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Improving fruit set and yield of tissue cultured date palm cv. Berhi by using a combined pollination technique

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Abstract: Tissue-cultured (TC) date palms produce no fruit or low yield due to abnormal fruit setting. To improve the yield of TC 'Berhi' palms, trees were pollinated using five pollen sources (Gantar, Ghannami, Mazafati, Zahedi, and Jarvis). The experiment was carried out in three replications for two successive years in a randomized complete blocks design. The fruit set, the fruit and seed physical traits at the Khalal stage, bunch weight at the Tamar and Khalal stages, ripeness of Tamar bunch, and the fruit quality at both Khalal and Tamar stages were measured and monitored. Year factor significantly affected the fruit set and the fruit and seed characteristics. Pollen sources affected fruit set and some seed characteristics significantly. Zahedi+Jarvis pollen treatment that induced 50% normal fruit set and the highest ratio of pulp to seed was found superior. It was also a top treatment in Khalal's bunch weight (3.11 Kg). Zahedi+Gantar treatment was realized superior in Tamar's bunch weight (6.00 Kg). Ghannami, Jarvis+Ghannami, and Zahedi+Jarvis treatments produced Khalal's fruits with higher quality indices but Zahedi+Jarvis treatment was superior in fruit quality at the Tamar stage. Overall, the combined application of Zahedi and Jarvis pollens yielded the most desired outcomes.

1. Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important commercial fruit crops of the arid and semi-arid subtropical regions of the world. The global total annual production reaches about 9.1 million tons. Iran with a production of about 1.3 million tons in 2020 is the world's third-largest date producer (FAO, 2020). Although many cultivars of dates grown in the country are popular in the domestic market, there has been a shift in recent years toward cultivars that are in high demand on both the local and international markets. Among such cultivars, 'Berhi' is considered a superior, elite cultivar that has earned high popularity in export markets. The international high demand for this cultivar is because of the rare characteristic of its fruits. Unlike most date cultivars, fruits from 'Berhi' are best marketed at the Khalal stage for their tannin-free, sweet and crispy flesh. However, 'Berhi' fruits are also consumed at Rutab and Tamar stages (Zaid and de Wet, 2002 a) though with lower frequency.

'Berhi' is comparatively a high-yielding cultivar (average of 200 Kg per palm) (Zaid and de Wet, 2002 a) ensuring higher income for the growers, which in turn is driving demand for its propagules to expand its plantations (Mohammadi *et al.,* 2017). The current demands for date palm trees cannot be satisfied with traditional propagation by offshoots (Ali-Dinar *et al.,* 2021), particularly for the cultivars whose offshoot production is low like 'Berhi' (Zaid and de Wet, 2002 a). Alternatively, micropropagation is an efficient method of propagating date palm clones on a large scale (Al-Khayri and Naik, 2017; Ali-Dinar *et al.,* 2021). Nevertheless, despite the numerous benefits of micropropagation, it has also resulted in various abnormalities in the growth and yield of TC (tissue culture)-derived palms.

The most economically damaging disorder, which occurs in high frequency, is floral fertilization failure resulting in abnormal fruit set with a great impact on productivity and crop yield, and predominantly leading to a very low proportion of normal fruitlets that ultimately affects the overall economics of the plantation (Al-Dinar *et al.,* 2021). The abnormal fruits, usually appearing in the form of triple parthenocarpic fruitlets, are of no commercial value. This abnormal fruiting has been reported in date palms in the past years frequently (Cohen *et al.*, 2004; Al-Kaabi *et al.,* 2007; Mohammadi *et al.,* 2017). TC-derived trees of 'Berhi' display a high prevalence of this phenotype, which has made it the most common unstable cultivar in this ground (Cohen *et al.,* 2004; Al-Wasel 2005; Mohammadi *et al.,* 2017; Al-Dinar *et al.,* 2021). The main cause of failure in normal fruit setting is failed fertilization originating from ineffective pollination of the female flowers. Although several reports have reviewed/evaluated the growth and yield of TC-derived date trees (Al-Wasel, 2000; Hajian, 2007; Kavand *et al.,* 2015) few attempts have been undertaken to overcome this shortcoming in the field-established TC-derived date trees, especially 'Berhi' (Mohammadi *et al.,* 2017; Al-Najm *et al.,* 2021). Pollination intervention can be an influential strategy to improve the fruit set and yield of such off-type palms (Mohammadi *et al.,* 2017). However, so far no optimized pollination procedure has been released. Thus, more research is required in this context.

Date palm is a dioecious species and artificial (hand) pollination by man is inevitably done to gain economic yield. There are several reports on the role of pollen source in the fruiting of non-tissue cultured date cultivars (Omar and El-Abd, 2014; Soliman *et al.,* 2020), but similar research on TC-derived date trees are few (Rezazadeh *et al.,* 2013; Mohammadi *et al.,* 2017).

Metaxenic effect reflected as changes in the physical and chemical characteristics of fruits under the direct influence of pollen source has been reported in many date cultivars previously (Nixon, 1934; Shafique *et al.*, 2011; Rezazadeh *et al.*, 2013; Mohammadi *et al.*, 2017; Al-Najm *et al.*, 2021). Exploiting this effect to improve fruit quality is of interest. Overall, despite several works carried out during the past several years, no clear-cut solution has been found for resolving abnormal fruiting and low yield in many TC-derived 'Berhi' trees yet.

The present study was performed to avoid abnormal fruit-setting and improve fruit yield and quality of TC-derived 'Berhi' trees through optimizing pollen source and pollination treatment.

2. Materials and Methods

Plant materials

Field pollination experiments were carried out at Date Palm and Tropical Fruits Research Station in Dashtestan (29°23´ N; 51°5´ E; altitude 50 m), Bushehr, Iran. Fourteen-year-old TC-derived trees of 'Berhi', uniform in growth, were selected as female parents.

Pollen collection and pollination

Pollen grains were collected from five clonally male selections preserved in the date palm germplasm collection at the research station, four locally superior selections *viz.* 'Gantar', 'Ghannami', 'Mazafati', and 'Zahedi (Zahidi)', and one internationally recognized elite clone "Jarvis". Pollen collection and storage were done in February 2018 and 2019 according to the procedure described by Mohammadi *et al*. (2017). To assess the viability of pollen grains, two tests was applied: acetocarmine staining test and *in vitro* germination test (Fig. 1) few days ahead of hand pollination. The pollens used for viability tests had been stored in a refrigerator at 4°C for 4-5 weeks.

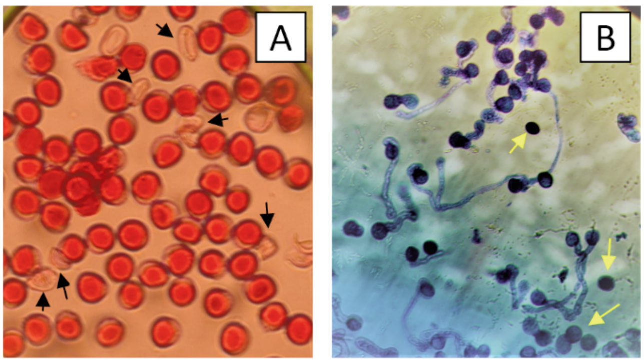

Fig. 1 - (A) Differential stainability of viable and non-viable pollen grains. Red-stained grains are viable and non-stained grains are non-viable. Arrows show abnormal pollen grains, which all are non-viable. (B) Germinated pollen grains with pollen tubes of various lengths. Arrows show non-germinated pollen grains.

