International Journal of Plant Breeding ©2011 Global Science Books

Estimation of Genetic Variability in Tamarind (*Tamarindus indica* L.) using RAPD Markers

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ABSTRACT

In this study, genetic diversity among 36 genotypes of tamarind (*Tamarindus indica* L.) was analysed using RAPD markers. PCR-amplifiable DNA was isolated using the CTAB method and 106 amplified fragments were obtained using 12 random primers. The genetic dissimilarity matrix, which was calculated based on Squared Euclidian Distances, revealed a maximum genetic distance of 9.6% between genotypes, 'NCBS1' and 'NB1', and 'PG2' and 'NB1'; the minimum genetic distance (4.2%) was between genotypes 'NCBS2' and 'NCBS3'. The Ward's method of cluster analysis grouped all the individuals on a dendrogram into two major clusters 'A' and 'B' at 19.5 linkage distance with two sub-clusters in cluster 'A'. Sub-cluster 'A1' consisted of 7 genotypes and sub-cluster 'A2' 9 linked together at 12 linkage distances. Cluster 'A' predominantly consisted of genotypes with semi-curved to curved shape fruits and a characteristic plagiotropic tree growth pattern in sub-cluster 'A1' and orthotropic tree growth pattern in sub-cluster 'A2'. Cluster 'B' consisted of two sub-clusters 'B1' and 'B2', clustered at 14.5 linkage distances with 7 and 13 genotypes, respectively. The genotypes of cluster 'B' was predominantly characterised by brown to dark brown coloured fruit pulp and the sub-clusters 'B1' with straight fruits and 'B2' with semi-curved to curved fruits. RAPD analysis proved to be a quick and simple testing method and resulted in a moderate level of genetic diversity among tamarind genotypes.

Keywords: cluster analysis, genetic diversity, RAPD-PCR

INTRODUCTION

Tamarind (*Tamarindus indica* L.) belongs to the family Fabaceae, is popularly known as 'Indian Date' that originated from India (Morton 1987) and is widely distributed in Africa and Asia. It is a highly cross pollinated crop with a wide variation in the species and the number of genotypes are estimated to be 19,327 (Lewis *et al.* 2005). The species has a wide geographical distribution in the subtropics and semi-arid tropics and is cultivated in numerous regions (El-Siddig *et al*. 2006). It is a multipurpose tropical fruit tree used primarily for its fruit, which are eaten fresh or processed, or used for seasoning or as a spice. India is the largest producer of tamarind with an annual production of over 300,000 tonnes most of which are locally consumed and 11,500 tonnes are exported to Europe and North America countries (Spice Board India 2009).

Tamarind is a perennial, slow-growing evergreen tree, up to 20-30 m tall and with a thick upright trunk. The bright-green foliage with a dense spreading crown makes it an attractive shade tree that can be used for fodder during the dry season (Kaitho *et al*. 1988). The tree is highly tolerant to drought and grows in a wide range of agro-climatic conditions. Tamarind is cultivated for its valuable fruit pulp which is slightly sweetish and more acidic in nature and is widely used as a spice (Purseglove 1981; Ishola *et al*. 1990). The pulp is rich in ascorbic and tartaric acids hence, used as a preservative in the pickle industry (Tsuda *et al*. 1995). The tree also provides valuable wood, and medicinally the leaves and fruit pulp are used as anti-inflammatory agent, against leucorrhoea, and skin disorders (Rimbau *et al*. 1999; Sen and Behera 2000; Punjani and Kumar 2002).

Although tamarind is one of the oldest domesticated crops, little is known about its genetic improvement. Cultivated populations are selected from desirable and observable characteristics, particularly based on fruit morphology and pulp quality. Identification of cultivar and estimation of genetic diversity using phenotypic markers have several limitations, especially in perennial crops (Purushotham *et al*. 2008) as molecular diversity, using DNA and protein-based markers, are more authentic and unaffected by environmental factors (Dhanraj *et al*. 2002). Among DNA-based molecular markers, RAPDs (randomly amplified polymorphic DNA) proved as an excellent tool to estimate genetic diversity and relationships among genotypes (Williams *et al*. 1990). It is simple, versatile, relatively inexpensive, and can detect slight genetic differences and help in identifying duplicates in the populations. RAPD markers also have been used successfully to study genetic diversity and relatedness among perennial crops by our research group such as mango (Kumar *et al*. 2001), guava (Prakash *et al*. 2002), cashew (Dhanraj *et al*. 2002), jackfruit (Simon *et al*. 2007), jasmine (Mukundan *et al*. 2007), areca nut (Purushotham *et al*. 2008), khat (Al-Thobhani *et al*. 2008), pomegranate (Narayanaswamy *et al*. 2008), tea (Ramakrishnan *et al*. 2009) and simarouba (Simon *et al*. 2009). To the best of our knowledge no research work on genetic studies has been carried out on tamarind genotypes despite its commercial importance. In the present study, RAPD markers were used to estimate genetic diversity and assess relationships among 36 genotypes of tamarind.

