Plant Archives Vol. 24, No. 1, 2024 pp. 309-318 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.1.044

RECENT ADVANCEMENT OF GENETIC MARKERS AND APPLICATION IN TREE IMPROVEMENT PROGRAMS : A REVIEW

Mexudhan Jaiswal

Department of Forestry, Mahatma Gandhi Horticulture and Forestry College, Sankra-Patan, Durg - 491 111 (C.G.), India.
E-mail : mj.mexu@gmail.com

(Date of Receiving-18-10-2023; Date of Acceptance-26-01-2024)

ABSTRACT

Molecular markers have emerged as indispensable tools in tree improvement programs, revolutionizing the field of forest genetics and breeding. The recent advancements in genetic markers have significantly transformed tree improvement programs, offering unprecedented precision and efficiency in enhancing the genetic attributes of forest trees. This abstract provides an overview of the latest developments in genetic marker technologies and their diverse applications in tree improvement programs. The advent of high-throughput sequencing and genotyping technologies has ushered in an era of unparalleled genomic insights, allowing researchers to delve into the intricacies of tree genomes with unprecedented resolution. Single Nucleotide Polymorphisms (SNPs), microsatellites and advanced sequencing techniques have become pivotal tools for assessing genetic diversity, unraveling population structures, and identifying genomic regions associated with economically and ecologically important traits. The applications of these genetic markers extend to marker-assisted selection (MAS), enabling the rapid identification and incorporation of desirable traits into breeding programs. The identification of Quantitative Trait Loci (QTL) has been greatly facilitated, providing a deeper understanding of the genetic basis of complex traits in forest trees. Recent innovations in gene editing technologies, such as CRISPR-Cas9, present promising avenues for targeted genetic modifications, further accelerating the pace of tree improvement. Moreover, genetic markers play a vital role in addressing contemporary challenges in tree improvement, including the development of disease-resistant varieties, increased stress tolerance and adaptation to changing climatic conditions.

Key words : Genetic Marker, SNPs, Marker assisted selection, Quantitative Trait Loci, CRISPR-Cas9.

Introduction

Forest genetic resources refer to the genetic material of trees and other woody plants in forests, including their seeds, pollen and other reproductive materials. These resources are essential for maintaining the biodiversity and health of forest ecosystems. However, various factors contribute to the decline of forest genetic resources, and molecular markers play a crucial role in understanding, conserving and utilizing these resources. Forest trees are an essential genetic subject as they are mostly undomesticated and long-lived, enabling them to "naturally" demonstrate genetic variation over a period of time (Ingvarsson and Dahlerg, 2019). However, over time, there have been subtle human impacts on forest ecosystems, more severely in the tropics through the increasing trends of deforestation and forest degradation, directly affecting their diverse genetic resources and associated functions (Finkeldey and Hattemer, 2007). Worldwide, multiple institutions, that through creation of gene banks and establishment of *ex-situ* collections, are designed to protect genetic resources and provide a valuable source of genetic diversity essential for tree breeding. In particular, for forest trees that are out crossing and largely undomesticated plant species, molecular markers have proven to be invaluable tools with applications in: (1) genetic conservation efforts by identification of genetic diversity hotspots; (2) the assembly of breeding populations in newly developed and advanced breeding programs; (3) the monitoring and characterization of population dynamics and gene flow;

(4) the proper delineation of species taxonomy for management issues associated with conservation; (5) assessment of gene flow (pollen contamination) in seed orchards and the authentication of "controlled crossings", the assessment of inbreeding occurrence in breeding programs and studies of mating systems in non-industrial tree species and (6) genetic fingerprinting in advanced breeding programs for the purpose of quality control to detect misidentified ramets in production and breeding populations Genetic variation within or among tree populations is assessed using gene markers. A gene marker, also known as gene locus or marker locus, is typically an environmentally stable trait whose variation is determined only by genetic factors. Different types of gene markers have been applied in genetic inventories of tropical forest trees. These include morphological or phenotypic, biochemical and molecular markers (Finkeldey and Hattemer, 2007) and cytogenetic markers (Chesnokov *et al.*, 2020). Several marker-based studies have investigated genetic variation in different tree species across the tropics. Finkeldey and Hattemer (2007), recent developments in molecular biology and biotechnology offer rapid characterization of genotypes and detection of genetic variation using a range of molecular markers from isozymes, restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), directed amplification of mini-satellite regions (DAMD), amplified fragment length polymorphisms (AFLPs) to simple sequence repeats (SSRs). The RAPD markers have been used for linkage mapping, genetic diversity, sex determination and disease detection in *Pinus radiata*, *Populus*, *Eucalyptus globulus*, *Abies alba*, *Juglans regia* etc.

Classification of markers : Classification of markers are given in Fig. 1.

Biochemical Markers

Monoterpenes

A subclass of terpenoid compounds present in plant resins and essential oils are called monoterpenes (Kozlowski and Pallardy, 1979). Although, the metabolic functions of monoterpenes are not fully understood, they probably play an important role in resistance to attack by diseases and insects (Hanover, 1992). Gas chromatography is used to detect the amounts of certain monoterpenes, which can be used as genetic markers. These include alpha- and beta-pinene, myrcene, 3-carene, and limonene (Hanover, 1966a, b, 1992; Squillace, 1971; Strauss and Critchfield, 1982).

Allozymes or isozymes

Allozymes have been the most important type of genetic marker in forestry and are used in many species for many different applications (Conkle, 1981a; Adams *et al.*, 1992a). Allozymes are allelic versions of enzymes that may be identified by an electrophoresis method. While the name "allozyme" suggests a genetic foundation for the variant form, the more generic term "isozymes" refers to any variable form of an enzyme. Most allozyme genetic markers have been derived from enzymes of intermediary metabolism, such as enzymes in the glycolytic pathway; however, conceivably an allozyme genetic marker could be developed from any enzyme. Allozyme analysis is fairly easy to apply and standard protocols (Fig. 2) for its use in trees are available (Conkle *et al.*, 1982; Cheliak and Pitel, 1984; Soltis and Soltis, 1989; Kephart, 1990).

