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Effect of pollen source on fruit set, yield, and physical properties of tissue culture-derived and offshoot-derived date palm, cv. 'Barhi' and' Piarom'

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ABSTRACT

The present study was carried out to evaluate the effects of different pollen sources, i.e. genotypes 7001 (control), 7005, 7013, 7030, and tissue culture-derived 'Boyer 11', on two maternal plant cultivars, i.e. 'Barhi' and 'Piarom' (tissue culture-derived and offshoot-derived). The quantitative properties of fruits were evaluated for a period of two years (2018-2019). A population of 30 ten-year-old trees was selected from each cultivar. Fruit physical properties were measured, including fruit length and diameter, the fruit length-to-diameter ratio, fruit volume, seed length and diameter, seed weight, and yield. The results showed that the highest percentage of natural fruit set was related to offshoot-derived trees of cv. 'Barhi', with pollen from genotypes 7030 and 7013. The lowest was found in tissue culture-derived trees of cv. 'Piarom', with pollen from the genotype 7001 (control). In general, offshoot-derived 'Barhi' and 'Piarom' cultivars were preferable to their tissue culture-derived trees, since they caused better quantitative and qualitative traits. Furthermore, genotypes 7013 and 7030 were selected as the most suitable pollinizers because of increasing the percentage of fruit set, reducing the number of parthenocarpic fruits, and improving the quantitative and qualitative properties of fruits in tissue culture-derived and offshoot-derived date palm cultivars ('Barhi' and 'Piarom').

Introduction

Date palm (*Phoenix dactylifera* L.) is a dioecious tree from Arecaceae and is one of the prominent members of this family (Elmeer et al., 2019). Date palm fruit is one of the first crops cultivated in the Middle East (Abdul-Hamid et al., 2020). As an important commercial product, it plays a significant role in the fruit industry of Iran (Hazzouri et al., 2015). Iran ranks first and third in the world in terms of area under cultivation and production of dates, respectively. According to FAO, in 2020, by producing about 1.3 million tons of dates, Iran allocated 10% of the global production share (FAO, 2020). The area under date palm cultivation in the world currently reaches more than 950 thousand hectares, of which about 268 thousand hectares belong to Iran (Ahmadi et al., 2019).

'Barhi' and 'Piarom' are desirable cultivars with commercial value because of their high-quality fruits. They are mainly grown in Khuzestan and Hormozgan Provinces, Iran (Ahmadi et al., 2019).

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Conventionally, the date palm is propagated through seeds and offshoots (Shah, 2014). Offshoots are outgrowths that develop from the base of date trees. They grow from both male and female trees. Using them is a common method of propagating date palm trees. Propagation of *Phoenix dactylifera* L. through seeds is not considered for commercial purposes due to heterozygosity (Fki et al., 2017). Normal vegetative propagation is done through offshoots, which have a slow growth rate, and the number of offshoots is limited (Fki et al., 2017). Date palm production through tissue culture is widely preferred for mass plant production (Aldaej et al., 2014; Aleid et al., 2015). The tissue culture method allows the rapid increase of female trees of the selected cultivars. However, there are some abnormalities in the structure of flowers and flowering, as well as the disruption of fruit set and the lack of commercial fruit set (Bouhouche et al., 2006; Awad, 2006), which have limited the commercial development of groves using tissue culture-derived trees. Despite the numerous advantages of date palm propagation through tissue culture, several drawbacks exist in the growth and yield of tissue culture-derived trees (Mohammadi et al., 2017). Awad (2006) showed that one of the most significant weaknesses of tissue culture techniques is the emergence of undesirable plants due to somaclonal variation. Al-Khalifah and Askari (2011) investigated plants that do not resemble their parents and reported that somaclonal variation occurred due to the variability of the genome, the ability to change in structure, and in response to internal and external conditions before tissue culture. They stated that all of them were due to somatic diversity caused by genetic or epigenetic changes during the tissue culture process. Somaclonal changes can be divided into genetic and epigenetic ones (Abass and Awad, 2019). In another study, various types of abnormalities were reported among tissue culture-propagated trees, which are often characterized by phenotypes with low fruit yield (Abd-Elhaleem et al., 2020). In such palms, most of the three-carpel flowers are formed as parthenocarpic fruits with three carpels, and in extreme cases, additional carpels are formed (Shair et al., 2016). In addition, incompatible pollen or ineffective pollination reportedly caused such abnormalities. The date palm is a dioecious species. Male and female flowers are produced separately on male and female trees (Abeed et al., 2020). Pollination is one of the most important and sensitive operations in an orchard for date palm trees. It plays a very important role in fruit production for improving fruit quality and quantity (El-Hamady et al., 2010).

Iqbal (2017) investigated the effects of pollen on the fruit set, as well as quantitative and qualitative features of fruit yield of two date palm cultivars ('Daki' and 'Zahedi'). The said research indicated that the amount of fruiting, fruit fall, fruit yield per bunch, and fruit size were significantly affected by the compatibility level between male and female cultivars. Many studies have been conducted on the effect of pollen grain type on fruit properties of date palm cultivars. The type of male pollen grain directly affects the quantitative and qualitative aspects of the fruit of the female cultivar (Merwad et al., 2015). It has been reported that one of the reasons for the reduction in fruit set in tissue culture-derived date seedlings is the improper growth of pollen grains (Cohen et al., 2004). In previous studies, pollinizer cultivars reportedly showed similar effects on fruit set rate, final date palm fruit yield (Omar et al., 2014), lower fruit set percentage, and higher parthenocarpic percentage in tissue culture-derived date palms compared to offshoot-derived ones (Cohen et al., 2004).

Shair et al. (2016) reported that unsuccessful pollination and little fruit set were observed for the 'Barhi' cultivar. Abd-Elhaleem et al. (2020) showed that in all pollinated bunches, 80 to 100% of the fruits were parthenocarpic and sometimes had more than three carpels, which are very common among date palm trees produced by tissue culture and are associated with a low fruit set. Although this phenomenon has been observed in other date palm cultivars, its incidence is more consequential in the 'Barhi' cultivar, which often reaches 59-86% (Shair et al., 2016). This abnormality in tissue culture-derived date seedlings is probably due to the slow growth of the pollen tube in the early stages of the fruit set and the high amount of abscisic acid at this stage (Ail-Dinar and Alkhateeb, 2005).

It has also been reported that the pollen grain source affects the fruit yield of date palm trees (El-Badawy et al., 2019). One of the most significant factors in successful pollination and fruit set is the degree of viability of pollen grains, which can directly affect the amount of fruit set (Munir et al., 2020). Some researchers also believe that low yields in trees are related to insufficient viability and germination ability of pollen grains (Giordani and Ferri, 2014).

