Lecture notes

Tree Improvement and Silviculture (SFB 451)
B.Sc 2nd Year 2nd semester

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Some terms and definitions

Additive genes -
A form of allelic interaction in which there is no dominance. The heterozygote is intermediate in phenotype between homozygotes for the alternative alleles. For multiple-gene traits, approximately equal contributions made by many loci.

Allele -
One of several alternative forms of a gene occupying the same locus on a particular chromosome.

Backward selection -
Selection of parent trees based on results from a progeny test.

Breeding orchard -
(breeding seed orchard, breeding arboretum) A planting of selected trees, usually clonally propagated, designed to ease breeding work.

Breeding population -
A group of individuals selected from a wild population for use in a breeding program. Usually phenotypically selected for desirable traits. In species with large natural ranges, there are usually several to many, more-or-less separate breeding populations, each designed to provide progeny suited to a particular geographic region.

Breeding value -
The genetic value of an individual determined by the mean value of its progeny. May be on the basis of individual traits or a selection index.

Breeding zone -
An area within which a single population of improved trees can be planted without fear of mis-adaptation.

Candidate tree -
A tree that has been tentatively selected for inclusion in a breeding program, but has not yet been measured or compared with surrounding trees.

Character (trait) -
A distinctive but not necessarily invariable feature exhibited by all individuals of a group and capable of being described or measured; e.g. color, size, performance. A character of a given individual will have a certain phenotype as determined by the individual's genotype and environment.

Cline -
An environmental gradient (temperature, rainfall, soil ph . . .) and a corresponding phenotypic gradient in a population of plants or animals. Where these have been evaluated by provenance tests, clines are often found to have a genetic basis.

Clonal test -
A field planting of several to many vegetatively propagated plants. Such tests furnish estimates of the relative performance of different genotypes, but do not necessarily provide information on breeding behavior.

Conelet -
An immature cone (strobilus) in the conifers.

Diallel, complete or full -
A mating design and subsequent progeny test resulting from the crossing of 'n' parents in all possible 'n2' combinations including selfs and reciprocals. (Because of severe inbreeding depression in the selfs, these are often skipped and the test still called a "full" diallel.)

Diallel, incomplete or partial
A partial sampling - any individual family or type of family may be omitted. In either the complete or incomplete diallel, identities of both seed and pollen parents are maintained for each family.

**Dysgenic** -
An action or process that is detrimental to the genetic qualities of a population. Usually applied to human actions, such as high grading or land clearing, which would reduce the local gene pool.

**Ecotype** -
A sub-population of a species that occurs in a particular well-defined environment, usually showing better adaptation to that environment than the species as a whole.

**Elite tree** -
A tree that has been shown by progeny testing to produce superior offspring

**Endemic** -
A plant or animal species or subspecies native to a small region.

**Forest genetics** -
The study of heredity in forest trees.

**Forest tree improvement** -
The application of genetic principles to the improvement and management of forest trees.

**Forward selection** -
Choosing the best individuals out of a progeny test for use in seed orchards and/or subsequent generations of breeding.

**Gene bank** -
A collection of germplasm (seeds, pollen, whole plants, extracted DNA) collected and maintained so as to sample as much as possible of the genetic variability in a population.

**Gene flow** -
The movement of specific alleles among different populations of a species or among related species.

**Gene pool** -
The sum total of all the genetic variation in the breeding population of a species and closely related species capable of crossing with the species.

**Gene** -
The basic unit of inheritance.

**Genetic drift** -
Changes in gene frequency in small populations due to random chance. Usually a loss of rare alleles.

**Genetic gain** -
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.

**Genotype** -
The specific set of genes possessed by an individual, both expressed and recessive.

**Genotype-environment interaction** -
Changes in rank or levels of performance among individuals when tested in different environments.

**Heritability** -
General concept: The degree to which progeny resemble their parents. Quantitative definition: A ratio of genetic relative to environmental factors that influence the expression of a trait.
**Heterozygous** -
Having two different alleles at a locus. When used to refer to the whole genotype, indicates that the individual has different alleles at most loci. When used to refer to a species as having low or high heterozygosity relative to other species, indicates that the species has a relatively high number of variable loci.

**Homozygous** -
Having two identical alleles at a locus, at most loci, or in the entire species. *(See heterozygous.)*

**Hybrid** -
Progeny from a cross among dissimilar genotypes. In forestry, the term is usually used for crosses between species.

**Ideotype** -
The ideal type or perfect specimen. A description or illustration of what the final goal of genetic improvement for a species would look like. The ideotype is developed without regard to heritabilities for the individual traits involved. It is not intended to be a practical field guide for selecting "+" trees, but as a starting point for developing a field-selection guide.

**Inbreeding depression** -
The reduction in vigor often observed in progeny from matings between close relatives. Inbreeding depression is due to the expression of recessive deleterious alleles and is usually severe in open-pollinated outcrossing species (most forest trees) that occur in fairly high population densities.

**Inbreeding** -
Matings between related individuals. In open pollinated tree species, inbreeding usually leads to mild to poor seed set, low germination, and severe growth reduction.

**Introgression** -
The movement of genes from one population into another through hybridization followed by backcrossing. Usually refers to movement of genes from one species to another or among sub-species that have been geographically isolated then brought back together by changes in the species ranges or planting of exotic populations.

**Land race** -
A population of trees of a non-native species that has undergone one or more generations of natural selection in a new environment, i.e., Norway spruce in New York State.

**Locus** -
The position of a gene on a chromosome.

**Mutation** -
A sudden change in the genotype, usually caused by a small change in the DNA sequences in the chromosomes. It may also be caused by changes in chromosome number or breakage of individual chromosomes.

**Ortet** -
The original plant from which a clone is started through rooted cuttings, grafting, or tissue culture, or other means of vegetative propagation. The original plus tree used to start a grafted clone for inclusion in a seed orchard is the ortet.

**Outcrossing** -
Matings (controlled or natural) among unrelated individuals. May also refer to a species that has specific barriers to selfing, or exhibits such inbreeding depression that inbred individuals never reach maturity.

**Pedigree** - Record of ancestry.
Phenotype-
The visible characteristics of a tree. The phenotype is determined by the genotype interacting with the environment in which it is grown.

Plus tree-
A phenotypically superior but untested tree.

Polyplloid-
A cell, tissue, individual, population, or whole species having more than twice the basic number of chromosomes of the ancestral species. Polyploidy may lead to increased growth rates (Populus) or severe dwarfing (Pinus). Three sets of chromosomes is called triploid, four sets-tetraploid, six sets-hexaploid, etc.

Progeny test-
A test to compare the offspring of different parents.

Propagule-
Any type of plant to be used for reproduction. It might be a seedling, a rooted or unrooted cutting, a graft, or a tissue-cultured plantlet.

Provenance test-
A test comparing trees grown from seed or cuttings collected in many parts of a species range.

Race-
A sub-population, usually fairly large, of a species exhibiting some degree of phenotypic (and presumably genotypic) uniformity among individuals within the population and distinct from the species as a whole.

Ramet-
A vegetatively reproduced copy of a plant. Each ramet will have almost precisely the same genotype as the original parent tree, known as the ortet.

Resilience-
The ability of a population to persist in a given environment despite disturbance or reduced population size. The resilience of a population is based upon the ability of individuals within the population to survive (fitness) and reproduce (fecundity) in a changed environment and the genetic variability of the population which allows the production of new genotypes.

Rogue-
To remove trees that have an undesirable phenotype, or that have been shown through progeny tests to have a less desirable genotype from a seed orchard, seed production area, or nursery bed.

Rootstock-
A rooted plant, usually a seedling, on which a scion is grafted.

Scion-
A twig, bud, or other vegetative cutting to be grafted onto the root-system of another plant.

Screening-
Selecting for a particular trait, often used when referring to the application of a specific treatment such as white pine blister rust spores or a herbicide, and then looking for resistant individuals. Usually implies checking large number of individuals at one time.

Seed orchard-
A plantation established for the production of tree seed.

Seed orchard, clonal-
A seed orchard established from vegetatively propagated trees, usually grafts. Usually clonal seed orchards are established with single-tree plots with different ramets of each clone located as far apart as possible to reduce self pollination.

**Seed orchard, seedling**-
A seed orchard established from seedlings (as opposed to grafts). Usually seedling seed orchards are established with multiple-tree plots so that selection can take place first among families and then among the individuals within each family plot, reducing each plot to a single tree.

**Seed production area**-
A stand or plantation not originally planted to produce seed, but rogued of inferior trees and treated in such a manner as to produce large quantities of seed. Usually the stand or plantation is from a known origin or selected seedlot.

**Seed zone**-
An area within which seed can be collected from any natural stand and planted in any new site without fear of mis-adaptation.

**Seedlot**-
A designation of a group of seeds with some factor in common, i.e., year of collection, stand or seed orchard, individual "+" trees, point of origin in a provenance test, a half- or full-sib family.

**Selection differential**-
The difference between a selected tree, family, or clone and the average of the population from which it is taken.

**Selection**-
Choosing individual trees or populations with desirable characters to obtain genetic improvement.

**Self pollination**-
The natural or artificial pollination of a female flower with pollen from the same genotype.

**Sib**-
(sibling) A term meaning brother or sister. Half sibs have one parent in common. Full sibs have both parents in common.

**Sublining**-
Dividing a breeding population into several to many smaller populations. All controlled crosses for forward selection are made within a subline, leading to inbreeding within sublines. Production seed orchards are planted with clones or seedlings from several to many sublines to minimize inbreeding in the production orchard.

**Tissue culture**-
A technique for cultivation cells, tissues, or organs of plants in a sterile, synthetic medium; includes the tissues excised from a plant and the culture of pollen or seeds.

**Variability**-
Differing from the average value. Absence of uniformity, usually referring to lack of genetic uniformity in a population.

**Variance**-
A statistical measure of variability.

**Viability**-
A population's ability to live, grow and develop. It is affected by physical habitat factors (climate, geology, topography, and aquatic features) and by biotic habitat factors (plant and animal populations and communities).
Unit-1: Introduction

- Historically, foresters generally did not view trees as typical plants having systems of heredity similar to all other living organisms.
- Genetic variability was ignored, and it was somehow felt that a tree’s development depended only upon the environment in which it was grown.
- It has only been in relatively recent years that there has been a general recognition that forest tree parentage is important and that changes and improvements in tree growth and quality can be brought about through breeding and parental control.
- Forest tree improvement activities were undertaken seriously on an operational scale only after this was recognized.
- In order to know what tree improvement is, it is necessary to know forest tree breeding, forest genetics and forest tree improvement.
- Activities that are restricted to genetics studies of forest trees are termed as forest genetics; here the objective is to determine the genetic relationship among trees and species.
- Forest genetic activity is the attempt to determine crossability patterns among species within a genus.
- The crosses are made to determine relationships, but otherwise they have no special breeding objective.
- In forest tree breeding activities are geared to solve some specific problems or to produce a specially desired product.
- Forest tree improvement is applied when control of parentage is combined with other forest management activities to improve the overall yields and quality of products from forests.

1.1 Introduction to tree improvement (Objective and important)

- Simply, tree improvement is an additional tool of Silviculture that deals with the kind and genetic make up of the trees used in forest operations.
- Tree improvement is the application of genetic principles to increase the value of tree crops.
- Tree improvement relies on understanding and using variation that naturally occurs in tree populations.
- Tree improvement increases the value of a tree species by
  1) selecting the most desirable trees from natural stands or plantations,
  2) breeding or mating these select trees and
  3) testing the resulting progeny.
- The trees involved in this process are referred to as the breeding population.
- This three-step process is then continuously repeated to further improve the average value of the breeding population.
- Each iteration of this improvement process is referred to as a generation.
- Tree improvement is concerned with how trees vary and how this variation can be utilized to improve forest productivity. All tree improvement programs consist of the following:
  1. A determination of the species, or geographic sources within a species, that should be used in a given area.
  2. Determination of the amount, kind and causes of variability with in the species.
  3. Packaging of the desired qualities into improved individuals, such as to develop trees with combinations of desired characters.
5. Developing and maintaining a genetic base population broad enough for needs in advanced generations.

