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Molecular Characterization for Genetic Diversity of Palmyrah (*Borassus flabellifer*) Accessions using Inter Simple Sequence Repeat (ISSR) Markers

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ABSTRACT

Palms are woody monocotyledons in the family Arecaceae which is placed in the order Arecales. They are a natural group of plants with fossil records dating from the late crustaceous and with a characteristic appearance that enables them to be recognized easily, despite occasional confusion with cycads or cordylines. Slow, tall-growing, hardy and non-branching, dioecious and perennial in nature, palmyrah palms have no distinguishing features to identify sex, stature and high *neera*-yielding types until flowering age of about 12 to 15 years. Thus tappers have shifted from the traditional risky job of palmyrah climbing and slowly shifted to other easy jobs. So trees whose sex can be determined and with higher quality post harvest qualities are desirable traits of palmyrah palm. Molecular markers can be effectively utilized to diagnose and select a genotype. In this study 20 palmyrah accessions were analysed using inter simple sequence repeats (ISSR) markers with 21 primers. A total of 130 ISSR markers were scored of which 65 were polymorphic, equivalent to 47.94% polymorphism. These markers were used to estimate the genetic similarity among accessions using Jaccard's similarity coefficient, with similarity values ranging from 71.6 to 95.7%. The average number of markers produced per primer was 6.11. For each of the 21 ISSR primers PIC value ranged between 0 and 0.46. Cluster analysis based on ISSR data grouped the 20 palmyrah accessions into two major clusters. PCA based on ISSR data clearly distinguish genotypes similar to the results of cluster analysis.

Keywords: genetic diversity, male and female, tree stature
Abbreviations: ISSR, inter simple sequence repeats; **PCA**, principal component analysis; **PIC**, polymorphism information content; **RAPD**, random amplified polymorphic DNA; **UPGMA**, unweighted pair group method with arithmetic mean

INTRODUCTION

Palmyrah palm is botanically known as *Borassus flabellifer* L., belongs to the family Arecaceae and is dioecious in nature with the great majority of its economic edible products such as immature endosperm (*nungu*), mesocarp pulp (fruit pulp), tuberous seedlings (tubers) obtained only from female palms (Sankaralingam *et al.* 1999). But sweet sap from the inflorescence (*neera*), fermented sap (*toddy*), palm sugar and non-edible products like brush fibre and wood are obtained irrespective of whether the palms are male or female (Kgazal *et al*. 1990). However, differences in their yield or quality have been reported. Thus female palms are supposed to yield more *neera* on tapping from the inflorescence (Davis and Johnson 1987); the female tree gives better and harder timber than the male tree and is also more expensive (**Fig. 1A-E**).

Slow-growing perennials have no distinguishing features to identify the sex until flowering (Mogea *et al*. 1991). The palm commences flowering only after 12 to 15 years of maturity (Kovoor *et al*. 1983). Hardy and irregular growth habit of the non-branching trunk makes it difficult to climb for collection of *neera* and *toddy* on a day-to-day basis, with climbers having to climb a single tree a minimum of twice a day (Cunningham 1990). Thus tappers move from traditional palmyrah-climbing jobs, with high risk to other easy-to-perform jobs. Thus knowledge of the tree's sex and higher post harvest qualities are desirable traits of palmyrah palm. Breeding and crop improvement work would be highly facilitated if the sex of the palm could be determined at the early seedling stage itself. This would help palmyrah growers to select seedlings and also maintain an optimum