MATERIALS AND METHODS

Plant material

The plant material used in this study comprised 36 genotypes of tamarind genotypes collected from South India and maintained at the University of Agricultural Sciences, Bangalore, India (**Table 1**). Fresh, young and healthy leaves that were free from pest and disease damage were harvested individually from the field, wiped with 70% ethanol and air-dried prior to the isolation of DNA.

Received: 1 April, 2010. Accepted: 3 May, 2010.

Original Research Paper

Table 1 List and source of collection of tamarind genotypes.

Name of genotype	Source of collection
P3	Department of Horticulture, GKVK, Bangalore
P10	Department of Horticulture, GKVK, Bangalore
P13	Department of Horticulture GKVK Bangalore
P11	Department of Horticulture, GKVK, Bangalore
P14	Department of Horticulture, GKVK, Bangalore
NB30	Department of Horticulture GKVK Bangalore
NB1	Department of Horticulture, GKVK, Bangalore
S16	Department of Horticulture, GKVK, Bangalore
S18	Department of Horticulture GKVK Bangalore
N22	Department of Horticulture, GKVK, Bangalore
NJ57	Department of Horticulture, GKVK, Bangalore
NB15	Department of Horticulture GKVK Bangalore
WIGAM	Department of Horticulture, GKVK, Bangalore
NO33	Department of Horticulture, GKVK, Bangalore
NO40	Department of Horticulture GKVK Bangalore
NO41	Department of Horticulture, GKVK, Bangalore
NO17	Department of Horticulture, GKVK, Bangalore
H1	Department of Horticulture GKVK Bangalore
H2	Department of Horticulture, GKVK, Bangalore
H3	Department of Horticulture, GKVK, Bangalore
H4	Department of Horticulture GKVK Bangalore
H5	Department of Horticulture, GKVK, Bangalore
PKM1	Department of Horticulture, GKVK, Bangalore
PKM2	Department of Horticulture, GKVK, Bangalore
BT1	Botanical Garden, GKVK, Bangalore
BT2	Botanical Garden, GKVK, Bangalore
BT3	Botanical Garden, GKVK, Bangalore
BT4	Botanical Garden, GKVK, Bangalore
PG1	Post Graduate Hostel, GKVK, Bangalore
PG2	Post Graduate Hostel, GKVK, Bangalore
MG1	Main Gate, GKVK, Bangalore
MG2	Main Gate, GKVK, Bangalore
MG3	Main Gate, GKVK, Bangalore
NCBS1	National Centre for Biological Sciences, GKVK, Bangalore
NCBS2	National Centre for Biological Sciences, GKVK, Bangalore
NCBS3	National Centre for Biological Sciences, GKVK, Bangalore

DNA isolation and purification

All the reagents and chemicals were obtained from Bangalore Genei, Bangalore, India of molecular biology grade. DNA was extracted from fresh leaves of tamarind by the modified cetyl trimethyl ammonium bromide (CTAB) protocol as described by Simon *et al*. (2007). Specifically, 1 g of leaf was powdered using motor and pestle, and was mixed with 12 ml extraction buffer, preheated to 65°C, containing 100 mM Tris-base, pH 8.0, 20 mM EDTA, pH 8.0, 1.4 M NaCl, 3% (w/v) CTAB, 2% polyvinyl pyrrolidone (MW = 40,000) and 1% β-mercaptoethanol, then incubated at 65°C for 1 h with gentle shaking. The mixture was cooled to room temperature and centrifuged at 7000 × *g* for 10 min at 4°C. To the supernatant 8 ml cold 24:1 (v/v) chloroform: isoamylalcohol was added and the contents were mixed well. After centrifugation at 8,000 × *g* for 10 min at 4°C, the supernatant was transferred to a fresh tube and the chloroform: isoamylalcohol step was repeated until a clear supernatant was obtained. To the supernatant, 5 M NaCl was added (0.5 v/v) and mixed gently followed by addition of 1 vol of cold isopropanol to precipitate the DNA. The mixture was incubated at -20°C for 30 min, and then centrifuged at 7,000 × *g* for 15 min. The resulting pellet was washed with 70% (v/v) ethanol, air-dried, and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Two µg RNase (bovine pancreatic ribonuclease) was added to each sample which was incubated for 1 h at 37°C, mixed with an equal volume of 1:1 (v/v) phenol: chloroform and centrifuged at 8,000 × *g* for 10 min at room temperature and then with chloroform alone. DNA was precipitated overnight at 4°C with 0.5 vol of 5 M NaCl and 1 vol of cold isopropanol and the resulting pellet obtained after centrifugation was dissolved in TE buffer, analysed on an agarose gel and quantified using a spectrophotometer (ND-8000, NanoDrop Technologies, Wilmington, USA).