In recent years, the production and cultivation of tissue culture-derived plants of 'Barhi' and 'Piarom' cultivars have increased significantly. There is no difference between offshoot-derived and tissue culture-derived plants at the vegetative growth stage. However, unnatural parthenocarpic multicarpel fruit production has highly reduced

the production and yield of date palm trees, which is a cause for concern for date palm growers. In addition to Iran, this problem has also been observed in other date-producing countries, which has created a challenging problem in cultivating tissue culture-derived cultivars by date palm growers. The present study aimed to evaluate the effects of pollen variation, sourced from different genotypes, on the percentage of fruit set, parthenocarpic fruits (seedless), and the quantitative properties of fruits in tissue culture-derived and offshoot-derived date palm cultivars ('Barhi' and 'Piarom').

Materials and Methods

Plant material and experiment site

The present study was conducted on 10-year-old date palm trees (tissue culture-derived and offshoot-derived date palm cultivars 'Barhi' and 'Piarom') in a research station field in Jahrom. The altitude of the research station is 1070 meters above sea level and the location is situated between a longitude of 52°45'4'' and a latitude of 28°19'10''.

In February 2018, female and male trees were selected and labeled by marking the tree trunks. Mature 'Barhi' and 'Piarom' cultivars were used as mother plants. For pollination, four genotypes originated from different seedlings, i.e. 7001 (control), 7005, 7013, 7030 genotypes, and tissue culture-derived 'Boyer 11' (imported) in the research station field in Jahrom.

Mature male spathes were selected from the pollinizer trees during the flowering season in March 2019 and 2020. After collecting and drying, their pollen was placed in a desiccator and stored in a refrigerator at 4-5 °C before pollination. Sufficient care was taken at all stages of pollen collection and pollination so that none of the pollen grains were mixed. Five spathes were selected from each female date palm (offshoot and tissue culture) to evaluate the effects of different pollen grains on the amount of fruiting. Then, each of the five fully-ripe spathes of each female date palm was pollinated with the maternal plant sources (offshoot and tissue culture) with selected pollen grains at the beginning of the second half of April. A perforated Kraft paper envelope was used to isolate the pollinated spathes for seven days to protect the inflorescences from unwanted pollen grains and to prevent mixing different pollen sources until the effective pollination period had passed. For all treatment groups, field maintenance criteria were considered uniformly, including irrigation, spraying, weeding, pruning, and fertilizer application (500 grams of ammonium phosphate, 700 grams of potassium sulfate, and 1,000 grams of urea). During the two years of the experiment (the second half of May, about a month after pollination), 10% of branches at the Hababok stage were selected from each tree (Zargari, 2005). Then, 50 naturally seeded fruits (inoculated flowers), parthenocarpic fruits (non-inoculated flowers), and fruit drops in each bunch were counted (Fig. 1).

Experimental design and treatments

The experiment was managed in a factorial experiment with three factors, laid out in a randomized complete block design (RCBD), with three replications (60 trees). Pollen factor occurred at five levels, including pollen of genotypes 7005, 7013, 7030, tissue culture-derived 'Boyer 11' (imported), and local pollen 7001 (control). The cultivar factor occurred at two levels ('Barhi' and 'Piarom'). Maternal plant sources occurred at two levels (tissue culture and offshoot).

Measurement of traits

Germination percentage of pollen grains

The pollen germination percentage test was done based on a method used by Ismail (2014) and Rigamoto and Tyagi (2002) to ensure the germination percentage of the pollen grains before pollination.

Measurement of the percentage of natural fruit set, parthenocarpic (seedless) fruit, and fruit drop.

In each bunch, fruit set, parthenocarpic (seedless) fruits, and fruit drops were counted and recorded using the following equations (Alasasfa, 2021).

(1)

$$\text{Fruit set}(\%) = \frac{\text{Total number of normal fruit set}}{\text{Total number of fruit}} \times 100$$

(2)

$$\text{Fruit parthenocarpic}(\%) = \frac{\text{Total number of fruit parthenocarpic}}{\text{Total number of fruit}} \times 100$$

(3)

$$\text{Fruit drop}(\%) = \frac{\text{Total number of fruit drop}}{\text{Total number of fruit}} \times 100$$

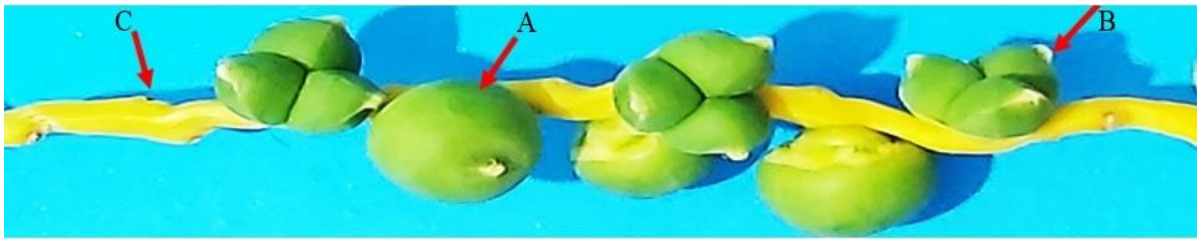

Fig. 1. A normally fertilized fruitlet (A), a triple parthenocarpic fruit (B), and fruit drop (C) in the tissue culture 'Barhi' date palm.

Weight measurement and physical characterization of date palm fruit

A digital caliper (0.01 mm accuracy) was used to measure date palm fruit dimensions (length and diameter). The weight of date palm fruit and seeds was measured using a scale at an accuracy of ± 0.0001 (Cunningham and Sobolewski, 2011). Moreover, after harvesting bunches, the weight of each bunch was measured using a large scale. Fruit volume was measured using a graduated cylinder, based on a water displacement method in cm3, following a method described by the Association of Official Analytical Chemists (A.O.A.C., 1995).

Statistical analysis

Data were collected and statistically analyzed using SPSS ver.23.0 software. Changes in the data were observed in response to pollen and maternal plant source factors. After two years, a combined ANOVA was performed, and their averages were compared using the LSD test at a 5% level ($P \leq 0.05$). The graphs were drawn using Microsoft Excel (2016).

Results

For the two-year analysis of variance, first, Bartlett's test was performed for homogeneity of variances in both maternal plant sources. According to the Chi-Square value and its statistical significance ($P \leq 0.05$), it was observed that the variances were homogeneous for all variables, and a combined analysis of variance was allowed.

In vitro germination of pollen grains

The results of the analysis of variance for the germination power of pollen grains (Table 1) showed a significant difference between different pollen grains ($P \leq 0.01$).

Table 1. Analysis of variance (ANOVA) of the percentage of pollen grain germination during 2018-2019.

