IDENTIFICATION OF MALE SPECIFIC MOLECULAR MARKERS IN DATE PALM SEWI CULTIVAR

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The date palm (*Phoenix dactylifera* L.) is a monocotyledoneus woody perennial tree belongs to the Arecaceae family, which comprises 200 genera and more than 2500 species (McCurrach, 1960). Date-palm (2n=36), cultivated mainly in North Africa and Middle East, has major economic, social and environmental importance. Date-palms constitute the principal financial resource and food source of oasis growers, and it contributes to the development of subjacent cultures (alfalfa, fig trees, pepper, tomato, saffron, etc.).

In Egypt, date palm trees are classified according to their fleshiness into three classes. The first class is the soft date such as Zaghloul, Samani, and Hayany. The second class is the semidry date such as Sewi, Aglany and Amry. The third class is the dry date like Sakoty and Malkaby. Sewi cultivar, a semi dry date palm, is considered one of the most important commodity items for export in Egypt. It is stored and processed throughout the year. The number of fruitful Sewi female palms in Egypt is 1.834 million. El-Wadi El-Gadid governorate cultivates the biggest number in Egypt, 0.661 million, followed by Giza governorate that cultivate 0.525 millions tree. (Ministry of Agriculture, 2009).

In the date palm, the dioecious mode (separate male and female individuals) and the late initial reproductive age (5-10 years) are major practical constraints for genetic improvement. Early selection on young seedlings could enhance breeding programmes and generate experimental male and female genetic stocks, but no easy robust cytogenetic protocol exists for sex determination in an immature date palm (Siljak-Yakovlev *et al.*, 1996). Genotype identification and cultivar identification, based on morphological character of date palm, is an intricate empirical exercise (Al-Khalifha *et al.*, 2011).

The development of the PCR-based fingerprinting techniques such as randomly amplified polymorphic DNAs (RAPDs) (Welsh and McClelland, 1990; Williams *et al.*, 1990), amplified fragment length polymorphisms (AFLPs) (Lin and Kuo, 1995) and simple sequence repeats (SSRs) (Powell *et al.*, 1996) has accelerated the

detection of distinct markers in plant genomes. RAPD method is straightforward and does not require previous genetic knowledge of the target organism. Furthermore, it is very quick and convenient to perform. The RAPD technique has been employed to develop sex-linked markers in *Silene latifolia* (Zhang *et al.*, 1998), *P. longum* (Banerjee *et al.*, 1999), *Pistacia vera* (Hormaza *et al.*, 1994), *Salix viminalis* (Alstrom-Rapaport *et al.*, 1998), *Cannabis sativa* (Mandolino *et al.*, 1999) and *Actinidia* species (Gill *et al.*, 1998). To address the problem of sensitivity, it has been suggested that RAPD marker should be converted to sequence characterized amplified region (SCAR) marker based on their DNA sequence (Paran and Michelmore, 1993). The SCAR marker is sequence-specific and can be used to amplify single bands corresponding to single genetic loci. The conversion of RAPD markers to sex-linked SCAR markers *et al.*, was performed in *Salix viminalis* (Gunter *et al.*, 2003), in *Actinidia chinensis* (Gill *et al.*, 1998; Geoffrey *et al.*, 1995) and in Papaya (Urasaki *et al.*, 2002).

The aims of this study are to 1- identify and discriminate between male and female date palm trees via vegetative virtual external morphological characteristics. 2- identify of male-associated SCAR markers in date palm in order to help for plant grower, in selecting the favorable male plant in their programs in fast cost-effective way.

MATERIAL AND METHODS

Plant material

This study was conducted at The Central Laboratory for Date Palm Researches, and Agricultural Genetic Engineering Research Institute, Agricultural Research Center, Giza, Egypt during 2009 and 2010. The study was performed on healthy productive male and female trees of date palm (*Phoenix dactylifera* L.) Sewi cultivar grown in Giza governorate, chosen among two orchards, their age ranged about between 10 and 15 years old and about 4-5 meters in height.

Morphological characteristics

The tested palm trees trunk girth (at one meter above the soil service) were measured for male (2.25 meter) and female trees (1.50 meter). The number and the length of new leaves formed during four seasons (Spring, Summer, Autumn, Winter) on male and female palm trees in two years 2009-2010 were recorded. Five leaves, three years old, per each tested palm were collected and randomly detached. The number and length of leaves blade, number and length spins blade and number of spaths of male and female palm trees were recorded.

Chemical analysis

The nitrogen content was determined using keldahl method according to A.O.A.C (1980). Phosphorus percentage was determined, according to the method adapted by Hucker and Catraux (1980).

