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Research Article

Genetic diversity in palmyrah genotypes using morphological and molecular markers

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Abstract :
Palms are woody monocotyledons in the family Arecaceae which is placed in the order Arecales. Slow and tall growing, hardy and non branching, dioecious and perennial in nature, palmyrah palm has no distinguishing features to identify sex, stature and high neera yielding types until flowering age of about 12 to 15 years. Under these circumstances molecular markers can be effectively utilized to diagnose and select a genotype. Twenty palmyrah accessions were analysed using RAPD and ISSR markers. In RAPD analysis, a total of 57 bands were obtained, among them 43 were polymorphic and rest of them were monomorphic. Amplification size ranged between 250 and 3200 bp. UPGMA based cluster diagram showed that all 20 different genotypes were grouped into four different clusters based on the stature, sex and high neera yielding types. The distance matrix between genotypes showed an average distance range from 0.54 to 0.91 with a mean of 0.70. 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 distinguished genotypes similar to the results of cluster analysis.

Key words: genetic diversity, ISSR, palmyrah, RAPD

Introduction

Palmyrah palm, botanically known as *Borassus flabellifer* L. belongs to the family Arecaceae, is dioecious in nature with a great majority of economic edible products such as immature endosperm (nungu), mesocarp pulp (fruit pulp), tuberous seedlings (tuber) obtained only from female palms. But sweet sap from the inflorescence (neera) toddy, palm sugar and non-edible products like brush fibre and wood are obtained irrespective of whether the palms are male or female. However, differences in their yield and quality have been reported. Thus female palms are supposed to yield more neera on tapping from the inflorescence and the female tree gives better and hard timber than the male tree and is also more expensive. Slow growing perennials have no distinguishing features to identify the sex until flowering. The palm commences flowering only after 12 to 15 years of maturity. Hardy and irregular growth habit of the non branching trunk makes it difficult for the climber to climb the tree for collection of neera and toddy. This makes the tappers to feel that the traditional palmyrah climbing is risky job and hence they have slowly shifted to other easy jobs. The female tree with dwarf stature and good post harvest qualities is desirable. Breeding and crop improvement work would be highly facilitated if sex of the palm could be determined at the early seedling stage itself. This would help palmyrah growers while selecting the seedlings and also to maintain an optimum sex ratio during plantation.

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1. Random Amplified Polymorphic DNA (RAPD)

Molecular markers have been successfully used to study the source of introduction and variability due to new environment. PCR – based random amplified polymorphic DNA (RAPD) has been widely used to survey genetic structure of populations. Among various molecular markers, the RAPD technique is a simple, rapid method and requires only a few nanograms of DNA, has no requirement of prior information of the DNA sequence and has feasibility of automation with higher frequency of polymorphism, which makes it suitable for routine

application for the analysis of genetic diversity. It is also proven to be quite efficient in detecting genetic variations, even in closely related organisms like two near isogenic lines of tomato. RAPD is a PCR-based fingerprinting technique that amplifies random DNA fragments with single short primers of arbitrary nucleotide sequences under low annealing stringency (Williams *et al.,*1990). This molecular marker is based on the PCR amplification of random locations in the genome. A single oligonucleotide (OGN) is used to prime the amplification of genomic DNA. Since these primers are decamers (10 nucleotide long), they have the possibility of annealing at a number of locations in the genome. These RAPD markers are dominant markers that bind to the inverted repeats, generally 150-4000 base pairs apart.

The RAPD technique has been used extensively in plants and animals for various purposes such as genetic diversity, DNA fingerprinting, classification, and phylogenetic studies. The modified version of this technique utilizes cDNA-RAPD, which amplifies the cDNA instead of genomic DNA. This cDNA modification has also been used to isolate novel genes and perform other genetic studies in plants. RAPDs are widely used as starting molecular markers for positional cloning of a gene of interest. They have been widely used in the reconstruction of phylogenetic relationships for many organisms and there has been general concordance among the results derived from RAPDs and other techniques. RAPD bands often originate from repetitive DNA sequences (Williams *et al.,* 1990), which have been shown to be valuable instruments for studying phylogeny and taxonomy within several families. RAPD markers have also been used in the identification of dwarf genome specific markers in rubber. Promoter isolation for a novel barley gene *lem 1* was performed using RAPD markers .

