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DNA Finger Printing Analysis in *Casurina equisetifolia* by Using RAPD Markers

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Abstract: The present investigation was to find out DNA finger printing analysis of *Casurina equisitifolia.* Randomly amplified Polymeric DNA is generally used for this present investigation by using PCR and which will be helpful to find out the results. *Casurina* is generally cultivated in India for timber purpose and also one of the raw materials for paper mills for the production of papers. This plant well grows in the sandy soil and has xerophytic adaptations and also cultivated for salt affected soil reclamation. In the present study, we can use RAPD markers for DNA fingerprinting analysis.

Key words: RAPD · DNA · PCR · *Casurina*

INTRODUCTION

RAPD markers are more suitable for clonal organisms than sexually reproducing organisms. As they breed asexually, a polymorphic fragment among individuals can be used to determine clonal identity. Randomly Ampliified Polymorphic DNA (RAPD) is the simplest of all PCR basedmarkers. RAPD is one of the dominant markers, which mean the dominant allele, will mask the presence of the recessive allele in such a way that hetrozygote will not be distinguishable from homozygote. Random pieces of the genome of 300 to 2000 bp long are amplified using a single oligonucleotide primer of arbitary sequence. RAPDs are well suited for use in the large sample throughout systems required for plant breeding, population genetics and studies of biodiversity.

Polymerase Chain Reaction (PCR) based molecular markers such as Randomly Amplified Polymorphic DNA (RAPDs), Sequence Tagged Sites (STS), Sequence Characterized Amplified Region (SCAR), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) and Inter Simple Sequence Repeats (ISSR) is preferred over hybridization based markers like RFLP [1]. The discovery of the RAPD marker technique [2] enabled dramatic improvements in genetic diversity analysis. Here we used a Polymerase Chain Reaction (PCR) technique, known as the directed amplification of Random primers. PCR based RAPD method used to amplify Random primers of 24 cloned species of *Casurina equisetifolia*. A total Random primers to assess polymorphism within 24 cloned *Casurina equisetifolia*. A total of 32 bands of amplified products were cloned and sequenced. RAPD markers are much valuable tool for plant breeding programs [3]. RAPD markers can be converted into Sequence Tagged Sites (STSs) and STS-Specific Primers [4]. Powerful tools in any genome mapping programme to identify DNA markers linked to economically important quantitative traits [5]. The first practical uses of RAPD markers was in the creation of high density genetic maps were able to place over 250 new genetic markers on a recombinant inbreed population of *Arabidopsis thaliana.* Many important crops system have suffered from a lack of genetic markers [6]. For example, genetic linkage analysis in conifers has been slow primarily due to the long size of the genome and the inherent difficulties involved in producing a segregating F_2 population [7]. The RAPD markers have also been used effectively to assess the amount of genetic diversity in germplasm collections. Using only 25 different decamer oligonucleotide primers [8]. *Casurina* species are of special biological characteristics and highly economic and have a wide range of social benefits. The molecular biology of *Casurina* was started in 20th century mainly for cellular basis of *Casurina*, nif gene isolation, cloning and genetic transformation were notable [9]. High yielding clones of *Casurinas equisetifolia* at the age of 12 months were screened [10].

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Estimates the genetic divergence among the selected 42 clones and were grouped into 12 clusters [11]. Cluster 2 had the fastest growing male clone while cluster 8 had the fastest growing female clone. Assessed genetic variation and population structure of 113 individuals of *Casurinas junghuhniana* sampled from 13 international provenances were examined using inter simple sequence repeat (ISSR) fingerprinting [12]. Thirteen RAPD primers amplified a total of 101 loci of which 15 were monomorphic. For all the 22 clones, unique bands were obtained for 5 clones and the remaining 17 clones were identified by the combination or absence of specific bands, in the case of C. *equisetifolia.* The morphlogical and genetic diversity among three species of *Casurinas (C. equisetifolia, C.glauca and C.Junghuhniana)* and two species of *AlloCasurina* (*A.huegeliana and A.liftoralis*) with 18 morphogenetic parameters and seven ISSR primers [13]. The genetic analyses of species in the genus *Casurina* is still for behind the other agronomically important species. Therefore it is very important to evaluate and adopt new DNA techniques for *Casurina* species. This study was conducted to investigate wheather PCR products contain, minisatelite evaluate the reproducibility of thetechnique and determine the level of polymorphisms within 24 *Casurina* species.

MATERIALS AND METHODS

Plant leaf tissues from 24 cloned *Casurina equisetifolia* were collected from model silviculture (Table 1) nursery at Jayankondam and washed in sterile water, dried with clean paper towels. Genomic DNA, were extracted according to the protocol of was followed [14]. It is commonly referred as the CTAB (Cetyl Trimethyl Ammonium Bromide) a cationic detergent that is used to get rid of protein Concentration and purity of the extracted DNAs were determined using a spectrophotometer and quality was determined using standard agarose gel electrophoresis technique. The RAPD is a technique that utilizes PCR to amplify DNA sequence by using random primers [15]. A total of 2µl of genomic DNAs, 2µl of random primer, 10mM-Taq assay buffer-1µl, 10mM $Mgcl_2$-1µl, 10mM dNTP-1µl, Taq DNA polymerse-0.5µl, Nuclease free water-2.5µl, in a 10µl of reaction volume. PCR was carried in a Corbett palm cycler. With the following a touchdown profile, 3minute hold at 94°C followed by initial Denaturation 94°C for 5 minutes, Denaturation 94°C for 1 minutes, Annealing of primers 37°C for 1 minutes. Repeating for 36 cycles at the end of the PCR sample in 72°C for final extension in 5 minutes and stored at 4°C.