Potassium percentage was determined using flame photometer according to Cottenie *et al*. (1982).

Molecular techniques

Genomic DNA was extracted from male and female date palm sewi cultivar (*Phoenix dactylifera* L.) leaves using the Nucleon PhytoPure Genomic DNA Extraction Kit (Amersham Bio-sciences). DNA quality, integrity and quantity of each sample were determined by running 2 µl of DNA from male and female samples. The purified genomic DNA was subjected to PCR for RAPD analysis using random primers each of twelve mer from BEX, Japan (Table 1). The PCR reaction mixture consisted of 50 ng genomic DNA, 200 µM each of dNTPs, 20-picomole primer, 1x Taq DNA polymerase buffer and 0.5 units of Taq DNA polymerase (Promega, WS, USA) in a final volume of 25 µl in sterile ultra-pure water. The PCR was performed in a Perkin Elmer 9700 thermal cycler for 40 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 1 min and extension at 72°C for 2 min followed by final extension at 72°C for 7 min. The Reaction products were submitted to electrophoresis in a 1.2% agarose gel in 1X TAE buffer as described by Sambrook *et al*. (2001). Eight reproducible male-specific bands were chosen for developing SCAR marker. The bands were purified from agarose gel using Montag gel extraction kit (Millipore). The purified bands were cloned in pGEM-T Easy plasmid (Promega) and transformed into *Escherichia coli* DH5α.

Sequence analysis

The recombinant clones were sequenced using a Big Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems) and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). A homology search was performed using BLASTX against the NCBI protein database (http://www.ncbi.nlm.nih.gov). Eight SCAR primers pairs were designed based on the DNA sequence of isolated fragments. The primers were designed using with OLIGO software (ver 6.44) (Molecular biology insights, CO, USA). Alignments of isolated sequences with the draft genome of date palm was performed using the matcher module of Jamboss software ver 1.5. The draft assembly of the Date Palm Genome was downloaded from the website of Cornell University in Qatar (http://qatar-weill.cornell.edu/research/datepalmGenome/index.html).

Experimental design

The experiments were performed utilizing randomized complete block design with factorial arrangement. The results were subject to analyzed for variance and the means were compared using LSD at 5% level according to Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

Number of new leaves

Table (2) shows the number of new leaves formed from male and female trees

of date palm (*Phoenix dactylifera* L.) during four seasons in two years. Data clearly showed that there was no significant difference between male and female trees of date palm under investigation. The number of new leaves formed in summer and spring were (6.375 and 6.125 leaves, respectively) without significant difference between male and female, followed significantly by Autumn (3.375 leaves). Number of new leaves formed in winter was the lowest (2.375 leaves).

Length of new leaves

Data in Table (3) shows that the length of new leaves formed from male and female trees of date palm (*Phoenix dactylifera* L.) during four seasons in two years. Data clearly showed that, male trees has higher length of new leaves formed (1.319 meter) than that of the female trees (1.250 meter) under investigation. Summer and spring recorded the highest length (1.437 and 1.312 meter, respectively) without significant difference between male and female. Winter recorded the lowest length of new leaves formed (1.125 meter). Our results are in accordance with the results of semi dry date palm cultivars grown in Libya **(**Alghool and Benismail, 2007).

Number and length of leaves blade, number and length of spins blade and number of spaths

Data tabulated in Table (4) shows the difference in number and length of leaves blade, number and length of spins blade and number of spaths between male and female trees of date palm. Regarding the difference in number of leaves blade, data indicated that, male trees of date palm was significantly higher in the number of leaves blade (141.25) than that of female trees (115.000) in the two years. With regard to the difference of length of leaves blade, data indicated that, male trees of date palm was the significantly higher (4.75 meter) than that of the female trees (3.50 meter) around in two years.

Number and length of spines in male trees of date palm was the significantly higher in the number of spines (36.50, 1.55 meter respectively), than those of the female trees (22.00, 1.1 meter, respectively) during the two years. In regard to the difference in the number of spaths, data indicated that, male trees of date palm was the significantly higher in the number of spaths 13.00/tree, than that of female trees that carries 7.75 /tree around in two years.

