Draw diagram of longitudinal section of flower with proper libelling

Practical No. 2

Objective: To study floral biology

Problem: Dissect and display the parts of the given flower, identify different parts of a flower and understand their function.

Material: ……

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1. Write the name of the flower you observed……..……...

2. Colour of the flower……..……..

3. Number of calyx……

4. Number of corolla……..……

5. Number of stamens……..

6. Number of Carpel (pistil)……

7. Position of ovary……..……

8. Draw diagram of longitudinal section of flower with proper libelling

Practical No. 3

Objective: To study of phenology of forest trees

Problem: To understand the phenology and record the phenological observations of given tree species

Material: …….

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1. Observe the phenological events of the given tree species
2. Name of the tree you observed……..……...
3. Write the botanical name of the tree: …….
4. Write its family: ……..
5. Habit of the tree…….
6. Habitat of tree: ……..
7. Period of first bud sprouting……
8. Period of first flower appeared……
9. Period of first fruit appeared……
10. Period of fruit matured……
11. Period of leaf shedding……..……
12. Period of leaf renewal……..…….

Practical No. 4

Objective: To study of phenology of forest trees

Problem: To understand the phenology and record the phenological observations of given tree species

Material: …….

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1. Observe the phenological events of the given tree species
2. Name of the tree you observed……..……...
3. Write the botanical name of the tree: …….
4. Write its family: ……...
5. Habit of the tree…….
6. Habitat of tree: ……..
7. Period of first bud sprouting……
8. Period of first flower appeared……
9. Period of first fruit appeared……
10. Period of fruit matured……
11. Period of leaf shedding……..……
12. Period of leaf renewal……..…….

Practical No. 5

Objective: To study of pollen morphology

Problem: Study pollen morphology and internal structure of pollen

Material: …….

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1. Observe the morphology of pollen grain of the given forest tree……..
2. Write the name of the plant you observed…….
3. Pollen size……..
4. Pollen shape……..
5. Dispersion unit of pollen grain……
6. Polarity……
7. Draw the neat and clean diagram of cross section of pollen grain

Practical No. 6

Objective: To study of pollen morphology

Problem: Study pollen morphology and internal structure of pollen

Material: …….

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1. Observe the morphology of pollen grain of the given forest tree……..
2. Write the name of the plant you observed…….
3. Pollen size……..
4. Pollen shape……..
5. Dispersion unit of pollen grain……
6. Polarity……
7. Draw the neat and clean diagram of cross section of pollen grain

Practical No. 7

Objective: To estimate pollen viability

Problem: How to estimate the viability of pollen

Material: …….

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Conduct pollen viability test in the laboratory and write the **procedure** of pollen viability test

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Observations:

a. Staining pattern in pollen……

b. Mention if any abnormality observed……..

c. Write down no. of viable pollen……..

d. Viability percentage……

e. Write down no. of sterile pollen……..

Practical No. 8

Objective: To study sexual reproduction in forest trees

Problem: To understand different methods of sexual reproduction

Exercise: Observe pollination in the given tree species

Type of pollination……..……

Observe any structural modification in flowers or any mechanism for cross pollination and draw diagram

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Mode of pollination……..……..……..

Practical No. 9

Objective: To study Emasculation of flower in forest tree species

Problem: To understand the method of emasculation flower for hybridization process

Material: ……

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Emasculate the flower of given tree species. Which procedure will you adopt for the emasculation

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Write the detail procedure of emasculation of flower

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Practical No. 10

Objective: To study hybridization in forest tree species

Problem: To learn the technique of hybridization of emasculated flower

Material: …….

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Hybridize the flower of the given forest tree species and write the stages for the hybridization

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Observation:

a. Number of parents selected for crossing…….

b. Number of crosses conducted…….

c. Number of fruit sets out of total crosses…….

Practical No. 11

Objective: To study tree breeding method

Problem: Draw flow diagram of conventional tree breeding methods

2. Describe suitable breeding technique for *Tectona grandis* tree breeding programme

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Practical No. 12

Objective: To study provenance trial

Problem: Identify provenance and plan provenance trial for important tree species

Material: …….

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Write the procedure to establish provenance trial

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Visit to nearby provenance trail and note down following observation

i) Species name:……..……

ii) Year of establishment: ……..…….

ii) Spacing: ……..…….

iii) Objective of provenance trail……..……..

Practical No. 13

Objective: To estimate genotypic coefficient of variation

Problem: Estimate genotypic coefficient of variation from given ANOVA

S. No.	Source	D.F	S.S	M.S	F	Expected mean square
1	Replication	2	12.800	6.400	4.69	
2	Genotype	9	1049.370	116.596	85.54	38.411
3	Error	18	24.534	1.363		1.363

Mean = 40.1

Practical No. 14

Objective: To estimate phenotypic coefficient of variation

Problem: Estimate the phenotypic coefficient of variation

S. No.	Source	D.F	S.S	M.S	F	Expected mean square
1	Replication	2	12.800	6.400	4.69	
2	Genotype	9	1049.370	116.596	85.54	38.411
3	Error	18	24.534	1.363		1.363

Mean = 40.1

Practical No. 15

Objective: To estimate general combining ability and specific combining ability

Problem: Estimate general combining ability (GCA) and specific combining ability (SCA) from given crossing data

Female parent	Male parent				Progeny means
	1	2	3	4	
5	9	17	12	14	13
6	10	16	12	10	12
7	11	20	10	15	14
8	14	15	6	17	12
Progeny means	11	17	30	14	Test mean= 13

Estimate General combining ability of parent 2 and 4

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Estimate Specific Combining Ability for parent 3 with 6, 7 and 8

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Practical No. 16

Objective: To estimate genetic advance and heritability

Problem: Estimate genetic advance and heritability from given ANOVA

S. No.	Source	D.F	S.S	M.S	F
1	Replication	2	3.22	1.6	
2	Treatment	24	2708.59	112.86	58.18
3	Error	48	93.28	1.94	
4	Total	74	2805.09		

Mean =50.76

Estimate genetic advance from the ANOVA given above-

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Estimate Heritability on the basis of above given ANOVA (Selection differential=2.06)

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Practical No. 17

Objective: To study the method for Plus tree selection

Problem: Understand the method of superior tree selection in natural and unimproved plantation

Material: …….

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Select the plus tree of *Tectona grandis* basis comparison method. Also mention growth traits used for identification of candidate tree

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Selection criteria (ideal characteristics) used for plus tree section

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How many candidate trees selected?