Source of variation	Degree of freedom	Mean squares
Year	1	9.63^{ns}
Error of the year	4	24.93
Pollen	4	2599.84^{**}
Year × pollen	4	0.34^{ns}
Error	16	32.64
CV (%)		8.29

^{ns}, **: non-significance and significance ($P \leq 0.01$), respectively.

The comparison of mean values (Fig. 2) showed that the highest germination percentage was related to pollen from genotype 7013 (93.92%), which was significantly higher than other pollinizer genotypes (Fig. 3). Then, pollinizer genotypes 7030 and 7005 were at the same statistical level at 77.75 and 74.67%, respectively, and did not show any significant difference. The percentage of pollen grain germination in the control trees was 58%. The lowest percentage of pollen grain germination was observed in tissue culture-derived 'Boyer 11' at 38.75%.

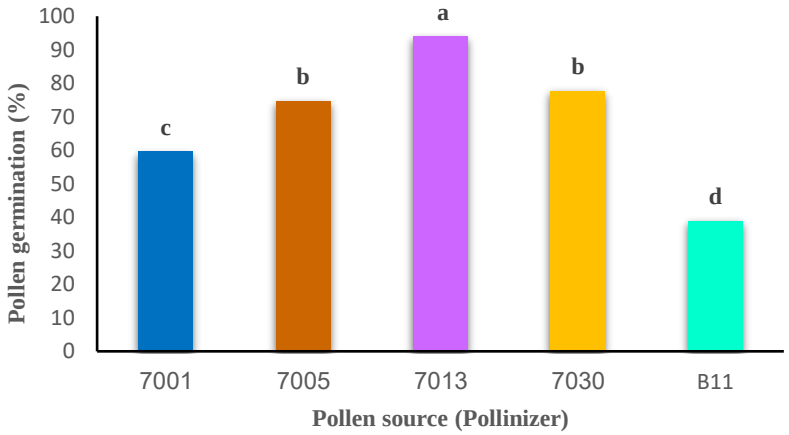

Fig. 2. Comparison of the two-year mean percentage of pollen grain germination. Different letters show a significant difference (P≤0.05) according to the LSD test.

Fig. 3. Schematic view of a male flower, genotype 7013. Image at 10X magnification using a research microscope.

Fruit set percentage (FSP)

According to the analysis of variance, the effects of different pollinizer genotypes, maternal plant sources, and cultivars on FSP were significant (P≤0.01) (Table 2). Furthermore, reciprocal effects of maternal plant source × cultivar, pollen × cultivar, and maternal plant source × pollen were significant on FSP (P≤0.01) (Table 3).

The results showed that pollinizer genotypes showed significant effects on FSP. The highest FSP was observed in the offshoot-derived 'Barhi' cultivar, pollinated with pollen 7013 (67.52%), which was at the same statistical level as pollinizer genotypes 'Boyer 11' and 7030, which did not show any significant difference. The control pollen showed the lowest FSP at 44.73% in the offshoot-derived 'Barhi' cultivar, which significantly differed from other pollinizer genotypes (Table 4).

The tissue culture-derived trees ('Barhi' cultivar) pollinated with pollen from genotype 7030 (42.20%) had the highest FSP, which was significantly higher than other pollinizer genotypes. The lowest FSP was also related to the control (19.37%) in the tissue culture-derived 'Barhi' cultivar. It seems that the use of all pollinizer genotypes increased FSP, compared to the control (Table 4). According to the overall average results, the 'Barhi' cultivar which was pollinated with pollinizer genotypes 7030 and 7013 had the highest FSP, compared to the control (Table 4 and Figs. 4 and 5).

Table 2. Results of a combined analysis of variance of the effect of cultivars, maternal plant source, and different pollinizer genotypes on date palm fruit length and diameter, seed length and diameter, and crop yield.

Mean square	Degree of freedom	Mean squares				
		Fruit length	Fruit diameter	Seed length	Seed weight	Yield
Year	1	12.59^{ns}	175.64**	60.79*	0.26**	14831473*
Error	4	5.67	1.98	3.31	0.00	1064088
Cultivar	1	2289.61**	678.01**	1395.71**	2.60**	465614233**
Year × cultivar	1	19.82*	98.71**	0.29^{ns}	0. 36**	146396173**
Maternal plant sources	1	75.09**	15.88**	75.54**	0.42**	1569990680**
Year × maternal plant sources	1	0.18^{ns}	45.21**	8.94*	0.18**	4297338^{ns}
Maternal plant sources × cultivar	1	0.26^{ns}	1.40^{ns}	3.61^{ns}	0.00^{ns}	105589910**
Year × maternal plant sources× cultivar	1	0.29 ^{ns}	13.60**	20.36**	0.01^{ns}	100045445**
pollen	4	3.63^{ns}	1.20^{ns}	0.72^{ns}	0.00^{ns}	22938492**
Year ×pollen	4	2.67^{ns}	6.77**	3.79^{ns}	0.01*	1346778^{ns}
pollen× Cultivar	4	3.44^{ns}	5.03*	2.27^{ns}	0.00^{ns}	6524454**
Year ×pollen × cultivar	4	5.77^{ns}	3.27^{ns}	1.67^{ns}	0.01*	1272943^{ns}
Maternal plant sources × pollen	4	0.46^{ns}	9.19**	1.44^{ns}	0.00^{ns}	10525397**
Year × maternal plant sources × pollen	4	0.17^{ns}	5.97**	1.93^{ns}	0.00^{ns}	2154242^{ns}
Maternal plant sources × pollen × cultivar	4	1.17^{ns}	5.72**	2.05^{ns}	0.00^{ns}	5093198**
Year × maternal plant sources × pollen × cultivar	4	0.25^{ns}	7.79**	3.91^{ns}	0.00^{ns}	334547^{ns}
Error	76	4.57	1.61	1.72	0.00	1271092
CV (%)		5.7	5.8	5.9	7.0	17.1

^{ns}, *, ** indicates non-significance and significance (P≤0.05) and (P≤0.01), respectively.

Table 3. Results of a combined analysis of variance of the effect of different factors on the percentage of natural fruit set, parthenocarpic fruit, as well as flower and fruit abscission.