Chemical analysis of macro-nutrient elements of male and female trees

Table (5) shows the chemical analysis of macro-nutrient elements percentage for nitrogen, phosphorus and potassium of male and female trees of date palms. Regarding the percentage of nitrogen, female trees showed higher percentage of nitrogen (0.62%), while male trees recorded significantly lower percentage (0.54%). With regard to the percentage of phosphorus, female trees showed higher percentage (0.838633%), while male trees recorded significantly lower percentage

(0.550298%). As for the percentage of potassium, female trees recorded higher percentage of potassium (0.838633%), while male trees exhibited significantly lower percentage (0.550298%). Our results are similar to those of Alghool and Benismail (2007) who studied the physical and chemical aspects of each cultivar of date palm trees grown in Libya. Also, our results are in accordance with Iranian studies (Mohebi, 2007).

Identification of male sex-associated RAPD bands

RAPD profiles of male and female date palm were generated using BEX primers (*BEX*, Japan). Twenty RAPD primers were tested and 11 of them showed reproducible and different RAPD profile between male and female (Table 1). The number of bands per primer ranged from two to nine, and a total of ninety nine polymorphic fragments were generated (Fig. 1).

Eight male-associated RAPD fragments were eluted and cloned using the pGEM T-Easy system (Promega). Plasmids were sequenced by ABI3100 genetic analyzer. DNA sequences were compared with the available database by BLAST search. A BLAST search did not reveal any significant similarity with the other known DNA sequences in the available database (e.g., EMBL, GenBank, DDBJ and PMB). A similar comparison with male-associated DNA sequences from other plant species also did not reveal any significant resemblance. The sequences were named MAD1 to MAD8 (male-associated DNA). Their size ranges were from 398 to 1180 (Table. 6). We could identify open reading frame (ORF) in both orientation, but none of them showed any similarity in GenBank, data not shown. Al-Dous *et al.,* (2011) assembled a draft genome for a Khalas variety female date palm, the first publicly available resource of its type for date palm in the world. We have aligned the sequence of the isolated fragments with the draft assembly of the Date Palm Genome generated by whole genome shotgun next generation DNA sequencing. The draft genome contain predicted genome size of ~650 Mbp, that contain about 60% of the genome sequence (remaining unassembled sequence is mostly highly repetitive). The isolated sequences in this study showed minor sequence similarity with the draft genome of date palm (Table 6). This may be explained as we isolated the fragments from the Sewi cultivar and the draft genome was performed on the variety 'Khalas' grown mainly in Saudi Arabia.

SCAR analysis

Eight SCAR primers pairs were constructed based on the DNA sequence of isolated fragments. The location primer sequences and product size of SCAR primers are listed in Table (6). The SCAR primers amplified intense fragments ranging from 338-955 bp at an annealing temperature of 55°C. Increasing the stringency by increasing the annealing temperature or the use of higher Mg concentration did not change the results.

After testing the eight SCAR primers, three of them, primers specific to MAD2, MAD3 and MAD4 fail to differentiate between male and female plants. On the other hand, four SCAR primers, MAD1, MAD5, MAD6 and MAD8 exhibited fragments in the male plants only. MAD 7 showed a different pattern between male and female plants. The different patterns were augmented as we pooled the DNA of ten male plants and ten female plants. Instead of sing single plant we pooled DNA to confirm the integrity of the marker and to confirm the validity of the marker to differentiate between male and female plants. We tested the male-specific marker on the DNA from single male plant, and it gives the same result of the pooled DNA.

In the present study, we have identified four male sex-associated SCAR markers, from the genomic DNA of date palm, sewi cultivar. These male-associated RAPD markers were reproducibly produced by the use of identical enzyme and reaction conditions at a high annealing temperature. Isolation of male-specific marker(s) will be of great help for palm tree grower, as it will help them to select the promising male trees and thus saving both time and efforts at an early stage of growth and so avoid cultivating too many male palms.

SUMMARY

Date palm is considered one of the most important commercial crops in the Middle East and Arab World. The entire tree of date palm is utilized to provide food, shelter, fiber, furniture and many other products. Moreover, the date palm tree successfully tolerates extremely adverse environmental conditions including drought, high temperature and salinity, which are the common criteria of desert lands. The aim of this study is to identify and discriminate between male and female date palm productive trees, Sewi cultivar, via virtual external morphological characteristics and molecular genetic analysis. Male trees of date palm were higher in length of new leaves, the number and the length of leaves blade, the number and length of spins blade, and the number of spaths when compared with the female trees of date palm under investigation. Female trees of date palm have higher percentage of nitrogen, phosphorus and potassium when compared with the male ones. It also describes the identification of male-associated SCAR markers in Sewi cultivar of date palm. Sex-linked RAPD markers were identified from date palm and were converted into four male specific SCARS marker. Isolation of male-specific marker(s) will be of great help for plant grower, as it will help them to avoid cultivating too many male palms, and selecting the favorable male plant in their tissue culture programs.