PCR amplification

The PCR amplification protocol followed was according to Williams *et al*. (1990) with minor modifications. Of the 27 primers screened using bulk DNA, 12 showing prominent bands were selected for RAPD-PCR analysis (**Table 2**). Reproducibility of the primers was tested by repeating the PCR amplification three times under similar conditions. PCR reactions were carried out in a volume of 25 µl containing 25 ŋg template DNA, 150 µM each dNTP, 1.5 mM $MgCl_2$, 1.5 unit *Taq* DNA polymerase (Hi Media, Mumbai, India), 5 pmol primer (OPA, OPB, OPC, OPD, OPE, OPF, OPG, OPH, OPI, OPJ and OPK series, Operon Technologies, Alameda, CA, US) in PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.05% (v/v) NP40 and 0.05% (v/v) Triton X-100). Amplifications were performed in a Corbett Research Thermocycler (Corbett Research Mortlake, New South Wales, Australia), programmed for an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, primer extension at 72°C for 2 min, and a final extension at 72°C for 10 min. PCR products were resolved in a 1.4% (w/v) agarose gel, visualised and documented using an Alpha Digidoc system (Alpha Innotech, San Leandro, CA, US).

RAPD profile analysis

Amplified fragments from each primer were manually scored for their presence (1) or absence (0) and a matrix of the different RAPD phenotypes of all eleven primers was assembled for statistical analysis by STATISTICA computer package (STATISTICA for Windows, StatSoft Inc, Tulsa. OK, USA, 1996). The sizes of the fragments were estimated using 500 bp standard DNA markers (Bangalore Genei, Bangalore, India), co-electrophoresed with the PCR products. A genetic dissimilarity matrix was developed using Squared Euclidean Distances, which estimates all pair-wise differences in the amplification products (Sokal and Sneath 1973) and a cluster analysis was based on Wards method using a minimum variance algorithm (Ward 1963). Principal Component Analysis (PCA) was used to make a multivariate statistical analysis of the RAPD data (Sokal and Sneath 1973).

Table 2 The sequence and level of polymorphism of selected polymorphic primers in tamarind.

Primers	Sequences (5′ - 3′)	Total No. of bands	No. of polymorphic shared bands	No. of polymorphic unique bands	No. of monomorphic bands
OPA-03	AGTCAGCCAT	8	6	2	0
OPA-13	CAGCACCCAC	8	4	3	1
OPC-02	GTGAGGCGTC	7	7	0	0
OPC-10	TGTCTGGGTG	9	9	0	0
OPD-07	TGCCCGTCGT	10	7	0	3
OPD-11	GAACCTGCGG	6	6	0	0
OPD-13	GGGGTGACGA	8	7	1	0
OPE-07	AGATGCAGCC	9	8	0	1
OPF-16	GGAGTACTGG	10	8	0	2
OPF-18	TTCCCGGGTT	10	7	0	3
OPJ-04	CCGAACACGG	10	8	2	0
OPJ-16	CTGCTTAGGG	11	7	4	0
Total		106	84	12	10

RESULTS AND DISCUSSION

Genetic diversity is an important aspect to study among cultivated crops as evolution has the ability to alter the species and populations (Ma *et al*. 2008). Maintenance of such genetic diversity in an economically important crop is essential to ensure the selection of valuable genotypes for breeding programme, as many of the species are becoming endangered due to genetic erosion (Porceddu *et al*. 1988). Understanding the spatial organization of genetic diversity within and among plant populations is of critical importance for the development of strategies designed to preserve genetic variation (Hamrick 1983; Brown and Briggs 1991). Evidently, RAPD technology is a rapid and sensitive technique, which could estimate relationship between closely and more distantly related species.

