Int J Pharm Bio Sci 2015 April; 6(2): (B) 244 - 250

Research Article **Molecular biology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

GENETIC DIVERSITY ANALYSIS IN PALMYRAH PALMS USING RAPD MARKERS

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ABSTRACT

Palmyrah palms (*Borassus flabellifer*), woody monocotyledons of the family Arecaceae is a common multipurpose tree of Tamilnadu state with great utility. 16 accessions from Tamil Nadu State of palmyrah were investigated for their genetic relationship using Random Amplified Polymorphic DNA markers. A total of 20 primersused for screening out of which 15 RAPD primers show consistency in giving polymorphic bands was selected for analysis. The fifteen primers produced a total of 112 reproducible bands and out of these amplified fragments, 82 (73.2%) were found to be polymorphic. The number of products produced by these arbitrary 10-mer primers was found to range from 1 to 14 with a maximum (14) by the primer UBC- 06 and primer UBC-15 produced the minimum (1) number of amplicons. The similarity matrix constructed using the WINDIST software showed the similarity index ranges from 0.60 to 0.93 with mean value 0.79 thereby suggesting high levels of genetic variability among the male and female plants of this species. Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped all male and female accessions separately into two major clusters. The relatively high polymorphism suggests a moderately high genetic diversity of palmyrah populations from which the present accessions have been derived and maintained over years.

KEY WORDS: ***Borassus flabellifer*** **;RAPD; Polymorphism; genetic diversity.**

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INTRODUCTION

Borassus flabellifer commonly known as Asian Palmyrah palm is a tropical dioecious palm native to Indian subcontinent and Southeast Asia. Attention has only recently been given to this tree because of the global assessment of the potential of underdeveloped plant resource having multifarious use. These slow– growing perennial palms have no distinct features to identify their gender until flowering and the plant commences flowering only after 12-15 years of maturity. Hence, farmers have hesitation in planting this multipurpose tree. Studying the genetic relationships of any plant species is very much essential for adapting suitable conservation strategies, effective management, and efficient utilization of plant genetic resources. Moreover, breeding and crop improvement would be highly facilitated if gender could be determined at the seedling stage itself in the case of dioecious plants. This would be a great help to farmers while selecting the seedling and maintain an optimal sex ratio at plantation. The information on existing genetic diversity in natural populations of plants is always of fundamental interest for basic science as well as applied aspects like improvement of plant genetic resources.During the past several years, a number of PCR-based DNA markers, such as RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), and ISSR (Intersimple Sequence Repeats), have been widely used to investigate population genetic structure because they overcome the limitations of allozyme markers [1,2,3,4]. Of these, the most popular marker is RAPD, which has been successfully used in a wide variety of fields [5].The genetic diversity and relationship between the different species of genus Aerides i.e, A.crispa, A. maculosa and A. ringens was determined using randomly amplifiedpolymorphic DNA (RAPD) analysis [6].The objective of this study was to find the genetic diversity among and between staminate and pistillate genotypes of Palmyrah collected from natural populations existing in Vilupuram district of Tamilnadu state, India, using RAPD markers.

MATERIALS AND METHODS

PLANT MATERIAL

A total of sixteen leaf samples with equal number of male and female plants of *Borassusflabellifer* L. were collected from a two populations that exists in Vilupuram district, Tamilnadu state, India were used for the extraction of good quality DNA. The total genomic DNA of each plant was extracted from young leaves using the cetyl trimethylammonium bromide (CTAB) method introduced by Murray and Thompson [7], with appropriate modifications. 1.2% PVP was added to the extraction buffer to remove phenolic contaminants and double chloroform extraction at 10000 rpm helped to remove polysaccharides. After ethanol precipitation DNA was resuspended in 0.1 cm3 of 1× TE buffer (pH 7.0). The quantification of DNA was done by spectrophotometric method by taking the absorbance at 260 nm.