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Table (1): Sequence of the selected primers.

Sequence I.D.	Sequence
A00	ATCAGCGCACCA
A01	AGCAGCGCCTCA
A02	GCCAGCTGTACG
A04	GCCCCGTTAGCA
A06	ACTGGCCGAGGG
A07	GATGGATTTGGG
A19	AAGGCGCGAACG
A21	GTGACCGATCCA
A23	AAGTGGTGGTAT
A24	GACGGTTCAAGC
A25	GGTCAGGCACCA

Table (2): Number of new leaves formed in male and female date palm trees during four seasons.

Season / Sex type	Spring	Summer	Autumn	Winter	Mean
Male	6.250	6.750	3.750	2.500	4.813
Female	6.000	6.000	3.000	2.250	4.313
Mean	6.125	6.375	3.375	2.375	
LSD 0.05	A=0.6923	B=0.9793	AB=1.385		

Table (3): Length of new leaves formed in male and female date palm trees during four seasons.

Season / Sex type	Spring	Summer	Autumn	Winter	Mean
Male	1.330	1.500	1.300	1.150	1.319
Female	1.300	1.380	1.230	1.100	1.250
Mean	1.312	1.437	1.262	1.125	
LSD 0.05	A=0.047	B=0.067	AB=0.093		

Table (4): Number and length of leaves blade, number and length of spins blade and number of spaths formed in male and female date palm trees during the year.

Characters / Sex type	Number of leaves blade	Length of leaves blade	Length of spins blade	Number of spines	Number of spaths
Male	141.25	4.75	1.55	36.50	13.00
Female	115.00	3.50	1.10	22.00	7.75
LSD 0.05	18.59	0.4612	0.2756	4.005	3.012

Table (5): Chemical analysis of macro-nutrient elements of male and female trees.

Macro-nutrient% / Sex type	N	P	K
Male	0.54	0.550298	0.322229
Female	0.62	0.838633	0.368179

Table (6): Sequences analysis of male associated DNA sequences isolated in this study. Locations of the SCARS primers are underlined. Alignment of the fragment with the Date palm genome are shown.

MAD1
Isolated MAD1 fragment size= 495
PCR size of the SCAR marker= 426
Alignment with Date palm "Khalas" database
Length: 123 # Score: 105
Identity: 73/123 (59.3%)
Gaps: 11/123 (8.9%)
Sequences
GCCTGCCTCACGGTGTCTGA**CAGATTGCCAGGCTGTAGGG**AACCATTCTCTCAGTGCTTTCGGGAA
ATTTTTAGCGTACTGGATTAGGATGGTCGTTATTGCCTCAGGATCAATGGGTAACTTCGCGTTGAT
GATGTTTTCTTTCAACTCAGCAGTATTTTCCAGCCCCAGTGCCTGTACATCGCTGTGTAGTAATGT
CAGGAGCAGCCGGTTCATTAATTTTTTCTCGTAAGCAGACTTTTGCCAACTGCGTTTAATTATCGC
AGCACAGTCTGGGTTGGGTGGGGTGATGACGAAGCTTAAGATTTCCATCTGTGGTTGCCGGGTATT
CATCAGCGTTTCAGGCTGAAGATAGGTAGGCCTGACAGAGGTAATCAATGAAGGCAGGACGGCTGC
GATGCCATCGTCATTGTATAACGTTATCGAT**GAGACATGCGGAACGGAAA**CCGGGAGGATATTCAT
AGACATTCCTTTGTTTAATACGTGAGGCAGGCA

MAD2
Isolated MAD2 fragment size= 507
PCR size of the SCAR marker = 414

Alignment with Date palm "Khalas" database

Length: 66 # Score: 117
Identity: 45/66 (68.2%)
Gaps: 3/66 (4.5%)

Sequences

TGCCTGCCTCACGGAGCCAAAAATATCGATAAC**AGCCAGTTCGCCTGCGTCAT**CAATGTAAGCTGA
TTCATTGCCTTTCATTTCAATTACCTCATAGCTGTAACAAAATGAGTGTAATTCACAACTCCACAT
GATGCTTTGGCAAAAGCTGCCATAAAAATATAAACCTGATTCAGTAGGTTAACTAAACTGTGGTTT
TTAATTCAGCCGCCTGCCCATCAACTGACGTTGCTGCTGCCAGCGCAGCCTTCAGTCGCGTCTGCA
CTTCAGCTTCAATCGCATCAGCTTTCTTCTGCGCTTTGGCTTTGAAGTAATCACGGATTTTTACCC
AGCCACCTGAAATAAGATAAAGGGCAACTCCGATGGCTGAGAACCACAACATGATAGTTTAATAAA
AGGTCATTTGGCTTGTTCCTGTCTGGATTGT**TCAATGTTACGGATGGCGGC**TTTGTCCTGATTGCA
TTTCTCTACCACGGCCAGCAGCTGCTCATTCAGCGTGAGGCAGGC

