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Short Communication

Genetic variation in date palm (*Phoenix dactylifera* L.) cultivars assessed using RAPD markers

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Date palm (*Phoenix dactylifera* L.) is oldest among cultivated fruit trees. Presently about 40 accessions are available at All India Coordinated Research Project (AICRP), Bikaner for evaluation and maintenance. Along with some local collections most of the stocks were procured from other countries like USA, Saudi Arabia, Egypt, Iraq and Oman. A few varieties have been found to be suitable for large-scale cultivation and are economically viable *viz*., Khuneizi, Halawi, Barhee, Medjool, Khalas etc. [1]. Though early evaluation work has proved agronomic and socio-economic significance of date palm in the Thar Desert, attempts to improve the understanding and the use of the available biodiversity have been limited.

A study of genetic variation in plant population is of considerable practical interest in crop improvement. Most of the descriptors for this purpose are based on fruit characteristics in perennial crops. Thus, evaluation of genetic diversity requires a large set of phenotypic data that are often difficult to assess and sometimes variable due to environmental influences [2]. Molecular markers can now be used to identify the most distantly related individuals in a collection. Molecular marker based determination of genetic relationships among different entries has been shown to be a useful guide to collection of plant genetic resources also. Among a large number of molecular markers available for variability studies, Random Amplified Polymorphic DNA (RAPD) markers have been widely applied [3]. A few studies have also been conducted using RAPD analysis for identification of date palm clones and genetic diversity studies [4-5] at international level.

To ascertain the applicability of RAPD analysis in identification and defining variability in date palm, the present study was undertaken on genotypes available with RAU, Bikaner. The information generated regarding the genetic distance of female lines from the male parent would help in selecting parents to generate further variability for selection and/or exploitation of heterosis.

Twenty accessions including 19 female and 1 male lines (only male clone maintained at the centre) of date palm, were analyzed using RAPD markers. Genomic DNA was extracted from the leaf tissue following the modified CTAB method [6]. RAPD amplification was carried out in a Biometra thermal cycler using 47 decamer random primers (Operon Technology, Inc. U.S.A.) representing OPA, OPB and OPC series. A total of 34 decamer primers were selected for final RAPD analysis screened on the basis of easily scorable and repeatable amplification products over two replications. A total of 191 amplicons were obtained with these 34 primers at an average of 5.61 bands per primer (Table 1). Out of these 191 bands, 159 (83.24%) were found to be polymorphic. Seventeen of total 34 primers, yielded all polymorphic bands. Most of the primers produced fragments below 1.5 kb range, though a few amplicons crossed 3 kb range. A typical example of RAPD pattern generated with primers OPB-15 has been shown in Fig 1. The level of polymorphism (83.24 %) obtained in this study is higher than the 66% reported by Sedra [4]. A few of the primers have generated all polymorphic bands (Table 1). Polymorphic Information content (PIC) values were also calculated based on the formula (PIC = 1 – n + ΣP_{ij}^2, where, P_{ij} is the frequency of jth pattern in the 1th marker summed over n patterns) suggested by Anderson *et al.*, [7]. The PIC values ranged from 0.0 to 0.48 with an average of 0.26. The primers generating high level of polymorphism generally had higher PIC value and could be useful for varietal identification [4-5].

Genetic similarity estimates using (*Jaccards coefficient*) based on RAPD banding patterns were used for cluster analysis to present genetic relationships in the form of dendrogram using NTSYS-pc 1.7 program [8]. The similarity coefficient matrix was subjected to unweighted pair group method using arithmetic average analysis (UPGMA). The range of genetic similarity was found to be between 0.433 (Medzool and Hamara) to

Table 1. Analysis of the polymorphism and polymorphic information content (PIC) obtained with random primers among various accessions of date palm (*Phoenix dactylifera* Z.)

