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An appraisal of pollen germination and viability of varied male pollen sources of date palm (*Phoenix dactylifera* L.)

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Abstract

Date palm is a dioecious fruit tree that permits cross pollination for fruit-setting. The germination of viable pollens to fertilize ovule is influenced by environmental cues such as temperature. The germination and viability of pollen grains collected for pollination purpose also varied with the male pollinizer source and the male spathe opening time. An *in vitro* study performed to determine the percentage of pollen germination at different temperatures (10, 15, 20, 25 and 30 °C) and germination times (4, 8, 12 and 24 h) taken from different male sources and to identify any variations in the germination and viability of pollen grains collected from spathes opened at different dates (20th February, 1st and 10th March). Pollens from different male sources showed a significant difference regarding germination percentage when incubated at different temperatures and for different germination times. Pollens incubated at 30 (81.07%) and 25°C (78.17%) had the highest germination percentage which decreased to 51.04, 16.23, and 5.90% when incubation temperature dropped to 20, 15, and 10 °C, respectively. Similarly, 71.11% pollens germinated after 4 h of incubation at 30°C, which decreased to 63.26 and 25.40% when incubation temperature decreased to 25 and 20°C, respectively after same time interval. Pollen germination and viability significantly differed when they were collected from spathes opened at three different dates. Early opened spathes had lowest germination (57.58%) and viability percentages (81.19%) than the middle (81.11 and 91.53%) or late (80.71 and 93.05%) opening spathes. It is therefore, concluded that the optimum temperature for date palm pollen grains germination is 25-30°C and at these temperatures maximum pollens germinated within 4 h. Moreover, pollen grains from early opened spathes were less superior compared to the middle or late opening ones.

Key words: Date palm, *Phoenix dactylifera* L., pollen grains, germination, viability

Introduction

Date palm is an ancient fruit tree in the Arabian Peninsula and is known as the 'Tree of Life'. It is an important fruit crop in many countries across the globe and cultivated in Africa, Asia, Europe, and American continent (Pintaud *et al*., 2013). Date palm is a dioecious species, which produces male and female flowers on separate trees. Pollen grains are produced on a male palm and applied onto female's fruit buds for pollination (Bekheet and Hanafy, 2011). The male and female flowers of date palm are arranged unsystematically on a spikelet (strand) and are enclosed in a hard sheath, which is collectively called as spathe (inflorescence). The spathes of both flowers emerge and open at different time of a year. The male spathes appear earlier than the female ones that makes cross pollination inevitable (Zaid and de Wet, 2002). Date palm growers usually collect current season male spathes randomly from different pollinizers for pollination, which have varied effect on fruit morphology, yield and biochemical attributes (Haider *et al*., 2013, 2014; Maryam *et al*., 2015b; Munir *et al*., 2020b). Moreover, the female receptivity to pollen grains also differ due to its physiological maturity (Munir *et al*., 2020a). In some regions, the availability of pollen grain is disrupted due to weather disorders, in this case date palm growers rely on the stored pollen grains (Maryam *et al*., 2015a). Pollen grains usually collected from mixed male population are applied on the female flowers using different pollination methods such as pollen strands placement, manual or mechanical dusting and liquid pollination (Zaid and de Wet, 2002; Hajian, 2005; Munir, 2019; Munir *et al*., 2020c).

The quality of date palm pollen grains is important for growers as it affects fruit characteristics. The assessment of functional quality of pollens facilitates to monitor pollen grains vigor of different pollinizers, pollen-stigma interaction studies, crop improvement, incompatibility and fertility studies (Dafni, 1992). The quality of pollen grains is evaluated on the basis of their viability and vigor. Pollen vigor means the speed of pollen grains germination and the rate of pollen tube growth (Ottaviano and Mulcahy, 1989). Pollen viability is generally referred as the ability of pollen grains to germinate and transfer the sperm nuclei to the embryo sac (Shivanna *et al*., 1991). The tube nuclei from stigma of a female flower germinates towards ovary to fertilize it. Two synergid cells in the ovary attract pollen tube to grow down towards the ovule for fertilization. The tip growth of pollen tube is guided precisely by female stimuli for a successful fertilization (Higashiyama and Takeuchi, 2015) and several female-secreted peptides are identified which control the direction of pollen tube growth (Okuda *et al.,* 2009; Takeuchi and Higashiyama, 2012).

Date palm growers generally apply fresh pollens during pollination. However, stored pollens are also used in case of their scarcity at the time of female spathe opening. Mesnoua *et*

al. (2018) worked on both fresh and stored date palm pollens and recorded up to 87% germination in fresh pollens in *cvs.* Bouhlesse, Deglat Beida, Deglet Nour, Ghars, Halwaya and Moch Deglat. Study conducted on stored pollens of date palm *cvs.* Dhakki, Khadrawy and Hillawi, Maryam *et al*. (2015a) reported that 30°C was the best for their germination followed by 25°C after 24 h. *In vitro* study conducted by Furr and Ream (1972) showed that the pollen germination of date palm increased with rising temperature from 7 to 32°C. At 26°C, they observed that maximum pollen germination rate was within 20 min and above 70% germination was recorded within 2 h which increased to 88% within 24 h of incubation. Overall, at 22 to 43°C, 50-70% of pollen germinated within 2 h and above 80% germinated after 4 h of incubation.

