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Study of Molecular Markers in Agroforestry

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ABSTRACT

Agroforestry is the combination of agriculture and forestry on farms and agricultural lands. The primary goal is to create a healthy and harmonious symbiosis with forest trees and agricultural crops using conventional farming techniques. Agroforestry is highly beneficial as it is a sustainable food production method. The main advantage of planting trees in between crops is to prevent water runoff and soil erosion. Plants undergo several unfavorable conditions during their growth cycle. Such conditions include abiotic stresses like drought, temperature, scarcity of nutrients and high levels of salt in the soil. This adversely affects the yield and productivity of crops. Molecular markers play a significant role in identifying stress tolerant genes in many crops. This helps in enhancing crop production, yield and most importantly improving food and nutrition security. Thus, it helps in improving the income of farmers. In this article, we will be studying the use of molecular markers to improve Agroforestry. Molecular markers are the most effective tools for the analysis of gene tags, genetic variability, phylogenetic evaluation and improvement of new and exquisite cultivars. The markers used in tree improvement are RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment length Polymorphism), SSR (Simple Sequence Repeats) or Microsatellites and SNP (Single Nucleotide Polymorphism). There are numerous advantages of using molecular markers in tree improvement as they been placed on the tree population, and it is been screened for a wide range of variety of physiological and quality traits. They play an important role in enhancing genetic gain for quantitative traits. With the help of molecular markers, they can do phenotyping, genotyping and also the construction on the tree populations. The selective markers are used to capture the extent of the genetic variation, as the diversity at the phenotypic level which is larger than the diversity at the marker level. Screening of controlled crosses in Neem tree, markers like M13, AP-PCR are used for germplasm identification; genetic mapping and breeding purposes are few among them. This project focuses on the molecular markers in tree improvement.

Keywords— Agroforestry, Gene tags, Genetic variability, Genotyping, Molecular Markers, Phylogenetic evaluation.

1. INTRODUCTION

Agriculture is a highly significant contributor to the national gross product. Since the land for agriculture is decreasing, a lot of forests are being changed into agricultural lands. This conversion is leading to forest degradation and global climate change. Hence this practice is considered unsustainable as the value of natural environment decreases in time. Understanding this development, various studies were done considering agroforestry system as a new paradigm.

Agroforestry is a collective name for efficient, integrated and sustainable development land management system that involves simultaneous cultivation of agricultural crops and forest crops. The main objective of agroforestry is to manage the land effectively so that its productivity is increased and restored. Agroforestry is highly beneficial for the environment and economy, as it produces more output and proves to be more sustainable. The Agroforestry systems can regulate the run off and soil erosion which in turn reduces loss of water, soil, organic matter and nutrients. Agroforestry makes maximum use of the land available which results in increased production and sustainability of small scale and large-scale agriculture. Soil fertility and nutrient status of the soil can be improved by the use of nitrogen fixing trees and shrubs in agroforestry system. The incidence of total crop failure is reduced which is very common in monoculture system. The physical, chemical and biological properties of soil can be

enriched by adding significant amount organic matter and recycling nutrients in agroforestry system. The high organic carbon content in soil is due to the presence of diverse plant species in agricultural land. The amount of soil carbon sequestration can be increased by incorporating trees in agroforestry system [1].

Incorporation of agricultural crops through agroforestry system is highly beneficial for improving the air and water quality of the surrounding environment [2]. Increment in the rotation age of crops will result in large amount of carbon sequestration. Therefore, it is the best alternative to replace unsustainable agricultural practices of sole cropping system.

Agroforestry being advantageous to the farmers, selection of the agroforestry species should be selected based on their personal needs.[9]

- (a) The various factors are taken into consideration like, The fast-growing tree species: As the trees are grown for commercial purpose, the fast-growing species will attain its average size within a short period. They also should have the short rotation.
- (b) Usually, the multipurpose trees are been selected because of having high economic values.
- (c) Selection of sparsely branched trees: The selected species should not block the sunlight to the crop plants which are growing under them. The tree species can withstand the pruning operation if it possesses dense canopy.
- (d) Depending according to the climate: the selected species should be well adapted to the climate and its changes of their locality.
- (e) Selection of the trees with deep roots: this is one of the most important aspect to be considered because, the agricultural crops will absorb the minerals, solutes and water from the surface. Hence selection of the species should have the ability to penetrate and little deep into the soil to absorb the necessary nutrients, solutes and mostly underground water.
- (f) adapt to the soil conditions: as the pH of the soil changes due to several factors like chemicals,
- (g) rain and so, the tree species should be adaptable to the local conditions and should grow vigorously in that particular soil type.
- (h) They should not interfere with the soil moisture as the species selected for the agroforestry should have least amount of water requirement.
- (i) They should help in building the soil fertility.
- (j) Soil erosion: it is again important to select the plant species that checks for the soil erosion.
- (k) The tree species should have the nutrient cycling and nitrogen fixation attributes. As they should convert the atmospheric nitrogen to organic nitrogen, by the use of the relationship between Rhizobium bacteria and their roots. Ex: *Acacia albida*, *Saraca indica*.