This is one of the most efficient genetic markers for assessing diversity (Lewontin and Hubby, 1966). In forestry, isozymes have been used to study genetic variation within and between populations, population structure, phylogeny and to elucidate mating patterns among natural populations as well as experimental populations (Mitton, 1983; Hamrick and Godt, 1989; El-Kassaby and Ritland, 1996a). Pollen contamination in the in seed orchards can also be detected using allozyme markers (El-Kassaby and Ritland, 1996b). Isozyme data is also generated for the comparison of genetic variation within and between populations and towards deduction of mating systems in many tropical forest tree species (Loveless, 1992).

Table 1 : Genetic markers and their features.

Feature	Marker			
	Phenotypic	**Biochemical**	**Molecular**	**Cytogenetic**
Description	Indicate morphological features of plant	Biochemical marker like protein based marker	Use of DNA fragments of plant	Study chromosome structure
Example	Colour, shape andsize of flowers, seeds, or leaves	**Isoenzyme gene loci**	SNP, SSR or microsatellites, RAPD, AFLP	FISH, GISH
Genomic coverage	**Low**	**Low**	**High**	**Average**

Source - Chesnokov *et al.* (2020).

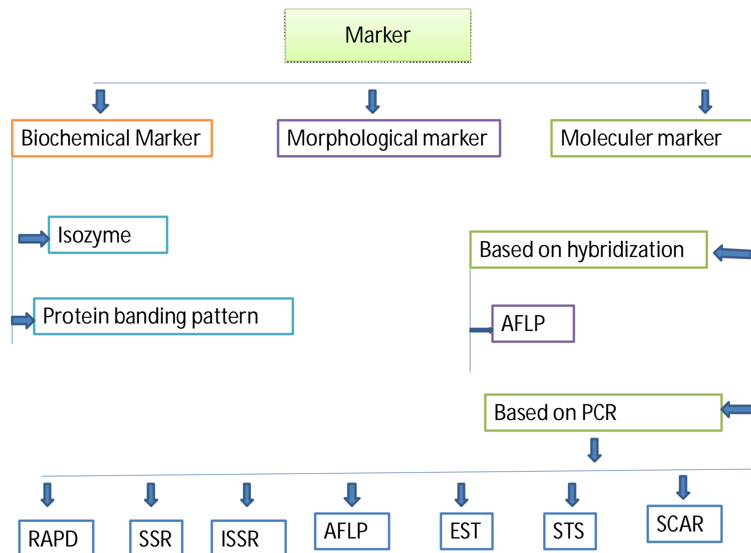

Fig. 1 : Classification of Markers.

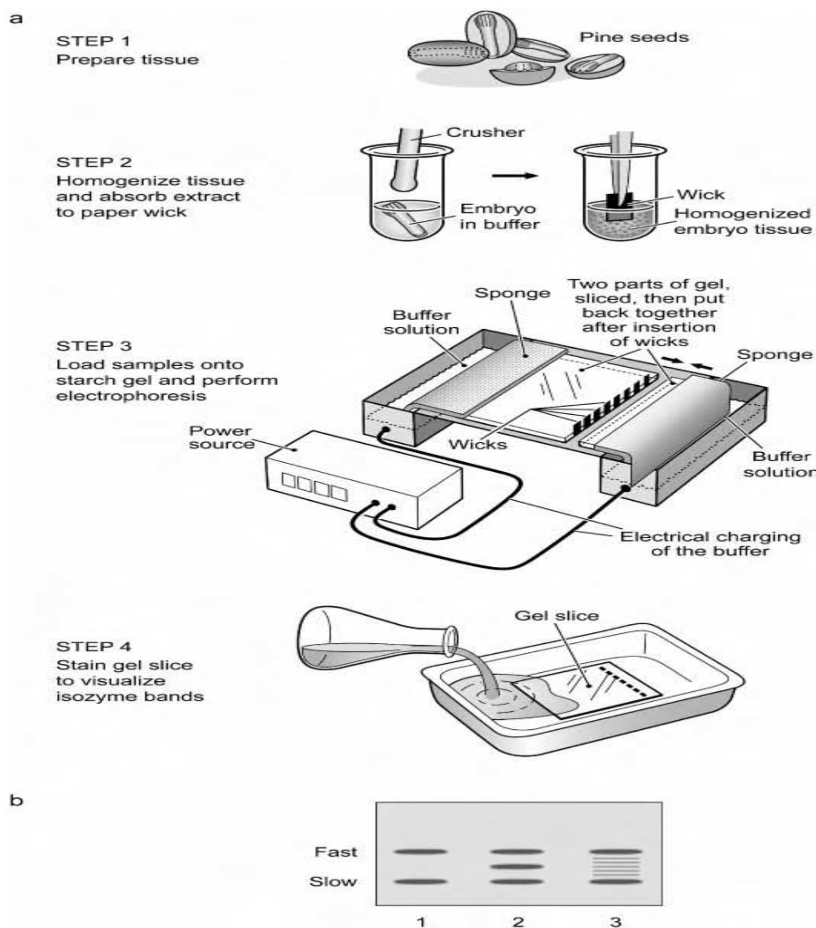

Fig. 2 : Allozyme analysis in forest trees. Source : White *et al*. (2007).

Classification of molecular markers

Molecular markers are tools used in molecular biology and genetics to identify and locate specific sequences of DNA or proteins within a genome. These markers play a crucial role in various applications, including genetic mapping, marker-assisted selection, and population genetics. Molecular marker is a DNA or gene sequence within a recognized location on a chromosome which is used as identification tool. In the pool of unknown DNA or in a whole chromosome, these molecular markers help in identification of particular sequence of DNA at particular location. Molecular markers can be classified based on PCR and hybridization.

Genetic marker can be classified as PCR and hybridization based

PCR based genetic markers : RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat), STR (Single Tendem Repeats), VNTR (Variable Number Tendem Repeat), STS (Sequence Tag Size), SNP (Single Nucleotide Polymorphism), EST (Expressed Sequence Tagged).

Hybridization based genetic markers : RFLP (Restriction Fragment Length Polymorphism)

Molecular markers can be also classified as :

- 1st generation markers (at the time of 1980-1990): RFLP, RAPD
- 2nd generation markers (1990-2000): AFLP, SSR, STR, VNTR, STS
- 3rd generation markers (After 2000): SNP, EST

First generation of markers was the hybridization based markers. These are so called because the DNA profile is visualized through hybridization of DNA with radioactively labeled probes of known sequence.