As mentioned previously, the common practice for date palm pollination is the use of fresh pollens collected from male pollinizers, few weeks to a month earlier opened spathes than the female spathes. However, the ability of these pollen sources to germinate varies with the male pollinizers and prevailing field temperatures. Moreover, the male flowers of one tree are not emerged and opened at same time due to climatic change (Hegland *et al*., 2009). The spathe cracking time of different date palm cultivars varied from a few days to weeks, which may affect pollen germination and viability. Therefore, the objectives of present study were (1) to determine the suitable temperature for pollen germination of different male pollen sources, (2) to estimate the optimum time of tube growth, and (3) to evaluate germination and viability of pollens obtained from the spathes of different male pollen sources emerged and opened at different time.

Materials and methods

The experiment was conducted during 2016 and 2017. Twelve-year-old date palm male trees were selected at different geographical locations of King Faisal University, Al-Ahsa, Saudi Arabia. The GPS location of each date palm male tree was taken by Nomad 900x handheld computer (Trimble Inc, USA), which were; Male 1 (25° 16' 26.6" N 49° 42' 22.8" E), Male 2 (25° 16' 03.1" N 49° 42' 30.4" E), Male 3 (25° 20' 40.9" N 49° 35' 32.2" E) and Male 4 (25° 20' 39.4" N 49° 35' 29.3" E). Five matured spathes were collected from each male tree. The protective sheath of spathes was removed with the help of a sharp bird's beak knife. These spathes were then placed on craft brown paper sheet at room temperature (16±2°C) for 48 h. Pollen grains were collected by gently shaking the strands of the spathes, sieved through 50 µm polyester mesh, dried in glass desiccator, and were kept in a refrigerator at 4ºC until the start of incubation experiment.

The synthetic medium for pollen grains germination was prepared with a slight modification as reported by Alcaraz *et al*. (2011). It contained calcium nitrate (0.3 $g.L^{-1}$), sucrose (15% w/v), boric acid (0.2 $g.L^{-1}$), potassium nitrate (0.1 $g.L^{-1}$), magnesium sulphate (0.2 $g.L^{-1}$) and agar (1%). The germination medium was sterilized in SterilElite™ autoclave for 20 min at 121°C and 10 mL medium was poured in 85 mm diameter glass Petri dishes, which were then placed in laminar airflow hood for 45 min for cooling. The dried pollen grains of each male were dusted on synthetic medium. The Petri dishes containing synthetic medium and pollen grains of each male were placed at five incubation temperatures *i.e.,* 10, 15, 20, 25 and 30°C (Heratherm refrigerated incubator, ThermoFisher Scientific, USA) for 4, 8, 12 and 24 h. After each specific time interval, the germination percentage of pollen grains, which grow pollen tube equal or larger than the size of pollen grain (Mortazavi *et al*., 2010), was counted as germinated one using compound microscope (BTW1-169, National Optical and Scientific Instruments, Inc., USA). The pollen germination percentage was calculated using following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated pollens}}{\text{Total number of pollens}} \times 100$$

The spathes of four male palm trees opened at different dates *i.e.,* 20th February, 1st and 10th March were also collected and their physical characteristics (number of strands per spathe, spathe length, spathe width, number of flowers per strand and pollen grains weight per spathe), germination percentage and viability percentage were estimated. For germination study, the pollen grains collected at different dates were cultured in Petri dishes containing the above-mentioned medium and their germination percentage was counted under microscope. Colorimetric staining test was done to determine pollen viability of four date palm male spathes opened at different dates using 1% acetocarmine (Stanley and Linskens, 1974). The red coloured pollen grains under microscope were considered as viable, whereas discoloured or colourless pollen grains were counted as non-viable. Similarly, absolute pollen viability was determined using below formula (Visser *et al*., 1977):

$$\text{APV (\%)} = \frac{\text{Stained pollens (\%)} \times \text{Germinated pollens (\%)}}{100}$$

APV=Absolute pollen viability

The experiment was laid out on two factorial completely randomized design having five replicates in each treatment. The collected data was statistically analyzed using Statistical Analysis Software, Release 9.4 (SAS Institute, North Carolina, USA) and the Duncan Multiple Range Test (DMRT) was applied to determine the least significance difference between the means at 5% level of probability.

Results and discussion

Germination percentage of different male sources significantly ($P \leq 0.05$) increased with the increase in incubation temperatures (Fig. 1). Pollen grains incubated at 25 and 30°C statistically behaved alike and had higher germination percentage (78.17 and 81.07%, respectively). Pollens germination percentage was linearly but significantly decreased at 20°C (51.04%), which was observed lowest at 10°C (5.89%) and 15°C (16.23%). Comparing male pollen sources, Male 4 indicated 48.71% germination followed by Male 3 (47.26%). However, Male 2 and Male 1 were statistically at par *i.e.,* 45.36 and 44.59%, respectively. The best combination of interaction was between 30°C × Male 4 (84.76%) followed by 30°C × Male 3 (81.22%), and 25°C × Male 4 (80.35%) whereas pollen from all four male sources very poorly germinated at 10°C and 15°C temperatures. Fresh pollens obtained from different date palm cultivars showed higher germination at 25°C (Mesnoua *et al*., 2018). *In vitro* pollen germination studies were conducted on detached date palm flowers and the fastest germination and pollen tube elongation in female flowers occurred at 25 or 28°C (Reuveni *et al*., 1986). Maryam *et al*. (2015a) incubated stored and fresh pollens of three date palm cultivars at different temperatures (20, 25