While selecting the agro-forestry species many factors should be taken into consideration as above [10]. The various species that are important and usually the choice of farmers are, Poplar (*Populus alba*), Ber (*Zizphus sp.*), Mahua (*Madhuca indica*), Eucalyptus (*Eucalyptus globulus*), Shisham (*Dalbergia sissoo*), Mango (*Mangifera indica*), Mahua (*Moringa sp.*), Teak (*Tectona grandis*), Kadamb (*Anthocephalus kadamba*), Semal (*Bombax malabaricum*), Gualr(*Ficus glomerata*), Bel (*Aeglemamelos*), Saijana (*Moringa sp.*) Neem, Bamboo etc.

Table 1: Selection of tree species based on climatic conditions

S. no	Climate type	Tree species
1	Hot desert	<i>Acacia tortilis</i> , <i>Capparis spp</i> , <i>Tecomella undulate</i> , <i>Prosopis cineraria</i> , <i>P. chinensis</i> ,
2	Cold desert	<i>P. tremula</i> , <i>P. euphretica</i> , <i>salix alba</i> , <i>S fragilis</i> , <i>Juniperus</i> , <i>Populus nigra</i> , <i>P. cilita</i> , <i>P. alba</i>
3	Tropical semi-arid	<i>Salvadora persica</i> , <i>Tamarix spp</i> Subtropical semi-arid: <i>Pinus roxburghii</i> , <i>Acacia modesta</i> , <i>Albizia procera</i> , <i>Bauhinia variegata</i> , <i>Prosopis spp</i> , <i>Acacia tortilis</i> , <i>A. nilotica</i> , <i>A. senegal</i> , <i>Albizia lebbeck</i> , <i>Eucalyptus camaldulensis</i> .
4	Temperate semi-arid	<i>Corylus colurna</i> , <i>Pinus gerardiana</i> , <i>Juniperus macropoda</i> .
5	Humid tropical	<i>Dipterocarpus macrocarpus</i> , <i>Cocus nucifera</i> , <i>areca catechu</i> , <i>Artocarpus heterophyllus</i> , <i>Pterocarpus santalinus</i> , <i>Chukrasia tubularis</i> , <i>Terminalia myriocarpa</i> , <i>Terminalia alata</i> , <i>Schima wallichii</i> .
6	Humid subtropical	<i>Pinus kesiya</i> , <i>Prunus spp</i> , <i>Quercus spp</i> , <i>Eucalyptus globulus</i> , <i>Acer oblungum</i> , <i>Acrocarpus fraxinifolius</i> , <i>Aesculus indica</i> .
7	Humid temperate	<i>Alnus nitida</i> , <i>Populus ciliata</i> , <i>Cryptomeria japonica</i> , <i>Acer campbellii</i> , <i>abies pindrow</i> , <i>Quercus spp</i> , <i>Robinia pseudacacia</i> , <i>Pinus alata</i> , <i>P. wallichiana</i>
8	Subtropical semi-humid	<i>Albizia chinensis</i> , <i>Pinus roxburghii</i> , <i>Eucalyptus grandis</i> , <i>E. globulus</i> , <i>Toona ciliate</i> , <i>P. kesiya</i> , <i>P. ellioti</i> , <i>Grewia optiva</i> .
9	Tropical sub-humid	<i>Eucalyptus teriticornis</i> , <i>E. citridora</i> , <i>Casuarina equisetifolia</i> <i>Anthocephalus chinensis</i> , <i>Adina cardifolia</i> , <i>populus deltoides</i> , <i>Moringa oleifera</i> , <i>Dalbergia latifolia</i> , <i>Bombax ceiba</i> , <i>Morus alba</i> , <i>Dalbergia sissoo</i> , <i>Leucaena leucocephala</i> ,
10	Subtropical semi-humid	<i>Albizia chinensis</i> , <i>Pinus roxburghii</i> , <i>Celtis australis</i> , <i>Morus indica</i> , <i>Toona ciliata</i> , <i>Eucalyptus grandis</i> ,
11	Temperate semi-humid	<i>Fraxinus spp</i> , <i>Quercus spp</i> , <i>Juglans regia</i> , <i>Alnus nepalensis</i> , <i>Cedrus deodara</i> , <i>Celtis australis</i> , <i>Acacia mearnsii</i> , <i>Acer oblungum</i> . <i>Eucalyptus globules</i>