MAD3

Isolated MAD3 fragment size= 398
PCR size of the SCAR marker = 338

Alignment with Date palm "Khalas" database

Length: 127 # Score: 122
Identity: 74/127 (58.3%)
Gaps: 11/127 (8.7%)

Sequences

GCCTGCCTCACGTATCATGCCGCCACCCCTCC**GGCCAGAGCGGAATCTATC**CTCTGCTGCCAGTCA
ATCTTTCCCTTTCCGCAACCATGCGGAGAGTCAATTTTCCCGTTCAATTCAGAAAATAGCGCCATT
TAGCATCGCGCAGAAAAGACCGTCTTCGGTTATGGAGATTACCCGTGGCCGCAAAGATCACGTTAA
AAGTAAACGTTTTGATTCTGGCTGGGGGGCGAGCGATATATTAAAATCAGGGAGCGAAGCGAGGCA
TAACGAAAAAGTAAAAAAATCGATCAAAAAAAATGCAACGGAGTTACTGCAAAAACTTTCATTAAA
AACGAAGGAAATCAGTGAA**AGTGAACGGGCAAAAGAAGA**TTTACTCAGTTGCATTCGTGAGGCAGG
CA

MAD4

Isolated MAD4 fragment size= 1180
PCR size of the SCAR marker = 939

Alignment with Date palm "Khalas" database

Length: 86 # Score: 141
Identity: 56/86 (65.1%)
Gaps: 9/86 (10.5%)

Sequences

GCCCCGTTAGCAGTAATTCAGGAGGGATATCAAATCCAGTGGAATTCGACACCACGCCCATGAAAA
TATCACCCCATAAAATTGAAGGCATTGGAAGACCGACTAGCAGTGGACGAAGCAGTATCGAAATTC
CTGGCTGCGG**GAATCATTGAGAAATCGCCTTC**TCAGRGCAGGGAGTATCTGTCAAATTTCTTCACA
ATTCAAGAAGAAACCAAAAGATGTCCGATTTTAGATTGCAGAGCATTGAACAAGTTTGTACAATGT
CATCACTTCAAAATGGAAGGTATCCCAGCTTTGAGATCAATCTTGGAGAAAAACGATTTTATCTGC
AAGATCGACCTGAAGGACGCATATGTAGTAGTTCCACTTCATCATCACTCCAGACCATTTTTAACT
TTCTTACATCAAGGTACGGTTTACCAGTACAAAACACTGGCATTTGGCCTCAGTGTAGCCCCCCCC
CCCCGAATTTTTTCAAAGCCAATGCGTTATGCAGTGGAGTCATTGATAGAGAAAGGCRTCCGACTA
GTATACAGTGGCCGTCAAAATTGGTCGCATGTTTTCATATTTTGCCTATACACGTTGCAGACTACT
TCATTTCTTAGTTTATGGCAATATAGTATACACCATTCGAAAGATCAAACTCTGGGTAATACTGTG
GTAGTACAAATATTGCAGTAGAGCCAGTGTATCGGTAGTAACTGGCCCTCAAAGTCCGACGGCACA
TGCGACCAATTTTGACGGCGTTTATAAGTGACATAATATAGTCACAAAAATGTGGCTTAATTGTAA
ATTTTTTATATTTTYTCTTTTCTTTCTTCTATTATTATAATATTGTTTACATGTAAGTGTTTTATT
GCTACATTGCGACACCCCATTACGAAATTACACATTGCAGATTAGACATGCGCGCAATTTCAACTA
ACAAAGTAAACAGCATTTCCTCATTGCTAAGATCTAAGAAGTCAAACAGCAACGTACAGTAAGGGA
CAAAATCAGTCCGTTTTTTGGTTTTTTCGCAATGCCTCGCCGGGAATTAAACTTAGGGCTTCGCTT
TGCTG**ACGAAACATTTGCACGCAAG**CTAGACAAAATTATTGGTGTAACCTAATTGCTTCTACCACC
ATTTTCTCTGTAGTTATGGCGGAAAAAGTGATTTTCCGTGATTGTTGCTAACGGGGCA

