



Study on the effects of pollen sources on the agronomic, biochemical, mineral, and pomological traits of date palm (*Phoenix dactylifera* L.) cv 'Deglet Nour' fruits in Degache Oases (Tunisia)

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Abstract Given the dioecious nature of date palms, selecting an effective pollinizer bearing a substantial quantity of high-quality pollen is imperative. In this study, we investigated the effects of 11 male date palm cultivars from Tunisia on the production of female trees, fruit sets, and the characteristics of 'Deglet Nour' cultivar dates. The comparison of pollen germination rates showed that ABD1 and P169 pollens exhibited outstanding viability, with 98.7% and 98.3%, respectively. On the other hand, P169 pollen had the highest germination rate, at 86.3%.

The most remarkable fruit set (90.7%) and retention rate (69.7%) were observed in response to P13 pollen. In response to the P165 pollinator, the fruit stood out among the resulting fruits for their notable weight, averaging 11.6 g. In addition, pollination with P90 increased total soluble solids (measured at 74.4°Brix in the Tamr stage), whereas P7-pollinated fruits recorded the highest levels of reducing sugars, reaching 41%. Notably, the highest acidity levels were found in P7-pollinated fruits (0.29%), and pollen

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sources significantly influenced the fruits' mineral content.

Moreover, regarding pomological characteristics, fruits pollinated with P4 attained maximum size, measuring 4.10 cm. Conversely, P7-pollinated fruits had a notable width of 2.02 cm. These findings illustrate the possibility of the selective application of high-value pollinizers and emphasize the direct influence of pollen sources on fruit quantity and quality.

Keywords Date palm · Pollen source · Fruit characteristics · Metazinia

Introduction

The date palm (*Phoenix dactylifera* L.) is a dioecious, perennial, diploid, monocotyledonous plant. Its dioecious reproduction has resulted in significant variability when propagated by seed (Alyafei et al. 2022; Mesnoua et al. 2020; Mesnoua et al. 2018a, b). The extensive genetic diversity of date palm has enabled the selection of numerous clones with differing morphological and physiological traits. Consequently, date-growing countries possess a rich genetic heritage, and two types of genetic resources can be distinguished: first, the millions of date palm hybrids obtained via seeds, and second, those derived from vegetative propagation. According to estimates, there are more than 500 recognized date palm cultivars in Iraq, 400 in Iran, 300 in Libya, 223 in Morocco, 940 in Algeria, and roughly 250 in Tunisia (Hannachi 1998; Rhouma et al. 2007). Thus, it is crucial to understand the genetic resources of cultivated species to increase productivity and efficiency in conservation and breeding efforts. Conversely, male pollinators are pivotal in the date production cycle due to the metaxenic impact of pollen on fruit-setting rates and yields, which underscores the significance of selecting and characterizing adequate male specimens (Karim et al. 2022a, b; Mesnoua et al. 2018a, b; Mesnoua et al. 2023). Furthermore, these male cultivars constitute exceptionally diverse gene pools, highlighting substantial biodiversity (Karim et al. 2022; Khouane et al. 2020).

In contrast to Middle Eastern countries where male date palm cultivars have been thoroughly studied (Hannachi 1998; Hannachi et al. 1998; Rhouma et al. 2007), The genetic makeup of male date palms

has not been extensively studied. Additionally, limited characterization research has been conducted in North African countries (Kadri et al. 2021; Karim et al. 2015; Khouane et al. 2020). In Tunisia, locally known as "Dokkars," these male trees are heterogeneous populations that are rarely cloned, with each individual exhibiting distinct traits (Bouguedoura et al. 2015). However, it is sometimes identified by the name of a phenotypically similar female cultivar.

Selecting quality pollen sources with abundant pollen production is essential in date palm cultivation. In Tunisia, male date palm trees are poorly characterized and often seed propagated, unlike in other date-growing countries (e.g., Iraq) where superior males are cloned via vegetative means and have designated names 'Fahl' such as 'Red Ganami,' 'Green Ganani,' and 'Gholami' (Bouguedoura et al. 2015). Multiple investigations have shown that pollen sources influence fruit quality (Frag et al. 2012; Mohammadi et al. 2017; Rezazadeh et al. 2013; Soliman 2020). Pollen can also affect seed size and shape (xenia effect) and the tissue surrounding the embryo and endosperm (metaxenia effect). This study, therefore, sought to investigate the effects of eleven different pollen sources on the agronomic characteristics and chemical composition of the 'Deglet Nour' date variety under the soil and climatic conditions of the Djerid region in southwestern Tunisia.

Materials and methods

Plant material, pollen collection, and pollination method

This research was conducted on male date palm trees that were 30 years old. Eleven pollinizers from Tunisian germplasm were selected based on their blooming period and pollen germination rate (as shown in Tables 1 and 2). These pollinizers were grown in the regional oasis agriculture research center located in the district of Degache, governorate Tozeur, southwestern Tunisia (E: 33°58'40.5"; N: 008°12'31.8"). The palm trees were healthy, had uniform vegetative growth and pollen production, and received regular agricultural practices such as irrigation and fertilization. A single male tree was used for each pollen source, and the pollen was collected during the second flowering

Table 1 Location and flowering period of the different pollen sources studied

Pollen source	Location	Flowering period	Number of spathes produced (2021)
ABD1	Degache experimental plot	Precocious	54
ABD2	Degache experimental plot	Medium	48
ABD3	Degache experimental plot	Medium	40
PS2	Tozeur experimental plot	Precocious	46
P4	Degache experimental plot	Medium	42
P7	Degache experimental plot	Precocious	64
P8	Degache experimental plot	Medium	40
P13	Degache experimental plot	Late	39
P90	Tozeur experimental plot	Late	47
P165	Tozeur experimental plot	Precocious	38
P169	Tozeur experimental plot	Medium	46

Table 2 Pollen in vitro germination and viability percentage of different pollen sources

Pollen source	Germination (%)	Viability (%)
ABD1	80.0 ± 1.00 b	98.6 ± 1.40 a
ABD2	63.0 ± 2.02 e	95.0 ± 1.10 bc
ABD3	75.3 ± 2.51 c	99.0 ± 1.17 a
PS2	50.3 ± 0.57 g	78.3 ± 1.50 g
P4	75.3 ± 1.52 c	91.6 ± 3.21 d
P7	76.0 ± 2.64 c	83.3 ± 4.16 f
P8	84.3 ± 3.05 a	88.0 ± 1.92 e
P13	83.7 ± 1.58 a	93.6 ± 0.95 cd
P90	59.3 ± 1.42 f	98.3 ± 1.37 a
P195	69.0 ± 1.05 d	98.0 ± 1.43 ab
P169	86.3 ± 1.57 a	98.3 ± 1.06 a
LSD	3.161	3.2823
<i>p</i> value	< 0.0001	98.6 ± 1.40 a

Values are the mean of five samples per treatment

Means followed by a different letter(s) are significantly different at 5% probability using the LSD test

period. "Pollination was conducted on 15-year-old 'Deglet Nour' female palms, which had been uniformly and consistently managed using standard cultivation techniques for fertilization and irrigation. These palms averaged an annual yield of 12 bunches each. We have chosen this variety because it is well known for its economic importance and accounts for 90% of Tunisia's date production (Ismail and Hassine 2021). Bunches were thinned at a ratio of one bunch per nine leaves. Male spadixes

that had just opened were harvested in the morning and allowed to dry in the open air for two days. This method allowed the anthers to dehisce without opening the flowers. The pollen grain was then collected, put in a tightly sealed glass jar, and stored at room temperature for immediate use.

