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The effect of pollen storage temperatures on fruit set and fruit quality of 'Deglet Nour' date palm (*Phoenix dactylifera* L.) cultivar

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Abstract: The aim of this research was to study the effect of three temperatures during the long-term storage of date palm pollen (25±2, 4, and -20 °C) on fruit set and the fruit quality of 'Deglet Nour' cultivar. The experimental design included pollen collected in different geographic regions. The overall comparison between temperatures showed that pollen stored at 4 °C had a significantly higher fruit set (40.6±16.7%) than pollen stored at 25±2 °C and -20 °C (31.9±16.8% and 34.8±19.4%, respectively). For each used storage temperature, the resulting fruit set was almost unaffected by the geographic origin of pollen. However, comparison between the aptitudes of pollens from each location to preserve their pollination potency under the different storage temperatures revealed some similarities among pollens from the same type of oasis and location. Pollen stored at 4 °C improved, significantly, the fruit water content (27.4±8.2%) compared to fruits obtained by flowers pollinated with frozen pollen (-20 °C) and stored at room temperature. However, other fruit traits (fresh weight, flesh weight to seeds weight ratio, juice total soluble solids, pH and titratable acidity) did not show any variation under the effect of different pollen storage temperatures.

Keywords: pollen storage temperature; geographic location; fruit set.

1. Introduction

Date palm (*Phoenix dactylifera* L.) trees are grown in hot, arid habitats, including desert oases, river valleys and well-irrigated plantations (Hazzouri et al., 2015) and their cultivation represent in many countries a principal source of life and the main food for local populations (Chao and Krueger, 2007). In Tunisia, date palm cultivation has great socio-economic importance. In 2019, it covered an area of 54,000 ha with nearly six million plants producing 327.9 thousand tons of dates. Almost a third of them, 113.9 thousand tons were exported with a value of 780.0 million dinars (ONAGRI, 2020). Actually, Tunisia is the world-leading country for the export value of dates and it is also the world-leading producer of the 'Deglet Nour' cultivar (ONFAA, 2017). This cultivar represents more than 65% of the total date palm planted area (DGPA, 2017), and more than 75% of the date crop in the country (Dawson, 2016). 'Deglet Nour' is a cultivar producing semi-soft dates (Hamza et al., 2015), known for their translucent blonde color and good ability to be kept in cold rooms which allows their marketing throughout the year (Dawson, 2016). This crop contributes significantly to the development of the desert areas of southern Tunisia and has a significant social impact, providing a livelihood for nearly 50,000 inhabitants in the oases, and is one of the main pillars of the regional and national economy. Dates in Tunisia represent 6% of national agricultural production and 12% of the total value of agricultural products exported from Tunisia (GIFruits, 2019).

In the date palm, a dioecious species, natural pollination, anemophilous or by bees and other insects, may not be enough for obtaining a commercially suitable fruit set (Al-Wusaibai et al., 2012).

Indeed, up to 50% of yield reduction is observed due to obstacles linked to the receptivity of the stigmas and the availability of pollen (Shafique et al., 2011; Shahid et al., 2017). Thus, artificial pollination is essential to overcome the disadvantages of dichogamy, reduce the number of male plants required, and achieve a commercial yield (Rezazadeh et al., 2013; El-Refaey and El-Dengawy, 2017) and a profitable and good quality of the dates (Iqbal et al., 2018). However, asynchronous flowering of male and female date palms is more and more frequently observed during the last years in southern Tunisian oases. This could be caused by climate change, which may disrupt the overlap in the seasonal timing of flower production as already observed in pistachio and almond in Tunisia (Ben Moussa et al. 2017; 2018) and in many other fruit trees (Memmott et al., 2007). Consequently, the conservation of date palm pollen is becoming progressively more important and necessary. Palm growers collect and store pollens according to the traditional method in cardboard boxes at room temperature for a short (one or two months) or a long (until the next season) period (El Kadri and Ben Mimoun, 2020). But the pollen potency of germination can be altered during storage. Pollen stored at room temperature (25-30 °C) or in a refrigerator (3-4 °C) retained less viability compared to the fresh pollen in date palm (Shaheen et al., 1986; Maryam et al., 2015). A recent work showed that storing pollen at -20 °C helped to better maintain the pollen in-vitro germination potency compared to storage at room temperature and at 4 °C (El Kadri and Ben Mimoun, 2020). Some others studies showed that the decrease of the in-vitro germination was significantly faster during storage at room temperature compared to storage at 4 °C or -20 °C (Mesnoua et al., 2018; Anushma et al., 2018). Mesnoua et al (2018) reported, also, that the percentage of fruit set for fresh pollen or pollen stored at 4 °C and -20 °C was more than 40%, and did not differ significantly between cultivars or seasons of pollination.