Molecular markers can be effectively 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 phase. With the advent of molecular markers, RAPD (Randomly Amplified Polymorphic DNA) markers have been extensively used in investigations aiming to reveal the level of genetic diversity and genetic relationships among species or subspecies, and sex identification of *Hippophae rhamnoides* (Persson and Nybom, 1998; Bartish, 2000; Ruan *et al.,* 2004). RAPD markers have been used for determining sex by bulk segregant analysis in *Pistacia vera*, *Atriplex garretti*, *Trichosanthes dioica* and *Salix viminalis* and DNA markers can be used to identify the high yielding genotypes of coconut, arecanut and date palm.

The present investigation was carried out to differentiate between male and female genotypes, stature and high yielding characters at the earlier stage of growth based on RAPD markers. Correct identification of palms is usually not possible until fruits are produced. In addition the characterization of cultivars and evaluation of genetic diversity require a large set of phenotypic data that are often difficult to assess and sometimes variable due to environmental influences. Earlier, isoenzyme markers have proved of some use in cultivar identification in date palms. However, they are limited by the number of informative markers and give no direct assessment of the genomic variation. Restriction Fragment length polymorphisms (RFLPs) have been evaluated for date palm clone identification, but the technique is laborious and not suited to studies of a large number of samples. Randomly amplified polymorphic DNA (RAPD) markers (Williams *et al*., 1990) are of particular interest. DNA profiles based on arbitrary primed PCR are both time- and cost-effective.

2. Inter Simple Sequence Repeats (ISSR)

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 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 the ISSR-PCR system as a tool for the identification of Palmyrah palm accessions.

3. Genetic diversity analysis

3.1. 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. Palm tree details and the special attributes of the trees are presented in Table 1. Fresh leaves from each tree were collected, cleaned and frozen quickly and stored at –80°C until DNA extraction.

3.2. DNA extraction

Genomic DNA was isolated from leaves using Hexadecyl Trimethyl- Ammonium Bromide (CTAB) (Aitchitt *et al.,* 1993). The quality and quantity of the DNA was assessed on a 0.7% agarose gel. DNA concentrations were determined by comparison with a serial dilution of standard λDNA cut with *EcoRI* and *HindIII* as molecular weight marker.

4. Molecular marker analysis

4.1. RAPD analysis

RAPD reactions were performed with 16 random decamer primers of the series OPZ (Operon, USA). Out of 16 primers only eight primers successfully amplified the products in all the templates used. The PCR reaction mixture consisted of 25 ng genomic DNA as template, 1 unit(0.3 µl) of *Taq* DNA polymerase, 2.5 µl of 10X PCR buffer (including 15 mM MgCl), 1 µl each of 20 p.mole primer, 0.5 µl of 10 mM dNTPs in a final volume of 25 µl using sterile ultra-pure water. Polymerase Chain Reaction (PCR) was performed in BIORAD – My cycler thermo cycler with initial denaturation at 94ºC for 5 minutes followed by 40 cycles of denaturation at 94 ºC for 1 minute, annealing at 42ºC for one minute and extension at 72 ºC for 1 minute, finally ending with one cycle of 72 ºC for 10 minutes. The amplified products were resolved by electrophoresis in a 2% agarose, 1X TAE (Trisacetate:EDTA) at 60V for 4 h. Bacteriophage λDNA cut with EcoR I and *Hind* III was used as molecular weight marker. The gel was stained with ethidium bromide and viewed under UV light. Each band was considered as a RAPD marker and was identified by its molecular weight.