Genetic studies are lacking in *T. indica* because they have not been the subject of much scientific investigations. However, medicinal properties of the species have been extensively explored in the past decade (Shivshankar and Devi 2004; Martinello *et al*. 2006; Ahmed *et al*. 2007; Havinga *et al*. 2010). Identification of salt-tolerant genotypes for a crop improvement programme has been evaluated by Gebauer *et al*. (2004). Investigations on tamarind fruit pulp have gained importance due to its commercial and economic importance (Siddhuraja *et al*. 1995; Librandi *et al*. 2007; Paula *et al*. 2009; Sivasankar *et al*. 2010). Extensive research on tamarind seed has been undertaken to identify antioxidant properties (Sudjaroen *et al*. 2005; Siddhuraju 2007), tannin content (Bhatta *et al*. 2000, 2001), bioremediation for aquatic pollution (Agarwal *et al*. 2006), cellular inhibitory effect (Komutarin *et al*. 2004; Fook *et al*. 2005), medicinal (Bhattacharya *et al*. 1991), nutritional (Bhattacharya *et al*. 1994) and X-ray diffraction, physical and engineering properties (Taylor and Atkins 1985; Bhattacharya *et al*. 1993).

The PCR amplification protocol that was followed was according to a standard protocol (Williams *et al*. 1990) with minor modifications and resulted in good amplification by the use of 25 ηg of template DNA. The PCR amplification resulted in intense and clear banding patterns. The use of 12 RAPD primers (10-mer) provided detectable polymorphism among the genotypes. A total of 106 amplified fragments between 250 bp and 3 kb were obtained from the 12 selected polymorphic primers from among the 36 genotypes with an average of 8.8 bands/primer. Of the 106 bands, 96 (90.6%) were polymorphic, only 10 (9.4%) were monomorphic and common to all the individuals. Twelve (11.3%) were polymorphic and unique. The number of fragments produced by a primer ranged from 6 (OPD 13) to 11 (OPJ 16). The pattern of RAPD fragments produced by the random primer OPD-13 and OPJ-16 are shown in **Figs. 1** and **2**, respectively. The dissimilarity matrix obtained using Euclidian Distance (Sokal and Sneath 1973) is shown in **Table 3**. The highest genetic dissimilarity (9.6%) was between genotypes 'NCBS1' and 'NB1', and 'PG2' and 'NB1', while the least genetic dissimilarity (4.2%) was noticed between genotypes 'NCBS2' and 'NCBS3'.

In the dendrogram (**Fig. 3**), the genotypes were divided into two major clusters 'A' and 'B' at 19.5 linkage distances and 16 and 20 genotypes, respectively. Cluster 'A' was segregated into two sub-clusters 'A1' and 'A2', at 12 linkage distances with two minor clusters each. The minor cluster 'A1a' with five genotypes ('N22', 'H3', 'H4', 'H5', 'NJ57') was characterised with trees of plagiotropic growth and dark green coloured leaves. In contrast, the two genotypes 'S16' and 'S18' in cluster 'Alb' were characterised by trees of orthotropic growth and light green coloured leaves. All the genotypes grouped under sub-cluster 'A1' were characterised with curved or semi-curved fruit shape.

Sub-cluster 'A2' consisted of 9 genotypes all with an orthotropic tree growth pattern and was segregated into two minor clusters 'A2a' and 'A2b' at 9 linkage distance. The genotypes of minor cluster 'A2a' showed semi-curved or straight fruit shape in genotypes 'NO40', 'NO33', and pulp colour was light to dark brown. However, two genotypes of the 'A2b' sub-cluster, 'NB1' and 'H2', were characterised with prominent semi-curved fruits with dark brown coloured pulp. In general, the majority of genotypes in cluster 'A' showed a semi-curved to curved fruit shape (**Fig. 4**) and characteristic plagiotropic tree growth pattern (**Fig. 5**) in sub-cluster 'A1' and orthotropic tree growth pattern in sub-cluster 'A2'.