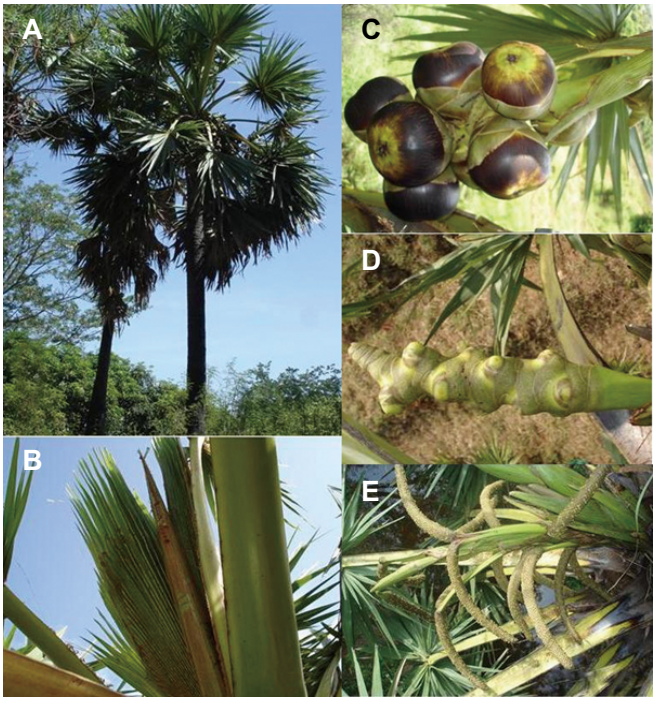

Fig. 1 Palmyrah. Tree (**A**), leaves (**B**), fruit (**C**), female inflorescence (**D**), male inflorescence (**E**).

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sex ratio at the plantation. Random amplified polymorphic DNA (RAPD) markers have been used to determine sex by bulk segregant analysis in *Pistacia vera* (Hormaza *et al*. 1994; Kafkas *et al*. 2001), *Carica papaya* (Macedo *et al.* 2002), *Trichosanthes dioica* (Singh *et al*. 2002) and *Borassus flabellifer* (Ponnuswami *et al*. 2008). Hormaza *et al.* (1994) screened 1000 primers of *P. vera* for sex determination and found only one female-associated marker, which was absent in males. They suggested that the low frequency of sex-linked bands may indicate that the DNA segment(s) involved in sex determination is small and probably involves a single or few genes. A female-specific DNA fragment of size 416 bp was identified in dioecious nutmeg (*Myristica fragrans*) by Ganeshaiah *et al*. (2000) after screening 60 RAPD primers.

Characterization based on horticultural traits needs complementation with molecular markers as they can contribute greatly to the utilization of genetic diversity through descriptive information of structure of genotypes, analyses of relatedness, the study of identity and location of diversity (Bhat and Karihaloo 2007). Understandably, these are the most powerful tools for evaluating diversity on the basis of fragment analyses which are indicators of potentially useful variability. In higher plants and animals, inter simple sequence repeat (ISSR) is a relatively novel technique that is more and more in demand because they it has proved to be powerful, rapid, simple, very reproducible, highly polymorphic, highly informative and quick to use (Zietkiewicz *et al*. 1994). ISSR markers reveal a much larger number of fragments per primer than RAPD analysis. These are mostly dominant markers, though occasionally a few of them exhibit codominance. ISSR permits detection of polymorphisms in inter-microsatellite loci, using a primer designed from di- or trinucleotide simple repeats (Gupta *et al*. 1994; Zietkiewicz *et al*. 1994).

Correct identification of palms is usually not possible until fruits are produced. Hence, in the present study, we tested the reliability of ISSR-PCR as a tool for the identification of palmyrah palm accessions.

MATERIALS AND METHODS

Plant material

Tender and soft-textured leaf samples from 4 dwarf males, 4 dwarf females, 4 tall males, 4 tall females, 2 high *neera*-yielding males and 2 high *neera*-yielding females maintained at the field gene bank at Agricultural College and Research Institute, Killikulam were used for this study. The details on the special attributes of the trees are presented in **Table 1**. Tender leaves from each plant were collected, and cleaned with distilled water and frozen quickly and stored at –80°C until DNA extraction.

Table 1 Palm trees (all 25-30 years old) used in this study details and their special features.