Tree improvement gradually developed along with the necessary basic disciplines and it is still developing. Tree improvement is considered to be an essential part of a forest management programme. Tree improvement is generally a regular part of the Silviculture management budget of most forestry organizations. Tree improvement is now realistically considered to be an essential tool of Silviculture. Tree improvement has really just started to make its contribution to forest production. It is without any question one of the best tools of Silviculture.

Observed characteristics such as growth rate are referred to as the phenotype of a tree.

- A tree’s phenotype is determined by both its genetic constitution, or genotype and the effect of the environment as described by the following equation:
  \[ P = G + E \]
  where,
  \[ P \] = phenotype
  \[ G \] = genotype
  \[ E \] = environment.

It is important that any tree improvement programme is planned and coordinated in accordance with the purposes and priorities of national tree planting programmes in such a way that emphasis is placed on species of present or expected future importance and duplication of trials is avoided.

The objective of any tree-improvement programme should be made clear and well-defined before the programme is initiated.

The purpose of a tree-improvement programme is to optimize one or more of the following points

1. the quality of end use as timber, fuelwood, fodder, shelter, etc.
2. survival (adaptation to climate and environment)
3. resistance to pests and diseases
4. growth rate.

A tree improvement programme is usually divided into different stages:

1. selection of desirable species
2. selection of desirable provenances within a species
3. selection of desirable families and individuals within a provenance
4. controlled breeding, incl. recombination and hybridization.

**Advantage of tree improvement**

i. Once a change is obtained, it can be kept over a number of generations.

ii. Genetic material that is developed can be kept essentially intact for an indefinite time through methods of vegetative propagation.

**Difficulties in tree improvement program**

1. Availability of seed with the known or desired genetic background is a frequent problem.
2. A lack of knowledge about what will be desired in the future can be a major deterrent to a tree improvement program.
3. Tree improvement program is a long term process, and as such it requires a good permanent records.
4. Tree improvement cannot respond to quick change in market demand.
1.2 Silvics and its importance

Definition

- Silvics is the study of life history and general characteristics of forest trees and crops with particular reference to environmental factors, as the basic for the practice of silviculture.
- Silvics implies the study of the trees and forests as biological units, the laws of their growth and development and the effect of the environment on them. It explains the natural laws of their growth and development and their behavior in a given set of environmental conditions.

Importance of silvics

1. Production of species of economic value
2. Production of large volume per unit area
3. Production of quality timber
4. Reduction of rotation
5. Raising forest in blank areas
6. Creation of man made forests in place of natural forest
7. Introduction to exotics
Unit-2: Tree improvement

- Tree improvement is the improvement of heritability of desirable features and their economic importance by breeding and genetic improvement programs.
- It is the application of genetic principles to increase the value of tree crops.
- Worldwide, tree improvement techniques are being employed on virtually all commercially important forest tree species to enhance economic returns from wood and paper products.
- Forest tree improvement is applied when control of parentage is combined with other forest management activities, such as site preparation or fertilization, to improve the overall yields and quality of products from forestlands.
- Tree improvement produces genetically superior trees which have better growth, tree form, site adaptability, wood quality, disease resistance, high productivity and product uniformity.
- Genetically superior trees are multiplied through techniques based on reproductive characteristics of a species (Dogra and Dhiman, 1998).

2.1 Provenance test

- Provenance tests is the plantation of nursery grown seedlings collected from the different identified seed stands of certain species of the country which have almost similar type of edapho climatic condition under tree improvement program to increase the overall production.
- For provenance tests, seeds are collected from genetically superior trees of selected natural forest or artificial plantation seed stands from different regions of the country and are germinated in the nursery, selected seedlings are planted in statistical design with not less than 100 seedling in each plot
- One or two extra rows plantation is also done around the trial plots as a wind break and for other protection point of view.
- Regular systematic recording of different parameters like height, diameter, branching patterns, bole form and disease pests is done for up to its rotation period or at least half of the rotation period.
- Finally statistical analysis is done and concludes on the basis of the significance results of the particular provenance.

The Phase, Sequence and time scale

- There are three phases of provenance trial; the choice depends on the current information available and extent of natural variations
The most commonly used design is the Randomized complete block design (RCBD). It is simple, flexible and robust but is less suitable if there are very many provenances to be evaluated.

### Important steps in provenance test
1. Survey of species distribution
2. Delineation of provenances
3. Seed collection
4. Plant production
5. Establishment of provenance trials in each major plantation site
6. Evaluation of trials
7. Information about best provenances for each site.

### Factors governing choice of provenance
- Climate and ecological matching between site of origin and potential site of introduction
- Growing season
- Day length and phenology
- Edaphic
- Latitude

### 2.2 Sources of variation (Biological and others)
- The first thing to do when starting a tree improvement program is to determine the amount, cause, and nature of the variation that is present in the species of interest and to learn how to use it.
- Most tree species possess greater variability than species of other organisms; it is reported to be almost double that of other plants (Hamerick et al., 1979).
- Forest tree improvers therefore possess a huge advantage by being able to draw on this variability in their breeding programs.
- Basically, the differences among trees are the result of three things; i. The differing environments in which the trees are growing.
ii. The genetic differences among trees,
iii. And the interactions between the tree genotypes and the environments
   ▪ Some genetic variations are predictable and useful, whereas other types are random
     and are more difficult for the tree breeder to use.
   ▪ In forest trees, a number of categories of variation exist that can be broadly grouped
     into species, geographic sources (provenances), stands, sites, individual trees, and the
     variability within individual trees (Zobel et al., 1960).
   ▪ For characteristics related to survival and adaptability (such as cold hardiness),
     geographic variation is often the most important, whereas for economic
     characteristics, which are not so obviously related to fitness (such as stem straightness
     or wood specific gravity), individual tree variability is generally the greatest.

A. Environmental variation
   ▪ Environmental variation is understood by most foresters, and its management is the
     basis of most silvicultural activities.
   ▪ Some environmental factors that influences tree growth can be controlled and
     manipulated, whereas others cannot.
   ▪ Things such as stocking levels and tree to tree competition can be handled by
     controlling plant spacing or by thinning. Within limits, nutrient deficiencies can be
     adjusted by fertilization, and soil moisture can be changed by drainage.
   ▪ But soil texture cannot be generally be altered, but site preparation can change the soil
     structure to a considerable extent.
   ▪ Operations such as subsoiling are sometimes useful to create an environment for
     better root development and tree growth.
   ▪ Other environmental variables such as rainfall, temperature, wind action, soil depth,
     aspect etc can be little influenced by humans but all these factors influence the
     phenotype of the tree.
   ▪ Variation among trees caused by environmental differences cannot be used in
     breeding program and is often not even predictable.
   ▪ However, environmental forces are the greatest cause of variability in some
     characteristics especially those related to growth.
   ▪ Forms and quality may also be strongly affected by environmental factors but
     generally, the quality characteristics in forest trees tend to be more highly inherited
     and less influenced by the environment than are growth characters. The various
     sources of environmental variations are;
     a. Geographic zones (provenance)
     b. Variability among sites
     c. Differences among stands within sites
     d. Tree differences within a stand
     e. Variation within a tree
   ▪ Although foresters generally cannot easily control the environment, it is frequently
     possible to develop strains of trees that will grow satisfactory under adverse
     conditions.
   ▪ The only method we can overcome adverse temperature, rainfall, wind action, pests or
     other strong environmental influences is to create strains of trees through breeding or
     to use those found in nature that are more tolerant to the adverse factors.
B. Genetic Variation

- Genetic variability is complex, but if its magnitude and types are known and if it is well used, genetic variation can be manipulated to obtain good gains in some tree characteristics. Genetic variation can be divided into:

1. **Additive variance**
   Additive variance is due to the cumulative effects of alleles at all gene loci influencing a trait.

2. **Non additive variance:** It can be divided into two types
   a. Dominance variance
      It is due to interaction of specific alleles at a gene locus.
   b. Epistasis variance
      It is due to interaction among gene loci.

- In most tree improvement programs, the non-additive types of genetic variability have generally been given little attention, because the additive portion of genetic variance is easier to utilize.

- Most characteristics of economic importance in forest trees are under some degree of additive genetic control. This is fortunate because additive variance can be successfully used in simple selection systems such as those that are most suitable to new tree improvement programs.

- Characteristics such as wood specific gravity, bole straightness, and other quality characteristics of trees have stronger additive variance components than do growth characteristics.

- Although growth traits are controlled to some degree by additive genetic effects, they also have considerable non-additive variance associated with them. Therefore, any selection program must include testing the progeny of selected phenotypes to determine the actual genetic worth of the tree.

- The response of selection of characteristics with considerable non-additive variance, such as growth, is generally less satisfactory than for the quality characteristics that are usually under strong additive genetic control (Stonecypher et al, 1973).

- There is little that the tree improver can do in the short term to improve the amount or kind of genetic variance available for use.

- The initial challenge to the tree improver is to determine the magnitude and kind of variance present from natural or unimproved populations and then to use it wisely.

- Genetic variations are the result of natural forces. Variability in natural stands is caused by four main forces, two that increase variation and two that decrease it.

- The forces such as Mutation and Gene flow increase variations whereas Natural selection and Genetic drift decreases variation.
Figure 1: There are numerous forces that alter the variation pattern within a population. These forces include genetic drift, which narrow the gene base, and genetic drift and selection. On the other hand, gene flow and mutation broaden the gene base.
C. Genotype × Environment interaction

- This type is used to describe the situation where there is a change in the performance ranking of given genotypes when grown in different environments. Such information must be known if maximum progress in breeding is to be obtained.
- Frequently, in tree improvement, a group of families are tested in a single environment, and their performance is then extrapolated to other environments, when in fact their relative performance might have been different when grown under other conditions.
- The challenge of forestry is to grow trees in variety of environments, some of which are greatly different from others.
- Strong genotype × environment interaction are more likely to occur when environment differs widely.
- As forestry operations become more intensive and as the productive forestland base decreases, there is need to establish plantations on sites that formerly were considered marginal or sub marginal for satisfactory tree growth.
- Knowledge is needed about how stable a given genotype is when grown in quite different environments (Hanson, 1970).

2.3 Seed production areas and seed stands

- All tree improvement programs must have seed production at some stage of their development if continued gains are to be achieved. This is true even for programs using vegetative propagules for large scale operational planting; seed is needed for the development of outstanding trees from which vegetative propagules can be obtained.
- Organizations with extensive planting programs need large quantities of genetically-improved seed to increase yield and productivity.
- There are several methods that can be used to obtain genetically-improved seed for planting. A simple and relatively low-cost method to produce slightly-improved seed is development of Seed Production Areas.
- The terms ‘seed stand’ and ‘seed production area’ are generally referred to as “A plus stand that is generally upgraded and opened by removal of undesirable trees and then cultured for early and abundant seed production”.
- These two terms are often treated in different context. In some countries, the term ‘seed stand’ refers to a planted forest (plantation) which has been converted to produce seed while ‘seed production area’ is a special area in natural forest which is managed for seed production.
- In some countries, however, seed production area is a preferred term for planted forest or plantation which is managed for seed production.
- Seed production areas are stands specifically managed for seed production.
- They are often used as interim sources until a more advanced seed production program such as that from seed orchards has been established. They are a very effective way of making available seed supply of an inexpensive but somewhat improved genetic quality.
- Although the amount of genetic improvement from a seed production area is expected to be small, the seed obtained from seed production areas still has better genetic qualities than seed from commercial collections in routine plantations, especially in adaptability, form and pest resistance.

2.3.1. Seed production Areas
Seed production areas can be stands specifically planted for seed production or existing stands specially managed for seed production, provided that their genetic origin is appropriate.

2.3.1.1 Development of seed production areas from existing planted stands

- The quickest way to make a seed production area is to convert existing mature stands of good quality trees for this purpose.
- A seed production area can also be developed from a progeny or provenance trial. There are no specific age limitations but the stand should be old enough to produce seed.
- A planted stand suitable for conversion to a seed production area should have the following specifications.