The genotypes of major cluster 'B' were segregated into

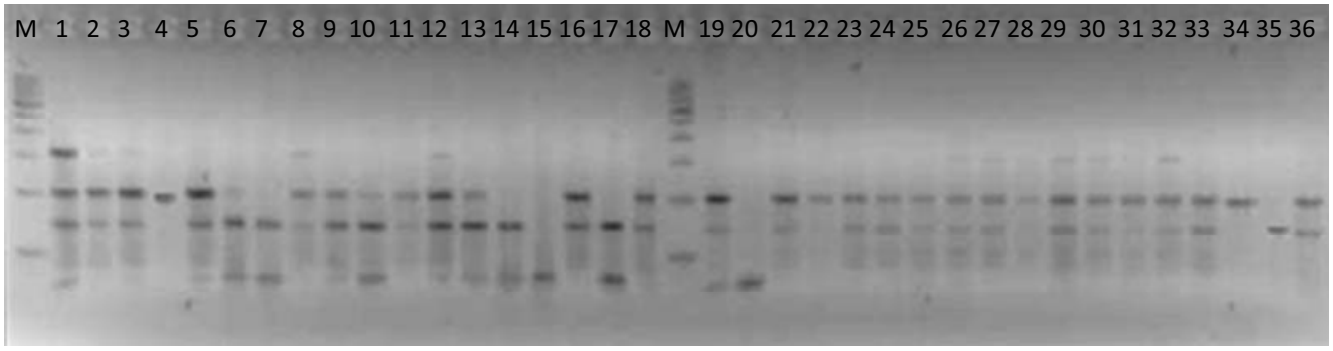

Fig. 1 Gel profile of tamarind according to OPD-13 primer. Lanes 1-36 contain the amplification profile obtained using the genotypes (P3, P10, P13, P11, P14, NB30, NB1, S16, S18, N22, NJ57, NB15, WIGAM, NO33, NO40, NO41, NO17, H1, H2, H3, H4, H5, PKM1, PKM2, BT1, BT2, BT3, BT4, PG1, PG2, MG1, MG2, MG3, NCGS1, NCBS2, NCBS3). Lane M contains 500 bp standard DNA markers.

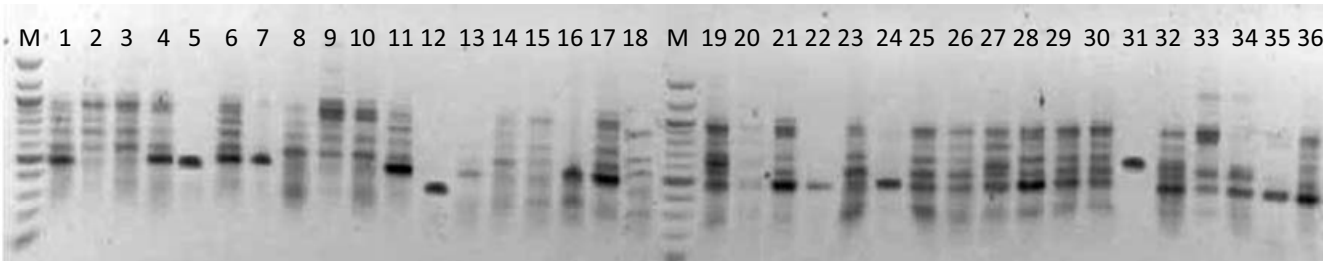

Fig. 2 Gel profile of tamarind according to OPJ-16 primer. Lanes 1-36 contain the amplification profile obtained using the genotypes (P3, P10, P13, P11, P14, NB30, NB1, S16, S18, N22, NJ57, NB15, WIGAM, NO33, NO40, NO41, NO17, H1, H2, H3, H4, H5, PKM1, PKM2, BT1, BT2, BT3, BT4, PG1, PG2, MG1, MG2, MG3, NCGS1, NCBS2, NCBS3). Lane M contains 500 bp standard DNA markers.

two sub-clusters *viz.*, 'B1' and 'B2' at 14.5 linkage distances. Sub-cluster 'B1' with 7 genotypes was segregated into two minor clusters 'B1a' and 'B1b'at 8.2 linkage distances, with 4 and 3 genotypes, respectively. The genotypes of minor group 'B1a' were characterised by an orthotropic tree growth pattern with straight fruit shape, except for 'NCBS1' with plagiotropic growth and 'NCBS2' with semi-curved fruit shape. Genotypes 'NCBS2' and 'NCBS3' were closely linked together at 4.3 map distances. The 3 genotypes of the minor cluster 'B1b' were characterised by an orthotropic pattern of tree growth and fruits with semi-curved shape. All genotypes of the group had a dark brown pulp and dark

