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Genetic diversity analysis in tamarind (*Tamarindus indica* L.)

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Abstract

The present study conducted at Shimoga (Karnataka) during 2005–2008 is an attempt to examine genetic relatedness and genetic diversity among 13 Indian collections of Tamarind. Twenty eight selected arbitrary primers were used for characterization using RAPD that generated 131 fragments, of which 116 (88.54%) were polymorphic. Two genotypes, NTI62 and NTI84 were distinguished by unique band specific to them. The genetic similarity coefficient values suggested a wide genetic base for genotypes considered for the study. Cluster analysis based on unweighted pair group method using arithmetic average (UPGMA) clearly indicated that genotypes did not cluster according to their site of collection. This could be attributed to highly cross pollinating nature, small distribution area and that most tamarind genotypes grown in India are from seed source.

Keywords: diversity, RAPD analysis, tamarind

Introduction

Tamarind *(Tamarindus indica* L) is a monotypic genus tree belonging to the family *Leguminosae*, sub family *Caesalpiniaceae* with somatic chromosome number of 2n=24 (Purseglove *et al*. 1981). It is indigenous to tropical Africa and Southern India (Nas 1979). The edible pulp of ripe fruit is used as flavoring agent in soups, jams, chutneys, sauces and juices (Isholoa *et al.* 1990). The fruit pulp is the richest natural source of tartaric acid (8–18%). It is the main acidulant used in preparation of foods in India and other Asian countries (Shankaracharya 1998). The major limitation to tree improvement has been lack of research in countries where it exists. There is a wide gene pool in different countries with variation in commercially important traits (Gunasena *et al.* 2000). The assessment of genetic diversity at DNA level is a desirable step in the process of developing taggable markers to aid genetic improvement and to develop desirable tamarind cultivars and identify superior elite genotypes. There is considerable evidence that valuable germplasm is gradually eroding and little effort is being made to collect, conserve and utilize it in tree improvement programs. Countries have overlooked the significance of tamarind despite its support for agro industry, in terms of food security, income and genetic conservation (Anon. 1982). This implies that it deserves immediate attention for genetic conservation and improvement. In order to identify the level of diversity, marker systems have almost become indispensable (Williams *et al*. 1993). The use of

morphological traits for identification of elite genotypes has not been of much success in trees. Isozyme based markers also have limited scope as these are known to be influenced by environmental factors and alter with developmental stages and are far few in number to define variation. DNA markers, on the other hand, are highly reliable and most preferred choice for germplasm characterization (Virk *et al.* 1995). Establishment of core collections based on field evaluation and molecular variation shown by accessions could be obviously advantageous, but clear and detailed assessment of molecular diversity in tamarind is not available. The present study is an effort to examine the extent of genetic diversity and assess genetic relationships among tamarind cultivars using DNA markers.

Materials and methods

The experimental material for present investigation (Table 1) comprised of 13 accessions (morphologically and geographically distinct) of tamarind collected from different locations in India. DNA was extracted following the CTAB extraction method (Lodhi *et al.* 1994). The DNA quantification was carried out using spectrophotometer at 260 nm. A 25 mL PCR mix reaction buffer consisted of 2.5 mL of 10X buffer (50 mM KCl, 50 mM $MgCl_2$, 100 mM Tris HCl pH 8.0), 25 mM $MgCl_2$, 100 mM of each dNTPs, 0.3 mM random primer (Operon Technologies Inc., Alameda, CA, USA), 0.5 units *Taq* polymerase (Bangalore Genei Pvt. Ltd, Bangalore) and 100 ng of template DNA. The PCR amplification was performed using a thermo cycler (Master Cycler gradient 5331-Eppendorf version 2.30. 31-09, Germany). The amplification profile was kept for initial denaturizing at 94°C for 5 min followed by 44 cycle denaturizing at 94°C for 4 min; primer annealing at 36°C for 1 min; primer extension at 72°C for 2 min; and a final primer extension at 72°C for 4 min. The PCR product was stored at 4°C till further electrophoresis. PCR amplified products were mixed with 2.5 mL of 10X loading dye (0.25%) bromophenol blue, was used for loading. PCR products were resolved by electrophoresis on 1.2% (w/v) agarose gels using 1X TAE buffer (40 mM Tris –acetate, pH 8.0, 1 mM EDTA), at 80 V for 2 hrs followed by staining with 0.5 mg mL^{-1} ethidium bromide and photographed using gel documentation system (UVi tech, England). The amplified products were scored as 1 for presence and 0 for absence at particular marker level generated by an accession and the marker. The scores for each accession and marker generated were recorded and genetic similarity for pair wise accessions was estimated based on Jaccard's similarity coefficient. A similarity matrix was constructed and subjected to cluster analysis following the UPGMA method using NTSYS PC version 2.0 software.

