Research Article

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Evaluation of molecular diversity of *ex situ* conserved germplasm of palmyrah (*Borassus flabellifer* L.) accessions using RAPD markers

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Abstract

The genetic relationship of 96 palmyrah palms, consisting of 24 indigenous accessions from Tamil Nadu and Andhra Pradesh, was investigated using Random Amplified Polymorphic DNA (RAPD) markers. Hundred and eighty primers were used initially to identify the polymorphic primers in six random samples and 10 polymorphic primers were selected to amplify the 96 palms. These 10 primers produced a total of 112 reproducible bands and out of them, 41 fragments (36.6 %) showed polymorphism. The number of bands produced with each primer varied from seven to 15 with an average of 11.2 bands per primer. The percent polymorphism ranged from 7.7 to 71.4 with an average of 37.4 per cent when all the primers were taken collectively. UPGMA grouped all the accessions into two major clusters at 0.85 similarity value. The highest similarity value (0.96) was observed between the accessions KLKM-8 and THY-54 and the lowest similarity value (0.782) was obtained between ANBI-17 and RCML-11. The relatively low polymorphism suggests a narrow genetic diversity of palmyrah populations from which the present accessions have been derived and maintained over the years.

Keywords:Borassus flabellifer, genetic diversity, germplasm, RAPD

Introduction

The genus *Borassus* is an extensively disseminated palm seen from western Africa and Madagascar to eastern Indonesia and Papua New Guinea (Davis and Johnson, 1987). *Borassus flabellifer* L., or palmyrah, is commonly distributed in India, SE Asia and rarely in other temperate regions of the world (Morton, 1988). It is a multipurpose tree of enormous use and the palm is dispersed along the coastal belts of India, northern Sri Lanka and SE Asia and eastern Indonesia (Davis and Johnson, 1987). The palm is located from sea level up to 760 m. The palms are scattered in Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Orissa, Maharashtra, Bihar, Madhya Pradesh and West Bengal. Limited numbers of palms are also seen in Gujarat, Assam and Uttar Pradesh (Anonymous, 1948). The palmyrah palm has been closely associated with human culture and tradition from prehistoric era. It is an economically important palm and is used widely as timber and firewood, for making fibres of various kinds, thatching material, furnitures and in food industry for making sugar, starch and many folk medicines (Morton, 1988).

The use of molecular markers to assess genetic diversity is essential to refine and complement classification based on morphological data. Understanding the genetic diversity and similarities existing within populations is vital for efficient management of germplasm in a breeding

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programme as the breeders can use the data in the development of breeding populations and crossing programmes to exploit hybrid vigour. In India, palmyrah germplasm collections are being maintained at Agricultural College and Research Institute (AC&RI), Tamil Nadu Agricultural University, Killikulam (TN) and Horticultural Research Station (HRS), Dr. YSR Horticultural University, Pandirimamidi (AP). The present study was carried out to explore the genetic diversity among indigenous palmyrah accessions, maintained in the above centres, using RAPD technique.

Materials and methods

The experimental materials comprised of 96 palms representing 24 accessions obtained from germplasm collections maintained at AC & RI, Killikulam (TN) and HRS, Pandirimamidi (AP). Four samples from each accession were pooled for genetic diversity studies (Table 1). The genomic DNA was extracted from immature leaflets of all palms using the SDS method. Initially 180 RAPD primers of the series OPBE, OPBA, OPAH, OPE, OPAF, OPC, OPA, OPM and OPAB (Operon, Germany) were used for screening six random samples to detect polymorphic primers. The PCR was carried out in a Biorad–DNA Engine thermocycler and the amplification reactions and electrophoresis were performed as reported by Jiji *et al*. (2007).

The reproducible and polymorphic RAPD profiles were scored as 1 for presence or 0 for absence of a band. The scored data in a binary matrix used for statistical analysis. Jaccard's (1908) similarity coefficient value was computed for each pair-wise comparison. The phylogenetic tree was constructed using similarity matrixes with the clustering algorithm, unweighted pair- group method using arithmetic averages (UPGMA). The genetic relationships among the selected germplasm were visualized as a phylogenetic tree using the SIMQUAL program of NTSYS-pc (Rohlf, 2000).