4.2. 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. 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 p.mol 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. 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).

4.3. Data scoring and analysis of RAPD

Scoring of RAPD bands was carried out by considering only the 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 RAPD marker were calculated based on the formula *Hn =1- 3pi2*, where *pi* is the allele frequency for the *i* – th allele (Nei, 1987). The data obtained by scoring the RAPD profiles of different primers were subject to cluster analysis. 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, 2005). The binary data for 20 palms was also subjected to principal component analysis (PCA) and scores for the first two components were plotted.

4.3.1. Degree of polymorphism

Among the twenty different palmyrah genotypes studied with 16 primers, only eight primers were used to assess genetic relationships in the tested accessions. The primers OPZ – 1, OPZ – 4, OPZ – 5, OPZ – 6, OPZ – 7, OPZ – 10, OPZ – 12 and OPZ – 13 were consistent and unambiguously scorable. A total of 43 reproducible polymorphic and 14 monomorphic bands were identified and scored as RAPD markers. Depending on the DNA template x primer combination, 3 – 11 reproducible bands were detected in the 250 – 3200 bp size range. Thus, maximum of 18 bands were produced when using OPZ – 7 and OPZ –13 primers. However only 6 amplified fragments were obtained with OPZ –1 primer. As shown in fig.1, RAPD banding patterns were typically generated from the included genotypes. The average polymorphic bands were obtained with 5.38 markers per primer. The experiments were repeated thrice with individual male and female, high and low neera yielding, and dwarf and tall types.

4.3.2. Cluster analysis

Jaccard's similarity coefficient matrix was used to generate a dendrogram using Unpaired Group with Mathematical Average (UPGMA) clustering method using NTSYS and later it was bootstrapped to confirm the grouping. The UPGMA analysis resulted in one phenogram shown in the figure 2. There is no clear clustering or grouping corresponding to the four groups. But, the majority of the genotypes were clustered based on the morphological similarities like male and female, dwarf and tall and high and low neera yielding pools of palmyrah. The cluster A comprises of two sub clusters namely A1 and A2. A1 comprises of two genotypes TNPO 7 and TNPO 8; A2 comprises of one genotype TNPO 10. The major cluster B was further divided into two subclusters. The sub cluster B1 comprises 14 genotypes and B2 comprises of three genotypes.

The distance matrix between genotypes shows an average distance range from 0.54 to 0.91with a mean of 0.70. Thus, the genotypes tested in the study are highly divergent at the DNA level. The smallest distance value was observed between the genotypes TNPO 1 and TNPO 8 (0.54) which appear to be the most similar genotypes and can be closely regrouped. The maximum distance value of 0.91, indicating a great dissimilarity was observed between the TNPO 7 and TNPO 9 genotypes. All other genotypes display different intermediate levels of similarity and are grouped with the other ones. It is noteworthy that the genotype TNPO 1 exhibited a very limited average distance range (0.56 to 0.73) with the genotypes other than TNPO 7. Thus, TNPO 1 was characterized by a slight divergence at the DNA level and could be unlikely regrouped with the other clusters.

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

Morphological markers for identifying the cultivars are limited in number and they do not often reflect genetic relationships because of interaction with the environment, epistasis and the largely unknown genetic control of the traits (Smith *et al.*, 1997). In contrast, DNA markers are found in abundance and are not influenced by the environment or developmental stage of a plant, making them ideal for genetic relationships studies (Reddy *et al.,* 2002). RAPD-based phylogenetic analyses have been conducted using neighbor-joining and/or UPGMA with Jaccard and raw-character-difference distances. RAPD markers have been extensively used in investigations aiming to reveal the level of genetic diversity, genetic relationships among species or subspecies, and sex identification. Obtaining a marker linked to a gene or genomic region through RAPD analysis depends to a larger extent on chance, because random sequences are used as PCR primers. Hormaza *et al. (*1994) screened 1000 primers in *Pistacia vera* for sex determination and identified one desirable trait associated marker. They suggested that the low frequency of desirable trait linked bands may indicate that the DNA segment(s) involved in trait determination is small and probably involves a single gene, or few genes.