Table 1: Nucleotide sequences of RAPD primers (OPB,OPE,OPM) showing amplification status with 24 clones of *C.equisetifolia*

CODE	Sequence 5-3	Amp status
OPM-05	GGGAACGTGT	+
OPm-13	GGTGGTAAG	+
OPB-18	CCACAGCAGT	+
OPE-06	AAGACCCCTC	+
OPE-07	GTGACATGCC	+
OPE-08	ACGCACAACC	+

Random primers used in present study were composed of 6 primers: OPM-05-GGGAACGTGT, OPM-13-GGTGGTAAG, OPB-18-CCACAGCAGT, OPE-06-AAGACCCCTC, OPE-07-GTGACATGCC and OPE-08-ACGCACACAACC.

The amplified products were loaded on a 1.8%(W/v) agarose gel containing 0.5µg/ml ethidium bromide in a loading buffer consisting of 0.25%(W/v) bromophenol blue, 0.25% (W/v) xylene cyanol. Samples were then electrophorised at v/cm at constant current for 1 hour in the presence of Tris-borate, 2mM EDTA (pH 8.0) Ethidium bromide stained PCR amplicons were visualized and photographed on an UV Transilluminator for data analysis. Bands (amplicons) were scored as present (1) or (0), respectively. A statistical analysis was carried out with the data generated using RAPD distance V1.04 and NTYSpc V2.02 Genetic similarity was calculated as SxY=2nxy, where nx and ny are the numbers of fragements in individuals x and y, respectively and nyx is the number of the amplicons shared [16].The dissimilarity (Dxy=1-Sxy) matices were analysed by unweighted pair group mean average (UPGMA) method of using NTSYS Software.

The input for tree construction is in the form of a rectangular data matrix which was converted to symmetrical similarity or dissimilarity matrix (SIMQ) with the values of SM which was then used to develop in to a Dendrogram using UPGMA clustering method. NTSYSpc can be used to discover pattern and structure in multivariate data. The input can be descriptive information about collections of objects or directly measured similarities or dissemilarites between all pairs of objects. The kinds of descriptors and objects used depend upon the application morphological characters, abundances of species, presence or absence of properties, etc. NTSYSpc can transform data, estimate dissimilarities among objects and prepare summaries of relationships using cluster, ordination and multiple factor analysis.

RESULTS AND DISCUSSION

Casurina equisetifolia extracts obtained from the 24 different cloned samples were checked for the presence of DNA by running the samples on a 0.8% Agarose gel using bromophenol blue as the tracking dye. Optimum quantity of DNA was present in all the extracts. The presence of high intensity bands indicates the presence of good quantity of DNA in all the extracts. The samples that were subjected to RAPD-PCR using 6 different primers were then checked for amplication using a 1.8% agarose gel. The results were obtained and documented.

Six operon primers were used for amplification, (Table 1). All the primers showed better results. Totally 32 scorable bands were identified and reveals a very high genetic variability of 24 clones in *Casurina equisetifolia.* Each of the random primers produced distinct polymorphic and monomorphic bands in all the clones examined. Reproducible results were obtained in this study using specific primer-template DNA combination. Extreme care was taken not to alter any experimental parameters. Ten major clusters are identified and 12 sub clusters are observed. Among twenty four clones APKKD-9 and APKKD-11 are genetically very similar and its coefficient variation value is 0.0435. The clones namely APVSP-15 and APVJM-35 are highly divergent among the rest of the clones. The genetic coefficient variation is 0.555. The genetic distance between APKKD-7 and APSKLM-26 are 0.0800, APVSP-16 and APSKLM-27 are 0.0820 and high genetic distance betweenAPVJM-31 and APVJM-33(0.3173), APKKD-6 and APKKD-10 (0.1667) and also in APKKD-3 and APKKD-12(0.1765) are observed.

Cluster analysis has also revealed high genetic variation among the Andhra Pradesh clones of *Casurina equisetifolia*. Ten major clusters and twelve sub-clusters were observed from the cluster analysis. Fig. 1 shows Genetic distance and similarity estimates between accessions revealed that clone APVJM-35, APVSP-15 and APVSP-14 is more divergent from all other clones and APPKD-04 has also shown the genetic distance of 55% among the other clones and the clone APKKD-09 and APKKD-11 shown resemblance of 92%. Hence the clones which shown the high genetic divergence can be recognized for tree breeding improvement. The similar work also analysed in *Casurina equisetifolia* [17].

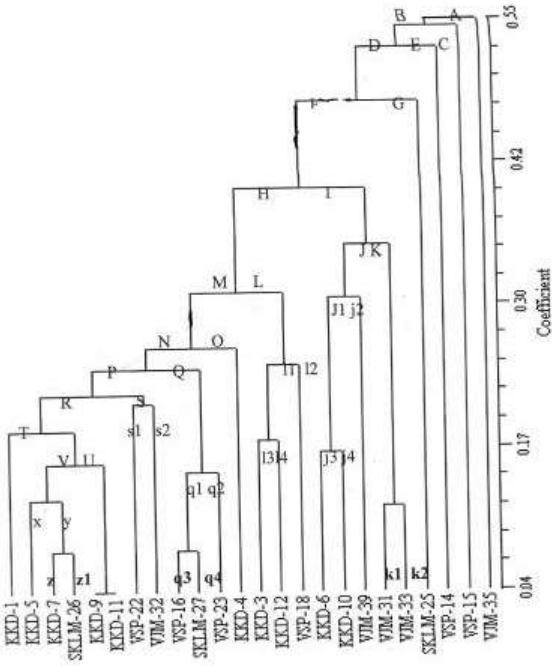

Fig. 1: Dendrogram showing the clustering between different clones of *Casurina equisitifolia*

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