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Species composition of selected area

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Base line value used for select candidate tree

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Practical No. 18

Objective: To study seed orchard

Problem: Visit nearby seed orchard and write procedure in detail for establishing seed orchard

Visit to nearby seed orchard and record observations

a. Name of the species……..

b. Seed source……..……

c. Locality of seed orchard……..…..

d. Size of seed orchard……..

e. Longevity of orchard……..

f. Spacing……

e. Pollen dilution zone……..

f. Year of establishment……

g. Experimental design……..……

Procedure: ……

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Appendix

FLORAL BIOLOGY

Flowers are the most complex structures of plants. Many plants (angiosperms) contain flowers where the sex cell is contained for the plant's reproduction. The biological function of a flower is to affect reproduction, usually by providing a mechanism for the union of sperm with eggs. Flowers may facilitate outcrossing (fusion of sperm and eggs from different individuals in a population) resulting from cross pollination or allow selfing (fusion of sperm and egg from the same flower) when self pollination occurs. Some flowers produce diaspores without fertilization (parthenocarpy). Flowers contain sporangia and are the site where gametophytes develop. Many flowers have evolved to be attractive to animals, so as to cause them to be vectors for the transfer of pollen. After fertilization, the ovary of the flower develops into fruit containing seeds.

Floral parts and their function

- **Take a flower of any colour and size growing in your area. Make basic observation of flower:** Flower is born on stalk called pedicle. Pedicle has swollen tip known as thalamus or receptacle on which two whorls (Accessory whorls and reproductive whorls) are born successively to definite order.

1. Accessory whorl:

a. Calyx (Collection of Sepals): The outer green part of flower formed by sepals; whose main function is protection.

b. Corolla (Collection of petals): The inner envelope of leaves of a flower, usually of delicate texture and some colour other than green. The next whorl of variously coloured petals. They help in attracting insect for pollination

2. Reproductive whorls:

a. Androecium: Is the aggregate of stamen *i.e* male reproductive part. A stamen has long stalk called filament. At the top of filament is a cluster of microsporangia called the anther. Anther produces pollen grain for pollination.

b. Gynoecium: Is composed of female reproductive unit called carpels. It consists of pistils or carpels and is typically surrounded by the pollen producing reproductive organ the stamen. It consists of

- Ovary - The structure that encloses the undeveloped seeds of a plant
- Ovules - Female reproductive cells of a plant
- Style - The stalk, or middle part, of the female organ in plants (connecting the stigma and ovary)
- Stigma - The tip of the female organ in plants, where the pollen lands
- Pollen - The male reproductive cells of plants

3. Pollen grain:

- Pollen grain, a course to powdery substance produced by matured flowering plants is a distinctive natural marker.
- Pollen grains develop from the diploid microspore mother cells in pollen sacs of anthers.
- Typically, pollen grain is a haploid, unicellular body with a single nucleus. Pollen grains are generally spherical measuring about 25-30 micrometeres in diameter.
- The outer surface of microspores may have spines, ridges or furrows which may vary in other ways in different species.
- There may be oval, ellipsoidal, triangular, lobed or even crescent-shaped pollen grains. The cytoplasm is surrounded by a two layered wall.
- The outer layer exine is thick and sculptured or smooth. It is cuticularized and the cutin is of special type called sporopollenin which is resistant to chemical and biological decomposition.

Common variation in flower and flower parts:

1. Number of stamens: Stamens may be free or united. If united they can be of the following type:

(i) Syngenesious: Filaments free and anthers united e.g. Sunflower

(ii) Synandrous: Stamens fused all through their length. e.g. Cucurbita

(iii) Adelphous: Anthers remain free and filaments are united.

(a) Monoadelphous - United to form 1 bundle. e.g. China rose

(b) Diadelphous - United to form 2 bundles. e.g. Pea

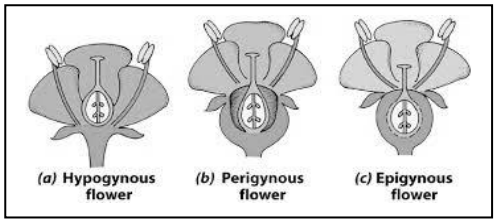
(c) Polyadelphous- United into more than two bundles. e.g. Lemon

2. Fusion of stamens with other parts of the flower.

(i) Epipetalous: Stamens fused with petals e.g. Sunflower, Dhatura

(ii) Epiphyllous: Stamens fused with perianth e.g. Lily
(iii) Didynamous: Four stamen two short and two long e.g. Tulsi
(iv) Tetradynamous: Six stamens inner four are long and outer two are short e.g. Mustard

3. Position of the ovary

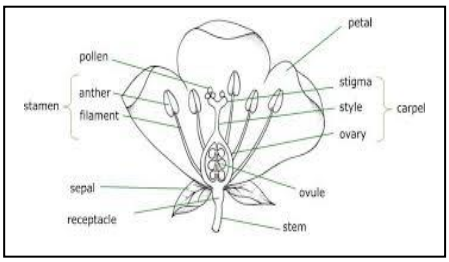


- **(i) Epigynous:** Position of ovary inferior to other floral parts. e.g., mustard, China rose.
- **(ii) Perigynous:** Other floral parts (organs) are attached around the ovary. e.g., apple, guava.
- **(iii) Hypogynous**: Position of ovary superior to other floral parts e.g., sunflower.

4. Number of carpels: If number of carpels is more than one, they may be

(i) Monocarpellary: Gynoecium having one carpel e.g. Pea
(ii) Polycarpellary: Gynoecium having many carpels e.g. china rose
(a) Apocarpous: Carpels are free. Each carpel has its own style and stigma. e.g., rose.
(b) Syncarpous: Carpels are united, e.g., lady finger, tomato

Cross Section of a typical flower:



PHENOLOGY OF TREE SPECIES

The study of the timing of seasonal biological activities in plant in known as phenology. The task of plant-phenology is to observe and record the periodically recurring growth stages and to study the regularities and dependency of the yearly cycles of development on environmental conditions. In the case of flowering plants, these life cycle events, or phenophases, include leaf budburst, first flower, last flower, first ripe fruit, and leaf shedding etc. The seasonal cycle of plants however is influenced to the greatest extent by temperature, photoperiod and precipitation.

1. Leaf budburst: The emergence of new leaves on a plant at the beginning of each growing season.
2. First flower: The act or state of producing flowers the period during which a plant produces blooms its first flowers. When the petals of its first flower begin to become visible as the bud opens, or is it when the flower is open enough to see the anthers or stigma inside
3. Last flower: The date on which the last flower has opened on the plant; any remaining buds remain closed.
4. First ripe fruit: When fruits change colour from unripe (usually green) to ripe-and-ready (often black, red, orange, or yellow), when capsules split open to reveal the seeds inside.
5. Leaf shedding (abscission): When a plant sheds one of its parts, such as leaves, flowers, and/or fruits

Phenological Monitoring:

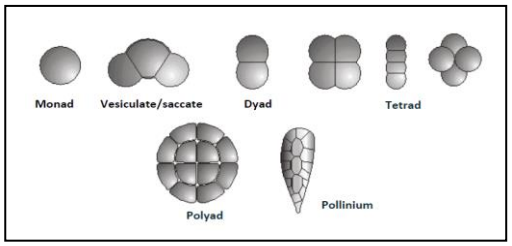
- The spring and autumn are particularly rewarding periods because the biosphere changes so quickly, but even in the middle of winter or during the long days of summer the fine details of plant development can be surprising and fascinating.
- The first step is to familiarize yourself with your natural surroundings and begin to observe day-to-day and week-to-week changes in the appearances of plants.
- Choose plants where you spend the majority of your time.
- Get into the habit of recording brief observations about phenological behaviour of selected plant.