The distance matrix was used to execute principal coordinate analysis (PCA) to emphasize the resolution of the ordination. The binary matrix data were used to calculate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. The polymorphism information content (PIC) was calculated using the formula: $PIC = 2fi\,(1\text{-}fi)$where, fi is the frequency estimated for the marker '*i'* in the study (Dubreuil *et al*., 2003). Marker Index (MI) was also calculated with the formulas given by Powell *et al*. (1996).

Table 1. Name of Accessions, code and place of collection

Place of collection	Sl. no.	Code	Name of the accession
Tamil Nadu accessions			
Killikulam	1	KLKM-1	ACC-1
	2	KLKM-2	ACC-2
	3	KLKM-3	ACC-3
	4	KLKM-5	ACC-5
	5	KLKM-8	ACC-8
	6	KIKM-11	ACC-11
	7	KIKM-12	ACC-12
	8	KIKM-13	ACC-16
Ananthanambikurichi	9	ANBI-17	ACC-17
	10	ANBI-18	ACC-18
	11	ANBI-19	ACC-19
	12	ANBI-21	ACC-21
	13	ANBI-22	ACC-22
Seervudaiyarpuram	14	SRBM-41	ACC-41
Thisyanvilai	15	THYL-54	ACC-54
Andhra Pradesh accessions			
Ethalapadu village	16	RCML-1	ACC-1
	17	RCML-2	ACC-2
	18	RCML-4	ACC-4
Chennuru	19	RCML-7	ACC-7
Bandapalli	20	RCML-8	ACC-8
Gokavaram	21	RCML-9	ACC-9
Veerlankapalli	22	RCML-10	ACC-10
	23	RCML-11	ACC-11
	24	RCML-12	ACC-12

Results and discussion

After screening with 180 primers, only 10 primers could detect polymorphism among the investigated genotypes and these primers were used for further analysis. The 10 primers produced a total of 112 consistent bands and out of these amplified fragments, 41 bands (36.6%) showed polymorphism. Amplification products were generated in the range of approximately 250 – 2100 bp. The total number of bands produced by each primer varied from seven (OPA-09, OPE-17 and OPAH-08) to fifteen

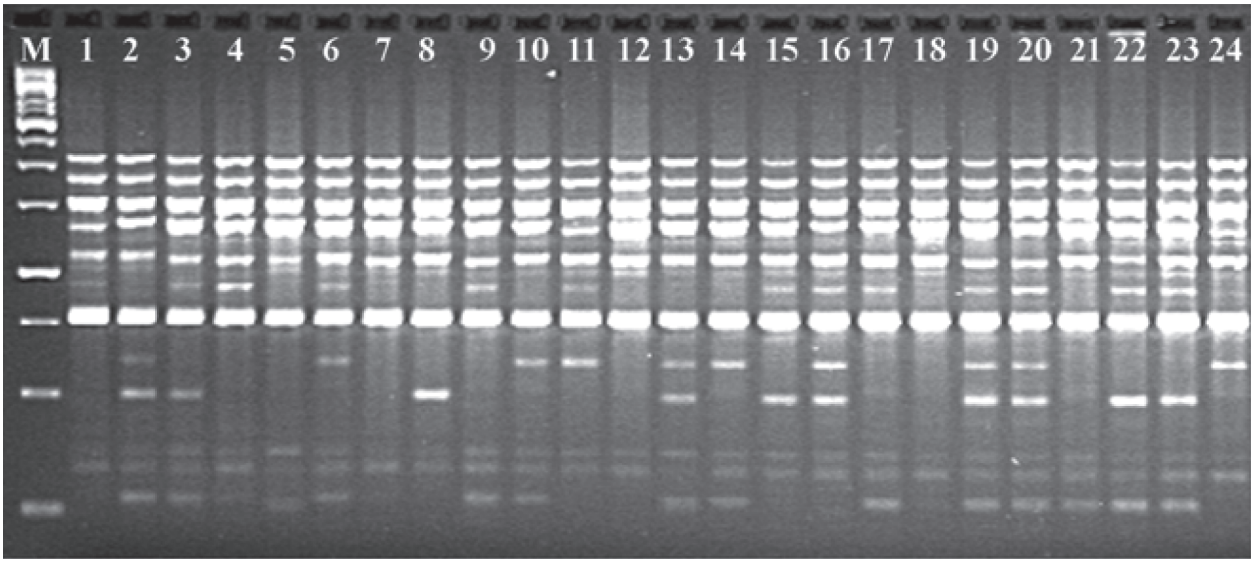

Fig. 1. RAPD marker profile of palmyrah accessions using the primer OPA-06. M: 1 kb ladder

