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Genotyping of some elite date palm germplasm using RAPD marker

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Abstract

In India wild groves of seedling date palms are found on the coastal belt from Anjar to Mandvi in Kachergtjchh district of Gujarat and these groves are believed to be 200 years old. A great diversity is existing in morphological characteristics of indigenous date palms of Kachchh, hence molecular characterization of this precious genetic resource is significant. Improvement of date palm through conventional breeding methods is very difficult due to its long life cycle, strongly heterogeneous nature and impossibility to determine the sex at early stages of development. Marker assisted selection based on molecular characterization can overcome these hurdles. The study was done at Date palm Research Station, Mundra and C.P. College of Agriculture, Sardarkrushinagar, Gujarat. RAPD fingerprints were performed on DNA extracted from 10 elite date palm germplasm. The analysis had two objectives - to detect polymorphism within cultivars of date palm and to investigate the genetic diversity of the indigenous date palm germplasm of Kachchh. Out of eight random primers screened, two primers OPD 16 and OPD 20 produced comparatively good polymorphic bands. The efficiency of RAPD as a simple molecular marker for cultivar identification and genetic relationships among different date palm germplasm are discussed.

Keywords: Date palm (*phoenix dactylifera* L.), RAPD polymorphism, genetic diversity.
Abbreviations: CTAB, Hexadecyltrimethylammonium bromide; EDTA, Disodium ethylene diamine tetraaceteate; PCR, Polymerase chain reaction.

Introduction

Date palm (*phoenix dactylifera* L.), is one of the important cultivated crops in arid regions of the world. The date belt stretches from the Indus valley in the east to Atlantic Ocean in the West. At present in India, commercial cultivation of this crop is restricted to Kachchh district of Gujarat. The wild date groves found in this area are rich in genetic diversity, which is due to the dioecious nature of date palm and cross-pollination. Besides its socio-economic importance the date groves contributes a lot to the stability of arid environment that have been tainted due to tremendous industrialization in the area. RAPD (Random Amplified Polymorphic DNA) technique (Williams *et al.*, 1990) has been widely used as a method to identify genetic polymorphism in several crops, which in turn helps to assess genetic diversity and to take measures to improve the existing varieties or to protect the genetic resource for further crop improvement researches. In the present study RAPD molecular marker was used to examine the genetic polymorphism in the available elite date palm germplasm which are suitable to agro-climatic conditions of Kachchh.

Materials and methods

Plant material

A set of 10 date palm germplasm (Table 1) has been used for the study of which four are exotic varieties (label 1-4) and the remaining six are local germplasm (label 5-10). The plant material consists of the young leaves collected from Date palm Research station, S. D. Agricultural University, Mundra, Kachchh.

Table 1. List of date palm germplasm

Germplasm	Label	Exotic/Indigenous
Barhee	1	Exotic
Halawy	2	Exotic
Khadrawy	3	Exotic
Khalas	4	Exotic
MDP - 02	5	Indigenous
MDP - 03	6	Indigenous
MDP - 05	7	Indigenous
MDP - 06	8	Indigenous
MDP - 09	9	Indigenous
MDP - 10	10	Indigenous

MDP = Mundra Date Palm

DNA dxtraction

DNA extraction performed in Biotechnology Laboratory, C. P. College of Agriculture, Sardarkrushinagar, Gujarat Total cellular DNA of 10 germplasm was extracted from 1 g leaflets according to Doyle and Doyle (1990) with some modifications. The leaves were first ground into fine powder with liquid nitrogen. DNA was extracted in 10 ml extraction buffer (3% CTAB, 1 M Tris pH 8, 0.2 M EDTA and 1.4 M NaCl) for one hour at 65°C. After complete extraction of DNA, it was purified by treating with RNase (5 mg/ml) and phenol : chloroform : isoamyl alcohol in 25 : 24 : 1 ratio (v/v). The purified DNA pellet was washed in 70 per cent ethnol and dissolved in TE buffer (10 mM Tris HCl, pH 8, 0.1 mM EDTA).

Primers and RAPD assay

A total of eight primers were tested to amplify the isolated DNA. These primers are listed in Table 2. For PCR amplifications, a 20 µl reaction mixture was used which contained 10x Taq DNA polymerase buffer, 1.5 mM $MgCl_2$, 0.25 mM each of dATP, dCTP, dGTP and dTTP, 30 ng template DNA, 50 pm oligonucleotide primer and 1 unit of Taq DNA polymerase.