Mean square	Degree of freedom	Mean squares		
		Fruit set	Seedless fruits	Fruits drop
Year	1	556.63*	1.16*	1064.81**
Error	4	57.49	0.06	13.32
Cultivar	1	553.02**	42.26**	152.81^{ns}
Year × cultivar	1	8.22^{ns}	0.44^{ns}	2.00^{ns}
Maternal plant sources	1	16299.05**	7.63**	12744.81**
Year × maternal plant sources	1	61.21^{ns}	1.46^{ns}	1.39^{ns}
Maternal plant sources × cultivar	1	670.38**	1.34^{ns}	406.79*
Year × maternal plant sources × cultivar	1	56.34^{ns}	2.10*	0.13^{ns}
pollen	4	465.05**	4.46**	251.15*
Year × pollen	4	26.08^{ns}	1.70**	21.63^{ns}
pollen × Cultivar	4	437.12**	2.49**	548.45**
Year × pollen × cultivar	4	34.79^{ns}	0.71^{ns}	51.71^{ns}
Maternal plant sources × pollen	4	365.51**	2.46**	375.19**
Year × maternal plant sources × pollen	4	28.87^{ns}	0.47^{ns}	47.36^{ns}
Maternal plant sources × pollen × cultivar	4	122.33^{ns}	1.33*	136.96^{ns}
Year × maternal plant sources × pollen × cultivar	4	3.40^{ns}	0.08^{ns}	7.81^{ns}
Error	76	63.77	0.45	76.81
CV (%)		19.3	25.1	17.3

^{ns}, *, ** indicates non-significance and significance ($P\leq0.05$) and ($P\leq0.01$), respectively.

Table 4. The interaction of cultivar and pollen on the percentage of biennial fruit set in the maternal plant source

Cultivars	Pollen	Fruit set (%)		
		Offshoot	Tissue Culture	Average
Barhi	7001	44.73^{b}	19.37^{fi}	32.05^{d}
	7005	49.96^{b}	31.54^{bcd}	40.75^{bc}
	7013	67.52^{a}	33.42^{bc}	50.47^{a}
	7030	61.16^{a}	42.20^{a}	51.68^{a}
	B11	64.41^{a}	21.07^{ef}	42.74^{b}
Piarom	7001	46.52^{b}	38.51^{ab}	42.51^{b}
	7005	45.97^{b}	24.39^{def}	35.18^{cd}
	7013	50.61^{b}	28.30^{cde}	39.45^{bc}
	7030	50.22^{b}	34.96^{abc}	42.59^{bc}
	B11	49.37^{b}	23.61^{def}	36.49^{bcd}

In each column, mean values with common letters are not significantly different (P≤0.05), according to the LSD test.

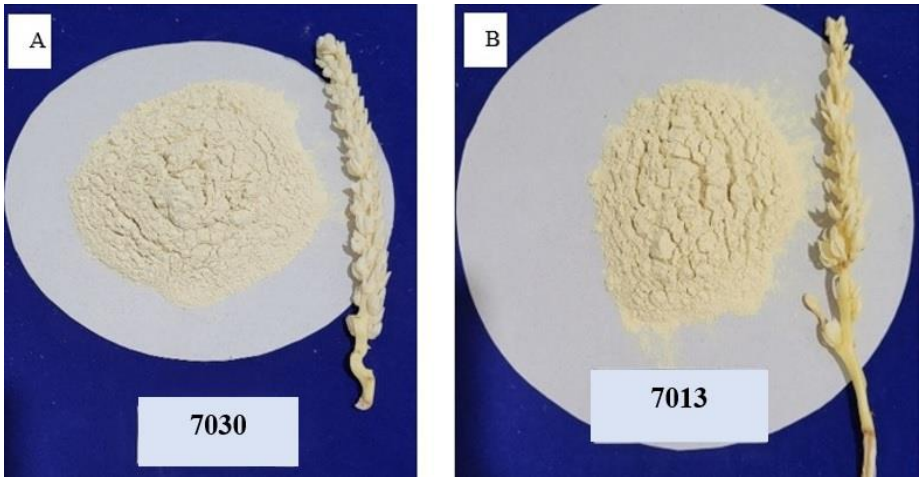

Fig. 4. Pollen grains and branches of male genotype 7030 (A), pollen grains and branches of male genotype 7013 (B).

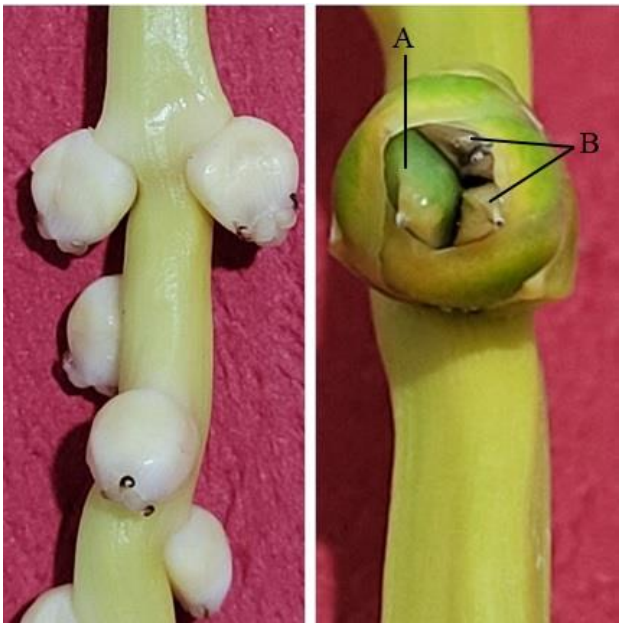

Fig. 5. Date palm flower of the 'Barhi' cultivar on the right, inoculated carpel (A), degenerated two-carpel (B), and unpollinated branches of the 'Barhi' cultivar on the left.

Percentage of parthenocarpic fruits (seedless)

The analysis of variance showed that the effects of cultivar, maternal plant source, and pollen on the percentage of parthenocarpic fruit (seedless) were significant (P≤0.01). Furthermore, pollen × cultivar and maternal plant source × pollen were significant too (P≤0.05) (Table 3). As shown in Table 5, the highest percentage of parthenocarpic fruit was found in the offshoot-derived 'Piarom' cultivar pollinated with the pollen of 'Boyer 11' (15.52%), and the lowest was in the offshoot-derived 'Barhi' cultivar pollinated with the pollen of genotype 7013 (1.36%). In addition, the highest percentage of parthenocarpic fruit was observed in the tissue culture-derived 'Piarom' cultivar pollinated with 'Boyer 11' (16.23%), which was at the same statistical level as pollen 7013 (14.42%) and did not show any significant difference. The lowest percentage of parthenocarpic fruit was related to the control trees (tissue culture-derived 'Barhi' cultivar) at 8.63%, which significantly differed from other pollinizer genotypes (Table 5). According to the results, pollinizer genotypes 'Boyer 11' and 7030 had the highest and lowest percentages of parthenocarpic fruit, respectively (Table 5).