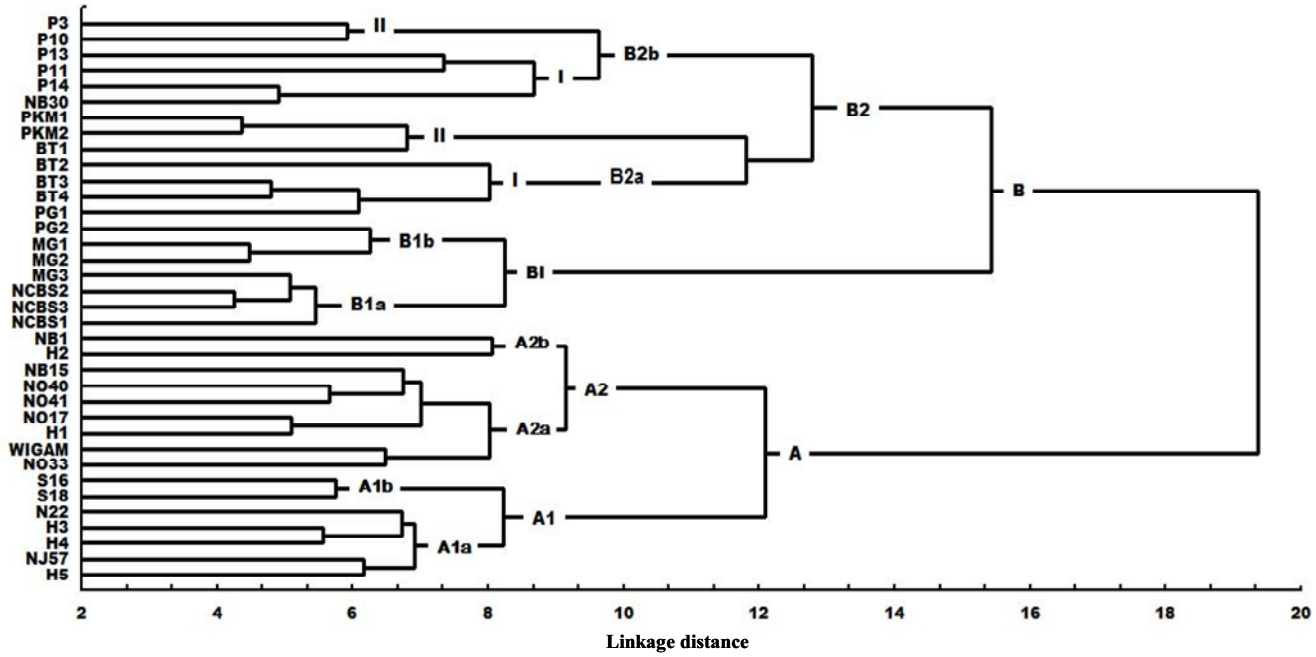

Fig. 3 Dendrogram showing RAPD marker-based genetic relationships among 36 tamarind genotypes. Two major clusters 'A' and 'B' with sub-clusters and minor clusters have formed.

Table 3 Genetic dissimilarity matrix of 36 tamarind genotypes based on polymorphism of RAPD markers.