Three bunches were chosen, with three repetitions per tree and six female trees selected from each bunch for pollination. Female bunches belonging to the second flowering stage were chosen as recipients. Each bunch was divided into twelve groups containing 5 spikelets, which were assigned to one of the treatments: 11 source pollens and a negative control not pollinated. This negative control will be employed to verify the sealing effectiveness of the envelopes used for enclosing various groups of spikelets pollinated with different pollen types. Each group consisted of five strands (as shown in Fig. 1). The groups of female strands were then covered with Kraft paper to prevent possible contamination with other sources of pollen. After three days, each female group received random pollens from the 11 different male pollinizers, and then each cluster was covered again with Kraft paper for 3 weeks. Each strand group was marked with a colored tape to track the development of fruits concerning their specific pollinizer. The experiment was organized in a complete randomized block design, comprising twelve treatments with three replicates. The negative control was used to verify the effectiveness of the pollination method.



Fig. 1 Various cross-pollination process steps include: **A:** Using Kraft paper to wrap the female bunches before flowering will help prevent pollen contamination. **B:** Separate each bunch into 12 groups, with 5 strands in each group, then wrap each group in Kraft paper. 11 different date palm cultivars' pollen sources, **C:** introduction of two male pollen strands into each

female group; **D:** closure of the envelopes following pollination. The worker cleans his hands with 95° alcohol after each pollination to get rid of any remaining pollen contaminants. **E–F:** In order to track the growth of fruits concerning their particular pollinator, each female strand group was marked with a different colored tape

In vitro pollen germination and viability

The in vitro *pollen* germination and viability percentages for 11 different male date palm pollen sources were assessed according to (El Kadri and Ben Mimoun 2020). Pollen was incubated in germination media for 24 h and examined under a microscope to calculate the proportion of pollen grains that successfully produced pollen tubes (germination percentage), then pollen viability was estimated using fluorescein diacetate staining and epifluorescent microscopy.

Fruits set, fruit retention, and fruit's physical characteristics

At different times, the fruit set and retention were assessed. The fruit set was evaluated at the "Kimir" stage, two months after pollination, using the technique described by (Chao and Krueger 2007; El Kadri and Ben Mimoun 2020). Four months following pollination, fruit retention was calculated in the meanwhile at the "Besser" stage. Each pollen source received five randomly chosen female strands, with three replicates in each group. The following formulas were used to determine the percentages: Fruit set (%) = $100 \times (N_{st}/N_t)$.

$$\text{Fruit retention (\%)} = 100 \times (N_{rt}/N_t).$$

Here are the definitions of the variables used:

N_{st} : number of setting flowers per strand.

N_t : number of total flowers per strand.

N_{rt} : number of retained fruit per strand.

N_s : number of flower scars per strand.

The morphometric analysis was conducted on 8 date fruits per pollen source at the Tamr harvest stage. Accordingly, ten fruits were randomly chosen from each strand group, and their pulp, weight, fruit, and seed measurements were recorded. We determined the average weight of each pollinator in grams by weighing the fruit on computerized scales. A caliper micrometer was also used to measure the millimeters' thickness, length, and width.

Biochemical and mineral composition analysis of fruits

The physicochemical analysis was carried out in triplicate for soluble solids (TSS), measured as °Brix in the fruit juice using a hand refractometer (Sopelem). Initially, 10 g of date pulp were hydrated in 100 mL of distilled water, and the resulting juice was collected separately. Afterward, it was centrifugated for 15 min at 4000 rpm before being filtered through Whatman No. 41 filter paper. The °Brix value of the juice was determined using a refractometer (Chang et al. 2002).

Total titratable acidity (TA) was determined in the juice by titration with 0.1N sodium hydroxide, with phenolphthalein as the indicator, and the results were expressed as a percentage of citric acid. For measuring titratable acidity, 25 g of fruit pulp were mixed with 50 mL of boiled distilled water in a blender and then filtered. Subsequently, 25 mL of the filtrate (juice) was titrated (Sadler and Murphy 2010).

Using a Consort C6010 electrochemical analyzer and following industry standards, the pH of the samples was determined (David et al. 2022). Water activity (WA) was assessed at 25 °C using an AW Sprint TH-500 apparatus. Moisture content was determined by drying 5 g of pulp at 103 °C until a constant weight was achieved. The results were expressed as a percentage of the fresh weight.

Using techniques from (El Arem et al. 2012), the ash content was calculated by burning 1 g of dry pulp in a porcelain container at approximately 530 °C for 5 h. Ash contents were calculated as a proportion of the dry matter.

Jackson (2005) prescribed the Na and K contents (mg/100g of fresh material), and accordingly, Na and K were determined using a flame photometer. The P content (mg/100g of fresh material) was assessed via the colorimetric method employing the yellow vanadate-molybdate assay, as outlined by Chapman and Pratt (1962). Mineral concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and expressed on a fresh weight basis.

Total sugars were quantified through the phenol–sulfuric acid reaction (Dubois 1956), while reducing sugars were determined utilizing the dinitrosalicylic acid method (Miller 1959). Glucose was used as the calibration standard for both total and reducing sugars.

Statistical analysis

The impact of the pollen source on the observed parameters was assessed using the analysis of variance (one-way ANOVA) (at least three replicates were used for each analysis). The least significant differences (LSD) at $p \leq 0.05$ were used to compare means. Using the Pearson correlation coefficient of SPSS Ver. 16.0 statistical software (SPSS Inc., Chicago, IL, USA), linear correlation was used to determine the associations among fruiting parameters.

The correlation coefficient plot was created using the "Corrplot" package (Team 2021).