Table 2: Selection of tree species based on the soil type

S. no	Soil condition	Tree species
1	Desert soil	<i>Prosopis cineraria</i> , <i>A. Senegal</i> , <i>P. chilensis</i> , <i>Acacia tortilis</i> etc.
2	Recent Alluvium	<i>Acacia catechu</i> , <i>Bombax ceiba</i> , <i>Dalbergia sissoo</i> etc.
3	Old alluvium	<i>Prosopis spp</i> , <i>Acacia nilotica</i> , <i>Ailanthus spp</i> , <i>Pongamia pinnata</i> , <i>Tamarix spp</i> , <i>Eucalyptus spp</i> .
4	Deltaic and coastal alluvium	<i>Casuarina equisetifolia</i> , <i>Cocus nucifera</i> , <i>Aviennia spp</i> .
5	Red soils	<i>Madhuca indica</i> , <i>Mangifera indica</i> , <i>Tectona grandia</i> , <i>Acacia nilotica</i> , <i>Eucalyptus hybrid</i> , <i>Adina cardifolia</i> , <i>Azadirachta indica</i> .
6	Black cotton soils	<i>Acacia nilotica</i> , <i>Hardwickia binnata</i> , <i>Adina cardifolia</i> , <i>Aegle marmelos</i> , <i>Bauhinia spp</i> , <i>A leucophloea</i> , <i>Dalbergia latifolia</i> , <i>Tectina grandis</i> .
7	Hill soils	<i>Cedrus deodra</i> , <i>Quercus spp</i> , <i>Juglans regia</i> , <i>Alnus nitida</i> , <i>Celtis australis</i> , <i>Grewia optiva</i> .
8	Peaty and organic soil	<i>Bischofia javanica</i> , <i>Lagerstromia speciosa</i> , <i>Glircidia sepium</i> , <i>Ficus glomerata</i> , <i>Bischofia javanica</i> , <i>Syzygium cuminii</i> .
9	Laterite and lateric soils	<i>Emblica officinalis</i> , <i>Tectona grndis</i> , <i>Eucalyptus spp</i> , , <i>Acacia auriculiformis</i> , <i>Azadirachta indica</i> , <i>Tamarindua indica</i> .

2. MOLECULAR MARKERS

Markers are used in plant breeding as it has high prevalence and expression in different stages of the organism. The use of genetic manipulation done at DNA molecular levels which in turn helps in improving the characters of interest in plants and animals which includes generic engineering, marker assisted selection, genomic selection etc. is known as molecular breeding. Breeding offers many challenges associated with outcrossing breeding [11]. There are many applications of using molecular markers in plant breeding like, genetic conservation efforts by identification of genetic diversity hotspots, assembly of breeding populations. Monitoring and characterization of population dynamics and gene flow, proper delineation of species taxonomy for conservation, assessment of gene flow in seed orchards, the assessment of inbreeding occurrence studies of mating systems and genetic fingerprinting in advanced breeding.