The second generation of markers were the PCR based markers, as their assay was carried out through amplification using either arbitrary or sequence specific primers.

The third generation markers are the most recent ones, called as SNPs. Their detection requires sequence information. With the advancement in the field of DNA sequencing, SNPs have become very popular in the last few years.

Features of an ideal molecular marker system

The properties of an ideal marker system would include the following:

- Should be able to use DNA of all qualities, including very degraded and old samples.
- Co-dominant vs. Dominant*:* Molecular markers can be co-dominant (both alleles are distinguishable) or dominant (only one allele is observed, and heterozygotes cannot be distinguished). The choice depends on the specific application..
- Polymorphic markers provide the basis for distinguishing different genotypes, facilitating genetic analyses and studies of diversity.
- Molecular markers should be applicable to a wide range of samples, from individual organisms to populations. Scalability allows for their use in various genetic studies. Molecular markers should produce reliable and consistent results when applied to the same DNA samples across different experiments or laboratories.
- *Genome Representation:* Molecular markers can provide high or low density across the genome. High-density markers are useful for fine-scale mapping, while low-density markers may be sufficient for broader genetic studies.

Moleculer markers based on hybridization system

A) RFLP stands for Restriction Fragment Length Polymorphism, and it is a type of molecular marker used in genetics to identify variations in DNA sequences. RFLP markers are based on the variations in the lengths of DNA fragments that result from the action of restriction enzymes. In 1980, Botstein *et al*. produced RFLP markers, which were the first DNA-based genetic markers**.** In order to reduce the genome to a sizable pool of restriction fragments of varying sizes, the whole cellular DNA is first digested using a restriction endonuclease (White *et al*., 2007). Hundreds of restriction endonucleases have been discovered that cleave DNA at specific recognition sites of varying length and sequence. However, just a few of these enzymes (*e.g. Hmdlll*, *EcoRI*, *BamHI*) are routinely used because they generally provide the best size distribution of DNA fragments and are inexpensive (White *et al*., 2007). As a potent tool for sampling DNA sequence diversity in the genome, restriction endonuclease recognition sites are distributed throughout the genome in both coding and non-coding regions. Next, an agarose gel is electrophoretically split into the restriction fragments according to their sizes. It is possible to visualize DNA within such a gel by staining it with ethidium bromide; however, because there are typically so many restriction fragments of all possible sizes, discrete fragments cannot be seen. To overcome this problem, the fractionated DNA is transferred and chemically bound to a nylon membrane by a process called Southern blotting**,** named after its inventor (Southern, 1975). Specific DNA fragments are visualized by hybridizing the DNA fragments bound to the nylon membrane with a radioactively- or fluorescently-labeled DNA probe (White *et al*., 2007).

The nuclear genome contains a significant quantity of repetitive DNA, making the development of probes for RFLP analysis of nDNA more difficult. Genomic DNA (gDNA) and complementary DNA (cDNA) probes are the two types of probes that are most often utilized. Probes are isolated from DNA libraries, which are a large collection of cloned fragments resulting from a single cloning experiment. Both cDNA and gDNA probes are equally easy to use and reveal abundant genetic variation in trees (Devey *et al*., 1991; Liu and Furnier, 1993; Bradshaw *et al*., 1994; Byrne *et al*., 1994; Jermstad *et al*., 1994). The gDNA probe libraries, however, are much easier to construct than cDNA probe libraries because the difficult task of mRNA isolation is not required. The cDNA probes are derived from expressed genes because cDNA is derived from mRNA, whereas gDNA probes generally are not; therefore, cDNA probes are often preferred for many applications of RFLP analysis in trees (Fig. 3).

For forest tree species, RFLPs have demonstrated inter population differentiation in *Gliricidia sepium* (Lavin *et al*., 1991), *Pinus attenuata, P. muricata* and *P. radiata* (Strauss *et al*., 1992), *Quercus robur* and *Q. petraea* (Kremer *et al*., 1991). Nuclear RFLPs have been used to distinguish *Populus tremuloides* and *P. grandidentata*. RFLPs have been also applied for fingerprinting. Calculations reported by Landry and Michelmore (1987) proved their effectiveness. Combined RFLP banding patterns of six probes could be used to individually identify each of 39 cultivars of peach examined (Ballard *et al*., 1992).

Moleculer marker based on PCR

Polymerase Chain Reaction (PCR) is a molecular biology technique used to amplify and replicate DNA sequences (Fig. 4). It was first developed by Kary Mullis in 1983 and has since become a fundamental tool in various areas of biological and medical research, as well as in diagnostics. PCR allows the selective and exponential amplification of specific DNA regions, enabling the generation of large quantities of a particular DNA sequence from a small starting amount. Polymerase

Chain Reaction (PCR) is widely employed in tree improvement programs for the identification and utilization of molecular markers.

The polymerase chain reaction enables the production of a large amount of a specific DNA sequence without cloning, starting with just a few molecules of the target sequence. One advantage of PCR-based marker methods over DNA-DNA hybridization marker methods is that the latter method requires isolation of large quantities of DNA (White *et al.*, 2007). The polymerase chain reaction has three basic steps: (1) Denaturing of the double stranded DNA template; (2) Annealing of a pair of primers to the region to be amplified; and (3) Amplification using a heat-resistant DNA polymerase called Taq polymerase.

A) RAPD (Random Amplified Polymorphic DNA) : Markers have been the most widely used molecular marker type in forest trees to date. They were the first of the PCR-based markers and were developed independently by Welsh and McClelland (1990) and Williams *et al.* (1990) (Fig. 5). The RAPD marker system is easy to apply as no prior DNA sequence information is needed for designing PCR primers as is required for other PCR-based genetic marker systems. In the RAPD marker system (Fig. 4), a PCR reaction is conducted using a very small amount of template DNA (usually less than 10 nano grams) and a single RAPD primer (White *et al.*, 2007). Primers are usually just 10 base pairs long (10-mers) and are of random sequence. There are several thousand primers commercially available, all with a different 10-base sequence, which in theory will all amplify different regions of the target genome. Therefore, the RAPD marker system has the potential to randomly survey a large portion of the genome for the presence of polymorphisms. The small amount of DNA needed is a big advantage of the RAPD technique *versus* RFLPs, because marker analysis can be applied to haploid conifer mega gametophytes as was discussed for allozyme markers (White *et al.*, 2007).