Results

Pollen germination and viability of the eleven male cultivars

The *in vitro* pollen germination and viability percentages for 11 different male date palm pollen sources are presented in Table 2. The table showed a substantial difference in pollen germination percentage for pollen sources, ranging from 50.3% (PS2) to 86.3% (P169) ($p < 0.0001$). The highest pollen germination was observed for P169, P13, P8, and ABD1, which did not differ significantly. Additionally, pollen viability varied significantly amongst sources, from 78.3% (PS2) to 99.0% (ABD3) ($p < 0.0001$). The pollen viability that showed out the most was found in ABD3, P90, P195, and P169.

Effect of pollen source on fruit set, fruit retention, and fruit characteristics

For the female cultivar Deglet Nour, the impact of 11 distinct male date palm pollen sources on fruit set % and fruit retention percentage is shown in (Table 3).

Table 3 Effect of pollen sources on fruit set, fruit retention of date palm cv. "Deglet Nour"

Pollen source	Fruit set (%)	Fruit retention (%)
ABD1	88.71 ± 3.46 a	68.92 ± 4.63 a
ABD2	81.72 ± 3.01 b	63.22 ± 1.43 ab
ABD3	71.65 ± 6.10 cd	65.95 ± 7.36 ab
PS2	78.22 ± 5.21 bc	52.63 ± 1.86 b
P4	77.87 ± 6.63 bc	63.74 ± 4.20 ab
P7	75.82 ± 7.68 bc	57.12 ± 5.06 b
P8	61.00 ± 3.72 e	41.77 ± 4.12 d
P13	90.64 ± 4.46 a	69.73 ± 1.12 a
P90	62.52 ± 6.82 e	44.25 ± 3.61 cd
P165	72.49 ± 4.82 cd	51.54 ± 2.16 bc
P169	67.02 ± 4.98 de	40.6 ± 6.76 d
LSD	7.0702	9.0361
<i>p</i> value	< 0.0001	< 0.0001

Values are the mean of five samples per treatment. Means followed by a different letter(s) are significantly different at 5% probability using the LSD test.

Significant variation was observed between pollen sources for fruit set and fruit retention ($p < 0.0001$). The highest fruit set was achieved with pollen from ABD1 (88.71%) and P13 (90.64%), which were not significantly different. The lowest fruit set resulted from crosses with P90 (62.52%) and P169 (67.02%). For fruit retention, pollination by ABD1 produced the maximum retention rate (68.92%), significantly exceeding all other pollen sources except P13 (69.73%). The lowest fruit retention percentage (40.6%) was obtained with P169 pollen.

Effect of pollen source on physical fruit characteristics

The physical properties of date fruits from the female cultivar Deglet Nour pollinated with 11 different male pollen sources are described in (Table 4), which revealed that fruit weight ranged from 9.16 g (ABD3) to 11.59 g (P165), with significant variation between pollen sources ($p = 0.0001$). The fruit from the P165 pollen donor was the heaviest, and the fruits from ABD1 and P165 were heavier than those from the other pollen donors. Pollen sources did not significantly affect fruit length. However, pollen source impacted fruit width ($p = 0.0195$), with ABD1, ABD2, P7, and P165 pollination producing the widest fruits. Additionally, there were significant differences in pulp weight ($p = 0.0015$), with ABD1, P165, and P13 pollination yielding the highest pulp weights.

Pollen supply impacted seed weight, size, and pulp thickness ($p < 0.0001$). The P90 pollen source produced the pulp that was the thickest.

Effect of pollen source on fruit's chemical composition

The effect of 11 different male date palm pollen sources on mineral content (sodium, potassium, phosphorus) in fruits of the female cultivar Deglet Nour is shown in (Table 5). Significant variation was observed between pollen sources for sodium, potassium, and phosphorus contents ($p < 0.0001$). The highest sodium concentration resulted from pollination with ABD2 (15.4 mg/100 g), while the lowest was obtained with P90 (9.6 mg/100 g). Potassium content ranged from 64.6 mg/100 g (PS2) to 129.6 mg/100 g (P169). Phosphorus concentration was maximized by ABD3 pollination (99.7 mg/100 g) and minimized by P13 (17.3 mg/100 g).

Chemical analysis results were performed on mature Tamr-stage fruits as described in (Table 6), which showed that the total soluble solids (TSS) varied from 60.66% (P4) to 70.80% (ABD3), depending on the pollen source ($p < 0.05$). Titratable acidity ranged from 0.16% (P4) to 0.30% (P7). Ash content was lowest with ABD3 pollination (2.12%) and highest with ABD2 (2.55%). Fruit moisture content was maximized by ABD1 pollination (24.2%) and minimized by P8 (17%). Water activity differed between

Table 4 Effect of pollen source on fruit physical proprieties of date palm cv. "Deglet Nour"

Pollen source	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Pulp weight (g)	Seed weight (g)	Pulp thickness (cm)	Seed length (cm)	Seed width (cm)
ABD1	11.13 ± 0.81 a	3.97 ± 0.18 a	1.99 ± 0.15 a	9.94 ± 0.56 a	1.11 ± 0.25ab	0.47 ± 0.08bc	2.57 ± 0.24ab	0.64 ± 0.06f
ABD2	9.72 ± 0.85 b	3.98 ± 0.22 a	2.01 ± 0.12 a	8.78 ± 0.71c	0.93 ± 0.22 bcd	0.36 ± 0.09 e	2.53 ± 0.25ab	0.83 ± 0.09 b
ABD3	9.16 ± 2.84 b	3.96 ± 0.16 a	1.95 ± 0.17 a	9.08 ± 0.77 b	1.03 ± 0.18 abc	0.44 ± 0.11 bcd	2.59 ± 0.27ab	0.79 ± 0.08bcd
PS2	9.66 ± 0.44 b	3.88 ± 0.25 b	1.95 ± 0.19 a	8.64 ± 0.52 c	1.02 ± 0.15abc	0.40 ± 0.07 cde	2.49 ± 0.11 ab	0.80 ± 0.09 bc
P4	9.87 ± 0.70 b	4.10 ± 0.81 a	1.75 ± 0.13 b	8.85 ± 0.54 c	1.01 ± 0.28abc	0.46 ± 0.08 bc	2.6 ± 0.23 ab	0.71 ± 0.06ef
P7	9.70 ± 0.46 b	3.93 ± 0.21 b	2.02 ± 0.22 a	8.68 ± 0.56 cd	1.00 ± 0.35 abc	0.45 ± 0.10 bc	2.55 ± 0.13ab	0.84 ± 0.07b
P8	9.62 ± 0.43 b	3.87 ± 0.11 c	1.94 ± 0.14a	8.77 ± 0.62 c	0.97 ± 0.10abcd	0.37 ± 0.04 de	2.41 ± 0.20ab	0.73 ± 0.10b
P13	10.03 ± 1.28 b	3.89 ± 0.15b	1.87 ± 0.24 ab	9.04 ± 1.02 b	0.9 ± 0.23 6bcd	0.48 ± 0.07 b	2.43 ± 0.26ab	0.84 ± 0.16cde
P90	9.77 ± 0.79 b	3.86 ± 0.81 a	1.88 ± 0.15 ab	8.87 ± 0.79 c	0.91 ± 0.10 cd	0.62 ± 0.06 a	2.68 ± 0.14a	0.72 ± 0.06 de
P165	11.59 ± 0.99 a	3.99 ± 0.11 a	2.00 ± 0.25 a	9.53 ± 2.87 ab	1.16 ± 0.13 a	0.40 ± 0.08 cde	2.50 ± 0.17 ab	0.93 ± 0.08a
P169	9.33 ± 0.57 b	3.83 ± 0.13 b	1.97 ± 0.11 a	8.53 ± 0.47 d	0.81 ± 0.11 d	0.48 ± 0.09 b	2.33 ± 0.09b	0.68 ± 0.02ef
LSD	1.0056	0.1641	0.1476	0.9573	0.186	0.07656	0.259	0.077
<i>p</i> value	0.0001	0.0769	0.0195	0.1515	0.0366	<0.0001	0.014	<0.0001