2.1 Molecular markers used in tree improvement:

2.1.1 RAPD (Random amplified polymorphic DNA): This is the dominant marker is mainly used for the estimation of heterozygosity of trees among different species [17]. To develop the DNA markers the most commonly used method is PCR (polymerase chain reaction) for taxonomic identification, genetic diversity and paternity analysis. This reaction as small pieces of about 10 base pairs short primers or oligonucleotides. The oligos hybridize randomly on any given genome resulting in production of many loci for one individual. Per agarose gel can continue 3 to 8 loci [14]. For staining of the DNA fragments ethidium bromide is used as it intercalates double stranded DNA, hence deforming it. Hence, RAPD markers can be used for diversity and phylogeny purposes. In this there is no prior knowledge of the Mathers required, no use of radioactive probes, simpler and relatively low-cost technique. It produces DNA primers of varied complexity depending on primer and template used. Some drawbacks of RAPD markers are the band formed can faint so to avoid inconsistency start with clean template DNA, consistent methodology and quantified concentration. Random nature of their generation, short primer length, poor reliability and reproducibility and sensitive to environmental conditions are the drawbacks of using RAPD. RAPD is widely used in tree genetics and breeding in *Eucalyptus* species, *Morus* species, neem, porch, olive etc., To overcome the problem of Dominance they are assayed from haploid megagametophyte tissue. Species specific RAPD markers are used in *Guerue rober* and *Q-petraea* to detect natural hybrids between species [31]. Genetic maps in poplus genome were also identified by RAPD.

2.2.2 RFLP (Restriction fragment length polymorphism): It is the co-dominant marker with very high reproducibility. These are Mendelian genetic markers which are formed due to mutations and rearrangements of the DNA. This is used to detect the coupling phase of the DNA molecules where DNA fragments from ask the homologous chromosomes are detected [11]. RFLP is the reliable market in linkage analysis and breeding which determines if the linked trait is present in the homozygous or heterozygous state in an individual which in turn desirable for the recessive traits. The samples are digested into pieces with the help of restriction enzymes and analysed these by gel electrophoresis [17]. Restriction endonucleases cleaves the DNA at specific palindromic sequences. Nucleic acid hybridization with radioactive or fluorescent probe is used to detect the individual fragments. The demerits of using RFLP is it is time consuming; level of polymorphism is low and it needs high quality and quantity of DNA. The requirement of radioactive isotope makes this method hazardous and expensive. These markers have demonstrated interpopulation differentiation in *Giricidia septum*, *Pinus attenuata*, *QTL* reported in some of the forestry trees by RFLP markers are *Acacia mangium* for disease resistance [18], *cryptomeria japonica* for vegetative propagation, *Eucalyptus vitens* for growth, *Pinus teada* for physical wood properties [31].

2.2.3 AFLP (Amplified fragment length polymorphism): These are carried out on cultivars like wheat, barley, rice, melon, corn and soyabean. It is used to generate linkage maps and fingerprinting regardless of complexity and origin of the genome. It is more effective than RAPD and RFLP as it produces large number of loci for any one of the reactions [14]. It has rare and frequent cutters which are the restriction enzymes. These are the dominant markers. It is highly repeatable and more loci. Primer's adapters are used in this which links to stick ends of the DNA fragments. The fragments are analysed using gel electrophoresis which generates fingerprints. Nucleotide motifs are used to know the number of bands produced. Obtained specific band differences are cloned and sequenced to identify source of polymorphism. In *Arabidopsis* the study of developmental genes by this marker is the advantage. Some disadvantages of this markers are: need of large amount of DNA, quality of DNA, irregular cuts which leads to

undesirable results. Besides these drawbacks they are popular in estimating outcrossing rates, study of genetic diversity, paternity analysis, dense mapping and germplasm collections [31]. In tree species it is mainly used for estimating out crossing rate like variation in Tall, intermediate and dwarf forms of *focus nucifera*.

2.2.4 SSR (Simple sequence repeats) or Microsatellites: This is a ideal genetic marker which provides rapidity and specificity in PCR [15]. They are multiallelic and codominant in nature. These are short segments of the DNA derived from tandemly repeated sequences of short motifs. Many are located in telomeric, centromeric regions and in roots of chromatin loops. They play important role in pairing and synapsis of chromosomes. They are amplified by PCR reaction using primers which flanks the tandem repeats. The fragments are separated on polyacrylamide gel or autoradiography. It is used for the study of noncommercial species for detection of markers in chromosomal regions of interest. They are considered to be highly polymorphic because the number of varies repeat due to slippage of DNA polymerase during replication. The frequency of SSR is higher in transcribed regions when compared to non-transcribed regions. They are used for genotyping in individuals and study of gene flow in forestry species. SSR's in tree species like eucalyptus, rubber tree, first developed were in *Pinus radiata*. They are developed from the nuclear genomes of range of temperate and tropical forest trees. SSR from chloroplast genomes has been isolated which is paternally linked in most gymnosperms which are used in gene flow and paternity testing.

