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GENETIC DIVERSITY ANALYSIS OF LOCAL DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS OF PAKISTAN THROUGH MOLECULAR MARKER (RAPD)

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ABSTRACT

Random amplified polymorphic DNA (RAPD) technique was applied to analyze the genetic diversity among six female date palm cultivars (Asseel, Fusli, Khupra, Kurblane, Punjmail, Pathri) and one male. Young leaves of these cultivars were collected from Khairpur. Seventeen arbitrary primers were used for this study. Among these primers, only 9 revealed polymorphic and unambiguously scorable bands. A total of 75 bands were generated with an average of 8.33 bands/primer. Out of 75 amplified fragments, 65 (86.66%) were polymorphic. Cluster analysis by unweighted paired group method of arithmetic mean (UPGMA) showed two groups Group 'A' and Group 'B'. Group 'A' consists of Khupra, Kurblane l, Fusli and Pathri while Group 'B' consists of Asseel and Punjmai. Maximum genetic similarity (77%) was present between Khupra and Kurblane while minimum genetic similarity (58%) was present between Asseel and Punjmail. Cultivar male did not belong to any group. It was 48% genetically similar to the rest of 6 cultivars. This study may be helpful to know the genetic difference present among local date palm cultivars cultivated in the province of Sindh.

Key-words: Date palm, *Phoenix dactylifera* L., genetic diversity analysis, RAPD.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a long living monocotyledonous woody perennial plant belongs to *Arecaceae* family, which comprises of 200 genera and more than 2500 species (Moore, 1973). Date palm is a rich source of nutrients containing 70% carbohydrates, 2% protein, and 2.4% fat in dried condition and is cultivated as early as 4000 B.C. (Zaid, 1999). Date palm is a crop of great economic importance in the agricultural countries like Pakistan and export wise ranks on 3rd position among major fruits. The main growing areas of date palm are provinces of Sindh and Balouchistan. The total production of date palm in Pakistan is about 426,800 tonnes (Anonymous, 2005) ranking fourth position among date palm growing countries of the world (Google Search; Jaradat and Zaid, 2004).

In Pakistan most of the date palm growing areas are cultivated by the elite varieties called "Asseel and Dhaki", inspite of presence of large genetic diversity among date palm. This trend would contribute significantly to the genetic erosion in this important phytogenetic patrimony and accelerate its vulnerability to biotic and abiotic stresses. Thus, it is imperative to elaborate a strategy aiming at the evaluation of the genetic diversity.

In this scope, many reports using either morphological traits or isozyme makers to identify the date palm varieties have been published (Rhouma, 1994; Reynes *et al*., 1994; Ould *et al*., 2001). Moreover, data based on molecular markers such as RFLPs and RAPDs have been used to characterize date palm genotypes (Sedra *et al.,* 1998, Ben Abdallah *et al.,* 2000, Trifi *et al.,* 2000, Trifi, 2001).

The random amplified polymorphic DNA (RAPD) technique, based on the polymerase chain reaction (PCR), offers a viable tool for genetic analysis. This technique utilizes arbitrary primers for the amplification of template DNA (Welsh and McClelland, 1990). The use of arbitrary primers for genetic analysis has been found effective in several plant species (Halward *et al.,* 1992; Carlson *et al.,* 1991). The objective of the present study was to determine genetic similarity among seven date palm genotypes (six female and one male plant) based on RAPD markers.

MATERIALS AND METHODS

Plant materials

Young leaves of different date palm cultivars were collected from the Khairpur/Sukhar sites of Sindh province during November 2005. The cultivars studied were six female clones of date palm (Asseel, Fusli, Khupra, Kurblane, Punjmail, Pathri) and one male clone.

DNA extraction:

DNA was extracted from fresh leaves of date palm cultivars using DNA isolating Kit (Gentra system, Minnesota, USA). Fresh leaves (200mg) were ground in liquid Nitrogen; 3 ml of the cell lysis solution (Tris

[hydroxymethyl] amino methane, ethylenediaminetetra acetic acid & sodiumdodecyl sulfate) was added with leaf sample to the 15 ml centrifuge tube and incubated at 65°C for 60 minutes. Fifteen µl of RNase A solution (Gentra Kit, Minnesota, USA) was then added to the cell lysate and incubated at 37°C for 30 minutes. Protein precipitation solution (Gentra Kit, Minnesota, USA) was added and vortex for 20 seconds and the tubes were placed on ice for 30 minutes. The mixture was centrifuged at 2000 x g for 10 minutes. Supernatant containing DNA was poured in the separate 15ml centrifuge tube and DNA was precipitated by centrifuging at 2000 x g with 3 ml of isopropanol absolute. Ethanol (70%) was used to wash the pellet and the DNA samples were then hydrated with TE buffer. DNA was quantified on spectrophotometer (BIOMATE 3).