(OPBA-16 and OPA-06) with an average of 11.2 bands per primer. The primer OPA-06 showed a maximum number of polymorphic bands (9). The per cent polymorphism ranged from 7.7 per cent (OPE-01) to a maximum of 71.4 per cent (OPAH-08) with an average of 37.4 per cent polymorphism when all primers were taken collectively. Five most informative primers (OPAH-08, OPAH-03, OPA-06, OPE-13 and OPM-18) were identified based on the polymorphism detected by individual primers. These primers generated 62 bands, out of which 31 (50%) showed polymorphism. The RAPD banding profile generated by the primer OPA-06 is shown in Figure 1.

Polymorphism Information Content and Marker Index

The polymorphism information content, computed from the average incidence of polymorphic bands across all the accessions, was 0.268 (Table 2). The primer OPE-13 revealed the highest value of PIC (0.458). MI was calculated

Table 2. Name of the primers with the number of amplified products, per cent polymorphism, polymorphism information content (PIC) and marker index (MI).

Sl. No.	Primer name	Sequence 5'-3'	Total amplified products	Polymorphic bands	Per cent polymorphism	PIC	MI
1	OPE-01	CCCAAGGTCC	13	1	7.7	0.079	0.079
2	OPE-13	CCCGATTCGG	14	5	35.7	0.458	2.290
3	OPE-17	CTACTGCCGT	7	1	14.2	0.125	0.125
4	OPA-06	GGTCCCTGAC	15	9	60.0	0.400	3.600
5	OPA-09	GGGTAACGCC	7	2	28.5	0.375	0.750
6	OPAF-06	CCGCAGTCTG	8	3	37.5	0.180	0.540
7	OPBA-16	CCACGCATCA	15	3	20.0	0.291	0.873
8	OPAH-03	GGTTACTGCC	13	8	61.5	0.296	2.368
9	OPAH-08	TTCCCGTGCC	7	5	71.4	0.167	0.835
10	OPM-18	CACCATCCGT	13	5	38.4	0.314	1.570
		Average	11.2	4.2	37.4	0.268	1.303

