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Propagation of Arabian Date Palm (*Phoenix dactylifera* L.) Seedling and DNA Fingerprinting by Using Molecular Marker

***Abul Kalam Azad[1], Lija Khatun[1], Mizanur Rahman[1], Mohammad Minnatul Karim[1] and S M Abdur Razzaque[2]**

[1]Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh.
[2]Department of Applied Chemistry and Chemical Engineering, Islamic University, Kushtia, Bangladesh

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ABSTRACT

The present research work was conducted on Arabian date palm (*Phoenix dactylifera* L.). Four Arabian date palm, that is Marium, Deglet Nur, Nagal and Lulu were used for study of germination and DNA fingerprinting among the varieties. One month is required for germination in poly bags of the varieties. The 92% seeds were germinated of Marium variety and length of seedlings was 20.5 (mean). 51.22% seeds were germinated of Deglet Noor variety and length of seedlings was 18.0 (mean). The Nagal Variety seeds were germinated 85% where the length of seedlings was 21.5 (mean), and finally, Lulu variety were germinated 69.58% where the length of seedlings was 19. 25 (mean). Sufficient amount of quality DNA was isolated by combined modified CTAB method from young leaves of Arabian date palms. Four different varieties were studied based on seven RAPD markers. RAPD analysis was revealed 118 scorable bands from seven primers. Among the 118 RAPD bands, 10 bands were monomorphic and 108 bands were polymorphic. Out of seven random primers, the maximum polymorphism was observed by primer OPG- 03 (96%). The RAPD based dendrogram generated cluster analysis among the verities was done by Origin Software. Cluster analysis revealed that it is consist of main two class C1(Nagal) and C2 (Deglet Nur, Lulu and Marium). C2 class is consist of two subclass C2S1(Lulu and Marium) and C2S2 (Deglet Nur) respectively. Overall results revealed that Nagal variety is distinct different from the other three varieties (Deglet Nur, Lulu and Marium).

Key words: Date palm, CTAB method, DNA fingerprinting, Polymorphism, RAPD marker.

INTRODUCTION

Arabian date palm is the Arecaceae family, widely cultivated in the Middle East and in North-Africa where it is thought to have been cultivated for over several thousands of years [1]. 70% of the 120 million date palms in the world are found in Arab countries, which also produce 67% of the world's dates. Date palm groves have suffered degradation over the past 50 years as a result of heavy exploitation brought on by an increase in the number of people and domestic animals [2]. Because they offer a full, wholesome meal, dates are a vital food for travelers in the desert or in the mountains. A very few attempts were made to cultivate Arabian novel date palm for fruits consumption in Bangladesh. Moreover, the Arabian dates was from Paradise and antidote against poison [3]. Moreover, Bangladesh imports dates from Arab countries before the month of Ramadan where consume a lot of currency in every year. If Bangladesh able to cultivate Arabian date palm garden and reached to production available then a lot of foreign currency would be saved. On the other hand, The Bangladesh imports a big lot of sugar from foreign countries in every year, because the production of sugar from sugarcane in Bangladesh is not enough on the basis of demand.

Date palm fruits contains a variety of vitamins which have a different function that help maintain a healthy body to metabolize carbohydrates and maintain blood glucose levels, fatty acids for energy, and they help make hemoglobin, the red and white blood cells. Magnesium and calcium are essential for healthy bone development

and for energy metabolism. Iron is essential to red blood cells production. Red blood cells carry all the nutrients to cells throughout the body. Today's healthy diets recommended eating foods that are low in sodium, fat, cholesterol and high in fiber [4].

Seed propagation, also called sexual propagation, although useful for breeding purposes, for the dioecious in nature, 50% progeny will be male and other 50% will be female trees that are not true- to- type fruits produced [5]. The first flowering of the trees takes place at the age of about 5–7 years of planting [6]. The fruits are variable and inferior quality compared to mother plants. Propagation through seeds has many limitations as well, like seed dormancy, low rate of germination and progeny variation [7]. However, in a seedling plantation it is rare that more than 10 percent of the palms produce fruit of satisfactory quality [6]. Problems of breeding and propagation of date palm arise from the fact that the tree has a long-life cycle [8]. To overcome these problems and fulfill the demand for planting material, it is necessary to develop the method of date palm propagation with the use of plant tissue culture [9]. There are very few Arabian Date Palm trees existed sporadically here and there in Bangladesh like Valuka in Mymensingh, Khulna, Bogra etc.