MAD5

Isolated MAD5 fragment size= 949

PCR size of the SCAR marker = 945

Alignment with Date palm "Khalas" database

Length: 104 # Score: 124
Identity: 64/104 (61.5%)
Gaps: 5/104 (4.8%)

Sequences

GATGGATTTGGGTGATTACAGGATGTTGTTGGGTTTTTGCTAAAATTTGATCNAAGATCATNCCGG
AATGGGCNGGAAGTGCCGATATGTCCTGTAAATGTTCTGCCTGCAAGCCAGTCCATTGATAAAACT
GGGCCTGACTTATTTTTAGGTTTGATTCAGCCTGAGTGACATCGGCCTGTCCCTGTGCGACCTGAG
CCAGAACCTGAGCCAGATCTGCCCGTGTAATGATGCCTGTGTCAAAGCGACGTTGCGCGTCATAAA
GGGAACGCTGCATGTTTTTCAGGACGTTATTTTTTAAAGCGAGTAAGGCCTGTTTTTTGAGTACAT
CCGTATAGGCGATGACGGTATTCAATATGGTGACTGCCGTTTGGTCGGTCAGGGACGCGCTGTTTG
CTGCCAGTTGTTTATTGGCGAGTTCGACCCCCAGCGCATGACGTCCTGATGTATAAAGAGGATAGT
CCAGTTGCAGCTGGGCACTTCGGCCTTTTCTATTGCCCGCTGTGGGAAATAAAACCCCTTCTGGTG
TTTCGACTCTCTCATAATCCAGTTTGGCTGACCAGATTAACTTTTCAGGCCATCCCGGGCTTTTGC
CTGACGAAGTGCTATAGAAGATTGCTTCAACATTGGAGCTGGCTCAATTTATAGCTGGTTTTCATA
ATTTAAACTGGCTTGAATTGCATCTTGCAGTGAAATGGCATACGCAGTGCAGGGAATGCATAAACT
GAATAAACAGGCACAGCCGAATAGTCTTTTTTTTAATTTACTCATCCTGAGCTCTCCTGTGCCTAA
GACCATTGTTCCAGACTATTCAAGGTGATTTTTTTCAAAGGCTCAGGTTTGATATTTCCTTCAATC
ATGCCATCGTGCATGACCAGTCGTTTATAATTTTCCCATCCATCTGAGGTATGTAAAATTCTGGGT
TTATCGAGCTGTCCCAAATCCATCA

MAD6

Isolated MAD6 fragment size= 766

PCR size of the SCAR marker = 653

Alignment with Date palm "Khalas" database

Length: 135 # Score: 123
Identity: 83/135 (61.5%)
Gaps: 12/135 (8.9%)

Sequences

GATGGATTTGGGCAGAACGTTATTATCCGTCACCCTTTCCATTATCATTATTGGCGTTGGTGTCGC
TGCGATGA**GAAAGAATGGCAAAGATAAACC**TTATTTGTTTATCATTTTTGTTTTCGCAGCGGCATT
ACCCAACTTACTGGTTAGTGAAAGTTGGGCGGCTTATCGCACGATAGTGGCTGTCGCATTGATCAC
CACCTCAGTCTTCCTCTTTGGATTGCTGATGATGTTTAGCAAGATTAAACACCCCCAAATACCTTA
TTTTATTTTCATTCTGTTGACGGTGTTTATCAGCAACAAGAATATCAGAGAGGGATTTTCTTCACC
TCAGCAGCAGGAATATAAACTGGTCACTTCAGCCATCATGAGTGCGGTGCCAAAAGAATTTACCGG
CAATGTGTATTACAAAATCGATGAAGATAATTTGACGAGAATTGCCAAATCCACTAAGTATGATGA
ATTCGGCGCACTTTCATTAGGCATGCCATGGACATTTGCTGGCATGGCTTATTCTGTCAAAAAAGA
GCACGCGATGAATTATGCCATTGCARAAKCGCCGGTTATTGGCATCAACAACCATTGCACTGAGCC
TTGCCTGATCATCAATGCCAGCGCCGTGCTCAATCATGGTGTGAAATAAGTTATTAGTCTTAAATC
AAATTCCGCTATTTTATACCACCACCAGGGAAGCCCCCTGGTGGT**GGTCTTACTCCAGTTCGGTGT**
TACAAATCAGCAATCACCCCTCATTGACCCAAATCCATCA