Pollen Morphology

Pollen: Pollen is a fine to coarse powdery substance comprising pollen grains which are male microgametophytes of seed plants, which produce male gametes (sperm cells). Pollen grains have a hard coat made of sporopollenin that protects the gametophytes during the process of their movement from the stamens to the pistil of flowering plants, or from the

male cone to the female cone of coniferous plants. Pollen grains come in a wide variety of shapes, sizes, and surface markings characteristic of the species

The pollen grain – dispersion units: Mature pollen disperse in shed units. When the post-meiotic products become separated the dispersal unit is a single pollen grain, a monad. Post-meiotic products also become partly separated or remain permanently united, resulting in dyads (a rare combination), tetrads or polyads. Pollinaria are dispersal units of two pollinia including a sterile, interconnecting appendage.



Polarity: Pollen shape and aperture location relate directly to pollen polarity. The polarity is determined by the spatial orientation of the microspore in the meiotic tetrad and can be examined in the tetrad stage. In monocots, due to the mostly distal position of apertures, there are four views: distal polar, proximal polar, and two different equatorial views.

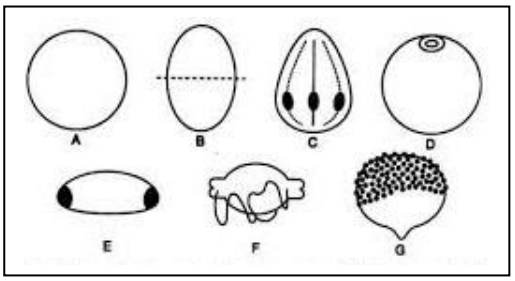
A) **Apolar:** this type of polar axis two polar areas cannot be identified.

B) **Isobar:** Isopolar pollen has identical proximal and distal poles, thus the equatorial plane is a symmetry plane.

C and D) **Hetrapolar:** heteropolar pollen the proximal and distal halves differ.

E and F) **Isodiametric:** In isodiametric pollen the polar axis is ± equal to the equatorial diameter.

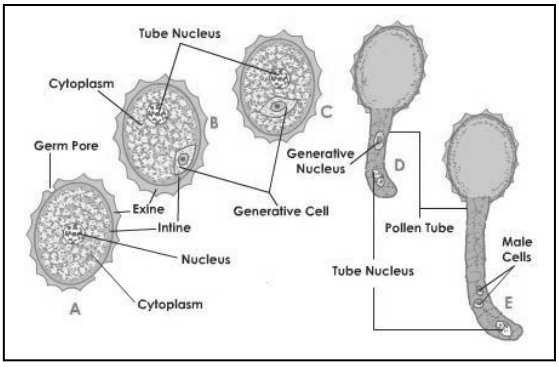
G) **Cryptopolar:** The distal and proximal faces have dissimilar sculpturing and lack of tetrade mark



Aperatures: An aperture is a region of the pollen wall that differs significantly from its surroundings in morphology and/or anatomy. The aperture is presumed to function as the site of germination and to play a role in harmomegathy. Pollen grains lacking apertures are called inaperturate. A circular aperture is termed a porus if situated equatorially or globally; if situated distally, it is called an ulcus. An elongated aperture is termed a colpus if situated equatorially or globally; if situated distally, it is termed a sulcus.

Pollen grain size: The size varies from small to gigantic. The small size is having diameter less than 10 µm. While, small have diameter ranges between 10 -24 µm, medium size diameter is in between 25 -49 µm, large diameter varies in between 50-99 µm, very large diameter in between 100- 200 µm and gigantic diameter is more than 200 µm.

Pollen grain shape: Pollen shape refers to the 3-dimensional form of a pollen grain in relation to the P/E ratio. A pollen grain can, for example, be spheroid-, cup-, boat-, cube-, tetrahedral-, triangular dipyramid-, hexafoil dipyramid-, triangular prism-, pentagonal prism-, or hexagonal prism shape. In pollen grains with three apertures, two types of aperture arrangement occur after simultaneous cytokinesis.



On the basis of the ratio between length of polar axis (P) and equatorial diameter shape of pollen grain varies. Peroblate shape is less than 0.50 µm, oblate is in between 0.50-0.75, suboblate is between 0.78 – 0.88, oblate –spheroidal in between 0.89-0.99, speherical is 1, prolate –spheroidal is between 1.01-1.14, subprolate in between 1.55-1.33. and prolate is in between 1.34 – 2.00 and perprolate is more than 2. In oblate pollen the polar axis is shorter than the equatorial diameter.

Pollen wall: The mature pollen grains have double walls. The vegetative and generative cells are surrounded by a thin delicate wall of unaltered cellulose called the endospore or intine, and a tough resistant outer cuticularized wall composed largely of sporopollenin called the exospore or exine. The exine often bears spines or warts, or is variously sculptured, and the character of the markings is often of value for identifying genus, species, or even cultivar or individual. The spines may be less than a micron in length (spinulus, plural spinuli) referred to as spinulose (scabrate), or longer than a micron (echina, echinae) referred to as echinate. The pollen wall protects the sperm while the pollen grain is moving from the anther to the stigma; it protects the vital genetic material from drying out and solar radiation.

Pollen viability: Pollen grain is a fine coarse powdery substance comprising grain which are male microgametophyte of seed plants which produce male gametes. Pollen viability refers to the ability of the pollen to perform its function of

delivering male gametes to the embryo sac. This functional property of the pollen after their release from the anther varies greatly from species to species and its quality is assessed on the basis of its viability. Pollen viability is an index of its quality and vigour. Pollen viability varies between minutes and years, and which primarily depends on the taxonomic status of the plant and on the abiotic environmental conditions. In order to maintain the viability and fertilizing ability of the pollen for a long period of time special storage conditions are needed. **Pollen** stored for up to three days at 25°C retained the ability to penetrate ovules following hand pollination, and of that stored for three years at 5°C, 19% of the grains fluoresced with FDA.

Pollen viability: It refers to the ability of the pollen to perform its function of delivering male gametes to the embryo sac. This functional property of the pollen after their release from the anther varies greatly from species to species and its quality is assessed on the basis of its viability.

Cryopreservation is the most efficient method for long-term preservation of partly dehydrated pollen grains. In vitro biotechnological techniques like isolation and fusion of reproductive cells, and DNA transformation of artificially produced zygotes and embryos, have opened new prospects for germplasm storage. Pollen viability has classified the examined plant taxa into three main groups, viz.:

a) Long-lived pollen (six months to a year), example, Ginkgoaceae, Pinaceae, Arecaceae, Saxifragaceae, Rosaceae, Fabaceae, Anacardiaceae, Vitaceae and Primulaceae.

b) Pollen with a medium life span (approximately 1-3 months), examples, Liliaceae, Amaryllidaceae, Salicaceae, Ranunculaceae, Brassicaceae, Rutaceae, Scrophulariaceae, and Solanaceae.

c) Short-lived pollen (from few minutes to a couple of days), examples, Alismataceae, Poaceae, Cyperaceae, Commelinaceae and Juncaceae.