Carlson *et al.* (1991) first demonstrated the use of RAPD markers in trees by showing the inheritance of RAPD markers in F_1 families of *Pseudotsuga menziesii* and *Picea glauca.* In a subsequent paper, Tulsieram *et al.* (1992) used RAPD markers and mega gametophyte segregation analysis to construct a partial genetic linkage map for *Picea glauca.* Random amplified polymorphic DNA markers have since been used for linkage mapping and marker analyses in dozens of tree species (Cervera *et al.*, 2000). However, as the popularity of RAPD markers increased, difficulty in establishing marker repeatability across laboratories slowly manifested itself. Therefore, although RAPD markers are easy and quick to use, they have less overall value than the earlier allozyme and RFLP markers because of the problems with repeatability.

RAPD (Random Amplified Polymorphic DNA), that uses single-10 base oligonucleotides to amplify short inverted repeats, distributed throughout the genome being assayed. Such inverted repeats are highly polymorphic, and provide large numbers of readily accessible dominant markers. The major advantages of these marker systems are that they can be applied to organisms with complex eukaryotic genomes without any prior information. A typical survey of pine DNA with 100 different arbitrary primers will provide 100 to 200 polymorphic markers. Carefully chosen markers are reliable and repeatable. However, control of reaction parameters and quality of reagents is essential, because amplification is extremely sensitive to initial conditions. Furthermore, the small amounts of DNA required (ng/reaction) makes possible the application of RAPD methods to the conifer haploid mega gametophyte (O'Malley *et al.*, 1996). Species specific RAPD markers have been sought in *Quercus robur* and *Q. petraea*, in order to detect natural hybrids between the species (Moreau *et al.*, 1992).

Jamnadass *et al.* used RAPDs to assess genetic variation in and among natural stands of the woody legume *Sesbania sesban*, a species important for 'improved fallow' plantings in agroforestry. Differentiation among nine *S. sesban* populations in sub-Saharan Africa was unusually high for a woody perennial, with all individuals stringently assigned to specific populations in cluster analysis.

Lowe *et al*. used RAPDs to assess genetic variation in the two bush mango species *Irvingia gabonensis* and *I. wombolu*, valuable multipurpose fruit trees from central and west Africa that are currently being domesticated. Significant genetic integrity was found within the two morphologically similar species, with no evidence of hybridisation between them, even between individuals from areas of contact where hybridisation was considered probable.

B) AFLP stands for Amplified Fragment Length Polymorphism and AFLP markers are a type of genetic marker commonly used in molecular biology and genetics. AFLP is a technique that allows researchers to analyze the genetic diversity and variations within populations of organisms. Markers are a recent development (Vos *et al.,* 1995). The AFLP process combines aspects of DNA digestion, ligation, and selective PCR to generate a highly polymorphic fingerprint of an organism's genome. They are like RAPDs in that many markers can be assayed

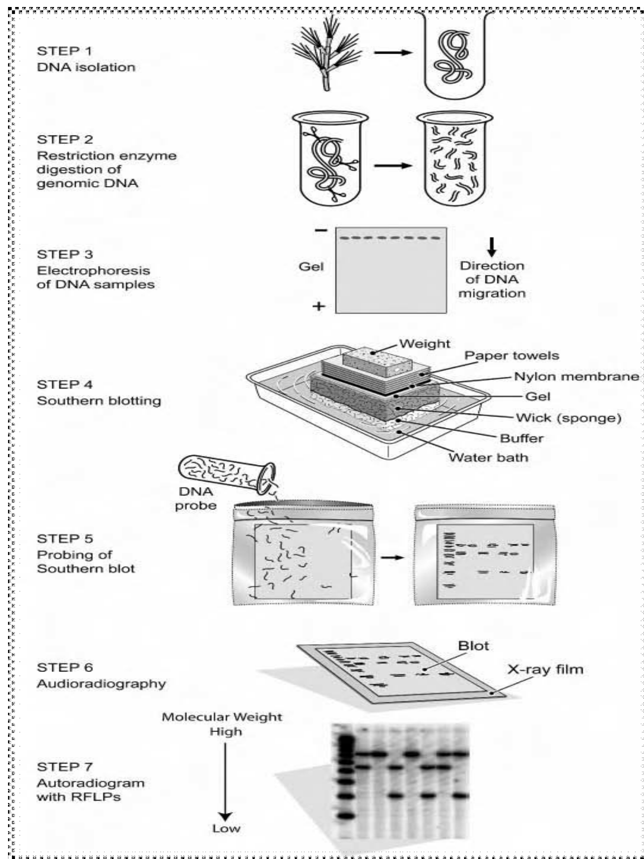

Fig. 3 : Restriction fragment length polymorphism (RFLP) analysis. Source : White *et al.* (2007).

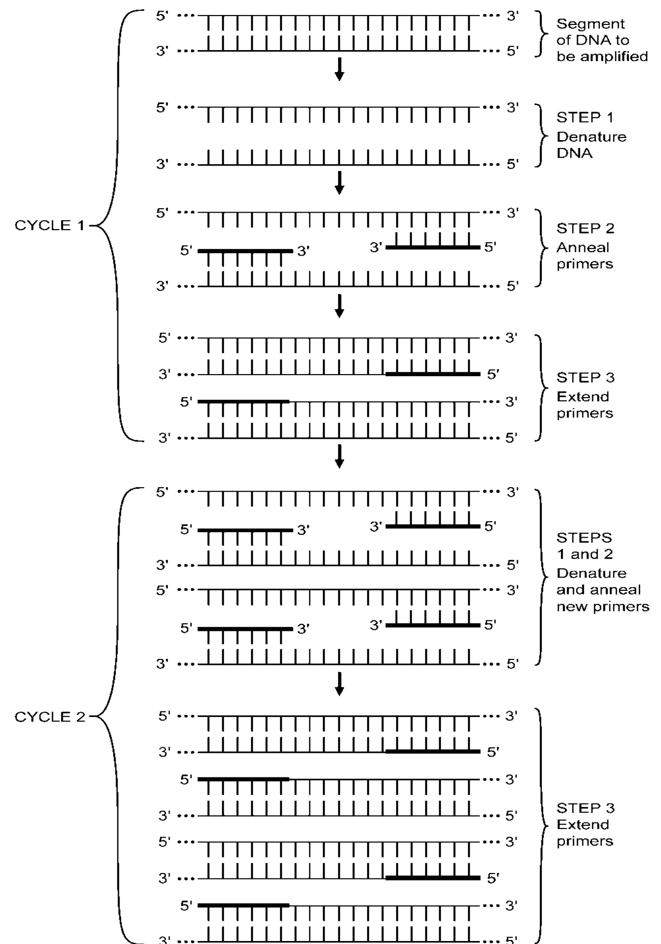

Fig. 4 : Polymerase Chain Reaction (PCR). Source : White *et al.* (2007).