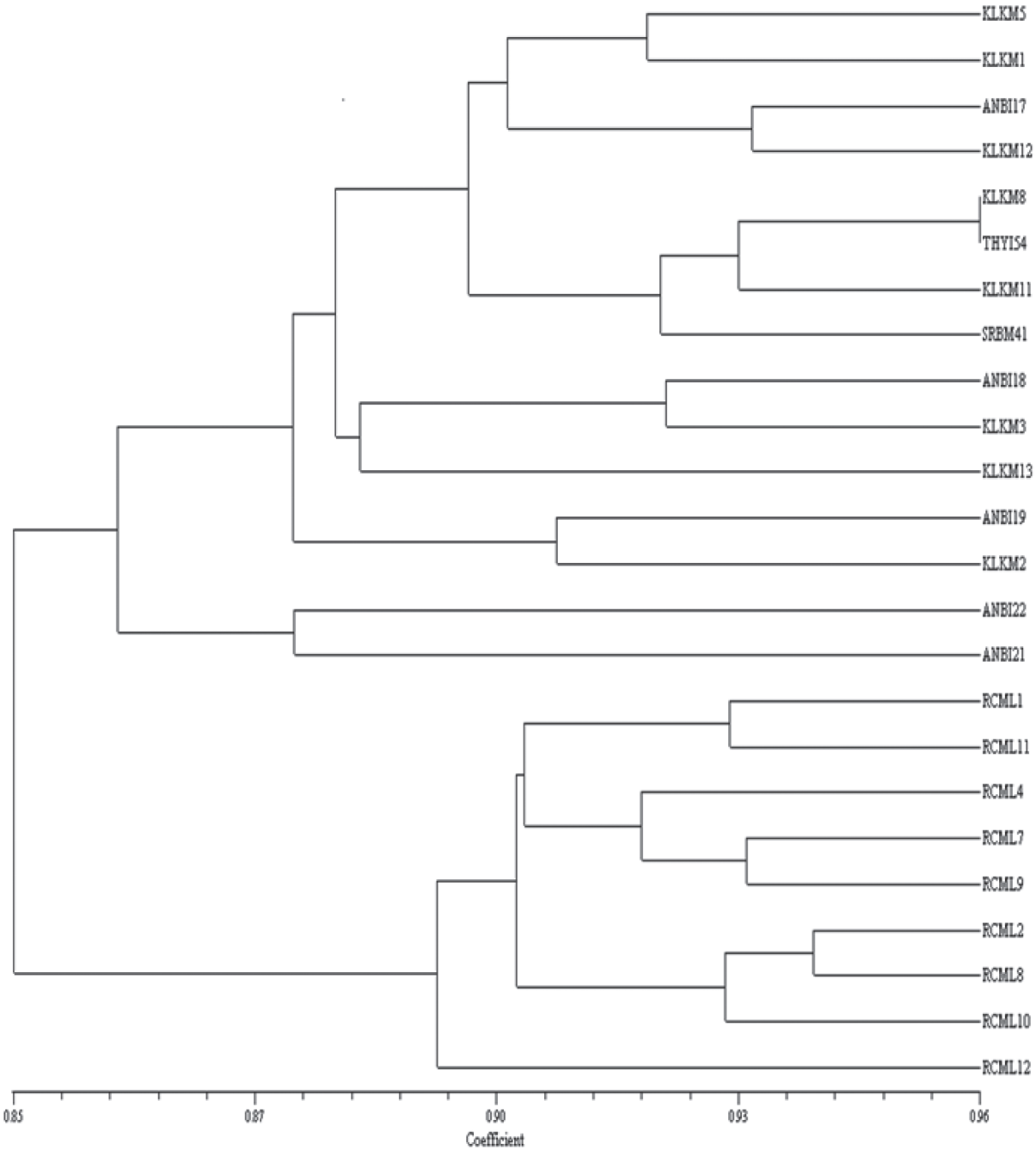

Fig. 2. A dendrogram of genetic relationship among 24 accessions of palmyrah palm based UPGMA cluster analysis

which reveals the amount of information that can be obtained from a particular primer. A primer with high MI value is more useful in revealing genomic differences. The marker index among RAPD primers was in the range of 0.079 to 3.6. The primers OPA-06 (3.6) and OPE-13 (2.29) recorded the highest MI and the lowest for the primer OPE-01 (0.079) with an average of 1.3.

Genetic similarity and cluster analysis

The pair-wise Jaccard's coefficient for the genetic similarities among the 24 accessions was