Causes for the loss of pollen viability: It has been extremely difficult to access the exact reasons behind the loss of viability among pollen grains within a span of short or long period. Changes in amino acid composition of stored pollen fail to explain the loss of viability. There are variable reasons to explain such inactivity as stated below.

Biochemical Alteration in Pollen: The major biochemical cause for the loss of viability during storage is basically due to the deficiency of respiratory metabolites, which is the result of continuous metabolic activity by the pollen. As a result of long-term storage there are reports of considerable changes in the amount of carbohydrate, amino acids and organic acid level in the pollen of different species.

Need of pollen viability test: For controlled pollination or hybridization there is need to test either pollen viable (able to germinate) or sterile.

Procedure for pollen viability test

Dye preparation: Preparation of Acetocarmine Glycerol Jelly stain Acetocarmine Glycerol Jelly stain is prepared using a solution of 100 ml of 45% acetic acid; boil this solution until boiling point, then add 2 g of carmine until the carmine is completely dissolved (approximately when the solution has a volume of 60 ml). Leave to cool, and filter; finally, add the same volume of glycerol as the final solution (approximately 60 ml). The whole process must be performed in a laminar flow chamber and under constant agitation.

Procedure:

- Extract the pollen from desired flower and collected it in glass bottle with proper lid. The collection of pollen must be early in the morning where pollens are most viable.
- Then the pollen is mixed with talcum power to reduce moisture content in it.
- After collecting desired pollens from different flowers must stored in ice box. It is needed to be stored in low temperature to retain its viability.
- The drop of dye, spread the pollen with light circular movements on a slide. One of the most well-known stain techniques is the Acetocarmine Glycerol Jelly. This test measures the integrity of the cytoplasm; the pollen grains get stained red when the cytoplasm membrane is integral.
- Place one or two drops of 2 percent acetocarmine glycerol jelly in the center of a slide. Using a wooden toothpick, take a small amount of pollen out of the capsule or bottle, and place it on the drop of dye, spreading the pollen with light circular movements.
- Let it stand for a minute, and then put the slide cover on. Mounted slides should lie flat for one or two days.
- If it is required that the samples be stored, place them in boxes designed for this purpose in a cold room or refrigerator at 4^{o} C.
- Afterwards, observe them under a light microscope at 200 or 400x magnification.
- A bright red staining of the cytoplasm of a pollen grain is indicative of viable pollen, while cytoplasm not red or pink indicates non-viable pollen. Compared with the viable pollen grains, the sterile ones are deformed; with a granular

cytoplasm and/or an unstained gap which is usually situated at one side of the pollen grains giving them an eclipse-like appearance (eclipse sterility).

- The observation of abnormalities such as tetrads or pollen grains with four cores indicates that the sample is infertile. Besides, propose a scale based on the range of pollen viability of promising genotypes in order to determine whether they can be used as male parents in breeding programs.

SEXUAL REPRODUCTION

Plant reproduction is the production of new offspring in plant which can be accomplished by sexual or asexual means. Sexual reproduction produces an offspring by fusion of gametes *i.e* by method of pollination and fertilization. In asexual reproduction produce new individual without the fusion of gametes *i.e*. by grafting, budding etc.

Pollination refers to the transfer of pollen from the male organ (anther) to the female organ (stigma).

Pollination and fertilization: for pollen sperm to successfully fertilize the egg, there must be pollination. For that, the pollen sticks to the stigma starts growing and produce pollen tube. Fertilization begins when pollen tube starts to grow toward the egg within the ovule.

Importance and need of pollination: Pollination is one of the fundamental steps in a sexual reproduction. Sexual reproduction produces variable offspring, crating diversity and variation among population. Pollination helps to natural selection. It helps in crossing or hybridization between two genotypes of same species or different species.

Types of Pollination:

Self-pollination: pollen from anther may fall on the stigma of the same flower leading to self-fertilization. Also known as autogamy. Pollen from anther may fall on the stigma of the same flower leading to the self-fertilization. Here the flower is perfect/ bisexual. Self-pollination leads to very rapid increase homozygosity. Therefore, the population of self-pollinated species are highly homozygous. Self-pollinated species do not show inbreeding.

Cross pollination: when pollen grains from flowers of one plant are transmitted to the stigma of flowers of another plants, is known as cross pollination or allogamy. Cross pollination preserves and promote heterozygosity in population. The breeding methods in such species aim at improving the species without reducing the heterozygocity to an appreciable degree. Usually, hybrids or synthetic varieties are aim of breeder. In many naturally cross-pollinated species, a small amount (up to 5-10 %) of selfing may occur. In cross pollinating species, the transfer of pollen from a flower to the stigma of other may be brought about by following agencies.

Mechanism promoting cross pollination

1. Dicliny: or Unisexual is a condition, in which the flowers are either staminate or pistilate.
   a. Monoecy: Staminate or pistilate flowers occurs on the same tree
      i. In same inflorescence: e.g. Castor, Mango, Jatropha
      ii. In different inflorescence: Walnut, Chestnut, Rubber
   b. Dioecy: male and female flowers are present on different trees. E.g. Poplar, willow, Casuarina, Papaya, Date palm
2. Dichogamy: Stamens and pistils of hermaphrodite flowers may mature at different times for facilitating cross pollination.
   a. Protogyny: Pistils mature before stamens. E.g. Bajra
   b. Protoandry: Stamens mature before pistil e.g. Maize
3. Physical Barrier: Waxy film covered stigma so that stigma is not receptive. Honey bees break this waxy film and let the stigmas become receptive. The pollen grains attached to the body parts of the honey bees here have more chance to pollinate such stigma.
4. Self-incompatibility: It refers to the failure of pollen from a flower to fertilize the same flower or other flower on the same plant. Here the flower does not set seed on selfing
   a. Sporophytic
   b. Gamatophytic
5. Male sterility: It refers to the absence of functional pollen grains in hermaphrodite flower.

Agents involve in pollination:

1. Wind pollination (anemophilous): Predominantly found in gymnosperms
2. Pollination by animals: **a. Insect** (entomophilous): Bees, Butterfly, moths, flies, beetles; **b. Birds** (ornithophilous): Hummingbird, Honey creepers; **c. Mammals:** Bat, Mice, Monkey; **d. Reptiles**
3. Pollination by water (Hydrophilous)

III. Geitonogamy: when the pollens from a flower of one plat fall on the stigma of other flowers of the same plant

EMASCULATION

The removal of stamens or anthers or killing the pollen of a flower without the female reproductive organ is known as emasculation. It is the essential method for hybridization in angiosperm tree species. In tree species of bisexual flowers, emasculation is essential to prevent of self-pollination. In monoecious trees, male flowers are removed or male inflorescence is removed. In tree species with large flowers hand emasculation is accurate and it is adequate. It is the first step of hybridization in bisexual flower where pollens or anthers are removed.