Research has developed experimental methods for date palm pollen storage such as freezing in liquid nitrogen and cryopreservation (Anushma et al, 2018), but these are still out of the reach of most of the Tunisian farmers and require complicated means and technicality. However, research about date palm pollen storage in Tunisia is very scarce and most of the studies focused on the influence of storage temperature on the laboratory potencies of the pollen grains (in-vitro germination percentage and viability rates using staining technique). Moreover, some researchers suggested that the percentage of fruit set was not correlated to the percentage of in-vitro pollen germination, since pollen with low percent viability (19%) stored at 4 °C induced similar percentages of fruit set as pollen with high viability (89%) stored at -20 °C (Mesnoua et al., 2018). Therefore, developing simple methods for date palm pollen storage and investigating the resulting fruit set and quality can contribute to help farmers to improve pollen storage and to improve date yield and quality.

For date palms, some research showed that pollen affects fruit size, quality, and time of maturation and these effects are known as metaxenia (Nixon, 1934 and 1936). These responses could be due to hormones or soluble substances that influence the development of adjacent ovarian tissues in the female flower (Chao and Krueger, 2007). However, the metaxenia effect is not always observed and seems to be related to specific male pollinators and growth conditions (Reuveni, 1986).

This study aimed to compare three storage temperatures for date palm pollen and to evaluate the resulting fruit set percentages and the obtained fruit characteristics, in order to determine the best method of pollen preservation that can be adopted by farmers to improve date production. The aptitude of different pollen sources, from different geographic locations to the storage temperatures was also investigated.

2. Materials and methods

2.1. Plant material

The study was carried out in 2016 and 2017. Pollen samples were collected from males plants located in different production areas in southern Tunisia and different types of oasis (insular, coastal, continental and mountainous) (Figure 1) with different geographic and climatic characteristics (Table

1). The eight selected sites for the study were distributed as follows:

- One insular oasis in the south-east of Tunisia: 'Djerba' in the Mednine governorate, characterized by the predominance of common varieties (Sghaier, 2010) and a significant number of spontaneous date palms (Zehdi-Azouzi et al., 2016).
- Two coastal oases in south-east of Tunisia: 'Zarat' and 'Ben Ghilouf' in the Gabes governorate, where common varieties are predominant (Sghaier, 2010).
- Three continental oases in the south-west of Tunisia: 'Jemna' and 'Gataya' in the Kebili governorate and 'Hezoua' in the Tozeur governorate, characterized a hot and dry climate and where 'Deglet Nour' is the predominant variety (Sghaier, 2010).
- Two mountainous oases in the south-west of Tunisia: 'Gtar' in the Gafsa governorate and 'Mides' in the Tozeur governorate, characterized by mild winters and the predominance of common varieties (Sghaier, 2010).

Pollinizers selected in this study were all from spontaneous seedling of date palm cultivars, and three plant replications were used in each area, and three mature male inflorescences from each male plant were used for pollen collection. The collection sites used in this study were chosen because they are the ones usually used by southern Tunisian date palm growers as pollen sources. Pollen from insular regions (Djerba) is often used when there is a lack of pollen in continental oases.

Pollen stored at the three temperatures and fresh pollen were used for cross pollination of three female uniform palm trees of 'Deglet Nour' cultivar, of about fifteen years old, receiving ordinary horticultural practices for date palm production, and located in the El Gataya oasis in the Kebili governorate (33°40.845'N, 8°52.258'E).