Manual pollination was performed on the day of natural spathe craking in early April in the first year and in late March in the second year. Pollens of 'Gantar', 'Ghannami', and 'Mazafati' were used both individually and in combination with each of the Zahedi and Jarvis pollens. A pollination treatment consisting of a combined application of 'Zahedi' and 'Jarvis' pollens was also included. The pollination operation was done according to the traditional method used by the local date growers. In this method, a traditional pollination device, made of cotton fabric and the dried midrib of date palm leaf and filled with fresh pollen, is tapped mildly on two sides of each selected receptive female inflorescence releasing pollen grains gently through the fabric onto the flowers (Fig. 2). The experimental inflorescences were selected on candidate female palms based on uniformity in length (approx. 80 cm) and the number of spikelets per inflorescence (14±2). For the pollination treatments that contained two different pollen sources, an equal quantity of each source was weighed, mixed well with each other, and poured into the pollination device. For each pollination treatment, a separate pollination device filled with the corresponding pollen source(s) was used. To prevent possible contamination of the flowers by foreign pollens, the pollinated inflorescences were then immediately covered with white cotton bags. The bags were removed three weeks later. All targeted inflorescence were tagged properly corresponding to the pollen treatment and the block number.

Fig. 2 - Pollinating female inflorescences through mild tapping of a traditional pollination device, releasing pollen grains gently through the cotton fabric onto the receptive florets.

Calculating fruit set traits

Five weeks after pollination, percentages of normal fruit set, parthenocarpic fruit set, and fruit drop were calculated for each tagged inflorescence. Five strands on each inflorescence were randomly selected. Numbers of normal fruits, parthenocarpic fruits, and flower scars as the representative of dropped flowers and fruitlets were counted separately, recorded, and each one divided by the total number of flowers in the chosen strands, then multiplied by 100.

Measuring bunch weight and bunch ripeness

In the first year of the study, each tagged bunch was harvested separately on September 18 and shaken off to collect the whole fruits. The weight of total fruits of each bunch was considered as the bunch weight, and the percentage of bunch ripeness was calculated based on the weight ratio of the bunch Tamar fruits to total fruits of the same bunch (including Tamar, Rutab, and Khalal fruits). In the second year, the weight of the whole Khalal fruits of each tagged bunch was considered as the bunch weight at the Khalal stage.

Measuring fruit and seed physical characteristics

During both experimental years, fruit sampling was done in mid-August at the Khalal stage. Fifteen single normal fruits were picked randomly from each tagged bunch, and the weight, length, diameter, and volume of the fruits and seeds were measured. In addition, pulp weight, the ratios of pulp weight to seed weight, and fruit length to fruit diameter were also calculated.

Measuring fruit phytochemical quality

Fruit phytochemical quality was measured twice; the first year at the Tamar stage and the second year at the Khalal stage. Fifteen normal fruits were harvested from each tagged bunch. Three replicated homogenized samples containing the pulp of five fruits were prepared and analyzed. To measure total soluble solids (TSS), fruit juice was extracted according to Aurand *et al*. (1987) and TSS was read using a digital refractometer (Atago PAL-3, Japan). The pH was measured directly in the extracted juice by a digital pH-meter (AZ 8686, Taiwan). The contents of reducing and total sugars were determined using the procedure described by Lane and Eynon (1923). Titratable acidity (TA) was quantified by titrating a known volume of juice with 0.01 N NaOH, using phenolphthalein as the indicator (AOAC, 2016) and expressed as the percent of acetic acid. The percentage of dry matter and moisture content were determined using 10 g of fruit pulp in a Petri dish dried in an oven at 72°C for 48 h.

Experimental design and Statistical analysis

The field experiment was conducted in a randomized complete blocks design with ten pollen treatments and three replications (blocks). Each block consisted of ten female inflorescences each of them pollinated with one of the ten pollen treatments. Inflorescences in each block were assigned to three female palms. Overall, nine female palms hosted 30 inflorescences uniform in length, and the number of spikelets were assigned for the experimental blocks. The current study was performed over two successive years. To determine the significance level of effect of pollen treatments on fruit-set parameters, and fruit and seed physical characteristics recorded for two years, a combined analysis was performed by GLM procedures of the SAS software package (SAS Institute, Cary, NC, USA). The mean comparison was also performed using Duncan's multiple range tests (DMRT) based on the averages of the two years data at a 5% probability level. For fruit qualitative traits, bunch weight at Khalal and Tamar stages and bunch ripeness at the Tamar stage, a one-way ANOVA was conducted for each year. The mean comparison was also executed for the fruit qualitative traits using DMRT at a 5% probability level.

3. Results

Pollen viability

The results have been illustrated in figure 3. Mean comparison for pollen viability by the staining test showed that the values had a ranging from a maximum of 98% for Zahedi+Mazafati pollens to a minimum of 79.3% for Ghannami pollen (Fig. 3). Apart from Ghannami pollen and Jarvis+Ghannami pollen source, there was not a significant difference among almost all other sources, which had pollen viability above 90%. Pollen viability assessed by the germination test ranged from 24.3 to 74%. The highest score was for Jarvis+Mazafati pollens though not significantly different from that of Zahedi+Gantar pollen source (69.7%) (Fig. 3). The lowest percentages of germinated pollens were observed with Ghannami male (24.3%) and Zahedi+Jarvis source (32.3%). The results revealed that combining Zahedi pollen with each of Gantar, Ghannami, and Mazafati pollens showed that the percentage of germinated pollens was increased significantly in cases of Gantar and Ghannami pollens but not for Mazafati pollen showing a reverse result. The results showed high differences among pollen sources in the ability of *in vitro* germination. Such significant differences can be due to genetic variation and storage conditions. Since the fresh pollen used in