	P3	P10	P13	P11	P14	NB30	NB1	S16	S18	N22	NJ57	NB15	WIGAM	NO33	NO40	NO41	NO17
P3	0																
P10	5.9	0															
P13	8.1	7.6	0														
P11	7.9	8.2	7.4	0													
P14	7.5	7.6	8	6.6	0												
NB30	7.4	7.6	7.6	7.4	4.9	0											
NB1	9.1	8	8.3	8.2	9	8.5	0										
S16	7.9	8.2	8.7	7.5	7.5	7.6	8.7	0									
S18	7.9	8.6	8.8	7.7	7	7.3	9.3	5.7	0								
N22	7.9	7.8	8.8	7.7	7.7	7.8	7.6	7	7.1	0							
NJ57	7.3	6.9	7.8	7.3	6.1	5.7	8.1	6.4	7.2	6.5	0						
NB15	7.8	8	8.8	7.4	7.3	7.7	6.9	6.7	8.1	6.8	6.6	0					
WIGAM	8.5	8.3	8.7	8.5	6.9	7.1	7.8	7.8	8.1	7.8	6.6	6.9	0				
NO33	8.8	9.3	8.5	8.3	7	7.8	8.6	7.3	6.3	7.5	7.6	7.6	6.5	0			
NO40	7.8	7.7	8.6	7.8	6.6	7.4	7.3	7.4	7.3	6.1	6.2	6.2	6.4	6.4	0		
NO41	8.3	8.2	8	7.1	7.2	7.9	7.9	7.2	7.8	7.4	6.7	6.7	6.2	6.7	5.7	0	
NO17	8.4	8.3	8.4	7.1	6.9	7.6	8	7.1	6.5	7.1	6.8	6.9	7.1	6.3	5.7	5.9	0
H1	8.1	8	8.3	7	7	7.6	7.9	7	7.5	7.6	7.2	6.3	7.5	7.6	6.2	6.4	5.1
H2	8.6	9	9.2	7.8	8	8.8	8.1	8.4	8.2	7.6	7.9	8.1	8.2	8.1	7.5	8.4	7
H3	7.8	8.5	8.9	7.3	7.1	7.7	8.1	6.7	7.4	6.2	6.6	7.4	7.4	7.4	6.2	7.3	6.8
H4	7.9	8.4	9.1	8	7.5	7.5	9.1	6.5	7.1	6.7	6.6	8.1	7.3	7.7	7.5	7.5	7.8
H5	7.7	8	8.4	8.3	6.9	7.4	8.5	6.7	6.5	6.8	6.2	7.8	7.4	7.5	7.3	7.6	8
PKM1	8.2	7.5	7.7	7.8	8.1	8.3	9.2	7.3	7.4	7.4	7.5	8.4	8.7	8.7	8.1	7.9	8.5
PKM2	8.5	7.7	7.8	7.6	8.1	8.4	9.1	7.9	7.7	7.4	7.8	8.7	8.5	8.9	8.4	8.5	8.7
BT1	9.4	7.7	8.4	8.3	8.6	8.9	8.7	8.6	8.9	8.4	8.7	8.8	8.5	9.2	8.4	8.5	8.9
BT2	8.8	8.4	8.8	8.1	8.5	8	8.5	8.3	7.6	8.4	8.2	8.5	8.5	8.5	8.5	8.5	7.8
BT3	8.9	7.9	8.6	7.6	7.4	7.8	9	8	8.2	8.7	7.7	8.7	8.2	8.4	8.1	8.3	8.1
BTM	8.5	7.9	8.5	8.1	6.9	6.7	9.3	7.6	7.9	8.5	7.2	7.8	7.5	8.4	8.1	8.2	7.8
PG1	7.9	7.9	8.6	7.2	5.7	6.8	9.4	7.5	7	7.6	6.7	7.8	7.8	7.3	7.2	7.6	6.7
PG2	8.4	9	8.5	8.3	7.4	7.9	9.6	7.4	7.8	9.1	8.3	8.8	7.8	6.6	8.1	7.9	8.1
MG1	7.7	8.3	8.5	7.4	6.6	7.1	8.8	7	6.8	8.1	7.4	7.9	7.2	6.9	7	7	7.1
MG2	8.7	8.3	8.5	7.8	7.3	7.9	9.1	7.9	7.9	7.8	8.1	8.6	7.8	8	7.3	7.6	7.9
MG3	8.7	8.1	8.7	7.9	6.9	7.4	8.4	8.4	8.2	8.5	7.8	7.9	7.4	8.2	7.9	7.8	8.2
NCBS1	8.4	8.5	9.5	8.5	6.9	7.4	9.6	7.6	7.4	8.1	7.9	8.3	7.2	7.2	7.4	7.6	7.8
NCBS2	8.3	7.9	9.3	8.3	6.6	6.9	9.1	8.4	8.1	8.1	7.6	8.4	6.3	7.8	7.3	7.4	8.3
NCBS3	8.5	7.9	9.1	8.5	6.9	7.4	9.3	8.4	7.8	7.5	7.6	8.8	7.1	7.6	7.1	7.6	7.5

Table 3 (Cont.)

