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Sex identification of high-quality date palm (*phoenix dactylifera* L.) cultivars from Wahat El-Baharya, Giza, Egypt

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Phoenix dactylifera (date palm) is an important fruit tree that flourishes in arid regions of Egypt, Middle East, and North Africa. The one million acres presidential initiative proposed the introduction of this strategic crop into many reclaimed lands in Egypt since 2019. The enigmatic divergence in gender prompted us to investigate easy and fast method to determine sex of immature tree. Three females: 1,2,3 and one male pollinator from selective export quality were collected from Wahat El-Baharya and were used in this study. These cultivars are coded as F1, F2, F3 and M, respectively to facilitate their tracking throughout this work. To determine a marker for distinguishing sex, this investigation used four specific and 27 RAPD molecular markers. Some of the markers showed inter-varietal differences while some were able to successfully differentiate between male and female trees and can be recommended to be used at the commercial level for seed propagated trees as will be further discussed.

Keywords: RAPD-PCR, Gender characterization, GPAT3, OPA-0 group, EOD group, OPAA group

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INTRODUCTION

The progress of date palm cultivation in Egypt is quite impressive in recent years. Egypt is considered one of top countries in exporting and producing dates worldwide. According to Bayoumi et al., 2022, the number of cultivated date palm still increasing every year since the presidential initiative in 2018 that aims to reclaim 1.5 million feddans of Egyptian land and plant 2.5 million top quality date palm trees for export trade. This is a practically important step to compete with many high qualities date producing countries such as Indonesia, Malaysia, Bangladesh and Morocco (Egypt presidency, 2018).

In Egypt, Delta region represents the highest production of dates (41 %) followed by Middle Egypt (27.4%), New Vally (17.6%) and Upper Egypt (13.5%) according to The Ministry of Agriculture and Land Reclamation (MALR, 2021). Some cultivated area recently such as Beheira, Nubaria, Kafr El-Shikh, Giza, Aswan, New Valley, Matrouh, North Sinai and Wahat El-Baharya added up to the production amount. Statistics predict that Egypt will contribute about 6.02 percent share of high-quality date during the period (2022-2034) as compared to the current 1.9 % of the world share (Mahmoued et al., 2022).

In the fourth quarter of 2023, date crop was selected as a special agricultural product in receiving more attention under the one country one priority project of the presidential initiative. This was held under the supervision of the ministry of Agriculture, the ministry of trade and industry in collaboration with FAO office in Egypt (FAO, 2023). Although Egypt is a producer of the largest quantity of dates among the world countries, yet it faces lots of challenges to enhance this product quality, productivity and market value. The detrimental effects of climate change, drought, pests (red weevil), lack of international certification and adequate national breeding programs hinder the value chain of the Egyptian dates (Hamed et al., 2022; FAO, 2023).

According to Dowson 1982, date palm tree is a dioeciously monocot (2*n*=36; family: Arecaceae) with 200 genera and 500 species worldwide. The genus *Phoenix* exert significant variation in their sex entity (Munier 1973; Dransfield et al. 2008). Early sex determination is a must in breeding program as it takes from five to ten years to determine the final sex of the tree (Bendiab et al. 1992). Interestingly, date palm composed of equal male and female components which emphasizes the hypothesis that sex is derived by genetics rather than the environment (Daher et al. 2010). Although many trials to determine the sex of immature date palm were done, yet it is quite a challenging task. To date, no publication is found to execute such a task in date palm tree cultivated in Egypt as general and Wahat El-Baharya in particular.

Determining the sex of date palm plants before they reach reproductive age (Cherif et al., 2013), which typically occurs between five to eight years after planting (Aberlenc-Bertossi et al., 2011; Bendiab et al., 1992) is essential to increase the production rate.

Several researchers have identified molecular makers that could segregate sex in date palm and methods have been developed for identifying the sex at an early stage, including the use of RAPD-PCR (George et al., 2007; Moghaieb et al., 2010 and Sharma et al., 2010), isozymes (Torres and Tisserat, 1980), peroxydases (Majourhat et al., 2002). AFLPs (Danilova and Karlov, 2006; Wang et al., 2020), ISSR-SCAR (Korpelainen et al., 2008), RAPD_SCAR (Dhawan et al., 2013), ISSR and RAPD (Younis et. al., 2008), SSR markers (Cherif et al.,2013) and FISH technique (Adawy et al., 2015). However, a major limitation is that none of these markers have been shown to work across a broad range of date palm cultivars (Al-Mahmoud et al., 2012), although some markers could identify sex in one or two cultivars (Younis et al., 2008). Sex determination in date palm has revealed a XX/XY (2n = 36) system with the male being heterogametic.

It also showed significant polymorphism between the male and female alleles in certain regions of their genome. Thus, this DNA variation could be used in distinguishing sexes at an early stage (Intha and Chaiprasart, 2018).

The objective of this research is to determine the sex from green leaves of immature date palm tree using RAPD and specific PCR marker approach focusing on four date palm tree cultivars in Wahat El-Baharya, Giza, Egypt.

MATERIAL AND METHOD

Plant materials

Fresh leaves of three female cultivars (F1, F2 and F3) and one male (M) were collected from immature off shoot trees on the month of October from Kenan Agricultural farm, Asmarco group limited, Wahat El-Baharya, Giza Governorate, Egypt (location is shown in Figure 1). Leaves were collected in the early morning and transferred on an ice box to the laboratory the same day. Samples were stored in the freezer until the time of DNA extraction.