On the basis of physical characteristics, date palm genotypes can be identified [10]. In most cases, only after fruiting, which occurs after 5-7 years, can identification be done. [11]. The molecular marker as well as Random Amplified Polymorphic DNA (RAPD) is a comparatively simple, quick and inexpensive procedure for generating genomic markers [12]. The technique has been successfully used for varietal identification of date palms [13]. DNA markers have been applied as a direct approach to detect the genetic variation of date palm cultivars. RAPD fingerprints have been used to identify date palm accessions in Saudi Arabia, Egypt [14]. Genetic material from male, female and unidentified date palm lines was compared using the RAPD approach. Since they have a large number of homologous bands, the two female cultures (adult and offspring) have a relatively close relationship [15]. RAPD analysis has been used as a reliable method to identify clones and cultivars and to assess somaclonal variation of date palm [16]. The advent of molecular techniques DNA-based procedures has been proposed for cultivar identification. RAPD markers are used for identification of cultivars [17].

The main objectives of this research in first phase that is- collection of date's samples, preparation of poly bags, germination of date seeds, plantation of germinated plants, DNA isolation, qualification, quantification, PCR amplification and dendrogram analysis.

MATERIALS AND METHODS

Collection of Seed Materials-The four Arabian dates that is DP-919 = Marium, DP-920 = Deglet Noor, DP-921 = Nagar and DP-922 = Lulu varieties were collected from the local market of Kushtia which has been collected from Arab Countries (Fig-1).

Fig. 1 Collection of seed materials

Table -1 Morphological fruit characteristics of Arabian date palm genotypes studied

Genotypes	Code No.	Fruit Characteristics	
		Color	Consistency
Marium	DP-919	Reddish	Semi-Dry
Deglet Noor	DP-920	Amber	Semi-Dry
Nagal	DP-921	Dark Brown	Semi-Dry
Lulu	DP-922	Honey	Dry

Preparation of Poly bags- The polybags were collected from loca l market of Kushtia and licked by punch machine and were sown in polybags containing 50% sand, 40% loam soil, 5% cow dung and 5% bagasse (Fig-2).

Fig. 2. Preparation of Poly bags

Germination of date seeds- Seeds were kept as straight in one inch depth on soil of poly bag in our faculty loan. Water was applied as and when necessary. After one-month seeds were germinated (Table-1).

Table -2 Germination of Arabian date palm in poly bags

Variety	No. of Seeds	Total Germination	% of Germination	Length of seedlings in mean value
Marium	500	460	92%	20
Deglet Noor	367	188	50.22%	18
Nagal	500	425	85%	21.5
Lulu	457	318	69.58%	19.25

Collection of young leaves- Four months old seedlings were used as plant materials i.e., young leaves for DNA isolation (Figure-3).

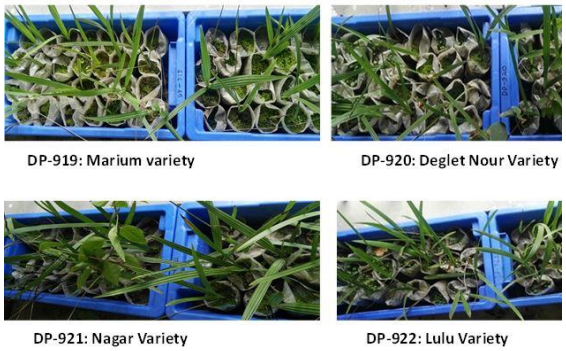

Fig. 3 Five months old seedlings of Arabian date palm germinated in polybags

Isolation of DNA -In the present investigation, modified method of Al-janbi et al. (1999) [18] reported by Hossain et al., (2006) [19], has been combined and used to isolate the total genomic DNA from Arabian date palm.

Quality of DNA- Spectrophotometric techniques and agarose gel electrophoresis was used to assess the DNA's quality. For each sample of the Arabian date palm, extracted genomic DNA was tested on a 1% agarose gel to assess the DNA's purity. (Figure-4).

Fig. 4 Electrophoregram of ethidium bromide-stained genomic DNA samples of 4 Arabian date palm varieties.

Quantification of DNA-By measuring absorbance at 260 nm, the amount of DNA was calculated using the spectrophotometric approach. Pure DNA was found to have a 260/280 ratio close to 1.8. 260/280 ratios below 1.8 frequently indicate the presence of contaminating protein or phenol. Elevated ratios typically indicate the presence of RNA, which can be verified by running a sample. Christopher and Clark concurred on this outcome. [20] (Table-2).