Values are the mean of eleven samples per treatment

Means followed by a different letter(s) are significantly different at a 5% probability using the LSD test.

Table 5 Effect of pollen sources on mineral content (mg/100 g fresh weight) of date palm cv "Deglet Nour"

Pollen source	Na	K	P
ABD1	11.7±0.41d	102.8±0.29f	6.4±0.95j
ABD2	15.4±0.50a	76.9±0.15 j	86.3±1.30b
ABD3	10.1±0.19ef	109.2±0.86e	99.7±0.47 a
PS2	12.8±0.32c	64.6±0.52 k	32.5±0.59 f
P4	14.7±0.26b	96.1±0.13 g	30.1±1.02 g
P7	15.8±0.27a	122.1±0.33c	48.9±1.32e
P8	11.4±0.47d	128.4±0.88b	72.7±1.09 c
P13	10.2±0.24e	83.5±0.47 i	17.3±1.42i
P90	13.3±0.33c	115.1±0.25d	24.8±1.52 h
P165	9.6±0.29f	89.7±0.44 h	56.0±1.32d
P169	11.1±0.17d	129.6±1.12a	49.4±1.78e
LSD	0.56301	0.94123	2.0772
<i>p</i> value	<0.0001	<0.0001	<0.0001

Values are the mean of ten samples per treatment

Means followed by a different letter(s) are significantly different at 5% probability using the LSD test

0.55 (P165) and 0.79 (ABD1). Among pollen sources, the pH ranged from 5.08 (P7) to 5.50 (ABD2).

Total sugar and reducing sugars also varied with pollen source. Total sugars were highest with P90 (75.13%) and lowest with PS2 (57.31%). For reducing sugars, P7 pollination resulted in maximal levels (41.65%), while ABD2 produced the lowest (17.82%).

A positive Pearson correlation coefficient (Fig. 2) indicates a favorable association between two variables between various fruit quality and pollen characteristics in date palms, while a negative value implies an inverse relationship. Several notable correlations are evident. Fruit acidity showed a strong negative correlation with pH (−0.812) and moisture content (−0.527) but a positive correlation with total sugars (0.460). Potassium content exhibited positive correlations with pollen germination rate (0.608) and pulp thickness (0.316), while sodium was negatively correlated with moisture (−0.354). Phosphorus level had a high positive correlation with Brix (0.535).

Fruit and seed morphometrics like fruit width, seed width, and pulp thickness were generally inter-correlated. Interesting negative correlations occurred between pulp thickness and pH (−0.504) and seed width and acidity (−0.531). Pollen viability positively correlated with phosphorus (0.363) and moisture content (0.184).

The principal component analysis (PCA) of pollinators, when combined with the analyzed variables, revealed that the first two axes accounted for the maximum variability, capturing 42.1% of the total (Fig. 2). This analysis categorized the pollinators into four distinct clusters. The first group, comprising the cultivars P165, PS2, and ABD2, is characterized positively by Axes 1 and 2. This group shows a positive correlation with variables such as Yield, Fruit Weight,

Table 6 Effect of pollen sources on fruit chemical characteristics of date palm cv. "Deglet Nour"

Pollen source	TSS (%)	Acidity (%)	Ash content (%)	Moisture content (%)	Water activity (a _w)	pH	Total sugar (%)	Reducing sugar (%)
ABD1	64.80±0.552 c	0.21±0.003 g	2.47±0.043b	24.20±0.408 a	0.79±0.0075a	5.12±0.0065 g	63.21±0.210e	24.00±0.259d
ABD2	61.91±0.365 f	0.26±0.0010 d	2.55±0.050 a	19.00±0.325f	0.72±0.0025ab	5.50±0.024a	69.38±0.631bc	19.62±0.333 h
ABD3	70.80±0.393a	0.23±0.0060f	2.12±0.034f	20.10±0.192d	0.66±0.010d	5.33±0.029c	68.33±0.701d	30.01±0.408b
PS2	64.75±0.531 cd	0.24±0.0014e	2.22±0.022 e	23.20±0.344b	0.67±0.0026d	5.47±0.022b	57.31±0.491 g	17.82±0.741i
P4	60.66±0.637 g	0.16±0.0007i	2.42±0.028 b	19.50±0.255 e	0.75±0.0078ab	5.28±0.011d	57.80±0.705 g	22.72±0.445e
P7	64.00±0.123de	0.30±0.0014 a	2.27±0.015de	19.30±0.324 ef	0.62±0.0024e	5.08±0.020 h	69.59±0.420b	41.65±0.074a
P8	68.25±0.157b	0.29±0.0058b	2.31±0.010 cd	17.00±0.120 g	0.65±0.0042d	5.23±0.0072e	63.56±0.426 e	20.30±0.338 g
P13	61.05±0.810 g	0.23±0.0053 f	2.32±0.017c	22.80±0.173b	0.71±0.0055bc	5.26±0.014d	69.31±0.561bc	29.34±0.370c
P90	63.75±0.559 e	0.24±0.0012e	2.13±0.024 f	21.30±0.370 c	0.70±0.0032c	5.19±0.016f	75.13±0.771a	22.11±0.074ef
P165	63.75±0.463e	0.27±0.0005c	2.33±0.027c	21.10±0.168c	0.55±0.0021 g	5.34±0.0050c	68.61±0.140 cd	21.65±0.111f
P169	62.00±0.263f	0.22±0.0008 g	2.52±0.023a	24.10±0.138a	0.61±0.0036f	5.06±0.0093 h	59.49±0.566f	29.34±0.296c
LSD	0.8196	0.0077	0.04677	0.46471	0.0085	0.02384	0.92289	0.61568
<i>p</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values are the mean of three samples per treatment