Table 2. List of RAPD primers with sequence

Primer	Nucleotide sequence (5' to 3')
OPA1	CAG GCC CTT C
OPA2	TGC CGA GCT G
OPA9	GGG TAA CGC C
OPC2	GTG AGG CGT C
OPD16	AGG GCG TAA G
OPD20	ACC CGG TCA C
OPF13	GGC TGC AGA A
OPF20	GGT CTA GAG G

Amplifications were performed in a thermal cycler (Eppendorf Master Cycler®) and the apparatus was programmed to execute the following conditions: an initial denaturation step of 4 min at 93°C, followed by 40 cycles composed of 1 min at 93°C, 1 min at the annealing temperature (37°C), and 2 min at 72°C. A final extension of 72°C for 8 minutes was included. The amplified DNA fragments were visualized along with a DNA marker on 1.8% agarose gel with 1X TAE buffer and detected by staining with ethidium bromide. Gels were photographed on polaroid films under UV light.

Data analysis

For each DNA sample, RAPD bands were transformed into a binary matrix where the presence of polymorphic DNA band at particular position on gels is scored 1, while a 0 denotes the absence. The index of similarity between each two cultivars was calculated using the formula: $B_{ab} = 2 N_{ab} / (N_a + N_b)$, where N_{ab} is the number of common fragments observed in individuals a and b, and N_a and N_b are the total number of fragments observed in individuals a and b respectively (Lynch, 1990). The genetic similarity among the different germplsam was calculated with the available banding patterns.

Results and discussion

A total of eight primers of arbitrary nucleotide sequence primers were screened for their ability to generate consistently amplified banding patterns and to assess polymorphism in the tested date palm cultivars. Among these primers, only two (OPD - 16 and OPD - 20) revealed polymorphic and unambiguously scorable bands. A set of four RAPD primers (Table 3) including OPD - 16 produced unique polymorphic bands useful to identify different germplasm. While smear or no amplified products were observed with the other primers. Details of RAPD profiles made by each primer are listed in Table 4. Examples of polymorphism are shown in Figure 1.

Table 3. Date palm cultivars identified using different RAPD primers.

Primer	No. of polymorphic bands	Varieties discriminated
OPA-2	1	MDP-02
OPD-16	3	MDP-09 and MDP-10
OPF-13	1	MDP-10
OPF-20	1	MDP-06
Total	6	Four

Table 4. Details of RAPD polymorphism detected using different primers.

Primer	Amplified bands	Total bands	No. of Polymorphic % of polymorphism
OPA-1	8	1	12.5
OPA-2	7	1	14.3
OPA-9	8	0	0
OPC-2	Smear	—	—
OPD-16	10	3	30.0
OPD-20	8	2	25.0
OPF-13	7	1	14.3
OPF-20	10	1	10.0

The matrix compiled using the data available from RAPD profile of primer OPD-16 showed a genetic similarity of 0.842 to 0.947 (Table 5) among the different local germplasm. Thus it may be assumed that these ecotypes emerged in Kachchh regions are characterized by a high degree of genetic similarity at the DNA level. The least similarity (0.842) was observed between MDP-10 and MDP-03 indicating that among the local germplasm these two

