Research Article

Genetic Diversity Assessed Through RAPD Markers in Shorea Tumbaggia

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ABSTRACT

Accurate assessment of genetic diversity is important for successful management and preservation of natural populations. Knowledge of genetic variability with in a population is significant for conservation of the species. The present study was formulated to assess the genetic diversity within *S. tumbaggia* population collected from six different accessions of Sesachalam hills, by employing random amplified polymorphic DNA (RAPD) marker. 20 decamer primers were screened, yielded a total of 22 bands from 8 reproducible primers, with a mean of 3.1amplified bands per primer. The plant species showed 54.8% polymorphism. Cluster analysis (UPGMA) was used to construct the dendrogram based on Jaccards similarity coefficients. Our results reveal that *S. tumbaggia* possess high level of genetic diversity, compared to other endangered plant species. These results also indicate that RAPD is a good molecular to study the genetic diversity of these species.

Keywords: *Shorea tumbaggia*, Endemic, Endangered, Genetic diversity, RAPD markers and Polymorphism

INTRODUCTION

Shorea tumbaggia Roxb. locally known as Tambajalari (in telugu) belongs to the family Dipterocarpaceae. It is an endemic and globally endangered semi-evergreen tree species, (Red list of threatened species IUCN-2006), mainly restricted to the Southern Eastern Ghats up to 100 m, distributed in Seshachalam and Veligonda Hills in Cuddapah, Tirupati in Chittoor District, Andhra pradesh and North Arcot and Chingleput districts[1]. *S. tumbaggia* is valued for its economical and medicinal importance. The tree bark is reported as a good source for phytochemicals[2] and also possesses antiulcer activity[3]. The traditional usage of *S. tumbuggaia* as folklore medicine, the plant parts are administered to counteract heavy sweating. The gum is used in indigenous medicine as an external stimulant and a substitute for arbutus. The Stem is used in marine yards as a substitute for pitch, and the use of tree trunk as flag poles for temples[4].

Intraspecies variation is prerequisite for adaptive change and it has important implications for conservation of the species[5-6]. Morphological markers are routinely used for estimating the genetic variations but are not successful due to strong influence of environment. Hence rapid use of molecular markers has complemented the classical strategies[7] and enabled the characterization of genotypes of all plant species. Now, the Molecular markers have been widely used in determining genetic diversity and to reconstruct evolutionary processes[8]. Polymerase Chain Reaction (PCR)-based methods such as RAPD marker system has been employed because of its simplicity, rapidity, low cost and non-requirement of sequence information for primer design[9]. RAPD polymorphism reflects the variation of the whole genomic DNA and would be a better parameter to measure the pattern of genetic diversity of the rare and endangered plants[10]. A large number of reports have appeared in the literature using RAPD patterns for differentiating varieties, species, etc. of, medicinal, endemic and endangered plants. These include, studies on *Asparagus* species[10], *Capparis decidua*[11], *Catharanthus roseus*[12], *Tylophora rotundifolia*[13], *Changium smyrinoides*[14], *Carum carvi*[15], *Draba dorneri*[16] etc. Wherein subtle differences in the banding patterns have been used as an index to differentiate varieties and assess genetic variability. Therefore, Random amplified polymorphic DNA (RAPD) is the marker of choice in present study. Thus, the prime objective of the present study was to assess the genetic diversity *S. tumbaggia* of by employing RAPD markers.

MATERIALS AND METHODS

Plant Material

A total of six accessions of *Shorea tumbaggia* were collected from six geographic locations of Sesachalam hills of Eastern Ghats, Andhra Pradesh, India. Fresh and young leaf samples were collected and stored in ziplock bags with silica gel and transported back to our laboratory for DNA extraction.

Genomic DNA Extraction

The genomic DNA was extracted from leaves of *S. tumbaggia* by CTAB extraction method[17] with slight modifications. Five grams of fresh leaves were crushed using liquid nitrogen using pre-sterilized mortar and pestle. The powder obtained was thoroughly mixed with 10 ml of pre warmed extraction buffer (0.1 MTris pH 8.0, 20 mM EDTA, 1.4 M NaCl, 1.5% CTAB). Just before the addition of extraction buffer to the crushed material 0.2% b-mercaptoethanol was added to it. The mixture was incubated at 65°C for 60 min with gentle mixing after every 15 min and centrifuged at 10,000 rpm for 10 min. In obtained supernatant equal volume of Chloroform:

