J. Korean Soc. For. Sci. Vol. 109, No. 4, pp. 421~428 (2020)
https://doi.org/10.14578/jkfs.2020.109.4.421

한국산림과학회지
JOURNAL OF KOREAN SOCIETY OF FOREST SCIENCE
ISSN 2586-6613(Print), ISSN 2586-6621(Online)
http://e-journal.kfs21.or.kr

RAPD Polymorphism and Genetic Distance among Phenotypic Variants of *Tamarindus indica*

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Abstract: Tamarind (*Tamarindus indica* L.) is one of the multipurpose tree species distributed in the tropical and sub-tropical climates. It is an important fruit yielding tree that supports the livelihood and has high social and cultural values for rural communities. The vegetative, reproductive, qualitative, and quantitative traits of tamarind vary widely. Characterization of phenotypic and genetic structure is essential for the selection of suitable accessions for sustainable cultivation and conservation. This study aimedto examine the genetic relationship among the collected accessions of sweet, red, and sour tamarind by using Random Amplified Polymorphic DNA (RAPD) primers. Nine accessions were collected from germplasm gene banks and subjected to marker analysis. Fifteen highly polymorphic primers generated a total of 169 fragments, out of which 138 bands were polymorphic. The polymorphic information content of RAPD markers varied from 0.10 to 0.44, and the Jaccard's similarity coefficient values ranged from 0.37 to 0.70. The genetic clustering showed a sizable genetic variation in the tamarind accessions at the molecular level. The molecular and biochemical variations in the selected accessions are very important for developing varieties with high sugar, anthocyanin, and acidity traits in the ongoing tamarind improvement program.

Key words: Tamarind*, genetic diversity, RAPD maker, polymorphism, similarity coefficient*

Introduction

Tamarind (*Tamarindus indica* L.) is a significant multi-purpose tree belonging to the Fabaceae (Leguminosae) family with a somatic chromosome number, 2n=24 (Purseglove, 1987). The species is widely distributed in Bangladesh, Sri Lanka, Myanmar, Australia, Malaysia, Thailand and also in African, South American and North American continents (Mishra, 1997). It has been naturalized in the southern part (Andhra Pradesh, Madhya Pradesh, Karnataka, and Tamil Nadu) of India where moist deciduous and tropical evergreen forests are commonly found (Champion and Seth, 1968). India is the largest producer of Tamarind in the globe with the production of 201,270 million tonnes at 48,420 ha during the year 2017-2018 (Government of India, 2018).

Larger geological distribution, higher cross pollination nature of the species adaptability to various climatic zones attributes the high degree of variations in a population from which the number of genotypes will be found to be large (Lewis, 1992; Engels and Ramanatha 1998). Based on the variation of biochemical contents, Tamarind has been categorized into three types such as Sweet Tamarind (low acidity and high sugar), Sour Tamarind (high acidity and low sugar) and Red Tamarind (high anthocyanin). The Sweet Tamarind pulp has rich minerals and vitamins and it is used for preparation of jam, jelly, candy and chocolate. The Red Tamarind has high scopes for utilizing as bio-colorant in food processing, pharmaceutical product, brewery, and confectionery. This helps to replace the use of carcinogenic inorganic colorants providing a large commercial utilization. The Red and Sweet Tamarind var-

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iants are rare mutants with scattered distribution preventing their commercial utilization. The Sour Tamarind is a common variant that occupies the largest Tamarind populations. The Sour pulp is the richest natural sources of tartaric acid and acidulant substance utilized for preparation of food nourishments (Shankaracharya, 1998).

The morphological characters are the oldest, most commonly utilized selection markers. They may in any case be ideal for management practices of germplasm and cultivars, where the cultivars have been recognized based on leaf, panicle, fruit and other physical attributes while environmental conditions have significant impact on these characteristics. In perennial woody plants, however, the identification of cultivar and assessing genetic diversity through morphological traits has several limitations (Purushotaman et al., 2008). Also, the estimation of genetic diversity through physical features is inefficient and inaccurate (Rahman et al., 2009). This can be overcome by direct recognition of genotypes with DNA-based marker system. The utilization of biochemical and genetic markers for the identification of variants offers a practical elective strategy (Williams et al., 1990).