Claudete *et al. (*1998) identified a 2075- bp male specific band after screening 158 RAPD primers in dioecious *Atriplex garrettii.* They tested DNA from ten male, ten female and hermaphrodite plants individually and reported that the male specific fragment was present in hermaphrodite, all but one of the male plants and was absent in all female plants. A female specific DNA fragment of size 416 bp was identified in dioecious nutmeg by Ganeshaiah *et al. (*2000) after screening 60 RAPD primers. This DNA marker is completely linked to sex, stature and high neera yielding types during planting. Kafkas *et al. (*2001) reported the possibility of some markers which are not tightly linked to a sex, stature and high neera yield determining locus. So the present study clearly revealed that the RAPD marker is tightly linked to the sex determination, tree stature and high neera yield. Hence Random Polymorphic DNA (RAPD) is a useful tool for identification of desirable traits in the palmyrah genotypes.

4.4. Data scoring and analysis of ISSR

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 ith allele (Nei, 1973). The data obtained by scoring the ISSR 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, 2002). The binary data for 20 palms was also subjected to principal component analysis (PCA) and scores for the first two components were plotted.

4.4.1. Degree of polymorphism

Among the twenty different palmyrah accessions studied with thirty five primers, only twenty one primers produced scorable markers and were used to assess genetic relationships in the tested accessions. A total of 130 markers were produced, of which 65 were polymorphic and this showed 47.94% polymorphism. 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-859 and UBC-886 primers yielded 2 bands, whereas UBC-840 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 TNPO 13 and TNPO 20) to 95.7% (TNPO 3 and TNPO 4).

4.4.2. Cluster analysis

UPGMA tree constructed on the basis of Jaccard's coefficient clustered the palmyrah accessions into two major groups. The UPGMA analysis resulted in one phenogram shown in the figure 5. The distance matrix between accessions showed an average distance range from 71.6% to 95.7%. Cluster A comprised of only one accession TNPO 13. Cluster B was divided into two sub groups *viz*., B1 and B2. Cluster B1 comprised of TNPO 20, separated from rest of the accessions. Cluster B2 was further divided into two sub clusters *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 showed maximum level of 95.7% similarity. In cluster B3, maximum similarity of 92.3% was found between TNPO 16 and TNPO 17 and minimum similarity of 78.2% was found between TNPO 6 and TNPO 18. In cluster B4, TNPO 3 showed maximum similarity of 95.7% with TNPO 4 while minimum similarity of 79.5% was found between TNPO 1 and TNPO 12. For each of 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.

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 germplasm 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 sequences; hence a variable number of polymorphic bands per primer were obtained. These results are consistent with the earlier reports on RAPD analysis (Connolly *et al*., 1994; Powell *et al*., 1996; Ashburner *et al*., 1997; Upadhyay *et al*., 2002). 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 (Ashburner *et al*., 1997; Rodriguez *et al*., 1997; Upadhyay *et al*., 2002). The level of polymorphism in terms of the number of polymorphic bands per primer (3.09) was also moderately high and found to be consistent 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 a useful technique for germplasm characterization in palmyrah.

The data on genetic similarity indicated that more variance existed among tall accessions (maximum SI of 90.3% between TNPO 14 and TNPO 18 and minimum SI of 75.4 % between TNPO 16 and TNPO 20) than among dwarf accessions (maximum SI of 95.7% between TNPO 3 and TNPO 4 and minimum of 77.8% between TNPO 5 and TNPO 9). These results were comparable with earlier studies, which demonstrated higher variation in tall than dwarf coconut (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.

