

Model Answer
AS-2316
M. Sc. IIIrd Semester Examination-2013
Forestry
Paper: Breeding Methods in Forest Trees
Maximum Marks: 60

Answer 1. (A) Multiple choice questions:

- (i) The important function of forest tree genetic tests is to explain:
(a) **Genetic architecture of forest tree's**
- (ii) A nested mating design is also called:
(a) **Hierarchial mating design**
- (iii) A tree determined superior both genetically and phenotypically after tests is called:
(d) **Elite tree**
- (iv) A population tends towards dissimilarity when:
(b) **Mutation rate are elevated**
- (v) The approach used to engineer as insect resistant tree of the following is:
(a) **Use of *Bt* genes from bacterium**

Answer 1. (B)

- (i) **Genetic thinning:** in seed orchards, it refers to the removal of orchard genotypes based on their supposed breeding value. Genetic thinning means to remove individuals that have an undesirable genotype/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.
- (ii) **Mutagens:** A mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level. As many mutations cause cancer, mutagens are therefore also likely to be carcinogens. Not all mutations are caused by

mutagens: so-called "spontaneous mutations" occur due to spontaneous hydrolysis, errors in DNA replication, repair and recombination.

Mutagens cause changes to the DNA that can affect the transcription and replication of the DNA, which in severe cases can lead to cell death. The mutagen produces mutations in the DNA, and deleterious mutation can result in aberrant, impaired or loss of function for a particular gene, and accumulation of mutations may lead to cancer.

In forest trees, different mutagens act on the DNA differently. Powerful mutagens may result in chromosomal instability, causing chromosomal breakages and rearrangement of the chromosomes such as translocation, deletion, and inversion. Such mutagens are called clastogens. Mutagens may also modify the DNA sequence; the changes in nucleic acid sequences by mutations include substitution of nucleotide base-pairs and insertions and deletions of one or more nucleotides in DNA sequences. Although some of these mutations are lethal or cause serious disease, many have minor effects as they do not result in residue changes that have significant effect on the structure and function of the proteins. Many mutations are silent mutations, causing no visible effects at all, either because they occur in non-coding or non-functional sequences, or they do not change the amino-acid sequence due to the redundancy of codons. Some mutagens can cause aneuploidy and change the number of chromosomes in the cell.

(iii) Transgenic trees: Genetically modified (GM) trees are called transgenic trees. The DNA of which has been modified using genetic engineering techniques. In most cases the aim is to introduce a new trait to the tree which does not occur naturally in the species. Examples include resistance to certain pests, diseases, or environmental conditions, or resistance to chemical treatments (e.g. resistance to a herbicide), or the production of a certain nutrient or pharmaceutical agent. Genetic engineering techniques are much more precise than mutagenesis (mutation breeding) where an organism is exposed to radiation or chemicals to create a non-specific but stable change. Other techniques by which humans modify plants include selective breeding; plant breeding, and somaclonal variation. There is broad

scientific consensus that food on the market derived from GM crops poses no greater risk to human health than conventional food. GM crops also provide a number of ecological benefits.

(iv) Family: Family has its own pedigree. The knowledge of pedigree lines is very much useful in tree breeding. Family may have pattern of self pollination or cross pollination. This makes the variability in each family lines. Family -based QTL mapping is a variant of QTL mapping where multiple-families are used. Pedigree in humans and wheat. Pedigree information includes information about ancestry. Keeping pedigree records is a centuries-old tradition. Pedigrees can also be verified using gene-marker data. The method has been discussed in the context of plant breeding populations. Pedigree records are kept by plants breeders and pedigree-based selection is popular in several plant species. Plant pedigrees are different from that of humans, particularly as plant are hermaphroditic - an individual can be male or female and mating can be performed in random combinations, with inbreeding loops. Also plant pedigrees may contain of "selfs", ie cloned offspring resulting from self-pollination of a plant.

(v) Ecophene: The variety of Phenotype (visible physical characteristics of behaviors) from a single Genotype (a specific combination of alleles in a gene) that can be observed in a population within a particular habitat, called the Ecophene. Ecophenic characters are not stable. They create error if they are used as a parents in tree breeding.