Isoamylalcohol mixture (24:1) was added and centrifuged at 10,000 rpm for about 10 min at 4°C. This step was performed thrice and to the obtained supernatant 0. 6 volume of chilled Isopropanol and 0.1 volume of 3M Sodium acetate (PH 5.2) was added to precipitate DNA and was incubated at -20 °C overnight. The mixture was centrifuged at 15000 rpm for about 15min and the precipitated DNA was sterilized by adding 500µl of 70% Ethanol and centrifuged at 7000 rpm for 5 min. The precipitated DNA was air dried and dissolved in TE buffer (10 mM Tris pH 8, 1 mM EDTA pH 8) at room temperature and stored at -20 °C until further use. RNA free DNA was obtained by the addition of 1 µl of RNAse (10 mg/ml) to 100 µl of extracted DNA and incubated at 37 °C for 30 min. The quality of RNase treated extracted DNA was determined by Ultraviolet absorbance at 260 nm and 280 nm using Spectrophotometer and rechecked by a running sample on 1% agarose gel containing 5 µg/ml of Ethidium bromide along with 1 Kb molecular weight marker.

RAPD PCR Amplification

The conditions to carry out amplification of *S. tumbaggia* using PCR were optimized. The best amplified products were obtained using 25 µl of reaction mixture which specifically contained 25 ng template DNA, 0.5mM primer, 0.5mM of each dNTP, 2.5 mM MgCl2, 1X Taq Polymerase buffer, 1 U Taq Polymerase. The amplification reaction was carried out using a CG palm thermal cycler. PCR amplification was performed ins three steps. Initial denaturation at 94 °C for 2 min, followed by 45 cycles of denaturation at 92 °C for 1 min, annealing at 37 °C for 1 min, extension at 72 °C for 2 min with final elongation at 72 °C for 5 min.

Agarose Gel Electrophoresis

RAPD–PCR amplification products were analyzed by electrophoresis on 1.2% agarose gel in 1X TBE buffer. The amplicon size was determined by comparison with standard ladder (1kb; MBI, Ferments, Germany,) containing 5ug/ml of Ethidium bromide. The gels were visualised on a Trans illuminator under UV, then photographed and analysed.

Data Analysis

RAPD patterns were analysed by scoring presence (1) or absence (0) for estimating the similarity among all the tested samples. Following the jaccard's method[18], similarity coefficients was calculated according to the unweighted pair-group method with arithmetic averages (UPGMA) using SPSS (11.0), software. Cluster analysis was done, to generate a dendrogram based on similarity coefficients. The polymorphism percentage was estimated by dividing the number of polymorphic bands over the total number of bands.

Result and Discussion

Six individual populations of *S. tumbaggia* were collected from six geographical locations of Sesachalam hills, represented in Table 1. In the investigation 20 random decamer oligonucleotide primers were screened, of these 20 primers, only 8 primers showed reproducible and scorable bands, 12 sub-optimal or non amplified primers were discarded. The amplification pattern of the reproducible primers is shown in Figure 1 and the details of the RAPD analysis were given in the Table 2. The RAPD profiles of 6 accessions were compared individually for each primer. All these 8 primers resulted in the amplification of 22 bands, of which 14 bands were polymorphic, and 8 bands were monomorphic. The mean number of amplified bands per primer was only 3.14. All primers obtained a wide range of amplicons, ranging from 250bp to 9000bp.The highest number of amplicons (7) was obtained for primer OPA13, followed by (4) for primer OPA3, (3) for primer OPA1 & OPA10, (2) for primer OPA4 and the lowest (1) for primer OPA2, OPA14,OPA18. The primer OPA13 produced a maximum number of polymorphic bands, and OPA3, OPA10 produce a minimum number. The highest level of polymorphism (100%) was produced by two primers OPA1 & OPA18 followed by (71.15%) by OPA3, (66.8%) by OPA10, (50%) by OPA3 &OPA4. The primers OPA2, OPA14 produced only monomorphic bands. RAPD analysis of *catharanthus roseus*[12], using a set of 25 primers, of which only 14 were reproducible. Out of 56 bands 46 polymorphic and remaining 10 were monomorphic. RAPD analysis of *Draba dorneri*[16] out of 52primers scored initially, 76 amplified fragments were obtained from 12 reproducible primers, of which 60 polymorphic and 16 were monomorphic. Jaccards similarity matrix was prepared on the basis of RAPD data, and the dendrogram clustering (Figure 2) based on similarity coefficients generated by the Unweighted pair group Method using arithmetic means (UPGMA), by using SPSS (11.0) software. Similarity coefficience represented in Table 3. A maximum similarity value of 88.2% was observed between plants of 5 and 3 accessions, followed by 81.3% between 6 and 3 and a minimum of 47% between 6 and 4. The dendrogram constructed (six accessions into a single cluster) based on the similarity co-efficient, showed plants from 3, 5 & 6 accessions seems to be more close to each other i.e., means genetically more similar, as compared to plants of 1, 2 & 4 accessions, which are genetically most diverse. The genetic similarity coefficients among all six populations varied from 0.471 (between 1 & 6) to 0.882 (between 3 & 5).