1. Details of the seed source used to establish the stand should be known.
   - Knowledge of the geographic origin and genetic base, which ideally will be broad, is important in determining the suitability of the stand for development into a seed production area.

2. The trees should be mature for seed production, but not too old.
   - There are no age limitations, other than that the stand must be old enough for reliable selection and be sexually mature to produce seed. Individual trees must have sufficient crown density to potentially produce large seed crops.
   - Seeds from very young or very old trees often are of inferior quality compared to those from middle age trees. In addition, old trees tend not to respond to thinning by further crown development.

3. The stand should be near full stocking and contain a large number of trees of good phenotype.
   - A suitable stand before conversion should be near full stocking to increase the selection intensity. The final stocking of a seed production is usually 150-200 stems per ha. An initial survey should reveal that the number of trees of good phenotypes is equivalent to that.

4. Free from pests and diseases.
   - Evidence of pests or diseases may be an indication of inferior adaptability of the seed source. If the stand shows widespread symptoms of attack by insects or diseases it should not be used for conversion.

5. The trees should have proven capacity to produce flowers and seeds in the area.
   - To avoid failure, a detailed survey of the candidate species’ ability to flower and seed in a particular environment should be a prerequisite to establishing seed production area. This is especially important for exotic species where flowering may fail or seeds are not produced due to incompatibility with the site. Non-favourable environments, such as drought prone areas, may be unsuitable for seed production area. Availability of pollinators can also be important.

6. The area should be easily accessible.
   - The conversion of a stand into a seed production area and subsequent management operations, such as harvest of seeds, requires that the area is accessible throughout the year. This implies that it should be relatively flat but well drained. Ideally it should
not be in a very remote area or too far from available labour for ease of maintenance and management.

(7) The selected stand should not be subject to commercial harvesting.
- Seed production areas will be maintained for many years. It is necessary, therefore, to ensure that the stand in question is safe from commercial harvesting operations. Good communication with the forest managers will help avoid the accidental loss of valuable seed production areas.

(8) Size of seed production areas.
- In general a minimum of 4 ha is recommended for practical management of seed production areas for most tree species. Managing small stands is inefficient, and there is a great danger of contamination from outside pollen. However, for some species such as eucalypts an area as small as 0.5 ha may be sufficient, due to prolific seed production. The area should be as close to square as possible, rather than a long linear block to facilitate more cross pollination among the trees.

(9) Isolation.
- Poor isolation will be a common disadvantage of seed production area developed from existing stands. Usually such stands are selected from mature plantations of the same species. It is virtually impossible to eliminate completely contamination by stray pollen. However, an isolation zone or pollen dilution zone surrounding the seed production area can reduce it. The dilution zone may be an open area of some 200 m. If trees are to be grown in the dilution zone they must be of a species that does not hybridise with the species of interest for seed production.

2.3.1.2 Development of seed production areas from specially planted stands
- When a seed production area is developed from a planted stand established specially for this purpose, certain factors should be given due consideration.

a. Site requirements
- The environment at the sites where seed production areas are to be established must be reasonably representative of those where the species are to be planted operationally in the region, as initial selection will be for superior individuals within such environments. It is important to select sites that are as uniform as practicable otherwise future management will be made more difficult.
- A check should be made to ensure that the species in question flowers and sets seed readily in the target environment. Examination of existing stands will establish whether this is so.
- Some specifications, such as the size of seed production area and isolation from pollen contamination, are the same as that recommended for development of seed production areas from existing planted stands.

b. Seed source
- It should be with seed derived from a large number of unrelated parents of appropriate provenance or many provenances. Using appropriate provenance(s) ensure the tree will be more adapted to the plantation site.
- Improved adaptability alone usually makes seed collection worthwhile. In most species, certain quality characteristics like stem straightness, and to some extent branch quality, will also be improved.

c. Site preparation
The planting site should be cleared of vegetative cover. This will be followed by disc ploughing of the area prior to planting to give the plants a weed-free start.

d. **Spacing**

- Planted seed production areas should be established at close spacing. Initial spacing of 3 m x 2 m (1666 stems per ha) is appropriate for most tropical eucalypts. A wider spacing of 4 m x 2 m (1250 stems per ha) or 3 m x 3 m (1100 stems per ha) is more suitable for most acacias.
- Initial high stocking rates allow heavy and early selective thinning so that the trees of best vigour and form can be retained. At the same time, a wide spacing is opened up to allow full crowns and good seed crops to develop.

e. **Fertilisation**

- Application of a starter-dose of balanced fertiliser to aid in successful establishment is highly recommended.
- Type of fertiliser and rates of application should where possible be based on local expertise. Otherwise a complete fertiliser NPK 15:15:15 is recommended at a rate of 100 g per seedling, in a ring of radius 30 cm around the stem. Fertiliser should be applied one month after planting.

2.3.2 **Selection of Trees for a Seed Production Area**

- Desired attributes of the trees left in a seed production area are similar to, but less rigorous than, the qualifications required for a select tree to be used in an intensive tree improvement program.
- Only trees in the dominant and co-dominant crown classes are considered for retention because of their growth and seed-producing potential. For existing mature stands that will be converted, trees showing potential for good seed production are given preference, although the evidence of past seed production is not essential if the trees have been growing in a tightly closed stand.
- Excellent seed crops are often produced after heavy thinning by trees that showed little seed production prior to thinning.
- Selection criteria may be different between species and end products but the following characteristics are typical in the case of timber species.
  1. Fast growth
  2. Relatively straight single cylindrical bole
  3. Good crown development
  4. Thin branches with wide branch angle
  5. Good self pruning
  6. Free from insects and diseases
- No tree below the desired standard should be kept, regardless of spacing. It is essential that the crowns of the trees be exposed to full sunlight of at least three sides if good seed production is to be realised. When several good phenotypes occur in a group, enough of them must be removed so that the remaining trees will receive enough light. In spots where the only trees available are inferior phenotypes, all trees must be removed, even if this results in a fairly large gap in the stand.
- Selection of trees should be done in small groups for ease of comparison. A group of 16 (4 x 4) trees each is convenient from a practical point of view. By dividing the stand into small plots, a more even spread of seed trees will be retained after final thinning. Trees that do not meet the desirable standard are marked for roguing. Colour flagging tapes can be used for this purpose.
- Some species such as casuarinas are dioecious, i.e. trees are unisexual with separate male and female trees. Care must be taken to ensure that there are sufficient male
trees left for pollination. There is no standard rule regarding the proportion of male and female trees. A ratio of 30:70 seems reasonable.

- The original identity of trees in a seed production area is generally not known. After the final thinning it is recommended to number all the remaining trees in the stand. This will provide useful reference to each tree in the future when assessment of flowering and seed production is required.

2.3.3 Thinning Seed Production Areas

- Thinning is an essential activity in the development of seed production areas. It removes poorly-performing individual trees in order to increase the genetic quality of seed produced by the mating of trees which have been retained. It should be noted that for some species heavy seed crops may not be obtained for several years after thinning because of the time required for development of large, vigorous crowns. A final stocking of 150-200 trees per ha is considered suitable for cross pollination and subsequent seed production in a wide range of commercial tree species.

a. Frequency of thinning

- Each seed production area should receive at least two-three successive thinnings (cullings) of poor individuals. Each thinning will selectively remove around 50% of trees.
- Opening a stand by thinning will expose trees to risks such as adverse weather conditions. Desiccation can be a problem in hot humid areas while wind-throw is a normal problem in strong wind areas. Thinning in several steps, not the total operation at one time, will allow the remaining trees to adjust and adapt to the new conditions. Illustration of the thinning process is shown in Figures. 1-6.
b. Timing
- The time of thinning should be chosen to avoid potential negative effects. For example, do not carry out thinning during typhoon season or strong winds as wind-throw and crown damage can occur. If desiccation is a major risk, carry out thinning just before the onset of the rainy season.
- For seed production areas developed from specially planted stands, the timing will depend on the growth rates achieved. The aim will be to carry out thinning just before strong competition between adjacent crowns would otherwise set in (i.e. just before the crowns touch one another).
- For many tropical acacias and eucalypts the early rapid growth rates will necessitate that the first thinning takes place about 24 months of planting. The second and final thinnings are carried out at 4 and 6 years-of-age respectively.
- Extreme care in conducting thinning operations is important because damage to the remaining trees can result in a degeneration of their seed production capacities. Careless thinning is a common cause of later problems in seed production areas.

2.3.4 Management of Seed Production Areas
- Seed production should be properly managed and maintained to ensure full potential of seed production capacity.
  a. Removal of cut material
- After thinning, it is necessary to remove all cut material that has accumulated on the ground. Removing the residue allows for easier access into the area for management activities, and reduces the potential dangers from pests and wildfires.
  b. Weeding
- If the seed production area is to function efficiently, vegetative growth under the seed trees must be controlled. Weed growth can be controlled by regular hand weeding and/or careful application of herbicides. Trees should not have their growth checked by competing weeds. Seed production areas will require ongoing weed control throughout their working life as selective thinning will leave large gaps between trees. Once the trees attain sufficient height (around 10 m) it may be possible to control weeds by grazing livestock within the areas.
  c. Control of coppice growth
For strong coppicing species like eucalypts, it is necessary to kill the stumps of cut trees to discourage regrowth. Glyphosate herbicides, such as Round-up, have proved to be effective if they are painted or sprayed onto the cut surface immediately after felling. Multiple applications may be necessary to ensure a 100% kill.

d. **Fertilization**
   - Fertilisation should be used in conjunction with the opening of the stand by thinning. The increase in tree vigour resulting from thinning and fertilisation enables the development of heavier and denser crowns that will produce more flowers. N containing fertilisers are usually applied only to promote vegetative growth. Prior to and during flowering, PK containing fertilisers with micronutrients should be applied.

e. **Stand demarcation**
   - The external boundaries of the seed production area should be marked with large permanent markers. A prominent sign should be constructed to explain the purpose of the stand and the agency responsible for its management.

f. **Protection**
   - Adequate fire breaks (10 m wide or more) should be established surrounding each seed production area and continually maintained. It may be necessary to fence the area if foraging domestic stock, wildlife or human activities are likely to cause damage to the trees.

2.3.5 Seed Harvesting
   - Prior to seed collection time, a rough inventory should be carried out to determine if sufficient quantity of seed is available to warrant collection. This can be done by checking the trees in several small sections within the seed production area. Collection should not be made if only a small number of trees are found to produce seed. Seed collected from these trees are likely to be of poor genetic quality because of the limited cross pollination among the small number of flowering trees. It is also recommended to avoid seed collection from very early- or late-flowering trees for the same reason.
   - There are various options for seed harvesting. The most common practice is to collect seed from standing trees in the whole the area, and bulk them up into one seedlot. Climbers are normally employed to lob branches on the trees. This can be expensive and should be considered only when there is a high demand for such seed. It should be noted that if the branches are heavily cut for seed harvesting, it may take a few years before the crown is fully developed and abundant crop of seed is produced again.
   - A seed production area can be divided into several sections. Each section is designated for seed collection in a particular year. By dividing the seed production area into several sections, the harvested trees will have sufficient time to recover and develop good grown density when they are revisited again for seed collection. This practice also ensures continuing supply of seed from the seed production area.

2.3.6 Record Keeping
   - It is important that full records on all aspects of seed production areas are maintained. The following information should be included:
     i. Species
     ii. Seed origin
     iii. Location
     iv. Year of establishment
v. Climate (rainfall, temperature)
v. Soil
vii. Silvicultural operations (time of thinning, weeding, fertiliser applications, etc)
ix. Flowering characteristics
ix. Annual seed collection (amount of seed, number of trees from which seed is collected)

- It is important to monitor flowering set and seed production as it is influenced by cultural practices.

Conclusions
- Seed production areas have three attributes that are very important.
  1. Seed collected from seed production areas will have better genetic qualities than seed from commercial collections in routine plantations, especially in adaptability, growth, stem form and pest and disease resistance.
  2. When seed production areas are developed from stand specially planted for this purpose, the geographic origins of the parent trees are known, thus producing seed from a suitable source.
  3. Seed production areas are reliable sources of well-adapted and seed at modest cost.

