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REVIEW ARTICLE

A review on Genetic markers with special reference to Genetic Diversity of Four Economically Important Trees of Myrtaceae Family of Braj Region, India

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Abstract

Callistemon viminalis (Sol. ex Gaertn.) Byrnes, *Psidium* guajava L., *Syzygium cumini* L. and *Eucalyptus grandis* W.Hill are members of the Myrtaceae family and are found growing in various locations throughout the Districts of Agra and Mathura. Although some trees can be found in tropically dry and xeric locations, these trees are found in tropical and subtropical regions. These plants have significant medical value, are frequently used customarily in the Braj region, and contain high amounts of secondary metabolites and polyphenols that prevent the separation of plant DNA. DNA isolation is a method for purifying DNA from plant elements by integrating various chemical and physical techniques. For the investigation of plant genetic variation, DNA markers like PCR (RAPD, SSLP, and AFLP) are often used methods that only need a minimal amount of DNA material. The most widely used method for examining genetic diversity is RAPD analysis. RAPD is utilized for a variety of tasks, including gene mapping, genetic identification, and investigations involving closely related species. This database review's main goal is to provide extensive, essential genetic information, methods, and applications. Significant connections between the history of a plant species and the makeup of its current population can be found thanks to genetic diversity. Researchers may utilize this information in the future to manage, cultivate, and improve the breeding programme for these plant species.

Keywords

Economic important trees; Molecular study; Myrtaceae; DNA marker; PCR; RAPDs.

Introduction

Fredrich Miescher carried out the first DNA isolation in 1869. Purification or refinement of DNA from a material using a combination of physical and chemical techniques is known as DNA isolation. The development of genetic or hereditary markers has allowed for the analysis and quantification of genetic multiplicity. For the purpose of carrying out plant domestication, protection, and specifically breeding programmes, genetic variety and genetic variation are particularly important (10). The cells are damaged and lysed as part of the isolation and purification process, then proteins and other impurities are removed, and finally the DNA is restored. Understanding how a plant species will react to changes in the environment depends mostly on the study of genetic variation within a species. Important sources about a species' ancestry and the makeup of its current populations can be made available thanks to genetic diversity (2). Genetic variation is the initial mechanism that provides assurance against genetic erosion for breeding programmers. DNA has a dual stranded helical structure, which is made up of two strands that wind around one another. It is arranged into chromosomes,

which are the primary source of genetic information in living things. Along with proteins, lipids, and carbohydrates (polysaccharides), DNA is made up of nucleic acids; the double strands of DNA are referred to as poly nucleotides. The cells are broken up and lysed as part of the analysis and purification process, then the proteins and other impurities are removed, and lastly the DNA is recovered. In order to increase genetic variety, genetic isolation with randomly amplified polymorphic DNA markers has been used.

One of the most crucial factors in the evolution of plant species is genetic diversity. A detailed grasp of genetic diversity is necessary for identification, clarity maintenance, the application of plant variety protection rights, and export in accordance with WTO criteria. To access genetic or hereditary variety, morphological traits, pedigree analysis, and molecular markers can all be used (3). Since they are numerous, unaffected by the environment, and do not require pedigree information, molecular markers, also known as genetic markers, are useful tools for evaluating genetic variation (4).

A DNA marker, often known as a molecular marker, is a specific DNA region that identifies genetic variations. Molecular markers shouldn't be handled like conventional genes because they frequently have no biological effect. Instead, think of them as enduring monuments in the genome. DNA markers are recognisable DNA segments that are present at particular locations across the genome and are passed down through the generations in accordance with accepted inheritance principles

Numerous different DNA marker types have been altered, and improvements in sequencing methods have increased yield. The advancements made in genomics, selection, genetics, and genome editing have improved our understanding of DNA markers and given us deep insights into the variety of crops and breeding techniques (5).

PCR-based methods are divided into two groups: sequencing-targeted PCR-based methods (RAPD, AFLP) and arbitrarily primed PCR-based methods (RAPD, AFLP) (SSLP, SNP). An easy-to-use, PCR-based DNA marker called RAPD only requires a little amount of DNA material. RAPD has demonstrated to be a useful approach for recognising germ plasm and discovering genetic variations in a range of bacterial, microbial, and plant species (6). Random amplified polymorphic DNA (RAPD) markers are utilized for a variety of purposes in plant heredity research because they are convenient for examination (7).