Table -3 Quality assessment of DNA isolated from young leaves of Arabian date palm varieties

Variety	Samples	OD at 260 nm	OD at 280nm	OD at 260/280	Total DNA (ng/µl)
Marium	Young Leaves	0.037	0.020	1.85	1850
Deglet Noor		0.072	0.039	1.84	2600
Nagal		0.072	0.036	2.0	3600
Lulu		0.045	0.023	1.95	2250

PCR Amplification- The PCR procedures were modified in the past [21][22]. The amplifications were performed in a thermal cycler (Genius, Techne, Cambridge Limited) set to 40 cycles for the entire DNA synthesis under the PCR-1 algorithm. The following ingredients were used for the PCR amplification: 2 µl (50 ng/l) of genomic DNA, 2 µl (10 M) primer, 1 µl (10X) PCR buffer, 0.6 µl (25 mM) MgCl2, 1 µl (2.5 mM) dNTPs, 0.2 µl (1 unit) Taq DNA polymerase (Promega, USA), and 3.2 µl deionized distilled water. The procedure involved three steps: 94°C for 1 min of denaturation of the DNA chain in shorter sequences (the initial denaturation took 2 min); 37°C for 1 min of primer hybridization to complemental sequence sites in the denatured DNA chain; and 72°C for 7 min of primer extension for the synthesis of the DNA complemental sequences, followed by 4°C cooling for an infinite amount of time. Following ethidium bromide staining, amplification products were electrophoresed in 1.5% agarose gels in TBE (1X/) solution and identified [23]. RAPD bands were seen on a transilluminator in the ultra violet, and they were recorded by snapping pictures with a digital camera. (Canon IXY Digital 220 IS, 12.1 Mega Pixel, Japan).

RESULTS AND DISCUSSION

RAPD-PCR Analysis-Using the computer program Alpha View 3.2, all RAPD bands were scored. Calculations were made for each primer's total number of bands, size ranges (bp), total number of bands per variety, number of polymorphic bands, and percentage of polymorphism. For each of the four date palm kinds tested, DNA fragments that were amplified by a specific primer were scored for presence ('1') and absence ('0'). By employing the un-weighted pair-group method with arithmetic averages (UPGMA) cluster analysis with the STATISTICA program, a dendrogram was created to identify the genetic relatedness among the genotypes based on the distance matrix [24]. Based on the analysis, fingerprinting keys were created. [25] (Table-3).

Table -4 List of 7 decamer RAPD primers with their sequences

S. No.	Primer Code	Sequence 5'→3'	%G+C Content
1.	OPA-10	GTGATCGCAG	60
2.	OPA-15	TTCCGAACCC	60
3.	OPG-03	GAGCCCTCCA	70
4.	UBC-283	CGGCCACCGT	80
5.	UBC-431	CTGCGGGTCA	70
6.	UBC-467	AGCACGGGCA	70
7.	OPB-18	CCACAGCAGT	60

Table 5 contains results from PCR-based RAPD study against four kinds of date palm. The four different date palm types' amplified bands ranged in size from 273 bp to 11825 bp. Among the seven RAPD primers, OPA-10 revealed bands with sizes ranging from 342 to 1825 bp, OPA-15 from 688 to 1442 bp, OPG-03 from 273 to 1407 bp, UBC-283 from 700 to 1732 bp, UBC-431 from 507 to 1393 bp, UBC-467 from 644 to 1403 bp, and OPB-18 from 289 to 873 bp. Four different varieties of date palms' DNA segments were amplified using seven RAPD primers, which were proven to be effective at amplifying the genome. On the tested types, these primers produced notable band profiles (shown in photo). The OPG-03 primer amplified the most bands (25) whereas the OPA-10 primer amplified the fewest bands (10) (Fig-5) (Table-4).