DNA extraction

DNA was extracted from date palm leaves following the method of Arif et al., 2010 except we replaced sterile sand by sterile crashed glass in the griding step. The purity and integrity of extracted DNA were checked using spectrophotometer (PG- instruments-T90+UV/VIS, UK) and agarose gel electrophoresis, respectively.

Specific and RAPD PCR

All primers used in this work were manufactured by metabion international (AG, Germany). Three specific date palm primers (GPAT3, PALM and SRY) were used to distinguish between male and female date palm. LOX5.1 primer pair was used as a positive control. The sequences of specific primers are shown in Table 1. The PCR program for specific primers was as follows: 3 min at 95°C then 30 cycles of 30 sec at 94°C, 45 sec at 58°C and 2 min at 72°C followed by final extension step for 10 min at 72°C then hold the samples at 4 °C until use.

Twenty-seven RAPD primers were used to determine more gender related polymorphism (Table 2). Ten primers from OPA-0 group, ten primers from EOD group and seven primers from OPAA group. The sequences of these primers are shown in Table 3. The PCR program for random primer was as follows: 3 min at 95°C then 25 cycles of 30 sec at 94°C, 30 sec at 33°C and 1 min at 72°C followed by final extension step for 10 min at 72°C then hold the samples at 4 °C until run onto 3 % agarose gel. All reaction components for both specific and RAPD primers were formulated as follows: 100 ng Genomic DNA, 2.5 mM ~~dNTPS,~~ ~~1.5 mM~~ $MgCl_2$, one picomole primer, 5 Units TAQ enzyme and its corresponding buffer. All PCR components were purchased from Promega Corporation, Madison, USA.

Twenty microliters of the PCR product were loaded and ran onto 2% agarose gel for specific primers and 3 % agarose gel for random primers at 100 V until the tracking dye reached the gel end. Gel bands were visualized using UV and pictures were captured using Gel Document system (GDS) (Syngene- Ingenius GDS, Thermo fisher scientific, USA) device. Samples were amplified using Techne thermal cycler (TC-4000, UK) and electrophoresed using Scie-Plas-TVG-SYS vari gel midi system (BioScience, Kuala Lumpur). Each sample was pooled from three different plants. To make sure that the outcomes are repeatable, the experiment was conducted twice. Bands were analyzed using *GELAnalyzer* 23.1.1 (at www.gelanalyzer.com).

Table 1. Sequences of specific primers used in this study

Primer name	Sequence (5`to 3`)
GPAT3 F	AGAAAACCTGATATGCTCTCTG
GPAT3 R	TGTGATGCACTTGGTAACTACT
SRYF	TCTCGAGATCCCCCAATGC
SRYR	ACCCTAAAGTGCCAACCAAT
PALM F	GCATTAGCACCATAGTAAATTGT
PALM R	GTCCCAATCAGAGTGCACTCAA
LOX5.1 F	CTACACCGCAGAGTTTGTCG
LOX5.1R	AGATTGGACCCATGAGTTGC

Table 2. The Sequence of RAPD primers used in this study

No	Name of the primer	Sequence (5`to 3`)
1	OPA-01	TCGGCGATAG
2	OPA-02	TGCCGAGCTC
3	OPA-03	AGTCAGCCAC
4	OPA-04	AATCGGGCTG
5	OPA-06	GGTCCCTGAC
6	OPA-07	GAAACGGGTG
7	OPA-08	GTGACGTAGG
8	OPA-09	GGGTAACGCC
9	OPA-10	GTGATCGCAG
10	OPA-11	CAATCGCCGT
11	EOD1	GGT GAC GCA G
12	EOD2	CTG CTG GGA C
13	EOD3	GTG AGG CGT C
14	EOD4	AAC GGT GAC C
15	EOD5	GGA TGA GAC C
16	EOD6	CCA GCT TAG G
17	EOD7	CAG CCC AGA G
18	EOD8	CAG GAT TCC C
19	EOD9	CTC TGC GCG T
20	EOD10	TGA GTG GGT G
21	OPAA-1	AGACGGCTCC
22	OPAA-2	GAGACCAGAC
23	OPAA-3	AGGACTGCTC
24	OPAA-4	ACCCGACCTG
25	OPAA-5	AACGGGCCAA
26	OPAA-6	GGACCTCTTG
27	OPAA-7	AGGGCGAATG

RESULTS AND DISCUSSION

The date palm is a very biodiverse plant that can be propagated through tissue culture, offshoots, or seeds. In a true breeding line, off shoot methods and tissue culture are frequently employed to preserve homogeneous yield (Jaradat, 2015; Abul-Soad et al., 2017; Naqvi et al., 2023). However, this can hinder biodiversity, which is necessary to overcome the changes in the ecosystem brought about by global warming (Alabdulkader et al., 2016; Alotaibi et al., 2023). In addition, tissue culture is a rather expensive procedure whereas branch propagation yields a limited number. Genetic biodiversity can only be preserved by seed propagation, which, regrettably, produces a large proportion of male plants (Al-Khalifah et al., 2012). It is well known that fifty female trees can be pollinated by one healthy male plant (Bekheet and El-Sharabasy 2015). Therefore, if there are too many male trees in the field, date production will become less profitable on an economic basis. Thus, in crops with long maturation periods such as dates, early sex determination could enhance breeding efforts by creating gender-specific genetic pools (Bekheet and Hanafy 2011).