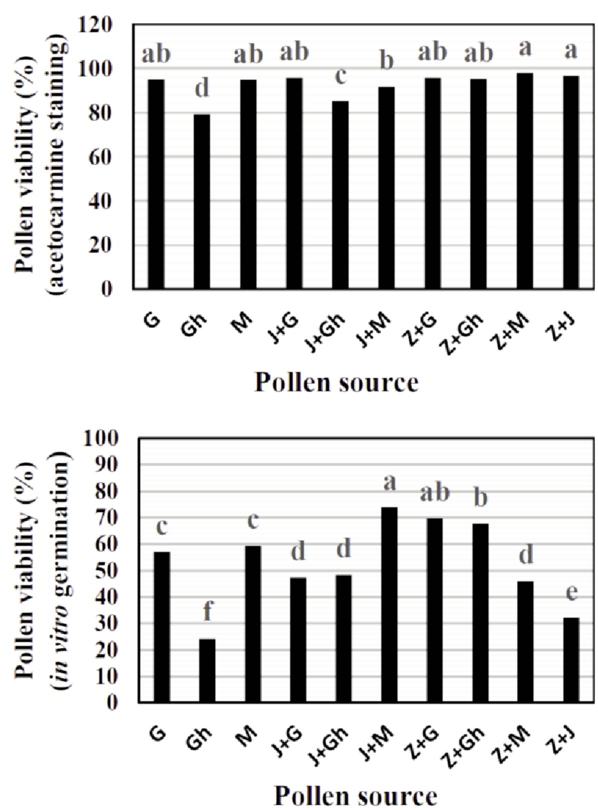

Fig. 3 - Mean comparison of pollen viability for 10 pollen sources tested by staining with acetocarmine and by *in vitro* germination. Means were calculated based on the two years data. Columns having same letter(s) do not show a significant difference with each other using Duncan's multiple range test at P≤0.01. G= Gantar, Gh= Ghannami, M= Mazafati, J= Jarvis, Z= Zahedi.

this study was kept under the refrigerator temperature for a few weeks before the date of hand pollination, the most important variable that differentiated these pollen sources based on their germination ability can be assumed as the genetic differences.

Fruit set

The results (Table 1) revealed that all the experimental variables including year, pollen source, and the interaction effect affected fruit set traits significantly at P≤0.01. Results of the mean comparison test conducted for the main factor (pollen source) have been illustrated in figure 4. The highest values of normal fruit set were achieved with the pollen treatments including Mazafati, Zahedi+Jarvis, and Zahedi+Gantar (53.1%, 49.2%, and 48.9%, respectively) while the lowest parthenocarpic fruit set was with the same pollen sources (18.1%, 10.3%, and 17.7%, respectively).

Mazafati pollen also caused the lowest fruit drop (28.8%) (Fig. 4). Gantar pollen and Jarvis+Gantar pollen treatment yielded the lowest percentage of normal fruits (28.9% and 34.6%, respectively). Gantar pollen also caused the highest percentage of parthenocarpy (36.7%) and a moderate fruit drop (34.4%). Meanwhile, the combined application of Jarvis and Gantar pollens acquired the first rank in fruit drop (43.5%). The interesting point was that the Zahedi+Jarvis pollen treatment caused the lowest parthenocarpy (10.3%) and the second-best normal fruit set (49.2%) which was statistically the same as the highest normal fruit set obtained with Mazafati (53.1%).

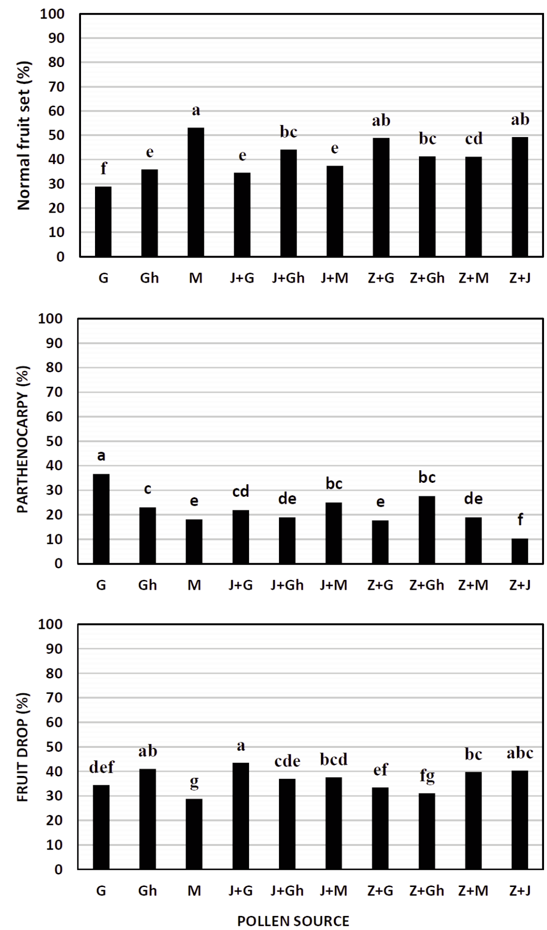

Fig. 4 - Mean comparison of fruit set traits for the ten pollen sources used. Means were calculated based on the two years data. Columns having the same letter(s) do not show a significant difference from each other using Duncan's multiple range test at P≤0.01 (G= Gantar, Gh= Ghannami, M= Mazafati, J= Jarvis, Z= Zahedi).

Table 1 - Combined analysis of variance for fruit set traits and some characteristics of Khalal fruit of TC-derived 'Berhi' date palms pollinated with 10 pollen sources

Fruiting characteristics	Sources of variation (Mean square)					CV%
	Year (df= 1)	Error (year) (df=4)	Pollen source (df=9)	Year × PS (df=9)	Error 2 (df= 36)	
Normal fruit set %	814.82 **	18.485	341.22 **	630.15 **	17.764	10.16
Parthenocarpy %	7863.40 **	20.90	297.24 **	717.87 **	6.712	11.86
Fruit drop %	3602.82**	15.66	132.31 **	129.91 **	8.321	7.86
Fruit weight (FW)	218.80 **	1.401	1.37 NS	3.25 **	0.865	10.47
Fruit length (FL)	49.5 **	1.58	2.02 NS	4.92 **	1.49	4.28
Fruit diameter (FD)	217.74 **	1.77	0.99 NS	3.56 *	1.42	5.22
Fruit volume (FV)	265.44 **	0.80	1.30 NS	3.68 **	1.04	11.71
Fruit volume (FV)	120.98 **	1.573	1.302 NS	19.73 **	0.957	11.22
Seed weight (SW)	0.308 **	0.004	0.016 **	0.024 **	0.003	7.58
Seed length (SL)	0.14 NS	3.135	1.389 **	1.308 **	0.402	3.21
Seed diameter (SD)	2.86 **	0.41	0.435 NS	0.918 **	0.250	5.46
Seed volume (SV)	3.10 **	0.076	0.165 NS	0.185 NS	0.113	23.28
Pulp weight (PW)	206.23 **	1.20	1.001 NS	2.82 **	0.817	11.1
PW/SW ratio	129.71 **	0.563	2.201 *	0.389 NS	1.018	9.45
FL/FD ratio	0.273 **	0.003	0.0028 NS	0.0007 NS	0.002	3.87

NS= not significant; ** significant at P≤0.01; * significant at P≤0.05.

Ghannami pollen that recorded the second-highest fruit drop also caused one of the lowest percentages of normal fruit set among all treatments (35.9%) with moderate parthenocarpy (23%). Overall, we concluded that the Zahedi+Jarvis pollen treatment followed by the Mazafati pollen respectively as the best and the second-best pollen treatments for the improvement of normal fruit set in TC-derived 'Berhi' dates.