quickly using PCR and they are generally dominant; but, AFLPs appear to be more repeatable than RAPDs. AFLP markers also are similar to RFLPs because they survey the genome for the presence of restriction fragment polymorphisms. The first report of the use of AFLPs in trees was by Cervera *et al*. (1996), who used this marker system to genetically map a disease resistance gene in *Populus.* Genetic linkage maps based on AFLPs have also been constructed in *Eucalyptus globulus* and *E. tereticornis* (Marques *et al*., 1998) and in *Pinus taeda.*

Russell *et al.* used AFLPs to assess genetic variation within and among nine populations of the riverine timber tree *Calycophyllum spruceanum* sampled along river tributaries in the Peruvian Amazon Basin. Most variation occurred among individuals within populations, although variation between stands was highly significant according to an Analysis of Molecular Variance

C) Simple sequence repeat (SSR) markers were first developed for use in genetic mapping in humans (Litt and Luty, 1989; Weber and May, 1989) and are another name for microsatellite DNA Short, tandemly-repeated sequences of two, three or four nucleotides are found throughout the genome. For example, the dinucleotide repeat AC is commonly found in *Pinus* genomes. Since the number of tandem repeats at a locus can vary greatly, SSR markers tend to be amongst the most polymorphic genetic marker types. For example, one allele might have 10 copies of the AC tandem repeat (AC)i0, whereas another allele would have 11 copies (AC)n, another 12 copies (AC)i2 and so forth.

Some of the first SSR markers developed in trees were from the chloroplast genome (Powell *et al*., 1995; Cato and Richardson, 1996; Vendramin *et al*., 1996). Development of these markers was made easier because the complete DNA sequence of the entire chloroplast genome *of Pinus thunbergii* was known (Wakasugi *et al*., 1994a,b). The SSR sequences were found by a computer search of the entire cpDNA sequence database Nuclear DNA SSRs have been developed for several forest trees, including species of *Pinus* (Smith and Devey, 1994; Kostia *et al*., 1995; Echt *et al*., 1996; Echt and May-Marquardt, 1997; Pfeiffer *et al*., 1997; Fisher *et al*., 1998), *Picea* (van de Yen and McNicol, 1996), *Quercus* (Dow *et al*., 1995) and *Populus* (Dayanandan *et al*., 1998). Each of these studies describes the isolation

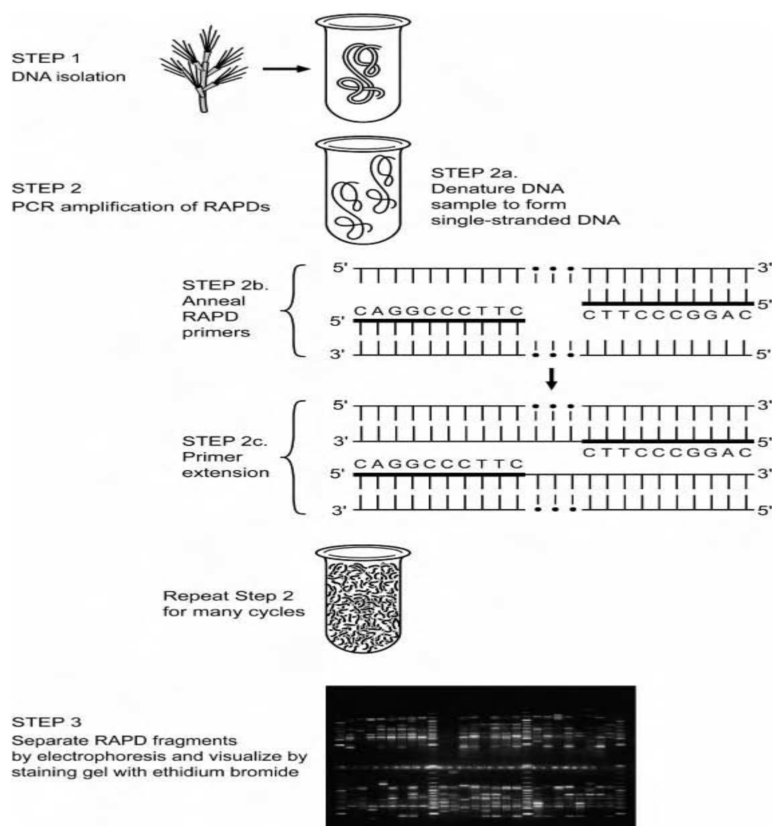

Fig. 5 : Random Amplified polymorphic DNA. Source : White, T.L. *et al.* (2007).

and cloning of a small number of SSRs, their inheritance patterns and their utility for related species.

D) Inter simple sequence repeats (ISSRs) : ISSR stands for Inter-Simple Sequence Repeat, and ISSR markers are a type of molecular marker used in genetic research to study genetic diversity, population structure, and phylogenetic relationships among individuals or populations. ISSR markers are based on the polymorphism in regions between simple sequence repeats or microsatellites. Approximately, 10 to 60 fragments are created simultaneously by this approach, which takes advantage of the presence of SSRs in genomes. Products are separated by gel electrophoresis and generally scored as the presence or absence of bands. ISSRs are generally assumed to have their origin in nuclear DNA. However, they may more rarely originate from organellar DNA.

Aga *et al.* used ISSRs to assess genetic variation in forest coffee trees (*Coffea arabica*) from 16 populations from four regions of Ethiopia.