In date palms, sex has been determined using Random Amplified Polymorphic DNA (RAPD) (Moghaieb et al. 2010). There is currently no trustworthy marker-based technique for determining the sex of date palms cultivated in Egypt. In this work, four specific markers (GPAT3, PALM, SRY and LOX5.1) were used in four of the top date palm cultivars with export quality in Wahat El-Baharya. *GPAT*3, a gene that encodes the glycerol-3-phosphate acyltransferase, is critical for male fertility not only in date palm but also in rice (Torres et al. 2018). In a previous test, *GPAT*3 was found to be highly effective in detecting sex in 315 date palm samples in India (Jani et al., 2022). According to our results, male plants generated one specific band whereas female plants generated heterozygous bands (Figure 2). As described by Jani et al 2022, these data were accurate and confirmed the effectiveness of this marker to be used with the selected Egyptian cultivars. However, the results of the PALM and SRY markers were inconclusive when it came to reliable gender determination. Figure 2 shows the heterozygous bands produced by palm in one of the female cultivars (F1) and the male cultivar. Only one band was visible on the other two female cultivars (F2 & F3) (Figure 2). This suggests that this marker cannot be used to accurately determine the gender from their date palm. *SRY* is a sex-determining gene that is present in both plants and mammals. It can be utilized to identify a fetus's gender as it develops (El-Din Solliman et al., 2019). Furthermore, SRY protein was employed as a molecular factor in date palm (Solliman et al., 2023) and papaya (Liu et al., 2004). It is important to note that the SRY primer sequence utilized in this study differs from that used in the solliman et al., 2023 study in that the primers are situated in the gene's coding region (Figure 6), whereas other SRY primers were created in the gene's flanking region. The *SRY* gene may function as a transcriptional factor since it possesses two High Mobility Group (HMG)-box domains (Wang et al., 2023). In this study, SRY primer was unable to definitively identify the gender of the date palm plants (Figure 2). This conclusion was consistent with that of Jani et al 2022 study, which found that this marker did not distinguish between male and female cultivars as well. The lipoxygenase gene *LOX*5.1, which produces a band in both male and female plants, can be utilized as a homomorphic DNA positive control (Jani et al., 2022). By producing a single band in both genders, LOX5.1 demonstrated that the genotypes utilized in this investigation for males and females were identical (Figure 2).

Table 3. Bands generated by RAPD PCR primers used in this study with their corresponding molecular size as compared with the DNA marker used. Data were retrieved using gel analyzer software.

RAPD Primer	Band Mwt (bp)	F1	F2	F3	M
OPA-01	931	1	1	0	1
	752	1	1	0	1
OPA-02	739	0	1	1	1
	694	1	1	1	1
	569	1	1	1	0
	498	1	1	1	1
	426	0	1	1	1
OPA-03	799	1	1	1	1
	732	1	1	1	1
	631	1	0	0	1
	471	1	1	1	1
OPA-04	1162	0	0	1	1
OPA-06	942	1	1	0	0
	886	1	1	0	0
	725	1	1	0	0
OPA-07	942	1	0	0	0
	886	0	1	0	0
	789	1	1	0	1
	677	1	1	0	0
	478	1	0	0	0
	429	0	1	0	0
OPA-08	875	1	1	0	0
	767	1	1	0	0
	725	1	1	0	0
	544	1	1	0	0
OPA-09	991	0	1	0	1
	968	1	1	0	1
	848	1	1	1	1
	805	1	1	1	1
	758	0	1	1	1
	713	1	0	0	0
	659	1	1	1	1
	609	1	0	0	0
	554	1	1	1	1
	401	1	1	1	1
OPA-10	950	0	1	0	1
	862	1	1	0	1
	799	1	1	1	1
	727	1	1	0	1
	667	1	1	0	1
	539	1	1	1	1
	345	1	1	1	1
OPA-11	972	1	0	1	0
	870	1	0	1	0
	747	1	1	1	0
	645	1	1	1	1
	551	1	0	1	0
	478	1	0	0	0
	308	1	0	0	0
EOD1	993	0	1	0	0
	976	1	0	0	0
	942	0	1	0	0
	892	0	1	1	1
	816	1	0	0	0
	789	0	1	1	1
	726	1	1	0	0
	677	1	1	1	1
	637	1	0	0	0
	536	1	1	1	1
	472	1	1	1	1
	418	0	1	0	0
	372	1	0	0	0
	312	1	1	1	1
	92	1	0	0	0
EOD2	1054	0	1	0	0
	904	1	1	1	1
	806	1	1	1	1
	716	1	1	1	1
	665	0	0	1	1
	575	1	1	1	1
	516	1	1	1	1
	472	1	0	0	0
	410	1	1	1	1
	254	1	1	1	1
	210	1	1	1	1
	106	1	1	1	1
EOD3	975	1	1	1	1
	918	1	1	1	1
	850	1	1	1	1
	800	1	1	1	1
	755	1	1	1	1
	699	1	1	1	0
	649	1	1	1	1
	601	0	1	1	0
	567	1	1	1	1
	513	1	1	1	1
	451	1	1	1	1
	405	1	1	1	1
	350	0	0	0	1
	135	0	1	1	1
EOD4	978	0	0	0	1
	928	1	1	1	1
	863	1	1	1	1
	820	0	0	0	1
	748	1	1	1	1
	672	1	1	1	1
	613	1	1	1	1
	559	1	1	1	1
	463	1	1	1	1
	403	1	1	1	1
	286	1	1	1	1
EOD5	1016	1	1	0	1
	963	1	1	0	1
	876	1	1	0	1
	836	1	1	1	1
	774	1	1	0	1
	710	1	1	0	1
	626	1	1	1	1
	473	1	1	1	1
	427	1	1	1	1
	346	1	1	1	1
	241	1	1	1	1
	158	1	1	1	1
	95	1	1	1	1
EOD6	887	1	1	1	1
	737	0	0	1	1
	680	0	0	1	1
	614	1	0	0	1
	532	1	0	1	1
	427	0	0	1	1
EOD7	1069	1	1	0	0
	1004	1	1	1	**0**
	921	1	1	0	1
	858	1	1	1	**0**
	837	1	0	0	1
	768	1	1	0	0
	737	1	1	0	0
	619	1	0	0	0
	562	1	1	1	**0**
	465	1	1	0	1
	357	1	0	0	0
	315	0	1	1	1
	112	1	0	0	0
EOD8	1054	0	1	0	0
	994	1	1	0	0
	919	0	1	0	0
	882	1	1	0	0
	792	0	1	0	0
	766	1	1	0	0
	680	0	1	0	0
	627	0	1	0	0
	564	0	1	0	1
	477	1	1	0	0
	365	1	1	0	0
EOD9	937	1	1	1	1
	456	1	1	1	1
EOD10	1032	0	0	0	1
	955	1	1	1	1
	899	1	1	1	1
	790	1	1	1	1
	698	1	1	1	1
	651	1	1	1	1
	608	1	1	1	1
	536	1	1	1	1
	465	1	1	1	1
	361	1	1	0	0
OPAA-1	1457	1	0	1	1
	741	1	1	1	1
	599	1	1	1	1
	459	1	1	1	1
	368	1	1	1	1
	264	1	1	1	1
OPAA-2	1017	1	0	1	1
	509	1	0	1	1
OPAA-3	1010	1	0	0	0
	666	1	1	0	1
	444	1	1	0	1
	222	1	1	0	0
OPAA-4	1449	1	1	1	1
	1046	1	1	0	0
	776	1	1	1	1
	611	1	1	1	1
	472	1	1	1	1
	432	1	0	0	0
	116	1	1	1	1
OPAA-5	1293	0	1	0	0
	819	1	0	0	1
	570	1	0	0	1
OPAA-6	1518	1	1	1	1
	1172	1	1	0	0
	1017	1	1	0	0
	761	1	1	1	1
	607	1	0	0	0
	341	1	1	1	1
OPAA-7	1330	1	0	0	0
	894	1	0	0	1
	689	1	1	1	1
	439	1	1	1	1