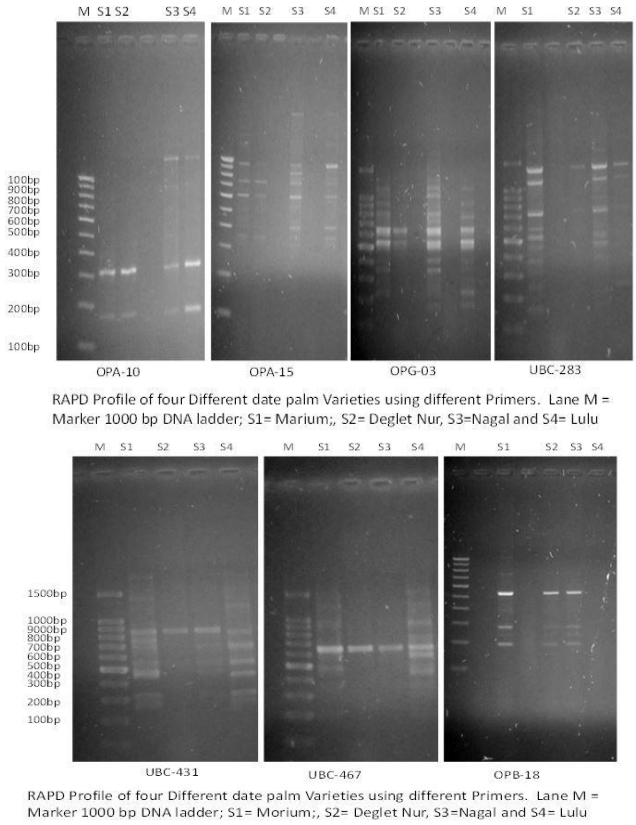

Fig. 5 Seven RAPD primers showed different bands against four date palm samples.

The mean square and means for the consideration traits in cluster analysis revealed that, there were significant differences among all the species. Genetic relationships among the varieties showed two major clusters (C1 and C2) presented in the Figure-6.

Table -5 RAPD primers with corresponding bands scored and their size ranges in selected Date palms

Primer Codes	Size Ranges (bp)	Total No of brands scored	No. of monomorphic brands	No. of polymorphic brands	Polymorphism %
OPA-10	342-1825	10	02	8	80.00%
OPA-15	688+1442	20	03	17	85.00%
OPG-03	273-1407	25	01	24	96.00%
UBC-283	700-1732	21	01	20	95.23%
UBC-431	507-1393	19	01	18	94.73%
UBC-467	644-1403	11	01	10	90.90%
OPB-18	289-873	12	01	11	91.66%
Total		**118**	**10**	**108**	

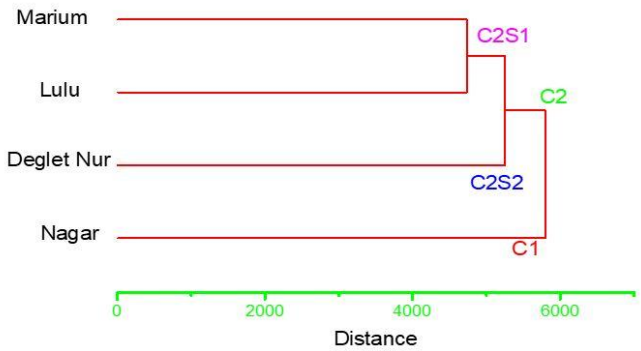

Fig. 6 UPGMA Cluster analysis of four date palm varieties based on RAPD markers. C = Cluster; SC = Sub-cluster

Cluster analysis indicated that it is consist of main two class C1(Nagar) and C2 (Degletnur, Lulu and Marium). C2 class is consist of two subclass C2S1(Lulu and Marium) and C2 S2 (Degletnur), respectively. Overall results revealed that Nagar variety is distinct different from the other three varieties (Degletnur, Lulu and Marium). Variety within the same subgroup demonstrated that these species' morphological behavior was comparable or that they may have shared ancestors, albeit morphological based categorization occasionally did not correspond to the species' molecular/genomic relationships [26]. The morphological features do not offer a useful framework for evolutionary classifications in this regard.

The main cluster C1 indicates that, it is completely different than all other varieties, because it formed separate / distinct cluster. While it can be assumed that an organism's basic DNA sequence remains unaltered by short-term environmental change, it should offer a more reliable alternative for strain and species identification. Consequently, the RAPD technique's random and genomic-wide character makes it better than morphological analysis in indicating overall genetic relatedness [27].

In order to determine the genetic diversity and relationships among the various date palm kinds, RAPD markers are particularly helpful. It can effectively produce polymorphism, which aids in distinguishing the closely related genotypes. The number of bands produced by the various primers in PCR varied. The primer OPG-03, with a polymorphism percentage of 96, produced the most polymorphism. The RAPD primers' ability to induce polymorphism may be owing to base substitution, insertion and deletion, or the gathering of genetic material from many sources [28] [29].

CONCLUSION

The Project work was conducted for plantation of Arabian date palm (Phoenix dactylifera L.) seedlings where four types of dates were used for seedling production; these seedlings were planted in Islamic University, Kushtia Campus. DNA fingerprinting of these four types date palm was done for their identification and make a genetic relationship among the varieties through RAPD analysis. The Nagal date palm variety revealed distinct different from the other three varieties (Deglet Nur, Lulu and Marium).

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