E) Cleaved amplified polymorphic sequences (CAPS) : CAPS are DNA fragments amplified using specific primers, which are afterwards digested by restriction enzymes. Sequence polymorphisms result in cutting of products in different places, and these variants are revealed as length differences when running reactions on agarose gels. The CAPS approach is sometimes known as restriction fragment length polymorphism (RFLP-) PCR and the technique bears similarities to the non-PCR-based older RFLP method. CAPS can be applied to organism-specific nuclear sequences, or to organellar DNA using universal primers. As with SSRs, sequencing is generally required in the former case in order to develop primer pairs. Similar to SSRs, CAPS assess variation at one locus only in a particular PCR.

Table 2 : Comparison of the five widely used DNA markers in forest tree.

Features	Molecular Marker				
	RFLP	**RAPD**	**AFLP**	**SSR**	**SNP**
Genomic coverage	Low copy coding region	Whole genome	Whole genome	Wholegenome	Whole genome
Quality of DNA required	High	Low	High	Medium high	High
Level of polymorphism	Medium	High	High	High	High
Inheritance	Co-dominant	Dominant	Co-dominant	Co-dominant	Co-dominant
Type of probes/ primers	Low copy DNA cDNA clone or	Usually 10bp random nucleotides	Specificsequence	Specificsequence	Allele-specific PCR primers
Suitable utility in diversity, genetics and breeding	Genetics	Diversity	Diversity and genetics	All purposes	All purposes

Source- Hasan *et al.* (2021).

Table 3 : Different molecular marker used in Forest tree species.

Species	Type of marker	Aim	References
Moringa oleifera	SSR	To study genetic diversity	Ganesan *et al.* (2014)
Eucalyptus tereticornis	ISSR	To study genetic diversity	Chezhian *et al.* (2010)
Mansonia altissima	AFLP	To study genetic diversity	Akinnagbe *et al.* (2019)
Pinus caribaea	SSR	To study genetic diversity	Sanchez *et al.* (2014)
Tectona grandis	SSR	To study genetic diversity	Minn *et al.* (2014)
Acacia mangium	SSR	To study genetic diversity	Yuskianti and Isoda (2012)

F) Expressed sequence tagged polymorphisms (ESTPs) are PCR-based genetic markers that are derived from Expressed Sequenced Tags (ESTs). Expressed sequenced tags are partial cDNA sequences that have been obtained by automated DNA sequencing methods The EST databases contain hundreds of thousands of entries from a variety of organisms, most notably *Arabidopsis thaliana,* rice, and maize in plants. In forest trees, there are EST databases for *Pinus, Populus* and *Eucalyptus*. The ESTs are routinely compared to DNA sequence databases to determine their biochemical function.

G) SNPs (Single Nucleotide Polymorphisms) : SNPs markers are known as the third generation markers, which are nowadays extensively used in various genomic studies for individual genotyping. Single nucleotide polymorphisms or SNPs (pro nounced "snips") are DNA sequence variations that occur due to point mutations when a single nucleotide (A, T, C, or G) in the genome sequence is altered (Gupta *et al*., 2001).

SNP markers are widely used in various applications, including population genetics, disease association studies, and personalized medicine. The availability of high-throughput genotyping technologies has facilitated the analysis of large numbers of SNPs in a cost-effective manner. The information obtained from SNP markers is valuable for understanding genetic variation within populations and its implications for health and disease.

Marker Assisted selection

Marker Assisted Selection [MAS] refers to indirect selection for a desired plant phenotype based on the banding pattern of linked molecular (DNA) markers. MAS is based on the concept that it is possible to infer the presence of a gene from the presence of a marker which is tightly linked to the gene of interest. Marker-Assisted Selection (MAS) is a breeding technique used in agriculture and tree breeding to select individuals with desirable traits based on the presence of specific genetic markers. This approach enables breeders to expedite the development of new varieties or breeds by directly selecting for traits of interest at the molecular level.

Conclusion

In forest trees, genetic markers are necessary for many different kinds of genetic studies. In order to understand mating systems and inbreeding, assess levels and patterns of genetic diversity, and investigate taxonomic and evolutionary links across species, genetic markers are frequently employed with natural populations of forest trees. The production of genetic maps and marker-aided breeding both require the use of genetic markers, which are regularly employed to assess the effectiveness of various tree improvement initiatives. There exist morphological genetic markers in forest trees, but their quantity is restricted and they are frequently linked to a detrimental trait. Nevertheless, mating mechanisms in several conifer species have been studied using seedling mutant traits.

There are several kinds of biochemical markers that have shown to be quite helpful for studying tree genetics. The majority of taxonomic research conducted on pines has utilized monoterpenes, which were the first biochemical markers found in trees.

The most recent type of marker to be produced is molecular, or DNA-based and it has numerous advantages over morphological and biochemical markers. The main benefits are as follows: (1) There may be an infinite number of molecular markers available; and (2) DNA markers are often unaffected by environmental factors or developmental changes. Molecular markers can be broadly classified into two groups: those based on polymerase chain reaction (PCR) and those based on DNA-DNA hybridization. Genetic linkage mapping in forest trees and organelle genetic analysis have both made use of restriction fragment length polymorphism (RFLP) markers, which are based on DNA-DNA hybridization. Amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD) are the three PCR-based

molecular marker types that are commonly utilized in forest trees. In non-coding sections of genomes, polymorphism is typically shown by all three of these marker types.

References

Aga, E., Bekele E. and Bryngelsson T. (2005). Inter-simple sequence repeat (ISSR) variation in forest coffee tree (*Coffea arabica* L.) populations from Ethiopia. *Genetica*, **124**, 213-221.

Akinnagbe, A., Gailing O., Finkeldey R. and Lawal A. (2019). Towards Conservation of Genetic Variation of Tropical Tree species with differing Successional Status: The case of *Mansonia altissima* A. Chev and *Triplochiton scleroxylon* K. Schum. *Trop. Conserv. Sci.*, **12**, 1-9. https://doi.org/10.1177/1940082919864267

Ballard R.E., Abbott He G, Mink A.G. and Belthoff L.E. (1992). DNA fingerprinting of *Prunus* varieties using low copy sequence probes. Proc. of IUFRO Working Party S2.04.06 Workshop "*Molecular Biology of Forest Trees*", Carcans-Maubuisson, France, June 1992. INRA, Bordeaux.