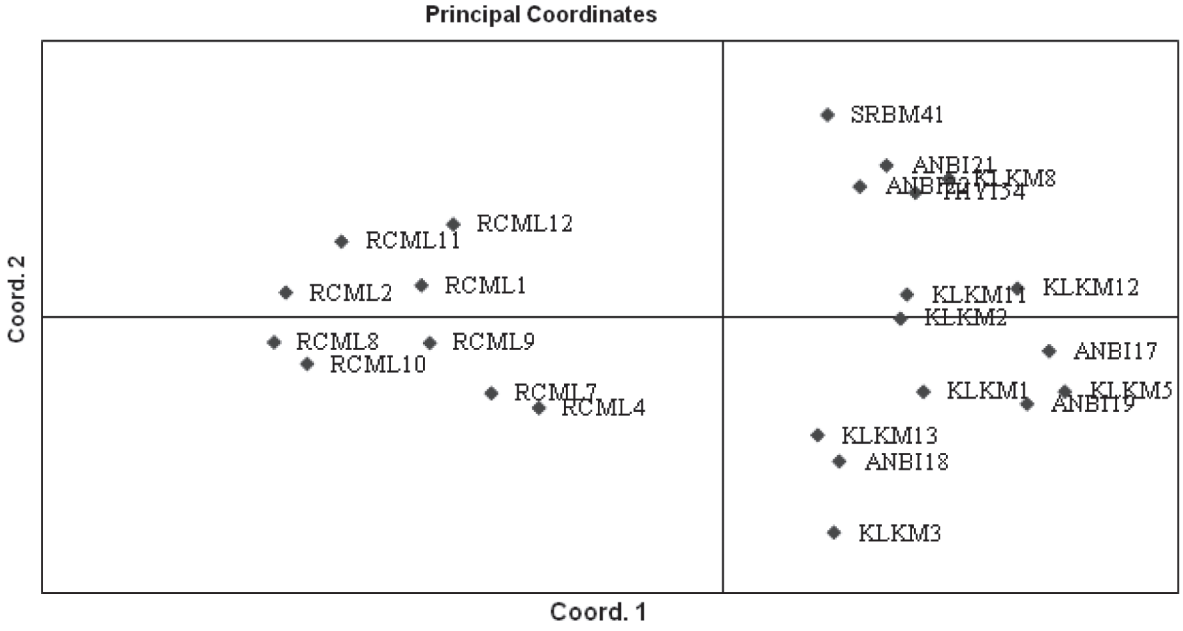

Fig. 3. Principal coordinate analysis of 24 accessions of *B. flabellifer* based on Jaccard's similarity matrix. Codes in the plot represent accession names listed in Table 1

analyzed with NTSYS-pc software (Table 3). The maximum similarity value (0.96) was observed between the accession KLKM-8 and THYI-54 and the lowest similarity value (0.782) was obtained between ANBI-17 and RCML-11. Cluster analysis of the genetic similarity values was executed to construct a dendrogram illustrating the overall genetic relationships among the accessions (Fig. 2). In the dendrogram, all of the accessions could be separated into two major clusters based on their geographic origin at 0.85 similarity value. Cluster I contained 15 accessions collected from Tamil Nadu and cluster II contains 9 accessions collected from Andhra Pradesh.

Principal coordinate analysis (PCA)

Principal coordinate analysis (PCA) was done based on Jaccard's similarity coefficient for comparisons among all the 24 accessions. The first principal coordinate accounted for 39.6 per cent of the variation, the second accounted for 15.5 per cent and the third for 12.8 per cent. A plot was made using the first two coordinates with each of the 24 accessions. The principal coordinate method based on the molecular similarity matrix discriminated the palmyrah accessions from Andhra Pradesh from those of Tamil Nadu (Fig. 3).

Knowledge about the genetic relationship and diversity among populations is vital in crop improvement strategies. Molecular markers disclose diversity at DNA level and thus provide direct, consistent and efficient tools for germplasm conservation and management. The palmyrah palm is one of the potentially useful, but underutilized palms of the world. Much of the palmyrah population is found in the wild in Cambodia (Kovoor, 1983), Burma (Lubeigt, 1977), Thailand, India and Indonesia (Tjitrosoepomo and Pudjoarinto, 1983 cited by Kovoor, 1983). Any palmyrah breeding programme can be effectively initiated by first assembling the widest possible collection of germplasm. The only formal collections of palmyrah maintained anywhere in the world are that of AC & RI, Killikulam (TN) and HRS, Pandirimamidi (AP).

In this study, only 36.6 per cent (41/112) of scored RAPD markers were polymorphic, showing a comparatively low level of polymorphism in Indian palmyrah accessions. The ancestral palmyrah, *Borassus aethiopum* was only exploited in the wild in Africa (Watt, 1908; Chevalier, 1949). This palm exhibits much more dissimilarity than the Asian palmyrah. Thus, the predictable trilocular fruit is common in *B. flabellifer*, while an abnormal