Table 5. The interaction effect between cultivar and pollen on the percentage of biennial parthenocarpic fruits in the maternal plant source

Cultivars	Pollen	Seedless fruit (%)		
		Offshoot	Tissue Culture	Average
Barhi	7001	3.63^{ef}	8.63^{cd}	6.13^{d}
	7005	3.15^{ef}	10.02^{bc}	6.58^{cd}
	7013	1.36^{f}	9.81^{bcd}	5.58^{d}
	7030	2.85^{ef}	0.77^{f}	1.81^{e}
	B11	2.36^{ef}	3.18^{ef}	2.77^{e}
Piarom	7001	12.43^{ab}	12.94^{ab}	12.69^{b}
	7005	8.20^{cd}	10.05^{bc}	9.13^{c}
	7013	9.83^{bc}	14.42^{a}	12.12^{b}
	7030	5.67^{de}	6.28^{de}	5.98^{d}
	B11	15.52^{a}	16.23^{a}	15.88^{a}

In each column, mean values with common letters are not significantly different (P≤0.05), according to the LSD test.

Fruit drop

The effect of maternal plant source and different pollinizer genotypes was significant at 1% and 5% levels, respectively. Furthermore, maternal plant source × cultivar was significant (P≤0.05), while pollen × cultivar and maternal plant source × pollen were significant too (P≤0.01) (Table 3). The highest percentage of fruit drop was related to tissue culture-derived trees ('Barhi' cultivar) pollinated with 'Boyer 11' (75.11%), which showed a significant difference with other pollinizer genotypes. The control (tissue culture-derived 'Piarom' cultivar) had the lowest percentage of fruit drop (Table 6). As shown in the table, the highest (51.6%) and lowest (31%) percentages of fruit drop, in the offshoot-derived 'Barhi' cultivar, were observed in the control and by the pollen of 7013, respectively. Experimental cultivars in terms of the percentage of fruit drop varied from 31 to 51% (Table 6).

The results indicated that the pollen of genotype 7013 caused a lower percentage of fruit drop than other pollinizer genotypes in this research (Table 6).

Yield

The analysis of variance showed that the effect of different pollinizer genotypes, mother plant sources, and cultivars on crop yield was significant (P≤0.05). Furthermore, maternal plant source × cultivar, pollen × cultivar, and maternal plant source × pollen and maternal plant source × pollen × cultivar were significant too (P≤0.01) (Table 2).

The results showed that the offshoot-derived 'Barhi' cultivar pollinated with pollen 7013 had the highest yield (15288.0 kg ha^{-1}), which did not differ significantly from pollen 7030 (15080.0 kg ha^{-1}). The lowest was observed in the offshoot-derived 'Barhi' cultivar (control) at 8028.8 kg ha^{-1} (Table 7). The tissue culture-derived 'Barhi' cultivar pollinated with pollen 7030 led to the highest fruit yield (4472.0 kg ha^{-1}), which did not significantly differ from pollen 7013 (4368.8 kg ha^{-1}). The lowest was found in the tissue culture-derived 'Piarom' cultivar pollinated with 'Boyer

11' (1539.2 kg ha^{-1}) (Table 7).
Based on the overall average, the results indicated that the 'Barhi' cultivar pollinated with pollinizer genotypes 7013 and 7030 had the highest yield compared to the control trees ('Piarom' cultivar) (Table 7).

Table 6. The interaction effect between cultivar and pollen on the percentage of biennial fruit drop in the maternal plant source

Cultivars	Pollen	Fruits drop (%)		
		Offshoot	Tissue Culture	Average
Barhi	7001	51.6^{a}	72.0^{ab}	61.8^{a}
	7005	46.6^{ab}	58.4^{cd}	52.5^{bcd}
	7013	31.1^{e}	56.8^{cd}	43.9^{f}
	7030	36.0^{cde}	57.0^{cd}	46.5^{def}
	B11	33.2^{e}	75.8^{a}	54.5^{bc}
Piarom	7001	41.1^{be}	48.6^{d}	44.8^{ef}
	7005	45.8^{abc}	65.6^{bc}	55.7^{ab}
	7013	39.6^{be}	57.3^{cd}	48.4^{cf}
	7030	44.1^{ad}	58.8^{c}	51.4^{be}
	B11	35.1^{de}	60.2^{c}	47.6^{cf}

In each column, mean values with common letters are not significantly different (P≤0.05), according to the LSD test.

Table 7. The interaction of cultivar and pollen on the percentage of biennial yield in the maternal plant source

Cultivars	Pollen	Yield (kg h^{-1})		
		Offshoot	Tissue Culture	Average
Barhi	7001	13624.0^{ab}	4056.0^{abc}	8840.0^{b}
	7005	12688.0^{b}	3744.0^{bc}	8216.0^{b}
	7013	15288.0^{a}	4368.8^{ab}	9828.4^{a}
	7030	15080.0^{a}	4472.0^{a}	9776.0^{a}
	B11	8944.0^{c}	3432.0^{c}	6188.0^{c}
Piarom	7001	8028.8^{cd}	1726.4^{de}	4877.6^{d}
	7005	6656.0^{d}	2142.4^{de}	4399.2^{de}
	7013	7883.2^{cd}	2329.6^{d}	5106.4^{d}
	7030	7696.0^{cd}	2017.6^{de}	4856.8^{d}
	B11	6281.6^{d}	1539.2^{e}	3910.4^{e}

In each column, mean values with common letters are not significantly different (P≤0.05), according to the LSD test.

Fruit length

The results showed that the effect of maternal plant source and cultivar on fruit length was significant (P≤0.01) (Table 2). As shown in Table 8, the highest fruit length (43.02 mm) was found in the offshoot-derived 'Piarom' cultivar pollinated with pollen 7030, which did not significantly differ from other pollinizer genotypes. The shortest fruit length (33.25 mm) was observed in the offshoot-derived 'Barhi' cultivar pollinated with pollen 7005 (Table 8).
In the tissue culture method, the highest fruit lengths (41.75 and 41.01 mm) were observed in the 'Piarom' cultivar pollinated with pollinizer genotypes 7005 and 7013, respectively, and the shortest (31.61 mm) occurred in the 'Barhi' cultivar pollinated with B11 (Table 8).
The results showed that the 'Piarom' cultivar pollinated with pollinizer genotypes 7005, 7013, and 7030 had the highest fruit length, compared to the effect of pollen 7001 (control) (Table 8).

Fruit diameter

The analysis of variance showed that the effect of cultivar and maternal plant source on fruit diameter had a significant effect (P≤0.01). Furthermore, pollen × cultivar and maternal plant source × pollen were significant too (P≤0.05) (Table 2).

