Review Article

PCR Study of Eucalyptus Hybrids using Random Primers for Hybrid Validation

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ABSTRACT

Eucalyptus is planed widely for its raw material in paper industry. Genetic markers have been used for validation and assessment of high density genotyping in Eucalyptus species. DNA marker based species identification and hybrid validation is an important tool in breeding program. Four molecular marker systems—RAPD random amplified polymorphic DNA), ISSR inter simple sequence repeat), SRAP sequence-related amplified polymorphism), and SSR simple sequence repeat)—were used to evaluate seed genetic purity of a hybrid. PCR amplification with marker resulted in polymorphism among the population of different species, were used to analyse the diversity of Eucalyptus species. To determine the genetic purity of hybrids, it is important to discriminate the genotype of parents and hybrids. This study shows that RAPD and ISSR markers are highly effective and reproducible for testing genetic purity of commercial hybrid seeds.

Keywords: Genetic markers, Hybrid, Amplification, Genetic purity, Genotype, Polymorphism.

INTRODUCTION

Eucalyptus plant species are the hardwood species, so this plant gives the pulpwood for the paper industry.[1] Brazil is the largest Eucalyptus plantation country where the wood is used in Iron and Steel production.[2] Also in Ethiopia, trees of eucalyptus dominate the plantation forestry.[3] In recent years world energy is going to decrease significantly; so far that eucalyptus plants are used as alternative energy plants. Species of Eucalyptus accounts for about 74% of forested area in which Eucalyptus globules is the main cultivar.[4] The sustainable thermal insulating materials development is a problem for the industry from this case, but natural fiber plays a major role. The eucalyptus plants are used as a natural fiber, because they contain the thermal insulating properties as well as low environmental impacts when compared to synthetic fibers.[5] Eucalyptus barks panel show lower carbon emissions than conventional insulation materials. It is also used as a sustainable material for the construction sector. Energy and exergo environmental analyses are developed for a eucalyptus plant. Overall energy efficiency is 16.89% due the high moisture content of the eucalyptus.[6]

Origin and Distribution

Eucalyptus is mainly endemic to Australia, but several species occur in part of Indonesia, New Guinea and the Philippines. They are grown not only across Australia, but also throughout Asia, South Africa, Southern Europe and Africa.[7] Among the available species, 30 are widely planted due to their broad range of adaptability and fast growth.[8] In today's new carbon economy, eucalyptus are receiving attention as fast growing, short – rotation, renewable biomass crop for energy production.[9] It is the most widely planted hardwood species in the tropical and subtropical region[10] and an important source of carbon neutral renewable energy and raw material for pulp, paper and solid wood.[11]

Eucalyptus plants are traditionally used as a medicine. They have antiseptic, antimicrobial, antioxidant and anti inflammatory activities. Eucalyptus spp. contain the component known as Eucalyptol, this component contribute the medicinal attributes.[12] All Eucalyptus is evergreen, but some tropical species shed their leaves at the end of the dry season.[13] These plant leaves and oil is famous for medical properties and also used in herbal products. They also used for health or therapeutic purposes.[14] This also acts as a host for endophytic fungi, which were found to be lignin degrading microorganism.[15] The plant has a high degree of drought resistance. In India this plant can grow in the temperature between in the range of 0°C to 47°C

Hybrid Production

Hybrid breeding is an important process among the methods used in the improvement of vegetable crops. Hybrids were made by cross between two genetically different parents. Male parent pollen Pollen parent) will pollinate, fertilize and set seeds in female seed parent) to produce F1 hybrids. Hybrid cultivar Fl) development is followed by Hybridization of genetically variable parents and molecular marker techniques are often used for fastening plant improvement using male parent-specific RAPD markers[16].

New hybrid breeding methods allow much bigger genetic diversity to be introduced to different varieties leading to increased levels of success in making desirable agronomic characteristics[17]. Elite inbred strains were taken which

express consistent phenotypes such as high crop yield, increased production, rich nutrient content) that are relatively good for inbred plants. From 1996 five strawberry F1-hybrid cultivars had been started to get cultivated. The recent introduction is the cultivar is 'Elan'[18].