2.2.5 ISSR (Inter simple sequence repeats): They ISSR marker is not emerging as an alternative for the SSR (microsatellites) and combines the advantages of amplified fragment length polymorphism (AFLP) and amplified polymorphic DNA (RAPD) [13]. This in technique the is amplification of the genomic segments is involved by inversely oriented and closely spaced microsatellites segments which are used as primers in a PCR to generate multilocus markers. It is a quick reliable method A single primer or a pair of primers which is based on SSR anchored 5 to 3, with around 1-4 pyrimidine residues. These markers are dominant markers and inherited in Mendelin mode. These markers are been widely been used on the field of plant genetics in plant breeding and studies of cultivator identification, gene tagging, phylogeny, genetic diversity, genetic mapping.

3. ADVANTAGES AND APPLICATIONS OF MOLECULAR MARKERS IN TREE IMPROVEMENT

- (a) Estimation of polymorphism, relatedness and mating system parameters and gene flow is the prerequisite for tree breeding and selection programs for conservation.
- (b) Estimation of mating system parameters like out crossing rates, inbreeding coefficients, extent of pollen dispersal are the factors for designing seed orchards for optimal seed yield. Isozyme is used for mating system and gene flow as they have co-dominant mode of inheritance.
- (c) Genetic identity of the individuals including inter, intra-inbreed lines and clones is done by genotype characterization by using different markers. Clone identification in forestry species by using markers in poplars and RAPD markers in neem tree. This also helps in screening of controlled crosses.
- (d) Marker assisted selection: use of RFLP and RAPD for quantitative trait loci mapping in trees. Mapping in forestry species include resistance to white pine blister sugar pine, wood density and volume and rust resistance in eucalyptus Francis.
- (e) Establishment of genetic diversity in domesticated population isozymes to access variability in breeding populations with wild from the selection. RFLP is better approach than RAPD in this application.
- (f) Germplasm identification: Forest trees have high levels of isozyme variation so markers are required for the germplasm identification problems. Markers like M13, AP-PCR, SSLPs are used for germplasm identification.
- (g) Controlled crossing is important for molecular breeding.
- (h) Genetic mapping and breeding purpose: RFLP and RAPD is used to develop genetic linkage maps.
- (i) Identification of clones and ramets in gene banks to avoid contamination and duplication.
- (j) Identifying their taxa and evolutionary histories.
- (k) As the genomic DNA is present in all the cell in any part of the plants it can be isolated easily.
- (l) It is time saving strategy for the plant breeders to carry out the genetic crosses, as the target trait information can be obtained with the linked DNA markers found before pollination.
- (m) Environmental factors play complicated role in the phenotypic characteristic of the traits, this making it difficult to evaluate the genetic traits.
- (n) Despite these complications, DNA markers remain neutral to the to the environmental variations.
- (o) Biosafety is a major concern in plant breeding. Several biochemical and diagnosis tests are been conducted in the laboratory conditions to check the presence or absence of the particular traits for disease resistance. It can be conducted the markers when it is rightly linked to the target gene of interest without resorting to the pathogen inoculation either in the field or greenhouse.
- (p) It helps the farmers and the breeders to reject the undesirable traits by selecting the desirable traits using molecular markers in the plant breeding of the early generations.

4. CURRENT RESEARCH OF MOLECULAR MARKERS IN AGROFORESTRY

In an era of dwindling research funding, it is critical that we prioritize the agroforestry research agenda for the twenty-first century. Agroforestry research should generate innovations for solving the land-management problems that we set out to solve more than 30 years ago, and research should be a means rather than an end in itself.

Molecular markers have proven to be useful tools for evaluating plant genetic resources by allowing us to better understand the distribution and a degree of genetic variation within and among organisms. Through expanded genome coverage, newly established marker technologies allow for unparalleled uncovering of the degree of genetic variation. Markers have a wide range of applications in plant sciences, but due to their inherent features, these marker forms have also shown their limitations. For an accurate assessment of the extent of intra- and inter-population genetic diversity of naturally distributed plant species from which

proper conservation directives for species in decline can be given, a combination of diverse marker types is generally recommended. Natural populations of forest trees are examined in this paper by summarizing existing studies in terms of genetic variation in pure organisms. In general, genetic diversity within populations of outbred forest tree species is greater than genetic diversity among populations of the same species., indicating a lack of spatial structure in the immediate vicinity. Furthermore, as with plants in general, phenotypic diversity is much greater than marker diversity, since selectively neutral markers are widely used to capture the degree of genetic variation. . However, nucleotide diversity within candidate genes underlying adaptive traits is increasingly being examined for single-site signatures of selection. This adaptive genetic diversity holds significant promise for forest management and conservation in the future.