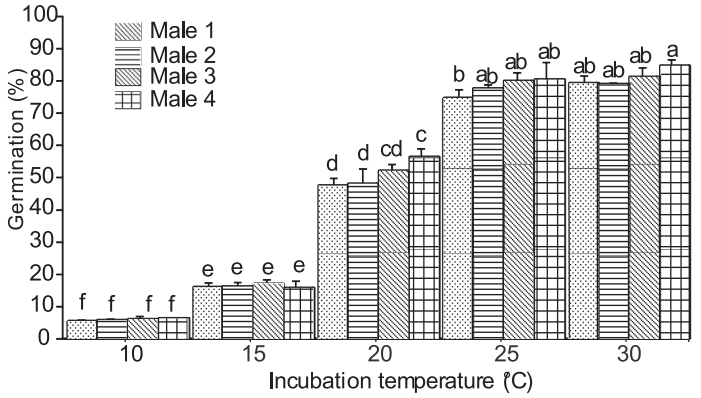

Fig. 1. Effect of five different incubation temperatures on pollen germination percentage of four date palm males. Error bars represent the variability within treatment.

and 30°C) and observed a linear increase when incubation temperature increased up to 30°C. Similarly, maximum germination percentage was reported in pomegranate pollens incubated at 28°C, which significantly declined at 10°C (Melgarejo *et al*., 2000). Increased incubation temperature (10, 20 and 30°C) had accelerating effects on germination pollen and pollen tube growth k vii) monitor the level of homozygosity inetics of different peach cultivars (Hedhly *et al*., 2005).

Highest germination percentage (60.79%) was observed after 24 h of incubation at 20°C temperature irrespective to male pollen sources, which significantly ($P \leq 0.05$) differed with other time intervals (Fig. 2). The interactional response between male pollen sources and incubation time indicated that Male 4 (63.05%), Male 3 (61.12%), and Male 1 (60.85%) had the best pollen germination at same temperature after 24 h. Similarly, Fig. 3 indicated that pollen germination percentage at 25°C at different time intervals (4, 8, 1 and 24 h) was highly significant regardless of male pollen sources and had highest value after 24 h (83.72%). The interaction data showed that all four male pollen sources had highest germination percentage after 12 h (79.84-81.63%) and 24 h (82.39-85.30%) incubation and were statistically at par. The data presented in Fig. 4 indicated that when pollens of four male trees placed in an incubator at 30°C for different time intervals, had the highest germination percentage after 12 h (83.44%) and 24 h (84.63%) incubation as both time intervals statistically behaved alike. Although, different male pollen sources were non-significant as it was at 20 and 25°C, however, the interaction between males and incubation time intervals was significantly differed as other two temperatures. Pollens obtained from Male 3 tree showed

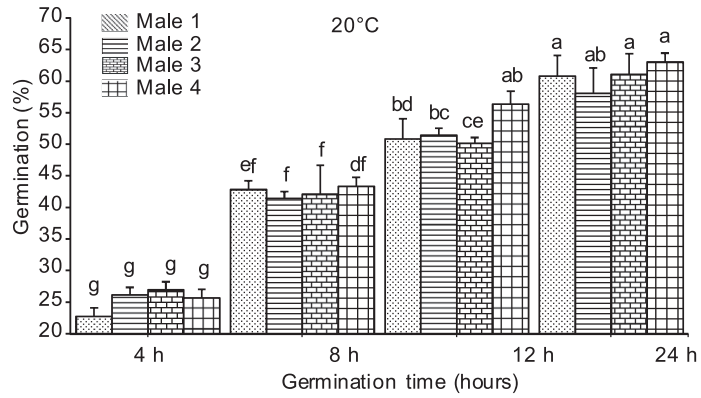

Fig. 2. Pollen germination response of four male date palm sources at varying time intervals incubated at 20 °C. Error bars represent the variability within treatment.

highest germination percentage (86.41%) after 24 h of incubation followed by Male 4 (85.36%). However, pollens from Male 4 (84.85%) and Male 3 (84.33%) incubated at 30°C for 12 h also showed promising results. Similarly, Male 1 (83.16%) and Male 2 (83.61%) at same temperature had maximum germination percentage after 24 h of incubation.

Table 1 indicated the pollen germination response of different male pollen sources with respect to varied time intervals (4, 8, 12, and 24 h) at different incubation temperatures (20, 25, and 30°C). In all four male sources of pollen, a more or less similar pattern of pollen germination was observed and showed a non-significant difference between them. However, there was a sharp and significant rise in pollen germination percentage from 20 to 25°C at all time intervals. An average increase in pollen germination of 60, 41, 35, and 27% was observed in all male pollen sources after 4, 8, 12 and 24 h, respectively between incubation temperatures of 20 to 25°C. However, it was 11, 10, 3, and 1% rise after 4, 8, 12, and 24 h germination time, respectively, between 25 and 30°C incubation temperatures in all male pollen sources. There are 55 genera where pollen germination occurred in 30 min or less time and their progamic phases completed in less than 60 h, however, 36 genera germinated pollen tube within 1 h or more (Nepi *et al*., 2001). Maryam *et al*. (2015a) observed that more than 40%