This study has established the ability of RAPD and 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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Table 1. Features of palm trees (all 25-30 years old) used in this study

Accession No	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

Table. 2: Details of Polymorphism among the RAPD primers in Palmyrah

S. No	Primer name	Nucleotide sequence 5' to 3'	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Per cent poly morphism	Amplified fragment size (bp)
1.	OPZ-1	TCTGTGCCAC	3	3	0	100	1000-2500
2.	OPZ-4	AGGCTGTGCT	10	7	3	70	500-3200
3.	OPZ-5	TCCCATGCTG	5	3	2	60	600-1400
4.	OPZ-6	GTGCCGTTCA	8	7	1	87.5	250-1500
5.	OPZ- 7	CCAGGAGGAC	10	8	2	80	250-1800
6.	OPZ-10	CCGACAAACC	4	3	1	75	550-1500
7.	OPZ-12	TCAACGGGAC	6	5	1	83.33	800-2000
8.	OPZ-13	GACTAAGCCC	11	7	4	63.63	500-3000
		Total	57	43	14		
		Mean	7.13	5.38	1.75		

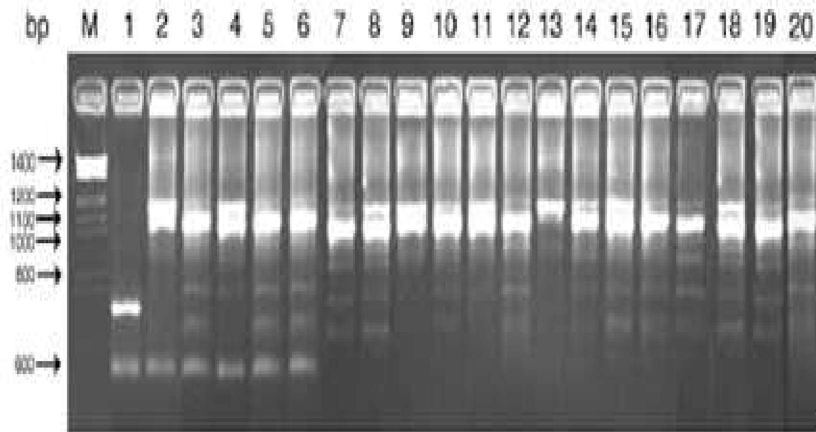

Fig. 1: RAPD marker profile of the primer OPZ -5 in palmyrah genotypes; 1 to 20 represents the genotypes