RAPD markers prove valuable genetic information when assessingthe genetic variation and relatedness among genotypes or variants. The RAPD markers have been widely used to assess the genetic relatedness in teak (Keiding et al., 1986), mango (Ravishankar et al., 2000), oil palm (Shah et al., 1994), plum (Shimida et al., 1999) and eucalyptus (Keil and Griffin, 1994). The genetic relatedness between flowering and non-flowering Tamarind cultivars (Kumar et al., 2015) and among 13 Indian collections of Tamarind (Gangaprasad et al., 2013) has been reported. On the other hand, the genetic diversity studies based on morphometric, quantitative and qualitative characters of Tamarind have been carried out (Divakara, 2008; Sharma et al., 2015; Fitriana and Chinawat, 2017; Mayavel et al., 2018).

Despite its commercial significance, Tamarind has been less explored in the genetic improvement because they have not been the subject of much scientific investigations (Algabal et al., 2011). Characterization of phenotypic and genetic diversity of Tamarind is necessary since it facilitates the selection of suitable accessions for domestication and cultivation. Understanding genetic structure of population is fundamental for conservation and sustainable utilization (Sun et al., 1998), which strengthens the comprehension on the origin, evolution, and variation pattern of gene pools (Engles, 1989). Estimation of genetic diversity is an important step for germplasm characterization and commercialization of elite Tamarind variants. The genetic improvement of Tamarind through conventional breeding methods has several limitations due to high heterozygosis of larger plant size, irregular flowering, and poor bearing habit. It is primarily out crossing species with long juvenile period, thus conventional breeding methods needs considerable time and money. Selection of parents with higher productivity with desirable quality will have higher implication in Tamarind improvement program. Pattern of genetic diversity present in the Tamarind genetics resources could be accessed with help of molecular markers.

The main purposes of the present study are 1) to assess the extent of genetic variation among Sour, Red and Sweet Tamarind variants at the molecular level using RAPD (Random Amplified Polymorphic DNA) makers, 2) to estimate the genetic distance and relatedness among three phenotypic Tamarind variants, and 3) to give molecular genetic information for future tree improvement programmes of Tamarind (*Tamarindus indica*). The genetic diversity and relationship among Sweet, Red and Sour Tamarind accessions could be used efficiently in genetic improvement of desirable cultivars and conservation of genetic resources in Tamarind.

Materials and Methods

1. Collection of samples and genetic resources

The tree improvement program of Tamarind is being implemented at the Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India to evolve high yielding variants of Tamarind for commercial cultivations at farm-lands. Selection of genetic resources for desirable variants such as Sour, Red and Sweet Tamarind has been the first initiative of the improvement programs. Extensive surveys were carried out in Tamil Nadu, Karnataka, Andhra Pradesh and Pondicherry in India. A total of ninety candidates of plus trees (30 Sour, 47 Red and 23 Sweet Tamarind) were selected based on superiority of fruit yield. The selected genetic resources were asexually multiplied through grafting and conserved in the germplasm banks.

The germplasm banks of Sour, Red and Sweet Tamarind were evaluated for quantitative and qualitative traits.

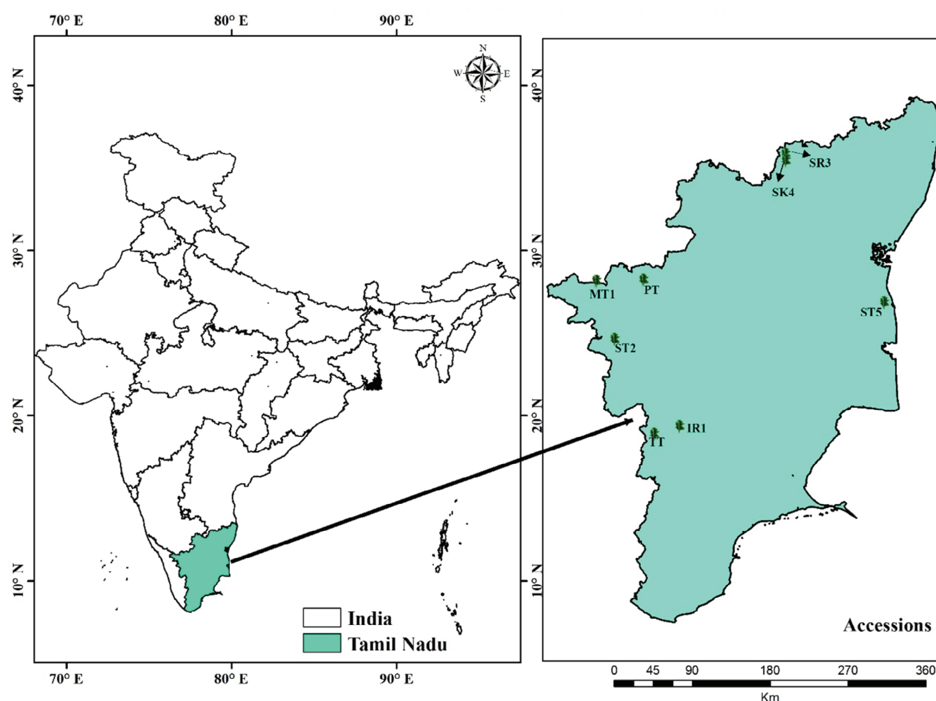

Figure 1. Geographical area of Tamarind accessions collected.