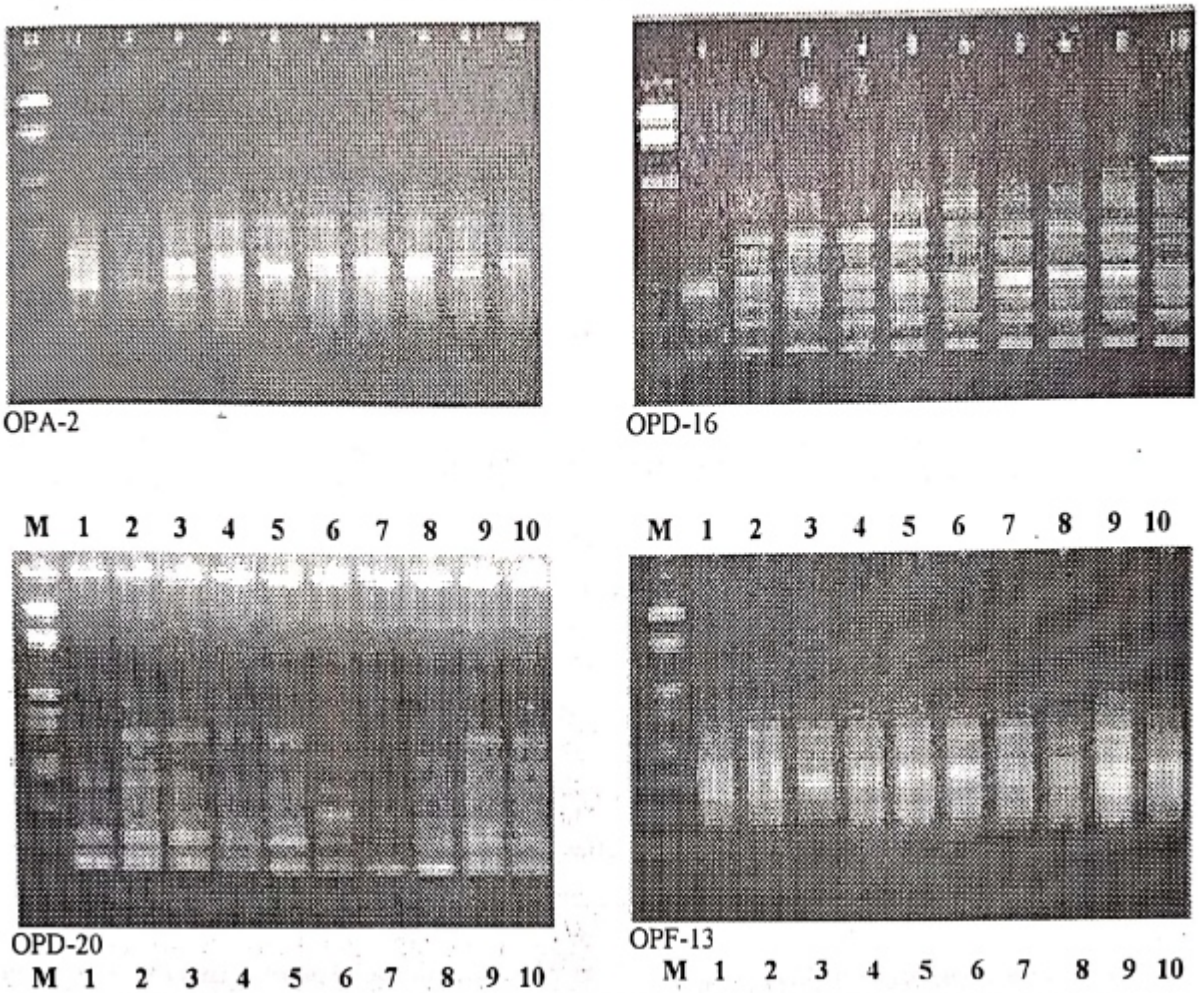

Figure 1: RAPD polymorphism in 10 samples of date palm revealed with the four random primers. M: Standard molecular size marker (2 kb); lanes 1-10 accessions described in materials and methods and Table 1.

have the greatest genetic difference, but the data is not sufficient to draw any conclusion regarding the genetic similarity between exotic and local date palm germplasms.

Table 5. Similarity index based on RAPD profile using primer OPD-16.

Cultivars	MDP-03	MDP-05	MDP-09
MDP 05	0.947		
MDP 09	0.947	0.947	
MDP 10	0.842	0.947	0.900

There may be reason to view with caution systematic conclusions based on RAPD analysis alone. As reported by Williams *et al.* (1993), the small alterations in PCR parameters or quality of target DNA can alter RAPD patterns. Further standardization of DNA extraction procedure, PCR parameters and gel documentation process may overcome the appearance of unclear bands and smear formation. Use of more number of primers will be useful for the complete varietal identification of all the tested date palm cultivars. This study can be extended for accurate identification of date palm varieties cultivated and wild relatives of *Phoenix* germplasm collection, which will accelerate the crop improvement research using marker assisted selection (MAS).

Collection, conservation and documentation variable genetic resource of the date palm available in Kachchh is significant for crop improvement work. The results from this study suggest that the use of RAPD markers to detect polymorphism is useful for identification of date palm germplasm.

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