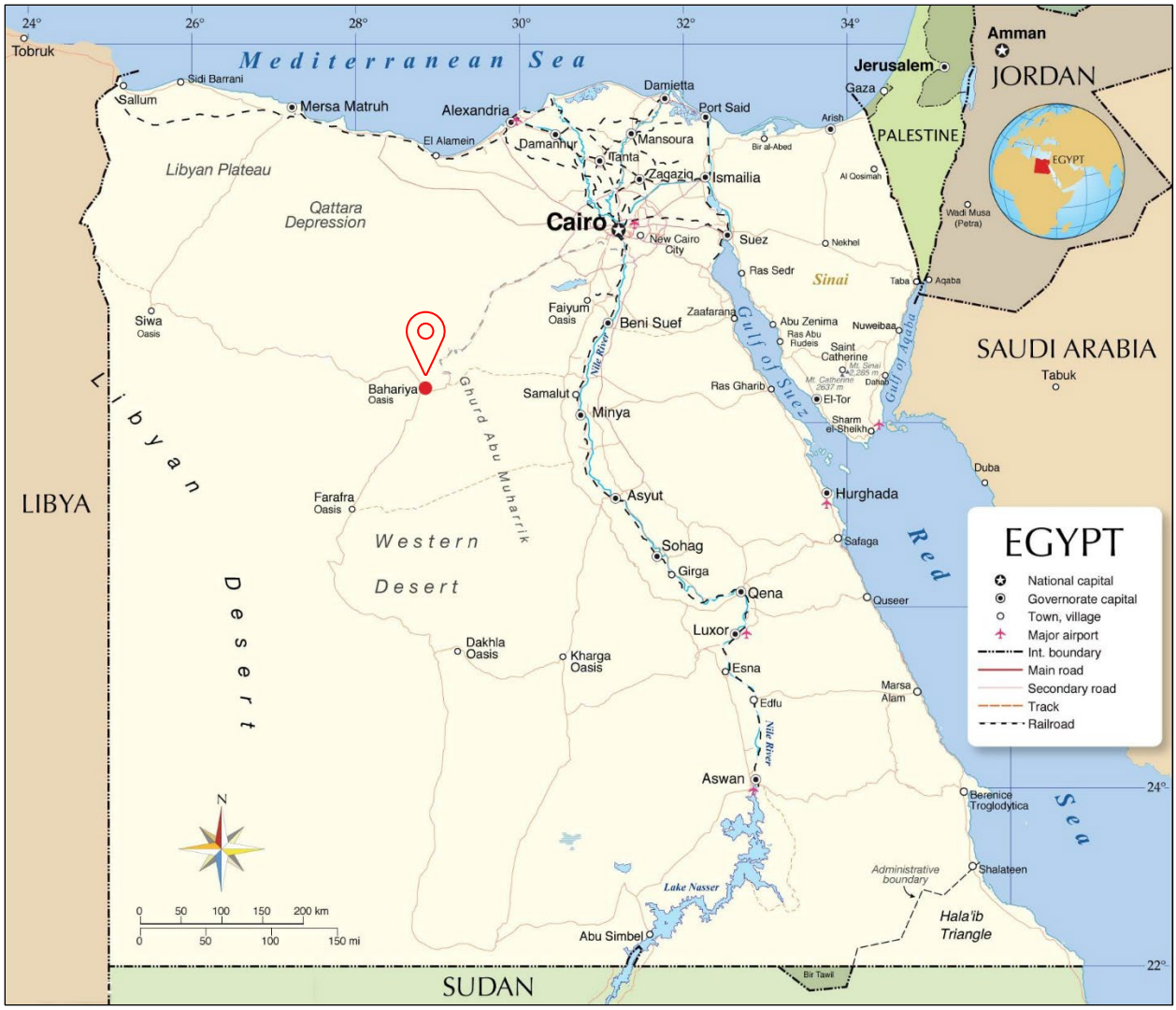

Figure 1. Location of sample collection of the four date palm tree cultivars from El Wahat El-Baharya, Egypt. Curtsey of google map navigator.

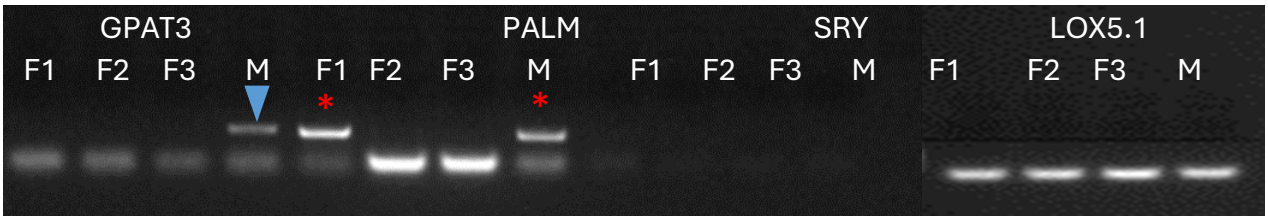

Figure 2. Specific PCR bands generated using four date palm male markers (GPAT3, PALM, SRY and LOX5.1 against genomic DNA extracted from the four cultivars from Wahat El- Baharya. Three females of different cultivars (F1, F2 and F3) and one common male as pollinatoir M.

This served also to exclude the possibility of any DNA from other plants in the field contaminating the sample. To find further polymorphism between males and females, three sets of RAPD primers—OPA, EOD, and OPAA—were employed. RAPD- PCR can generate consistent fingerprint patterns irrespective of the plant source or age (Welsh et al., 1990). Bands of RAPD primers were analyzed using Gel analyzer