Table 1. Date palm pollen grains germination response of different male pollen sources to varied time intervals incubated at different temperatures

Treatments	Germination (%)		
	20 °C	25 °C	30 °C
Factor A: Pollen source			
Male 1	44.33^{A}	73.68^{A}	79.01^{A}
Male 2	44.30^{A}	74.16^{A}	78.86^{A}
Male 3	45.10^{A}	75.42^{A}	80.71^{A}
Male 4	47.13^{A}	76.61^{A}	81.24^{A}
DMRT (P=0.05)	2.96^{NS}	3.16^{NS}	2.51*
Factor B: Incubation time			
4 hours	25.39^{D}	63.26^{D}	71.10^{C}
8 hours	42.45^{C}	72.22^{C}	80.64^{B}
12 hours	52.23^{B}	80.66^{B}	83.44^{A}
24 hours	60.79^{A}	83.71^{A}	84.63^{A}
DMRT (P=0.05)	5.51*	2.84*	2.52*
Interaction: A × B			
Male 1 × 4 hours	22.75^{G}	60.86^{D}	70.99^{E}
Male 1 × 8 hours	42.86^{EF}	70.87^{BC}	79.98^{CD}
Male 1 × 12 hours	50.86^{BD}	80.58^{A}	81.93^{AD}
Male 1 × 24 hours	60.86^{A}	82.39^{A}	83.16^{AD}
Male 2 × 4 hours	26.16^{G}	62.46^{D}	70.28^{E}
Male 2 × 8 hours	41.46^{F}	71.46^{BC}	78.91^{D}
Male 2 × 12 hours	51.46^{BC}	79.83^{A}	82.64^{AD}
Male 2 × 24 hours	58.12^{AB}	82.89^{A}	83.61^{AD}
Male 3 × 4 hours	26.97^{G}	63.33^{D}	71.36^{E}
Male 3 × 8 hours	42.11^{F}	73.43^{B}	80.73^{BD}
Male 3 × 12 hours	50.22^{CE}	80.62^{A}	84.33^{AC}
Male 3 × 24 hours	61.12^{A}	84.28^{A}	86.41^{A}
Male 4 × 4 hours	25.69^{G}	66.39^{CD}	71.79^{E}
Male 4 × 8 hours	43.39^{DF}	73.13^{B}	82.96^{AD}
Male 4 × 12 hours	56.39^{AB}	81.62^{A}	84.85^{AC}
Male 4 × 24 hours	63.05^{A}	85.30^{A}	85.36^{AB}
DMRT (P=0.05)	5.92*	6.33*	5.03*

Means showing common letter(s) in a column are non-significant statistically at 5% probability. DMRT represents the least significance difference obtained from DMRT among column means whereas * and NS indicates significant and non-significant difference among means in a same column, respectively.

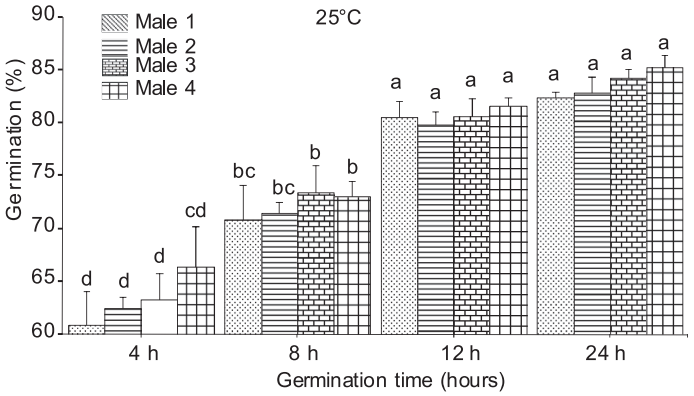

Fig. 3. Pollen germination response of four male date palm sources at varying time intervals incubated at 25 °C. Error bars represent the variability within treatment.

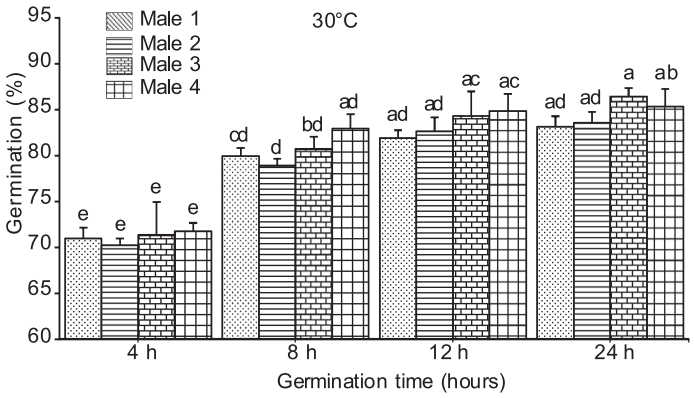

Fig. 4. Pollen germination response of four male date palm sources at varying time intervals incubated at 30 °C. Error bars represent the variability within treatment.