Historically, two major areas have always been essential for molecular marker applications in plant improvement:

- (a) Determining genetic diversity within, within, and among populations.
- (b) Genotype verification and characterization

Example of use of molecular markers in Barely:

- (a) In barley, an AFLP marker has been associated to water-stress-tolerant bulks (*Hordeum vulgare* L.) The amplified fragment length polymorphism (AFLP) assay is a quick and easy way to identify molecular markers that can help you enhance a variety of crops.
- (b) SSR markers revealed a lot of variation among *Chrysanthemum* cultivars, which might be exploited in breeding operations to improve the plant.
- (c) A SNP marker for the dwarfing gene is among the SNP markers related with essential rice genes. The SNP was discovered within an SSR flanking sequence and is employed in a variety of crosses for selection. Rice-blast resistance genes have also been given SNP-based markers.
- (d) In the development of genetic maps, RAPD markers are used. Genetic maps have been created for various plants, including the model plant *Arabidopsis* and tobacco. In coffee, RAPD markers were used to create 15 linkage groupings. Probes were made from both genomic and chloroplast DNA.
- (e) The RFLP marker aids in the transmission of specific fruit quality traits from wild to domesticated plants. From wild tomato, a tightly connected RFLP marker for the sucrose accumulator gene has been introduced to tomato (*Lycopersicon esculentum*) (*Lycopersicon chmielewski*).

The following is a summary of the potential of molecular markers for forest gene conservation management:

- (a) To determine the taxa's identity and relatedness, as well as infer their evolutionary histories.
- (b) To avoid mislabeling, duplication, and contamination, correct the correct clones and ramets in gene banks.
- (c) Determine the amount, scope, and distribution of genetic variation within and between populations.
- (d) Calculate the mating system (selfing and out-crossing rates) as well as gene flow.
- (e) Using genetic information, assess the status of genetic resources as criteria for ex situ and in situ conservation.
- (f) To maximize gene conservation management by combining adaptive traits, ecogeographical, and genetic surveys for both ex-situ collecting programs and identifying in situ conservation sites.

5. SUMMARY AND CONCLUSION

Agro-forestry is advantageous in food production and water management. In this paper, the main objective is to prove the advantage of molecular markers in agro-forestry. The use of molecular markers in tree improvement can be helpful in improving the desired characteristics in plants at a DNA molecular level. They can also be useful in genetic conservation attempts by identifying the genetic diversity hotspots, monitoring and characterization of population dynamics and gene flow, assessment of gene flow in seed orchards and genetic fingerprinting in advanced breeding.

The most common markers used in tree improvement are RAPD, RFLP, AFLP and SSR. RAPD is the most reliable marker used for estimation of heterozygosity of trees among different species. The most common method used to develop DNA markers is PCR; for taxonomic identification, genetic diversity and paternity analysis.

RFLP is a co-dominant marker with very high reproducibility; they are Mendelian genetic markers resulting from mutations and rearrangements of the DNA. It is one of the most reliable markers in the linkage analysis and breeding to find out heterozygous or homozygous state in an individual.

AFLP is used to generate linkage maps and fingerprinting regardless of complexity and origin of the genome. It is more effective than RAPD and RFLP as it produces large number of loci for any one of the reactions. It has rare and frequent cutters which are the restriction enzymes. These are the dominant markers. It is highly repeatable and more loci.

SSR is an ideal genetic marker which provides rapidity and specificity in PCR. They are multi-allelic and co-dominant in nature. These are short segments of the DNA derived from tandem repeated sequences of short motifs.

Molecular markers in agro-forestry prove to be advantageous in various ways. It can be used to estimate polymorphism, mating parameters and gene flow for tree breeding. Inter and intra- inbred lines and clones can be identified by genotype characterisation by using various types of markers. This method has provided solution for germ plasm identification of isozyme in tree species. RFLP proves to be a better technique than RAPD for this. RFLP and RAPD are helpful in developing genetic linkage maps, identifying taxa and evolutionary histories. The most important agricultural application is farmers and breeders can reject the undesirable traits using molecular markers in plant breeding in early generations. There are multiple disadvantages of molecular markers as well, beyond these, the DNA markers remain neutral to the environment variations.

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