From studies involving closely related species to individual study, RAPDs have been used for a number of purposes. In gene mapping studies, RAPDs have been utilized to fill in the blanks that no other markers could. DNA Amplification Fingerprinting and Arbitrarily Primed Polymerase Chain Reaction (APPCR), which use deeper arbitrary primers than RAPDs, are two RAPD approaches (DAF). The Multiple Arbitrary Amplicon Profiling (MAAP) technique is a method for profiling numerous. The main advantage of RAPDs is how quickly and easily they can be tested. Little amounts of template DNA are needed because PCR is utilized. The genome has a large number of RAPDs that are dispersed randomly. In order to reduce DNA sample contamination, RAPD investigations frequently call for purified, high molecular weight DNA and synthesis because few random primers are used to amplify DNA fragments in a variety of organisms.

The use of DNA-based molecular markers, also referred to as DNA markers, has increased as a versatile tool in industries including nomenclature, plant breeding, seed production, and genetic manipulation. There are numerous uses for using molecular methods to identify DNA diversity in individual plants. Molecular markers are no longer regarded as basic DNA fingerprinting in variability study, but they are updated regularly to increase their value and automate the genome analysis method (8).

The pattern of variation present in the population of economically significant trees must be understood in order to use the DNA markers for domestication, protection, management, and tree reproduction. Considering that they are unaffected by ecological factors and reveal changes across the entire genome, molecular markers are one of the most important and quite reliable signals. Some of the molecular markers used in genetic fingerprinting include random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), restriction fragment length polymorphism (RFLP), and sequence tag sites (STS) (9).

Most frequently used in RFLP (Restriction Fragment Length Polymorphisms) are DNA markers (RFLP, 3). Genomic clones with an anonymous modest copy number are frequently used to study polymorphisms. Target DNA sequence information is needed for the creation of amplification primers in other polymorphism experiments centred on the PCR (polymerase chain reaction). The time and cost involved in gathering this sequence information make it impractical for many large-scale genetic mapping applications. In the amplification products, polymorphisms are frequently found and can be found using an agarose gel stained with ethidium bromide. This method is not appropriate for everyone because it reads random sections of genomic DNA to look for polymorphisms using primers of any nucleotide sequence. The combined use of several single locus detection techniques is known as DNA fingerprinting. The description of genome fingerprinting, genetic variability, gene localisation, genome mapping, analysis of genome evolution, population genetics, taxonomy, etc. are all included in this technique (10). An authoritative way to comprehending the evolution and passing on of genetic possessions in unmanaged and managed populations can be obtained by genetic analysis employing DNA marker technologies. For systematic conservation, evolutionary, and ecological investigations, it is crucial to identify taxonomic units and observe the uniqueness of plant species (11). RAPD analysis is the quickest and most straightforward molecular technique utilised for genetic similarity research, according to (7).

In the past ten years, DNA markers have emerged and integrated fast into the arsenal of common laboratory tools available for genome research. Among the various uses of RAPD technology are the determination of parentage, cultivar identification, evaluation of genetic relationships, and calculation of population genetic diversity (12-15).These DNA RAPD markers are most frequently used for biosystematic study, cultivar identification and genetic diversity assessment (16). Regardless of chromosome position or unique nucleotide sequences, both RAPD and AFLP markers may provide a similar evaluation of DNA sequences throughout the genome.

Genetic diversity can be used to better understand the molecular origins of numerous biological processes in plants. DNA or genetic marker techniques including RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), and SSR (Simple Sequence Repeats) are employed in evolutionary, taxonomic, ecological, phylogenic, and genetic studies of plants. These tactics have been identified, along with how they can be used and their restrictions. Recent years have seen the emergence of advanced approaches, primarily as a result of the addition of prior important methods. On cDNA-based templates, the RAPD and AFLP approaches are also being utilised to examine gene expression patterns and identify the genetic underpinnings of biological responses.

Genetic markers might be improved for a variety of functions thanks to the introduction of random primers, which overcame the restriction of prior sequence knowledge for PCR observation. Sequence focused PCR-based methods and arbitrarily primed PCR-based methods, also known as sequence nonspecific methods, are the two different types of PCR-based procedures. Retrotransposon-based DNA markers IRAP, REMAP, and S SAP facilitate the investigation of genome-wide discontinuities among strongly related people (17). Additionally, molecular markers have been utilized to identify new genes responsible for the differential gene expression profile and to explain the origins of many biological phenomena.

Even though immature, flaccid leaves that have just begun to grow are the ideal starting material for the DNA isolation process for two reasons. First of all, mature leaves are more difficult and more difficult to ground. The removal of the DNA band is also made more challenging when using mature leaves since a wide white band appears immediately beneath the DNA band in the CsC1 density gradient. A notable technique to prevent polyphenol contamination of the finished DNA synthesis is to isolate nuclei before lysis.