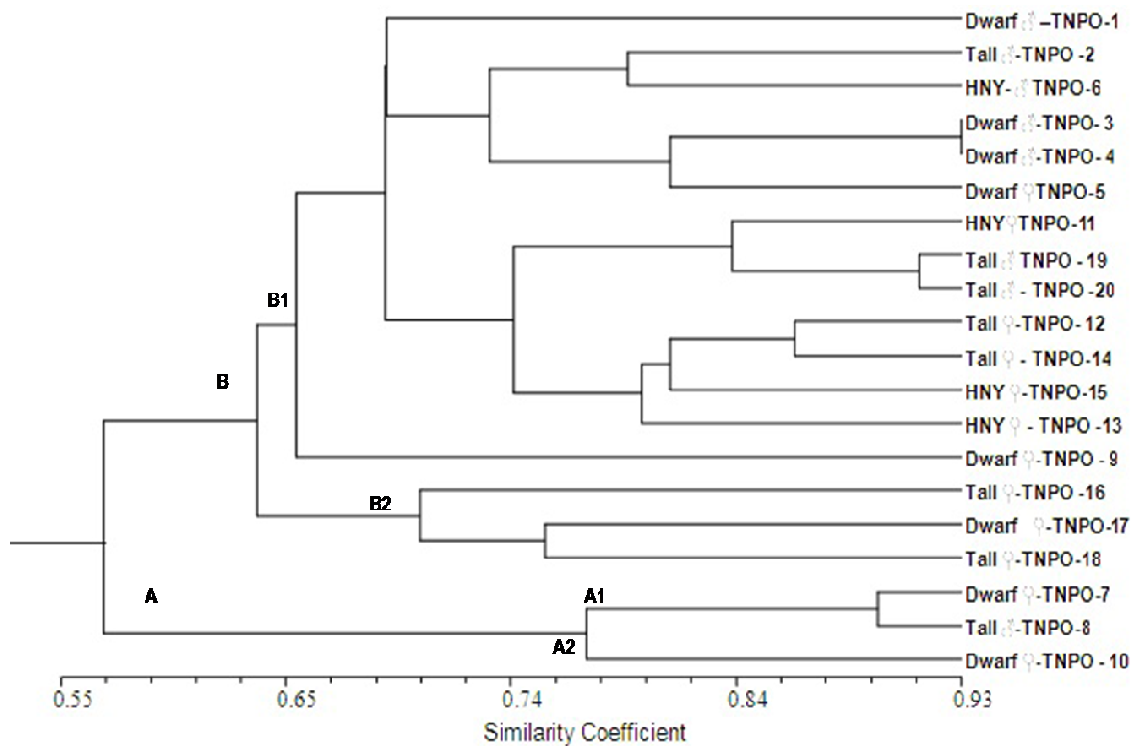

Fig. 2: A UPGMA phenogram based on RAPD data for the studied genotypes of *Borassus flabellifer*. L. palm

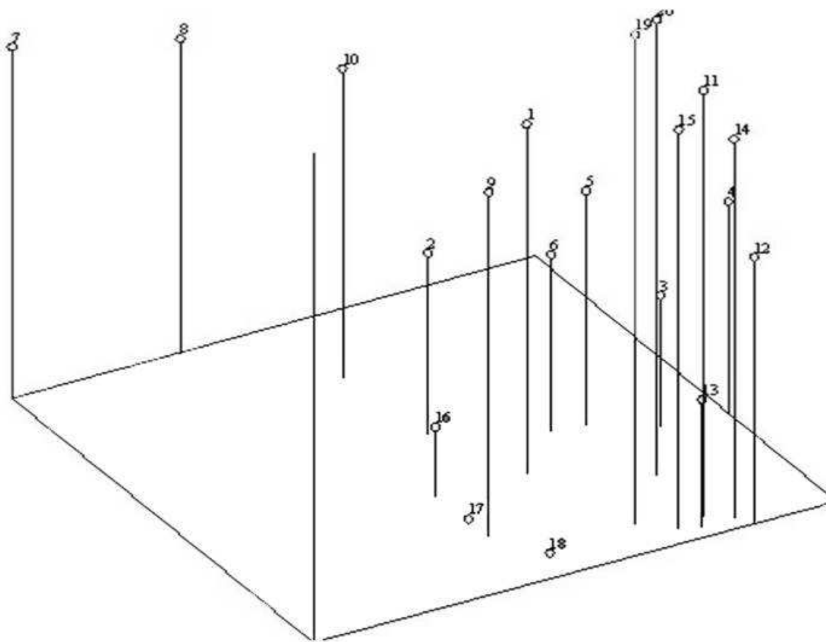

Fig.3: A PCA plot based on RAPD data for the studied populations of *Borassus flabellifer*. L. palm; 1 to 20 represents the genotypes