Means followed by a different letter(s) are significantly different at 5% probability using the LSD test

TSS: Total soluble solids (Brix); WA: Water Activity; pH: hydrogen potential

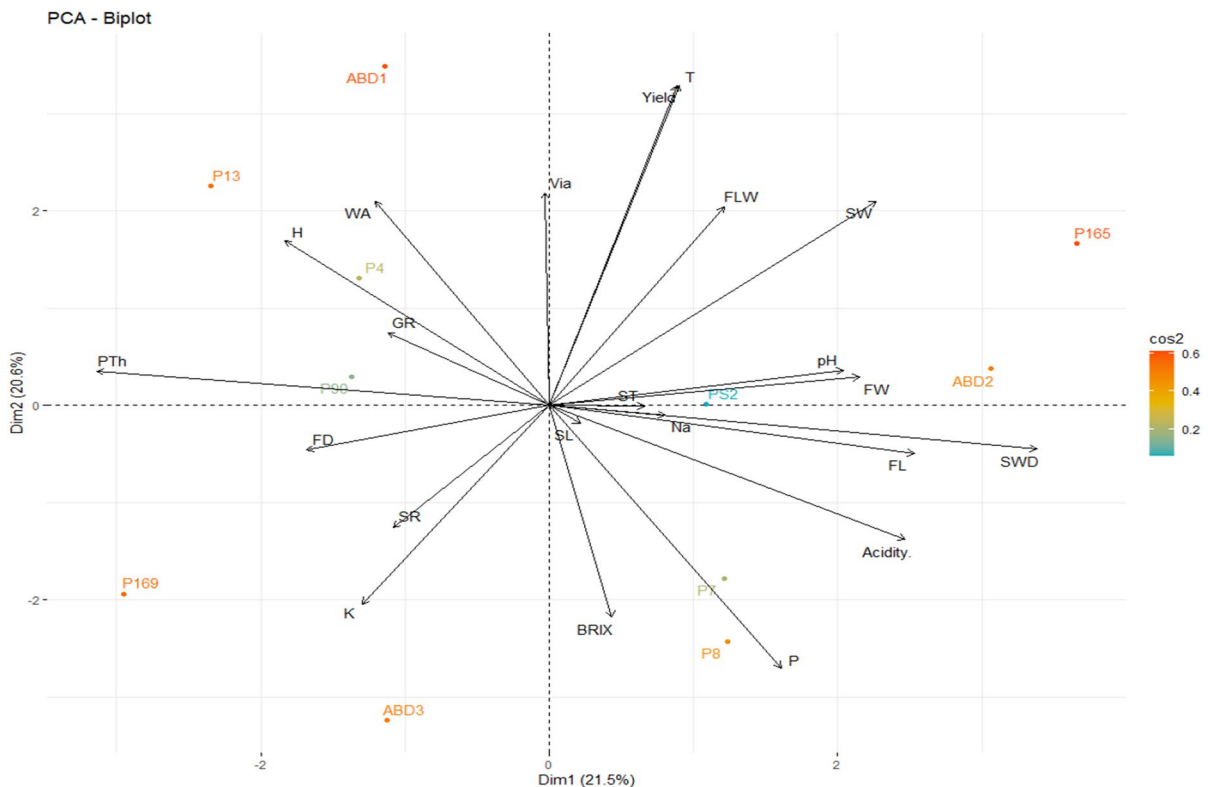


Fig. 2 Multivariate analysis of pollinators according to the variables studied on the plan defined by axis 1 and 2. Via: Viability, T: Ash content, FLW: Fruitweight, SW: Seed width, ST: Total sugar, FW: Fruit width, SWD: Seed width, FL: Fruit

length, SL: Seed length, SR: reducing sugar, FD: Fruit diameter, PTh: Pulp thickness, GR: Germinability, H: Moisture content, WA: Water activity

Seed Weight, pH, Fruit Width, Ash Content, and Total Sugar. The second group, consisting solely of cultivars P7 and P9, correlates negatively with Axis 1 and positively with Axis 2. It is defined by variables like Seed Length, Sodium Content, Fruit Length, Acidity, Seed Width, and TSS Content. The third cluster includes cultivars P169 and ABD3, correlating negatively with both axes and defined by Fruit Diameter, Reducing Sugar, and Potassium Content. The final group, containing the pollinators ABD1, P4, P13, and P90, correlates positively with Axis 1 and negatively with Axis 2. This group is characterized by variables such as Water Activity, Water Content, Germination Rate, Fruit Thickness, and Viability. The correlation coefficients (Fig. 3) show several strong correlations between the variables. For example, there is a strong positive correlation between water activity and TSS ($r=0.80$), between Total Sugar and Reducing sugar ($r=0.70$), and between Fruit weight

and TSS ($r=0.60$). Acidity and TSS also strongly correlate negatively ($r=-0.60$).

Discussion

The present investigation was initiated to delve into the intricate dynamics governing the impact of 11 distinct Tunisian pollen sources on the pivotal aspects of fruit set, fruit retention, and fruit quality within the esteemed date palm cultivar, 'Deglet Nour.' The viability and germination of pollen grains, which are crucial criteria for date palm cultivation success, are of the utmost importance in this endeavor. It is well-established in the scientific literature that there is a considerable variance in pollen viability across different species and cultivars, a phenomenon readily observable in date palms (Mesnoua et al. 2018a, b). This observed variation may be attributed, in part,