Methods of Emasculation:

Hand Emasculation: In species with large flowers, removal of anthers is possible with the help of forceps. It is done before anther dehiscence. It is generally done between 4 and 6 PM one day before anthers dehisce. It is always desirable to remove other young flowers located close to the emasculated flower to avoid confusion. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed with the help of forceps. Sometimes corolla may be totally removed along with epipetalous stamens. In all cases, gynoecium should not be injured. An efficient emasculation technique should prevent self pollination and produce high percentage of seed set on cross pollination.

Suction Method: It is useful in species with small flowers. Emasculation is done in the morning immediately after the flowers open. A thin rubber or a glass tube attached to a suction hose is used to suck the anthers from the flowers. The amount of suction used is very important which should be sufficient to suck the pollen and anthers but not gynoecium. In this method considerable self-pollination, upto 10% is like to occur. Washing the stigma with a jet of water may help in reducing self-pollination, however self-pollination cannot be eliminated in this method.

Hot Water Treatment: Pollen grains are more sensitive than female reproductive organs to both genetic and environmental factors. In case of hot water emasculation, the temperature of water and duration of treatment vary from crop to crop. It is determined for every species. For sorghum 42-48^{0}C for 10 minutes is found to be suitable. In the case of rice, 10 minutes treatments with 40-44^{0}C is adequate. Treatment is given before the anther's dehiscence and prior to the opening of the flower. Hot water is generally carried in thermos flask and whole inflorescence is immersed in hot water.

Alcohol Treatment: It is not commonly used. The method consists of immersing the inflorescence in alcohol of suitable concentration for a brief period followed by rinsing with water. In Lucerne the inflorescence immersed in 57% alcohol for 10 second was highly effective. It is better method of emasculation than suction method.

Cold Treatment: Cold treatment like hot water treatment kills the pollen grains without damaging gynoecium. In the case of rice, treatment with cold water 0.6^{0}C kills the pollen grains without affecting the gynoecium. This is less effective than hot water treatment.

Genetic Emasculation: Genetic/ cytoplasmic male sterility may be used to eliminate the process of emasculation. This is useful in the commercial production of hybrids in tree species. In many species of self-incompatible cases, emasculation is not necessary, because self-fertilization will not take place. Protogyny will also facilitate crossing without emasculation.

Use of Gametocide: Also known as chemical hybridizing agents (CHA) chemicals which selectively kills the male gamete without affecting the female gamete. *e.g.* Ethrel, Sodium methyl arsenate, Zinc methyl arsenate, Maleic hydrazide.

HYBRIDIZATION

Artificial hybridization is defined as the process of crossing two genetically different individuals having desirable traits to obtain an offspring having superior traits than the parents. It can be achieved by emasculation in plants.

Stages of hybridization:

1) **Labelling and Bagging:** After planning to schedule for the species to be hand pollinated or crossed or hybridized during particular period of the year, when the trees flower abundantly, the female and male trees should be labelled properly. The labels should be durable and ink used should be waterproof. Daily observations should be taken during the flowering period for bud- break of the trees included in the hybridization, because bud is to be covered and tied with bags before anthesis takes place to check selfing. When flowering buds have appeared, these should be covered with 'pollination bag' and tied, having transparent plastic window and material that allows aeration at appropriate time.
2) **Emasculation:** The removal of stamens or anthers or killing the pollen of a flower without the female reproductive organ is known as emasculation. The care must be taken not to injure the young bud or the ovary, style and stigma. After removing male portion of the young buds, again the bag should be tied carefully so that emasculated bud is not damaged. The date of emasculation should be noted on the label as well as field note book.
3) **Pollen collection:** Pollen collection is tedious process in the case of angiosperms tree species, as enough pollen is not

available and even if it is there, in most of the species it is sticky. Pollen should be collected when it matures and fertility or viability tests conducted by staining. Storage is required only when there is no synchronization in the flowering time of species.

4) **Application of pollen on receptive stigma:** The pollen of the male parent is applied only when the stigma becomes receptive, which can be recognized after seeing nectar or other droplets on the stigma. The pollen is collected in sterilized petri-dishes and it is applied on the stigma with the help of a sterilized camel-hair brush No. 4, 6 or 8 as required. After application of pollen, the bag should be immediately tied again. Then the painting brush must be sterile with the help of ethanol before use for other pollination. This is normally done in the morning hours during anthesis. Pollen should be applied thrice a day on 3 alternate days. Date should be recorded on label and in field note book.
5) **Fertilization:** After the pollen has been applied, the pollen tube will grow through the style to reach the egg cell to fertilize it. This natural process is reflected by withering of the stigma and style. The daily observation through the plastic window of the bag is essential.
6.) **Removal of bags:** After fertilization the bag should be removed but the labels should be updated and kept intact. As these labels have to stay for longer period, they should preferably be aluminium or hard thick drawing paper, which should be waxed properly.
7) **Harvesting of crosses:** Crosses should be harvested only when the seed is mature. Each cross should be harvested separately and stored under ideal conditions till the sowing season approaches.
8) **Sowing:** As the hybrid seed is very precious and small in quantity, it should be sown under controlled condition, and utmost care should be taken for its healthy growth, in laboratory, nursery and field etc. by adopting good seed-testing rules and cultural practices.

TREE BREEDING

Tree breeding programs can be thought of sequentially, first selecting the best species for a particular environment, then selecting the best provenances (geographic sources), then the best families, and then the best individual trees in the best families. The selected genotypes can then be crossed among themselves to produce the next generation, when the selection and breeding cycle can begin again.

Tree breeding programs can be thought of sequentially, first selecting the best species for a particular environment, then selecting the best provenances (geographic sources), then the best families, and then the best individual trees in the best families. The selected genotypes can then be crossed among themselves to produce the next generation, when the selection and breeding cycle can begin again. Typically, within a species, there is variation in volume growth among provenances. The best provenances may grow 10% to 30% faster than the average of the species (and the worst 10% to 30% slower). Similarly, the best families within a provenance may grow 2 to 25% faster than the average of the provenance. Finally, the breeder can select outstanding individual trees within the best families to make additional genetic gain.