Accession №	Phenotypic description	Sex
TNPO 1	Dwarf	Male
TNPO 2	Tall	Female
TNPO 3	Dwarf	Male
TNPO 4	Dwarf	Male
TNPO 5	Dwarf	Female
TNPO 6	High *neera*-yielding	Male
TNPO 7	Dwarf	Female
TNPO 8	Tall	Male
TNPO 9	Dwarf	Male
TNPO 10	Dwarf	Female
TNPO 11	High *neera*-yielding	Female
TNPO 12	Tall	Female
TNPO 13	High *neera*-yielding	Male
TNPO 14	Tall	Female
TNPO 15	High *neera*-yielding	Female
TNPO 16	Tall	Female
TNPO 17	Dwarf	Female
TNPO 18	Tall	Female
TNPO 19	Tall	Male
TNPO 20	Tall	Male

DNA extraction

Genomic DNA was isolated from leaves using Cetyl trimethyl ammonium bromide (CTAB) (Aitchitt *et al.* 1993). The quality and quantity of the DNA was assessed on a 0.8% agarose (Bangalore Genei Ltd., India) gel. DNA concentration for PCR amplification was estimated by comparing the band intensity of a sample with the band intensities of known dilutions of 1 kb λDNA Marker (Fermentas, Germany). Based on the band intensity the DNA was further fractionated to the required concentration (25-50 ng) using sterile double distilled water.

Table 2 Details of polymorphism among the ISSR primers in palmyrah.

Primer	Nucleotide sequence (5′- 3′)	Annealing temperature (T_a)	Number of polymorphic markers	Total number of markers	Polymorphism percentage	Polymorphism information content (PIC)	Amplified fragment size (bp)
UBC-812	GAGAGAGAGAGAGAGAA	48	4	10	40	0.0582	250-1200
UBC-813	CTCTCTCTCTCTCTCTT	47	1	4	25	0.0488	400-750
UBC-815	CTCTCTCTCTCTCTCTG	48	1	7	14.28	0.1448	200-650
UBC-816	CACACACACACACACAT	50	2	3	66.66	0.1244	300-550
UBC-817	CACACACACACACACAA	50	5	8	62.50	0.2462	300-1200
UBC-818	CACACACACACACACAG	53	5	7	71.43	0.3113	250-750
UBC-822	TCTCTCTCTCTCTCTCA	50	2	3	66.66	0.1800	400-750
UBC-823	TCTCTCTCTCTCTCTCC	51	6	8	75	0.2462	300-1000
UBC-835	AGAGAGAGAGAGAGAGYC*	53	4	7	57.143	0.4550	200-1300
UBC-840	GAGAGAGAGAGAGAGAYT*	51	3	13	23.08	0.1982	100-650
UBC-841	GAGAGAGAGAGAGAGAYC*	52	3	5	60	0.1958	250-750
UBC-842	GAGAGAGAGAGAGAGAYG*	51	5	6	83.33	0.3832	200-750
UBC-843	CTCTCTCTCTCTCTCTRA*	50	1	6	16.67	0.1528	250-1000
UBC-844	CTCTCTCTCTCTCTCTRC*	51	5	7	71.43	0.3367	250-1500
UBC-855	ACACACACACACACACYT*	55	2	7	28.57	0.2346	250-750
UBC-857	ACACACACACACACACYG*	56	8	10	80	0.4118	250-1500
UBC-859	TGTGTGTGTGTGTGTGRC*	55	0	2	-	-	600-1200
UBC-885	BHBGAGAGAGAGAGAGA*	52	3	4	75	0.4550	350-900
UBC-886	VDVCTCTCTCTCTCTCT*	50	0	2	-	-	350-400
UBC-887	DVDTCTCTCTCTCTCTC*	49	3	6	50	0.0644	350-850
UBC-888	BDBCACACACACACACA*	56	2	5	40	0.1800	850-1300

*SINGLE LETTER ABBREVIATIONS FOR MIXED BASE POSITIONS
B = (C,G,T) (i.e. not A), D = (A,G,T) (i.e. not C), H = (A,C,T) (i.e. not G), N = (A,G,C,T), R = (A,G), V = (A,C,G) (i.e. not T), Y = (C,T)