Table 2. Spathe traits of four date palm males at different opening dates

Treatments	Strands per spathe	Spathe length (cm)	Spathe width (cm)	Flowers per strand	Pollen weight (g)
Factor A: Pollen source					
Male 1	82.88^{A}	38.66^{A}	11.77^{A}	68.66^{A}	15.44^{B}
Male 2	82.00^{A}	41.00^{A}	12.22^{A}	68.55^{A}	15.11^{B}
Male 3	82.00^{A}	42.33^{A}	12.55^{A}	69.77^{A}	16.88^{AB}
Male 4	84.66^{A}	42.22^{A}	13.00^{A}	71.33^{A}	17.77^{A}
DMRT (P=0.05)	2.72^{NS}	4.62^{NS}	2.13^{NS}	3.11^{NS}	1.98^{NS}
Factor B: Spathe opening date					
20th February	62.92^{C}	32.50^{C}	9.33^{C}	52.25^{C}	8.16^{C}
1st March	81.67^{B}	39.08^{B}	13.25^{B}	71.66^{B}	17.75^{B}
10th March	104.08^{A}	51.58^{A}	14.58^{A}	84.83^{A}	23.00^{A}
DMRT (P=0.05)	2.92*	2.98*	1.29*	2.78*	2.94*
Interaction: A × B					
Male 1 × 20th February	63.33^{C}	29.33^{D}	8.33^{C}	50.66^{C}	8.33^{E}
Male 1 × 1st March	82.67^{B}	38.33^{BC}	12.66^{AB}	72.66^{B}	16.66^{CD}
Male 1 × 10th March	102.67^{A}	48.33^{A}	14.33^{A}	82.66^{A}	21.33^{AC}
Male 2 × 20th February	60.67^{C}	33.00^{CD}	9.33^{C}	50.66^{C}	7.33^{E}
Male 2 × 1st March	80.67^{B}	38.33^{BC}	12.66^{AB}	70.66^{B}	15.33^{D}
Male 2 × 10th March	104.67^{A}	51.66^{A}	14.66^{A}	84.33^{A}	22.66^{AB}
Male 3 × 20th February	63.00^{C}	34.33^{BD}	9.66^{BC}	53.00^{C}	8.33^{E}
Male 3 × 1st March	80.00^{B}	39.66^{BC}	13.33^{A}	70.00^{B}	18.66^{BD}
Male 3 × 10th March	103.00^{A}	53.00^{A}	14.66^{A}	86.33^{A}	23.66^{AB}
Male 4 × 20th February	64.67^{C}	33.33^{CD}	10.00^{BC}	54.66^{C}	8.66^{E}
Male 4 × 1st March	83.33^{B}	40.00^{B}	14.33^{A}	73.33^{B}	20.33^{AD}
Male 4 × 10th March	106.00^{A}	53.33^{A}	14.66^{A}	86.00^{A}	24.33^{A}
DMRT (P=0.05)	5.85*	5.96*	2.59*	5.57*	5.89*

Means showing common letter(s) in a column are non-significant statistically at 5% probability. DMRT represents the least significance difference obtained from DMRT among column means whereas * and NS indicates significant and non-significant difference among means in a same column, respectively.

germination occurred after 6 h in date palm *cv.* Khadrawy, which reached to more than 70% after 24 h. However, the germination response varied with the cultivar. Similarly, Reuveni *et al*. (1986) reported that at 25 and 28°C *in vitro* temperatures most of the pollen tubes of date palm *cv.* Hayani reached the base of the female ovary within 6 h. However, Furr and Ream (1972) stated that more than 70% date palm pollens germination occurred after 2 h of incubation at 26°C. The germination of cherry pollens accelerated more than 80% at higher temperature (30°C) within 1 h in *cv.* Moret, however, *cv.* Rose Diamond and P.A. Tolosa took 2 and 4 h, respectively to exhibit similar response (Hedhly *et al*., 2005). *In vivo* pollen germination of *Austrobaileya* elevated from 28% after 1 h of pollination to 97% after 12 h (Williams, 2012). In present study, 61-66% germination occurred after 4 h at 25°C, which accelerated to 70-72% at 30°C after same time interval.

In another part of the study, pollen spathes were collected at different opening dates (20th February, 1st and 10th March) from four male sources at the same geographical location and their physical characteristics were recorded (Table 2). There was a non-significant difference between the four males regarding number of strands per spathe, length and width of strand and number of flowers per strand. However, Male 4 had significantly higher weight of pollen grains (17.77 g) followed by Male 3 (16.88 g). A significant variation was recorded regarding all these parameters when spathes were collected at different opening dates. The highest number of strands per spathe (104.08), length (51.58 cm) and width (14.58 cm) of strand, number of flowers per strand (84.83) and weight of pollen grains (23.00 g) were calculated in spathes opened on 10th March followed by 1st March. These parameters were at lowest in early spathe opening date (20th February). More or less similar trend was observed in interaction data where all four males had significantly higher values of these characters when their spathes were opened on 10th March. Similarly, in early emerging spathes in date palm *cv.* Dhakki, the minimum pollen grain weight was noted (Iqbal *et al*., 2011). Shaheen (2004) stated that the amount of pollen grains produced per spathe varied greatly from one male to another (0.02-82.92 g.spathe^{-1}). Moreover, spathes collected from different males at different opening times showed significant variation regarding spathe length and width, number of strands per spathe, number of male flowers per strand and pollen weight (Iqbal *et al*., 2009; Shahid *et al*., 2017).