Table 1. Selected tamarind accessions used for RAPD analysis

Sl. No.	Accession name	Place of collection	Number of amplified bands
1.	NTI-13	Belgaum	61
2.	NTI-75	North Karnataka	69
3.	NTI-80	North Karnataka	66
4.	NTI-84	North Karnataka	65
5.	NTI-32	Karwar (Yellapur)	60
6.	NTI-19	Dharwad	58
7.	NTI-62	Belgaum	64
8.	NTI-82	North Karnataka	64
9.	PKM-1	Coimbatore	64
10.	SMG-13	Shimoga	65
11.	NTI-15	Belgaum	66
12.	NTI-5	Bagalkot	74
13.	SMG-4	Shimoga	79
		Total	855

Results and discussion

Random amplified polymorphic DNA (RAPD) analysis carried out on all the accessions produced a large number of distinct fragments for each primer. Twenty-eight selected arbitrary primers (of 80 primers tested) yielded amplification products in most of the accessions (Table 2). RAPD analysis of 13 accessions representing different regions generated 131 levels of distinct scorable marker with an average 4.68 amplicon per primer. Of the 131 fragments, 116 were found to be polymorphic

Table 2. Selected arbitrary primers along with their sequences and polymorphism information in selected tamarind accessions

Sl. No	Primer name	Sequence of Primer (5′ to 3′)	Total no. of bands generated by the primer	Total no. of polymorphic bands	Polymorphism (%)
1	AB7	GTAAACCGCC	7	5	71.43
2	H18	GAATCGGCCA	8	8	100.00
3	AC12	GGCGAGTGTG	9	9	100.00
4	AT9	CCGTTAGCGT	2	0	0.00
5	H1	GGTCGGAGAA	5	5	100.00
6	H5	AGTCCTCCCC	4	3	75.00
7	AB4	GGCACGCGTT	2	2	100.00
8	B18	CCACAGCAGT	7	6	85.71
9	G06	GTGCCTAACC	3	2	66.67
10	G20	GAGCCCTCCA	7	7	100.00
11	B10	TCTCCCTCAG	5	5	100.00
12	H4	CTGCTGGGAC	8	6	75.00
13	AC5	GTTAGTGCGG	2	0	0.00
14	GO3	GGAAGTCGGC	3	3	100.00
15	K19	AGGTGAGCGT	3	2	66.67
16	B15	GGAGGGTGTT	3	3	100.00
17	K11	AATGCCCCAG	4	4	100.00
18	B20	GGACCCTTAC	5	5	100.00
19	B03	CATCCCCCTG	7	7	100.00
20	B17	AGGGAACGAG	4	4	100.00
21	G02	CTGAGGTCCT	5	5	100.00
22	G14	CTCTCGGCGA	3	3	100.00
23	L16	AGGTTGCAGG	3	2	66.67
24	AK4	AGGGTCGGTC	4	4	100.00
25	AK9	AGGTGAGCGT	5	3	60.00
26	K9	CCCTACCGAC	5	5	100.00
27	H3	AGACGTCCAC	3	3	100.00

(88.54%). Figs. 1A and 1B shows RAPD profile for the 13 accessions generated by AC12 and AC5 primers. The number of amplicons obtained was in the range of 2 to 9, primers AB4, AT5 and AT9 produced minimum number of bands (2), while primer AC12 produced the maximum number of bands (9) with an average of 4.14% polymorphic bands per primer (Table 3).

The number of amplicons of all the accessions were 58 (NTI 19) to 79 (SMG-4) and two primers AT9 and AC5 were monomorphic. Although the majority of primers produced polymorphic bands, no single primer could

Table 3. Summary statistics of RAPD analysis of selected 13 accessions of tamarind

Sl. No.	Particulars of Amplicon	
1	Total marker levels	131
2	Total number of polymorphic levels	116
3	Average number of marker per primer	4.68
4	Maximum number of bands generated by primer	9
5	Minimum number of bands generated by primer	2
6	Average number of polymorphic bands per primer	4.14