RAPD ANALYSIS

RAPD assay was carried out in 25µl reaction mixture containing 0.2mM dNTP's, 10mM Tris-Hcl, 1.5mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 1.0U Taq DNA polymerase (Finnzymes, Finland,USA), 15 p mol primer (University of British Columbia, UK) and genomic DNA(50 ng). The amplification performed in an Eppendorf ESP Gradient DNA Thermal Cycler (Eppendorf, Germany). After the initial cycle of 2 min at 94 °C, 2 min at 36 °C and 2 min at 72 °C, 30 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C were performed. 7 min extension was given after last cycle at 72 °C. Reaction mixture wherein template DNA replaced by distilled water was used as negative control. 1.4% agarose gel (1x TBE) were used for resolving the Amplified products followed by EtBr staining.

GENETIC DATA ANALYSIS

Amplification with each random primer was repeated three times and those primers that resulted reproducible and consistent bands

were selected for data generation. Reproducible RAPD products were scored against the presence or absence of a fragment and denoted as ' + ' or ' - ' respectively. Dice coefficient of similarity is defined as 2a/2a+u, where 'a' is the number of positive matches and 'u' is the number of non matches was computed using the Popgene 1.32 software [8] was used to calculate the genetic parameters: (1) percentage of polymorphic loci (PPL); (2)number of alleles (Na) and number of effective alleles (Ne);(3) expected heterozygosity (He); (4) Shannon's Information Index (I). The scored binary was analyzed for the construction of phenogram following the unweighted pair- group method with arithmetic averages (UPGMA) and determination of confidence limits by bootstrap analysis using the WINBOOT software.

RESULTS AND DISCUSSION

In a majority of dioecious plants, the females are agronomically superior to the males. Hence, male plants under the mentioned category may be under threat due to the selective upholding of females in the field. A wide range of genetic base always gives an opportunity to select genotypes with a trait of interest. Hence, it is imperative to understand the extent and distribution of genetic variation before any selection or plant breeding programmes. A decreased heterozygous nature of populations will certainly decrease vigor and productivity. So the information on the genetic make up of palmyrah is particularly important because of its dioecious nature and the so called threat to the male plants.Among 32 random primers tried with sixteen accessions of *Borassus flabellifer*, 15 primers that showed reproducibility in amplification and consistency in band formation were selected to use them for the estimation of intra specific and intra population variation studies. The selected primers (Table.1) altogether generated 112 products out of which 82 were found to be polymorphic (73.2% polymorphism). On an average, the primers generated 7.5 bands and 5.5 polymorphic bands per primer (Fig.1). The number of products generated by these arbitrary 10-mer primers was found to range from 1 to 14 with primer UBC 06 giving the maximum (14) and primer UBC 15 giving the minimum (1) number of amplicons (Table I). Though there were primers viz; UBC 14 and 15, that produced 100% polymorphism, not even a single primer could reveal 100% monomorphism across the accessions especially between the male and female accessions. The high number of polymorphic products generated by certain primers in this analysis might be attributed to the fact that in RAPD can deduce DNA polymorphisms produced by ''rearrangements or deletions at or between oligonucleotide primer binding sites in the genome'' using short random oligonucleotide sequences [9] .The reproducibility of RAPD profiles for the all the samples as observed in the present study may indicates genetic stability of the accessions.The similarity matrix developed using the WINDIST software showed that similarity index ranges from 0.60 to 0.93 with a mean value of 0.79 thereby suggesting high levels of genetic variability among the male and female plants of this species (Table 2). At gender specific level, extent of similarity was more within male/ female plants as mean genetic similarity index obtained was 0.86 and 0.89 respectively. However, the genetic diversity observed was slightly higher in male plants in compared to female plants. This may be due to either slightly greater number of male plants in the natural populations or even distribution of male plants in compared to females in the area of the present study. This corroborates the results obtained in another dioecious species *Pistacia* atlantica [10].In dioecious plant species, obligate out crossing should naturally maintain a high genetic diversity within populations and low genetic differentiation among populations compared to self-compatible plants [11] .Though the sex chromosomes in plants were discovered more than a century ago, [12] the sex determination mechanism in dioeciousplants is not well understood [13]. It is also observed that the sex ratio in dioecious plant species iscontrolled by the expression of alleles at from one to several loci [14]. Genetic marker systems based on direct analysis of the