- However, it is emphasised here that seed production areas are interim sources of seed of somewhat improved genetic quality to meet short-term needs. It is expected that seed production areas will be obsolete when seed of higher genetic quality of the species of interest becomes available from seed orchards.

2.4 Plus and elite tree selection

What is a plus tree?

**Plus Tree, Superior Tree or Select Tree:**
A tree that has been recommended for production or breeding orchards following grading. It has a superior phenotype for growth, form, wood quality, or other desired characteristics and appears to be adaptable. It has not yet been tested for its genetic worth.

**Candidate Tree:**
A tree that has been selected for grading because of its desirable phenotypic qualities, but has not yet been graded or tested.

**Comparison or Check Trees:**
Trees that are located in the same stand, are of nearly the same age, are growing on the same or better site as the select tree and against which the select tree is graded. Trees chosen as comparison trees are the best ones in the stand, with characteristics similar to “crop” trees that would be chosen in a silvicultural operation.

**Elite Tree:**
The term is reserved for selected trees that have proven to be generally superior by means of progeny testing. An elite tree is the “winner” from a selection program and it is the kind of tree that is most desired for used in mass production of seeds or vegetative propagules.

**Selection Intensity:**
The number of trees selected among an exact number of trees in the stand, e.g., 1 per 1000 trees.

Applied tree improvement program begins with the selection of the base breeding population it will determine how much gain will obtained in first and successive generation. The gains can be no greater than the quality of the base breeding population used and the best way to obtain maximize gain is through efficient selection.

A common problem with many tree breeding programs is too little emphasis given on method used to chose plus trees or superior trees and too much emphasis has placed on the design employed to breed these selected individuals. For first generation, the base breeding populations are selected from natural stand or generically unimproved plantations. In these circumstances there is no choice other than selecting by eye, however, in advance generations it is possible to implement far more efficient methods of selection. Selection methods used for trees from stands where there is no pedigree information are almost and always different from genetic test where parentage is known.

The basis for selection

The degree of genetic improvement in desired traits through selection is largely depends on three components.
- Variation
- Heritability
- Intensity of selection

Selection methods

1. Mass selection-trees selected from plantations based on their phenotypes. Used when the heritage of the tree is unknown.
2. Family selection-entire families are selected based on their average phenotypic performance.
3. Sib selection-individuals selected on the basis of their siblings. Used when destructive sampling must be used to measure the trait.
4. Progeny testing-parent trees are selected based on the performance of their progeny.
5. Within family selection-individuals selected on the basis of their deviation from the family mean. Rarely used.

Selection criteria

Different species have by nature different architecture. Selection traits may vary between different species and improvement programmes. However, timber species to be cultivated in plantations share a number of desired features. The ideal plantation tree has following characteristics:

1) Straight, cylindrical, non-forking, non-twisting bole.
2) Fast growth
3) Narrow crown
4) Thin branches with wide branch angles
5) High wood density and long fibres
6) Resistance to pest and diseases.
7) It should be genetically and phenotypically superior.
8) High yielding, high productive and very good in health.
9) Should be straight, less branching with handsome crown.
10) Should be mature (middle aged), not be stag headed.
11) Vigorous flowering and fruiting
Where to select
Selection is carried out in natural stands or preferably in plantations. Certain considerations of importance in the choice of the site for selection are identified below:

1) Selection should be made from stands that are as pure in species composition as possible.
2) Selection should be concentrated on stands or plantations that are average or better in traits of interest.
3) Selection works better in an even aged stand, since the age difference can then be eliminated from the evaluation.
4) Selection is best carried out in a mature stand, i.e near to maximum height.
5) Selection in natural forests where selective logging has taken place should be avoided since that may imply that the best trees have been logged, leaving the poorer (genetic material) behind. Logging may also have influenced crown competition.

How many to selects per stand (selection intensity)
- The number of selects in a stand is evaluated after the grading. Candidates should not be selected too close to each other, since closely growing trees may be related, e.g. same parent(s).
- Selection intensity depends on the variation of the stand.
A rule of thumb suggests 1 tree / hectare, i.e. one per 1000 trees with initial spacing of 3 x 3m

Steps in plus tree selection
1. Mapping of area and stand
   - Selected trees will be demarcated on the map.
   - The map is covered with plastic sheets with coordinates to facilitate location and demarcation of selects.

2. Site description.
   - In case of homogenous environment this may be carried out as representative for the whole area.
   - In case of a heterogeneous area, site evaluation is conducted for each selected tree. Parameters include.
3. Selection and marking of trees
   - Candidate trees are marked and graded. The mark should be distinct and conspicuous.
   - The tree is marked with a number, which corresponds to that in the grading sheet and on the map.
   - Yellow, red or white paint should be used for numbers.

4. Grading of the trees
   The candidate trees are measured and graded against comparison/check trees
   a) Height
   b) DBH (Diameter at Breast Height, 1.3m)
   c) Crown diameter
      - A narrow crown is desired. The diameter of the crown is estimated by projecting the outermost branches to the ground.
   d) Bole form
      - Deviation from the desired ideal straight, cylindrical bole is evaluated. Following observations are made.
        1. Basal sweep
        2. Bole swellings
        3. Bends and twists
        4. Leaning
        5. Trunk curves
        6. Circularity
   e) The branch angle
      - A wide angle is desired

   ![Branch Angles](image)

   f) Branch diameter.
      - Small branch diameters are desired.
   g) Self pruning ability
      - A long clear bole is desired. The presence of old branches, epicormic branches are assessed
   h) Forking
A non-forking clear bole is desired.

i) **Tree health:** If the tree shows any major signs of the following pests, diseases or attack, it should be rejected

1. Sign of dead top or thin crowns
2. Nibbles, galls and discoloring of leaves and shoots
3. Major leaf or needle fall not coinciding with natural shedding.
4. Knots or tumors on trunk and branches.
5. Scars, soft (rotten) spots, discoloring etc. bark
6. Any visible fungus attack.
7. Insect borings of wood
8. Any other visible insect or pest attack

j) **Wood properties**

k) **Photos of trees**

Some tree grading sheets include photos of the selected trees. If photos are taken, following measured should be observed.

1. Photographs should be carefully taken as the light conditions in the forest are generally poor.
2. The photo should be taken as close to the tree as possible in order to avoid disturbance of the picture from other trees or vegetation.
3. Light conditions in the forest are usually adverse. Especially on sunny days the contracts are pronounced. Pictures should never be taken against the sun.
4. A photo of a plus tree should show a picture of that one tree only: as sharp and clear as possible and excluding as much of the neighboring trees and vegetation as possible. In general a “scale object” should be pictured next to the tree, e.g. a person.

**Some desired characters for timber species**

- Fast growth: The tree is more vigorous than surrounding comparison trees
- Resistance to diseases: There are no sings of attack from insects, fungi or other pests.
- Growth form: The tree has a long, straight, clear bole without branches. There are no signs of twisting, forking or crooking. The branches are fine with a wide angle to the main stem.
- High specific gravity: The wood is dense and heavy.
- Long wood fibres: Long tracheid fibres appearing in microslides indicate wood strength.

**2.5 Seed orchard**

- **Seed Orchard (SO):** A plantation area or garden raised/tested specifically for the production of high quality seeds and in which the seeds are of high quality.
- **Seedling Seed Orchard (SSO):** Seed orchard which has been raised from the seedlings from seeds of plus trees.
- **Vegetative/Clonal Seed Orchard (CSO):** Seed orchard which has been raised by grafting clones of the plus tree.

**Definition**

A seed orchard is a plantation of selected clones or progenies which is isolated or managed to avoid or reduce pollination from outside sources, and managed to produce frequent, abundant, and easily harvested crops of seed (Feiberg and Soegaard, 1975).

A seed orchard is an area where seeds are mass-produced to obtain the greatest genetic gain as quickly and inexpensive as possible (Zobel et al, 1958).
- In some cases, seed orchards are established to mass-produce seed of some population of which it is impossible to obtain seed in adequate quantities, without too much attention being paid to the genetic superiority of the individuals.
- Seed orchards are the most important means the tree breeder has for mass producing seed for large improved plantations, based on the best selected tree. (Faulkner 1975).
- Seed orchard is defined as a plantation of genetically superior trees isolated to reduce pollination from genetically inferior ones, and intensively managed to produce frequent abundant and easily harvested seed. It is established by setting out clones or seedling progeny of plus trees (Khanna 1993).
- Seed orchard is different from seed production area, which is a good natural or planted stand managed intensively after removal of phenotypically inferior trees to increase seed production. Clones or seedlings used for desired characteristics.

Types of Orchard

1. **Clonal seed orchard** - Orchard established with vegetative propagules such as grafts, cuttings or tissue culture raised plantlets. Such orchard established with untested clones is known as **first generation orchard**. Clonal orchard developed with genetically tested clones (elite clone) is called **advanced generation orchard**.

2. **Seedling seed orchard** - Orchard established with seedling progeny (half-sib or full-sib) followed by rouging of inferior families as well as inferior individuals within family.

- Both type of orchards are established depending on the facilities, particularly in terms of trained personnel, and the genetic gain sought by the organization. However, clonal orchards are most commonly used.

A. Establishment of Clonal Seed Orchard

Production of clonal material

- The production of clonal material is through different vegetative propagation techniques.
- Commonly used vegetative propagation techniques are grafting, cutting, air layering, tissue culture etc., in case of grafting two individuals, rootstock and scion are involved. These may interact positively or negatively. The selected tree with desired characters is called scion, which donates bud material that is grafted on rootstock. The selected tree with desired characteristics, which donates bud material, is also called ortet.

Selection of orchard site and its preparation.

- An area which is easily accessible and near to main/regional station should preferably be selected.
- The selection of site is also influenced by the regional and local seed needs. Nearness to the center helps in easy monitoring of the programme.
- An area that will not be diverted for some other purpose like construction of road, market, dam, etc. in future should be selected.
- Select orchard site, which favours profuse flowering and fruiting. Favourable conditions are essential for regular, reliable and higher production of seed.
- Severe drought, wind and frost may all have an adverse effect on the orchard trees particularly on flowering and seed setting.
- Select an orchard site where problems due to destructive animals are less.
Poor sites are unsuitable for clonal seed orchard development. Abandoned agricultural lands with average fertility are the best suited for orchard establishment. Highly fertile land often delays flowering because of heavy vegetative growth.

Flat land is highly suitable for orchard development.

Establish orchard in an area with good drainage.

Remove all weeds and other bushes.

Plough the orchard site and level properly. Gently sloping land needs some sort of soil conservation measure.

Fence the area properly before field planting clonal materials.

Dig pits of optimum size, the size of the pits will vary (0.45 x 0.45 x 0.45 to 0.9 x 0.9 x 0.9 m³) according to the soil type.

Fill the pits with good soil, sand and FYM in the required proportion. Field plant grafts in a particular design during rainy season.

All plants should be labeled with plastic or aluminium tags for proper identification at a later date.

Size of orchard

The actual size of orchard depends on the total seed or seedling requirement.

The other factors affecting orchard size are location, importance of the species, availability of land in a particular locality and facilities.

In most cases the minimum size of the orchard should be 2.5 to 5 hectare.

This helps in planting minimum/optimum required number of clones and ramets of each clone to minimize related mating and also to have broad genetic base.

Clonal placement in seed orchard

Proper placement of clones in an orchard is highly essential to minimize selfing, relatedness among progeny and to increase chances of complete mixing.

The actual spacing between ramets of different clones for most of the tropical species varies from 4 x 4 m² to 8 x 8 m². The spacing also depends on the fertility level of the soil. Good soil promotes good growth.

A square or rectangular arrangement of ramets provides access for machinery between rows. First generation clonal orchard can be established with 40 to 50 untested clones.

Rouging of inferior clones will reduce this number to 20 or so. Advanced generation orchard may be composed of even less than 10 clones.

A number of field designs ranging from simple to most sophisticated computer designs are available for clonal deployment in the orchard.