Answer 2. Pure-line selection generally involves three more or less distinct steps:

- (1) Numerous superior appearing plants are selected from a genetically variable population;
- (2) Progenies of the individual plant selections are grown and evaluated by simple observation, frequently over a period of several years; and
- (3) When selection can no longer be made on the basis of observation alone.

Mass selection: In mass selection, seeds are collected from (usually a few dozen to a few hundred) desirable appearing individuals in a population, and the next generation is sown from the stock of mixed seed. This procedure, sometimes referred to as phenotypic selection, is based on how each individual looks. Mass

selection has been used widely to improve old “land” varieties, varieties that have been passed down from one generation of farmers to the next over long periods. An alternative approach that has no doubt been practiced for thousands of years is simply to eliminate undesirable types by destroying them in the field. The results are similar whether superior plants are saved or inferior plants are eliminated: seeds of the better plants become the planting stock for the next season. Mass selection, with or without progeny test, is perhaps the simplest and least expensive of plant-breeding procedures. It finds wide use in the breeding of certain forage species, which are not important enough economically to justify more detailed attention.

Clonal selection: The clonal selection hypothesis has become a widely accepted model for how the immune system responds to infection and how certain types of B and T lymphocytes are selected for destruction of specific antigens invading the body.

Recurrent selection refers to selecting for certain traits generation after generation. With the interbreeding of reselected plants, the breeder can access favorable recombinations as well as stabilize traits within the genepool. Select your ideotype in each IBL, but don't be totally reliant on the phenotype because it's not always indicative of the actual genotype. Make yield and quality trials with test cross and select the best ten lines. Intercross and repeat. After recurrent selection is done, select new individuals to be the new parents of IBLs. These are then recurrently selected for four or five generations. After recurrent selection has been done in two separate programs, an F1 single cross of the two lines (A X B) is then produced. In reciprocal recurrent selection (RRS), pollen of multiple A males is used to pollinate ideal B females and pollen of B used to pollinate ideal plants of A. Thus A is used as a tester to select for the combining ability of B plants, and B is a tester for A. At the same time, inbred seedlots (A X A) and (B X B) are made, using mixed male pollen and the best females of each population. Store the resulting seed-- the seedlines with the best combining ability will be used as parents of the next RRS cycle. The (A X B) hybrid progeny are simply used as visual indicators of the combining ability that lies in the saved seeds.

These specific inbred parental lines are kept in reserve until the progeny testing of the different (A X B) hybrids has shown which has better SCA and will make the better hybrids. Since this is such a complicated strategy, good note taking and organization are definitely required.

Answer 3. Provenance: the geographic locality of a stand of trees from where the seed was collected. When species (or provenances) are to be selected, it is important to remember the purpose of tree planting and the priorities laid down in the national forest policy. Choice of right species/provenance is a vital decision in any planting as well as improvement programme. The aim is to provide species/provenances that:

- (1) Will fulfil the purpose of planting,
- (2) are well-adapted to the planting sites in question.

A provenance trial, where many sources of a promising species are tested, is an essential tool for clarifying the extent of variation within a species. For species with wide distribution, the selection of provenances may go through a number of phases:

- (1) A range-wide provenance sampling phase,
- (2) A restricted provenance sampling phase and
- (3) A provenance proving stage.

Provenance improves the genetic diversity of forest trees by selection of superior seed sources of the trees which have been identified in provenance trials, new stands of these should be established for future seed supply in order to provide enough seeds of the desirable provenances and to avoid contamination from inferior trees nearby, but also to secure the conservation of the good seed sources for the future.

Answer 4. The primary objective of a breeding program is to increase the frequency of desirable alleles found in the breeding population. While breeders know the traits they wish to improve, they do not know which alleles (genes) favorably impact the traits or their distribution in the native population. Breeding programs must maintain sufficient genetic variation to allow for continued genetic gains over

multiple generations. Complicating matters is the fact that traits of interest change over time in response to new pests or changes in markets. Population sizes needed to maintain gains in polygenic traits of current interest are much smaller than population sizes needed to find potentially rare traits that may be desired in the future.