Hybridization Program in Eucalyptus

In the Eucalyptus hybridization program, key traits which have been identified for improvement by hybridization include clonal propagation, coppicing ability, frost, drought and salt resistance, resistance to pests, wood density and pulp yield[19]. Presently, breeding for traits improvement in eucalyptus has been mainly obtained by controlled crossing programs between species with better pulping traits like *E.grandis* and *E.globulus*. These crosses are basically designed based on the inherent nature of the species like *E.camaldulensis*×*E.tereticornis* for better rooting ability, *E.grandis* and *E.globulus* for better cellulose quality, *E.camaldulensis* for salt tolerance and *E.nitiens* for frost tolerance. Currently, the area under Eucalyptus hybrid throughout the world is more than 3.9 lakh hectare.[20]

In India interspecific hybridization was attempted between *E.tereticornis* ×*E.camaldulensis, C.citriodra* × *E.E.teorelliana, E.torelliana* × *E.camaldulensis* and *E.tereticornis* × *E.grandis* in 90 and 80s and a series of controlled and natural hybrids with considerable superiority biomass) over the parents were produced. At IFGTB, an exhaustive hybridization program was initiated to enhance productivity in *E.tereticornis*. Hybrids of *E.tereticornis*× *E.camaldulensis, E.grandis, E.pellita, E.urophylla and E.abla* have been developed and deployed in field trialsmin coastal and inner wet agro climatic regions. Similar inter specific hybrids between *E.urophylla*×*E. grandis, E.tereticornis, E.camaldulensis, E.pellita*were also developed by Mysore paper mills.[21]

Molecular markers are useful for plant genome analysis and have now become an important tool in genetic diversity analysis. DNA based markers are popular means for identification and authentication of plant species. They are less affected by age, physiological condition of samples are environmental factors and are not tissue specific and thus can be detected at any phase of development. The power of discrimination of DNA-based markers is comparatively high and hence very closely related varieties can be differentiated.

The most commonly used DNA based marker are Random Amplified Polymorphic DNA RAPDs), Inter simple sequence repeats ISSR), Amplified Fragment Length Polymorphism AFLPs), Restriction Fragment Length Polymorphism RFLPs) and Microsatellites or Simple Sequence Repeats SSRs).These markers vary in the amount of DNA required, the cost of development and assay, the amount of genetic information revealed and transferability across taxa.[22] Most of these techniques rely on the advantages of PCR detection, including speed, sensitivity and selectivity, except RFLP.

Marker for Genetic Studies

Microsatellite markers were used for fingerprinting of DNA of the hybrids to accesses variation within parental and provenience, also the genetic purity.[23] They were also used to identify genetic diversity and population genetic structure.[24] In genetic studies DNA based molecular markers are used as the popular genetic engineering tool and to produce the linkage maps.[25] RAPD is the one of the efficient tool used for the identification of marker linked important traits. It is used to detect hybrid separation, create specific probes, taxonomic identity and detect interspecific gene flow. It is one of the low expensive techniques then the limited DNA is required for the analysis.[26] SSR is called simple sequence repeat. It is a short nucleotide repeats it is contain high polymorphic level. This marker is highly informative, stable and it is the power full tool.[27]

RFLP is the one of the rapid technique. PCR based molecular methods are used to find the sea food authentification.[28] PCR method are used to detect the species specific variation which are not close knit organisms because of the highly similarity of the DNA which can be overcome by the RFLP, due to destroy of the PCR amplicon by using the different restriction enzymes. The other molecular markers are known as next generation sequencing NGS), DNA micro array techniques; single stranded conformational polymorphism SSCP). For differentiate the species based variation the RFLP method is commonly used [29].