TREE BREEDING METHODS: Different breeding methods followed in tree improvement programs are as follows:

1. **Surveying and identification of superior phenotypes/ candidate plus tree:** This is the first breeding method and important one that is undertaken to start tree breeding programme. This is non- random differential reproduction of genotypes, which is targeted to utilize the best among the existing population for the breeding purpose. The objective of this breeding method is to mark the potential tree, although on phenotype basis, to be subsequently evaluated for selection of elite trees among them for further use in production orchard or in breeding programme. This selection, however, is done after conducting genetic tests. The selection of candidate plus trees (CPTs) has to be done very carefully, preferably by a team and by an individual. The basic assumption behind this selection is that a sizable environmental variance is eliminated, whereas selection is done following comparison method, in which, in addition to the candidate tree, four to five comparison trees are also marked with which the comparison of the candidate tree is done, is done and the latter is graded accordingly. Other methods are used for selection of CPTs like regression method or “baseline method” for grading the selected tree remains the same. The quality of these trees therefore required to be carried out comprehensively, maintaining high selection intensities, which would eventually result into higher genetic gains. The genetic gain is proportional to the selection differential and the selection differential is governed directly by the selection intensity, having positive relationship.
2. **Establishment of clonal bank:** This is the second important component in tree breeding programme. This involves vegetative reproduction of all these individuals that have been selected as candidate trees following comprehensive surveying, and raising them within the organization/ Institutes/University area these trees are true genotypes replicas of CPTs. To establish these clonal banks, any workable method of asexual reproduction can be applied. This method assures the availability of the selected individuals for further selection or use in the breeding progamme. In the absence of such clonal banks, sometimes original the original trees get lost on account of logging etc., and thus their back selection after the progeny test is jeopardized and the whole method of genetic testing is rendered meaningless. In

addition to the above purpose, this clonal bank serves as a gene conservation source for maintaining *ex-situ* preservation of biodiversity.

3. **Carrying out clonal mating:** There are number of mating designs, classified under incomplete pedigree and complete pedigree mating systems. With the use of appropriate mating design, a system of recurrent selection can be carried out to ensure continuous upgrading of the propagules. It is a way of making step-wise changes in gene frequencies within a population while maintaining sufficient genetic variability for continued selection. Many a times, when a single ramet is not sufficient to meet the requirements, a number of ramets from the original ortet are used to carry out crossing, and hence the term clonal mating sometimes takes more than one flowering cycle. Pollen management is important part of this method. After having decided the mating schemes and selection of the parents, the pollen of the male parent is made available to carry out pollination. The pollen management becomes more important when in the crossing schedule the male and female parents are placed at different locations or when there is difference in the maturity time of male and female flowers of the parent tree selected for the mating.
4. **Carrying out genetic test or progeny test:** After having completed the exercise of mating different clones, the next component of the breeding programme is to carry out genetic tests or progeny tests, following proper methods. The genetic test conducted with multi-point objectives. In addition to the genetic information that these tests provide to help identify the superior parents or elite trees, these tests offer future breeding population and sometimes these tests themselves are converted to seedling seed orchards after making proper between/ within family selections. It is from here that nature of variance understood. Specific combining ability and general combining ability values of different crosses and parents respectively are obtained, which have a significant role in further decision making in the breeding strategy. These genetic tests are conducted at multi- locations to better understand Genotype x Environment interaction, especially to understand impact of G x E on index or composite traits. Progeny test or genetic test have significant role in the multi-generation tree breeding programme.
5. **Manipulation of time in breeding cycle:** This is indirect component of breeding methods. In trees rotation age is quite long as in most of the pines and hardwoods the time management is equal of importance. Normally, to complete breeding cycle the time requirement is felt when the controlled crossing work is to be carried out after selecting the parent. The crossing is done normally in clonal bank itself. However, to reduce the time to grow the parents to the flowering stage, the parents are required to be raised under controlled condition. Again, time is required to test progenies. It is therefore, at both stages that efforts are made to reduce the time. For early flowering, use of suitable growth- regulators is adopted and the requirements of dormancy are managed. Similarly, in progeny testing evaluation of the progenies is being standardized to be taken up much earlier than the rotation age or half of the rotation age.
6. **Establishment of seed orchards:** A seed orchard is essential component of tree breeding methods. Broadly two types, clonal seed orchard and seedling seed orchards. Clonal seed orchard is established using ramets of the phenotypically superior trees with comparatively higher score values. After genetic test or progeny test seed orchards are established. Seedling seed orchards are established from seeds.
7. **Production of quality planting stock:** Although the production of planting stock is a specialized job of a nursery technologist, in many tree-improvement programmes, this method is taken as a part of the whole strategy. Many times, seed orchard management to produce quality seeds and then subsequently the quality planting stock is taken care by plant production specialist. Some minimum standard is required to be fixed for this planting stock, so that full genetic worth of the superior seeds/ cuttings could be exploited.
8. **Maintenance of broad genetic base:** For indigenous species, where unlimited scope for selecting plus trees fresh entries can be made after effecting the programme for about initial two generations. A system of sub-lining, maintaining several independent groups for future sue, is quite effective to maintain a broad genetic base. The maintenance of a sizeable breeding population offers the scope of attempting desirable crossing schemes and provides ample material to enter into subsequent generations. Avoidance of environmental hazard is needed to maintain broad genetic base.
9. **Management of inbreeding in breeding and production population:** Severe inbreeding depression after self-fertilization has been found in many species. Inbreeding will occur in limited number of generations in the breeding programs of multiple generations. The amount of inbreeding will be determined by the intensity of family selection, size of the initial breeding population and the choice of the mating design. The relatedness could increase rapidly if complete pedigrees are not maintained while attempting crosses or planting seed orchards. Sub-lining the breeding population into breeding groups significantly helps avoiding inbreeding.
10. **Continuity in Improvement programme:** The continuity of programme is needed. But due failure to continuity of programme, all the efforts are done to go waste. The breeding related activities have been carried out in many trees, but unfortunately, they have never been working in isolation without a strategy. It is therefore, important to well understand the fact that a breeding strategy means a continuous process planned for number of years. A planning of work with written document helpful to maintain continuity in improvement programme.

PROVENANCE TRIAL

The term **provenance** refers to the geographic source of seed or plant material or to the plants from such a source. The main practical object of provenance trials is to locate as quickly and as economically as possible those provenances yielding well-adapted and productive forests. Productivity itself may not always imply rapid growth; important criteria could be survival, resistance to adverse environmental factors or pests, wood quality, seed production. The second major object is to establish local seed production stands. Spare seed may be sown in separate provenance plots or as a bulk mixture but in either case the best individuals should be selected for future breeding.

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Phenotypic variation: It is the total in a particular trait that is recorded in a population. P = G+ E

Where, P= Phenotypic variation, G = Genotypic variation and E = environmental variation

Genotypic variation: Genetic variation is due to genetic differences among the individuals of a population. This type of variations forms the basis of any tree- improvement programme. In a cross- pollinated tree species every individual in a population has different genetic make-up. Genetic variation cannot seen always be seen directly and it must be distinguished from environmental variation. Genetic variation is created through changes in genetic constitution in an individual.

Environmental variation: All factors, excluding genotype, that affect the trait values are included in environmental factors.

Types of provenance trails: Various types and stages of provenance trial may be necessary to locate the best provenance or provenances of a species for a given site, country, or region. The choice depends on the objective, current information available, the extent of natural variation in the species, and the variation among the potential planting sites. The following stages may therefore be used on each major site type:

1. Range-wide sampling phase: It contains reproductive material from widely scattered provenance throughout the natural range. In initial phase very little is known about the pattern of variation of the species in question and guesses have to be made on which provenance should be include based on environmental and distributional factors. 10-30 provenances are suggested in this phase, which are usually ¼ to ½ rotations. Samples from areas where the species shows optimum performance should be included as well as areas with extreme environment and insular occurrences. Additional samples should be taken where there are obvious and unusual phenotypic variations. After selecting the provenance, the seed should be collected from at least 25-30 unrelated trees which should be at least 100-300 m from each other.