It was observed that the percentage of pollen germination in early opening spathes (20th February) decreased significantly (P≤0.05) by 29% compared to other dates (1st and 10th March), which were statistically non-significant (Fig. 5). It was noted that there was significantly higher pollen germination in Male 4 (79.51%) and Male 3 (75.82%), which decreased significantly in Male 1 (69.67%) and Male 2 (67.53%). Male pairs in both groups were at par statistically. The mean fresh pollen viability percentage showed that the viable pollen percentage (81.19%) in early spathe opening date (20th February) was 13% lower than the other dates *i.e.,* 1st (91.53%) and 10th March (93.05%) (Fig. 6). Pollen

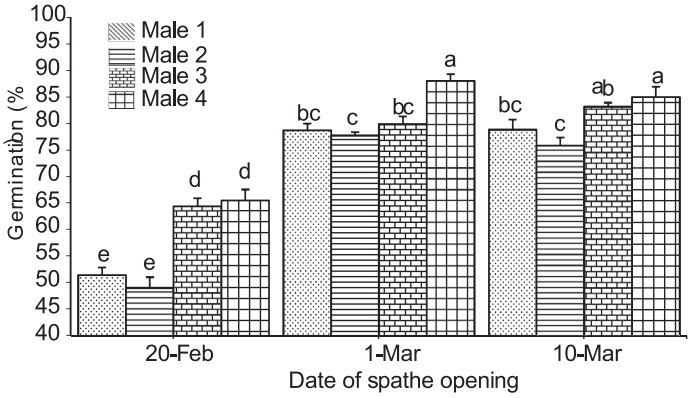

Fig. 5. Pollen germination response of four date palm male spathes opened at different dates. Error bars represent the variability within treatment.

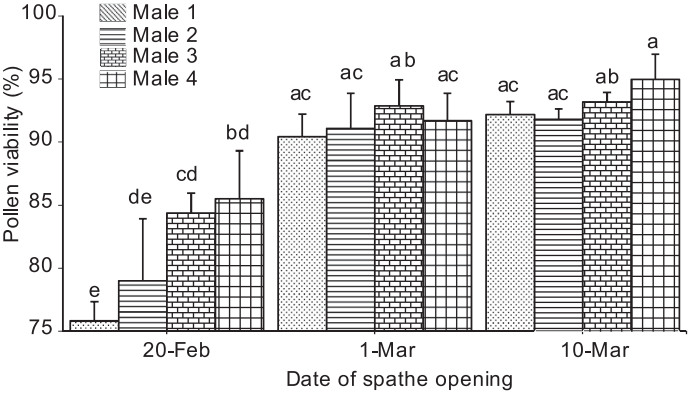

Fig. 6. Pollen viability response of four date palm male spathes opened at different dates. Error bars represent the variability within treatment.

viability also significantly varied between male pollen sources and Male 4 (90.73%) and Male 3 (90.15%) had higher pollen viability values than others. Interaction effect of date of spathe opening and male pollen sources yielded the maximum viability in 10th March × Male 4 (94.99%). However, pollens from all four males showed higher viability (90.42-94.99%) when collected from 1st and 10th March opening spathes. Absolute pollen viability, which is a measure of effective germination capacity of pollen grains, was significantly ($P \leq 0.05$) higher in spathes opened on 1st (74.22%) and 10th March (75.17%) (Fig. 7). Similarly, all four male pollen sources showed a significant difference among them. The interaction of both factors (date of spathe opening and male pollen sources) indicated the highest (80.81%) and lowest (38.68%) absolute pollen viabilities in Male 4 spathes opened on 10th March and Male 1 and Male 2 spathes opened on

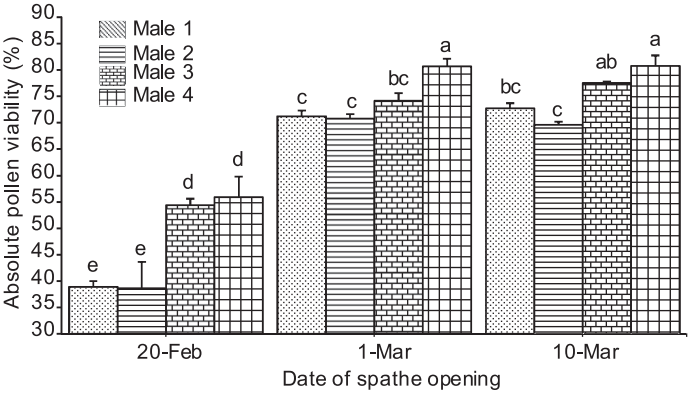

Fig. 7. Absolute pollen viability response of four date palm male spathes opened at different dates. Y-bars represent the variability within treatment.

20th February, respectively. Mesnoua *et al*. (2018) and Shaheen (1986) reported highest pollen viability in fresh pollens of date palm compared to stored pollens. Present results are in line with Iqbal *et al*. (2009, 2011) and Shahid *et al*. (2017) who reported that pollens viability varied with male source and time of spathe emergence. Similarly, the viability of date palm stored pollen varies from 65 to 87% at 1 and 12 months storage periods, respectively (Maryam *et al*., 2017). Shaheen (2004) studied 61 male pollen sources and affirmed that the pollen viability varied between 44.60-100% using acetocarmine test.