DNA amplification:

Seventeen primers from Gene Link (New York, U.S.A), each ten bases in length, were used to amplify the DNA. PCR reaction was carried out in 25µl reaction mixture containing 13ng of template (genomic DNA), 2.5mM $MgCl_2$ (Eppendorf, Hamburg, Germany), 0.33mM of each dNTPs (Eppendorf, Hamburg, Germany), 2.5U of Taq polymerase (Eppendorf, Hamburg, Germany) and 1µM of primer in a 1xPCR reaction buffer (Eppendorf, Hamburg, Germany). The amplification reaction was performed in the Eppendorf Master cycler with an initial denaturation for 5 min at 94°C, then 32 cycles:1 min denaturation at 94°C; 1 min annealing at 52°C; 2min extension at 72°C. Final extension was carried out at 72°C for 10 min. Amplified products were analyzed through electrophoresis on 1.5% agarose gel containing 0.5X TBE (Tris Borate EDTA) at 72 Volts for 2 hours, the gel contained 0.5µg/ml ethidium bromide to stain the DNA and photograph was taken under UV light using gel documentation system (Vilber Lourmat, France).

Data analysis:

Data was scored as presence of bands as (1) and absence of band as (0) from RAPD of amplification profile. Coefficient of similarity among cultivars was calculated according to Nei and Li (1979):

$$S_{ij} = \frac{2Nij}{N_i+N_j}$$

Where:

N_{ij} = number of bands common in between cultivar of i and j and

N_i and N_j = total number of bands for cultivars i and j

RESULTS AND DISCUSSION

A total of seventeen primers were used to assess polymorphism in the tested varieties. Among these primers, only 9 revealed polymorphic and unambiguously scorable bands while smear or no amplified product were observed with other primers (Table 1).

Table 1. Sequence of primers

Primer	Sequence	Primer	Sequence
A-01	CAGGCCCTTC	A-20	GTTGCGATCC
A-03	AGTCAGCCAC	B-06	TGCTCTGCCC
A-11	CAATCGCCGT	B-17	AGGGAACGAG
A-13	CAGCACCCAC	B-19	ACCCCCGAAG
A-18	AGGTGACCGT		

These 9 primers generated 3 to 11 polymorphic bands with an average of 8.33 bands with a range of 292 to 3054 bp (Fig 1). The polymorphic patterns obtained suggested that RAPD procedure constitutes an alternative approach that is suitable to examine the date palm's genetic diversity at the DNA level. A total of 65 polymorphic RAPD bands were obtained.

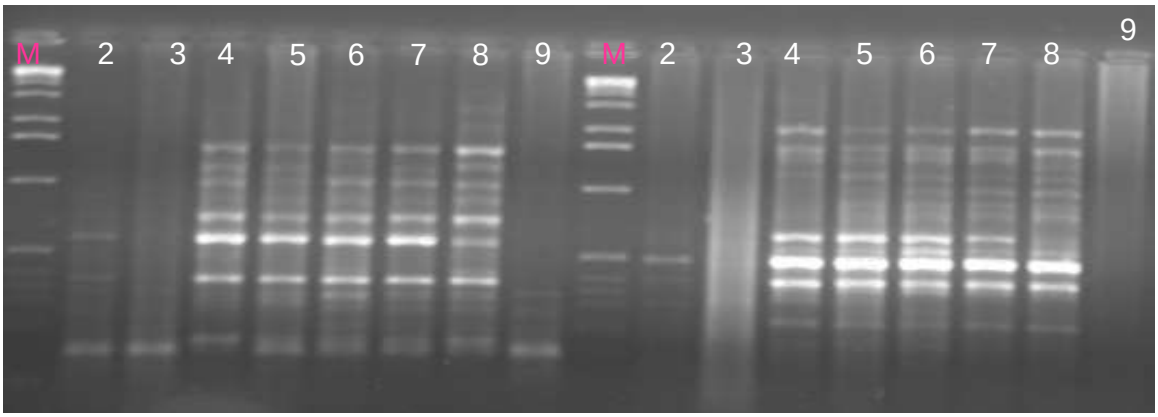

Fig 1. RAPD Profiles of date palm genotypes using Primers A-18 and A-20 respectively. Molecular weight marker (Lane M), Male plant (L2), Asseel (L3) Pathri (L4), Fusli (L5), Khupra (L6), Kurblane (L7), Punjmail (L8), Control (L9).