Bunch weight and bunch ripeness percentage

Investigating bunch characteristics showed that pollen source affected (P≤0.01) bunch weight at Tamar and Khalal stages as well as the percentage of bunch ripeness. Means comparison for these traits has been depicted in figure 5. The Jarvis+Ghannami treatment caused the highest percentage of bunch ripeness with an average of 92.98%. The lowest bunch ripeness was recorded with Gantar and Jarvis+Gantar sources indicating a significant delaying effect of Gantar pollen on fruit maturation and bunch ripeness compared to other pollen sources.

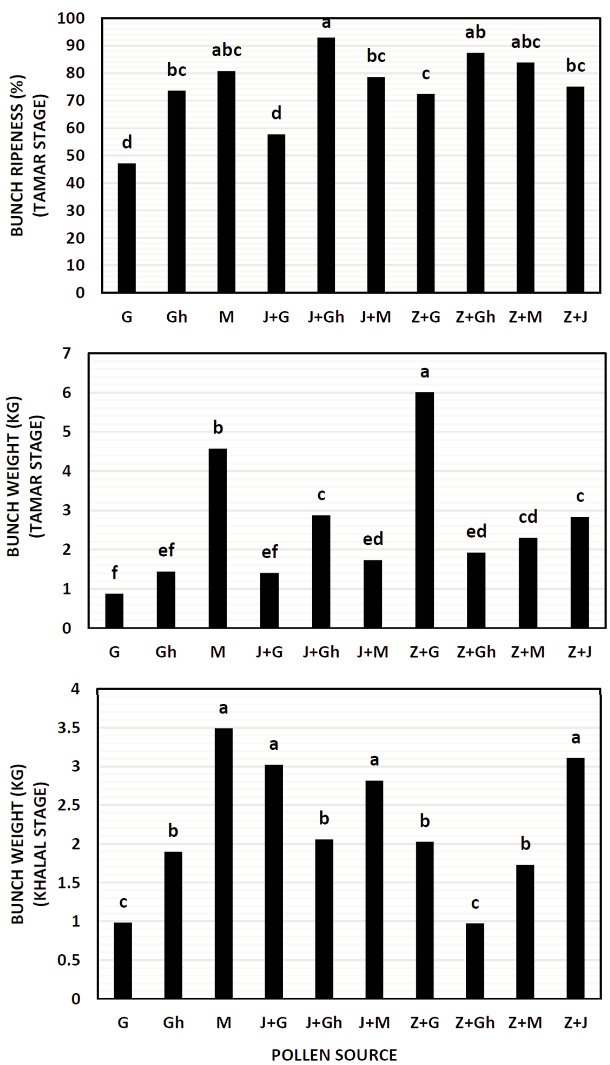

Fig. 5 - Means comparison of bunch ripeness and bunch weight (at Tamar and Khalal stages) for the ten pollen sources used. Columns having the same letter(s) do not show a significant difference from each other using Duncan's multiple range test at P≤0.05 (G= Gantar, Gh= Ghannami, M= Mazafati, J= Jarvis, Z= Zahedi).

The Zahedi+Gantar treatment yielded the Tamar bunches with an average of 6 Kg which were heavier than those of all other treatments (P≤0.05), followed by the Mazafati pollen that yielded the bunches carrying averagely 4.58 Kg fruits (Fig. 5). Gantar pollen appeared as the poorest source since it produced bunches with averagely 0.88 Kg of fruits. Measurement of bunch weight at the Khalal stage (in the second year) showed four pollen treatments including Mazafati (3.49 Kg), Zahedi+Jarvis (3.11 Kg), Jarvis+Gantar (3.02 Kg), and Jarvis+Mazafati (2.82 Kg) were the top-yielding treatments. The poorest pollen sources in Khalal's bunch weight were Gantar (0.98 Kg) and Zahedi+Ghannami (0.97 Kg).

Fruit and seed characteristics

Combined ANOVA showed that the year factor affected (P≤0.01) all the Khalal fruit and seed characteristics except seed length (Table 1). Pollen sources could not affect fruit physical characteristics significantly (Tables 1 and 2), but seed weight and length were affected (P≤0.01) (Table 1). In addition, the pollen source influenced the ratio of pulp weight to seed weight at P≤ 0.05 (Table 1). The means for the fruit and seed characteristics of the normal fruits at the Khalal stage have been presented in Table 2. As the table shows, though pollen sources have influenced these characteristics and some sources have improved them, the differences among the pollen sources have not been significant (P≤0.01) in anyone.

As illustrated in figure 6, the mean comparison for seed weight showed that the lightest seed was produced with Zahedi+Ghannami pollen (0.66 g) though not significantly different from that of Zahedi+Jarvis and Gantar sources. The smallest seeds were obtained with Zahedi+Ghannami pollen (18.7 mm long) and the longest ones with Jarvis+Mazafati source (20.3 mm). The highest weight ratio of fruit pulp to seed (11.94) was obtained in the fruits produced through pollination with the Zahedi+Jarvis pollen treatment.

Fruit phytochemical characteristics

ANOVA revealed that all measured qualitative traits of the Tamar fruits were affected by pollen sources (P≤0.01). In Khalal fruits, however, pollen sources affected TSS, reducing sugars and total sugars at P≤0.01 and titratable acidity, pH, dry matter,

Table 2 - Mean comparison of fruit and seed characteristics for the ten pollen sources used. Means were calculated based on the data from the two year

Pollen sources	Fruiting characteristics							
	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Fruit length/ fruit diameter	Fruit volume (cm^2)	Seed diameter (mm)	Seed volume (cm^3)	Pulp weight (g)
G	8.40	28.4	22.4	1.28	8.20	9.12	0.61	7.67
Gh	9.17	28.4	23.0	1.23	8.77	9.35	0.88	8.38
M	9.58	28.8	22.9	1.27	9.25	9.33	0.68	8.78
J+G	8.92	28.4	23.2	1.23	9.25	8.92	0.81	8.16
J+Gh	8.65	28.3	22.6	1.26	8.37	9.37	0.84	7.86
J+M	9.28	29.2	22.9	1.28	8.95	9.43	0.82	8.45
Z+G	9.03	29.3	23.1	1.27	8.72	9.10	0.83	8.30
Z+Gh	7.88	27.3	22.0	1.25	7.80	8.62	0.68	7.37
Z+M	8.90	28.6	23.4	1.22	8.98	9.48	0.70	8.12
Z+J	9.01	29.1	23.0	1.27 a	8.88	9.07	0.77	8.32

G= Gantar, Gh= Ghannami, M= Mazafati, J= Jarvis, Z= Zahedi
Means in each column are statistically the same at P≤ 0.01 by DMRT.