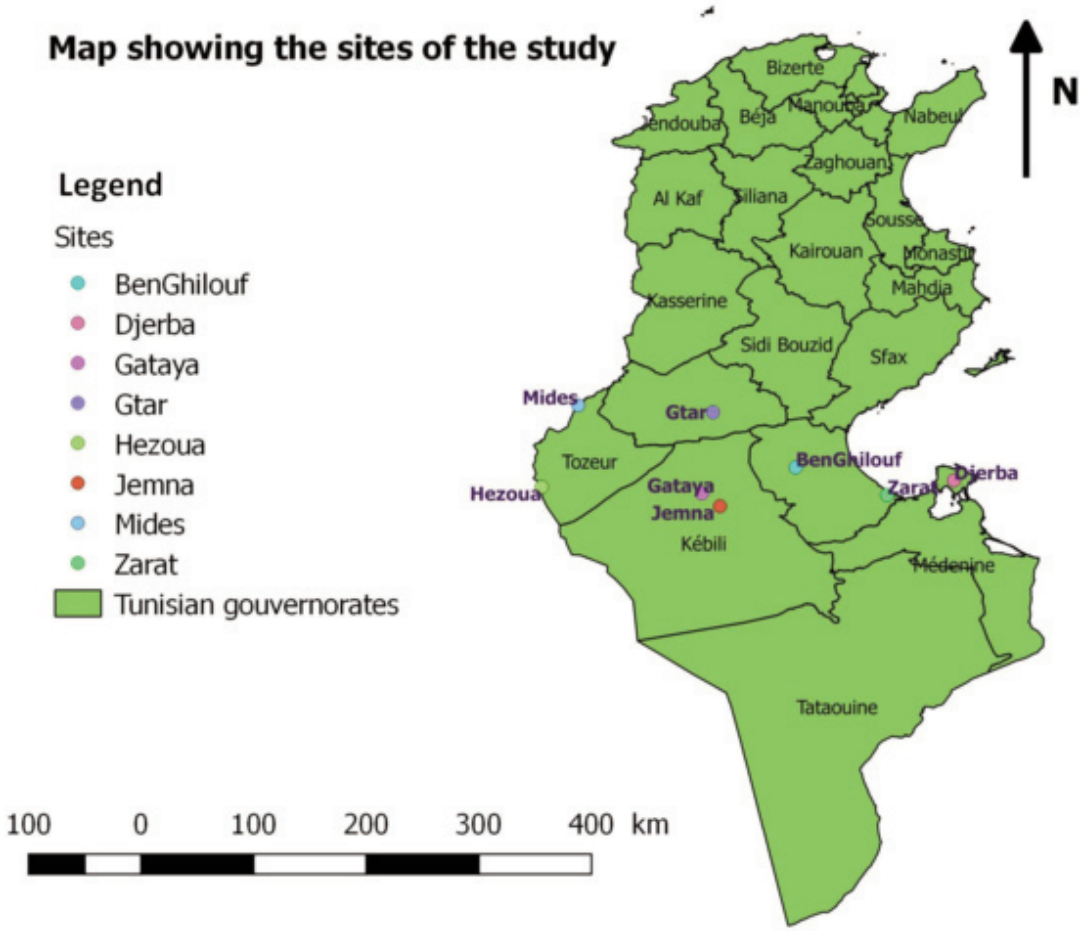

Figure 1. Geographic localization of the eight pollen sources of the study in Southern Tunisia (Qgis software version 3.6).

Table 1. Weather data at the collection sites from 2007 to 2017: PP: average annual total precipitation. Tmean: average annual temperature; Tmax: average maximum temperatures; Tmin: average minimum temperatures (INM, 2017).

Governorates	PP (mm)	Tmean (°C)	Tmax (°C)	Tmin (°C)
Medenine	240.1	21.4	25.8	17.2
Gabes	105.3	20.5	26.3	14.9
Kebili	97.1	22.7	28.6	16.9
Tozeur	96.9	22.6	28.5	15.2
Gafsa	148.9	20.5	27.0	13.78

2.2. Pollen collection and storage conditions

In 2016, in each site, fresh pollen was collected from three mature, newly opened, inflorescences. Collected pollens were stored at three storage temperatures (Boughediri et al, 1995) for 12 months. Then, during 2017, stored and fresh (newly collected from each site) pollens were used for pollination. Female plants were pollinated once at mid April.

Storage temperatures were chosen to be simple and inexpensive, so that they can be easily adopted by farmers. In previous studies, pollen storage at 4 and -20 °C was reported to be effective for preserving pollen potencies (Mesnoua et al., 2018; El Kadri and Ben Mimoun, 2020). The following four treatments were compared:

- Fresh pollens (collected in 2017): control treatment
- Room temperature at 25± 2 °C: (the method adopted by most of the farmers): pollens were kept inside the flowers, dried at room temperature and stored in cardboard boxes.
- Pollen stored at 4 °C.
- Pollen stored at -20 °C.