By isolating nuclei in a buffer containing glucose salt and citrate, Katterman and Shattuck (18]) successfully isolated DNA from Gossypium plant, which contains large levels of polyphenols. The solution's chemical composition and pH prevent the subsequent procedures from producing oxidised polyphenolic chemicals (19). Both small and large areas can be isolated using this technique. It is quick and easy to extract DNA from a readily available source, and it yields restriction endonuclease-digestible DNA. The degree of genetic alterations in plant populations was evaluated and homozygous and heterozygous individuals were distinguished using biochemical markers (20).

Isozyme analysis was utilised by Wendel et al. (21) to look into the genetic distances between numerous upland cotton accessions from various locales. The isozyme observation has several limitations because marker loci are readily available, elite propagation sources typically contain few polymorphisms, and banding patterns may change as a result of plant improvement (22). An endless number of markers can be produced by the RAPD (random amplified polymorphic DNA) method, which can be used for a variety of purposes (23). Because of its technological simplicity and speed, the RAPD technique (24) has a similar level of genetic resolution to restriction fragment length polymorphism (RFLP) for identifying genetic communication between Brassica oleracea L. genotypes breeding lines (25).

RAPD markers have been successfully utilized on a variety of plant species, including rice, brassica, and lycopersicon, to examine genetic diversity and cultivar assessments (26-27). Multani and Lyon (28) examined 14 Australian cotton varieties using RAPD markers and silver staining and showed that closely related kinds could be distinguished. The genetic gaps discovered by RAPD markers of 16 elite US cotton cultivars were linked to the taxonomy gaps discovered by morphological criteria. The RAPD technique works well for identifying differences between strains of the same species. Due to how fast and readily sample can be processed, the RAPD technique is advantageous for determining population genetic features (7).

In the last ten years, a number of techniques using PCR-based methods have been created, each with a unique combination of advantages and disadvantages. Using randomly amplified polymorphic DNA (RAPD) markers is a quick, easy method that doesn't require any prior sequence knowledge. Using a single primer of any nucleotide sequence, it exposes polymorphisms in nucleotide sequences (7). The polymerase chain reaction (PCR) method, which is most frequently used for these reasons, is dependent on these molecular markers. They are useful for differentiating different plant species genetically. The RAPD marker has been widely used in population genetic research and DNA fingerprinting (29-31). DNA (RAPD) is widely used for determining genetic diversity due to its speed, simplicity, and low cost when compared to other molecular markers (7, 32).

Collection of Data

For this database review study, the systematic scientific data on genetic markers technology, such as RAPD, SSLP, and AFLP etc. were gathered from several books and scientific reports as well as Google Scholar, PubMed, Science Direct, Scopus, and Web of Sciences. These trees were chosen for this review because to their medicinal and

economic value, and also because they are all members of the same family

Family Myrtaceae

Over 3000 species and 130 genera of shrubs and trees belong to the family Myrtaceae, which are mostly found in the tropics and subtropics of the world. The Myrtaceae family includes the plants *Callistemon viminalis* (Bottle brush), *Psidium guajava* (Guava), *Syzygium cumini* L. (Jamun), and *Eucalyptus grandis*. (Fig.1 A–D). All plant species are woody, have flower parts that are multiples of four or five, and contain essential oils. Selected plants have alternate, evergreen, primarily opposite, simple, and toothless leaves. Five petals make up a flower. Typically, the stamens are large, obvious, many, and brightly colored.The majority of hardwood trees are eucalypts, which come in over 700 different kinds (33). Most of the species are native to Australia and were introduced to South Africa, France, Brazil, and India. Seven species, including *Corymbia citriodora, Eucalyptus globules, Eucalyptus tereticornis, Eucalyptus camaldulensis, Eucalyptus grandis, Eucalyptus pellita*, and *Eucalyptus globules*, have been demonstrated to be suitable for Indian climatic conditions. They are widely planted throughout the subcontinent (34,35). In Braj region of western Uttar Pradesh the different variety of these 4 plants are planted in Mathura, Agra, and Aligarh areas. Based on their economic importance, these four plants were chosen for this review study because they are all members of the same family and found in Braj region and are commonly utilized in many traditional health systems.