The breeder needs to consider both short- and long-term objectives when structuring a breeding program.

Short-term objectives usually include obtaining substantial gains in current traits of interest in the first few generations of breeding while maintaining well-adapted trees.

Long-term objectives include the maintenance of low frequency alleles and control of inbreeding.

A major conflict arises between short- and long-term objectives. Selection intensity must be high to obtain substantial genetic gains, yet maintaining rare alleles requires keeping a large breeding population in subsequent generations. However, there are ways to structure the breeding population and make selections to reduce this conflict.

Answer 5.

(i) **Agrobacterium:** Agrobacterium is a genus of Gram-negative bacteria established by H. J.

Conn that uses horizontal gene transfer to cause tumors in plants. Agrobacterium tumefaciens is the most commonly studied species in this genus. Agrobacterium is well known for its ability to transfer DNA between itself and plants, and for this reason it has become an important tool for genetic engineering. The Agrobacterium genus is quite heterogeneous. Recent taxonomic studies have reclassified all of the Agrobacterium species into new genera, such as Ruegeria, Pseudorhodobacter and Stappia, but most species have been reclassified as Rhizobium species.

(ii) **Genetic erosion:** Genetic erosion is a process whereby an already limited gene pool of an endangered species of plant or animal diminishes even more when individuals from the surviving population die off without getting a chance to meet and breed with others in their endangered low population. The term is sometimes used in a

narrow sense, such as when describing the loss of particular alleles or genes, as well as being used more broadly, as when referring to the loss of varieties or even whole species. Genetic erosion occurs because each individual organism has many unique genes which get lost when it dies without getting a chance to breed. Low genetic diversity in a population of wild animals and plants leads to a further diminishing gene pool – inbreeding and a weakening immune system can then "fast track" that species towards eventual extinction.

(iii) **Haploid tree:** The nucleus of a eukaryotic cell is haploid if it has a single set of chromosomes, each one not being part of a pair. By extension a cell may be called haploid if its nucleus is haploid, and an organism may be called haploid if its body cells (somatic cells) are haploid. The number of chromosomes in a single set is called the haploid number, given the symbol n . Haploid tree can be produced by pollen/ egg culture. This can be used as a parent for tree breeding.

(iv) **Polyploidy:** Polyploidy is the state where all cells have multiple sets of chromosomes beyond the basic set, usually 3 or more. Specific terms are triploid (3 sets), tetraploid (4 sets), pentaploid (5 sets), hexaploid (6 sets), heptaploid[17] or septaploid[5] (7 sets) octoploid (8 sets), nonaploid (9 sets), decaploid (10 sets), undecaploid (11 sets), dodecaploid (12 sets), tridecaploid (13 sets), tetradeaploid (14 sets) etc. Polytene chromosomes of plants and fruit flies can be 1024-ploid.[23][24] Ploidy of systems such as the salivary gland, elaiosome, endosperm, and trophoblast can exceed this, up to 1048576-ploid in the silk glands of the commercial silkworm *Bombyx mori*. The chromosome sets may be from the same species or from closely related species. In the latter case, these are known as allopolyploids (or amphidiploids, which are allopolyploids that behave as if they were normal diploids).

(v) **Breeding population:** a population within which free interbreeding takes place and evolutionary change may appear and be preserved. Breeding population size varies from provenance to provenance and from forest to forest depending upon the forest quantitative structure of a species.

Answer 6. Molecular markers: A molecular marker (identified as genetic marker) is a fragment of DNA that is associated with a certain location within the genome. Molecular

markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA. The characterization of cellular molecules and their variants, as an alternative to assessment of morphological and quantitative traits, has its attraction in the fact that such molecular markers are less subject to the influences of environmental factors and developmental stage.

Molecular markers are basically helpful in three areas of forest tree breeding programme specifically: quantification of genetic diversity, genotype verification and delineation, and marker assisted selection.

The use of molecular markers for the rapid determination of the extent of genetic variation within and among populations is of value in guiding. Gene conservation activities, which are aimed at maintaining genetic diversity with respect to traits of both known and unknown importance. Development of breeding populations, which is aimed at defining populations with appropriately large genetic variation around high means with respect to traits of known importance.