The results showed that the highest fruit diameter (27.81 mm) was found in the offshoot-derived 'Barhi' cultivar pollinated with 'Boyer 11', and the lowest (19.55 mm) occurred in the offshoot-derived 'Piarom' cultivar pollinated with 'Boyer 11'. As shown in Table 8, the tissue culture-derived 'Barhi' cultivar, pollinated with pollen 7005, had the highest fruit diameter (24.20 mm), which had no significant difference with the effects of pollinizer genotypes 7030, 7013, and 7001 (control). The lowest fruit diameter (18.52 mm) occurred in tissue culture-derived 'Piarom' cultivar pollinated with 'Boyer 11' compared to pollen 7001 (control) (19.49 mm) in the same cultivar.

The results indicated that 'Boyer 11' caused a smaller fruit diameter than other pollinizer genotypes in this study (Table 8).

Table 8. The interaction of cultivar and pollen on the biennial fruit length and diameter in the maternal plant source

Cultivars	Pollen	Fruit length (mm)			Fruit diameter (mm)		
		Offshoot	Tissue Culture	Average	Offshoot	Tissue Culture	Average
Barhi	7001	33.53^{b}	32.48^{c}	33.00^{c}	24.15^{b}	23.70^{a}	23.93^{b}
	7005	33.25^{b}	32.00^{c}	32.63^{c}	24.23^{b}	24.20^{a}	24.21^{b}
	7013	33.64^{b}	32.31^{c}	32.98^{c}	23.62^{b}	23.88^{a}	23.75^{b}
	7030	33.91^{b}	32.53^{c}	33.22^{c}	23.79^{b}	24.12^{a}	23.95^{b}
	B11	34.05^{b}	31.61^{c}	32.83^{c}	27.81^{a}	22.98^{a}	25.39^{a}
Piarom	7001	42.80^{a}	40.85^{ab}	41.82^{ab}	19.70^{c}	19.49^{b}	19.59^{c}
	7005	42.82^{a}	41.75^{a}	42.28^{a}	19.60^{c}	19.52^{b}	19.56^{c}
	7013	42.88^{a}	41.01^{ab}	41.94^{ab}	19.70^{c}	19.23^{b}	19.46^{c}
	7030	43.02^{a}	40.78^{ab}	41.90^{ab}	20.18^{c}	19.43^{b}	19.81^{c}
	B11	41.02^{a}	39.76^{b}	40.39^{b}	19.55^{c}	18.52^{b}	19.03^{c}

In each column, the means with common letters are not significantly different (P≤0.05), according to the LSD test.

Seed length

According to the analysis of variance, the effects of maternal plant source and cultivar on seed length were significant (P≤0.01) (Table 2). The results showed that the offshoot-derived 'Piarom' cultivar pollinated with pollen 7030 had the greatest seed length at 26.33 mm, which did not significantly differ from pollinizer genotypes 7001, 7005, 7013, and 'Boyer 11' in the same cultivar. The lowest seed length was observed in the 'Barhi' cultivar pollinated with pollen 7001 (control) at 19.40 mm (Table 9).

As shown in Table 9, the maximum seed length (25.80 mm) was recorded in the tissue culture-derived 'Piarom' cultivar pollinated with 'Boyer 11'. The lowest was related to the tissue culture-derived 'Barhi' cultivar pollinated with 'Boyer 11' pollen at 17.42 mm.

The results showed that pollinizer genotypes 7005, 7030, 'Boyer 11', and 7013 had the greatest seed length, respectively (Table 9).

Seed weight

The results showed that the effect of maternal plant source and cultivar on seed weight was significant (Table 2). The results showed that the highest seed weight was observed in the offshoot-derived 'Piarom' cultivar pollinated with pollen 7001 (control) at 1.14 mm, which did not significantly differ from male genotypes 7030, 7013, 'Boyer 11', and 7005 at 1.12, 1.10, 1.10, and 1.09, respectively. The lowest seed weight was recorded in the offshoot-derived 'Barhi' cultivar with male genotypes 'Boyer 11' and 7001 at 0.81 mm (Table 9).

As shown in Table 9, the highest seed weight was observed in the tissue culture-derived 'Piarom' cultivar, pollinated with the male genotype 'Boyer 11' (1.03 mm), which did not significantly differ from the effect of pollen from male genotypes 7030 and 7001 (control). The lowest seed weight was recorded in the tissue culture-derived 'Barhi' cultivar pollinated with pollen 7005 at 0.67 mm. According to the overall average, the pollen of

genotypes 7001 and 'Boyer 11' had the highest effect on seed weight in the 'Piarom' cultivar, and the lowest seed weight was observed in the 'Barhi' cultivar, pollinated with pollen from genotype 7005 (Table 9).

Table 9. The interaction of cultivar and pollen on the biennial seed length and weight in the maternal plant source

Cultivars	Pollen	Seed length (mm)			Seed weight (g)		
		Offshoot	Tissue Culture	Average	Offshoot	Tissue Culture	Average
Barhi	7001	19.40^{b}	17.66^{d}	18.52^{b}	0/81^{b}	0/71^{c}	0/76^{c}
	7005	19.35^{b}	17.51^{d}	18.42^{b}	0/82^{b}	0/67^{c}	0/74^{c}
	7013	20.29^{b}	18.28^{d}	19.28^{b}	0/86^{b}	0/72^{c}	0/79^{c}
	7030	19.91^{b}	17.89^{d}	18.90^{b}	0/86^{b}	0/70^{c}	0/78^{c}
	B11	19.48^{b}	17.42^{d}	18.45^{b}	0/81^{b}	0/69^{c}	0/75^{c}
Piarom	7001	26.59^{a}	23.80 ^{c}	25.19^{a}	1/14^{a}	1/02^{ab}	1/08^{a}
	7005	26.21^{a}	25.46 ^{ab}	25.83^{a}	1/09^{a}	0/96^{bc}	1/02^{b}
	7013	25.96^{a}	24.34 ^{bc}	25.15^{a}	1/10^{a}	0/97^{bc}	1/03^{ab}
	7030	26.33^{a}	25.19 ^{abc}	25.75^{a}	1/12^{a}	1/03^{ab}	1/07^{ab}
	B11	25.71^{a}	25.80 ^{a}	25.75^{a}	1/10^{a}	1/06^{a}	1/08^{a}

In each column, mean values with common letters are not significantly different ($P \leq 0.05$), according to the LSD test.