There are many therapeutic applications for the small woody tree or shrub known as *Callistemon viminalis*, which has pendulous leaves. Ten of the 34 plants of the Callistemon genus, 10 of which are indigenous to India, are recognized for their cylindrical, brush-like flowers that resemble classic bottlebrushes (36). With the exception of extremely dry and cold areas, this lovely shrub, often called bottle brush or red bottle brush, may be found in many places and has a major medicinal potential. Additionally, it is present in botanical gardens and on the streets. Various kinds of callistemon are grown as farm trees for forestry operations or for ornamental purposes. The bulk of Callistemon species are found in Australia's southeast and east. Common uses for Callistemon species include forestry and the production of essential oils.

The guava, or *Psidiumguajava* L., is a subtropical and tropical longstanding and evergreen fruit tree that reaches a height of 25 feet. The tiny, pear-shaped, reddish-yellow fruit of this plant is 3 to 5 cm long when fully ripe. Eatable *P. guajava* fruit is used to treat intestinal spasms, severe diarrhoea, and coughing. Vitamins A and C, fibre, and folic acid are all present in guavas in good amounts. The leaves and bark of *P. guajava* are used to cure and prevent illnesses like headache, spasm, cough, inflammation, acute diarrhoea, pyrexia, colic, gastric pain, and flatulence.

A tree with great therapeutic value, *Syzygium cumini*, sometimes called *Syzygium jambolanum*, black plum, or Jamun, is used in many conventional medical systems. Other names for *S. cumini* include Indian Blackberry, Black Plum, Java Plum, and Jambul. *S. cumini* is a domesticated diploid tree with chromosomal number 2n=66. These trees are now spread across Eastern South America, Africa, and Asia. Only once a year does *S. cuminiy*ield fruit, and the berries have a sweet-sour flavour. The ripe fruits can be used to make jams, jellies, health drinks, squashes, and wine. Since ancient times, the fruits, leaves, seeds, and bark of Jambul have all been used in Ayurvedic treatment.In folk medicine, the leaves, fruits, bark, and seeds of *S. cumini* are used to cure diabetes because they have a hypoglycemic effect (37).

Shakya et al. (38) looked at Jamun's (*Syzygium cumini* L. Skeels) molecular identification. Whether through natural selection or through the efforts of breeders, plant improvement has always focused on creating, evaluating, and choosing the best combination of alleles. A huge number of genes must be changed in order to change even the most basic features. These choices revealed some tiny variations when OPZ9 and OPA12 primers were used. Asif et al. (39) sequenced and analysed the chloroplast genome of *Syzygium cumini* (L.), a member of the Myrtaceae family. These polymorphism traits may be employed as intra-specific indicators to assess whether lineage sorting has taken place as a result of polymorphic heritage. The intergenic spacer between the IRA/large single copy (LSC) boundary and the first or opening gene of the LSC area, which was determined by a 54-bp length,

Fig. 1.A: Plants of *Callistemon viminalis* **B:** *Psidium guajava,* **C:** *Syzygium cumini* L., **E:** *Eucalyptus grandi*.

was expanded in the chloroplast genomes of *S. cumini* and related dicots. Investigations on the phylogeography of *Syzygium* and other members of the Myrtaceae family may benefit from this region's variation. The cp genome has been well conserved in plants and algae despite duplications, mutations, gene deletions, and rearrangements (40).

Ahmed et al. (41) investigated the molecular characterisation of the guava (*Psidium guajava* L.) germplasm using RAPD analysis. With *P. guajava*, molecular and morphological characterizations were employed to demonstrate different levels of diversity. The 10 mer and 12 mer oligonucleotide primers were amplified using RAPD. Identification of uncultivated, high-yield cultivars of *P. guajava* is aided by RAPD. Microsatellite markers were studied by Viji et al. (42) to define Guava germplasm genetic study (*Psidium guajava*). Microsatellite markers were employed to investigate the guava variety. Seven PCR primers amplifying fourteen previously created microsatellite loci were used to characterize and identify 13 guava accessions. Polymorphic microsatellite can be employed to identify genetic varieties or as a DNA marker, which is crucial for the parent recruitment process of the guava improvement programme.

In order to better understand the genetic diversity and germplasm structure of the guava plant, Kareem et al. (43) explored the morphogenetic classification of 37 guava accessions. For the 18 microsatellites analysed, repeatable and scorable bands ranged in size from 150 to 320 bp. numerous types of variability were examined in the genotypes with distinctive morphogenetic characteristics. The DNA analysis identified four groups of the selected Guava accessions based on a 50% difference index. SNPs produced for Eucalyptus were used by Costa & Santos et al. (44) to look into the genetic diversity of *Psidium* accessions. The SNP transferability of Eucalyptus to *Psidium* species has been quite reliable since the signal amplification of Eucalyptus SNP sequences in the *Psidium* genome occurred, correlating with earlier studies of microsatellite genetic divergence.

