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Assessment of Genetic Diversity among *Terminalia* Species Using RAPD Markers

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Abstract: Genus *Terminalia* is considered as complex group having many problems associated with taxonomic identification. Five taxonomically critical *Terminalia* L. species were analysed with 31 random primers to evaluate genetic diversity and species relationships. From the total 31 primers screened, 26 primers amplified across all species scoring 336 bands of which 305 were polymorphic. On average 12.92 bands per primer were scored. The average polymorphism was 90.77% and eight primers were reported to produce 100% polymorphism. Dendrogram based on RAPD data grouped five *Terminalia* species in two distinct clusters. Cluster-I comprised 3 species viz. *T. arjuna*, *T. bellerica* and *T. chebula,* while cluster-II comprised 2 species *T. tomentosa* and *T. catappa.* Clustering of species was discussed with previous morphological studies.

Key words: Genetic diversity · RAPD markers · *Terminalia*

INTRODUCTION

Genus *Terminalia* L. belongs to family Combretaceae comprising of nearly 200 species distributed throughout the humid, semi-humid, tropical and subtropical regions of the world. Nearly 24 species of *Terminalia* have been reported from various states/union Territories of India[1]. *Terminalia* has various uses in pharmaceutical, indigenous medicine therapies, silk and other chemical industries Srivastav [2] and Srivastav [1]. Amongst all *Terminalia* species, *T. arjuna*, *T. bellerica*, *T. chebula*, *T. tomentosa* and *T. catappa* exhibited very high medicinal and economic properties and are rich source of Non Wood Forest Product [3]. Though *Terminalia* species are very important multipurpose trees (MPTs), their taxonomic status has been very controversial as they exhibit various morphotypes scattered in various regions which may be distinct species, sub-species/varieties, ecotypes and natural hybrids Hooker [4], Haines [5], Parker [6], Parkinson [7], Bahadur and Gaur [8], Srivastav [2], Srivastava *et al*.[9]. The sub-division of the genus into section or by some taxonomist into distinct genera are made on the basis of fruit characteristic alone and although line of demarcation is often indefinite, no better character has been represented so far in *Terminalia* species [1]. An understanding of the extent and organization of genetic diversity among these species could be useful for both its genetic improvement and conservation [10].

Recently the development of polymerase chain reaction (PCR) technique has revolutionized the field of molecular biology [11]. Polymerase chain reaction (PCR) derived markers with non-specific primers have been exceedingly popular since they do not request sequence information from target species. RAPD Williams *et al*. [12] is simple polymerase chain reaction (PCR)-based technique and used extensively in studying genetic relationships among accessions of various plant species Manimekalai and Nagarjan [13], Ahmad [14].

As per literature concerned despite having taxonomical identification problems hitherto no work was reported earlier from India on genetic diversity in these five species of *Terminalia*. Earlier studies in genus *Terminalia* based on fruit morphology Biswas and Kukreti [15], Leaf characters [16], nectarines [17], karyotype analysis by Ohri [18, 19] and cytology [20]. Further, before attempting interspecific hybridization to transfer useful traits, it is essential to establish the relationship among these species of concerned. Considering this facts present study was under taken to evaluate genetic diversity among five tree species of *Terminalia* based on RAPD markers.

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MATERIALS AND METHODS

Plant Material and DNA Isolation: Plant leaves of *T. arjuna*, *T. bellerica*, *T.chebula*, *T. tomentosa* and *T. catappa* were used as material for DNA isolation. Juvenile and tender leaves from 10 individuals of each *Terminalia* species were collected from various locations of Melghat Tiger Reserve, Maharashtra, Central India (Latitudes 21° 15'N and 21° 45'N, Longitudes 76° 57'E and 77° 33'E and altitude 312 M to 1178 M above mean sea level) and brought to the laboratory in ice bags. DNA was isolated by method of Deshmukh *et al*. [21].

RAPD Analysis: Thirty one different 10-mer primers were tested across five *Terminalia* species. RAPD reaction and procedures were carried out as described by Williams *et al*. [12]. The reaction mixture (25 µl) containing 25-50 ng of DNA, 2.5 U *Taq* DNA polymerase enzyme (Fermentas, USA), 0.4 µM each dNTPs (Fermentas, USA), 2.5mM $MgCl_2$(Fermentas, USA), 1X *Taq* DNA polymerase buffer supplied with enzyme (Fermentas, USA) and 0.4 µM decamer primer (Operon, USA). The plant DNA was amplified in a programmable thermocycler (T Personal Biometra, Germany) using the following conditions: Initial denaturation at 94°C for 5 min, followed by 45 cycles; denaturation at 94°C for 1 min, annealing of primer at 38°C for 1 min and extending primer at 72°C for 3 min and final extension at 72°C for 7 min. The amplified PCR products were fractionated on 1.2% agarose gel using 1X TAE buffer containing 5µg/ml ethidium bromide. Gel was photographed on Gel documentation system (Alpha Innotech, USA).