For storage at 4 °C and -20 °C, hand-extracted pollen powders were collected and separated from flower parts using fine sieves and dehydrated in an oven at 40 °C for 24 hours (Boughediri et al, 1995) and placed in dry, transparent and closed vials before storage.

2.3. Fruit set percentages (pollen in-vivo fertility)

For the three female date palm trees used in this study, the chosen female inflorescences were divided in groups of three spikelets and covered with kraft paper to avoid cross pollination. Pollination was carried out on the same day for all treatments, according to the traditional method for fresh pollen and pollen stored at 25±2 °C (by introducing three male strands in the middle of female bunches previously bagged together). For stored pollen, the traditional method used for pollination with pollen powder was adopted. Pollen was mixed with talcum powder (Ullah et al., 2018) at a ratio of 1:5 parts (El Mardi et al., 2007; Al-Wusaibai et al., 2012), then cotton pieces were soaked in the mixture and introduced into the middle of the female bunches (Zaid and de Wet, 2002). The 1:5 ratio was chosen as the lowest rate reported by researchers to minimize the effect of the pollen mixture with talcum (El Mardi et al., 2007).

The kraft paper was removed four weeks after pollination (Figure 2). The fruit set percentage was measured after about 7-8 weeks after pollination according to the following equation:

$$\text{Fruit set percentage (\%)} = [RF / (RF + RS + NF)] \times 100$$

where RF is the number of retained fruits, RS is number of flower scars on strand replicates, and NF is number of non-pollinated fruits (parthenocarpic fruits) (Awad, 2011; Alkhalifah, 2006).

Figure 2. Covered spikelets after pollination with labels (chosen female inflorescences were devised in groups of three bunches, labeled and covered with kraft paper to avoid cross pollination).

2.4. Fruit characteristics

Fruits from each treatment were harvested in the last week of October 2017. Samples of 30 fruits were taken at random from each treatment. Data on the following characteristics were recorded:

- Fruit length /width ratio (L/W): fruit dimensions of fifteen fruits from each treatment were measured with a manual caliper and the L/W ratio was calculated.
- Fresh fruit weight (PF) (g/fruit): fresh weight of fifteen fruits by treatment was determined with an analytic balance (WTB-200, RADWAG, Radom, Poland).
- Flesh weight (FLW) (g/fruit): the pulp of fifteen fruits was weighed after separation of pulp from seeds.
- Weight of seed (SW) (g/seed): seeds of fifteen fruits from each treatment were weighed by analytic balance.
- Fruit juice pH: the determination of the pH for fruit juice was carried out according to the AFNOR standard (1982) using a pH meter (BPH-231, Lutron, Taipei, Taiwan).
- Total soluble solids (TSS) (°Brix): the determination of the soluble solid content was carried out as recommended by AOAC (1995) using a digital refractometer (95200-002 ATC Digital Refractometer, Alla France, Chemillé en Anjou, France).
- Moisture content (%): the water content was determined by desiccation of a 2 g sample of date

pulp in an oven (WGL-65B, Huanghua Faithful Instrument, Huanghua City, China) at a temperature of 65 °C for 48 hours (Booij et al., 1992).

- Titratable acidity (AT) (%): the determination of titratable acidity was carried out using phenolphthalein as an indicator by titrating the date juice against NaOH (0.1 N) (Shafique et al., 2011).

The fruit characterization was carried at the Fruit Trees Laboratory in Agronomy and Plant Biotechnology Department in the National Agronomic Institute of Tunisia.

2.5. Statistical Analysis

The experiment was arranged in a completely randomized design with a total of 32 treatments (8 pollen sources × 4 storage methods) and three replications. Data were subjected to two-way ANOVA using General Linear Model (GLM) in order to check the significance of the effects of the pollen source and the storage method and their interactions on the measured parameters. Duncan's multiple comparisons test was used to separate means at α=0.05. All statistical analyses were performed using IBM SPSS statistics software (Version 20, IBM, USA).