MAD7

Isolated MAD7 fragment size= 853

PCR size of the SCAR marker = 744

Alignment with Date palm "Khalas" database

Length: 82 # Score: 113
Identity: 53/82 (64.6%)
Gaps: 7/82 (8.5%)

Sequences

TGATGGATTTGGGTACTTAGGAACATCCTTGTGGGAAAAATCTCGCTTTTGTCCTCGGCAATGTGT
TGAG**CTGAGACTTCAAGCCGAACCAC**GCCAGAAGACCACATTGCTTCTTCAACAACACCAGCGCCG
TATCTTCTGGGAATGCTATGTTCTCGATCGATATAGTTCAGGTGTGCTGGGCCGACCATTTGCGAT
AGCCGAAGCAGATATCAGCGTTCCGTTACCAGTAGATGCCTCTGATGAAGCAATCATGGGATGTAC
TGGAACGAACTCCTTGTCTGATGCTGCGATAGTAGTCGAGTCATACCCCACGGAACTATCTGTGTT
CATATTCTGCATCAAGCTCCGCAAGATCACCTCAAGCATTCATCTGGAGTTCTATAATGGCCGCAG
CGCGGGTCCTAACAGCGGTGAAGCCACGCCAATCCAGCATACATCCGGCCATACATATGTCAAGCT

CTATCACTTTTTGGACGAGCTCGACAAATGGCTGCAAGAAGCGCCCATATTTCCACAGCCTCGGTC
ACTATATGAGAGGCTTGAATGGTATGAGTTCCTCATGGAAAAGGATAAGCTCCTACTTGTCCGAGG
AGCTATTCATAAAGCCCCAAGAAGAGAGGGACGGCCCCCTCCAGATCTGTTGGAGCTCTGTCTTGC
CTCTGCCTCGCGAATCATTGTGCTATATGATCAGATGATACAGATTAAACATTACATGGGACTCGG
GGTTACTTTCAAGTCATATTTGCGGCTGGCCTCTCGATCATCTACTGCGTCTCGATTGGNGTTGGC
TCGA**AAACGTCACCAGGGCCAG**AGAACGAGCGAGAAGCAGCATCCATTCAAAAACCCTAGC

MAD8
Isolated MAD8 fragment size= 1165
PCR size of the SCAR marker = 955

Alignment with Date palm "Khalas" database

Length: 52 # Score: 113
Identity: 37/52 (71.2%)
Gaps: 1/52 (1.9%)

Sequences

GATGGATTTGGGGATTACAGGGGTATCTTTTTAAATCTGTCCCCTTTAAAAACGTGGATTTATTGC
TGTAGTGTTGAAACCATCAAATAGGGGCATACGCCCCTTTGATCTATCACCCA**CCTGTTGATGACT**
GACCGCTACTCACTTGTAGCGTTAAGCTCTGCATAAAGCTTGCCTGACTTGCTTTAGCCTGGAAAT
CAACATAATCAGCAAGACGTGAGGTGTCACTTTGGATAGTTAGAGGTACGGTAGGCTCAACCGTCA
GGTTGAATATCGGGTTTAGTGTGTAAGCTGGCTCGTTGCTGCTGCTGAGTGACATTGCCGAATCAC
GTAATACCTCACCATTCAGGCCGTAACCACTTGCCGCCGTTCCTGCTTGCTGATTACCGAAGCCCA
GCCAATCTTTGATAGCCGGAGCCACAGAGCGAGTATCAAATCCGGTTTGTTGTTGAATCCAATCGG
CTGCACTGTTCGCACTTCCTGAAACAGCACCATCATAGAGAGCCTGGGCCGCTGGTTTATCGCTAC
CAGTGAACCATTGAGGGAAACGTTCTTTCAGCCCTGCATTTAGATCCGAGACAACACCGGAGATTT
CAGAAACAAATCCTGTCATCTGAGATGCCAGATTACCTAATCCCTCACCTAACAGCCCTGCACTCT
GATTGATTGCCTGTGTATTTTTCACCAGCTCATCGCCGCTATCGGGCAAAACTGGACATAAAGCCA
GTGAATAGACTGTTATCAACGCCATTCANTCAGATTACCCATCCGCTACACTCAGGTCACCATATG
CCAGAGTTTGNCCTTATTTGCTCATCGTTAAGAGTTTGAACCACTATCGACAAGTTTTCTCAGTGT
AGATATAAACTGCTCACCGTTNATTTTTGAAAAGAATCGAGGGTAGTGCATCCCATCATCAAAAGT
ATTTTCGAGAAGATGCCCGATTTCACCGTCTTTCATCCCGCGACGCTGACCTTCATTTACCACCGA
TTGTAGGTAATCGAGCGGATTATCTGCATACTGTGAAAGCTGCGATTTGTTCCAACCAAACTTA**TT**
TAACACTTCGTCAACGGCGCTATTTCCACCAGACCATTTACCGCCTTTGAACTGGCTATTTAATAA
TGTTTCGTTGATTCTCTCGCGGGTATCTTTCCCAAATCCATCA