ISSR analysis

A total of 35 ISSR primers (as described by the University of British Columbia, Canada) synthesized at Sigma-Aldrich, Bangalore, were used for the present study (**Table 2**). Amplification reactions were in volumes of 25 µl containing 30 ng of genomic DNA, 2.5 µl of 10X PCR buffer (including 15 mM $MgCl_2$), 0.5 µl of 10 mM each of dATP, dTTP, dGTP and dCTP, 2 µl of 20 pMol of primer, 1 U of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore). Amplifications were performed in Bio-Rad (MyCycler thermal cycler) programmed for an initial denaturation at 94°C for 5 min, 40 cycles of 1 min denaturation at 94°C, 1 min at a specific annealing temperature for each primer and 1 min extension at 72°C and a final extension of 5 min at 72°C and then at 4°C until storage. PCR-amplified products (12.5 µl) were then subjected to electrophoresis in a 1.5% agarose gel in 1X TBE buffer at 100 V for 3.5 hrs using a submarine electrophoresis unit (Maxi Sub System, Bangalore Genei). The ethidium bromide (0.001%)-stained gels were documented using the Alpha Imager TM 1200-Documentation and Analysis system of Alpha Innotech Corp., USA. Sizes of the identified bands were determined relative to 1 kb ladder (Fermentas, Germany).

Data scoring and analysis

Scoring of ISSR bands was carried out by considering only clear and unambiguous bands. Markers were scored for the presence and absence of the corresponding band among the different genotypes. The scores '1' and '0' were given for the presence and absence of bands, respectively. Polymorphism information content (PIC) or expected heterozygosity scores for each ISSR marker were calculated based on the formula $Hn = 1- \sum pi^2$, where pi is the allele frequency for the i^{th} allele (Nei 1973). The data obtained by scoring the RAPD profiles of different primers were subject to cluster analysis. A similarity matrix was constructed using Jaccard's coefficient and the similarity values were used for cluster analysis. Sequential agglomerative hierarchical non–overlapping (SAHN) clustering was done using unweighted pair group method with arithmetic averages (UPGMA) method. Data analysis was done using NTSYS software package version 2.02 (Rohlf 1994). The binary data for 20 palms was also subjected to principal component analysis (PCA) and scores for the first two components were plotted.

RESULTS AND DISCUSSION

Degree of polymorphism

Among the 20 different palmyrah accessions studied with thirty five primers, only twenty one primers produced scorable markers and used to assess genetic relationships in the tested accessions. A total of 130 markers were produced of which 65 markers produced polymorphic bands and this showed 47.94% polymorphism (**Table 2**). The number of polymorphic bands ranged from 1 (UBC-813, UBC-815 and UBC-843) to 8 (UBC-857). The average number of markers per primer was 6.19. UBC-828 and UBC-830 primers yielded 2 bands whereas UBC-821 primer amplified 13 bands. The band size ranged from 100-1500 bp. Primer UBC-842 showed the maximum level of 83.3% polymorphism. Primers UBC-859 and UBC-886 produced only monomorphic bands. Jaccard's similarity coefficient varied from 71.6% (between TNOP 13 and TNOP 20) to 95.7% (TNOP 3 and TNOP 4).

Cluster analysis

An UPGMA tree constructed on the basis of Jaccard's coefficient clustered the palmyrah accessions into 2 major groups. The UPGMA analysis resulted in pone phenogram shown in **Fig. 2**. The distance matrix between accessions (**Table 3**) shows an average distance range from 71.6% to 95.7%. Cluster A comprised of only one accession TNPO 13 (HNY-M). Cluster B divided into two sub groups viz., B1 and B2. Cluster B1 comprised of TNPO 20 (T-M). It separated from rest of the accessions. Cluster B2 was further divided into two sub cluster viz., B3 and B4. Cluster B3 consisted of nine accessions. TNPO 6 and TNPO 8 grouped in same sub cluster and showed 89.2% similarity. TNPO 16 and TNPO 17 showed 92.3% similarity and grouped in same sub cluster. In cluster B4 nine accessions were grouped together. Accessions TNPO 3 and TNPO 4