Data Analysis: Data was scored as '1'for the presence and '0' for the absence of a DNA band of each species. The similarity matrix was prepared by using Nei and Li [22] formula. The average of similarity matrix was used to generate a tree for cluster analysis by UPGMA (Unweighted Pair Group Method with Arithmetic Average) method using NTSYS 2.1 [23].

RESULTS

A total of 31 decamer random primers were tested in five *Terminalia* species for examining RAPD pattern (Fig. 1), of these 26 primers amplified across all species

Table 1: RAPD primers, their sequences and % polymorphism

S.N.	Primer	Seq 5' to 3'	Total bands	Polymorphic bands	Monomorphic bands	Polynmorphism (%)
1	OPP-1	GTAGCACTCC	19	19	00	100
2	OPP-2	TCGGCACGAC	16	13	03	81.25
3	OPP-3	GTGATACGCC	17	15	02	88.23
4	OPP-4	GTGTCTCAGG	15	11	04	73.33
5	OPP-5	CCCCGGTAAG	13	13	00	100
6	OPP-8	ACATCGCCTA	14	13	01	92.85
7	OPP-10	TCCCGCCTAC	21	20	01	95.23
8	OPP-12	AAGGGCGAGT	15	13	02	86.66
9	OPP-15	GGAAGCCAAC	09	08	01	88.88
10	OPP-17	TGACCCGCCT	12	11	01	91.66
11	OPP-18	GGCTTGGCCT	--	--	--	--
12	OPP-19	GGGAAGGACA	15	15	00	100
13	OPAP-1	AACTGGCCCC	14	14	00	100
14	OPAP-4	CTCTTGGGCT	16	15	01	93.75
15	OPAP-5	GACTTCAGGG	--	--	--	--
16	OPAP-6	GTCACGTCTC	13	11	02	84.61
17	OPAP-8	ACCCCCACAC	13	13	00	100
18	OPAP-10	CTGGCTTCTC	09	08	01	88.88
19	OPW-1	CTCAGTGTCC	08	07	01	87.5
20	OPW-2	ACCCCGCCAA	07	04	03	57.14
21	OPW-5	GGCGGATAAG	14	13	01	92.85
22	OPW-6	AGGCCCGATG	07	04	03	57.14
23	OPW-8	GACTGCCTCT	12	11	01	91.66
24	OPW-12	TGGGCAGAAG	07	05	02	71.42
25	OPW-14	CTGCTGAGCA	10	09	01	90
26	OPW-15	ACACCGGAAC	19	19	00	100
27	OPW-16	CAGCCTACCA	10	10	00	100
28	OPW-17	GTCCTGGGTT	11	11	00	100
29	OPW-18	TTCAGGGCAC	--	--	--	--
30	OPQ-4	AGTGCGCTGA	--	--	--	--
31	OPB-1	GTTTCGCTCC	--	--	--	--
Total			336	305	31	90.77

Table 2: Similarity matrix of *Terminalia* species based on RAPD data

	T. arjuna	*T. bellerica*	*T. chebula*	*T. tomentosa*	*T. catappa*
T. arjuna	1				
T. bellerica	0.804	1			
T. chebula	0.785	0.782	1		
T. tomentosa	0.393	0.400	0.412	1	
T. catappa	0.425	0.424	0.440	0.484	1

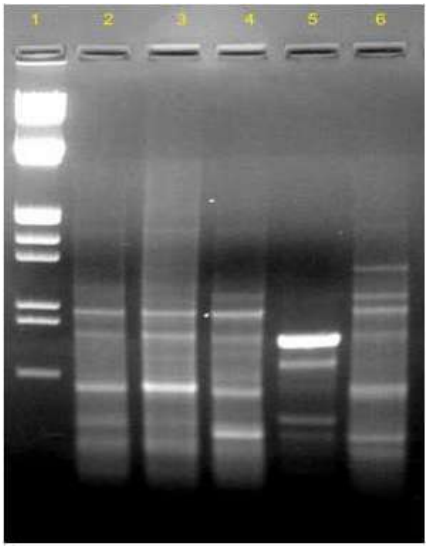

Fig. 1: RAPD banding pattern produce by primer OPP-3 (5'GTGATACGCC 3').
Lane 1-Lambda DNA *Eco* RI/*Hind* III double digest, 2- *T. arjuna*, 3- *T. bellerica*, 4- *T. chebula*, 5- *T. tomentosa*, 6- *T. catappa*.