Some of the commonly used designs for clonal dispersal are as follows.

i. Completely Randomized design:

The basic principle of randomized design is that no two ramets of same clone are planted in adjacent positions within rows or column, or that at least two different ramets must separate ramets of the same clone.

Such restrictions are imposed by manipulating the positions of ramets.

The complete randomization of all ramets of all clones between all planting positions are achieved by the process of randomization.

The table below shows randomization of 10 clones ( A to J), each clone being randomized 10 times.

The table below shows randomization of 10 clones ( A to J), each clone being randomized 10 times.
ii. Randomized Complete Block Design:
- In this design, the whole area is divided in equal blocks each sufficiently large to contain all the ramets of all clones.
- All ramets are completely randomized within each block, and also each block is randomized independently.
- This design is also simple to use and preferred commonly, but it required large area because of making blocks.

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| Block 1 | Block II |

iii. Fixed Block Design:
- In this design a systematic layout is replicated over the whole area as a fixed block. The major limitation of this design is that its size, total number of ramets and clones, and their arrangement are fixed.

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Pollen dilution zone
- The orchard should be protected from contamination by outside inferior pollen sources. This can be achieved either by establishing the orchard on sites where contamination by pollen of the same or related species is likely to be negligible or by creating a pollen dilution zone at least 100-200 meter wide.
- The actual distance will depend on the reproductive biology and breeding systems of the species.
- The pollen dilution zone is created by leaving 100-200m wide-open space or by developing 100-200m wide strip by planning other species. It is difficult to completely isolate the orchard as pollen moves with the wind and insects.
- Economic loss from pollen contamination can be considerable. Though it is costly proposition, proper maintenance of pollen dilution zone is always profitable.

Management of Clonal Seed Orchard
- Management procedures should be directed towards early establishment and healthy development of the clones and the promotion of sustained fruit yield.
- Subsoiling the orchard site to prune surface roots, helps greater root penetration and proliferation, and reduce surface water runoff. It also helps alleviate conditions of soil compaction.
Subsoil two side of the tree in the first year or depending on the establishment of the
grafts. Repeat the process after two years at right angle to the original direction of
subsoiling. Also subsoil the orchard just before flower initiation.

- Protect the floor of the orchard from wind and water erosion.
- Maintain adequate level of organic matter for proper nutrient. Establish good ground
cover to achieve these objectives. Any leguminous crop which grows fast may be
grown.
- Remove weed growth to benefit the tree from fertilization and irrigation.
- Avoid burning and grazing in the orchard as these results in damage to grafts and soil
compaction.
- Keep the floor of the orchard leveled and clean for ease of collection of seeds.
- Apply fertilizer to the orchard for promoting growth and vigour of clones when young
and to induce flowering at a later date. Use both nitrogenous and phosphorous
fertilizer to promote growth and flowering. Application of any compound fertilizer
like Di-ammonium phosphate (DAP) will supply both the nutrients. Apply fertilizer in
split doses around the periphery of the plant after proper soil working.
- Watering/irrigation should follow fertilizer application. Irrigate orchard at young age
too maintain good growth and vigour.
- Control diseases and pests as and when they appear in the orchard by spraying
insecticide and fungicide.
- Fire may be fatal to grafts and loss of complete orchard.

### Record on orchard site

1. Range
2. Block
3. Compartment
4. Area
5. Soil type
6. Topography
7. Site quality

### Record on site preparation

1. Ploughing
2. Leveling
3. Pit size
4. No. of pits
5. Fertilizer/manure applied
6. Quantity applied
7. Method of application

### Record on clonal materials

1. No. of clones
2. No. of ramets
3. List of clones
4. Tested/untested clones
5. Methods of clonal propagation

### Record on planting

1. Date of planting
2. Design used
3. Spacing

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Lecture notes on Tree improvement and Silviculture by Bishnu P Devkota, 2010
Establishment and Management of Seedling Seed Orchard

- The basic principles and methods of establishment of seedling seed orchard are same as clonal seed orchard.
- Collect open pollinated seed (half-sib) from selected plus tree in their original locality or from clones of these trees assembled in a first generation orchard.
- Maintain identify of seed lots by individual tree/ clone. Each lot will represent a family.
- Raise seedling (progeny) and establish seedling orchard
- The methods remain same except spacing. Use close spacing, say 4 x 4 m² for teak. This will help in keeping optimum spacing at a later date when inferior families and inferior individuals within family are removed on the basis of genetic test results.
- Follow the same methods of maintenance, management, seed harvesting and record keeping as described in case of clonal seed orchard.

Comparing Clonal and Seedling Seed Orchards

- Clonal seed orchard is a costly proposition than seedling orchard
- Clonal orchard flowers and fruits early than seedling orchard. However in species which flower early, seedling seed orchard is advantageous.
- If there is graft incompatibility or flowering and root deformation problem in rooted cuttings, seedling orchard is beneficial.
- More number of families can be accommodated in seedling orchard as compared to number of clones in clonal orchard. Thus, seedling orchard has wider genetic base but increases the probability of inferior families.
- Genetic gain from clonal seed orchard is higher than seedling seed orchard.

2.6 BSO’s in Nepal

- Breeding orchard, clone bank or research seed orchard are used to preserve and test large number of genotypes, not to produce massive quantities of seed for operational planting.
- The main objective is to obtain and retain a broad genetic base and to combine desired characteristics into suitable trees that will be valuable for future generations.
- In conventional genetic improvement includes selection, testing and breeding from species down to the clonal level. It demands high cost and long term technical and financial commitment.
- Additionally tree improvement programs in tropical countries are often required to work with many provenances of many species for many sites.
- A Multiple Population Breeding Strategy (MPBS) was proposed in response to this need and the BSO devised to combine the conventional hierarchy of sequential testing, selection and seed production populations in a single planting.
- Breeding Seedling orchard is an appropriate alternative to conventional breeding strategies, which are based on recurrent selections from a single breeding population and seed production from progeny tested clonal seed orchards.
- The BSO design is characterised by planting at a close initial spacing, followed by progressive and highly selective thinning, using available genetic information, until only the seed producing trees remain. Founder members of the next generation are selected from the final population.
- BSO can be established at three levels:
1. **Simple level BSO**
   - At this level, the design is fairly simple. In a simple-level BSO, family identity is not maintained.
   - An equal number of seedlings from each family (single tree collection) in a fully randomised mixture should be planted.
   - The randomisation is usually done prior to sowing, by mixing equal amount of seed from each family.
   - Where seeds from a lesser number of families is to be used, or where germination rates vary markedly from family to family, seedlings are propagated in family lots and an equal number of seedlings from each family should be taken and planted at random.
   - The size of the BSO should be minimum of two hectare.
   - Where simple level BSO’s are being established solely for seed production, and where there is no intention to base further breeding cycles solely on the material contained in the BSO, the selection of between 30 and 50 unrelated plus trees should be sufficient.
   - Higher the number, the better it is for both gene conservation and seed production.

2. **Intermediate (open pollinated family) and complex level (control pollinated families) BSO**
   - For intermediate and complex level BSO, the design is a randomised complete block, with a plot of each family (single tree collection) allocated at random within each block.
   - The minimum number of blocks should be three and will be that number required to meet estimated seed needs.

**Breeding material**
- Germplasm (seed or cuttings) from the selected candidate plus trees should be used to plant the BSOs.
- The genetic resources contained in these candidates plus trees dictate the first generation gains and the options that are available for further breeding.
- It is essential that the selection of these trees captures the best possible genetic material.
- Genetic gain could be achieved by selecting trees for their desirable morphological characteristics and then allowing them to cross.
- For most species there is a lack of information regarding genetic variation and rules of thumb for the minimum population size necessary to avoid inbreeding have to be used.

**BSO establishment in Nepal**
- BSO’s have been found as a robust, efficient means of implementing seed delivery oriented tree improvement activities in Nepal.
- Simple level BSO’s are suitable for most of the commonly used species.
- In Nepal, presently BSO’s of *Dalbergia sissoo*, *Choerospondias axillaries* and *Eucalyptus camaldulensis* have been established at various locations using seedlings raised from 84(at Sauraha, Chitwan district) plus trees.
- The number of plus trees should be sufficient to allow selection at both individual and family levels, while maintaining enough variation to avoid inbreeding depression.

**Selection and thinning regimes**
Selection and thinning regimes varies according to the growth characteristics of the species.

The guiding principle is that competition should not compromise growth, and regular selections and thinnings are required to maximise quality and quantities of seed production.

In ordinary regime, the best performing individual in each plot remains as a seed tree, but in the other regime there is also an allowance to remove some of the families.

In simple level BSO where main objective is for gene resource conservation, mechanical thinning will be applied regardless of its appearance.

Selection method

- The selection system used and the selection criteria applied differ from species to species, according to the characteristics being selected.
- If the BSO is principally established for ex-situ conservation the selection should be designed mechanical, i.e the removal of alternative trees at each thinning, regardless of tree’s appearance.

Intermediate level BSO management regime in *Dalbergia sissoo*

<table>
<thead>
<tr>
<th>Age</th>
<th>Average spacing (m)</th>
<th>Trees/plot</th>
<th>Trees/ha</th>
<th>Operations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.5</td>
<td>30</td>
<td>4444</td>
<td>Plant and assess survival</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>30</td>
<td>4444</td>
<td>Replanting as required</td>
</tr>
<tr>
<td>4</td>
<td>2.1</td>
<td>15</td>
<td>2222</td>
<td>Assess height, diameter and form. Thin 50%</td>
</tr>
<tr>
<td>6</td>
<td>2.9</td>
<td>8</td>
<td>1185</td>
<td>Assess height, diameter and form. Thin 50%</td>
</tr>
<tr>
<td>8</td>
<td>4.1</td>
<td>4</td>
<td>593</td>
<td>Assess height, diameter and form. Thin 50%</td>
</tr>
<tr>
<td>10</td>
<td>5.8</td>
<td>2</td>
<td>297</td>
<td>Assess height, diameter and form. Thin 50%</td>
</tr>
<tr>
<td>11+</td>
<td>8.2</td>
<td>1</td>
<td>149</td>
<td>Assess height, diameter and form. Select and thin 50%. Operational and production, collection and/or crosses for the next generation</td>
</tr>
</tbody>
</table>
2.7 Hybrids and hybridization techniques

2.7.1 Definition

**Hybrid**-Progeny from a cross among dissimilar genotypes. In forestry, the term is usually used for crosses between species.

**Hybrid Vigor**-growth superiority (wood qualities, cold hardiness, pest resistance, etc) in which hybrid exceeds that of both parents.

**Hybridization**-is the method of producing new plant varieties in which two or more plants of unlike genetical constitution are crossed together (Chaudhari, 2000).

- The plants which are crossed together may belong to the same species, different species or different genera.
- The main purpose of hybridization is to create variation. Although no new genes are produced by hybridization but, variation is created by bringing in new combinations of genes already present in the parental stock.
- During the course of hybridization work, many plants each possessing a separate combination of different characters are produced. Out of them some may be selected possessing all the possible good characters together.
- By further selections a variety may be produced from these plants and thus hybridization a variety containing as many economically valuable characters as possible may be produced.

2.7.2 Types of Hybridization

According to the relation of parental plants, the hybridization is divided into following categories.

a. **Intravarietal Hybridization**

   The crosses made between the plants of same variety. Such crosses are useful only in self pollinated crops, if the variety is mixture of different genotypes, which on hybridization produces new plants showing better combinations of economic characters.

b. **Intervarietal Hybridization**

   The crosses made between the plants belonging to two different varieties of the same species and are also known as intraspecific hybridization. This hybridization has been the basis of improving self pollinated crops as well as certain cross pollinated crops.

c. **Interspecific or Intrageneric hybridization**

   The plants of two different species belonging to the same genus are crossed together. This hybridization is between the species and with in the same genus. It is commonly used for transferring the genes of disease, insect pests and drought resistance varieties. All the interspecific hybrids of two homozygous plants are uniform as a consequence of their identity in genetic constitution.

d. **Intergeneric hybridization**: The crosses made between the plants belonging to two different genera. It is usually used for transferring the characters like disease, insect and drought resistance from wild genera into the cultivated plants.

e. **Introgressive hybridization**: In this type of hybridization one species is completely replaced by another in nature. If there is free intercrossing among the plants of two species and population of both the species is equal at the beginning of hybridization, theoretically the hybrid swarm should be distributed according to normal frequency curve. However, if one species is more abundant than the other, the F1 and subsequent
hybrids will have more opportunities of backcrossing to that species than to the less abundant species. After a few such backcrosses, most of the individuals of hybrid population will appear as pure species rather than hybrids. This absorbing of one species by another is known as introgressive hybridization.