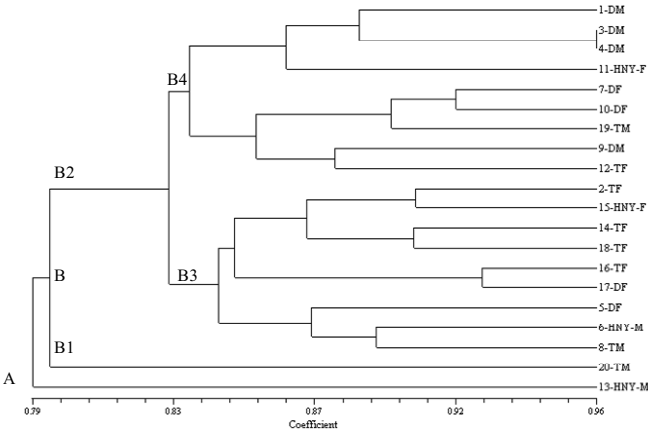

Fig. 2 UPGMA phenogram based on ISSR data for the studied genotypes of *Borassus flabellifer* L. palm.

Table 3 Genetic similarity coefficient among the palmyrah accessions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1																			
2	0.863	1																		
3	0.874	0.827	1																	
4	0.899	0.850	0.957	1																
5	0.844	0.872	0.852	0.862	1															
6	0.813	0.871	0.851	0.861	0.868	1														
7	0.828	0.856	0.851	0.846	0.823	0.851	1													
8	0.837	0.880	0.861	0.840	0.877	0.892	0.861	1												
9	0.811	0.811	0.850	0.860	0.778	0.850	0.850	0.829	1											
10	0.815	0.857	0.883	0.862	0.839	0.883	0.915	0.893	0.851	1										
11	0.840	0.795	0.880	0.874	0.820	0.864	0.833	0.813	0.832	0.835	1									
12	0.795	0.795	0.849	0.843	0.805	0.833	0.849	0.798	0.879	0.835	0.846	1								
13	0.793	0.808	0.802	0.797	0.789	0.832	0.817	0.782	0.785	0.833	0.798	0.829	1							
14	0.872	0.892	0.879	0.889	0.849	0.847	0.888	0.873	0.846	0.857	0.853	0.838	0.805	1						
15	0.816	0.903	0.795	0.819	0.855	0.824	0.839	0.863	0.766	0.825	0.778	0.778	0.762	0.858	1					
16	0.823	0.850	0.846	0.855	0.862	0.816	0.816	0.825	0.815	0.832	0.798	0.813	0.782	0.857	0.848	1				
17	0.811	0.855	0.805	0.815	0.851	0.820	0.835	0.844	0.789	0.836	0.758	0.787	0.770	0.831	0.852	0.923	1			
18	0.847	0.861	0.842	0.836	0.835	0.782	0.818	0.843	0.772	0.813	0.817	0.802	0.732	0.903	0.874	0.866	0.839	1		
19	0.800	0.823	0.844	0.845	0.820	0.827	0.913	0.862	0.867	0.879	0.800	0.886	0.778	0.892	0.821	0.827	0.832	0.813	1	
20	0.761	0.790	0.807	0.817	0.784	0.814	0.767	0.800	0.855	0.776	0.813	0.804	0.716	0.826	0.748	0.754	0.757	0.781	0.857	1