Some common types of genetic markers used in forest tree breeding programmes are:

- **RFLP** (or Restriction fragment length polymorphism)
- **SSLP** (or Simple sequence length polymorphism)
- **AFLP** (or Amplified fragment length polymorphism)
- **RAPD** (or Random amplification of polymorphic DNA)
- **VNTR** (or Variable number tandem repeat)
- **SSR** Microsatellite polymorphism, (or Simple sequence repeat)
- **SNP** (or Single nucleotide polymorphism)
- **STR** (or Short tandem repeat)
- **SFP** (or Single feature polymorphism)
- **DArT** (or Diversity Arrays Technology)
- **RAD** markers (or Restriction site associated DNA markers)

Molecular markers can be used to study the relationship between an inherited disease and its genetic cause (for example, a particular mutation of a gene that results in a defective protein). It is known that pieces of DNA that lie near each other on a chromosome tend to be inherited together. This property enables the use of a marker, which can then be used to

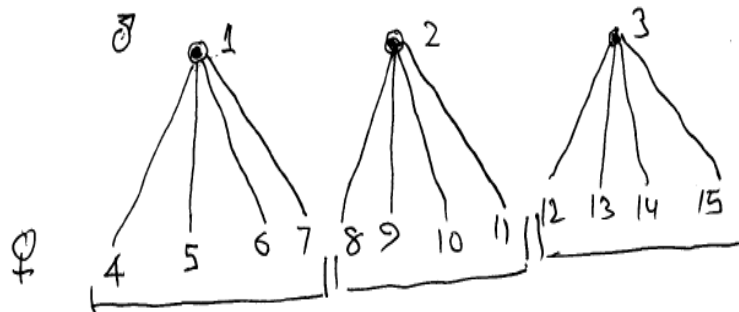
determine the precise inheritance pattern of the gene that has not yet been exactly localized in forest tree species.

Molecular markers are employed in genealogical DNA testing for genetic genealogy to determine genetic distance between individuals or populations. Uniparental markers (on mitochondrial or Y chromosomal DNA) are studied for assessing maternal or paternal lineages in forest trees.

Molecular markers have to be easily identifiable, associated with a specific locus, and highly polymorphic, because homozygotes do not provide any information. Detection of the marker can be direct by RNA sequencing, or indirect using allozymes will help in development of forest trees.

Some of the methods used to study the genome or phylogenetics are RFLP, Amplified fragment length polymorphism (AFLP), RAPD, SSR. They can be used to create genetic maps of whatever forest trees are being studied.

Answer 7. (a) Nested mating design: Each of male parents is mated to a different subset of female parents .



This mating design;

- Provide information for parents and full-sib families
- Provide estimates of both additive and dominance effects
- Not efficient for selection
- Low cost for controlled mating

(b) **Factorial mating design:** Each member of a group of males is mated to each member of group of females.

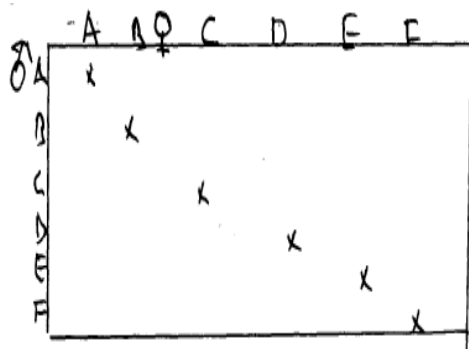
Male Parents

	1	2	3	4	5
6	x	x	x	x	x
7	x	x	x	x	x
8	x	x	x	x	x
9	x	x	x	x	x
10	x	x	x	x	x
11	x	x	x	x	x
12	x	x	x	x	x

This mating design;

- Provide good information for parents and full-sib families
- Provide estimates of both additive and dominance effects
- Provide estimates of genetic gains from both VA and VD
- Limited selection intensity
- High cost

(C) **Single pair mating design:** Parents are mated only once by pairs P parents generate $\frac{1}{2}$ P full-sib families .