Table 3 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	PIC	Amplified fragment size (bp)
UBC-812	GAGAGAGAGAGAGAGAA	48	4	10	40.00	0.06	250-1200
UBC-813	CTCTCTCTCTCTCTCTT	47	1	4	25.00	0.05	400-750
UBC-815	CTCTCTCTCTCTCTCTG	48	1	7	14.28	0.14	200-650
UBC-816	CACACACACACACACAT	50	2	3	66.66	0.12	300-550
UBC-817	CACACACACACACACAA	50	5	8	62.50	0.25	300-1200
UBC-818	CACACACACACACACAG	53	5	7	71.43	0.31	250-750
UBC-822	TCTCTCTCTCTCTCTCA	50	2	3	66.66	0.18	400-750
UBC-823	TCTCTCTCTCTCTCTCC	51	6	8	75.00	0.25	300-1000
UBC-835	AGAGAGAGAGAGAGAGYC*	53	4	7	57.14	0.46	200-1300
UBC-840	GAGAGAGAGAGAGAGAYT*	51	3	13	23.08	0.20	100-650
UBC-841	GAGAGAGAGAGAGAGAYC*	52	3	5	60.00	0.20	250-750
UBC-842	GAGAGAGAGAGAGAGAYG*	51	5	6	83.33	0.38	200-750
UBC-843	CTCTCTCTCTCTCTCTRA*	50	1	6	16.67	0.15	250-1000
UBC-844	CTCTCTCTCTCTCTCTRC*	51	5	7	71.43	0.34	250-1500
UBC-855	ACACACACACACACACYT*	55	2	7	28.57	0.23	250-750
UBC-857	ACACACACACACACACYG*	56	8	10	80.00	0.41	250-1500
UBC-859	TGTGTGTGTGTGTGTGRC*	55	0	2	-	-	600-1200
UBC-885	BHBGAGAGAGAGAGAGA*	52	3	4	75.00	0.46	350-900
UBC-886	VDVCTCTCTCTCTCTCT*	50	0	2	-	-	350-400
UBC-887	DVDTCTCTCTCTCTCTC*	49	3	6	50.00	0.06	350-850
UBC-888	BDBCACACACACACACA*	56	2	5	40.00	0.18	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)

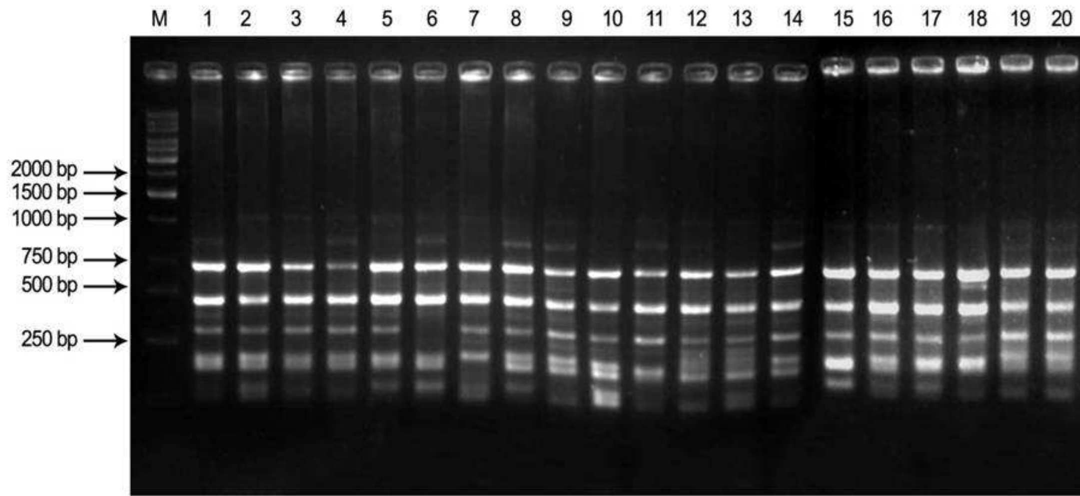

Fig.4: ISSR marker profile of 20 Palmyrah genotypes produced by primer UBC – 857; 1-20 represents genotypes