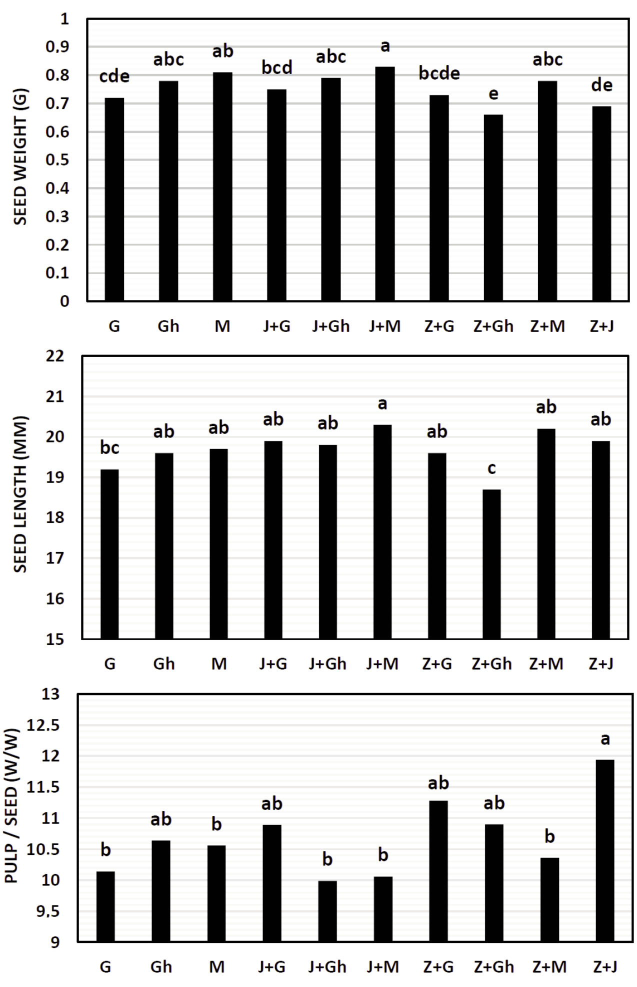

Fig. 6 - Mean comparison of seed weight, seed length, and pulp weight/seed weight ratio for the ten pollen sources used at the Khalal stage. Means were calculated based on the two years data. Columns having the same letter(s) do not show a significant difference from each other using Duncan's multiple range test at P≤0.01 (G= Gantar, Gh= Ghannami, M= Mazafati, J= Jarvis, Z= Zahedi).

and moisture content at P≤0.05.

In Tamar fruits, TSS ranged from 52 to 64 percent so the highest value was obtained by Ghannami pollen and the lowest with Zahedi+Jarvis pollen source (Table 3). Fruit juice pH was in the range of 6.58 to 6.87. The lowest pH belonged to the fruits set by Ghannami pollen (6.58) the same source that earned the highest TSS. The highest titratable acidity (0.028%) was recorded with the Zahedi+Jarvis pollen treatment. Total sugars ranged from 38.86 to 53.74 percent, the highest contents obtained by Zahedi+Mazafati and Jarvis+Ghannami pollen treatments (>53%), and the lowest remained for Zahedi+Ghannami pollen source (38.68%). The content of reducing sugars was significantly the highest with the Jarvis+Ghannami pollen treatment (46.5%). Application of Zahedi pollen in combination with either Ghannami, Mazafati, or Jarvis pollens caused maximum pulp dry matter (~88%) and minimum moisture content (11-12%). This is while the lowest dry matter (76.9%) and the highest moisture content (23.1%) were recorded in the fruits produced by Zahedi+Gantar pollen treatment.

In Khalal fruits, TSS ranged from a maximum of 34.03% induced by Jarvis+Gantar pollen to a minimum of 21.77% made by Gantar pollen (Table 4). Fruit juice pH was not different among most of the pollen sources used (P≤0.05). Titratable acidity showed a narrow range so the lowest value (0.47%) was for the Zahedi+Jarvis source and the highest one (0.58%) for the Zahedi+Ghannami pollen treatment. While the highest amount of total sugars was recorded with Zahedi+Mazafati pollens as well as with Jarvis+Ghannami pollen treatment (53.74% and 53.55%, respectively), the maximum amount of

Table 3 - Mean comparison for some fruit qualitative characteristics of TC-derived 'Berhi' date palms at Tamar stage pollinated by 10 pollen treatments

Pollen sources	Fruit quality characteristics						
	TSS (%)	pH	Titratable acidity (%)	Total sugars (%)	Reducing sugars (%)	Dry matter (%)	Moisture (%)
G	59.3 cde	6.85 a	0.012 cd	45.47 cd	36.42 bc	85.6 abc	14.4 cde
Gh	64.0 a	6.58 c	0.014 cd	47.18 bc	35.63 c	81.9 cd	18.1 bc
M	61.5 abc	6.63 bc	0.016 c	42.73 de	38.81 b	80.6 de	19.4 ab
J+G	54.9 f	6.70 abc	0.011 d	41.11 ef	31.11 d	83.8 bcd	16.2 bcd
J+Gh	62.5 ab	6.60 bc	0.013 cd	53.55 a	46.50 a	86.9 ab	13.1 de
J+M	56.9 def	6.86 a	0.021 b	44.85 cd	35.62 c	86.3 abc	13.7 cde
Z+G	59.0 cde	6.77 abc	0.013 cd	44.32 cd	37.15 bc	76.9 e	23.1 a
Z+Gh	56.5 ef	6.85 a	0.022 b	38.86 f	32.84 d	88.1 ab	11.9 de
Z+M	60.0 bcd	6.87 a	0.022 b	53.74 a	38.11 bc	88.7 a	11.3 e
Z+J	52.0 g	6.79 ab	0.028 a	49.59 b	36.83 bc	88.1 ab	11.9 de

G= Gantar, Gh= Ghannami, M= Mazafati, J= Jarvis, Z= Zahedi
Means in each column are statistically the same at P≤0.01 by DMRT.

Table 4 - Mean comparison for some fruit qualitative characteristics of TC-derived 'Berhi' date palms at Khalal stage pollinated by 10 pollen sources

Pollen sources	Fruit quality characteristics						
	TSS (%)	pH	Titratable acidity (%)	Total sugars (%)	Reducing sugars (%)	Dry matter (%)	Moisture (%)
G	21.77 e	5.74 c	0.55 ab	20.63 c	15.00 d	22.7 abc	77.3 abc
Gh	30.17 bc	5.83 abc	0.57 a	29.55 a	18.19 abc	20.3 c	79.7 a
M	27.57 bc	5.90 abc	0.49 bc	26.23 b	17.93 abc	26.4 a	73.6 c
J+G	34.03 a	6.02 ab	0.48 c	26.51 b	16.46 bcd	23.2 abc	76.8 abc
J+Gh	30.57 b	5.82 abc	0.55 ab	28.05 ab	18.61 ab	20.5 c	79.5 a
J+M	29.33 bc	5.89 abc	0.51 abc	28.69 ab	18.89 a	21.8 bc	78.2 ab
Z+G	26.87 cd	5.80 bc	0.51 abc	26.61 ab	18.20 abc	19.1 c	80.9 a
Z+Gh	28.33 bc	5.99 ab	0.58 a	27.65 ab	18.25 abc	21.0 bc	79.0 ab
Z+M	23.9 de	5.86 abc	0.56 a	22.74 c	16.13 cd	21.6 bc	78.4 ab
Z+J	30.87 b	6.04 a	0.47 c	28.38 ab	17.93 abc	25.3 ab	74.7 bc

G= Gantar, Gh= Ghannami, M= Mazafati, J= Jarvis, Z= Zahedi
Means in each column are statistically the same at P≤0.01 by DMRT.

reducing sugars (46.50%) was obtained only with Jarvis+Ghannami source. Mazafati pollen caused the highest pulp dry matter (26.4%) and the lowest pulp moisture (73.6%). The highest fruit moisture was made by Zahedi+Gantar pollen (80.9%).