2.7.3 Object of Hybridization
Hybridization is practiced in every type of crop, especially when no further improvement can be achieved in the local as introduced material by any other method. The following of are the main aims of hybridization.
- To combine all the good characters into a single variety
- To increase the range of genetic variability by introducing various recombination of characters
- To exploit and utilize the hybrid vigor.

2.7.4 Prerequisites for Hybridization
Before implementing hybridization programme, the following requisites are to be considered and the plant breeder must be quite familiar with them.
- All the requirement of the tract either from agriculturists’, foresters’, local people’s test or industrialists’ point of view.
- All the local conditions, i.e., variations in the soil, climate, agronomic practices and market and industrial conditions.
- Facilities of fund, land, labour and equipment to grow and evaluate parental material as well as hybrids.
- Existing varieties of crops, both local as well as introduced as to know how far they satisfy the requirements and then their utility as parents in the crossing programme.
- Plant material, i.e., a thorough comparative information of different varieties of a crop with regard to original habitat (soil, water requirement and cultural practices); morphological and physiological peculiarities, floral biology (sex, habit and time of flowering, anthesis and dehiscence); crossing (pollination, fertilization, compatibility, sterility, inheritance of characters, etc); adaptability to locality; resistance to diseases, insects and drought; etc, must be kept in mind.
- Objectives to be attained, i.e., yield quality, hardiness, resistance, thrashability, etc.

2.7.5 Hybridization Procedure
Practical hybridization is a technical operation and requires skilled hands. The various steps involved in this operation are serially described below.

1. Selection of parents: The first step in hybridization is to select the plants which are to be used as parents and can supply all the desired important characters which lack in a good standard variety. This requires the collection of material and its thoroughly testing.

2. Self pollination or selfing of parents: This is the second step consisting in artificial self pollination of parents. It is therefore very essential for eliminating the undesirable characters and obtaining inbreeds. Selfing is automatically performed in self pollinated crops when they are allowed to follow their natural mode of pollination. In them seeds of individual plants are harvested separately to isolate different inbreeds from the mixed heterogenous populations. In cross pollinated crops, the selfing is comparitively cumbersome and the parents are artificially selfed in different ways. Whatever may be the way of selfing its operation must be quick, safe and inexpensive.
3. **Emasculation**: this is the third step in hybridization and defined as the removal of stamens from female parent before they burst and have shed their pollens. The purpose of emasculation is to prevent self fertilization, and therefore, it is usually performed a few hours before the anther ripe, dehisce and self pollinate their stigmas. Emasculation is not needed at all in unisexual, i.e., monoceious plants but it is very essential in bisexual plants of self as well as cross pollination. It can be done variously as follows:

- **Forcep or Scissor method**: It consists in opening of flower and removing of the stamen which can be accomplished easily by using a pair of forceps or scissor. This technique of emasculation is generally used with those plants which bear large flowers.
- **Hot or cold water and alcohol emasculation**: this is done by dipping the panicles in hot water having a desired temperature (45º to 53ºC) for a definite time (1 to 10 minutes) period. Similarly, cold water or alcohol emasculation is carried out.
- **Male sterility method**: in some self pollinated crops emasculation may be eliminated by the use of male sterile plants which have sterile anthers and do not produce any viable pollen. The male sterility conditioned by recessive genes is first introduced into the plants to be used as females by backcrossing and the emasculation in them is then needed at all. Male sterility may also be induced by spraying some chemicals such as 2, 4 D, naphthalene acetic acid (NAA), maleic hydrazide (MA) and tri-iodobenzoic acid.

4. **Bagging**: this is the fourth step and is completed with the emasculation in bisexual plants and before the stigma receptivity and dehiscence of the anthers in unisexual plants. Both male and female flowers are bagged separately to prevent contamination in staminate flowers and cross pollination in pistillate flowers. The pollens are also collected only from already bagged males for crossing purpose.

5. **Crossing**: It is the fifth step and is anthesis of inbreeding. It can be defined as the artificial cross pollination between the genetically unlike plants. This operation consists of collection of the viable pollens or anthers from the desired male parent and transferring them on to the stigma of desired emasculated female parent.

6. **Labelling**: the crossed flowers are properly tagged and labeled. The labeling is done either on the bag itself or on the labels specially designed for this purpose. They are tagged to bags with the help of threads. The labeling on them must be as brief as possible but complete, bearing the following information:

- Number referring to the field record
- Date of emasculation
- Date of crossing
- Details of parents, male and female

All other necessary particulars must be entered in a handy field record book in which the observations are also recorded from time to time.

7. **Harvesting hybrid seeds and raising F1 generation**: in this step bags are removed and the crossed heads of desirable characters are harvested and collected with their attached labels separately in envelopes. After complete drying, they are threshed individually and preserved as such. In the coming season, these seeds are sown separately to the F1 generation. All the plants of F1 generations, although heterozygous, have similar genetical constitution and look exactly alike. They may or may not show hybrid vigour. The term hybrid variety was used for many years to identify many years to identify varieties developed by hybridization. This term is now used only for those varieties that
express hybrid vigour, i.e., increased growth, size, yield or function over the mean of parents.

8. **Trials, multiplication and distribution**: In this step, testing, multiplication and distribution of so produced hybrid varieties is carried out. The testing is done at various regional research stations by research workers. The seeds are multiplied at seed multiplication farms situated at different localities and distributed.

2.7.6 Methods of Hybridization

2.7.6.1 Hybridization in Self Pollinated Crops
- **Pedigree method**: The individual plants are selected from the F1 population on the basis of desired characters. The number of plants to be selected depends upon the number of important characters involved, the importance of breeding problem, and the assistance and facilities available. These plants are harvested and threshed separately, and sown in the separate rows in the next year to raise the F2 generation. This process is continued up to F4 or F5 generation. The plants uniform in desired characters is harvested and bulked together to constitute a variety.
- **Bulk Method**: In this method F3 plants are not maintained separately but are bulked together to form a single F3 population. In F3, again the suitable plants are selected, collected and bulked together. This bulking is done for six generations. In F6, the desired individuals are selected and harvested separately and new variety is released.
- **Back cross method**: This method is employed in improvement of both self and cross pollinated crops where varieties are deficient in one or two aspects particularly for transferring a single simply inherited characters like disease, frost or drought resistance. In this method F1 plants, instead of permitting to self pollinate as in pedigree or bulk method, are crossed with the recurrent parent and therefore it is called as backcross method. The purpose of backcrossing is to recover the genotype of recurrent parent with disease resistance. Back cross method has got many advantages as
  a) Independence from environment, ie, it can be used in any environment where the plant will grow and character under transfer will express itself.
  b) No need of evaluation of performance, ie, back cross derived varieties can be safely released to growers without evaluation of yield, adoption or quality
  c) It is rapid
  d) It requires a small number of plants
  e) It is repeatable
  f) It is predictable
- **Multiple / composite cross method**: It consists in crossing of several pure-lines together. The selected pure-lines are first combined into crosses as A x B, C x D, E x F, G x H, and so on. F1 of these single crosses are then combined into double crosses as (A x B) X (C x D) and (E x F) X (G x H). Finally the F1s of double crosses are crossed with each other to produce the hybrids [(A x B) X (C x D) X (E x F) X (G x H)]. This cross is known as multiple cross and further breeding in these hybrids are carried out according to either pedigree or bulk method.

2.7.6.2 Hybridization in Cross Pollinated Crops
- **Single cross**: This is the cross between two inbreeds, such as A x B or C x D and its hybrid seed is distributed to growers. This gives the maximum degree of hybrid vigour and produce most uniform plants.
- **Double cross:** This is the cross between two single crosses involving four different inbreeds as (A x B) X (C x D). It is also advisable to combine the similar or closely related inbreeds in the single crosses and the different or distantly related inbreeds in double crosses. Double crosses are the most widely used commercial hybrids. Some double crosses were made in Pinus in Queensland, e.g., \((P. \text{elliottii var. elliottii} \times P. \text{caribaea var. hondurensis}) \times (P. \text{elliottii var. elliottii} \times P. \text{caribaea})\).

- **Three way cross:** This is a cross between a single cross used as female and an inbred used as male, i.e., it involves three inbreeds, as \((A \times B) \times C\). The advantage of three way cross is the use of vigorous hybrid of first generation as female in order to maximize the yield of hybrid seed as well as obtain seeds of normal kernel size. It is intermediate between single and double crosses in its characteristics. Some three way crosses were made in Pinus in Queensland, e.g., \((P. \text{caribaea var. hondurensis} \times P. \text{tecunumanii}) \times P. \text{elliottii}; (P. \text{elliottii var. elliottii} \times P. \text{caribaea var. hondurensis}) \times P. \text{tecunumanii}\).

- **Top cross or inbred variety cross:** this is a combination between an open pollinated variety and inbred line. Either the inbred or the variety may be used as female parent, but to use variety as female parent is preferable. The top crosses are used mostly for testing the combining ability of inbreds and not for commercial hybrid seed production.

- **Synthetic cross:** This is the combination of a number of inbreds, sibbed lines, or clones to utilize the desirable characters from different sources. It eliminates the necessity of producing seeds in an isolated plot because they are allowed to pollinate naturally. The synthetic crosses have been utilized in forage crops where floral structure causes difficulties in artificial pollination.

### 2.7.7 Difficulties and Precautions in Hybridization

1. **Isolation of suitable parents and hybrids:** Most difficult part of hybridization is to recognize and isolate the desirable inbreds to be used as parents and hybrids to constitute the variety finally. This require careful observation, through testing of all selected plants and their progenies, subjection of selected lines to diversities such as disease, drought or cold, accurate and exhaustive record keeping and finally up to date available information and trained plant breeder for identifying the potentially desirable lines with very high degree of accuracy.

2. **Different times of maturity:** Always the plants grown in the same season are selected for crossing, but they may not flower together due to difference in their time of maturity.

3. **Susceptibility to mutilations:** During emasculation and crossing, the floral parts are touched with hands; anthers are removed totally to facilitate the hybridization. Due to these, the flowers are subjected to mutilations and the species vary in the amount of mutilations they can tolerate.

4. **Incompatibility and sterility:** The incompatibility and hybrid sterility are common in both interspecific and intergeneric crosses. Incompatibility may be due to many causes ranging from simple morphological differences to complex physiological relations. The simplest probable cause of unsuccessful crosses is failure of viable pollens to germinate and this trouble can be controlled by the application of a film of water of weak sugar solution to the surface of stigma before pollination. The causes of sterility are numerous, all originating from genetic imbalance which is responsible for meiotic irregularities causing hybrid inviability.
2.7.8 Bases of Hybrid Superiority

- In tree improvement, a single, large population may be chosen as a base for recurrent breeding, or one or more episodes of crossing between a few populations within a species may be used to initiate a single, diverse base population which is then managed for population improvement by means of recurrent selection.
- Hybridization is an alternative breeding strategy, in which at least two separate, conspecific or other breeding populations are maintained as separate entities and managed in such a way as to attempt to maximize the merit (hybrid superiority) of interpopulation cross.
- Phenomena which may be associated with hybrid superiority are heterosis and complementarity. Heterosis occurs as a result of non-additive gene actions for the characters of interest, and the level of heterosis increases with increasing difference in gene frequencies between the two populations being crossed. Some cases of apparent heterosis in forest tree hybrids might be due to complementarity in different pairs of some traits, e.g., frost resistance and drought hardiness.
- In forest trees, a variety of characters - e.g., adaptabilities, growth potential, disease and insect resistance, ease of vegetative propagation, stem and branch quality and wood properties - appear to show complementarity among species or provenances in some genera, e.g., tropical eucalyptus in the Congo (Vigneron, 1991 cited by Nikles, 1993).
- Complementarity is also evident in many eucalypt hybrids produced in Brazil, China and South Africa. It is also apparently demonstrated in the performance of F1 hybrid of Pinus elliottii var. elliottii and P. caribaea var. hondurensis in Australia (Nikles et al. 1987 cited by Nikles 1993).
- These parental varieties are moderately crossable, and are characterized by a number of complementary characters, including seasonal patterns of growth, tolerance of poorly-drained and droughty soil conditions, stem and branch quality, wood properties and stability in response to strong winds.
- Because of this performance capability, and the development of mass propagation technologies, the hybrid is the preferred taxon for plantation establishment in a wide variety of environmental conditions in Australia (Queensland and New South Wales), and it is promising in a number of other countries where it has been tested (Barret et al. 1991, Haines 1992, Nikles 1991 cited by Nikles 1993).