Table 1. Information on the genetic resources of Tamarind used in the present study.

Variant	Accession Code	Latitude and Longitude	Elevation (m)	Location
Sweet	ST2	N11°00.25', E76°55.51'	421	Coimbatore, Tamil Nadu
”	ST3	N11°02.26', E76°03.41'	384	Chinnayampalayam, Tamil Nadu
”	ST5	N11°23.16', E79°43.53'	26	Karaikal, Puducherry
Sour	TT	N10°01.25', E77°20.34'	349	Theni, Tamil Nadu
”	PT	N10°96.84', E76°73.56'	507	Poondi, Tamil Nadu
”	MT1	N10°95.97', E76°43.93'	444	Mullangaddu, Tamil Nadu
Red	IR1	N10°06.16', E77°36.18'	352	Jayamangalam Tamil Nadu
”	SR3	N12°56.05', E78°42.24'	364	Pernampattu, Tamil Nadu
”	SR4	N12°51.48', E78°42.39'	360	Chengalpattu, Tamil Nadu

Totally, nine accessions from Sour (3), Red (3) and Sweet (3) Tamarind were sampled based on fruit productivity and quality. Detail information of the samples including variants, accession code, collection elevation and origin are given in Figure 1 & Table 1. Young, healthy leaves from the selected accessions were harvested individually and used for DNA isolation.

2. DNA extraction, PCR and genetic distance analysis

Genomic DNA from leaf samples were isolated using ArborEasy® DNA isolation kit following manufacturer's instruction with minor modification. The isolated DNA was digested with RNase A (10 mg/ml) and stored for further use at -20°C. The integrity of isolated DNA was checked on agarose gel electrophoresis (0.8%) and quantified using NanoDrop 2000 UV-VIS Spectrophotometer (Thermo Scientific, USA) by measuring the absorbance at 260 nm and 280 nm.

RAPD analysis was carried out by amplifying 15 different highly polymorphic RAPD primers (Operon technologies, USA). The PCR reaction was undertaken in 10μl reactions having 30 ng of template DNA, 1X Taq buffer A, 1mM dNTP Mix, 1.2 mM $MgCl_2$, 2 μM RAPD primer and 1U Taq DNA polymerase. The PCR mixture was am-

plified in a thermal cycler (Bio-Rad, USA) with initial denaturation at 94°C for 4 min followed by 45 cycles of 1 min denaturation at 94°C, 1 min annealing at 37°C and 2 min extension at 72°C. At last, extended polymerization at 72°C was done for 7 min. The PCR products were resolved on 1.5% agarose gel and electrophoresed for 3-4 hours at 90 V for complete separation of the RAPD bands. The gel was visualized under UV light and the photographs were documented.

RAPD profiles were scored visually for their absence(0) or presence (1) of bands and the binary data (0, 1) was statistically analysed using NTSYS software version 2.02 (Rohlf, 1998). The unweighted pair–grouping method (UPGMA) was employed with mathematical averaging of simple matching coefficients. A dendrogram was generated using the sequential agglomerative hierarchical nested (SAHN) algorithm in theNeighbour-Joining method (Sneath et al., 1975). The genetic distance values for the similarity matrix were calculated using Jaccard's coefficient (Jaccard, 1901). The polymorphism information content (PIC) of RAPD primers was calculated by the formula PIC = $2P_i(1-P_i)$ (Bhat, 2002) where P_i is the frequency of occurrence of polymorphic bands in different primers.

Results and Discussion

The concentration of extracted DNA varied from 89 ng/µl to 1,168 ng/µl across nine Tamarind accessions and the ratio of A260/280 ranged from 1.82 and 1.90. The DNA was diluted and amplified using 15 highly polymorphic primers (Table 2). A sum of 169 RAPD bands was generated, and out of which 81.2% (138 bands) were polymorphic in nine Tamarind accessions. The number of fragments produced per primer ranged from 7 (OPE 04) to 20 (OPN18) with the average of 11.3. On average, the number of polymorphic bands per primer was 9.2. The polymorphism percentage of RAPD primers varied from 42.9% to 100%, and the average of PIC value was 0.31 (Table 2).