4. Discussion and Conclusions

In the present study, different pollen sources revealed various potentials for the improvement of normal fruit set, fruit quantitative and qualitative characteristics, and the bunch yield. The results approved that certain pollen sources were more effective in reducing the problems of abnormal fruit setting. The pollen sources also influenced fruit physical characteristics and bunch yield variably, some with promising performance in tree yield. The effect of pollen source on the fruit set and fruit traits was variable between the two years of the study suggesting a significant interaction with climatic factors. Screening pollen sources for improved fruit yield and quality has been done earlier for some horticultural crops (Fattahi *et al.*, 2014; Kuroki *et al.*, 2017) including date palm (Omar and El-Abd, 2014; Mohammadi *et al.*, 2017; Soliman *et al.*, 2020).

Fruit set characteristics

The results unveiled that the Mazafati pollen was the only pollen source that could set normal fruits averagely beyond 50%. Other tops were Zahedi+Jarvis and Zahedi+Gantar sources both with about 49% normal fruit set. Previously, Mohammadi *et al.* (2017) announced Zahedi pollen as the most promising pollen source with an average of 50.1% normal fruit set for low fruiting TC-derived 'Berhi' date palms. Our results are following theirs confirming the desirable potential of Zahedi pollen in improving normal fruit set in such trees. However, in our work, the percentage of survived parthenocarpic fruitlets was less than 20% in all three superior pollen treatments. The Zahedi+Jarvis pollen treatment caused reduced parthenocarpic fruitlets to 10% whereas Mohammadi *et al*. (2017) reported 32% parthenocarpy for their top pollen treatment (Zahedi pollen). This outstanding reduction in the percentage of parthenocarpy was achieved by performing combined pollination consisting of two elite pollen sources simultaneously *i.e.* Zahedi and Jarvis.

Comparing the singular and combined applications of pollen sources discloses that singular pollination with the Gantar source yielded the lowest percentage of normal fruit set but it behaved variably when it was applied in combination with each of the two prominent pollen sources; Zahedi and Jarvis. These two male sources had been known as superior sources from our previous work (Mohammadi *et al.*, 2017). The combined application of Gantar pollen with Jarvis pollen improved the normal fruit set slightly while its combined application with Zahedi pollen achieved the third rank, not significantly different from the first and the second ranks of pollen treatments in this parameter. Regarding Ghannami pollen, its application in combination with Jarvis source as well as with Zahedi source improved the normal fruit set compared to its singular application. In the case of Mazafati pollen, the opposite results were observed compared to the Gantar and Ghannami source's behaviors. Singular application of Mazafati pollen performed better in normal fruit set than its combined application with each of the Jarvis and Zahedi pollens.

Having looked from another point of view, those pollen treatments that recorded the highest figures for normal fruit set *i.e.* Mazafati, Zahedi+Jarvis, and Zahedi+Gantar, though caused a lower percentage of parthenocarpy compared to their corresponding fruit drop percentages but acted differently in the values of these two traits. This means that a more favorable fruit set was obtained with Zahedi+Jarvis pollen treatment, which reduced the surviving parthenocarpic fruits to a minimum rate at the cost of a high rate of fruit drop. This is while the other two superior pollen sources (Mazafati and Zahedi+Gantar) reduced fruit drop more, instead, they added proportionally to the rate of parthenocarpy. Since the dropped organs have mainly consisted of the unfertilized flowers and the parthenocarpic fruitlets, a higher percentage of fruit drop accompanied by a lower rate of surviving parthenocarpic fruits allows the normal fruits on the bunch to receive more metabolites. This situation is more beneficial as it would result in improvement of the fruit's physical and phytochemical characteristics, ultimately leading to an improved bunch weight with more marketable fruits. Therefore, the Zahedi+Jarvis pollen source was concluded as the best pollination treatment for the improved fruit set. Various factors have been suggested to cause variation in date fruit set such as pollen viability, growth of the pollen tube, or fertilization (Zaid and de Wet, 2002 b), differences in compatibility barriers (Al-Obeed and Abdul-Rahman, 2002), and compatibility levels between pollen variety and female tree (Mohammadi *et al.*, 2017).

It has been shown that growth-promoting phytohormones play a significant role in pollen germination and pollen tube growth (Kojima, 2005), the processes that are initially critical for successful ovule fertilization leading to a normal fruit set. The hormonal content of pollen grain can play a key role in this context. In date palms, parthenocarpic fruit growth is mostly triggered by hormonal imbalance in certain tissues. Auxins (IAA), gibberellins (GA_3), and abscisic acid (ABA) have been considered the major hormones in parthenocarpic fruits (Al-Dinar *et al.*, 2021). Pollens from various male sources possess different hormonal profiles. This variability can be attributed to the genetic makeup of pollen-producing mother plants and the environmental conditions in which the male palms grow and produce pollens. Shahsavar and Shahhosseini (2021) found the hormonal content of the pollen grains from different male sources variable. They reported the highest amounts of cytokinin (zeatin) in Jarvis pollen, gibberellin in pollens of Zahedi and Jarvis males, and auxins in Fard and Zahedi pollen sources. Interestingly, in our study, one of the highest figures of normal fruit set was achieved with the combined application of pollens of Zahedi and Jarvis males, the two sources that contained the highest amounts of growth-promoting phytohormones in the report of Shahsavar and Shahhosseini (2021). It is well known that though *in vivo* germination of pollen grain on the stigma is critical for the process of normal fruit set, it must be followed by healthy directional growth of a pollen tube in the style to deliver the male gametes to the embryo sac (Salomón-Torres *et al.*, 2021).

Bunch characteristics

The results indicated that by choosing a more compatible pollen source, the bunch weight (both Khalal and Tamar stage) could be improved. For Tamar bunch weight, Zahedi+Gantar pollen treatment was seen as the top source. On the other side, this treatment showed about 72% ripened fruits per bunch at the time of bunch harvest whereas four other treatments showed bunch ripeness of over 80%, the highest belonging to the Jarvis+Ghannami treatment. Therefore, according to our results, for the production of maximum Tamar fruits in TC-derived 'Berhi' palms, pollination by Zahedi+Gantar pollen source is recommended but the harvest time should be postponed probably for two more weeks to get maximum Tamar fruits. For Khalal bunch weight, Mazafati, Zahedi+Jarvis, and Jarvis+Gantar sources produced the weightiest bunches (3-3.5 Kg). Through these pollen treatments Khalal bunch weight was doubled and even tripled compared to the other pollen sources like Gantar pollen. Khalal bunch weight in offshoot-originated Berhi palms has been reported up to about 20 Kg (Abd-Elhaleem *et al.*, 2020). This indicates that more research is needed to optimize the pollination issue for tissue-cultured Berhi palms.