3. Results

Data analysis indicated that the effects of storage temperature, pollen source and the interaction between storage method and pollen source on fruit set percentage were highly significant (Table 2). On the other hand, the pollen source showed significant effect on fruit L/W ratio, flesh weight/seed weight ratio (FLW/SW), fruit juice pH, water content and total acidity (AT). However, the interaction between pollen source and the storage method did not show any significant effects on all measured fruit characteristics.

Table 2. Two-way ANOVA showing the effect of pollen storage method (ST), pollen source (PS) and the ST×PS interaction on fruit set, and seven fruit characteristics: length/width ratio (L/W), fresh weight (FW), flesh weight/seed weight (FLW/SW), juice pH, total soluble solids (TSS), water content (%) and titratable acidity (TA).

Source	F-value							
	Fruit set	L/W	FW	FLW/SW	pH	TSS	Water content	TA
ST	19.77**	4.75**	0.79	2.28**	1.87	0.39	1.23	0.39
PS	2.77*	11.06**	1.37	74.24**	10.80**	2.12	5.21**	664.89**
ST×PS	1.86*	1.43	0.92	0.81	0.78	1.40	0.71	0.58

*Significant at p≤0.05; **Significant at p≤0.01.

Statistical analysis showed that fruit set rate was significantly influenced by the pollen storage method (Figure 3). The comparison between storage methods showed that the fruit set ranged between 31.9±16.8% (25±2 °C) and 52.1±18.2% for fresh pollen (Figure 2). Pollen stored at 4 °C significantly improved fruit set percentage (40.6±16.7%) compared with storage at 25±2 °C (Figure 3).

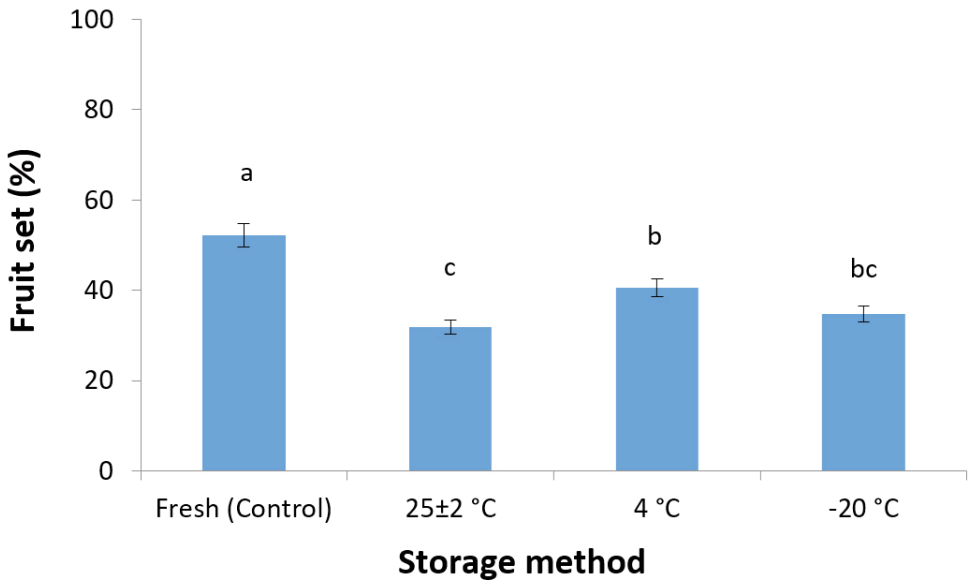

Figure 3. Fruit set (%) under the effect of fresh pollen (Control), and pollen stored at 25±2, 4 and −20 °C of eight pollen sources. Each value represents mean ± standard deviation. Different letters indicate that means are significantly different (Duncan test, $P < 0.05$).

When fresh pollen was applied, fruit set was not affected by the geographic origin of pollen. For pollen stored at 25±2 °C, the use of Djerba, Jemna and Mides pollens induced a significantly higher fruit set (41.3±12.1, 42.1±11.9, and 41.1±24.1%, respectively) than pollen collected in other locations (Table 3). Those pollens are from different locations and type of oasis. Regarding stored pollen at 4 °C, fruit set was also affected by the pollen geographic origin. Indeed, fruit set was significantly higher for Gataya and Gtar pollens (51.0±20.0 and 47.8±9.2%, respectively), whereas the lower rate was found for pollen from Benghilouf (23.5±22.2%). Concerning pollen stored at -20 °C, fruit set rates were not affected by the pollen geographic origin.