The primer OPN18 produced the highest number of polymorphic bands and the primers OPE05, OPJ19, OPP18 and OPP19 showed 100% polymorphism (Table 2). Among the fifteen polymorphic primers, the highest PIC value (0.44) was observed from the primer OPP19 and the lowest value (0.10) from the primers OPE04 and OPN18, which indicated that the primers were competent enough for detecting RAPD polymorphism among nine Tamarind accessions.

Table 2. Primer sequence, polymorphism percentage and polymorphism information content (PIC) value for RAPD marker analysis of Tamarind variants.

No.	Primer	Sequence (5'-3')	Total no. of products	No. of polymorphic bands	Polymorphism percentage	PIC value
1	OPB20	GGACCCTTAC	11	6	54.6	0.21
2	OPC10	TGTCTGGGTG	16	12	75.0	0.29
3	OPE04	TGTCTGGGTG	7	3	42.9	0.10
4	OPE05	TCAGGGAGGT	7	7	100	0.42
5	OPE20	AACGGTGACC	15	11	73.3	0.24
6	OPJ19	GGACACCACT	8	8	100	0.35
7	OPM02	ACAACGCCTC	9	8	88.9	0.31
8	OPN02	ACCAGGGGCA	12	11	91.7	0.36
9	OPN03	GGTACTCCCC	9	7	77.8	0.32
10	OPN05	ACTGAACGCC	12	11	91.7	0.29
11	OPN16	AAGCGACCTG	8	6	62.5	0.19
12	OPN18	GGTGAGGTCA	20	15	75.0	0.30
13	OPP18	GGCTTGGCCT	12	12	100	0.41
14	OPP19	GGGAAGGACA	10	10	100	0.44
15	OPX01	CTGGGCACGA	13	11	84.6	0.35
Mean			11.3	9.2	81.2	0.31

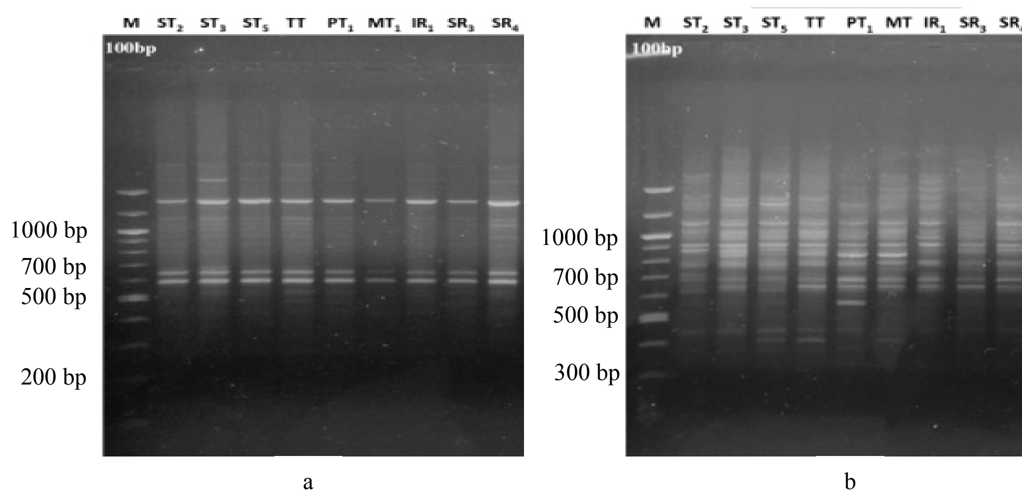

Figure 2. RAPD profile generated by primers OPN18 (a) and OPX01 (b) in the nine accessions of *Tamarindus indica*. M: size marker, Sweet Tamarind: ST_2, ST_3 and ST_5, Sour Tamarind: TT, PT_1 and MT_1, and Red Tamarind: IR_1, SR_3 and SR_4.

Variant-specific bands were observed in the primer OPB20 at 970 bp in Red Tamarind and the primer OPC10 generated unique bands at 430 bp in Sour Tamarind accessions (TT, PT1 and MT1). The unique bands were observed in all accessions of Sweet Tamarind by the primers OPE20 at 590 bp and 1170 bp, and the primer OPN02 at 430 bp. The primer OPM02 produced unique bands at 500 bp and 1,230 bp in only ST5 and ST2 accessions of Sweet Tamarind.