2.7.9 Advantages of Hybridization

Hybridization provides the following advantages, so it is used in tree improvement.

1. Creation of heritable variation: The crossing of unlike individuals gives rise to entirely new plants showing variations in their characters as compared to their parents. This variation is not due to creation of any new genes but simply due to Mendelian recombination of the genes already present in the population.

2. Production of superior varieties: Most of the economic characters of crop plants result from interactions of many genes scattered over several chromosomes in different plants. Hybridization brings all these useful factors together and concentrates them into a single variety. Thus,
   - A variety possessing all the desirable characters such as high yield, fast growth, good quality, resistance to disease, insects, frost, drought, fire etc. is produced.
   - The varieties suited to every condition (environmental and others) and need of men can be evolved.
• Thirdly, the varieties produced by hybridization are more vigorous exhibiting hybrid vigor.

2.7.10 Disadvantages of Hybridizations
1. **Tedious, time consuming and expensive procedure:** Usually hundreds of crosses are made before obtaining an individual possessing the desired combinations of characters. If only one or two characters are required to be brought into a variety, the task is much less difficult. The crossing and handling of crosses need a high technical training and practical experiences. It is a painstaking task and when carried out extensively, it is time consuming and therefore, most expensive among all the breeding procedure. Mass production of F1(specially interspecific) hybrids may be constrained by a degree of incompatibility, problems of pollen handling, high cost of emasculation and controlled crossing, or difficulties of seedling or clonal propagation.

2. **Hybrid sterility:** Sterility is the main handicap in achievements to be made by hybridization. It is more or less absolute in wide crosses and comparatively very less in intervarietal crosses.

3. **Technical procedure:** Hybridization, being highly technical and complicated, can only be adopted by experienced plant breeders and can not be advised to farmers/growers for adoption because the farmers accept only the achievement and not the experiments.

2.7.11 Application of Hybridization in Tree Improvement
Genetically modified forest trees are used commercially; the use of materials with controlled reproduction should be a prerequisite. The traits most likely to be targeted for improvement are insect resistant and wood quality (changes in the composition and amount of lignin) for use in commercial plantation forestry. The aim would be to reduce inputs and increase yields to meet increasing demand of wood, pulp and paper based industry. Hybridization has the potential to deliver large gains in productivity more quickly than population improvement. Such gain may result primarily from hybrid superiority of growth and/or of more favorable trait combinations. This can be so even when there is great viability within the populations being considered for hybridization which is normally exploited first by population improvement (Nikles, 1993).

2.8 Vegetative propagation and tree improvement
- The use of vegetative propagation is rapidly increasing and its vital importance to tree improvement.
- Vegetative propagation has been successfully for several centuries by horticulturists. The older horticultural practices as well as the new methodology are being increasingly applied in tree improvement program.
- Vegetative propagation has been employed in forestry for more than 100 years.

2.8.1 Uses of vegetative propagation
- Preservation of genotypes through use of clone banks.
- Multiplication of desired genotypes for special uses such as in seed orchards or breeding orchards.
- Evaluation of genotypes and their interaction with the environment through clonal testing.
- Capture of maximum genetic gains when used for regeneration in operational planting programs.
Use of vegetative propagation can be separated into research and production phases.

A. Research uses of vegetative propagation
1. Genetic evaluation of plant material
2. Determining magnitude and control of common environmental effects
3. Preserving genotypes and gene complexes in clone banks and arboreta for scientific purposes and for possible later use in operational programs
4. Bring valuable plants to a centralized area for further study
5. Speeding up the reproductive cycle for accelerated breeding and testing
6. For nongenetic studies—reduce genetic variability (or to obtain the information to handle it statistically) in expt that will reduce “error variation”

B. Production (operational) uses of vegetative propagation.
- Preservation of genotypes through use of clone banks
- Multiplication of desired genotypes for special uses such as in seed orchards or breeding orchards
- Evaluation of genotypes and their interaction with the environment through clonal testing
- Capture of maximum genetic gains when used for regeneration in operational planting programs

Advantages of vegetative propagation in operational plantations.
- Greater genetic gains are possible.
- More uniform tree crop or stand results.
- Provides an opportunity to get faster results from tree improvement activities under certain situations.
- All the genetic potential of donor tree is captured and transferred to the new tree.

2.8.2 Methods of vegetative propagation

A) Propagation by cuttings
Vegetative propagation is commonly carried out from cutting of vegetative parts of the donor plant. The cuttings of vegetative parts are of three types.

Types of cutting
- Stem cutting
- Root cutting
- Leaf cutting

Stem cutting
- A segment of stem branches can be rooted to produce full plant.
- Size of cutting varies from plant species to species.
- Presence of buds on cutting is very essential for propagation
- Presence or absence of leaves affect rooting system.

Leafless stem cutting
- Leafless cutting are commonly known as ‘hardwood cutting.
- These cutting are branches with minimum if 2 nodes.
- Lower cut is made below the lower node and upper cut is made above the upper node.
- Hard woody cutting do not dry up fast and can root without much difficulty.
The hard wood cuttings are of three types:

1. Mallet: A segment of older branches is present at the base of cuttings and its appearance is somewhat like the inverted ‘T’.
2. Heel: It contains small chip of wood from older.
3. Straight: It is a branch segment and does not contain a branch of its base.

Leaf stem cutting:
- One or two terminal leaves are left in the cuttings.
- The presence of leaves is advantageous to rooting because they become the source of carbohydrates and auxins.
- Presence of leaves also prone to desiccation.
- Thus leafy cutting require special propagating structures like mist chamber or poly tunnel to maintain humidity.
- Semi leafy cutting require special propagating structures like mist chamber or poly tunnel to maintain humidity.

Root cuttings:
- Root segments are capable of regenerating into full plant.
- Root cuttings are collected at active growth period. At this time they have sufficient stored food material.
- First adventitious shoots are produced from the root cutting and then roots are formed from new shoot.
- Those plants which propagate from root suckers can be propagate by this method.
- The proximal end of the cutting should always face up.

Leaf cutting:
- Leaf cuttings are formed by two mechanisms:
  - From pre-formed embryos present on the margin of leaves.
  - Through induction of meristem.
- Propagation by leaf cuttings has limited utility.
- Only small group of species can be propagated e.g. *Saintpaulia* and *Begonia* rex.
- Leaf bud cuttings are also a kind of leaf cutting, where leaf blade petiole and a bud is the starting material this structure is rooted and a full plant is produced e.g. *Rhododendron*, *rubber*.

Factors affecting Rooting in cuttings

Rooting Phenomenon is influenced by several plant (internal) and environmental (external) factors.

Plant Factors
- Physiological state Juvenile or mature cuttings.
- Hormones – It is organic compound helps in regulate rooting in plants cuttings. Auxin is most important root hormones.
  1. Quick dip method: Auxins are dissolved in ethyl alcohol and cutting is dipped for 1-5 second.
  2. Talcum- powder method: Auxin is diluted with talcum powder and base of the cutting is put into the powder.
  3. K- salt Formulations: Such formulations are water soluble for treatment the basal ends of cuttings are soaked in auxin.
solution for 4-12 hrs.
Cytokinins- It is responsible for cell division and helps for carbohydrate loading which helps for rooting phenomenon.

**Environmental Factors**
- Humidity – RH – 90%  
- Temperature- Temperate- 25 degree C, Sub tropical – 35 degree C  
- Light – Low light irradiance is required.  
- Diseases and pests.

**Other Factors:**
- Rooting medium sand, saw dust, pest moss, coconut dust, composted coir piths and perlite.  
- Rooting contains  
  - Trays  
  - Pots  
  - Poly bags.

**B) Propagation by Layering**
In layering adventitious roots are initiated on the stem or branch of a tree, Which after induction of roots is detached and planted or roots a small part of ring of bark is removed. The area is kept moist by covering it with peat moss, soil etc. and wrapping it with polythene covering to avoid moisture loss. After some time the roots are produced and at this stage the branch is cut and planted. In layering there xylem connection, and hence there is adequate supply of nutrients and water to the branch and leaved, which contributes sufficient amount of carbohydrate production through photosynthesis. In addition, this helps in better rooting in the layering. In contrast, is a cutting is detached from the mother plant and is kept for rooting, the plant utilizes stored food and if leaves are left the cutting may dry up fast because of excessive loss of water through transpiration.

**Types of layering**

1. **Simple layering**
   - Flexible lower branch is bent and part of it is buried in the soil.  
   - After sometime, roots are termed on the buried area and then cut it and plant.  
   - To increase the removal of rooting bark, girdling or wiring can be done in buried part

2. **Serpentine layering:**
   - Long flexible branch is buried at more than one point giving it appearance of a snake.  
   - more than one plant can be obtained from a single branch

3. **Air Layering**
   - More useful in forestry.  
   - Bark removed from a small area of the branch  
   - Debarked area is covered with moist moss and again covered with polyethylene.  
   - After sometime roots developed and at this stage cut the branch and plant it.

4. **Mould layering**
   - It is also known as stool layering
- Stump is cut close to the ground and new shoots came out and grow into a certain height.
- The base of the shoots is covered with rooting medium.
- After the induction of the roots the shoots are removed and planted. It is practiced in *Santalum album* and *Pterocarpus santalinus*.

5. **Trench layering**
- Mother plant is laid down flat in the trench and covered with rooting medium.
- New vertical shoots come out from nodes and develop roots.
- The rooted shoots are removed and planted

**Advantage of layering:**
- Layering do not experience water stress and chances of drying are less
- Root development is much better in layering
- Larger plants are obtained for planting and being mature they can flower and thus useful for ornamental tree propagation.
- It is important for large planting materials with less number.

**Disadvantage:**
- Labor intensive and need large nursery space.
- Only few plants can be produced.
- This technique is not suitable for mass propagation programs.

C) **Propagation by Grafting**

Grafting is a technique of a joining two or more plants species in a manner that they start growing together and subsequently results in a complete plant. The piece of plant that contains dormant buds is joined to another piece of plant, which contains its own root. The former is known as scion and the latter is known rootstock. Scion grows into a shoot system, where as rootstock developed into a root system of a grafted tree. Grafting in forestry is done only when the species is difficult to propagate through cuttings. It is extremely useful in raising clonal seed orchards where superior clones are grafted on to the local rootstock.

**Mechanism of union formation:**

Union formation can be divided into three steps:
- Adhesion of scion to the root stock.
- Callus formation between scion and root stock.
- Formation of vascular tissues (Xylem and phloem)

It is essential that the cambium of scion and root stock touch each other for union. If the cambium of scion is not in contact with the rootstock, union of the graft is not possible. Grafting is easy in dicotyledonous plant than in monocotyledonous because cambium layer in dicotyledonous plants is present in the form of a sing between phloem and xylem, thus it is quite easy to match the cambium layer of scion with that of root stock. To make scion and root stock intact they are tied together firmly. The cells from both scion and root stock divide and intermingle with each other the space between scion and root stock gets filled with callus. Some cells nearer to cambium differentiate into new cambium differentiate into new cambium and this forms new secondary xylem and phloem. The formation of vascular connection then completed the formation of graft union.
Types of Grafting
There are major two types of grafting
1. Detached grafting
2. Approach grafting

1. Detached grafting
The scion is cut from the mother plant and joined to the root stock is a process of detached grafting. The following are the methods of detached grafting:

Whip/ Splice Grafting
- Scion is removed from the mother plant by oblique int.
- The opposite oblique cut is made into the rootstock and the top portion is removed.
- Then scion is replaced into the rootstock ensuring cambium contact and then tied firmly with tape or rope.