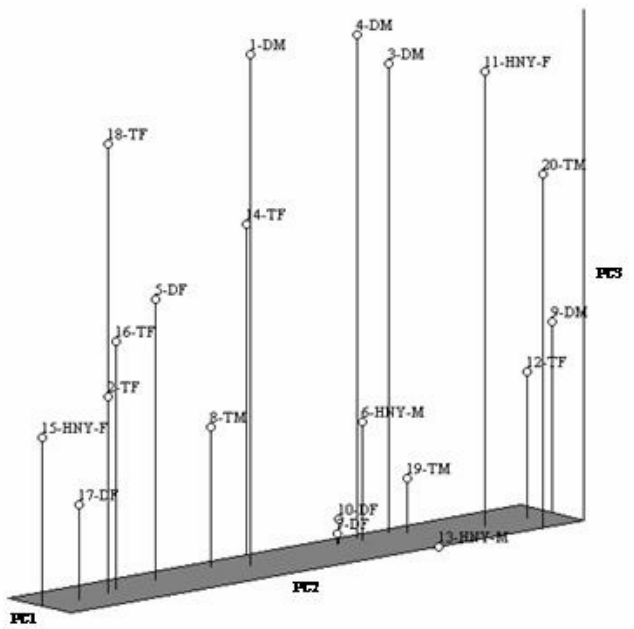

Fig. 3 3D distribution of palmyrah accessions revealed by first two principal components based on ISSR data.

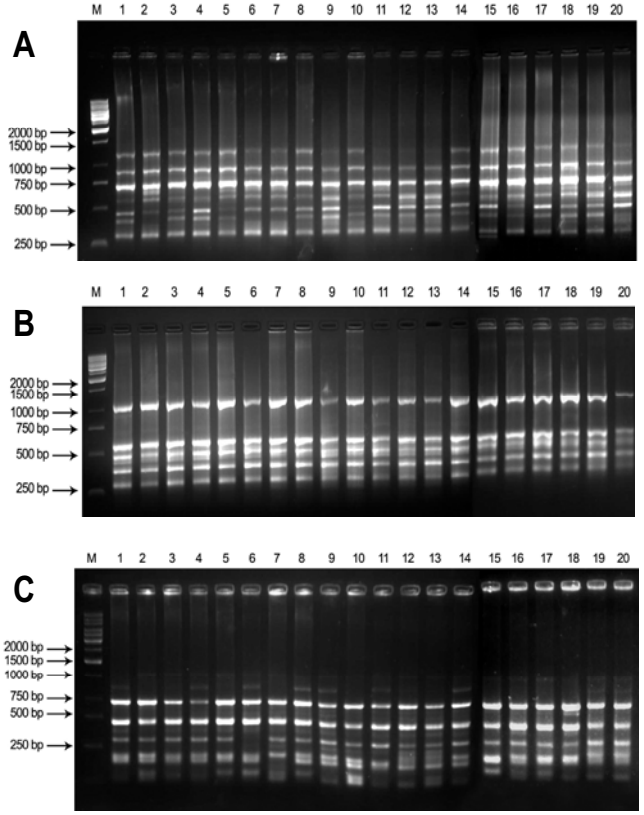

Fig. 4 ISSR marker profile of 20 palmyrah genotypes produced by primers UBC-817 (A), UBC-843 (B) and UBC-857 (C).

showed maximum level of 95.7% similarity. In cluster B3 maximum similarity of 92.3% was found between TNOP 16 and TNOP 17 and minimum similarity of 78.2% was found between TNOP 6 and TNOP 18. In cluster B4, TNOP 3 showed maximum similarity of 95.7% with TNOP 4 while minimum similarity of 79.5% was found between TNOP 1 and TNOP 12. For each of the 21 ISSR primers PIC value ranged between 0 and 0.46

A 3D score plot was generated using extracted PCA scores. Principal component analysis based on ISSR data clearly distinguished genotypes similar to the results of cluster analysis (**Fig. 3**).