In addition, the accession-specific bands were generated by the primers OPE 20 in SR3 at 800 bp, and the primer OPN02 at 610 bp and 1,050 bp in ST2 and ST3 accessions, respectively. The primer OPN03 produced unique fragment in the accession ST2 at 2,000 bp. The RAPD fragments observed in the accessions ST3 and TT were found to be unique at two loci of 500 bp and 390 bp by tThe primer OPN16 generated unique bands in the accessions SR3 and PT1 at 500 bp and 1,040 bp, respectively. The unique RAPD fragments were also generated in the accession PT1 by the primer OPN18 at 570 bp and in the accession IR1 at 1,080 bp by the primer OPP18 (Figure 2).

The UPGMA dendrogram was generated in order to evaluate the level of RAPD polymorphism among nine Tamarind accessions (Figure 3). The cluster analysis placed nine Tamarind accessions into two major clusters (i.e., clusters A and B). The cluster A was the large group with seven accessions which subdivided into two sub-clusters (A1 and A2) at different similarity coefficients. The sub-clusters of A1 and A2 were further segregated into four

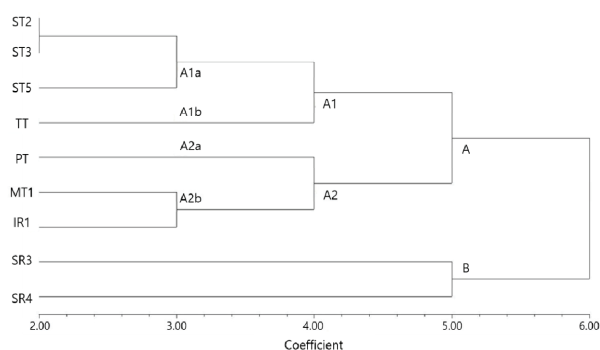

Figure 3. Dendrogram for genetic distance and clustering of nine accessions from three Tamarind variants.

small groups such as A1a consisting of three accessions from Sweet Tamarind (ST2, ST3 and ST5), A1b consisting of single Sour Tamarind accession (TT), A2a clustering two accessions (MT1 and IR1) from different variants of Sour and Red Tamarind, and A2b with single accession (PT) belonging to Sour Tamarind. The cluster B grouped two remaining accessions (SR3 and SR4) of Red Tamarind (Figure 3).

Genetic similarity matrix was constructed by Jaccard's coefficients from the RAPD bands, and used to estimate the genetic diversity among nine Tamarind accessions (Table 3). The similarity coefficient values among nine accessions of Tamarind varied from 0.37 to 0.70. The higher value of similarity coefficient (0.70) was observed between accessions MT1 from sour Tamarind and IR1 from red Tamarind. The lower similarity coefficient (0.37) was observed between ST5 from Sweet and SR3 from Red

Table 3. Genetic similarity matrix among nine accessions of Tamarind variants using Jaccard's coefficient based on the RAPD band pattern.

Variant Accession	Sweet			Sour			Red		
	ST_2	ST_3	ST_5	TT	PT_1	MT_1	IR_1	SR_3	SR_4
ST_2	-								
ST_3	0.67	-							
ST_5	0.62	0.65	-						
TT	0.55	0.60	0.65	-					
PT_1	0.45	0.47	0.49	0.59	-				
MT_1	0.56	0.49	0.51	0.65	0.60	-			
IR_1	0.56	0.50	0.53	0.61	0.59	0.70	-		
SR_3	0.42	0.41	0.37	0.40	0.45	0.43	0.44	-	
SR_4	0.57	0.60	0.51	0.55	0.46	0.55	0.58	0.59	-

Tamarind variants (Table 3).

The estimation of genetic relatedness at DNA level is one of the alluring steps in the process of developing taggable markers to identify elite accessions for development of desirable variants. The average polymorphism of three variants (Sour, Red and Sweet) of Tamarind (*Tamarindus indica*) was observed as 81.2% (Table 2), which was demonstrating that a wide genetic variation is existing among Tamarind accessions. This variation might be because of the geographical isolation of accessions and somewhat its cross-pollinating nature. Similar studies on RAPD markers in determining of Tamarind genotypes was reported by Gangaprasad et al. (2013) where 88.5% RAPD polymorphism was found among 13 accessions of Tamarind selected in various parts of Karnataka, India.