software. Detectable bands were represented as 1 while absent bands or those below the detection level were represented as (Table 3). Common bands among the four cultivars under investigation were

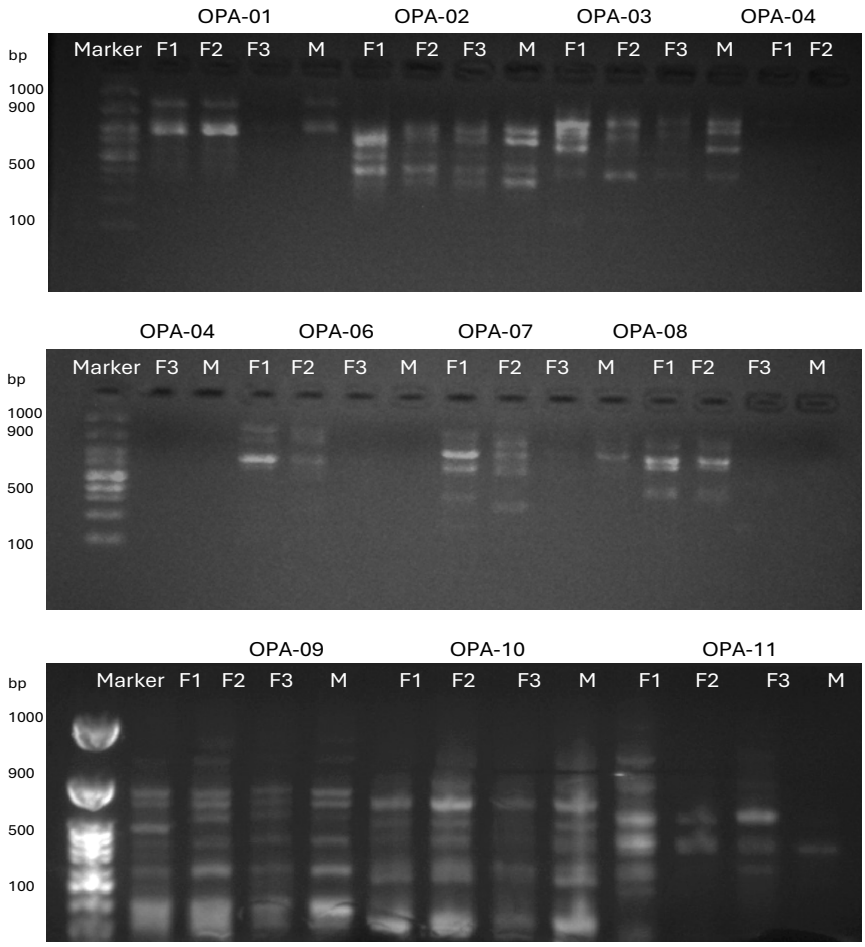

Figure 3. Gel image shows band regenerated against genomic DNA of date palm using RAPD primer OPA 01-011. Highlighted primers are those with polymorphic differences among the cultivars used; green corresponds to general polymorphism while red corresponds to polymorphism due to gender.

highlighted in grey. Unique bands for the male cultivar were highlighted in red and in yellow for the other cultivars (Table 3). Bands represented in red zero are those absent in the male cultivar and present in all other female cultivars (Table 3). On contrary, bands represented in green zero are those absent in one female cultivar while present in all other male and female cultivars.