STMS markers play a huge role in genetic studies. Among various DNA based markers, genetically mapped sequence tagged microsatellite sites are vital because of their co-dominance, abundance, and uniform distribution throughout the genome.[30]To test the purity of genome in hybrid seed the STMS marker linked to the fertility restorer gene at a distance of 9.5 cM[31] in the pollen parent. The microsatellite markers are considered more reliable because of their ability to produce high fidelity profiles as a result of their co-dominant nature and chromosome specificity.[32]

RAPD Marker

DNA polymorphism can be used as molecular marker for identification. In a study, it had been reported that RAPD is a novel technique based on random DNA sequence by PCR with primers to discriminate the cultivars and detect the disease resistant gene.[33] RAPD and ISSR were used to check seed purity of a commercial cabbage F1 hybrid.[34] According to this study, Between 'Zaoxia 16'hybrid) and its parental species, 126 out of 157 screened RAPD primers produced 347 polymorphic bands, in that 44 and 49 primers produced male and female parent specific bands, respectively. As per the study, out of 17 decamer primers used for RAPD analysis only 6 primers i.e., M-188, M-119,M-31, M-33, M-122 AND M-191 yielded good amplified products.[35]

RAPD technique is applied for to identify the subspecies variation.[36]RAPD is used to create the linkage map. In this technique the arbitrary sequences are used for amplification in a low annealing temperature about 35°C.[37] Primers are bind randomly by underlying the genomic sequence. If we want to get a successful amplification the primers should be bind in both strands. This method is a low reproducibility so for that we have to choose the primers.[38] These techniques contain the low cost and the time saving workflow and it is one of the common tools in genetics.[39] The paternity exclusion allows a precise level of individual species identification, indicating that these microsatellites marker will be novel for population genetic and parentage type studies.[40]

ISSR

Literature discussed the work on ISSR primer that, among Fifty-four ISSR primers screened for 'Zaoxia 16' hybrid) and its parental species, only 42 primers detected 112 polymorphic locus between the F1 provenance and its parents, 14 primers produced only Female Parent Specific band, and 20 primers alone generated Male Parent Specific band.[41] In his study he concluded that, twenty ISSR primers produced, on an average, 308 bands in the accessions examined, of which 211 were polymorphic. An author in his work concluded that among ten primers checked, only seven of those produced unmistakable DNA fragments. All the selected individuals of six Eucalyptus species were extensively amplified using seven ISSR primers and produced 583 fragments varying from 265 to 1535 bp.[42]

The Genetic homogeneity is essential to validate the micropropagated plants to determine the genetic quality in hybrid plants. For genetic validation PCR based molecular markers are used such as ISSR Inter Simple Sequence Repeats), AFLP Amplified Fragment Length Polymorphism), SCOT Start Codon Targeted) and RAPD Random amplified polymorphic DNA)these are the main markers that are used for the validation process.[43] This marker is reported as high polymorphic, reproducible and informative when compared to the RAPD markers.[44] The assay for genetic diversity play the most important role in diverse aspects as well as genomic differentiation, designing the breeding programs, improvement of accurate populations, increase the genotype adaptability in a various environment.[45] ISSR is DNA based marker and it is constructed by the bases of the penta nucleotide, dinucleotide, or tetra nucleotide repeats.[46, 47] ISSR marker is used in different studies like genetic variation, fingerprinting, germplasm identification, gene mapping.[48,49]

Advantages and Disadvantages of Commonly Used DNA Markers

Molecular Marker	Dominance	Advantages	Disadvantages	References
Random Amplified Polymorphic DNA	Dominant	Quick simple, Inexpensive, Multiple loci from a single primer possible and small amount of DNA required	Problems with reproducibility and Generally not transferable	50
Inter Simple Sequence Repeats	Dominant	Quick simple, reproducible, inexpensive and small amount of DNA required	Increased number of bands per primer	51, 52

In trees species, DNA markers have been used for clonal fingerprinting, phylogenetic and diversity analysis, hybrid confirmation, genome mapping, gene tagging and marker assisted selection for wood uniformity, specific gravity, fiber quality, insect and disease resistance and adoption to stress. In hybridization programs also it is important to determine the best choice of individuals for crossing to optimize the expression of genes of interest.[53]

Traditionally, morphological observations and progeny tests were used as descriptors of genetic diversity, but they failed to reveal the exact taxonomic affinity, since most of the morphological characters are plastic and influenced by environmental factors. For the past two decades, DNA markers have become a key strategy for the study of genetic diversity in tree species and they provide an increasingly accurate assessment of taxonomic relationship and history of gene flow.

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