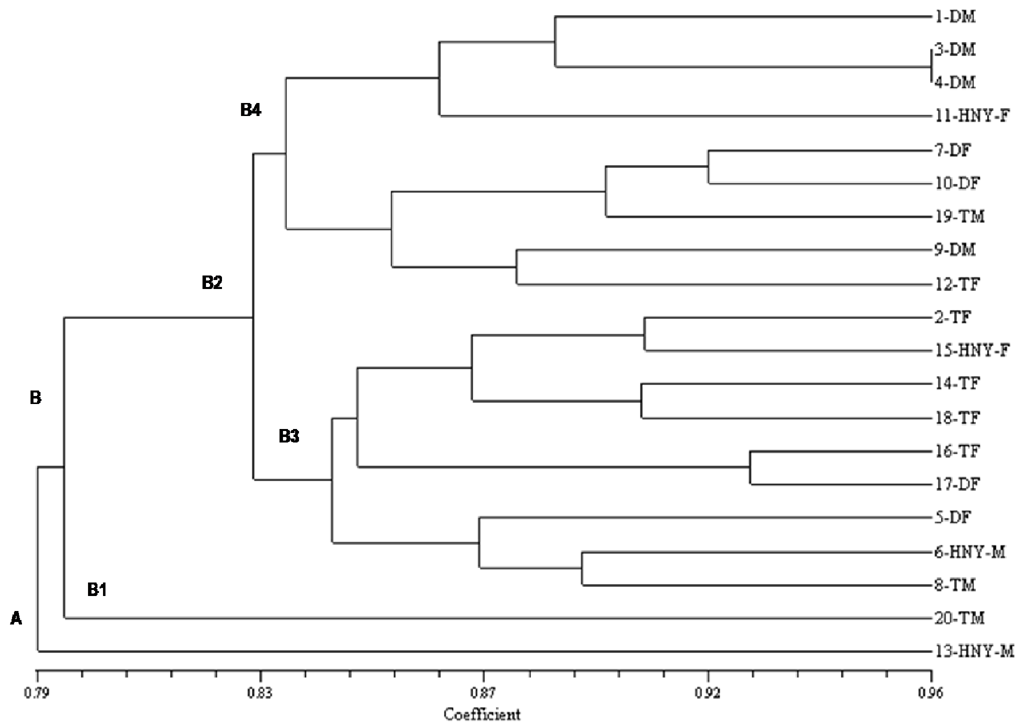

Fig.5: UPGMA phenogram based on ISSR data for 20 genotypes of Palmyrah

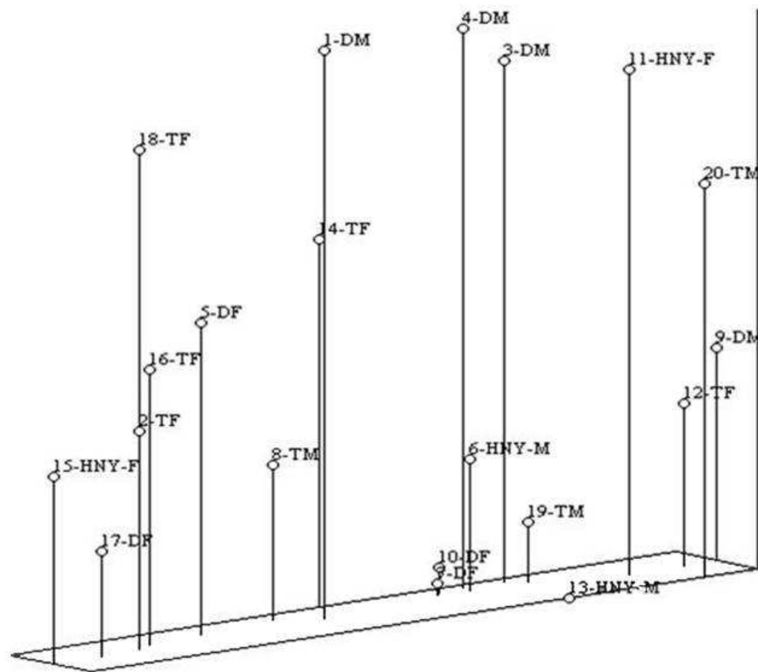

Fig.6: 3D distribution of palmyrah accessions revealed by first two principal components based on ISSR data