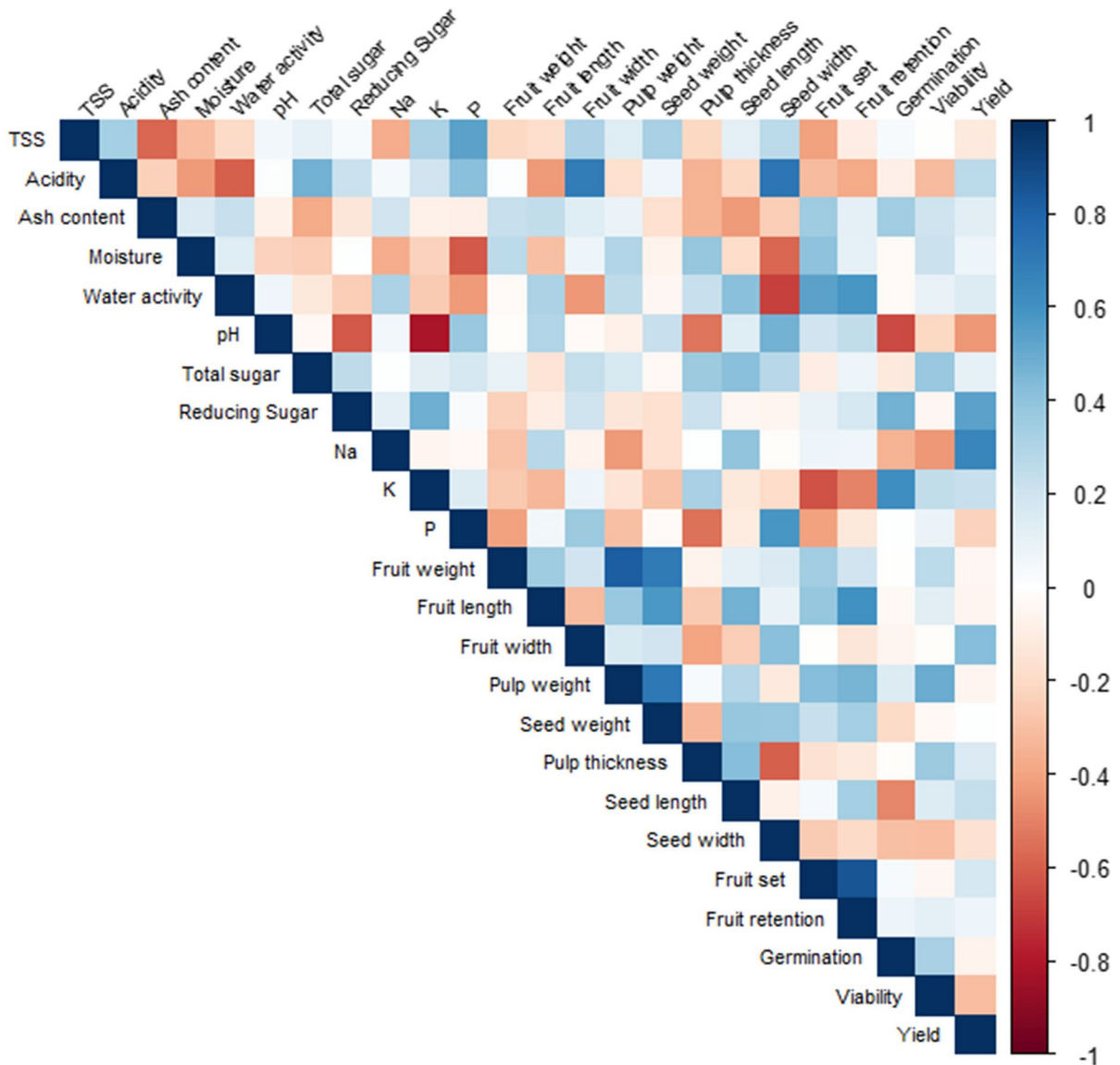


Fig. 3 Pearson correlation coefficients between various physical, chemical, and yield traits of date palm fruits pollinated with different male sources

to the genetic diversity inherent in different cultivars (Kadri et al. 2017; Soliman and Al-Obeed 2013). This variation may manifest within the same cultivar when cultivated under diverse pedoclimatic conditions.

Furthermore, it is essential to recognize that pollen quality can vary throughout a flowering period (Karim et al. 2022a, b). Munier (1973) notably suggested that the pollen from the initial and terminal inflorescences often exhibits lower quality. In our study, the average pollen viability, assessed by

the in-vitro germination test and staining method, exceeded 60% and reached approximately 73% and 93%, respectively. These values surpass the threshold proposed by Dollé and Peyron (2000), who suggested a minimum in-vitro germination rate of 60% to ensure a successful fruit set. The variances observed in fruit setting and retention among different pollen sources are likely attributed to genetic disparities. These findings align harmoniously with the conclusions drawn by Hafez et al. (2014), who reported variations in

fruit sets between the 'Siwi' and 'Zaghloul' varieties. However, the percentages of fruit set recorded in our study surpassed those documented by Salomon-Torres et al. (2017), who reported fruit set values of less than 50% in 'Medjool' trees pollinated with derived males from the most common cultivars 'Deglet Noor,' 'Khadrawy,' 'Zahidi,' and 'Medjool' pollen. The influence of pollen source on fruit weight, a phenomenon well-documented in research by Omar and El-Abd (2014), Kadri et al. (2017), and Salomon-Torres et al. (2017), which was consistent with our findings.

In contrast, our study did not reveal any remarkable effect of pollen sources on fruit length, with most pollen-yielding fruits of similar lengths. These outcomes resonate with prior research by Al-Muhtaseb and Ghnaim (2006) and Omar and El-Abd (2014), indicating that pollen sources generally do not significantly impact fruit length. Conversely, our investigation revealed that fruit width was influenced by pollen sources, in agreement with the conclusions of Abdel-Hamid (2000). Pulp weight, however, exhibited minimal variation, which contradicts the findings of Rezazadeh et al. (2013) and Salomon-Torres et al. (2017), who reported a direct effect of pollen sources on pulp weight. In contrast, pollen sources significantly affected pulp thickness in our study, which is consistent with the results of Mustafa et al. (2014).

Our findings also indicate that different pollen sources can affect the weight and width of seeds. However, the analysis of the variance concerning the effect of different pollen sources on seed length parameters showed that the differences were not statistically significant. Thus, our results align with those of previous research, including Mustafa et al. (2014), Islam (2017), Salomon-Torres et al. (2017) and Soliman (2020). Moreover, our study elucidated that pollen sources significantly impacted fruit Na, K, and P contents, an observation at odds with the findings of Soliman (2020), who reported significant effects of pollen sources solely on Fe and Mn contents. Salomón-Torres et al. (2018) discovered significant differences in macrominerals (potassium, magnesium, phosphorus, sodium, and sulfur) when using four different pollen sources. Additionally, the same study reported significant variations in microelements, including silicon, selenium, copper, iron, strontium, manganese, and zinc content. Borchani et al. (2010) found that the Deglet Noor variety exhibited the highest potassium level compared to 11 other

cultivars analyzed for their chemical properties. The variation in total soluble solids (TSS) values observed can be attributed to the inherent genetic diversity among male cultivars, which aligns with the findings of Mustafa et al. (2014), Omar and El-Abd (2014), and Shafique et al. (2011).