Botstein, D., White R.L., Skolnick M. and Davis R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Amer. J. Hum. Gen.*, **32**, 314-331.

Bradshaw, H.D. Jr., Villar M., Watson B.D., Otto K.G., Stewart S. and Stettler R.F. (1994). Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS and RAPD markers. *Theoretical and Applied Genetics* **89**, 167-178.

Byrne, M., Moran G.F., Murrell J.C. and Tibbits W.N. (1994). Detection and inheritance of RFLPs *in Eucalyptus nitens. Theoretical and Applied Genetics*, **89(4)**, 397-402.

Carlson, J.E., Tulsieram L.K., Glaubitz J.C., Luk V.W.K., Kauffeldt C. and Rutledge R. (1991). Segregation of random amplified DNA markers in F_1 progeny of conifers. *Theoretical and Applied Genetics* **83**,194-200.

Cato, S.A. and Richardson I.E. (1996). Inter- and intraspecific polymorphism at chloroplast SSR loci and the inheritance of plastids *in Pinus radiata* D. Don. *Theoretical and Applied Genetics* **93**, 587-592.

Cervera, M.T., Gusmao J., Steenackers M., Peleman J., Storme V., Broeck A.V., Montagu M.V. and Boerjan W. (1996). Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus. Theoretical and Applied Genetics*, **93**, 733-737.

Cervera, M.T., Plomion C. and Malpica C. (2000). Molecular markers and genome mapping in woody plants. In: Jain, S.M. and Minocha S.C. (eds.). *Molecular biology of-woody plants. Forestry Sciences*, Volume 64. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 375-394.

Cheliak, W.M. and Pitel JA. (1984). Techniques for starch gel electrophoresis of enzymes from forest tree species. Canadian Forestry Service, Petawawa National Forestry Institute, Information Report PI-X-42.

Chesnokov, Y.V., Kosolapov V.M. and Savchenko I.V. (2020). Morphological Genetic Markers in Plants. *Russian J. Gen.*, **56**, 1406-1415. https://doi.org/10.1134/S1022795420120042

Chezhian, P., Yasodha R. and Ghosh M. (2010). Genetic Diversity Analysis in a Seed Orchard of *Eucalyptus tereticornis*. *New Forests*, **40**, 85-99. https://doi.org/10.1007/s11056-009-9184-1

Conkle, M.T. (Technical Coordinator) (1981a). *Proceedings of the symposium on isozymes of North American forest trees and forest insects.* USDA Forest Service General Technical Report PSW-48.

Conkle, M.T., Hodgskiss P.D., Nunnaly L.B. and Hunter S.C. (1982). Starch gel electrophoresis of conifer seeds: A laboratory manual. *USDA Forest Service General Technical Report* PSW-64

Devey, M.E., Jermstad K.D., Tauer C.G. and Neale D.B. (1991). Inheritance of RFLP loci in a loblolly pine three-generation pedigree. *Theoretical and Applied Genetics*, **83**, 238-242.

Echt, C.S. and May-Marquardt P. (1997). Survey of microsatellite DNA in pine. *Genome*, **40**, 9-17.

Echt, C.S., May-Marquardt P., Hseih M. and Zahorchak R. (1996). Characterization of microsatellite markers in eastern white pine. *Genome*, **39**, 1102-1108.

El-Kassaby, Y.A. and Ritland K. (1996a). Genetic variation in low elevation Douglas-fir of British Columbia and its relevance to gene conservation. *Biodiver. Conserv.*, **5**, 779-794.

El-Kassaby, Y.A. and Ritland K. (1996b). Impact of selection and breeding on the genetic diversity in Douglas-fir. *Biodiv. Conserv*., **6**, 795-813.

Ganesan, S., Singh R., Choudhury D.R., Bharadwaj J., Gupta V. and Singode A. (2014). Genetic Diversity and Population Structure study of Drumstick (*Moringa oleifera* Lam.) using Morphological and SSR Markers. *Industrial Crops and Products*, **60**, 316-325. https://doi.org/10.1016/j.indcrop.2014.06.033

Hamrick, J.L. and Godt M.J.W. (1989). Allozyme diversity in plant species. IN: *Differentiation patterns in higher plants* (Urbanska, K. ed.) pp. 53-67. New York: Academic Press.

Hanover, J.W. (1966a). Inheritance of 3-carene concentration in *Pinus monticola. Forest Sci*., **12**, 447-450.

Hanover, J.W. (1966b). Genetics of terpenes. I. Gene control of monoterpene levels in *Pinus monticola* Doug. *Heredity*, **21**, 73-84.

Hanover, J.W. (1992). Applications of terpene analysis in forest genetics. *New Forests*, **6**, 159-178.

Hasan, N., Choudhary S., Naaz N., Sharma N. and Laskar R.A. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *J. Gen. Engg. Biotech.*, **19(1)**, 1-26.

Hattemer, H.H. (1995). Concepts and Requirements in the Conservation of Forest Genetic Resources. *Int. J. Forest Gen.*, **2**, 125-134.

Ingvarsson, P.K. and Dahlberg H. (2019). The Effects of Clonal Forestry on Genetic Diversity in Wild and Domesticated Stands of Forest Trees. *Scandinavian J. Forest Res.*, **34**, 370-379. https://doi.org/10.1080/02827581.2018.1469665

Jamnadass, R., Hanson J., Poole J., Hanotte O., Simons T.J. and Dawson I.K. (2005). High differentiation among

populations of the woody legume *Sesbania sesban* in sub-Saharan Africa: Implications for conservation and cultivation during germplasm introduction into agroforestry systems. *Forest Ecol. Manage.*, **210**, 225-238.

Jermstad, K.D., Reem A.M., Henifin J.R., Wheeler N.C. and Neale D.B. (1994). Inheritance of restriction fragment length polymorphisms and random amplified polymorphic DNAs in coastal Douglas-fir. *Theoretical and Applied Genetics*, **89**, 758-766.

Kephart, S.R. (1990). Starch gel electrophoresis of plant isozymes: A comparative analysis of techniques. *Amer. J. Bot.*, **77**, 693-712.