Fruit and seed characteristics

Pollen sources used in this study manifested xenic as well as metaxenic effects. The Zahedi+Jarvis pollen treatment gained the most superior place in the ratio of pulp to seed weight. This source also produced fruits with acceptable dimensions, causing one of the lowest seed weights. Previously, Mohammadi *et al*. (2017) obtained the highest value for the weight ratio of pulp to seed by Jarvis pollen in TC-derived 'Berhi' trees. Several reports have confirmed the effect of different pollinizers on the fruit characteristics of dates (Helail and El-Kholey, 2000; Awad and Al-Qurashi, 2012; Rezazadeh *et al.*, 2013).

It seems variation in the performance of pollen sources in fruit and seed characteristics can be attributed to the differences in content and composition of the phytohormones that are synthesized within the embryo. As the genome of each pollen source is different from the other sources, the genetic make-up of the embryo produced by each pollen source would be different compared to the others. This distinction in seed genetics would cause the synthesis of a unique hormonal profile by the embryo that consequently will regulate seed and fruit characteristics (Shafique *et al.*, 2011). In a study on the Hayany date cultivar, it was reported that different pollen sources change the amounts of various phytohormones such as auxin and gibberellins at various stages of fruit development. It was also indicated that larger fruits were obtained by the pollen sources that had higher gibberellin content comparatively (El-Hamady *et al.*, 2010). Shahsavar and Shahhosseini (2021) emphasized that pollen source has a key role in fruit growth and development with a special focus on the hormonal content of each pollen source.

Overall, considering the highest values in the normal fruit set and pulp-to-seed ratio, the Zahedi+Jarvis source was realized as the best pollination treatment for the production of Khalal fruits. We suggest Zahedi male as an elite pollen source to be included in future works aiming to improve the yield of low-fruiting TC-derived 'Berhi' date palms. Earlier, improvement in the yield of the 'Berhi' cultivar was reported by the use of Saki and Maktoumy pollen sources (Al-Obeed and Abdul-Rahman, 2002) and several Iranian local male selections (Rezazadeh *et al.*, 2013). The exploitation of the potential of pollen source in other fruit and nut species such as hazelnut (Fattahi *et al.*, 2014) and Japanese pear (Kuroki *et al.*, 2017) has been reported earlier.

Fruit quality

Pollen source affected fruit quality significantly both at Khalal and at Tamar stages. These results are compatible with those reported earlier in date (Awad and Al-Qurashi, 2012; Shafique *et al.*, 2011) and in fruit species such as mandarin (Wallace and Lee, 1999), and fig (Gaaliche *et al.*, 2011) confirming the critical role of pollen source in fruit quality. However, there are also reports that state the influence of pollen sources on some chemical traits of date fruits is low (Awad and Al-Qurashi, 2012; Rezazadeh *et al.*, 2013). This variation in the findings may indicate that other factor(s) such as crop load, local weather conditions, soil fertility, irrigation, etc. can also influence the fruit quality.

In the Tamar stage, we observed the highest TSS in the fruits set by the Ghannami pollen, the pollen source that caused one of the lowest bunch weights. This indicates a reverse relationship between crop load and TSS. Such a relationship was more apparent when we realized that the Zahedi+Jarvis pollen source that induced one of the top rates in normal fruit set and bunch weight (both at Tamar and Khalal stage), possessed the lowest TSS value and the highest amount of TA. The results also indicated that the Zahedi pollen tends to accumulate more dry matter in Tamar fruits when it is used in combination with the other pollen sources but the Gantar source. Overall, Zahedi+Jarvis pollen treatment was concluded as the most preferable pollen treatment for the quality of fruit at the Tamar stage. It brought moderate sweetness (concerning amounts of TSS, titratable acidity, total and reducing sugars) and drier pulp tissue. Concerning the quality of Khalal fruits, Ghannami and Jarvis+Ghannami pollen sources appeared as the best pollen treatments. They caused the highest amounts in TSS, TA, total sugars, reducing sugars, and the moisture content, which are important in making the Khalal fruits sweeter, tastier, crispier, and more palatable to the consumer. Zahedi+Jarvis pollen treatment can also be considered as another candidate treatment though the fruits set by this treatment were slightly less crispy.

The results of the present study proved that the fruit set and yield are affected differently in different years probably due to variations in environmental conditions. However, based on the average means of the two experimental years, the combined application of Zahedi and Jarvis pollens yielded the most desired results. This pollen treatment obtained about 50% normal fruit set with significantly the lowest parthenocarpy (10%). It also brought about the highest ratio of fruit pulp to seed weight. This pollen treatment was seen as the top pollen source in Khalal's bunch weight. Although the Zahedi+Gantar pollen source was superior in Tamar's bunch weight, the presence of Zahedi pollen in this latter pollen treatment indicates the potential of the Zahedi source over other pollen sources used in the study. Regarding fruit quality, Ghannami, Jarvis+Ghannami, and Zahedi+Jarvis were the pollen treatments that produced Khalal fruits with higher quality indices. However, for the Tamar fruit quality, only Zahedi+Jarvis pollen treatment was chosen as it induced the fruits with moderate sweetness and drier pulp. Overall, considering all fruiting and fruit quality indices, the Zahedi+Jarvis pollen treatment can be drawn as the most desired pollination treatment for low-fruiting TC-derived 'Berhi' date palms. Our results revealed that pollination with elite pollen sources in combination could emerge as a more successful pollination strategy over the singular application of different pollen sources. In addition, the results suggest that relevant future pollination studies utilize the Zahedi pollen source as a potent male source in combination with other elite pollen sources.

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References

ABD-ELHALEEM S.A.M., ABD El-LATIF F.M., EL-BADAWY H.E.M., EL-GIOUSHY S.F., ABDALLA B.M., 2020 - *Impact of pollen grain sources on productivity and fruit characteristics of Date Palm (*Phoenix dactylifera *L.) cv. Barhi*. - Asian J. Res. Bot., 3(2): 45-48.

ALI-DINAR H., MOHAMMED M., MUNIR M., 2021 - *Effects of pollination interventions, plant age and source on hormonal patterns and fruit set of date palm (*Phoenix dactylifera *L.).* - Horticulturae, 7(11): 427.

AL-KAABI H.H., ZAID A., AINSWORTH C., 2007 - *Plant-off-types in tissue culture-derived date palm (*Phoenix dactylifera *L.) plants.* - Acta Horticulturae, 736: 267-281.

AL-KHAYRI J.M., NAIK P.M., 2017 - *Date palm micropropagation: Advances and applications*. - Ciência e Agrotecnologia, 41(4): 347-358.

AL-NAJM A., BRAUER S., TRETHOWAN R., MERCHANT A., AHMAD N., 2021 - *Optimization of* in vitro *pollen germination and viability testing of some Australian selections of date palm (*Phoenix dactylifera *L.) and their xenic and metaxenic effects on the tissue culture-derived female cultivar "Barhee"*. - In Vitro Cell. Dev. Biol. - Plant, 57: 771-785.

AL-OBEED R.S., ABDUL-RAHMAN A.O., 2002 - *Compatibility relationships within and between ten date palm cultivars (*Phoenix dactylifera *L). I- Fruit Set and Yield.* - J. Adv. Agri. Res., 7(4): 809-820.