Evaluation and identification of germplasms using ISSR markers are playing an important role in studies of genetics and breeding. Generally endemic species have lower genetic diversity than widespread species. Other factors such as breeding systems, vegetative reproduction, dispersal pattern, sample size, etc., also significantly influence the genetic diversity of a species. Characterization and documentation of genetic resource is an active area of germplasms conservation. Genetic diversity research is needed in perennial crop like palmyrah because of the constraints in implementing the collection of diversity from wider gene pools. Though there were published reports on the use of other molecular marker techniques such as ISTR, RFLP, RAPD, AFLP and SSR to analyze various palms in the past, this is the first report of the use of ISSR markers in analyzing palmyrah germplasm.

Majority of the accessions were clustered based on the morphological similarities like male and female, dwarf and tall palmyrah. In cluster B3 majority of female palmyrah accessions were grouped together. Currently there is no method to distinguish between male and female plants prior to flowering in palmyrah. Molecular markers can be utilized to diagnose and select a genotype based on linked DNA markers, long before the phenotype is apparent. This is particularly important in palmyrah palm, which has a long juvenile period.

The number of polymorphic bands detected by each primer depends on the primer sequence; hence a variable number of polymorphic bands per primer were obtained. These results are consistent with the earlier reports on RAPD analysis (Upadhyay *et al*. 2002; Connolly *et al*. 1994; Powell *et al*. 1996; Ashburner *et al*. 1997). The percentage (47.94%) of polymorphic bands between the palmyrah accessions indicated a moderate level of polymorphism and was comparable with earlier reports in coconut (Upadhyay *et al*. 2002; Ashburner *et al*. 1997; Rodriguez *et al*. 1997). The level of polymorphism in terms of the number of polymorphic bands per primer (3.09) was also moderately high and found to be consisting with the earlier reports on soybean (1.56) (Powell *et al.* 1996) and sweet potato (3.7) (Connolly *et al*. 1994). These results indicate that ISSR markers can be useful technique for germplasm characterization in palmyrah (**Fig. 4A-C**).

The data on genetic similarity indicated that more variance exist among tall accessions (maximum SI of 90.3% between TNOP 14 and TNOP 18 and minimum SI of 75.4% between TNOP 16 and TNOP 20) than among dwarf accessions (Maximum SI of 95.7% between TNOP 3 and TNOP 4 and minimum of 77.8% between TNOP 5 and TNOP 9). These results were comparable with earlier studies, which demonstrated higher variation in tall than dwarf (Ashburner and Rohde 1994; Perera *et al*. 1998). In cluster B2 three dwarf palmyrah accessions were grouped closer to tall palmyrah accessions. Similar results were obtained by Everard (1999) in coconut.

CONCLUSION

In conclusion, this study has established the ability of ISSR markers to distinguish palmyrah accessions with moderate efficiency. This information will form the base for analysis of intra-population variation. Extensive use of this technique and other molecular markers for characterization of palmyrah accessions is envisaged. Such a study will help in planning further germplasm collection and the selection of parents in future breeding programmes.

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REFERENCES

Aitchitt M, Ainsowrth C, Thongavelu M (1993) A rapid and efficient method

for the extraction of total DNA from mature leaves of date palm. *Plant Molecular Biology Reporter* **11**, 317

Ashburner GR, Rohde W (1994) Coconut germplasms characterization using DNA marker technology. *ACIAR Proceedings* **53**, 44-46

Ashburner GR, Thompson WK, Halloran GM (1997) RAPD analysis of South Pacific coconut palm populations. *Crop Science* **37**, 992-997

Barreveld WH (1993) *Date Palms Products*, FAO Agricultural Services Bulletin No 101, FAO, Rome, 262 pp

Bhat KV, Karihaloo JL (2007) Novel approach for characterization of genetic resource and search for new gene. In: Chopra V-L, Sharma R-P, Bhat S-R, Prasanna B-M (Eds) *Search for New Genes*, NAAS, New Delhi, pp 93-106

Connolly AG, Godwin ID, Cooper M, Delacy IH (1994) Interpretation of randomly amplified DNA marker data for fingerprinting sweet potato (*Ipomoea batatas* L.) genotypes. *Theoretical and Applied Genetics* **88**, 332-336