Kozlowski, T.T. and Pallardy S.G. (1979). *Physiology of Woody Plants.* Academic Press, London, UK.

Kremer, A., Petit R., Zanetto A., Fougere V., Ducousso A., Wagran D. and Chauvin C. (1991). Nuclear and organelle gene diversity in *Quercus robur* and *Q. petraea.* In: Muller-Starck, G. and Ziehe M. (eds.). *Genetic Variation in European Populations of Forest Trees.* Sauerlander's Verlag, Frankfurt, Germany, pp. 141-166.

Landry, B.S and Michelmore R.W. (1987). Methods and applications of restriction fragment length polymorphism analysis to plants. pp. 25–44. In : Bruening, G., Harada J. and Hollaender A. (eds). *Tailoring genes for crop improvement: an agricultural perspective*. Plenum Press, N.Y.

Lavin, M., Mathews S. and Hughes C. (1991). Chloroplast DNA variation in *Gliricidia sepium* (Leguminosae): Intraspecific phylogeny and tokogeny. *Amer. J. Bot.*, **78**, 1576–1585.

Lewontin, R.C. and Hubby J. (1966). A molecular approach to the study of genic heterozygosity in natural populations. II Amounts of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura. Genetics,* **54**, 595-609.

Litt, M. and Luty J.A. (1989). A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Amer. J. Hum. Gen.*, **44**, 397-401.

Liu, Z. and Furnier G.R. (1993). Inheritance and linkage of allozymes and restriction fragment length polymorphisms in trembling aspen. *J. Heredity*, **84**, 419-424.

Loveless, M.D. (1992). Isozyme variation in tropical trees:patterns of genetic organization. *New Forests*, **6**, 67-94.

Lowe, A.J., Gillies A.C.M., Wilson J. and Dawson I.K. (2000). Conservation genetics of bush mango from central/west Africa: implications from random amplified polymorphic DNA analysis. *Mole. Ecol.*, **9**, 831-841.

Marques, C.M., Araujo J.A., Ferreira J.G., Whetten R., O'Malley D.M., Liu B.-H. and Sederoff R. (1998). AFLP genetic maps *of Eucalyptus globulus* and *E. tereticornis. Theoretical and Applied Genetics*, **96**, 727-737.

Minn, Y., Prinz K. and Finkeldey R. (2014). Genetic Variation of Teak (*Tectona grandis* Linn. f.) in Myanmar Revealed by Microsatellites. *Tree Genetics & Genomes*, **10**, 1435-1449. https://doi.org/10.1007/s11295-014-0772-7

Mitton, J.B. (1983). Conifers. In Isozymes in plant genetics and breeding. Part B. Edited by Tanksley, S. D. and Orton T.J. Elservier Science Publishers, Amsterdam.

O'Malley, D.M., Porter S. and Sederoff R.R. (1992). Purification, characterization, and cloning of cinnamyl alcohol dehydrogenase in loblolly pine (*Pinus taeda* L.). *Plant Physiology*, **98**, 1364- 1371.

Powell, W., Morgante M., Andre C., McNicol J.W., Machray G.C., Doyle J.J., Tingey S.V. and Rafalski JA. (1995). Hypervariable microsatellites provide a general source of polymorphic DNA markers for the chloroplast genome. *Curr. Biol.*, **5**, 1023-1029.

Russell, J.R., Weber J.C., Booth A., Powell W., Sotelo-Montes C. and Dawson I.K. (1999). Genetic variation of *Calycophyllum spruceanum* in the Peruvian Amazon Basin, revealed by amplified fragment length polymorphism (AFLP) analysis. *Mole. Ecol.*, **8**, 199-204.

Sanchez, M., Ingrouille M.J., Cowan R.S., Hamilton M.A. and Fay M.F. (2014). Spatial Structure and Genetic Diversity of Natural Populations of the Caribbean Pine, *Pinus caribaea* var. *bahamensis* (Pinaceae), in the Bahaman Archipelago. *Botanical J. Linnean Soc*., **174**, 359-383. https://doi.org/10.1111/boj.12146

Smith, D.N. and Devey M.E. (1994). Occurrence and inheritance of microsatellites in *Pinus radiata. Genome*, **37**, 977-983.

Soltis, D.E. and Soltis P.S. (1989). *Isozymes in Plant Biology*. Dioscorides Press, Portland,

Squillace, A.E. (1971). Inheritance of monoterpene composition in cortical oleoresin of slash pine. *Forest Sci*., **17**, 381-387.

Strauss, S., Hong Y.P. and Hipkins V. (1992). Chloroplast and mitochondrial DNA subdivision in the California closed-cone pines. Proc. of IUFRO Working Party S2.04.06 Workshop Molecular Biology of Forest Trees. Carcans-Maubuisson, France, June 1992. INRA, Bordeaux.

Strauss, S.H. and Critchfield W.B. (1982). Inheritance of p-pinene in xylem oleoresin of knobcone X Monterey pine hybrids. *Forest Science* **28**, 687-696.

Tulsieram, L., Glaubitz J., Kiss G. and Carlson J. (1992). Single tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. *BioTechnology,* **1**0, 686-690.

Vendramin, G.G., Lelli L., Rossi P. and Morgante M. (1996). A set of primers for the amplification of 20 chloroplast microsatellites in Pinaceae. *Mole. Ecol.* **5**, 595-598.

Vos, P., Hogers R., Bleeker M., Reijans M., van de Lee T, Homes M., Frijters A., Pot J., Peleman J., Kuiper M. and Zabeau M. (1995). AFLP: a new technique for DNA fingerprinting. *Nuc. Acids Res*., **23**, 4407-4414.

White, T.L., Adams W.T. and Neale D.B. (2007). *Forest Genetics*. CABI.

Williams, J.G.K., Kubelik A.R., livak K.J., Rafalski J.A. and Tingey S.V. (1990). DNA polymorphoisms amplified by arbitrary primers are useful as genetic markers. *Nuc. Acids Res*., **18(22)**, 6531-6535.

Yuskianti, V. and Isoda K. (2012). Genetic Diversity of Acacia Mangium Seed Orchard in Wonogiri Indonesia Using Microsatellite Markers. *HAYATI J. Biosci.*, **19**, 141-144. https://doi.org/10.4308/hjb.19.3.141