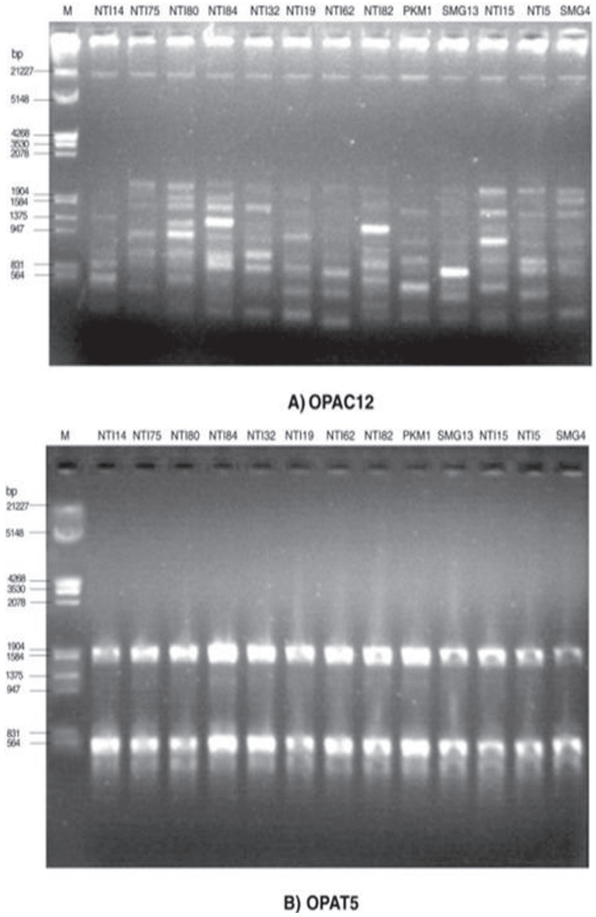

Fig. 1. RAPD profile obtained with random primer A) OPAC12 and B) OPAT5 for 13 genotypes of tamarind (M: ë *Eco*RI + *Hind*lll digest Marker)

clearly distinguish all the genotypes. The polymorphic fragment that was present in only one genotype was considered to be a unique fragment. Of the 28 primers, G06 and G14 were able to generate unique bands for NTI62 and NTI84 genotypes, respectively to yield genotypic finger prints.

Based on simple matching coefficient (Table 4), a genetic similarity matrix was constructed using the RAPD bands to assess the genetic relatedness among 13 genotypes. The similarity coefficient values ranged from 0.52 to 0.85. The highest value of similarity coefficient (0.85) was found between the genotypes NTI82 and NTI84, both collected from Northern Karnataka. However, the lowest value of

Table 4. Genetic similarity of Dice coefficients among 13 accessions of tamarind based on RAPD banding pattern using UPGMA cluster analysis

Genotypes	SMG13	NTI19	NTI75	NTI32	SMG4	NTI62	NTI15	NTI5	NTI14	NTI80	PKM1	NTI82	NTI84
SMG13	1												
NTI19	0.65	1											
NTI75	0.68	0.74	1										
NTI32	0.66	0.71	0.75	1									
SMG4	0.61	0.71	0.69	0.73	1								
NTI62	0.58	0.67	0.70	0.78	0.75	1							
NTI15	0.52	0.63	0.63	0.67	0.7	0.77	1						
NTI5	0.70	0.69	0.72	0.70	0.72	0.68	0.74	1					
NTI13	0.69	0.68	0.66	0.69	0.7	0.72	0.67	0.80	1				
NTI80	0.62	0.70	0.76	0.70	0.71	0.76	0.72	0.76	0.70	1			
PKM1	0.61	0.70	0.68	0.68	0.71	0.72	0.63	0.69	0.71	0.75	1		
NTI82	0.69	0.74	0.70	0.73	0.70	0.70	0.65	0.74	0.74	0.74	0.75	1	
NTI84	0.69	0.76	0.77	0.74	0.70	0.71	0.68	0.73	0.71	0.79	0.76	0.85	1

similarity coefficient (0.52) was observed among the genotypes NTI-15 from Belgum and SMG-13 from Shimoga.

In order to quantify the level of polymorphism between the genotypes, Jaccard's similarity matrix was used to generate dendrogram by selecting the unweighted pair group method with arithmetic average (UPGMA) algorithm using NTSYS PC (Fig. 2). The dendrogram showed that all the 13 accessions were grouped into three major clusters at 72.0% similarity coefficient. The accession SMG13 formed a distinct cluster I, as it did not group with other