Table 3. Fruit set obtained using pollen collected in eight locations and used fresh (Control) or stored at 25±2 °C, 4 °C and −20 °C.

Location	Storage type				F-value	ANOVA p
	Control	25±2 °C	4 °C	-20 °C		
Djerba	63.5±16.0 a/A	41.3±12.1 a/B	41.6±11.1 ab/B	42.0±12.4 a/B	6.22	<0.01
Zarat	52.9±16.8 a/A	22.3±12.6 b/B	44.8±6.3 ab/A	41.8 ±20.2 a/A	6.25	<0.01
Benghilouf	44.4±14.2 a/A	28.3±9.6 ab/A	23.5±22.2 c/A	23.8±21.4 a/A	2.81	0.06
Gataya	47.2±24.8 a/A	32.2±15.6 ab/A	51.0±20.0 a/A	31.3±16.6 a/A	2.42	0.08
Jemna	56.0±15.5 a/A	42.1±11.9 a/A	43.0±12.1ab/A	27.0±20.3 a/B	5.32	<0.01
Hezoua	49.5±23.9 a/A	20.0±15.1 b/B	42.6±13.4 ab/A	48.6±21.3 a/A	4.79	0.01
Mides	55.9±14.0 a/A	41.1±24.1 a/AB	30.5±19.0 bc/B	32.1±15.2 a/B	3.57	0.03
Gtar	47.8±16.3 a/A	29.0±17.6 ab/B	47.8 ±9.2a/A	31.4±19.6 a/B	3.60	0.02
F-value	1.05	2.66	3.28	1.88	-	-
ANOVA p	0.41	0.02	<0.01	0.09	-	-

Each value is an average of three replications ± standard deviation. Means followed by different lowercase (within each column) or uppercase (within each row) letters are significantly different at 5% level of probability (Duncan test).

Comparing the response of each pollen source to different storage methods, most of studied pollens allowed significant higher fruit set percentages when stored at 4 °C and -20 °C (Table 3).

However, Gataya and Benghilouf pollens induced similar levels of fruit set independently of the storage method (Table 3), Jemna pollen induced significant lower fruit set only for pollen stored at -20 °C (27.0±20.3%), while Gtar pollen stored at -20 °C induced a fruit set (31.4±19.6%) similar to that of pollen stored at 25±2 °C (28.9±17.6%). Pollens collected at Gataya and Jemna, which are both continental oases with similar geographic characteristics, showed better aptitude to be stored at 25±2 °C than pollens from the similar oasis of Hezoua. (Table 3).

The results concerning the study of the effect of the three methods of pollen preservation on the quality of the produced dates showed significant differences in fruit size, pH and titratable acidity between the fresh pollen and the stored pollen (Table 4). Fruit size and fruit juice pH were significantly higher in fruit obtained using fresh pollen compared to the other methods (2.1±0.1 and 5.4 ±0.2, respectively), while the acidity was lower. These parameters indicate that a better fruit quality can be obtained when fresh pollen is used (Table 4). On the other hand, fruit characteristics were not significantly different between storage treatments. Only fruit water content (%) increased significantly when pollen was stored at 4 °C (27.4%) compared to fruits obtained using fresh, frozen or stored at room temperature pollen (Table 4). On the other hand, fresh weight (FW), flesh weight to seed weight ratio (FLW/SW), total soluble solids (°Brix), fruit juice pH and titratable acidity (AT) were not affected by the storage treatments (Table 4).

Table 4. Length/width ratio (L/W), fresh weight (FW), flesh weight/seed weight (FLW/SW), juice pH, total soluble solids (TSS), water content (%) and titratable acidity (TA) of fruits obtained using fresh pollen (Control) or pollen stored at 25±2 °C, 4 °C and −20 °C.