Whip and Tongue Grafting
- The tongue is made in scion which exactly fit in the rootstock having the similar opposite cut. This gives better cambium contact.

Cleft Grafting
- The top portion is sawed off and care is taken that the cut is made smooth.
- Cleft is made with sharp knife
- The scion is cut in the same way.
- 2 scions are inserted into the cleft at 2 edges.
- Graft is covered and tied firmly.

Saddle grafting
- Two cuts are given either side on the rootstock and it looks like inverted ‘v’ and similar cut is made on the scion. The scion is kept on the rootstock and tied.

Side- Stub Grafting
- The oblique cut is made in the side of rootstock by applying slight pressure on the branch with the hand above the cut.
- Scion of similar size and with a wedge shaped base in inserted into on opening and the pressure is released.
- The scion is inserted with maximum cambium matching.
- Then tied firmly and covered with the help of grafting wax.

Side –tongue grafting
- The scion is prepared like-that whip and tongue grafting
- Similar but opposite cut is made on the side of root stock
- The scion and root stock are joined and tied.
- After the union formation top portion of the rootstock is removed and scion allowed growing.

Side – Veneer Grafting
- Slanting cut is made at the base of scion and a similar cut is made at the side of rootstock
- Scion is joined and tied.
- A piece of stem from the side of the root stock is replaced by the scion from another plant.

Bark grafting
- Rootstock is cut horizontally and vertically downward cut is given from the cut end and bark is lifted.
- The scion is prepared by giving a long slanting cut on one side of the base.
- The scion is inserted under the lifted bark the cut facing to the exposed wood of the root stock and is nailed.
- The area is then covered with wax.

2. Approach grafting

In such grafting the scion is not removed from the donor plant for grafting. The scion branch and root stocked are grafted and after the union formation the scion branch is cut below the union and rootstock just above the union. This method is relatively easy, because both rootstock and scion have their root system and both are self- sustained plants.

**Splice Approach grafting**
- A thin slice bark along with wood is removed from the side of root stock and scion branch.
- The branches should be flexible and of same thickness
- The exposed area of scion and rootstock joined and tied together.
- After union formation scion branch is cut just below the graft union and root stock just above graft union.

**Tongue Approach grafting**
- The tongue is made on both scion and root stock which leads a better interlock.
- Then tied firmly.

**Repair Grafting**
The repair grafting is done to save or repair the damaged tree and is not associated directly with the propagation of trees.

D) Propagation by budding

Budding is technically similar to grafting scion is a single bud where as it is small segment of short consisting of more than one dominant bud. Budding is alone when plant is at active growth state, because at this time bark with bud can be easily separated from the shoot to break the dormancy of the bud the shoot is removed just above the place where budding has been done which is done just before the advent of active growth period, when budding is done in active growth period, the shoot is removed after union formation.

**Union Formation**
The basic steps of union formation are similar to that of grafting as the bud is inserted between the flaps of bark of rootstock. Callus formation takes place mainly from the vascular cambium of the rootstock and it covers the bud as well as fills the space between scion and rootstock.

**Types of Budding**
The following are different types of budding:

**Patch Budding**
- Rectangular patch of bark is removed from the rootstock and this piece is replaced by a patch of same size, containing single bud from the donor plant.
- The patch bud is then covered tightly with the help of polythene tape.
**Forkert budding**
- Rectangular bark is not completely removed from the rootstock and it remains attached as a flap at the lower end.
- Rectangular patch of scion bud is placed over the removed bark of rootstock, covered with the lower flap and tied.
- After union formation the flap is cut and removed.
- This method is success in teak and rubber.

**Chip Budding**
- The bud is removed along with a piece of wood and similar piece is removed from the rootstock and replaced by chip bud
- The two pieces than tied.

**T- Budding**
- T- Shaped cut is made in rootstock deep enough until the wood is reached.
- Then the flaps are opened and shield shaped scion bud is inserted and tied.
- When the incision is made in the shape of an inverted T, the duding is called inverted 'T' budding, it is usually done to prevent water entering the grafted area.

**I – Budding**
- I – shaped cut is made on the bark of rootstock
- A rectangular patch of scion bud inserted between the opened flap on the rootstock

**Ring Budding**
- A complete ring bark is removed from rootstock and replaced by a ring of bark containing a bud from the donor plant. The bud is tied as usual.

**Flute Budding**
- Instead of removing full bark ring, a small strip of bark is left, it is because not to disturb the phloem connection
- The removed part is replaced by a similar patch of scion bud.

**Advantages of budding**
- Budding is more economical than grafting
- If the scion material is in limited quantity, more number of plants can be obtained through budding than through grafting, because each bud can be utilized to produce single plant.
- Budding is easy to perform
- Union formation is better.
E) Tissue Culture

Plant cell have the potential to grow as a plant. This ability of the plant cell is called totipotency. Because of this ability any plant part can develop a plant as a whole. These plant part cells can be grown artificially in the laboratory condition. Thus, the plant tissue culture is defined as 'a technique of growing plant cells, tissues or organs in an artificially prepared nutrient medium under an aseptic condition in the laboratory'.

Types of Tissue Culture:
- Each and every part of the plant has got potential to develop a plant. Plant parts such as root, bud, shoot, leaf, anther ovule, etc can be used as explants for plant tissue culture. On the basis of the explants used for tissue culture plant tissue culture can be of following types.

Shoot Culture
- Various adventitious, apical and axillaries shoots can be used as explants for Tissue Culture.
- Shoots can be cultured on to a plant tissue culture medium to develop plant.
- This technique is known as clonal propagation
- Shoots to plants.

Callus Culture
- In this process, a large number of plantlets can be obtained from callus.
- Callus is an undifferentiated mass of cells.
- It is formed around the cut edges of the segments (explants) of plant organs when placed on solidified culture medium
- These callus developed into new plants
- Large number of plants can be obtained in a short period of time
- Tissue to callus to plants.

Meristem Culture
- Meristem is the apical growing part of plant which is always multiplying.
- It is the technique to culture meristem aseptically in on artificial medium in the laboratory.
- It produces virus free plant.
- This technique has its major application in eliminating various diseases.

Embryo Culture
- Ovules are excised and embryos are dissected out and transferred to culture medium.
- Knudson, 1922, grew orchid embryos into plantlets by culturing them on agar medium containing sugar.
- Embryo to plant.
Anther Culture
- It is a means of obtaining haploid plants from pollen grains
- Anther is used as explants
- This process is called 'androgenesis'.
- Bourgin and Nitsch, 1967, obtain flowering haploid plants in *Nicotianum tabaccum* and *N. sylvestris*.
- It produces haploid and homozygous plant so there is a special interest to geneticists and plant breeder.
- Anther to callus to plant.

Protoplast culture
- Protoplast is a cell without cell wall.
- The cell wall is dissolved by enzymes.
- Protoplast can be isolated from leaves, stem, callus, and from pollen grains.
- This can be fused with the protoplast of other compatible species and new cell with characters different from the parent can be obtained.
- This material is important tool for genetic engineering.
- Introduction of foreign DNA, gene can be achieved at this stage very easily.
- The protoplast can be cultured and regenerated into plantlets.
- This process is protoplast culture.

Explant Selection
- Explant is a part of a plant or tree, which is used for Tissue Culture.
- Explant must contain meristematic cells, capable of division and differentiation.
- It is better to use juvenile material as an explant.
- Stem pieces with buds, leaf pieces, root lips, floral parts embryo, cotyledons, isolated cells protoplast etc, can be used as explant.
- Stem tip explant found good for rapid propagation
- Explant should always be collected from a superior mother tree that is free from diseases.
- The season also affects the success of the culture.
- If the explant is collected in dormant season , it is necessary to break the dormancy of explant
- Removal of bud scale and gibberelic acid treatment are some treatments to break dormancy

Methods of Plant Tissue Culture

Media for Culture
It is an important component of Tissue Culture. The medium is the source of macronutrient micronutrients, vitamins, growth regulators, carbon etc, which are needed by the cells for normal growth. Plants when grow *in-vitro*, solely depends on the nutrients contained in the medium. Nutrition requirement of different species may vary. So a wide range of culture media differing in their elemental compositions has been formulated for culture of plant cells.

Tissue culture can be maintained either in liquid medium or semi-solid medium. In the liquid medium, plant material is immerse in the medium partially or completely while in the solid medium plant material is placed on the surface. The medium contain inorganic nutrients, organic nutrients and growth hormones.

Inorganic nutrients
- Macronutrients- nitrogen, calcium, potassium , phosphorus, sulphur, magnesium (6)
- Micronutrients – iron, manganese, copper, boron, zinc, molybdeum (6)
Organic nutrients
- Supplement of organic nutrients for better growth is required
- Organic nutrients are amino acids and vitamins
- Commonly used vitamin is thiamine.
- Others are nicotinic acid, calcium pantothenate and pyridoxine.
- In order to promote callus growth some substances are added in the medium, these are yeast extract, coconut milk, tomato juice and malt extract.

Growth hormones
- Hormones used are: Auxins, cytokinins and gibberellins.
- Auxins help to promote cell division and root differentiation
- Cytokinins help to cell division and shoot differentiation.

Agar
- It is used to solidified the medium
- This prevents the diffusion of nutrients into the tissues

pH
- Adjust pH between 5 to 5.8 by adding NaOH or HCL

Media Selection
There is not a single medium suitable for culturing all cells, tissues and organs. It is necessary that most suitable medium be determined to accommodate specific requirements of the plant material. Any media developed must free from any contamination by sterilization.

Sterilization
- The media used for culture also provides suitable environment for the growth of microorganism like bacteria and fungi.
- These organisms grow faster than the cultured tissues and produce toxic metabolites.
- It inhibits the growth of explant or even kills it.
- Therefore, it is necessary to maintain a completely aseptic environment inside culture vessels.

Sources of contamination
There are a number of source through which medium may get contaminated. They are discussed below:

Medium itself:
- Medium itself may be present microbes right from the very beginning
- To eliminate them, vessel containing the medium is sterilization with autoclaved or in pressure cookers at 12 degree c for 15-20 minutes.

Culture vessels and instruments:
- Glassware and metal instruments are sterilized by placing them in an oven at 160 -180 degree centigrade for 3-4 hours;
- The instruments like scalpels, forceps and needles are dipped in 95% ethyl alcohol and flamed to sterilize before use.

Plant material:
Explant material must be surface sterilized before placing on the medium.
Calcium or sodium hypochlorite solutions are used for surface sterilization.

**Transfer area:**
- Aseptic condition is made in a closed transfer area fitted with gloves and ultra violet lamps.
- Ultra violet sterilizes and eliminates atmospheric contamination.
- In tissue culture laboratories, laminar air flow cabinets are used. Air passes through ultra filters and flows in the working areas for protection.

**Role of Plant Tissue Culture in Plant Improvement**
Tissue culture application is critically needed in the country to increase crop productivity which lower cost, improve quality and abundance, and provide for protection and maintenance of natural habitat.
- Tissue culture technique helps to propagate plants of economic importance such as orchids, vegetable, medicinal plants etc.
- Tissue culture technique helps to propagate virus free plants.
- *In-vitro* propagation is a powerful and attractive tool for the rapid cloning of desirable plants. In tissue culture plant multiplications can continue throughout the year irrespective of the season.
- It has great value as a potential system of germplasm storage.

In some crops production of hybrids seeds is very expensive. Cost of such seeds may be reduced by *in-vitro* multiplication of selected parents.