In this study, some primers failed to characterize cultivars either at the gender or the variety level such as OPA-03, OPA-04, OPA-06, OPA-08 and EOD9 (Table 3). This might suggest that these primers most likely annealed to common areas in the date palm genome distant from the sex region. The above results are out of the scope of this study which aims to distinguish the cultivars at the gender level. Primers OPA-01, OPA-07, OPA-09, OPA-10, EOD1, EOD2, EOD5, EOD6, EOD8, OPAA-1, OPAA-2, OPAA-3, OPAA-4, OPAA-5, OPAA-6, OPAA-7 produced inter-cultivar variation (Table 3 and Figures 3-5). OPA-01 primer generated two bands at molecular sizes 931&752 bp in all cultivars except for the F3 cultivar (Table 3).

OPA-07 primer generated six bands that were all absent in F3 cultivar. The 789 bp band was unique for the male cultivar. The 942 & 478 bp bands were unique for the F1 cultivar, whereas the 886 &429 bp bands were unique for the F2 cultivar (Table 3). OPA-09 primer generated ten bands with five common bands at molecular sizes 848, 805, 659, 554 & 401 bp. The 968 & 758 bp bands were absent in the F3 & F1 cultivars, respectively. On the other hand, bands having molecular sizes 713& 609 bp were only present in the F1 cultivar (Tabel 3 and Figure 3). OPA-10 primer generated seven bands with three common bands at 799, 539 & 345 bp. The band of 950 bp was absent in the F1 and F3 cultivars and present in the F2 & male cultivars. The bands at molecular sizes 862, 727 & 667 bp were present in all the cultivars used except the F3 cultivar (Table 3, Figure 3).

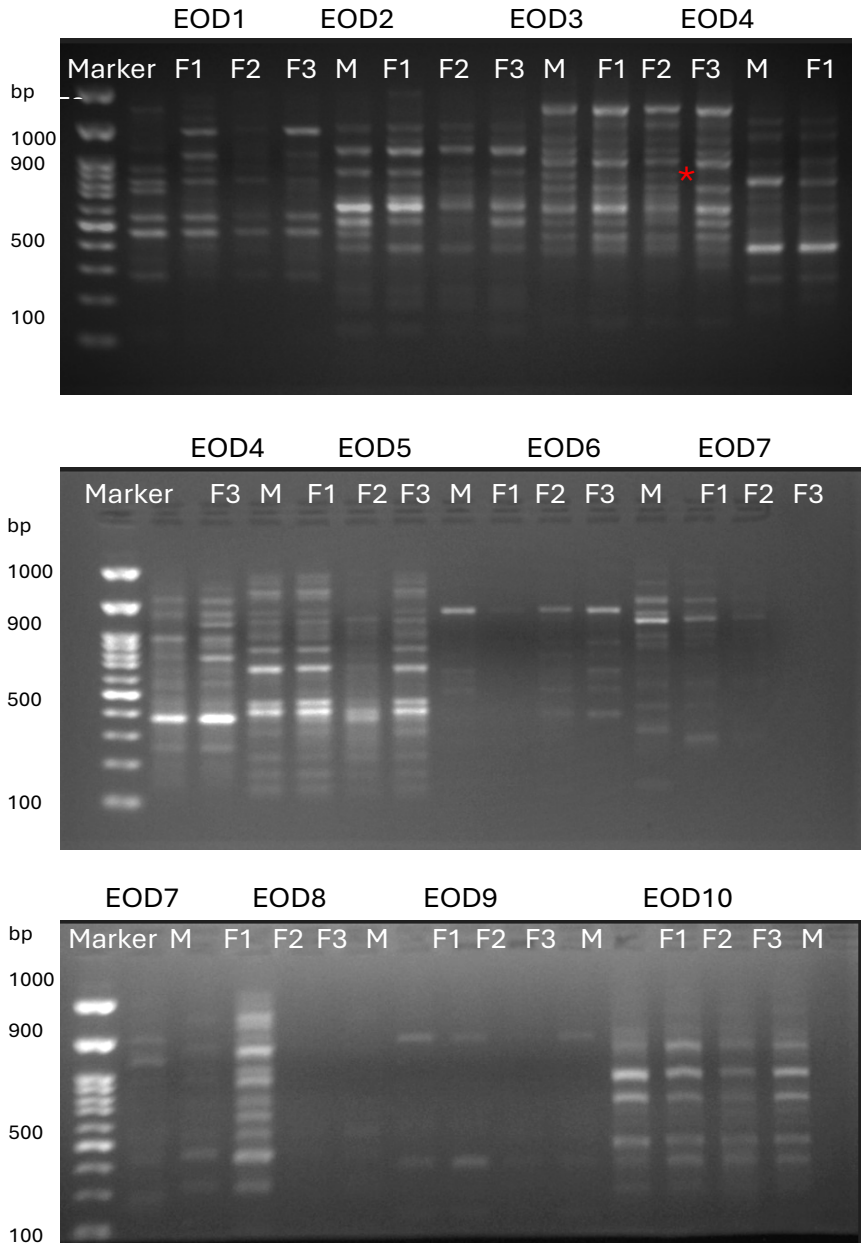

Figure 4. Gel image shows band regenerated against genomic DNA of date palm using RAPD primer EOD 1-10. Highlighted primers are those with polymorphic differences among the cultivars used; green corresponds to general polymorphism while red corresponds to polymorphism due to gender.