Storage type	L/W	FW (g)	FLW/SW	pH	TSS (°Brix)	Water content (%)	TA (%)
Fresh (control)	2.1 ±0.1 a	11.0 ±1.4 a	11.1 ±2.1 a	5.4 ±0.2 a	18.3 ±3.5 a	23.9 ± 5.8 b	0.4 ±0.2 b
25±2°C	2.0 ±0.1 b	11.4 ±1.1 a	11.3 ±2.3 a	5.1 ±0.2 b	18.3 ± 2.6a	24.4 ± 4.8 b	0.8 ±0.7
4°C	2.0 ±0.1 b	11.2 ±1.3 a	12.9 ±6.4 a	5.1 ±0.2 b	19.1 ± 2.8 a	27.4 ±8.2 a	0.8 ± 0.8 a
-20°C	1.9 ±0.1 b	11.3 ±0.8 a	13.0 ±6.2 a	5.1 ±0.2 b	17.8 ±2.3 a	22.6 ±5.3 b	0.9 ± 0.6 a
ANOVA p	<0.01	0.2	0.1	<0.01	0.1	<0.01	<0.01

Each value is an average of three replications ± standard deviation. Within each column, means followed by different letters are significantly different at 5% level of probability according to the Duncan test.

4. Discussion

Results showed that fruit set was higher when fresh pollen was used compared to that stored at the three storage temperatures. Modifications of the chemical and physical pollen characteristics occurring during the storage at 4 °C and -20 °C can cause a decline in pollen germination (Mesnoua et al., 2018; Akond et al., 2012). On the other hand, storing pollen at 4 °C, as a simple method, help to maintain pollen potency for pollination. Fruit set obtained using pollen stored at 4 °C was higher than the other two temperatures (25±2 °C and -20 °C). These results are consistent with previous findings on Baydir cultivar (Babahani and Bouguedoura, 2009). Similarly in Bahlani and Medjahdil cultivars, the storage at 4-5 °C was the best condition for retaining pollen potency compared with pollen kept at room temperature and in freeze-dryer (El Mardi and Bakheit, 1996). Previous studies showed that storage temperature had a significant effect on date palm pollen potency (El Mardi and Bakheit, 1996; Mortazavi et al., 2010; Mesnoua et al., 2018). For instance, the fruit set responses of pollen from continental oases showed different aptitudes for the storage methods. On the other hand, the comparison between pollens collected in different locations revealed some response similarities among pollens from similar types of

oasis and location. Some researchers explained the differences in pollen germination by the genetic variability among male genotypes (Chao and Krueger. 2007; Mortazavi et al. 2010). This hypothesis is in line with a high level of genetic variation reported for male date palm pollinizers in Tunisia (El Kadri et al., 2019). Other authors hypothesized that the differences in desiccation tolerance of the pollen can cause variation in pollen longevity (Akond et al. 2012).

Regarding the study of the effect of the three methods of pollen storage on fruit quality showed that only the water content was higher in fruits obtained using pollens stored at 4 °C compared to the other treatments. All the other fruit traits (the fresh weight, the flesh weight to seeds weight ratio, juice total soluble solids, pH and titratable acidity) were not affected by the pollen storage method. These results are, in part, in accordance with the findings of Mesnoua et al. (2018) who found that fruit length and diameter, seed length and diameter were less affected by the pollen storage method, while fruit weight and pulp weight were the most influenced characteristics. However, the authors also reported that the use of pollen stored at 4 °C lead to poor fruit quality. This may be due to the low viability of pollen recorded at 4 °C compared to fresh pollen and pollen stored at -20 °C. As a semi dry fruit cultivar, the optimal water content for "Deglet Nour" is 25±1% (Booij et al., 1992) which was obtained with the different pollens used in this experiments. This character is important to ensure the good ability of 'Deglet Nour' cultivar to be stored in cold rooms and be marketing throughout the year (Hamza et al., 2015; Dawson, 2016). On the other hand, the interaction between pollen source and the storage method significantly affected only fruit set percentage, but did not have any significant effect on all measured fruit characteristics.

The overall results of this study showed that most of pollens lost an important part of their potency at 25±2 °C, whereas pollen stored at 4 °C showed better fruit set percentage for most of pollen sources, independently of their geographic origins. Pollen storage method did not affect fruit characteristics.

5. Conclusions

One-year long pollen storage at 4 °C can be, safely, recommended to farmer. This can be considered as a simple and an inexpensive storage method. Indeed, the resulting fruit set and fruit characteristics for this temperature were almost similar to those obtained with the fresh pollen. Additional investigations are required to study how different pollination techniques with stored pollen powder can help improving fruit set.

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Conflicts of Interest: The authors declare no conflict of interest.

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