This mating design;

- Provide good information for full-sib family performance
- Provide estimates of some genetic parameters
- Not for estimating genetic gains from VA
- Maximum unrelated ness but not optimum for selection
- Low cost

(d) **Full diallel and half diallel mating design:**

Full diallel – In this mating design all parent are crossed to all other in every combination .It involves direct crosses, reciprocal crosses and selfed parents. All possible combination of crosses. Parents may be individuals, clones, homozygous line, open pollinated varieties. It involves direct crosses, reciprocal crosses and selfed parent.

		♂ Parents				
		1	2	3	4	5
♀ Parents	1	x	x	x	x	x
	2	x	x	x	x	x
	3	x	x	x	x	x
	4	x	x	x	x	x
	5	x	x	x	x	x

Half diallel – It is similar to full diallels in this mating design each parent is mated with every other parent in the population, excluding selfs and reciprocal. In this design reciprocal crosses are not included. this design is Less expensive than full diallel but requires extra care for handling also this design may not be accurate as a full diallel.

		♂ Parents					
		1	2	3	4	5	6
♀ Parents	1	x	x	x	x	x	x
	2		x	x	x	x	x
	3			x	x	x	x
	4				x	x	x
	5					x	x
	6						x

(e) **Partial diallel mating design:** This mating design is a modification of the diallels. These are called partial diallel Systematic or progressive mating scheme. In this mating design cross are made that fall on particular diagonals.

		1	2	3	4	5	6
1	x	x	x			x	x
2		x	x	x			
3			x	x			
4				x			x
5					x		
6						x	x

Answer 8. Resistance to infection is influenced for the most part by two factors, the genetic qualities of the host tree and parasite, and the environmental conditions which for various reasons can be more or less propitious for the occurrence of an attack by parasites. In general, an invasion of dead tissues such as heartwood is not governed by the same genetic factors as those that determine resistance. The hereditary occurrence in the heartwood of growing trees of substances which have a toxic effect on decay fungi and increase wood durability is, however, an important branch of resistance research. In some cases the same rot fungus may appear as saprophyte in one tree species and as a parasite in another.

Breeding for resistance to disease involves recognition of the disease organism and an understanding of the relation between tree and parasite, the genetics of both tree and parasite and the environmental conditions under which the attack develops. Disease resistance is governed by morphological or physiological variation of the host. Thus, such morphological variations as a slightly thickened epidermis, cork formation, or lignin or cutin development may prevent the germ tube of a fungus spore from entering the host plant. Physiological variation may prevent the development of the disease. For example, attack is prevented when the cell sap of a host is so concentrated or so acid that a fungus cannot thrive or live, or when substances toxic to the fungus are present in the cell protoplasm, or when growth substances essential to the fungus are lacking.

There are four major types of diseases in tree species: wood rots, leaf diseases, cankers, and diebacks and wilts.

Wood rots.--Heart rots cause more volume loss in southern hardwoods than all other diseases combined. Tree species of first importance include eastern cottonwood. (*Populus deltoides* Bartr.), sweetgum (*Liquidambar styraciflua* L.), and the oaks (*Quercus spp.*). The fact that several dozen fungi are involved will make the development of resistant clones particularly difficult. Furthermore, direct tests of resistance must be delayed until selections have become old enough to form heartwood.

Leaf diseases.--Heavy infections of *Melampsora* rust on cottonwood, leaf blister on Oak, anthracnose on sycamore and oak, and other leaf fungi occur periodically in the South. The possibilities of breeding for resistance to these leaf diseases appear

excellent. At Stoneville a start has been made on *Melampsora* rust. This disease has long been a serious pest of various *Populus* species throughout the world.

Pest resistance often is the major consideration in tree breeding and, always must be taken into account. Although little breeding for pest resistance in southern hardwoods has been undertaken the need for it has been recognized. It should receive increased attention because of:

- (1) The disadvantages of pesticides
- (2) The probable increase in pests with increase in hardwood. planting; and
- (3) The ease of vegetative propagating some species.

In breeding for pest resistance, the first step often is to select resistant individuals out of a host population that is heavily attacked by the pest.