Table 1: *Shorea tumbaggia* accessions collected from different localities

S. No	Locality	Area of Study
1	LOC 1	Talakona
2	LOC2	Nelakona
3	LOC3	Papavinasanam
4	LOC4	Akkagarla gudi
5	LOC5	Japalitheertham
6	LOC6	Balapalli

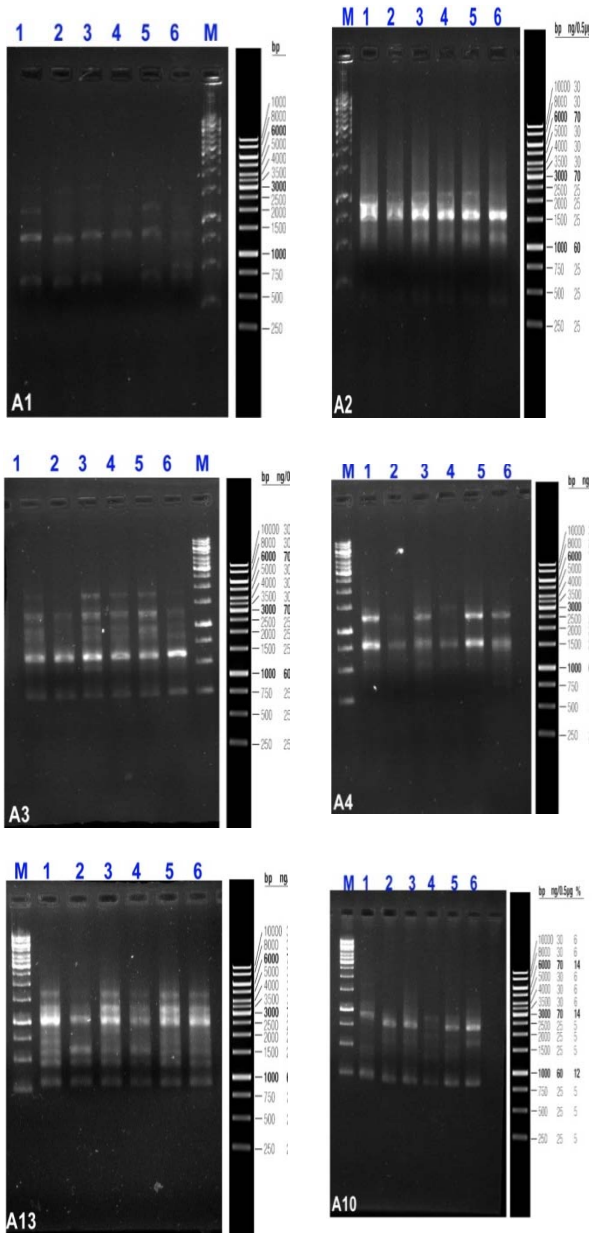

Figure 1: The RAPD profiles of *S. tumbaggaiav* Samples 1 - 6 *S. tumbaggaia* individuals. M-1 kb DNA ladder

Table 2: Primers with their sequences used for RAPD analysis of *Shorea thumbaggia*, the total number of bands, polymorphic bands and the percentage of polymorphism yielded by each primer.

Name	Sequence 5	Total	Polymorphic	% of
A1	CAGGCCCTTC	3	3	100
A2	TGCCGAGCTG	1	0	0
A3	AGTCAGCCAC	4	2	50
A4	AATCGGGCTG	2	1	50
A13	GTGATCGCAG	7	5	71.15
A14	CAGCACCCAC	1	0	0
A18	TCTGTGCTGG	1	1	100
A10	AGGTGACCGT	3	2	66.8
		22	14	54.8%

Table 3: Jaccard's similarity coefficients of polymorphism obtained in *Shorea thumbaggia* with 20 random decamer primers.