Cunningham AB (1990) Income, sap yield and effects of sap tapping on palms in south-eastern Africa. *South African Journal of Botany* **56**, 137-144

Davis TA, Johnson DV (1987) Current utilization and further development of the palmyrah palm (*Borassus flabellifer* L.) Arecaceae in Tamil Nadu State, India. *Economic Botany* **41**, 247-266

Everard JMDT (1999) An investigation towards developing a molecular approach to improve the efficiency of coconut breeding by RAPD marker assisted selection. *CORD* **XV 2**, 115-130

Ganeshaiah KN, Shibu MP, Lalitha Anand, Ravishankar KV, Uma Shanker R (2000) Identification of sex-specific DNA markers in the dioecious tree, nutmeg (*Myristica fragrans* Houtt.). *Plant Genetic Resources Newsletter* **121**, 59-61

Gupta MY, Chyi SJ, Romero Severson J (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple sequence repeat. *Theoretical and Applied Genetics* **89**, 998-1006

Hormaza JL, Dollo L, Polito VS (1994) Identification of a RAPD marker to sex determination in *Pistacia vera* using bulked segregant analysis. *Theoretical and Applied Genetics* **89**, 9-13

Jambulingam R, Fernandes ECM (1986) Multipurpose trees and shrubs on farmlands in Tamil Nadu State (India). *Agro Forestry Systems* **4,** 17-32

Kafkas S, Cetiner S, Perl Treves R (2001) Development of sex-associated RAPD markers in wild *Pistacia* species. *Journal of Horticultural Science and Biotechnology* **76**, 242-246

Kgazal MA, **Salbi MI**, **Alsaadawi IS**, **Fattah FA**, **Al-Jibouri AAM** (1990) Bisexuality in date palm in Iraq. *Journal of Islamic Academy of Sciences* **3 (2)**, 131-133

Kovoor A (1983) *The Palmyrah Palm: Potential and Perspectives*, FAO Plant Production and Protection Paper No 52, FAO, Rome, 77 pp

Macedo LEG, Lacerda SPSC, Actis ZH (2002) Identification of sex in *Carica papaya* L. using RAPD markers. *Euphytica* **127**, 179-184

Mogea J, Seibert B, Smits W (1991) Multipurpose palms: the sugar palm. *Agro Forestry Systems* **13**, 111-129

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA* **70**, 3321-3323

Perera L, Russell JR, Proven J, McNicol JW, Powell W (1998) Evaluating genetic relationship between indigenous coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. *Theoretical and Applied Genetics* **96**, 545-550

Ponnuswami V, Jagadeesan R, Ramesh Kumar A, Prabhu M, Makesh S (2008) Genetic relationship and diversity in palmyrah accessions based on RAPD markers. *American-Eurasian Journal of Sustainable Agriculture* **2**, 165-171

Powell W, Morgante M, Andre C, Hanafey M, Voger J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasms analysis. *Molecular Breeding* **2**, 225-238

Rodriguez MJB, Estioko LP, Namia TI, Soniega JA (1997) Analysis of genetic diversity in coconut by RAPD. *The Philippine Journal of Coconut Studies* **22**, 1-7

Rohlf FJ (1994) NTSYS-PC: Numerical taxonomy and multivariate analysis system. Version 2.02. Exeter Publications Setauket, New York

Sankaralingam A, Hemalatha G, Mohamed Ali A (1999) *Report on All India Coordinated Research Project on Palms*, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, 40 pp

Singh M, Sanjeev Kumar, Singh AK, Ram D, Kalloo G (2002) Female sex-associated RAPD marker in pointed gourd (*Trichosanthes dioica* Roxb.). *Current Science* **82**, 131-132

Upadhaya A, Jose J, Manimekalai R, Parthasarathy VA (2002) Molecular analysis of phylogenetic relationships among coconut accessions. In: IPGRI (Ed) *Managing Plant Genetic Diversity*, pp 61-66

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* **20**, 176-183