AL-WASEL A.S., 2000 - *Tissue culture technique: Is it a safe* *method to micropropagate elite date palm,* Phoenix dactylifra *L. cultivars?*. - Arab J. Biotech., 3: 245-256.

AL-WASEL A.S.A.A., 2005 - *Survey study on somaclonal variations in in vitro-derived date palm trees. - Proceedings of international workshop on true-to-typeness of date palm tissue culture-derived plants.* Institut National de Recherche Agronomique, Morocco, 23-25 May.

AOAC, 2016 - *Official methods of analysis*, 20th edition. - Association of Official Analytical Chemists., Washington D.C., USA.

AURAND L.W., WOODS A.E., WELLS M.R., 1987 - *Food composition and analysis.* - Van Nostrand Reinhold Company, USA.

AWAD M., AL-QURASHI A.D., 2012 - *Partial fruit set failure phenomenon in 'Nabbut-Ali' and 'Sabbaka' date palm cultivars under hot arid climate as affected by pollinator type and pollination method.* - Sci. Hortic., 135: 157-163.

COHEN B.Y., KORCHINSKY R., TRIPLER E., 2004 - *Flower abnormalities cause abnormal fruit setting in tissue culture-propagated date palm (*Phoenix dactylifera *L.).* - J. Hort. Sci. Biotech., 79(6): 1007-1013.

EL-HAMADY M., HAMIDA M., AYAAD M., SALAMA M.E., OMAR A.K.H., 2010 - *Metaxenic effects as related to hormonal changes during date Palm (*Phoenix dactylifera *L.) fruit growth and development*. - Acta Horticulturae, 882: 155-164.

FAO, 2020 - *FAOSTAT*. - Food and Agriculture Organization of the United Nations, Rome, Italy, https://www.fao.org/faostat/en/#data/QCL.

FATTAHI R., MOHAMMADZADEH M., KHADIVI-KHUB A., 2014 - *Influence of different pollen sources on nut and kernel characteristics of hazelnut.* - Sci. Hortic., 173: 15-19.

GAALICHE B., TRAD M., MARS M., 2011 - *Effect of pollination intensity, frequency and pollen source on fig (*Ficus carica *L.) productivity and fruit quality*. - Sci. Hortic., 130: 737-742.

HAJIAN S., 2007 - *Quantity and quality comparison of offshoot and tissue cultured Barhee date palm trees.* - Acta Horticulturae, 736: 293-297.

HELAIL B.M., EL-KHOLEY L.A., 2000 - *Effect of pollen grain source on palm fruiting and date quality of Hallawy and Khadrawy date palm*. - Ann. Agric. Sci., 38: 479-494.

KAVAND A., EBADI A., DEHGHANI SHURAKI Y., ABDOSSI V., MOSTAFAVY M., 2015 - *Evaluation of stability and uniformity in tissue culture-date palm (*Phoenix dactylifera *L.) plants of cv. Berhee by using morphological characteristics.* - Intl. J. Biosciences, 6(1): 117-122.

KOJIMA K., 2005 - *Phytohormones in shoots and fruits of tomato; apoplast solution and seedless fruit.* - J. Japan Agricult. Res. Quarterly, 39(2): 77-81.

KUROKI K., TAKEMURA Y., MINGFENG J., MARUMORI H., TERATANI N., MATSUMOTO K., MATSUMOTO T., TAMURA F., 2017 - *Pear pollen selection using higher germination properties at low temperatures and the effect on the fruit set and quality of Japanese pear cultivars*. - Sci. Hortic., 216: 200-204.

LANE J.H., EYNON L., 1923 - *Determination of reducing sugars by means of Fehling's solutio, with methylene blue as internal indicator*. - J. Soc. Chem. Ind. Trans., pp. 32-36.

MOHAMMADI N., RASTGOO S., IZADI M., 2017 - *The strong effect of pollen source and pollination time on fruit set and the yield of tissue culture-derived date palm (*Phoenix dactylifera *L.) trees cv. Barhee.* - Sci. Hortic., 224: 343-350.

NIXON R.W., 1934 - *Metaxenia in dates.* - Proc. Am. Soc. Hortic. Sci., 32: 221-226.

OMAR A.E.K., EL-ABD A.E.N., 2014 - *Enhancing date palm (*Phoenix dactylifera *L.) productivity, ripening and fruit quality using selected male palms*. - Acta Adv. Agri. Sci., 2(6): 11-19.

REZAZADEH R., HASSANZADEH H., HOSSEINIA Y., KARAMIYA Y., WILLIAMS R.R., 2013 - *Influence of pollen source on fruit production of date palm (*Phoenix dactylifera *L.) cv. Barhi in humid coastal regions of southern Iran.* - Sci. Hortic., 160: 182-188.

SALOMON-TORRES R., KRUEGER R., GARCIA-VAZQUEZ J.P., VILLA-ANGULO R., VILLA-ANGULO C., ORTIZ-URIBE N., SOL-URIBE J.A., SAMANIEGO-SANDOVAL L., 2021 - *Date palm pollen: Features, production, extraction and pollination methods.* - Agronomy, 11: 504.

SAS INSTITUTE, 2001- *SAS for Windows, SAS User's Guide: Statistics. Version 8.0 e*. - SAS Inst., Inc., Cary, North Carolina, USA.

SHAFIQUE M., KHAN A., MALIK A.U., SHAHID M., RAJVANA I.A., SALEEM B.A., AMIN M., AHMAD E., 2011 - *Influence of pollen source and pollination frequency on fruit drop, yield and quality of date palm (*Phoenix dactylifera *L.) cv. Dhakki.* - Pak. J. Bot., 43: 831-839.

SHAHSAVAR A.R., SHAHHOSSEINI A., 2021 - *Pollen grain hormones of date palm pollinator cultivars and their relationship with hormones of different stages of 'Piarom' date fruit growth*. - Sci. Hortic., 288: 110389.

SOLIMAN S.S., KASSEM H.A., AL-OBEED R.S., JEMNI M., 2020 - *Influence of pollen source and its mineral content on fruit retention and quality of Kadary date palm cultivar (*Phoenix dactylifera *L.).* - J. Chem. Biol. Physic. Sci., 10(3): 437-446.

WALLACE H.M., LEE L.S., 1999 - *Pollen source, fruit set and xenia in mandarins*. - J. Hortic. Sci. Biotechnol., 74(1): 82-86.

ZAID A., DE WET P.F., 2002 a - *Botanical and systematic description of the date palm,* pp. 1-28. - In: ZAID A., and E.J. ARIAS-JIMENEZ (eds.) *Date palm cultivation*. FAO Plant Production and Protection Papers No. 156, Rev.1, Rome, Italy.

ZAID A., DE WET P.F., 2002 b - *Pollination and bunch management*, pp. 145-175. - In: ZAID A., and E.J. ARIAS-JIMENEZ (eds.) *Date palm cultivation*. FAO Plant Production and Protection Papers No. 156, Rev. 1, Rome, Italy.