Titratable acidity percentages were also observed to change, in agreement with Farag et al. (2012), Mustafa et al. (2014) and Salomón-Torres et al. (2018). Our results unveiled that fruits with the highest flesh weight (ABD1) were associated with the highest moisture content, a trend corroborated by the findings of Omar and El-Abd (2014). Notably, water activity was influenced by the pollen source, with the highest value recorded for ABD1 and P4. This value, however, remains below the threshold recommended to prevent pathogenic microorganism proliferation ($WA < 0.86$). Elevated water activity has been previously associated with increased fungal proliferation in fruits (Blaha et al. 2006).

Furthermore, the variation in pollen sources also manifested in pH values, a critical factor, as acidic pH values in dates pose a heightened risk for fungal contaminations, as noted by Nasser (2017). Sugar content is a crucial indicator of the nutritional value of date fruits, and our results indicate that pollen sources substantially influence it. However, this variation in sugar content may be attributed to the enzyme systems initiated by the metaxenial effect, which subsequently passes into extracellular sites and dissolves readily into the water, leading to the inversion of sugars (Shafique et al. 2011). Hydrolytic enzymes, such as polygalacturonase and cellulase, are believed to play a role in this biochemical process by solubilizing pectin and cellulose from the cell wall (Omar and El-Abd 2014). Reducing sugars, comprising a mixture of glucose and fructose formed during the hydrolysis of sucrose by invertase, were notably influenced by pollen sources, a phenomenon supported by the findings of Mustafa et al. (2014), and Soliman and Al-Obeed (2013).

In addition to these insights, the correlation coefficients derived from our study provide further evidence of the interrelatedness of variables, hinting at their potential role in shaping fruit quality and yield. For instance, the strong positive correlation between water activity (a_w) and total soluble solids (TSS) underscores the pivotal role of (a_w) in determining the sugar content of fruits. On the other hand,

the strong negative correlation between acidity (Ac) and TSS suggests that acidity plays a crucial role in determining the sweetness of fruits (Salomón-Torres et al. 2018). However, by selecting the best pollinators, these findings and insights may help create strategies to improve fruit quality and yield in date palm cultivation.

Conclusion

The findings of this study reveal that the choice of pollen sources exerts a significant influence on various physiological parameters, including germination and viability, as well as on crucial agronomic indicators such as fruit set, retention rate, and fruit weight. Moreover, these sources of pollen significantly impact the physico-chemical characteristics of dates. However, among the tested pollen sources, P13, ABD1, and P165 emerged as the most effective in enhancing fruit set, fruit retention, and date weight, and notably, fruit quality also demonstrated sensitivity to the choice of pollen sources. For instance, P13 exhibited the highest levels of total and reducing sugars, underscoring its potential to enhance the sweetness of the fruit. Conversely, P165 was associated with the lowest water activity values, a crucial factor in fruit preservation, while ABD3 induced the highest total soluble solids (TSS) content elsewhere in the study. As a result, these findings provide insightful information for in vitro propagation initiatives. In order to start cloning the selected male trees in Tunisia, farmers are looking for practical recommendations that will help them maximize fruit quality. Future research should focus on examining the effects of other pollen sources, particularly from coastal oases, in this selection process. Additionally, their compatibility with the Deglet Nour variety, as well as other varieties, warrants investigation. It is also advisable to replicate this study across different seasons to establish reliable conclusions and stable indicators for selecting pollinators with a metaxenic effect.

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Author contributions KK: Shaping the study's conception, drafting the manuscript and design, SS, MA: Visualization, MJ, SM: Methodology, Data curation, MM: Statistical analysis, ME: Writing, review, editing, statistical analysis, and visualization.

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Data availability The datasets produced and/or analyzed during the present study can be obtained from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors affirm that they do not possess any competing interests.

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References

- Abdel-Hamid N (2000) Effect of time, rate and patterns of thinning, leaf/bunch ratio and male type on "Zaghloul" date yield and quality. *Arab Univ J Agric Sci* 8(1):305–317
- Al-Muhtaseb JA, Ghnaim HD (2006) Effect of pollen source on yield, quality and maturity of "Barhi" date palm. *Jordan J Agricul Sci* 2:9–14
- Alyafei MA, Al Dakheel A, Almoosa M, Ahmed ZF (2022) Innovative and effective spray method for artificial pollination of date palm using drone. *HortScience* 57(10):1298–1305
- Blaaha J, Baloch E, Grube M (2006) High photobiont diversity associated with the euryoecious lichen-forming ascomycete *Lecanora rupicola* (Lecanoraceae, Ascomycota). *Biol J Lin Soc* 88(2):283–293
- Borchani C, Besbes S, Blecker C, Masmoudi M, Baati R, Attia H (2010) Chemical properties of 11 date cultivars