OPA-11 primer generated seven bands with one common band at 645 bp. Bands at the molecular sizes 972, 870& 551 bp were present in the F1 and F3 cultivars and absent in the F2 and male cultivars. The band at the size 747 bp was detectable in all cultivars except the male cultivar, thus could be further used for sex determination. Bands of 478& 308 bp were unique for the F1 cultivar (Table 3, Figure 3). EOD1 primer generated fifteen bands with three common bands at the sizes 536, 472& 312 bp. Bands with molecular sizes 976, 816, 637, 372 & 92 bp were unique in the F1 cultivar and could be used for its characterization. Bands with sizes 993, 942& 418 bp were unique for the F2 cultivar and could be used as well for its characterization. The bands of 892& 789 bp were detectable in all cultivars except the F1 cultivar. Although the EOD1 primer generated the highest number of bands, it could not be used to distinguish the cultivars at the gender level (Table 3, Figure 4).

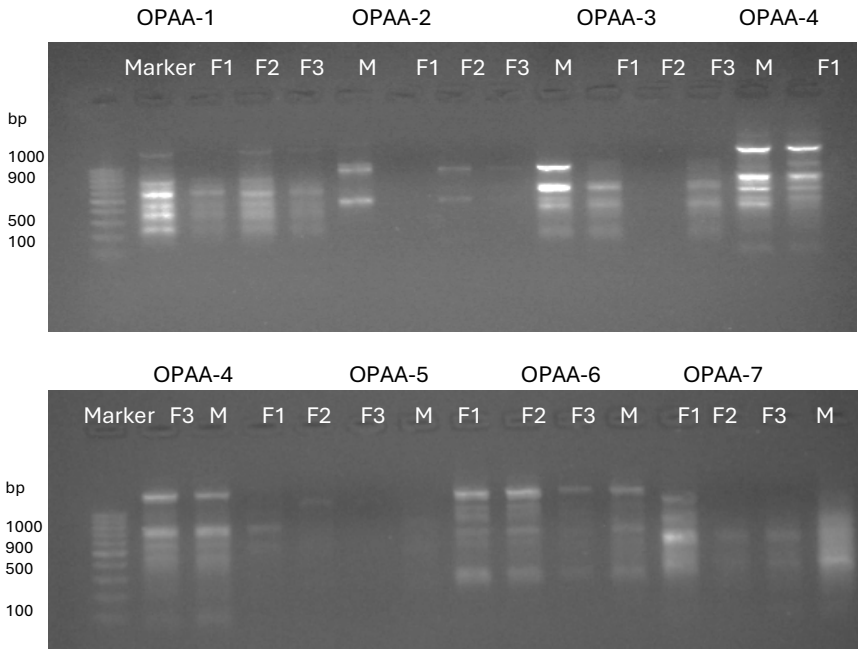

Figure 5. Gel image shows band regenerated against genomic DNA of date palm using RAPD primer OPAA 1-7. Highlighted primers are those with polymorphic differences among the cultivars used; green corresponds to general polymorphism while red corresponds to polymorphism due to gender.

GGGGGGATACCAGTTACCCAAATCGGCCCTCTAAGTATCTGTGCGCAACGGCCAGACATCTTTAGAGGCC
ACTTCTGCGATTCTTGAAGCGACCCTTGAGAGCATTCATCGAGTGGTCTCCGTCCTCACGCGTGGATGGC
TCTCGAGATCCCCCAATGCGACACTCTGAGATCACCTATCGACTGGGATACCGGTGATAAAATGCTCACT
GGAGCCAAGGGATGGACCTTCTTCCCGGAGGTTCAGAGATTTCAGGCCATGCACATAAATAAATGCACGA
GTTATAAATATCCACCTCCTCTGAAGGCGAAAATACTGCCGAAGACTTGCAGTTTGCTTCCCGCTCATCC
CGCTTCGTTACTCTGCTTCGAAGTGCAAACTGGACAACAGGTAAGCGGGGGAAATGGAGGGAACAGGGTA
TGCATAGATTTCGGAGATTCGAACAAATTGGTTGGCACTTTAGGGTTATACAATATATCTTTTCCGTTAT
AAGCCTTAATGGAAGGAAAGGTGGGGGAAATCCATTTTGGATTGAGAAATCGATGTGCCGGTGCTTAACT
TATAATCCAGATTGAAACTTCGTGTTCGTGATCAGGACCCAGCACCAACACCAGCGATGGGGGCGGGCGA
CAATACCACCATAGCGGTTCTAGTAGCAGCAAAAAATAGTGGCATAACAAACATAAGTAGTAGAACATGA
GTAGCAGAAGTTTACCCGGCGCAATAACCGGTGGCACTAGTAGCAGAAGCATAGGCTTTTTGGGTACCGG
GCAGAGGCAAGGCATCGAATCCCTCATCAAGCCCATCCACATCAATAGCTCACTACCCACCAACATCGAA
ATAAGGGGATGAGTCGACTGGCGAATCAGCTGCAATAATTGGACTCGACCAGTGCAACTGGAACAACAGG
TAAA

Figure 6. SRY gene sequence of date palm genome. The figure represents the location of forward and reverse primers which were used in this study. The primer sequences are present within the coding region of the gene. Sequence as retrieved from NCBI data base.