genomic DNA have been used widely for disease diagnostics, genetic mapping and evolutionary studies, and this could prove very useful in the study of sexual determination in dioecious plants such as papaya [15] , asparagus [16] , hemp [17], and palms [18]. Though many molecular markers linked to sex have been generated, the molecular approaches have not yet identified any primary sex determining genes in any dioecious plant species. As Ponnuswami [19] reported, two RAPD (UBC 13 and UBC 06) markers were found to be linked to the sex in the present study also. Hence it may be feasible to identify sex at the early stages of plant life, which is useful for improving breeding programmes and also for the maximum utilization of this bioresource.In the cluster analysis done by WINBOOT software, the accessions of *Borassusflabellifer* clustered broadly under two major groups with sub grouping in cluster I (Fig.2). Accessions of male plants and female plants tend to group separately though they were collected from the same population. All male accessions of *Borassus flabellifer* grouped as Cluster I while female plant accessions were clustered separately as cluster II.

Fig.II Phenogram based on UPGMA analysis. M01-M08 – *Borassus flabellifer* male and F01-F08- *Borassus flabellifer* female plant accessions. Numbers at the fork indicate boot strap values

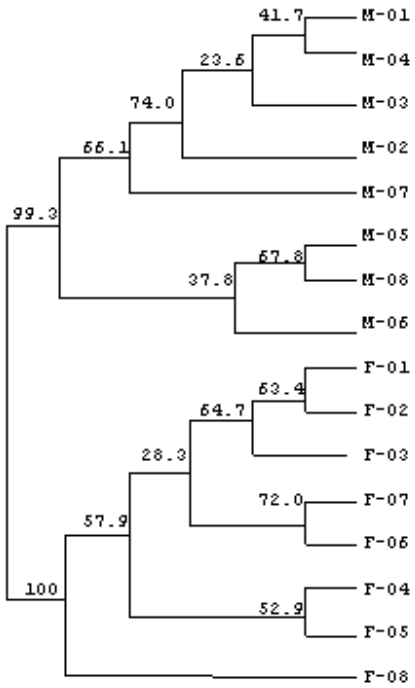

Table 1
List of primers and its sequence used for RAPD analysis

SLNo	Primers	Primer sequence 5'→3'	No of bands	No of polymorphic bands
1	UCB 6	CCT GGG CCT A	14	13
2	UCB 7	CCT GGG GGT T	4	3
3	UCB 8	CCT GGC GGT A	10	9
4	UCB 9	CCT GCG CTT A	7	3
5	UCB 10	GGG GGG ATT A	6	3
6	UCB 11	CCC CCC TTT A	8	7
7	UCB 12	CCT GGG TCC A	7	6
8	UCB 13	CCT GGG TGG A	11	10
9	UCB 14	CCT GGG TTT C	13	13
10	UCB 15	CCT GGG TTT G	1	1
11	UCB 17	CCT GGG CCT C	4	3
12	UCB 19	GCC CGG TTT A	8	3
13	UCB 20	TCC GGG TTT G	4	1
14	UCB 30	CCG GCC TTA G	9	5
15	UCB 55	TCC CTC GTG C	6	2
	Total no of bands		112	82
	Mean per primer		7.5	5.5