	H1	H2	H3	H4	H5	PKM 1	PKM 2	BT1	BT2	BT3	BTM	PG1	PG2	MG1	MG2	MG3	NCB S1	NCB S2	NCB S3
P3																			
P10																			
P13																			
P11																			
P14																			
NB30																			
NB1																			
S16																			
S18																			
N22																			
NJ57																			
NB15																			
WIGAM																			
NO33																			
NO40																			
NO41																			
NO17																			
H1	0																		
H2	6.9	0																	
H3	6.3	6.7	0																
H4	7.9	7.5	5.6	0															
H5	8.4	8.7	6.6	5.7	0														
PKM1	8.5	9	8.3	7.1	5.8	0													
PKM2	8.5	8.6	7.9	7.2	6.6	4.4	0												
BT1	8.4	9.1	8.4	8.1	8.3	6.6	5.8	0											
BT2	8.4	8.5	8.4	8.5	8.5	8.2	7.6	7.2	0										
BT3	8.2	8.8	8.2	8.3	7.8	7.8	7.5	6.8	6.3	0									
BTM	8	9.3	8.3	7.7	7.8	8	7.9	7.4	7.3	4.8	0								
PG1	7.3	8.3	7.3	7.4	7.6	7.9	7.6	8	7.9	6.3	5.2	0							
PG2	8.1	8.7	7.3	7.4	7.8	8.3	8.5	8.8	8.6	7.9	7.8	6.8	0						
MG1	7.4	8.7	7.1	7.1	6.6	7.1	7.3	7.6	7.9	6.9	6.6	5.7	5.4	0					
MG2	7.9	8.7	7.2	7.3	7.4	7.1	7	7.1	8.2	7	7.2	6.4	6.2	4.5	0				
MG3	8.1	8.7	8.3	7.8	7.1	7.3	6.9	6.9	7.6	7.1	6.7	6.6	7.4	5.6	5.9	0			
NCBS1	7.9	8.8	7.5	6.4	7.2	8	7.8	7.7	8.2	7.3	6.6	6.7	6.4	5.7	6.2	5.6	0		
NCBS2	8.4	9.3	7.9	7.1	7.1	7.6	7.4	7.3	7.9	7	6.3	6.4	7	5.3	5.5	4.4	4.9	0	
NCBS3	8.4	9.3	8	7.7	7.4	7.6	7.7	7	7.9	6.7	6.5	6.1	7.3	5.3	5.1	5.4	5.1	4.2	0

Fig. 4 Three types of fruit shape in tamarind.

green leaves except for 'MG1' with light brown pulp and 'PG2' with light green coloured leaves.

The genotypes of sub-cluster 'B2' were divided into two minor clusters 'B2a' and 'B2b' at 12.8 linkage map distances. The seven genotypes of minor cluster 'B2a' could be segregated into two groups (I and II) with 4 and 3 genotypes, respectively linked at 11.8 linkage distance. The 4 genotypes of group I ('BT2', 'BT3', 'BT4' and 'PG1') were characterised by orthotropic tree growth and semi-curved fruit shape. In contrast, the 3 genotypes in group II ('PKM1', 'PKM2' and 'BT1') predominantly showed plagiotropic tree growth pattern and curved fruit shape. However, both groups shared brown coloured fruit pulp and dark green coloured leaves except for 'PKM1', which had a light brown pulp and 'BT4', which had a dark brown pulp.

The 7 genotypes of minor cluster 'B2b' were grouped

Fig. 5 Two types of tree growth character in tamarind.

together at 9.7 linkage distances and could be segregated into two groups (I and II) with 5 and 2 genotypes, respectively. However, both groups shared common morphological features such as orthotropic growth pattern and semi-curved fruit shape except for genotype 'P11' with a straight fruit shape. The pulp colour in the cluster 'B2b' varied from light brown to dark brown and the leaf colour varied from light green to dark green. In general, the genotypes of the major cluster 'B' was predominantly characterised with brown to dark brown fruit pulp and the sub clusters 'B1' with straight fruit shape and 'B2' with semi-curved to curved fruit shape (**Fig. 4**).

All the tamarind genotypes analysed in the present studies were collected from Southern India with variable growth and fruit morphologies. RAPD analysis revealed a high level of polymorphism (92%), proving their wide origin and as a cross pollinated species. Since tamarind is a perennial tree crop, the *ex situ* collection could accommodate only a limited number of accessions. Based on the RAPD analysis genetically closely associated genotypes could be identified such as 'H3' and 'H4', 'S16' and 'S18', 'NO17' and 'H1', 'NCBS2' and 'NCBS3', 'BT3' and 'BT4', and 'PKM1' and 'PKM2', which could be avoided for further breeding programmes, thus proving the potential of DNA-based markers to determine the genetic relationship among genotypes and could have a practical application in breeding hybrids (Jain *et al*. 1999). In summary, the use of RAPD markers is a useful tool for germplasm analysis and for detection of genetic relationships within tamarind genotypes. Knowledge on genetic diversity will help in the efficient management of tamarind germplasm and future hybridization programmes.

ACKNOWLEDGEMENTS

The authors acknowledge the University of Agricultural Sciences, Bangalore, India for furnishing lab and other facilities for conducting the experiments.

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