EOD2 generated twelve bands, nine of which were common at the molecular sizes 904, 806, 716, 575, 516, 410, 254, 210&106 bp. The 1054& 472 bp bands were unique for the F2& F1 cultivars, respectively. The 665 bp band was present in the F3& male cultivars and absent in the F1& F2 cultivars (Table 3 and Figure 4). EOD5 generated thirteen bands with eight common bands. Bands with sizes 1016, 963, 876, 774& 710 bp were detectable in all cultivars except the F3, which indicated that this primer (EOD5) could distinguish the F3 cultivar from the male and other females (F1& F2) cultivars. EOD6 primer generated six bands with only one common band at 887 bp. Bands of 737, 680& 427 bp were absent in the F1& F2 and present in the F3 and male cultivars. A 614 bp band was present in the F1& male cultivars and absent in the F2& F3 cultivars. A 532 bp band was present in all cultivars except the F2 (Table 3 and Figure 4). EOD8 primer generated eleven bands. Bands at molecular sizes 1054, 919, 792, 680& 627 bp were detected in the F2 cultivar only. Bands at molecular sizes 994, 882, 766, 477& 365 bp were present in the F1&F2 cultivars and absent in the F3 and male cultivars. The band at the size 564 bp was detected in F2& male cultivars but absent in the F1& F3 (Table 3, Figure 4).

OPAA-1 primer generated six bands with five common bands. A band of 1457 bp was detectable in all cultivars except the F2. OPAA-2 generated two bands with molecular sizes 1017& 509 bp and were present in all cultivars except the F2 cultivar (Table 3 and Figure 5). OPAA-3 generated four bands. A 1010 bp band was unique for the F1 cultivar while the bands at 666 & 444 bp were detectable in all cultivars except F3. A 222 bp band was present in the F1&F2 cultivars and was absent in the F3 & male cultivars (Table 3 and Figure 5). OPAA-4 generated seven bands with five common bands among all cultivars. The band at the molecular size 1046 was present only in the F1& F2 cultivars while the 432 bp band was present only in the F1 cultivar (Table 3 and Figure 5). OPAA-5 primer generated three bands at molecular sizes 1293, 819& 570 bp. The largest band was unique in the F2 cultivar while the other two bands were only detectable in the F1 and male cultivars (Table 3 and Figure 5). OPAA-6 generated six bands with three common ones. A 607 bp band was unique in the F1 cultivar while the bands at the sizes 1172& 1017 bp were present in the F1&F2 cultivars and absent in the F3 & male cultivars (Table 3 and Figure 5). OPAA-7 generated four bands: two common bands at sizes 689& 439 bp, unique band for the F1 cultivar at 1330 bp and an 894 bp band that was detected only in the F1& male cultivar (Table 3 and Figure 5).

The primers that produced intervarietal and gender variation were OPA-02, OPA-11, EOD3, EOD7 (Table 3, Figures 3& 4). OPA-02 primer generated five bands. The bands exhibiting molecular sizes 694 & 498 bp were common bands that have been detected in all cultivars. The 739 & 426 bp bands were absent in the F1 cultivar. The absence of a 569 bp band in the male cultivar might indicate a deletion within the male genome that could be used for sex differentiation (Table 3 and Figure 3). OPA-11 primer generated seven bands including one common band. A 747 bp band was absent in the male cultivar but present in other female cultivars. The bands at 478& 308 bp were unique for the F1 cultivar (Table 3 and Figure 3). EOD3 primer generated fourteen bands with ten common bands. The band of 699 bp was detectable in all cultivars except the male, while the band of 350 bp was detectable only in the male cultivar. Thus, EOD3 primer could be used to distinguish the gender of date palm tree among the cultivars used in this study. In addition, the band of 135 bp was detectable in all cultivars except the F1 (Table 3 Figure 4). EOD7 generated thirteen bands. The bands at molecular sizes1004, 858& 562 bp were detectable in all female cultivars except for the male. Thus, these bands could be used to distinguish the male cultivar from other females. Bands at the size 619, 357& 112 bp were unique in the F1 cultivar. The bands 921& 465 bp were detectable in all cultivars except F3 (Table 3, Figure 4).

The primers that can distinguish the cultivars at the gender level were EOD4 and EOD10 (Table 3, Figure 4). EOD4 primer generated eleven bands with nine common bands. Interestingly, bands at molecular sizes 978& 820 bp were unique in the male cultivar. This could indicate that this marker is ideal to distinguish the gender of date palm cultivars (Table 3 and Figure 4). EOD10 generated ten bands with eight common bands. The band at 1032 bp was unique for male cultivar and can be used to distinguish the gender. A 361 bp band was present in the F1& F2 cultivars and absent in F3 & M cultivars (Table 3 and Figure 4).

CONCLUSIONS

This study used four specific primers and twenty seven RAPD molecular markers to identify the sex of date palm in four cultivars collected from Wahat El-Baharya, Giza, Egypt. The four specific primers were GPAT3, PALM, SRY and LOX5.1. GPAT3 primer was able to distinguish between male and female cultivars. On the other hand, PALM and SRY failed to execute this task. LOX5.1 is a palm specific marker and consequently gave one specific band in all male and female cultivars. From RAPD- PCR markers, EOD4 and EOD10 were robust at distinguishing the gender of date palm cultivars used in this study.

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