Table 2
Similarity matrix of male and female plants of Borassus flabellifer

M-01	1.000															
M-02	0.902	1.000														
M-03	0.919	0.917	1.000													
M-04	0.934	0.932	0.917	1.000												
M-05	0.729	0.772	0.793	0.754	1.000											
M-06	0.824	0.787	0.791	0.787	0.813	1.000										
M-07	0.894	0.874	0.843	0.857	0.748	0.844	1.000									
M-08	0.810	0.820	0.871	0.836	0.864	0.824	0.797	1.000								
F-01	0.734	0.726	0.759	0.756	0.641	0.722	0.691	0.748	1.000							
F-02	0.730	0.752	0.741	0.752	0.636	0.718	0.687	0.730	0.947	1.000						
F-03	0.730	0.752	0.741	0.752	0.620	0.704	0.687	0.730	0.933	0.932	1.000					
F-04	0.731	0.738	0.742	0.769	0.619	0.691	0.687	0.701	0.898	0.869	0.924	1.000				
F-05	0.733	0.709	0.713	0.756	0.602	0.706	0.703	0.702	0.875	0.859	0.845	0.921	1.000			
F-06	0.711	0.733	0.722	0.748	0.630	0.700	0.697	0.726	0.905	0.890	0.877	0.881	0.900	1.000		
F-07	0.676	0.712	0.701	0.712	0.656	0.723	0.677	0.721	0.886	0.871	0.857	0.861	0.837	0.924	1.000	
F-08	0.686	0.677	0.696	0.692	0.651	0.676	0.642	0.686	0.867	0.824	0.838	0.883	0.845	0.822	0.830	1.000
M-01	***M-02***	***M-03***	***M-04***	***M-05***	***M-06***	***M-07***	***M-08***	***F-01***	***F-02***	***F-03***	***F-04***	***F-05***	***F-06***	***F-07***	***F-08***	

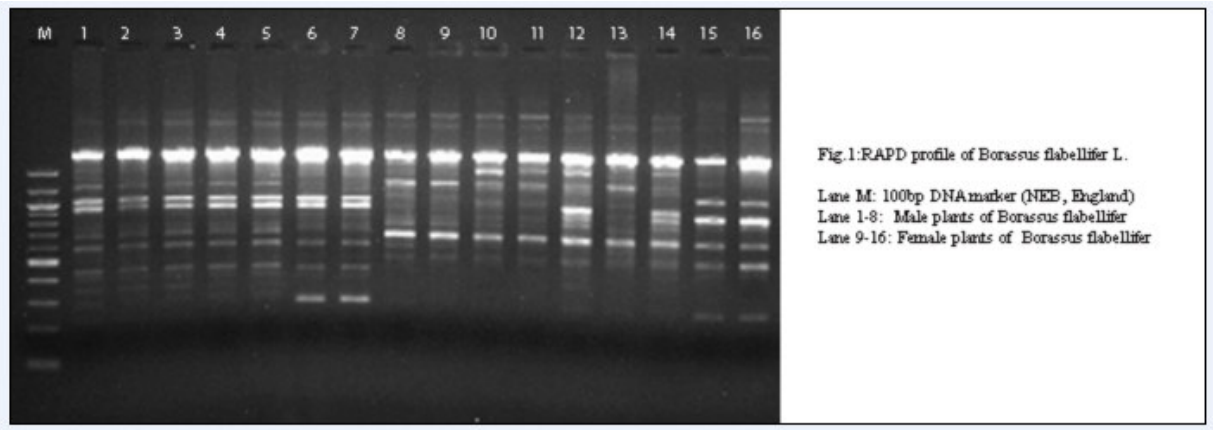

Fig.1:RAPD profile of Borassus flabellifer L.

Lane M: 100bp DNA marker (NEB, England)
Lane 1-8: Male plants of Borassus flabellifer
Lane 9-16: Female plants of Borassus flabellifer

CONCLUSION

The results of this study indicate that RAPD analysis could be successfully used for the estimation of genetic diversity among palmyrah palms. However, a detailed study to get further information on patterns of gene flow within and between population is needed to understand the population structure in this plant because of the human interference in the formation of population with more female plants in many localities.

ACKNOWLEDGEMENT

The authors thank the management of Sathyabama University for the facilities and their support for carrying out the study.

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