Table 3. Similarity matrix of 24 accessions of *B. fabellifer* based on RAPD profile.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	KLKM5	1.000																							
2	ANBI18	0.913	1.000																						
3	ANBI22	0.845	0.858	1.000																					
4	KLKM1	0.921	0.914	0.864	1.000																				
5	KLKM3	0.892	0.923	0.819	0.893	1.000																			
6	ANBI21	0.880	0.857	0.879	0.881	0.817	1.000																		
7	ANBI17	0.912	0.888	0.838	0.876	0.867	0.854	1.000																	
8	ANBI19	0.910	0.886	0.835	0.892	0.864	0.870	0.883	1.000																
9	KLKM2	0.864	0.895	0.845	0.883	0.874	0.861	0.875	0.910	1.000															
10	KLKM8	0.911	0.905	0.873	0.875	0.865	0.871	0.903	0.864	0.892	1.000														
11	KLKM11	0.931	0.906	0.874	0.913	0.885	0.891	0.886	0.902	0.893	0.922	1.000													
12	KLKM12	0.923	0.899	0.868	0.906	0.879	0.867	0.933	0.895	0.887	0.914	0.933	1.000												
13	KLKM13	0.885	0.897	0.830	0.886	0.876	0.846	0.860	0.857	0.849	0.858	0.895	0.889	1.000											
14	SRBM41	0.857	0.870	0.838	0.894	0.832	0.891	0.850	0.848	0.875	0.922	0.904	0.897	0.860	1.000										
15	THYI54	0.913	0.907	0.857	0.895	0.868	0.874	0.887	0.867	0.876	0.960	0.942	0.934	0.896	0.942	1.000									
16	RCML1	0.830	0.861	0.846	0.867	0.857	0.845	0.807	0.821	0.848	0.857	0.876	0.870	0.868	0.858	0.877	1.000								
17	RCML2	0.821	0.852	0.819	0.840	0.830	0.835	0.832	0.811	0.838	0.848	0.849	0.827	0.841	0.849	0.850	0.931	1.000							
18	RCML4	0.869	0.917	0.850	0.906	0.861	0.867	0.862	0.860	0.852	0.844	0.897	0.874	0.872	0.862	0.864	0.888	0.914	1.000						
19	RCML7	0.858	0.907	0.822	0.877	0.868	0.838	0.835	0.867	0.876	0.868	0.887	0.881	0.896	0.869	0.870	0.932	0.922	0.916	1.000					
20	RCML8	0.806	0.870	0.804	0.824	0.832	0.802	0.800	0.796	0.822	0.832	0.833	0.813	0.843	0.850	0.852	0.894	0.941	0.915	0.905	1.000				
21	RCML9	0.849	0.897	0.813	0.886	0.858	0.864	0.809	0.822	0.832	0.858	0.895	0.855	0.887	0.877	0.879	0.922	0.913	0.925	0.933	0.913	1.000			
22	RCML10	0.802	0.850	0.817	0.821	0.846	0.798	0.813	0.792	0.819	0.829	0.865	0.809	0.822	0.813	0.832	0.892	0.939	0.895	0.885	0.921	0.893	1.000		
23	RCML11	0.804	0.835	0.837	0.840	0.848	0.835	0.782	0.811	0.838	0.830	0.867	0.844	0.824	0.849	0.850	0.931	0.921	0.879	0.904	0.885	0.913	0.901	1.000	
24	RCML12	0.840	0.870	0.804	0.824	0.815	0.837	0.850	0.813	0.857	0.867	0.850	0.862	0.826	0.868	0.869	0.876	0.922	0.897	0.905	0.904	0.895	0.865	0.903	1.000

number of seeds (one, two or four), are more frequently seen in *B. aethiopum*. But *B. flabellifer* also exhibits more or less similar pattern of seed characters such as one seeded, two seeded, three seeded and rarely four seeded fruits.

Realizing the potential source of sugar, Indian voyagers took back seed and naturally selected them for the purpose. *B. aethiopum*, on prolonged cultivation in India, could have speciated in to *B. flabellifer*. From India, palmyrah palm spread eastward subsequently, the dispersal being principally effected by man (Kovoor, 1983).

In this study, accessions from the two states formed distinct clusters in the UPGMA dendrogram at 0.85 similarity value further revealing the narrow genetic base of Indian palmyrah, in spite of the cross-pollinated nature of the crop. In our study, we have screened 180 primers and only 10 primers could detect polymorphism. The other primers also worked well and showed an average of 11 bands which were all monomorphic so not included in the study. The low polymorphism could be related to the mode of introduction and maintenance of Indian palmyrah germplasm involving limited foundation germplasm and exchange of germplasm between farmers. Genetic diversity studies in palmyrah using RAPD markers were also reported by Ponnuswami (2010) and Raju and Reji (2015), where they obtained a similarity index of 0.70 and 0.79 respectively. They have used different set of samples and RAPD primers for their studies. To conclude, the results obtained in this study disclose the utility of RAPD markers in assessing the variability of palmyrah palms. Considering the low amount of variability among the accessions studied, sampling of many accessions from different agroecological regions would be an effective approach to capture genetic diversity for future conservation efforts in palmyrah.

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