The similarity matrix showed genetic distance of 0.351 to 0.77, which may indicate that the varieties are characterized by a high degree of genetic diversity at the DNA level (Table 2).

Table 2. Similarity coefficients among date palm genotypes calculated according to Nei & Lei's (1979).

Varieties	Male plant	Asseel	Pathri	Fusli	Khupra	Kurblane	Punjmail
Male plant	1						
Asseel	0.560	1					
Pathri	0.456	0492	1				
Fusli	0.506	0.450	0.660	1			
Khupra	0.452	0.450	0.600	0.750	1		
Kurblane	0.431	0.351	0.450	0.623	0.770	1	
Punjmail	0.602	0.580	0.443	0.608	0.576	0.430	1

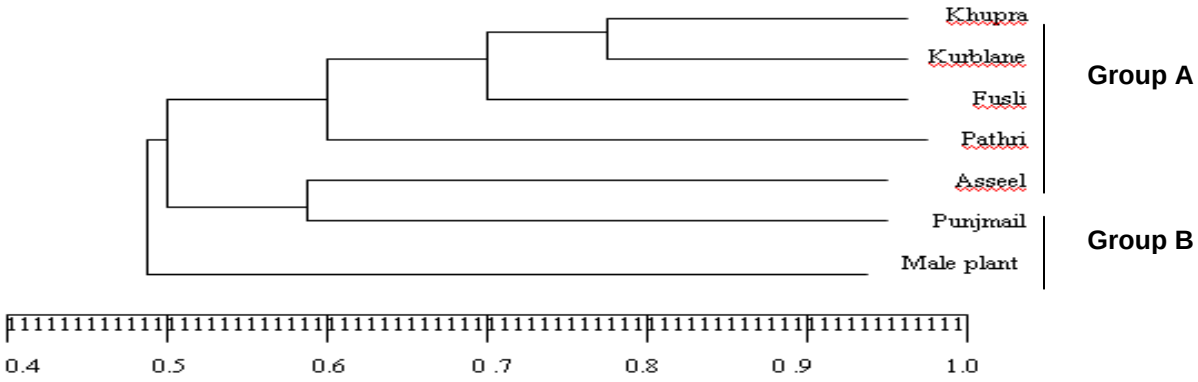

Fig 2. A dendrogram of phylogenetic relationships among date palm cultivars based on RAPD analysis using 9 primers

Cluster analysis using UPGMA resulted in two groups (Group A and Group B) as shown in Fig 2. Group 'A' consists of four cultivars Khupra, Kurblane, Fusli and Pathri. Out of four cultivars Khupra and Kurbalne are more closely related (77%) as compare to all the rest of the 7 cultivars. Fusli is 70% genomically similar to Khupra and Kurblane. Pathri is 60% genomically similar to the Fusli, Khupra and Kurblane.

Group 'B' consist of only two cultivars Asseel and Punjmail which are 58% genomically similar to each other and 49% similar to Group 'A'. Cultivar male does not belong to any cluster. It is 48% genetically similar to the rest

of 6 cultivars.

Our studies provide evidence of a genetic diversity between the tested varieties indicating the interrelationship between the date palm ecotypes in spite of their agronomic divergence, which may be supported by Zehdi *et al*., 2004; Askari *et al*., 2003; Diaz *et al.,* 2003.

The grouping-association identified by cluster analysis, clustered morphologically similar varieties together. Group 'A' consists of Khupra and Kurblane, which may tolerate the rain due to hardness of fruit while Asseel, which has high exportable value due to its fruit quality, clustered in Group 'B'.

Relatively high polymorphisms are observed among the tested date palm cultivars grown in Pakistan. This could be related to the introduction, exchange of cultivars between plantations, and periodic development of new recombinant cultivars following sexual reproduction. On the whole, our data augment those describing the application of molecular tools in date palm variability analysis and previously reported (Sedra *et al*., 1998; Trifi *et al*., 2000; Askari *et al*., 2003; Diaz *et al*., 2003).

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