The unique polymorphic fragments in a particular variant or genotype can be used to develop the genotypic fingerprinting. The variant-specific bands were generated by primers of OPB20 and OPC10 in Red and Sour Tamarind variants. More numbers of unique bands were generated in Sweet Tamarind by primers of OPE20, OPN02 and OPM02. In addition, the accession-specific RAPD bands were observed in most of accessions including SR3, ST2, ST3, TT, PT1 and IR1. This result was in accordance with the genotype-specific bands reported by Gangaprasad et al. (2013). On the contrary, complete absence of amplicons for some primers (OPP18 and OPJ19) was observed in the accessions SR3 and SR4 from Sweet Tamarind. These variant-specific and accession-specific bands could be converted to SCAR (Sequence Characterized Amplified Region) markers which can be tagged to identify highly divergent variants and accessions.

The polymorphic information content (PIC) is an important factor in choosing the RAPD marker for genetic studies. In the assessment of genetic variation among Sour, Red and Sweet Tamarind accessions, the values of PIC ranged from 0.10 to 0.44, which was lower compared to ISSR analysis yet competent for the polymorphic determinant of RAPD marker reported by Chesnokov and Artemyeva (2015). Based on the PIC values from our study, the primers exhibiting 100% (OPP05 and OPP19) and high polymorphism are recommended for further studies of genetic variation in Tamarind. This elevated level of polymorphism could be interpreted that the elite accessions have considerable genetic variation due to the collection from various environments and locations of southern India.

The phylogenetic tree gave clusters of different variants grouped in to two major clusters implying that the Tamarind accessions collected from different geographical locations did not fall into clear-cut distinct groups despite of variants. The sub-cluster A1a comprised only accessions of Sweet Tamarind. The accession TT (Sour Tamarind) was not grouped with any other accessions at the minimum genetic distance, and clustered as A1b under the cluster A1 together with all Sweet Tamarind accessions at the maximum genetic distance. Meanwhile the Red variants SR3 and SR4 were grouped in single cluster B. A comparative perception was made Kumar et al. (2015) reported that the tamarind cultivar grouped variedly irrespective of colour and taste with different coefficient levels.

The similarity coefficient value was ranging from 0.37 to 0.70, indicating that the genetic variation was large among the accessions. However, the highest value was found between MT1 from sour and IR1 from red originated from different geographic origins locations. In such cases, the existence of common ancestors might have determined the similarity among variants or cultivars (Morales et al., 2011). This result indicates that Sour Tamarind might have been a chance of having primitive accessions of Red Tamarind. When there is a chance of free pollen flow and random fertilization, this circumstance could emerge in natural populations of cross-pollinated species like Tamarind.

The lowest similarity coefficient value was observed between the accessions ST5 (Sweet) and SR3 (Red Tamarind), showing the possibility that the two accessions have wide genetic variation as they were originated from different parents and geographical locations. Similar studies in Tamarind were reported by Gangaprasad *et al.* (2013) where high similarity coefficient was found between accessions from Northern Karnataka and low value was among genotypes from Belgaum and Shimoga. Likewise, Kumar et al. (2015) reported a moderate degree of genetic diversity with Jaccard's similarity coefficient and 87.5% similarity between PKM1 (Sour) and Red Tamarind cultivars. The Tamarind accessions ST5 (Sweet) and SR3 (Red Tamarind) will have a great scope in utilizing as parents in Tamarind breeding programme for improving qualitative traits.

Conclusions

DNA and protein-based markers are increasingly genuine and unaffected by environmental factors (Dhanraj et al., 2002). Molecular characterization of variants at genetic level provides the fundamental basis for efficient maintenance, utilization, and conservation of existing genetic resources (Prakash et al., 2002). The DNA-based markers are coordinated to assume a significant role in the future Tamarind tree improvement programs. The current investigation revealed an insight for Tamarind breeders to choose the accessions based on genetic relationship and strong implications on identification of Sweet, Sour and Red Tamarind accessions. Therefore, the Tamarind germplasm had a significant role in the conservation of Tamarind cultivars for future improvement program. It also provides direction for conversion of variant-specific bands to SCAR markers which can be tagged to identify highly divergent and rare variants.

Acknowledgement

Authors express their deepest gratitude to the Director General, Indian Council of Forestry Research and Education, Dehradun and the Director, Institute of Forest Genetics and Tree Breeding, Coimbatore for providing research facilities.

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Manuscript Received : September 29, 2020
First Revision : November 4, 2020
Second Revision : November 19, 2020
Accepted : November 19, 2020