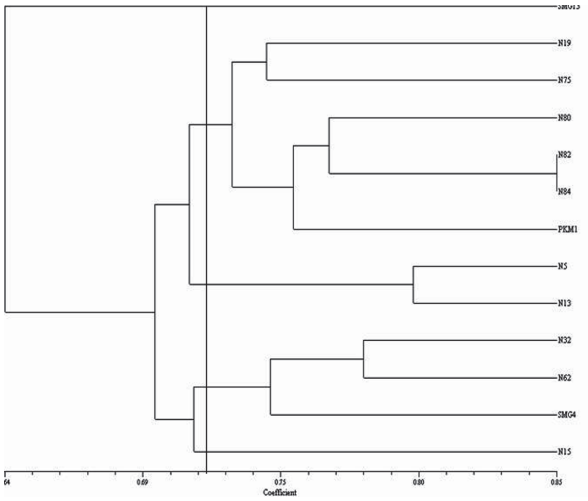

Fig. 2. Dendrogram clustering based on similarity co-efficient among selected genotypes of tamarind

genotypes. Cluster II was largest group with seven accessions, further subdivided into two sub clusters at similarity coefficient of 73%, genotypes NTI19, NTI75, NTI80, NTI82, NTI84 and PKM1 grouped in sub cluster IIA whereas genotypes NTI5 and NTI13 in sub cluster IIB and cluster III consisting of accessions NTI32, NTI62, SMG4 and NTI15.

This is the first report in India describing the genetic diversity in tamarind using DNA markers. RAPD has been successfully used to study the genetic diversity in trees *viz*., eucalyptus (Keil & Griffin 1994), mango (Ravishankar *et al.* 2000) and plum (Shimida *et al*. 1999), teak (Keiding *et al.* 1986) and oil palm (Shah *et al.* 1994). RAPD markers proved to be very informative and useful in monitoring the genetic diversity present in samples of selected genotypes.

Tamarind was found to have genetic heterozygosity of 0.15 and the level of polymorphism observed in present study was fairly high (0.52-0.85) indicating that a wide and diverse genetic base existed between genotypes of tamarind. This variation may be due to the diverse genotypes used and partly due to its cross pollinating nature. The variation between genotypes observed with 28 of the 80 selected primers, can be used for identification of superior types and also for genotype specific DNA markers. The 28 primers showing repeatable amplification indicated that out of 131 bands, 116 were polymorphic indicating considerable genetic variation among the tamarind genotypes. The results in coffee by Lashermes *et al.* (1993) showed the ability of RAPD to discriminate among genotypes and suggested their application in cultivar identification. Though the majority of primers produced polymorphic bands, no single primer could clearly distinguish all the genotypes.

Considering the level of diversity, an effort was made to identify genotype specific bands, which could distinguish the genotypes from various locations. Two primers *viz*., GO6 and G14 generated unique bands for genotypes NTI62 and NTI84 respectively. Such informative primer either singly or in combination may be of use in establishment of identities of unknown genotypes and short listing of such primers will be useful for documentation of germplasm and biodiversity conservation (Vidal *et al.* 1999).

There was complete absence of amplicon for some primers (B17, B20, H1 and AB4) in some genotypes. Further, some of the genotypes possessed less number of bands. The less number of bands in accessions NTI19 and NTI32 could be due to selection, which is known to result in narrowing genetic base of cultivated species or duplication of the genotype

spread across locations.

Based on Dice coefficient the mean similarity indices of 13 accessions ranged from 0.52 to 0.85 (mean 0.68) indicating that on an average 68% of their RAPD amplified fragments are common. This wide range of similarity indices indicated a level of polymorphism at the DNA level among accessions. The least genetic similarity observed between the genotypes NTI15 from Belgaum and SMG13 from Shimoga, supported the idea that the genotypes in these regions possess wide genetic variation and greatest similarity between the genotypes NTI82 and NTI84 was obvious as they are in the same region of North Karnataka. Also, there are collections as close as 80% (NTI5 from Bagalkot and NTI13 from Belgaum) in terms of similarity matrix, even though they may belong to different distinct locations. This situation can arise in natural populations, when there is a possibility of free or random pollen flow and fertilization as in the case of most cross pollinated species like tamarind.

Dendrogram drawn separated the genotypes into four major clusters, wherein intermix of genotypes in each cluster was observed. This clearly indicated that collections made from various parts of India did fall into well defined distinct groups, indicating the loose association of marker and geographical locations. Interestingly, the dendrogram revealed that cluster IIA comprised of all the genotypes from North Karnataka except PKM1. Similar observation was made by Ishii *et al.* (1996) in rice wherein accessions with geographical proximity clustered together more frequently as compared to the one from different geographical locations.

The findings of the present study indicated that tamarind possesses large genetic diversity. This study also revealed that there is no significant association between RAPD pattern and geographic location of accessions. This study provides a preliminary basis for tamarind breeders to select on the basis of genetic diversity. Further studies using additional accessions and co-dominant marker from a broader geographical distribution would provide more valuable information on available genetic diversity in this species. RAPD profile detected in this study forms a preliminary documentation of the tamarind genotypes and their diversity. This information could be used in identification of diverse genotypes for tamarind improvement.

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