	Matrix File Input					
Case	Loc 1	Loc 2	Loc 3	Loc 4	Loc 5	Loc 6
Loc 1	1.000					
Loc 2	.550	1.000				
Loc 3	.667	.647	1.000			
Loc 4	.550	.600	.647	1.000		
Loc 5	.667	.556	.882	.647	1.000	
Loc 6	.600	.667	.813	.471	.706	1.000

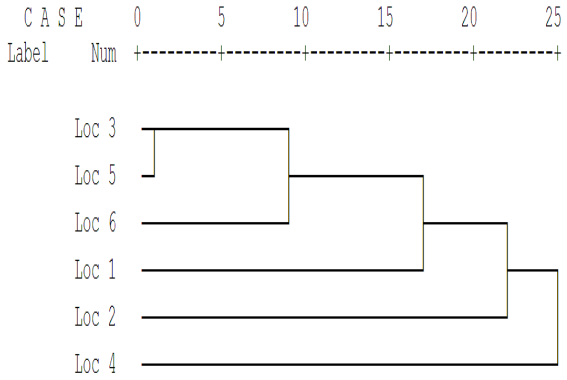

Figure 2: Dendrogram depicting the variation among *Shorea tumbaggia* accessions based on RAPD

Medicinal plants are increasingly endangered due to diverse environmental factors, natural hazards and anthropogenic disturbances. Characterization of the genetic variation within populations is very important for the establishment of efficient conservation strategies for endemic and endangered plant species. The maintenance of genetic diversity in rare or endangered species is a major goal of conservation programs, as the long-time survival of species in a changing environment depends on the preservation of sufficient genetic variation within and among populations[19]. Among the different type of molecular markers RAPD can be considered to be essential tool for assessment of genetic variability and to study the phylogenetic relationships with in, and among the populations of varieties, species. Several reports are available regarding, the use of RAPD markers for determination of genetic variation in plants. Jain[20] studied molecular diversity in *Phyllanthus amarus* by RAPD profiling of 33 collections from different location using MAP primers. Naugzemys[21] reported genetic variation and relationship among 39 accessions of *Lonicera caerulea* and one accession of *L. xylosteum*. Batitini[22] evaluated the genetic diversity of seven populations of *Anemopaegma arvense*, using random amplified polymorphic DNA markers. Rodica canata[16] reported genetic variation within seven individual populations of *Draba dorneri.* A total of 77 reproducible bands with an average of 6.41 bands per primer were obtained from the 12 primers and the polymorphism was 78.94%. Genetic

diversity of a plant species could be affected by many factors such as distribution range, life form, breeding system, genetic drift, restricted gene flow and the way that its seeds disperse. A species which has had a long life, a high frequency of gene flow and produce many seeds tends to have high genetic diversity. High diversity would be expected in population of *S. tumbaggia* since it shows anemophily and dispersal of seeds by wind. But the species exhibited low genetic variation which is attributed due to its self compatible nature of the plant[23].

Many studies have demonstrated that endangered and endemic species tend to possess low levels of genetic diversity based on ISSR data[24-25], some others have showed opposite findings[26]. The percentage of polymorphism in *S. tumbaggia* was higher compared to other endangered plants, such as *Lactoris fernandeziana* (Lactoridaceae) 24.5%[27], *Cathaya argyrophylla* 32%[28], *Paeonia suffruticosa* 22.5% and *P. rockii* 27.6%[29]. This shows that the species, Genetic diversity is not low, and it should be able to adopt the environmental variation. In the present study, the plants in 3, 5, 6 accessions exhibits high genetic similarity compared to 1, 2, 4 accessions, which are genetically more diverse.

CONCLUSION

Based on our results obtained, we concluded that *S. tumbaggia* possess high level of genetic diversity, as compared to other endangered plant species. The present study is the very first report that provides genetic information of *S. tumbaggia* population. It is suggested that RAPD markers could be successfully applied for detecting genetic variability in natural population of *S. tumbaggia.* Moreover RAPD marker will have a major impact on the conservation and improvement of the endemic and endangered tree species *S. tumbaggia.*

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