For the two intermediate genetic density linkage maps for Eucalyptus species, Grattapaglia and Sederoff (45) presented the joint pseudotestcross mapping approach in grouping with DNA (RAPD) testing and evaluated a total of 558 markers. Using RAPD markers, gametic and meiosis segregation in each individual may be directly monitored. 48 randomly mapped random amplified polymorphic DNA (RAPD) markers that show a 53% amplify from small copy areas are used to characterise the complexity of the genome. These are the earliest period documented heavy treatment connection maps for hardwood tree species other than *Eucalyptus* species.

In order to identify the ideal hybrid combinations, Abad et al. (46) researched the crucial stage of a breeding programme where heterosis is employed. For the purpose of researching genetic diversity, 40 *Eucalyptus grandis* and *E. urophylla* plants were observed. Microsatellite and RAPD markers were more useful for identifying different species. On the other side, there was a weak and negative correlation between RAPD and microsatellite markers. Microsatellite markers were successful in differentiating the parents of *E. grandis* and *E. urophylla.*

Inter-simple sequence repeat (ISSR) markers were employed by Balasaravanan et al. (47) to examine the genetic interactions between and within the six species of Eucalyptus. 583 loci from 149 individuals of the Eucalyptus species were amplified using seven ISSR primers. Using ISSR fragments, individuals with high levels of polymorphism and genetic variation were identified. They claim that by using this knowledge, sampling techniques can be developed that efficiently capture genetic variation for use in selection experiments and the subsequent dispersion of clonal planting material. Eucalyptus plant species' genetic diversity enables breeding practises to produce offspring with superior genetics.

Verhaegen and Plomion (48) looked into two single tree linkage maps for *Eucalyptus urophylla* and *Eucalyptus grandis* using 480 Random Amplified Polymorphic DNA (RAPD) markers. Relative mapping studies of two *E. urophylla* and *E. grandis* linkage maps, according to their argument, showed that RAPDs are reliable markers for the beginning of a consensus species map.The developing field of eucalyptus genomics has been examined by Ribeiro et al. (49). They claim that although the molecular characteristics of *E. globulus* and *E. grandis* are well understood, there has been very little observation of the genome and chromosome organisation. The karyotypes of *E. globulus*, *E. grandis,* and *E. calmadulensis* were compared and described. To develop modified breeding techniques for this genus, understanding the arrangement of the Eucalyptus genome is absolutely essential.

With a focus on regulatory sequences, transcriptomes, and genetic material families in Eucalypts involved in the manufacture secondary cell wall, Hefer et al. (50) reported on the genetic regulation of wood development. Three *Eucalyptus grandis* trees' primary and secondary tissues underwent stable mRNA sequencing in order to study transcriptional regulation of cellulose production and lumber development. They were able to identify the genes that are co-regulated and differently expressed during various stages of wood production.Six polymorphic microsatellite markers created for the Eucalyptus species were utilised by Kirst et al. (51) to examine the genetics of 192 individuals in a breeding population of Eucalyptus grandis. The set of microsatellites is an efficient method for identifying Eucalyptus for fingerprinting and parentage testing due to its high level of multiallelism and simple co-dominant Mendelian inheritance.Using 454 pyrosequencing technology, Novaes et al. (52) reported the gene sequences in *Eucalyptus grandis*. An assortment of genotypes and tissues were included in the cDNA pool used to produce the expressed sequence technology (EST) sequences. Estimates of synonymous and non-synonymous nucleotide diversity were often lower, suggesting purifying selection.

Conclusion

Improvement of plant genetics depends on the existence, nature, and range of manipulable genetic variables. Using genetic experiments with random amplification of polymorphic DNA markers, it has been generally shown how genetic diversity of plant species is determined. The most widely used techniques for examining the genetic diversity of organisms are RAPDs. The conservation and development of these plants may be significantly impacted by RAPD markers. The primary importance in determining how a species will react to environmental changes is the study of genetic variety within a species. The main benefit of RAPDs is that they are rapid and simple because little DNA template is needed for PCR. Numerous tools for analysing genetic diversity at the genome level and evolutionary relationships between species have been developed by the area of molecular biology. The primary goal of this review is to act as a database for detailed information and economic significance of four Myrtaceae family plants: *Eucalyptus grandis* W.Hill, *Psidium guajava* L., *Callistemon viminalis* (Sol. ex Gaertn.) Byrnes and *Syzygium cumini* L. Researchers may utilize this genetic information in the future to manage, cultivate, and improve plant species' breeding programmes.

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