- and their corresponding fiber extracts. *Afr J Biotech* 9(26):4096–4105
- Bouguedoura N, Bennaceur M, Babahani S, Benzouche SE (2015) Date palm status and perspective in Algeria. *Date Palm Genet Resour Utiliz* 1:125–168
- El Arem A, Saafi EB, Flamini G, Issaoui M, Ferchichi A, Hammami M, Achour L (2012) Volatile and nonvolatile chemical composition of some date fruits (*Phoenix dactylifera* L.) harvested at different stages of maturity. *Int J Food Sci Tech* 47(3):549–555
- Chang W-K, Chen M-Z, Chao Y-C (2002) Use of the refractometer as a tool to monitor dietary formula concentration in gastric juice. *Clin Nutr* 21(6):521–525
- Chao CT, Krueger RR (2007) The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. *HortScience* 42(5):1077–1082
- Chapman HD, Pratt PF (1962) Methods of analysis for soils, plants and waters. *Soil Sci* 93(1):68
- David IG, Buleandră M, Popa DE, Bercea AM, Ciucu AA (2022) Simple electrochemical chloramphenicol assay at a disposable pencil graphite electrode by square wave voltammetry and linear sweep voltammetry. *Anal Lett* 55(10):1531–1548
- Dollé V, Peyron G (2000) Cultiver le palmier-dattier: guide illustré de formation. Montpellier CIRAD, 110. ISBN 2–87614–399–2
- El Kadri N, Ben Mimoun M (2020) In vitro germination of different date palm (*Phoenix dactylifera* L.) pollen sources from Southern Tunisia under the effect of three storage temperatures. *Int J Fruit Sci* 20(sup3):S1519–S1529
- Dubois K (1956) The phenol-sulfuric acid reaction for carbohydrates. *Anal Chem* 28:3350
- Farag K, Elsabagh A, ElAshry H (2012) Fruit characteristics of “Zaghloul” date palm in relation to metaxenic influences of used pollinator. *Am Euras J Agric Environ Sci* 12(7):842–855
- Hafez OM, Saleh MA, Mostafa E, Naguib M, Ashour N (2014) Effect of pollen grain sources on yield and fruit quality of Samany date palm. *Int J Agric Res* 9(3):164–168
- Hannachi S, Khitri D, Benkhalifa A, Brac de la Perrière RA (1998) Inventaire variétal de la palmeraie algérienne. Ministère de l’agriculture et de la pêche, Ministère de l’enseignement supérieur et de la recherche scientifique. Ed. ANEP Rouiba, 225
- Hannachi S (1998) Inventaire variétal de la palmeraie algérienne. (*No Title*).
- Islam S (2017) Evaluation of date palm males types as pollinators for Zaghloul and Samany date palm cvs. grown in Qalyubia governorate. *Mid East J Agric Res* 6(4):1049–1056
- Ismail HB, Hassine DB (2021) Tunisian date cultivars: Economical aspect, physicochemical properties, sensory characterization and potential valorization. In: *Agriculture productivity in Tunisia under stressed environment* pp 57–71. Springer.
- Jackson M (2005) *Soil chemical analysis: advanced course*. Madison, WI, UW-Madison Libraries Parallel Press
- Kadri K, Abdelhafidh M, Aounallah K (2021) Analysis of the morphological diversity of inflorescence of six Tunisian date palm (*Phoenix dactylifera* L.) pollinators. *Eur J Agric Food Sci* 3(4):56–62
- Kadri K, Boaaga K, Makhlof S (2017) Physiological characterization of some male pollinators in Tunisia and study of the effect of conditioning temperature on the viability and germination of pollen. *New Sci* 48:2907–2920
- Karim K, Awad MA, Manar A, Monia J, Karim A, Mohammed E (2022a) Effect of flowering stage and storage conditions on pollen quality of six male date palm genotypes. *Saudi J Biol Sci* 29(4):2564–2572
- Karim K, Halima B, Khaled C, Nabila K, Foued B (2015) Genetic diversity analysis of a local date palm pollinators collection (*Phoenix dactylifera* L.) using SSR markers and study of their metaxenic effects on the maturation and quality of dates obtained. *Acad J Agric Res* 3(12):381–394
- Karim K, Hamza H, Tiba H, Elsafy M (2022) The genetic diversity analysis of tunisian male date palm cultivars (*Phoenix dactylifera* L.) revealed by phenotypic and molecular markers. *Eur J Biol Biotechnol* 3(5):6–16
- Khouane AC, Akkak A, Benbouza H (2020) Molecular identification of date palm (*Phoenix dactylifera* L.) “Deglet noor” pollinator through analysis of genetic diversity of Algerian male and female ecotypes using SSRs markers. *Sci Hortic* 274:109668
- Mesnoua M, Roumani M, Bensalah M, Salem A, Benaziza A (2018) Optimization of conditions for in vitro pollen germination and pollen tube growth of date palm (*Phoenix dactylifera* L.). *J Fund Appl Sci* 10(1):158–167
- Mesnoua M, Roumani M, Mizab O, Zeguerrou R (2020) Heavy metals differentially affect date palm pollen germination and tube elongation. *Italus Hortus* 27:64–71
- Mesnoua M, Roumani M, Salem A (2018b) The effect of pollen storage temperatures on pollen viability, fruit set and fruit quality of six date palm cultivars. *Sci Hortic* 236:279–283
- Mesnoua M, Roumani M, Tahirine M et al (2023) Effect of pollen quantity on fruit set, seed germination and plantlet vigor of date palm cv. Deglet Nour. *Agric Res*. <https://doi.org/10.1007/s40003-023-00682-7>
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31(3):426–428
- 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 *Scientia Horticulturae* 224:343–350
- Munier P (1973) *Le palmier dattier*. Ed. G. P. Maisonneuve & Larousse. Paris, 49–50
- Mustafa E, Heiba S, Saleh M, Ashour N, Mohamed DA, El-Migeed M (2014) Effect of different pollinizer sources on yield, fruit characteristics and phylogenetic relationships with Amhat cv date palm (*Phoenix dactylifera* L.) in Egypt using RAPD markers. *Int J Agric Res* 9(7):331–343
- Nasser L (2017) Fungal contamination and invertase activity in dates and date products in Saudi Arabia. *Am J Food Technol* 12:295–300
- Omar A, El-Abd A (2014) Enhancing date palm (*Phoenix dactylifera*, L.) productivity, ripening and fruit quality using selected male palms. *Acta Adv Agric Sci* 2(6):11–19
- Rezazadeh R, Hassanzadeh H, Hosseini Y, Karami Y, Williams RR (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
- Rhouma S, Zehdi-Azouzi S, Ould Mohamed Salem A, Rhouma A, Marrakchi M, Trifi M (2007) Genetic diversity in ecotypes of Tunisian date-palm (*Phoenix dactylifera* L.) assessed by AFLP markers. *J Hortic Sci Biotechnol* 82(6):929–933
- Sadler GD, Murphy PA (2010) pH and titratable acidity. *Food Anal* 4:219–238
- Salomon-Torres R, Ortiz-Uribe N, Villa-Angulo R, Villa-Angulo C, Norzagaray-Plasencia S, Garcia-Verdugo C (2017) Effect of pollenizers on production and fruit characteristics of date palm (*Phoenix dactylifera* L.) cultivar Medjool in Mexico. *Turkish J Agric For* 41(5):338–347
- Salomón-Torres R, Ortiz-Uribe N, Sol-Uribe JA, Villa-Angulo C, Villa-Angulo R, Valdez-Salas B, García-González C, Iniguez Monroy CG, Norzagaray-Plasencia S (2018) Influence of different sources of pollen on the chemical composition of date (*Phoenix dactylifera* L.) cultivar Medjool in México. *Aust J Crop Sci* 12(6):1008–1015
- Shafique M, Khan AS, Malik AU, Shahid M, Rajwana IA, Saleem BA, Amin M, Ahmad I (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(2):831–839
- Soliman S, Al-Obeed R (2013) Investigations on the pollen morphology of some date palm males (*Phoenix dactylifera* L.) in Saudi Arabia. *Aust J Crop Sci* 7(9):1355–1360
- Soliman SS, Al-Saif AM, Al-Obeed RS (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 Phys Sciences* 10(3):437–446. <https://doi.org/10.24214/jcbps.B.10.3.33746>
- Team R (2021) R: A language and environment for statistical computing v. 3.6. 1 (R Foundation for Statistical Computing, Vienna, Austria, 2019). *Sci Rep*, 11:12957

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