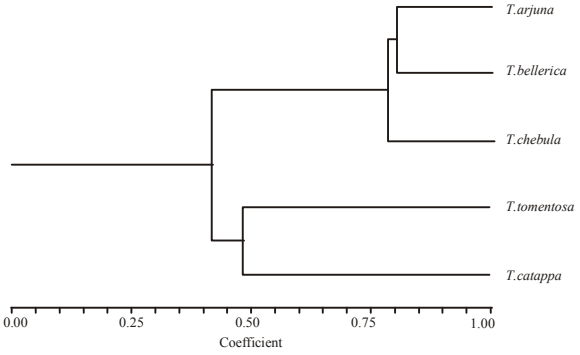

Fig. 2: Dendrogram of five *Terminalia* species based on RAPD data

(Table 1). In all, 336 amplified bands were obtained of which 305 bands were polymorphic, while 31 bands were monomorphic. On average 12.92 bands per primer were scored. The average polymorphism was 90.77%. Out of 26 primers, OPP-10 primer produced maximum 21 bands, while primer OPW-2, OPW-6, OPW-12 produced minimum 7 bands each. In all 8 primers (OPP-1, OPP-5, OPP-19, OPAP-1, OPAP-8, OPW-15, OPW-16 and OPW-17) produced 100% polymorphism while, primer OPW-2 and OPW-6 showed least polymorphism (57.14%). The values of similarity coefficient (Table 2) indicated that *T. arjuna* and *T. bellerica* had highest similarity (80.4%), while *T. arjuna* and *T. tomentosa* had least (39.3%) similarity. Dendrogram generated (Fig. 2) from average similarity coefficient of 26 primers grouped five *Terminalia* species in two distinct clusters. Cluster-I comprised 3 species viz. *T. arjuna*, *T. bellerica* and *T. chebula,* while cluster-II comprised 2 species viz *T. tomentosa* and *T. catappa.* Cluster-I was sub divided into two sub-clusters. Sub-cluster I consisted of two closely related species viz. *T. arjuna* and *T. bellerica,* while sub-cluster II had single species *T. chebula*.

DISCUSSION

Assessment of genetic diversity in wild tree species is very critical thing to undertake. The main problem associated in estimating genetic diversity in wild tree species is their long life cycle and the secondary metabolites synthesized by them. Genus *Terminalia* is a complex group and showed wide variations in morphological characters but still today beside fruit no better character was found to discriminate the species [1]. Previous morphological studies on *Terminalia* by Rao and Ramayya [16], Tilney and Van Wyk [17] were inadequate to reveal genetic relationships as morphological characters are influenced by environmental conditions [24].

Dendrogram based on RAPD data grouped five *Terminalia* species into two distinct clusters. Position of *T. arjuna*, *T. bellerica* and *T. chebula* in one cluster on dendrogram was in good agreement with Anonymous [25], where DNA based markers grouped these three species in one cluster. The positions of *T. arjuna* and *T. catappa* were at extreme ends on dendrogram indicating them as most divergent species. The reason for divergence of *T. catappa* with other *Terminalia* species is its origin (Andaman Islands), which is quite different from tropical conditions.

On dendrogram *T. arjuna* and *T. tomentosa* were placed in separate clusters; can be used as parents in tree

breeding programme, as some spontaneous hybrids of these two species are available in field Srivastav *et al*. [26], Srivastava *et al*.[9]. Thawaites (quoted by Hooker [4]) were opinion to merge *T. arjuna* and *T. tomentosa* in to single species, but present results strongly regret to do so, as both were found to be genetically much divergent species showing least (39.3%) of genetic similarity. Although these two species having wing type fruit pattern [15] but genetically they are very much divergent species. The percentage of polymorphism generated by decamer primers indicates that RAPD is the most suitable marker to assess genetic diversity and is applicable to those cases where no or meager genomic information is available, which is very true about genus *Terminalia*

In conclusion, the present study was the very first report on molecular analysis of these five important tree species and it had opened new avenues for genetic improvement of *Terminalia,* which is the need of time for conservation of its forest genetic resources.

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