2. Restricted sampling phase: This phase of provenance trials is designed to identify smaller regions and finally individual provenances that have the greatest productivity. The number of provenances tested may be between 5 and 10 and it may be valuable to represent each by seed from superior and randomly selected parent trees. This depends upon the resources of the seed collecting agency.

3. Proving phase: A small number of probably useful provenances are tested at this stage under normal plantation conditions. Whereas in the first two phases careful attention must be given to experimental design, in the proving phase this aspect is less important. Replication of sample plots within and between proving plots is essential. Proving plots are large enough to support stander/1 mensuration assessments (0.5 to 1.0 hectare) or to facilitate costing and silvicultural studies (2 to 5 hectares). In this phase only 1-2 provenance will usually be tested in plantation.

Experimental design and layout

- The purpose of experimental design is to minimize environmental heterogeneity and to increase the precision of treatment (provenance) comparisons
- A randomized plot design could be useful for the range-wide sampling phase but for all stages the most favoured design is the randomized complete block layout (RCB) in which each replication contains one plot of each provenance.

Management and assessment: It is not possible to generalize on the management and assessment of provenance trials but details should be included in each trial plan. It is difficult to determine in advance whether silvicultural operations such as thinning and pruning will be undertaken at prescribed times for the trial as a whole or for provenances differentially. Thinning may be systematic, random or selective, depending on the objects of the trial. Generally, however, management should be similar to that currently used or anticipated to be optimum for the test material.

GENOTYPIC COEFFICIENT OF VARIATION

Gene can be brought as functional unit of inheritance. Genotype is genetic component of an individual consist of DNA inherited from parents or transformed from parents in the case of clone. DNA acts as blueprint for construction of protein and enzymes which controls the development and functions of individual. Variability in the

Genotype: genetic composition of an individual with no regard of environmental influence

Genetic variability: Genetic variability is complex, genetic variation generally divided into additive and nonadditive components.

Additive variance: Its due to cumulative effect of alleles at all gene loci influencing the trait.

Nonadditive variance again divided into *dominance variance* is due to interaction of specific gene loci. Whereas, *epistatis variance* is due to interaction among gene loci.

Genotype of progeny that are produced when two parents are crossed depends the type of gametes produced by each parent e.g. an *AA* genotype would produce only A gametes, where as *aa* individual will produce only gametes with a genetic constitution. Heterozygous genotype (*Aa*) produce both *A* and *a* type gamete.

		Male parent	
		A	A
Female parent	A	AA	Aa
	a	Aa	Aa
Ratio: 1AA:2Aa:1aa			

Factors controlling genotypic variation:

1. Mutation: Is heritable changes in genetic constitution of organism. Mutation is the ultimate source of all genetic variation

2. Selection: Also known as natural selection. Is strong force that reduces variability. It determines which tree will grow and reproduce. It has direct effect on the genetic makeup of the tree.

3. Gene Migration: It is the migration of alleles from one population or species in to another where they may be absent of with different frequency. Generally, cause due to the movement of pollen or seed from one place to another.

4. Genetic drift: Is a complex mechanism that operates trough chance fluctuation in the allele frequencies within population.

Genotypic coefficient of variation (GCV): Information on the variability was majored by genotypic coefficient of variation (GCV) for individual quantitative characters and through equilibrium distance over the characters.
Study of variability parameters is important before starting any breeding programme in a particular plant to know the variability status of the population under study. Variability parameters are represented in coefficient to avoid the difference due to various units of characters. Based on these parameters characters are identified having maximum selection response.

Phenotypic coefficient of variation: Phenotypic variation refers to differences within species for particular growth traits. Variation due to genotypical and environment interaction may be refer as phenotypic variation. As we know two trees are identical.

Phenotype: It refers to Physical manifestation of genotype in interaction with local environment.

Environmental variation: Some environmental factors that influence on tree growth that includes soil nutrition, temperature water availability, wind, rainfall, soil depth, aspect etc. (Abiotic factors) and disease insect, animals, biotic competition (Biotic factors). Environmental forces are the greatest cause of variability in some characteristics of growth.

Genotype and environmental interaction: The term is used to describe the situation where there is a change in the performance ranking of given genotype when grown in different environment. Strong Genotype x Environment interaction are more likely to occur when environments differ widely.

General combining ability (GCA) and specific combining ability (SCA): Genetic variation that occurs in living organisms is inherited in a way that is common to all species. Almost all-important traits in forest trees are influenced be several or many gene loci, each of which has a relatively small effect on the phenotype. This results in a large array of genotype for traits influenced by many genes if there is genetic variation at the influential gene loci. When environmental effects are added to this array, a continuum of phenotype results. Genetic differences in their genetic composition and environment in which they were raised. Genetic inheritance may be computed in different mechanism like Genetic values, General combining ability (GCA), specific combining ability (SCA) etc.

Genetic value: Genetic value is genetic quality of individual is to compare of its offspring against the offspring of other parent trees. The genetic value of parents is expressed in terms of combining abilities. There are two type of combining abilities

a) General Combining ability: Is defined as average performance of progeny of an individual when it is mated to a number of other individuals in the population. Although GCA may be expressed in absolute unit, it is usually more convenient and meaning full to express them as deviations from the overall mean. Thus, parent with GCA of 0 has an average GCA. A positive GCA indicates a parent that produces above average progeny, whereas parent with negative GCA produces progeny that perform below average for the population.

b) Specific combining ability: is a term that refers to the average performance of the progeny of a cross between two specific parents that are different from what would be expected on the basis of their general combining abilities alone. It can be either negative or positive. SCA always refers to a specific cross and never to particular parent itself.

Heritability: The concept of heritability is one of the most used in quantitative genetics. Heritability values express the proportion of variation in the population that is attributable to genetic differences among individuals. It is therefore a ratio indicating the degree to which parents pass their characteristics along to their offspring. heritability is of key importance in estimating gains that can be obtained from selection programme. There are two type of individual tree heritability

i.) Broad sense heritability: It is the ratio of total genetic variation in a population to phenotypic variation. Broad sense heritability can range from 0 to 1. A Lower limit of 0 occur if none of the variation in a population was attributable to genetics. If all variation was due to genetics, then broad sense heritability would be equal to 1.

$$H^2 = \frac{\sigma^2 G}{\sigma^2 P} = \frac{\sigma^2 A + \sigma^2 NA}{\sigma^2 A + \sigma^2 NA + \sigma^2 p}$$

H^2 = Genotypic variance / phenotypic variance

In % = Genotypic variance / phenotypic variance X 100

ii.) Narrow sense heritability: It is the ratio of additive genetic variance to total variance. Lower limit for narrow-sense heritability is also 0 (no additive variance) and the upper limit is 1 (no environmental or nonadditive). Narrow sense heritability is never greater than broad sense heritability.

$$h^2 = \frac{\sigma^2 A}{\sigma^2 P} = \frac{\sigma^2 A}{\sigma^2 A + \sigma^2 NA + \sigma^2 E}$$

h^2= Additive variance/ phenotypic variance

h^2% = Additive variance/ phenotypic variance X 100

Genetic advance: It is measure of genetic gain under selection. The change achieved by artificial selection in a specific trait. Gain is usually expressed as the change per generation or the change per year. Gain is influenced by selection intensity, parental variation and heritability. Average improvement in a progeny over the mean of the parents. Gain is achieved by selection in the parental generation; the amount depends on selection intensity, parental variation and heritability.