In conclusion, encouraging findings emerged regarding the pollen grains germination and their viability. It appeared that pollen germination and viability varied from one male source to another. The increase in temperature significantly accelerated pollen germination and 25-30°C was found optimum for pollen tube growth. The pace of pollen tube formation is temperature dependent that is higher the temperature (25-30°C), higher would be the speed of pollen germination. In present study, 63 and 71% pollens of four male sources germinated within 4 h at 25 and 30°C, respectively. It is also observed that pollen viability varied with the spathe opening times that means early opening date palm spathes contained less viable pollens than the subsequent emerging ones.

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References

Alcaraz, M.L., M. Montserrat and J.I. Hormaza, 2011. *In vitro* pollen germination in avocado (*Persea americana* Mill.): optimization of the method and effect of temperature. *Sci. Hort*., 130: 152-156. doi.org/10.1016/j.scienta.2011.06.030.

Bekheet, S.A. and M.S. Hanafy, 2011. Towards sex determination of date palm. p. 551-566. In: *Date Palm Biotechnology*, S.M. Jain, J.M. Al-Khayri and D.V. Johnson (eds.). Springer, Dordrecht.

Dafni, A. 1992. *Pollination Biology: A Practical Approach*, Oxford University Press, Oxford, UK.

Furr, J.R. and C.L. Ream, 1972. The influence of temperature on germination of date palm. *Punjab Fruit J*., XXXIII-IV: 109-112.

Haider, M.S., I.A. Khan, M.J. Jaskani, S.A. Naqvi and M.M. Khan, 2014. Biochemical attributes of dates at three maturation stages. *Emir. J. Food Agr*., 26: 953-962.

Haider, M.S., I.A. Khan, S.A. Naqvi, M.J. Jaskani and R.W. Khan, 2013. Fruit developmental stages effects on biochemical attributes in date palm. *Pak. J. Agr. Sci*., 50: 577-583.

Hajian, S. 2005. Fundamentals of pollination in date palm plantations in Iran. *Proceedings of First International Conference on Mango and Date Palm: Culture and Export*. University of Agriculture, Faisalabad, Pakistan, 2005, p. 252-259.

Hedhly, A., J.I. Hormaza and M. Herrero, 2005. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in Peach. *Plant Biol*., 7: 476-483.

Hegland, S.J., A. Nielsen, A. Lázaro, A.L. Bjerknes and O. Totland, 2009. How does climate warming affect plant-pollinator interactions? *Ecol. Lett*., 12: 184-195. doi.org/10.1111/j.1461-0248.2008.01269.x.

Higashiyama, T. and H. Takeuchi, 2015. The mechanism and key molecules involved in pollen tube guidance. *Annu. Rev. Plant Biol*., 66: 393-413.

Iqbal, M., Jalal-ud-Din, M. Munir and M. Khan, 2009. Floral characteristics of the different male date palms and their response to fruit-setting and yield of *cv.* Dhakki. *Pak. J. Agr. Res*., 22(1-2): 36-41.

Iqbal, M., M. Munir and M. Niamatullah, 2011. Effect of different dactylifera males and their whorl pollen grain on fruit-set, fruit drop and fruit characteristics of Dhakki date palm. *J. Agr. Res*., 49(4): 507-516.

Kavand, A., A. Ebadi1, Y. Shuraki and V. Abdosi1, 2014. Effect of calcium nitrate and boric acid on pollen germination of some date palm male cultivars. *Euro. J. Exp. Biol*., 4(3):10-14.

Maryam, M.J. Jaskani and S.A. Naqvi, 2017. Storage and viability assessment of date palm pollen. p. 3-13. In: *Date Palm Biotechnology Protocols*, Volume II: Methods in Molecular Biology, J. Al-Khayri, S. Jain and D. Johnson (eds.). Humana Press, New York, USA. doi. org/10.1007/978-1-4939-7159-6_1

Maryam, M.J. Jaskani, B. Fatima, M.S. Haider, S.A. Naqvi, M. Nafees, R. Ahmad and I.A. Khan, 2015a. Evaluation of pollen viability in date palm cultivars under different storage temperatures. *Pak. J. Bot*., 47(1): 377-381.

Maryam, M.J. Jaskani, S. Ahmad and F.S. Awan, 2015b. Metaxenial effects on morphological attributes in date palm cvs. Hillawi and Khadrawy. *Pak. J. Agr. Sci*., 52(2): 385-391.

Melgarejo, P., J.J. Martínez and F. Hernández, 2000. A study of different culture media for pomegranate (*Punica granatum* L.) pollen. *Proceeding of the Symposium: Production Processing and Marketing of Pomegranate in the Mediterranean Region: Advances in Research and Technology*, Orihuela, Spain, 1998, 42: 63-69.

Mesnoua, M., M. Roumani and A. Salem, 2018. The effect of pollen storage temperatures on pollen viability, fruit-set and fruit quality of six date palm cultivars. *Sci. Hort*., 236: 279-283. doi.org/10.1016/j. scienta.2018.03.053

Mortazavi, S.M.H., K. Arzani and A. Moini, 2010. Optimizing storage and *in vitro* germination of date palm (*Phoenix dactylifera*) pollen. *J. Agr. Sci. Technol*., 12: 181-189.

Munir, M. 2019. Influence of liquid pollination technique on fruit yield and physico-chemical characteristics of date palm cultivars Khadrawy and Zahidi. *J. Biodiv. Environ. Sci*., 15(2): 41-49.