Discussion

The present study aimed to evaluate the effects of pollen, sourced from several genotypes, on the natural fruit set percentage, parthenocarpic fruits (seedless), and quantitative properties of offshoot-derived and tissue culture-derived date palm fruits from two commercial cultivars, 'Barhi' and 'Piarom'. Based on the results, different pollen grains affected the quantitative traits of date palm fruits and the commercial cultivar seeds of 'Barhi' and 'Piarom' which are sometimes called metaxenia and xenia. In addition to the pollen source, the maternal plant source plays a central role in the quantitative traits of date palm fruits. One of the most significant factors in successful pollination and fruit set is the degree of viability of pollen grains, which can directly affect the amount of fruit set (Munir et al., 2020). Some researchers believe that a low yield in trees is related to insufficient viability and germination ability of pollen grains (Giordani and Ferri, 2014; Romano, 2006). Therefore, it can be expected that using pollen grains with high germination power in 'Barhi' and 'Piarom' cultivars can increase fruit production. In this study, the germination percentage of the pollen grains was above 92.93%, which highly affected the yield of date palms in both 'Barhi' and 'Piarom' cultivars. Our results showed that the superiority of pollinizer genotypes 7030 and 7013 in the offshoot-derived 'Barhi' cultivar was due to the metaxenia effect of male cultivars 7030 and 7013, which increased the maximum percentage of fruit set percentage. As reported in previous studies, some males have higher fertility than others, and the difference in the fruit set percentage is due to the difference in the amount of pollen grain viability or the difference in the amount of mutual compatibility (Iqbal, 2012). In addition, the low fruit set percentage in tissue culture-derived trees, with different pollen sources, suggested that there are differences in compatibility levels between cultivars and pollinizers. The pollen source significantly affects the fruit set percentage, and the extent of the effect depends on the cultivar (Islam, 2017). A similar study showed that the pollen source affected the fruit set and quality of some date palm cultivars (El-Hamady et al., 2010; Mustafa et al., 2014). Our findings supported the results of similar studies (Omar et al., 2014; Iqbal et al., 2012; Mohammadi et al., 2017; Omaima et al., 2015; Shafique et al., 2011) which showed that the pollen source significantly affected fruit set percentage. According to Sarrwy et al. (2014), the pollen source had a metaxenia effect on fruit set percentage, and this effect differed both between years and between male sources. Similar results were also obtained by Omar et al. (2014), Iqbal et al. (2012), and Mohammadi et al. (2017), which were in line with our results. Abd-Elhaleem et al.

(2020) stated that various abnormality types were common among tissue culture-derived date palm trees, often characterized by a low fruit set. According to the results, among the 'Barhi' and 'Piarom' cultivars, the lowest natural fruit set percentage was found in the tissue culture-derived 'Barhi' cultivar. Attaha and Al-Saadi (2015) reported similar results in this regard. In the current research, it seems that the presence of reproductive abnormalities, such as the decrease in the natural fruit set percentage and the increase in the percentage of parthenocarpic fruit in tissue culture-derived trees, is far more than in offshoot-derived ones, the reason for which can be attributed to environmental or endogenous factors or pollen type. The pollen type can be one of the factors in the production of parthenocarpic fruits. Several reproductive abnormalities were observed in tissue culture-derived date palm trees. The abnormalities included low fruit set, production of multicotyledonous fruits, and parthenocarpic fruits. These observations led researchers to perform assessments on tissue culture-derived date trees. Several researchers attributed the problem to how the seedlings are produced in the laboratory while other researchers examined environmental factors in field conditions to assess various causes such as the percentage of pollen germination, pollen tube growth, lack of compatibility between pollinizer (male plant) and pollen receiver (maternal plant), and genetic diversity. In this study, the effects of pollen grains and cultivars were reported as one of the influencing factors on these reproductive abnormalities. The mechanism of fruiting in date trees largely relies on the fact that date palm trees are dioecious. In April, pollination of female trees is usually done with the flowers of male trees. The fruit usually has three carpels. In case of successful fertilization, one of the three carpels becomes a full fruit and the other two carpels are lost. In the case of no successful fertilization, the three carpels grow at the same time but eventually become unsuitable for human consumption.

Tissue culture-derived date palm trees had a different phenotype than offshoot-derived trees, such as the multi-carpel and parthenocarpic fruit set, and these results indicated the effect of pollen grain type and mother plant sources on the percentage of fruit set and parthenocarpic fruits. The impact of the type of pollen grain and maternal plant source on fruit set percentage and parthenocarpic fruits can be attributed to the difference in genetic diversity, pollen grain viability, pollen tube growth, fertilization, as well as different levels of compatibility between pollen types and female trees, which, in turn, are responsible for the ability of pollen grains to germinate. Other researchers similarly reported the effect of the type of pollinizer cultivar on lower levels of fruit set, the final yield of date fruit (Mohammadi et al., 2017; Omar et al., 2014), FSP, and a higher percentage of parthenocarpic fruits in tissue culture-derived date palms, compared to offshoot-derived ones (Cohen et al., 2004). The tissue culture-derived trees had much more early flowers than the offshoot-derived ones, but an increase in the number of seedless fruits or, on the other hand, a high percentage of parthenocarpic fruits was observed (Abd-Elhaleem et al., 2020). It has also been reported that an improper fruit set may be due to the incompatibility of pollen grains (Cohen et al., 2004). Regarding these abnormalities, researchers have stated that many flowers on unnatural trees cause disturbances in the elongation of the pollen tube, and its growth is limited, or there are areas near its connection to the carpel where the direct growth of the pollen tube is stopped, and the tubes grow in different directions, or the growth is stopped completely (Attaha and Al-Saadi, 2015). In another research, it was reported that a low level of fruit set might occur due to insufficient pollination and abnormalities in the structure of flowers (e.g. carpel, style, and stigma). As a result, all three non-fertilized carpels become abnormal, and, thus, small fruits have acquired local names such as Shees or Meg (Zargari et al., 2021). According to Cohen et al. (2016), the flower of date palm fruit consists of three carpels. Usually, after successful pollination and fertilization, one of the carpels grows and turns into a fruit, and the other two carpels are lost. If pollination is inadequate and fertilization fails, or if no fertilization occurs, the carpels may continue to develop into seedless fruits, even if they are not pollinated. These fruits either fall or remain in bunches until the stage of maturity. Such fruits are called parthenocarpic or multicarpel fruits, which have no commercial value (Cohen et al., 2016). Our results supported the findings of previous research (Cohen et al., 2004; Shair et al., 2016). Although this phenomenon was observed in other date palm cultivars, its incidence was higher in the tissue culture-derived 'Piarom' cultivar than in the 'Barhi'. In the present study, a significant amount of parthenocarpic and fruit drop may be attributed to the tissue culturing of 'Barhi' and 'Piarom' cultivars and pollination with pollinizer genotypes 'Boyer 11', 7001, and 7005. Fruit fall is genetically, physiologically, and environmentally regulated, but plant stress and ethylene production are the basis of actual physiological fall (Farkhondeh, 2016). Iqbal et al. (2012) showed that the percentage of fruit fall is different