Genetic gain = heritability x selection differential

$$GA = h^2 S$$

Selection differential: The difference between mean of population and mean of selected individual to be parent of next generation.

PLUS TREE SELECTION

Tree breeding programme consist of selection of trees with desirable genes, packing of desirable genes, multiplication of genetically improved planting material and developing and maintaining of base population with a broad genetic base for advanced generation breeding. The first step in the programme is the selection of plus tree, the tree with desirable genes.

Methods of selection: this method allows selection of a plus tree by comparing its value with those of base population. This method minimizes the confounding of environmental effects with those of genetic ones. It includes three methods

A) Comparison tree method: Selection should be done on uniform sites, and the observation on the characters of economic interest on candidate trees and next best five trees in the vicinity of the candidate tree should be recorded. The comparison tree or check tree are normally dominant in crown position with the candidate tree, found in identical

environmental conditions. The growing conditions and age of the candidate tree and the check trees must be the same. The superiority percentage of each candidate tree over the mean value of the comparison trees is worked out for each trait.

B) Base value method: Certain characters like branching habit, disease resistance, wood density and bole straightness are known to be under strong genetic control with high heritability. For such traits, a base value (average value) is prepared by recording observations of 5-10 % sample in the base population. The base values are prepared for each trait and each stand. candidate trees are selected and superiority percentage is worked out for each character over the base value.

C) Regression method: this is useful in uneven aged stands, for selection of traits which are more affected by age. This method is of particular value for the growth characteristics like diameter and height, as the quantitative traits can be easily accessed on the basis of phenotype alone without the of check tree. Sample plots are laid out at the base of population and regression curves are built for the desired character against age. Different regressions are developed for different sites. For selection of candidate tree, the trait value is plotted on the regression graph, and if the candidate tree falls at some defined distance above the regression line, it is acceptable and the higher above, the more desirable it becomes. When the value falls below the regression line the tree is rejected. When the value fall on the line, its use depends on the other characters. The drawback of this method is that it could not be used to species whose age cannot be determined.

Selection of plus tree: After selection candidate tree, plus tree select on the basis of arbitrary fixed selection standard. The score of each trait of candidate tree are calculated by multiplying the superiority percentage with economic score of the receptive trait. The minimum selection standard is fixed for each trait and all those candidate trees which meet the minimum selection standard are considered as plus tree. The selection standard can either be lowered or raised depending on the number of plus trees required for the breeding programme or depending on economic important of the trait.

SEED ORCHARD

Is an area where seed are mass produced to obtain the greatest genetic gain quickly and inexpensively as possible. Plantation of selected clones or progenies which is isolated or managed to avoid or reduce pollination from outside source and managed to produce frequent, abundant and easily harvested crops of seed. Seed orchard are not always solely for genetic improvement of specific characteristics but can be used to produce quantities of seed that are adapted to planting location.

General steps in seed orchard

1. Three are intensively selected in natural stand or unimproved plantation
2. Clones or progenies of these selected tree are established together in seed orchard.
3. Breeding value of each tree is evaluated by progeny testing
4. Genetically undesirable individuals are thinned out from orchard on basis of progeny test results
5. Remaining trees are then allowed to cross pollinate for commercial production of genetically improved seed.
6. Best individuals within progeny test are selected and established together in an advance generation orchard.

Types of seed orchard:

1. **Seedling seed orchard (SSOs):** SSOs are the progeny test that are rouged so that the remaining trees can cross pollinate and produce seed.
2. **Clonal Seed Orchard (CSO):** CSOs are the collections of vegetative propagules of selected trees. The propagule is established together, progeny tested when they flower and then rouged based on the progeny test results. Most CSOs have been established by grafting.

Seed orchard Location:

- Selection of the appropriate site for the seed orchard will ease the management during and after the establishment.
- The location should accomplish species specific requirements e.g. Soil texture, Soil fertility, Soil pH, drainage, topography, exposure, altitude, climatic factors like temperature, precipitation, wing etc.
- The location should also fulfil the other management aspects like Accessibility, labour availability, close to administration, close to nursery, gentle topography.

Size and longevity of seed orchard

- It is determined by seed demand and expected production from the orchard
- A minimum of 15-25 clones or families to assure a sufficient genetic base and limit selfing should be used.
- A seed orchard will only operative until a new improved seed orchard has been established based on next generation progeny test.
- Seed orchard usually referred by generation e. g, 1st, 2nd, 3rd

Protection: Seed orchard should be protected from strong wind, fire, illegal logging, fuel wood collection.

Site preparation and establishment of seed orchard:

1. **Clearing:** Left woody material may attract pest, disease, increase risk of fire and impede mechanical management.
2. **Soil preparation:** Proper soil preparation improves the growth of the tree plants and promotes their competition with other plants
3. **Promotion of pollination:** Promotion of pollination is easiest for wind-pollinated species. Spacing is a method of improving the condition for wind pollination. The effect on insect pollinated species is probably different from species to species according to pollinator, but details are poorly known. Promoting pollination by insect by putting up beehives is widely used in fruit orchard and agriculture and is applicable to seed orchard.
4. **Demarcation of plots and rows:** The orchard design is clearly demarcated. replication blocks and plots should be distinctly demarcated. The material and way of putting up should be so that it will be difficult to removed.
5. **Demarcation of pits and pitting:** Follows the normal silvicultural practices. Depending on the expected thinning the initial spacing may be 2-6 meter.
6. **Planting:** Extra care should be taken with grafted and budded material since the grafting or budding site may still be vulnerable. Sometimes the root stock is planted in the field before grafting and consequently carried out in the field. The planting usually involves several clones or families and it is important that the planting is done according to prescribed plan, so that the identity of the individual tree is clear.
7. A plan of the orchard design should be brought to the field and used when planting spot are documented distinctly on the plan with the identity of the plant to be planted on the spot.
8. The plants, scion in field grafting should be distinctly marked and kept separately
9. The plants are marked with a tag when planted. The tag carries a number/symbol of the plant similar to that of the map.
10. **Beating up:** Correct replacement of dead seedlings is crucial. If wilted, seedlings or grafted plants are replaced and care should be taken that the replacement will not disturb the orchard design i.e. plants should be replacing with same clones.
11. **Cover crops:** A temporary legume cover crops may be planted between the trees. The cover crop is beneficial to the soil and may facilitate weed control and diminish erosion.