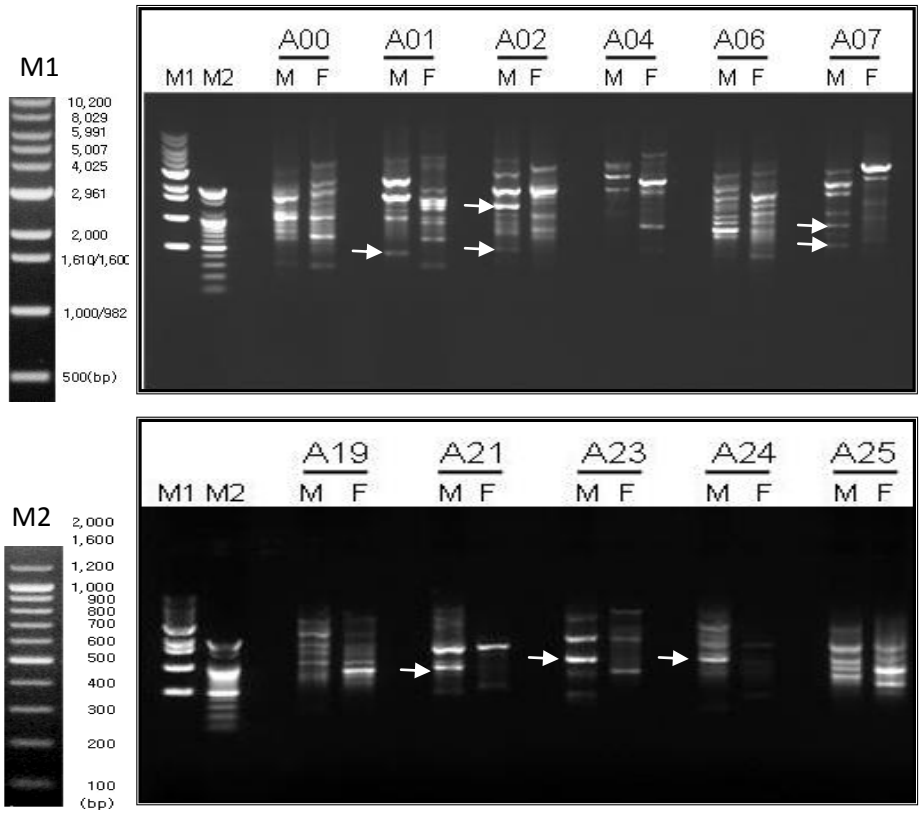

Fig. (1): DNA polymorphisms of (M) male and (F) female cultivars amplified with Bex RAPD primers A00, A01, A 02, A 04, A06, A07, A19, A21, A23, A24 and A25 using RAPD PCR. M1 is 1KB ladder ladder, M2 is 100 bp ladder. Selected fragments for further analysis are arrowed.

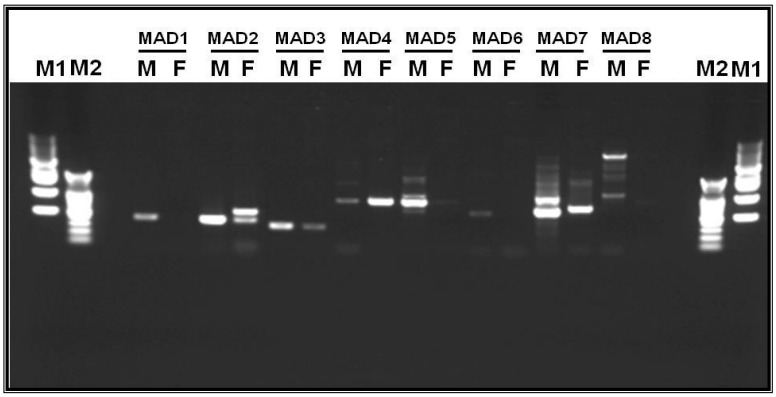

Fig. (2): SCAR analysis with the male-specific primers.DNA from ten male plants and ten female plants of *Phoenix dactylifera* L were used. M1 is 1KB ladder ladder, M2 is 100 bp ladder.