Primers	Sequences	Total no of bands (a)	Polymorphic bands (b)	Polymorphism (%) b/a × 100	PIC Value
OPA-4	AATCGGGCTG	6	6	100.0	0.29
OPA-7	GAAACGGGTG	5	4	80.0	0.36
OPA-9	GGGTAACGCC	3	2	66.6	0.22
OPA-10	CTGATCGCAG	3	2	66.6	0.06
OPA-18	AGGTGACCGT	5	5	100.0	0.32
OPA-20	GTTGCGATCC	3	2	66.6	0.09
OPB-4	GGACTGGAGT	11	11	100.0	0.31
OPB-6	TGCTCTGCCC	8	8	100.0	0.26
OPB-7	GGTGACGCAG	12	10	83.3	0.24
OPB-10	CTGCTGGGAC	8	6	75.0	0.17
OPB-11	GTAGACCCGT	9	9	100.0	0.28
OPB-12	CCTTGACGCA	9	7	77.7	0.22
OPB-13	TTCCCCCGCT	1	0	0.0	0.00
OPB-15	GGAGGGTGTT	5	3	75.0	0.21
OPB-16	TTTGCCCGGA	3	2	66.6	0.22
OPB-17	AGGGAACGAG	4	3	75.0	0.26
OPB-18	CCACAGCAGT	6	5	83.3	0.26
OPB-20	GGACCCTTAC	7	7	100.0	0.37
OPC-1	TTCGAGCCAG	13	9	69.2	0.22
OPC-2	GTGAGGCGTC	5	2	40.0	0.16
OPC-3	GGGGGTCTTT	8	6	75.0	0.30
OPC-4	CCGCATCTAC	4	4	100.0	0.36
OPC-6	GAACGGACTC	7	5	71.4	0.19
OPC-7	GTCCCGACGA	4	4	100.0	0.46
OPC-8	TGGACCGGTG	3	1	33.3	0.14
OPC-9	CTCACCGTCC	4	4	100.0	0.25
OPC-10	TGTCTGGGTG	7	7	100.0	0.30
OPC-12	TGTCATCCCC	5	5	100.0	0.47
OPC-13	AAGCCTCGTA	3	3	100.0	0.48
OPC-15	GACGGATCAG	7	7	100.0	0.23
OPC-16	CACACTCCAG	4	4	100.0	0.27
OPC-17	TTCCCCCCAG	2	2	100.0	0.32
OPC-18	TGAGTGGGTG	3	3	100.0	0.47
OPC-20	ACTTCGCCAC	4	1	25.0	0.13
	Average	5.61	4.67	83.24	0.26

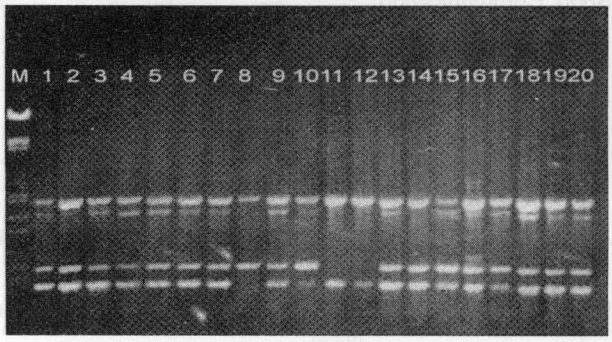

Fig. 1. RAPD profile of samples from datepalm plants amplified by Primer OPB-15 Lane 1-21. M- Marker, lambda DNA uncut (~50kb), 1-Halawi, 2-Zahidi, 3-Medzool, 4-Barhee, 5-Khalas, 6-Sayar, 7-Khadrawy, 8-Barshi, 9-Khasab, 10-Hatemi, 11-Ruziz, 12-Sakloti, 13-Agolani, 14-Shamran, 15-Hayani, 16-Hamara, 17-Muscat, 18-Umshok, 19-Sriganganagar, 20-Male

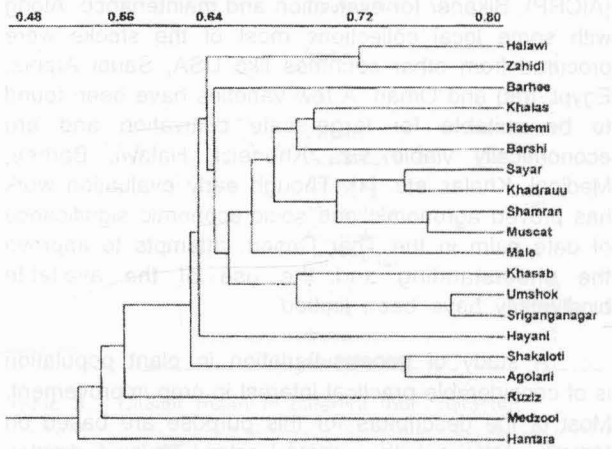

Fig. 2. Dendrogram showing relationship among detepalm accessions generated by UPGMA analysis based on RAPD data

0.760 (Umshok and Sriganganagar) which was lesser than the 0.33 to 0.87 reported by Sedra [4]. The average genetic similarity calculated over all the genotypes was 61.8%. The dendrogram reveals a major group consisting of 11 genotypes, of different origins (Fig. 2). The sub-cluster consisting of *viz.*, Khalas, Hatemi, Barhee, Sayar, Khadrawy, Shamran and Muscat had representation of genotypes from four places. Though some of the varieties collected from one place paired together clustering was not always according to the place of collection of variety. Thus, varieties collected from same place, not clustering in one group, and larger distances within groups than between groups indicate their origin from one or related populations, which further migrated to various places.

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