based on the cultivar. Shafique et al. (2011) reported that the pollen source and the frequency of pollination significantly affected the fruit fall in the 'Daki' cultivar. Zargari (2022) showed that different pollinizer genotypes significantly affected the percentage of fruit drop in tissue culture-derived 'Medjool' cultivar, which is in line with the results of the present study. Therefore, the pollinizer genotypes 7013 and 7030 caused more yields than other pollinizers, which could be attributed to the germination percentage, pollen tube growth, and the compatibility of these male genotypes with the 'Barhi' cultivar. It seems that there is a direct relationship between FSP and yield. With the increase in fruit set, the yield also increases. In this study, males (pollinizers) were variable in pollination response to fruit set and yield. The pollen type in fruit trees was based on the characteristics of the male parent (pollinizers) which can have different effects on the yield and fruit quality of the female cultivars (pollen receiver). In addition, the genetic pollen structure affects crop yield. One of the best potentials for increasing the productivity of date palms is the selection of pollen grains to be compatible with female flowers to improve the yield and quality of the fruit. Many factors affect the yield and fertility of date palm trees in terms of fruit quantity and quality, and one of these factors is the quality of pollen grains, which varies among males (Zargari et al., 2021). The difference in the product can be due to pollen grain quality, germination percentage, and pollen tube growth (Shafique et al., 2011). It has also been reported that the pollen source affects the yield of date palm trees (El-Badawy et al., 2019). A significant difference exists between the percentage of pollen survival among male cultivars. Cultivars with a high percentage of pollen survival reportedly led to maximum fruit formation and yield (Shahid et al., 2017). Similar results were reported by Salomon-Torres et al. (2017), Omar and El-Ashry (2015), Omaima et al. (2015), and Mohammadi et al. (2017), which were in line with the current findings. According to the results, the positive effect of pollen on fruit length was evident. Good pollen can increase the fruit length by affecting the growth of the ovary and embryo. It is concluded that the compatibility between the pollinizer cultivars with the 'Piarom' cultivar was more than the 'Barhi', and the effect of the level of hormones formed in the fruit affected the fruit size. Pollen grains can be effective in forming and growing embryos. They can also define the structure of dendrons and make their size small, large, incomplete, or complete. The emergence of any of these states can affect the growth and development of the fruit. Some fruit growth hormones, such as gibberellin, are supplied from dendrons, and if the supply source of this type of hormone is incomplete or does not work well, fruit growth becomes limited, the fruit remains small in size and does not reach a normal size (Dezhampour and Gerigourian, 2004). Different pollinizer genotypes affect the levels of cytokinin and gibberellin, and the difference in fruit size can be related to the difference between the levels of IAA hormone release. The superiority of pollen in genotypes 7005, 7013, and 7030, compared to others, can be attributed to the genetics of these pollinizer genotypes. Mustafa et al. (2014) and Ashour et al. (2008) reported that the physical properties of the fruit, including the length, are affected by pollen type. Heydari and Abbasi (2011) suggested that the pollen grain type significantly affected the fruit length ('Barhi' cultivar), which was in line with the results of the present study. Based on the results, different pollinizer genotypes showed various effects on fruit diameter. This difference was due to the metaxenia effect of different pollinizer genotypes on fruit diameter. In addition, the results indicated that the fruits of offshoot plants had the highest diameter compared to the tissue-cultured plants. It seems that the superiority of the pollen type to other male pollinizer cultivars can be attributed to the genetics of that pollinizer cultivar. KhajehPortadvani et al. (2016) reported that fruit diameter in the Shahani cultivar was affected by the pollen source of tissue culture-derived 'Boyer 11'. Al-Muhtaseb and Ghnam (2006) and Salomon-Torres et al. (2017) showed that different pollinizer genotypes affected the fruit length and diameter, and this difference was due to the metaxenia effect. Mustafa et al. (2014) and Sakr et al. (2010) reported that the fruit diameter was affected by the pollen grain type. The present study was aligned with previous ones, including research by Siyahsar et al. (2018). The results showed that pollen positively affected the seed length and weight. There is a direct relationship between the increase in the fruit and seed length, and the rise in fruit length is a positive trait in date palm fruit, while this increase in seed length reduces the fruit quality and marketability. The effect of pollen grains on date palm fruit seed characteristics may be related to the type of pollen grains and partial incompatibility between pollinizer cultivars and female cultivars. In this study, the xenia effect of pollinizer genotypes and the type of female cultivar affected the properties of seed length and weight. Nixon (1956) reported that the seed size was influenced by the pollen type, which was in line with the results of this research. It has also been reported that the pollen grain source

significantly affected traits related to the fruit and seed of date palm cultivars ('Barhi' and 'Nabat Seif') (Al-Khalifah, 2006). This finding was not in line with the results of the current study. The effect of pollen on seed weight may be due to the xenia effect, which was in line with the results of previous studies by Krueger (2001) and Mustafa (2001). In general, the maternal plant source and the pollen type were effective in increasing the natural FSP and yield. They were effective in reducing parthenocarpic fruits and fruit drop, thereby improving the quantity of the fruit. The results of this research can be a solution to the big problem of low fruit set percentage in tissue culture-derived trees in areas where date palm cultivation is subject to a significant loss.

Conclusion

The use of suitable pollen is an essential factor for increasing fruit set percentage and reducing parthenocarpic fruits in female date palms (tissue culture-derived 'Barhi' and 'Piarom' cultivars). The results showed significant effects of pollen type on two female date palm cultivars (tissue culture-derived 'Barhi' and 'Piarom'). Based on the results, tissue culture-derived trees had a higher percentage of parthenocarpic fruits and fruit drop, but a lower percentage of natural fruit set, fruit length and diameter, seed length and weight, and yield. In terms of the average fruit length, there was no significant difference between the two maternal plant sources (offshoot-derived and tissue culture-derived). Offshoot-derived trees had a higher fruit set percentage, yield, fruit length, and diameter, as well as seed length and weight. The highest yield and fruit set percentage were observed in the 'Barhi' cultivar. The highest germination percentage was related to pollinizer genotypes 7013 and 7030, and the lowest was observed in tissue culture-derived 'Boyer 11' in both maternal plant sources regarding fruit set percentage and crop production. Therefore, according to these results, it is possible to understand the significant effects of the maternal plant source of 'Barhi' and 'Piarom' cultivars and the pollen type on the quantitative properties of date palm fruits. On the other hand, the use of pollinizer genotypes 7030 and 7013 in offshoot- and tissue culture-derived date palm cultivars ('Barhi' and 'Piarom') was suitable for increasing the highest natural fruit set percentage and reducing parthenocarpic fruits and fruit drop percentage, thereby making the possibility of higher yield and fruit quality.

Conflict of interest

The authors indicate no conflict of interest for this work.

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