Munir, M., M.R. Alhajhoj, A.A.M. Sallam, H.S. Ghazzawy and A.M. Al-Bahigan, 2020a. Fruit yield and quality response of date palm cultivar Khalas to female inflorescence receptivity varied by pollination days. *Plant Arch*., 20(2): 4007-4014.

Munir, M., M.R. Alhajhoj, A.A.M. Sallam, H.S. Ghazzawy and A.M. Al-Bahigan, 2020b. Effects of indigenous and foreign pollinizers on the yield and fruit characteristics of date palm cultivar Khalas. *Iraqi J. Agr. Sci*., 51(1): 356-365. doi.org/10.36103/ijas.v51i1.935

Munir, M., M.R. Al-Hajhoj, H.S. Ghazzawy, A.K.M. Sallam, A.M. Al-Bahigan and M.A. Al-Muiweed, 2020c. A comparative study of pollination methods effect on the changes in fruit yield and quality of date palm cultivar Khalas. *Asian J. Agr. Biol*., 8(2): 147-157. doi. org/10.35495/ajab.2019.11.537

Nepi, M., G.G. Franchi and E. Pacini, 2001. Pollen hydration status at dispersal: Cytophysiological features and strategies, *Protoplasma*, 216: 171-180.

Okuda, S., H. Tsutsui, K. Shiina, S. Sprunck, H. Takeuchi, R. Yui, R.D. Kasahara, Y. Hamamura, A. Mizukami, D. Susaki, N. Kawano, T. Sakakibara, S. Namiki, K. Itoh, K. Otsuka, M. Matsuzaki, H. Nozaki, T. Kuroiwa, A. Nakano, M.M. Kanaoka, T. Dresselhaus, N. Sasaki and T. Higashiyama, 2009. *Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. Nature*, 458: 357-361.

Ottaviano, E. and D.L. Mulcahy, 1989. Genetics of angiosperm pollen. p. 1-65. In: *Advances in Genetics*, Volume 26, J.G. Scandalios and T.R.F. Wright (eds.). Academic Press Inc., UK.

Pintaud, J.C., B. Ludeña, F. Aberlenc-Bertossi, S. Zehdi, M. Gros-Balthazard, S. Ivorra, J.-F. Terral, C. Newton, M. Tengberg, S. Abdoulkader, A. Daher and M. Nabil, I. Saro Hernández, M.A. González-Pérezand P. Sosa, S. Santoni, S. Moussouni, F. Si-Dehbi and N. Bouguedoura, 2013. Biogeography of the date palm (*Phoenix dactylifera* L., Arecaceae): Insights on the origin and on the structure of modern diversity. *Acta Hort*., 994: 19-38. doi.org/10.17660/ ActaHort.2013.994.1

Reuveni, O., S. Abu and S. Golobovitz, 1986. Date palm pollen germination and tube elongation on pistillate flowers cultured at different temperatures. *Acta Hort*., 175: 91-96. doi.org/10.17660/ ActaHort.1986.175.13

Rizk, R.M., S.F. El-Sharabasy and K.A. Soliman, 2007. Characterization and evaluation of sex males date palm (*Phoenix dactylifera* L.) genotypes in Egypt. *Proceedings of the Fourth Symposium on the Date Palm*. King Faisal University, Saudi Arabia, Al-Hassa. p. 238.

Shaheen, M.A. 1986. Pistil receptivity in three cultivars of date palm (*Phoenix dactylifera* L.). *Proceedings of the First Horticultural Science Conference*, Tanta Univ. Egypt. p. 489-499.

Shaheen, M.A. 2004. Evaluation of date palm males using pollen viability and ultrastructure. *Acta Hort*., 632: 37-43. doi.org/10.17660/ ActaHort.2004.632.3

Shahid, M.A., M. Iqbal and M. Niamatullah, 2017. Response of male pollinizers in fruit-set, yield and quality of date palm (*Phoenix dactylifera* L.) *cv.* Dhakki. *Sarhad J. Agr*., 33(1): 108-116. doi. org/10.17582/journal.sja/2017.33.1.108.116

Shivanna, K.R., H.F. Linkens and M. Cresti, 1991. Pollen viability and pollen vigor. *Theor. Appl. Genet*., 81: 38-42. doi.org/10.1007/ BF00226109

Stanley, R.G. and H.F. Linskens, 1974. *Pollen: Biology, Biochemistry, Management*, Springer, New York, NY, USA.

Takeuchi, H. and T.A. Higashiyama, *2012. Species-specific cluster of defensin-like genes encodes diffusible pollen tube attractants in Arabidopsis*. *PLOS Biol*., 10: e1001449.

Visser, T., D.P. De Varies, G.W.H. Welles and J.W.H. Scheurink, 1977. A hybrid tea rose pollen. I. Germination and storage. *Euphytica*, 26: 729-732.

Williams, J.H. 2012. The evolution of pollen germination timing in flowering plants: *Austrobaileya scandens* (Austrobaileyaceae). *AoB Plants*, 2012: pls010. doi.org/10.1093/aobpla/pls010

Zaid, A. and P.F. de Wet, 2002. Pollination and Bunch Management. In: *Date Palm Cultivation*, A. Zaid and E.J. Arias-Jiménez (eds.). FAO Plant Production and Protection Paper 156 Rev. 1. Food and Agricultural Organization of the United Nations, Rome, Italy. http:// www.fao.org/3/Y4360E/y4360e0c.htm

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