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EVALUATION OF POLLEN VIABILITY IN DATE PALM CULTIVARS UNDER DIFFERENT STORAGE TEMPERATURES

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Abstract

Date palm is a dioecious monocotyledonous plant which belongs to *Arecaceae* family. Date palm pollen retains viability for a single growing season. Air dried pollen was enclosed in sealed capsules and stored at 4°C, -20°C and -80°C for different storage periods (1, 3, 6, 12 months). Pollen viability of fresh and stored pollen was determined by in vitro germination and staining test. After thawing, stored pollens were cultured in petri dishes and incubated at three different temperature i.e., 20°C, 25°C and 30°C for different time periods (3, 6, 12 and 24 hours). Pollen grains looking normal and stained red were considered viable, whereas weakly stained or colorless were recorded as non viable. Germination test showed that pollen of cultivar Khadrawy stored at -20°C had higher germination (71.22%) when incubated at 30°C for 24 hours after 12 months of storage while least was in Hillawi i.e. 34.86%.

Key words: Pollen, Viability, *Phoenix dactylifera*, *Arecaceae*.

Introduction

Date palm (*Phoenix dactylifera* L.) has been an important crop in the desert regions of Asia and North Africa for centuries. Alexander the great and Arab conquerors introduced date fruit in Indo-Pak subcontinent after that date spread as a food article. Date plays an important role in the economic development of many countries of world, so it is considered as important commercial crop. Date is a world agricultural industry with total production of 7.5 million tons (MT) of fruit and Pakistan contribution is 0.6 million tons (FAO, 2012). Pakistan exports fresh (12 thousand tons) as well as dried (225 thousand tons) dates all over the world. Bangladesh, Canada, Denmark, Germany, India, Indonesia, Malaysia, South Africa, Sri Lanka, USA and UK comprehends the fresh markets, whereas dried dates are exported to Afghanistan, Bangladesh, Canada, Denmark, Germany, India and Japan (Amin *et al*., 2007).

Pakistan has low date productivity which is related with floral biology. Typically there is asynchronous maturation of male and female flowers and staminate flowers are not available at the time of pollination, usually producing dates without kernels make it necessary to preserve the pollen from one year to next (Boughediri *et al*., 1995). Late frost may also destroy the seasonal supply of male pollen (Popenoe, 1973). Scarcity of pollen results due to the absence of adequate number of male spathes at the time of early emergence of female spathes, so the growers have to use the pollens without knowing the characterization of fertility and compatibility. Similarly pollen parents influence on dates morphology and biochemical attributes (Haider *et al*., 2013, 2014). Consequently, pollen conservation from one season to another or within the pollination period (2-3) months is a necessity (Mortazvi, 2010).

Pollen viability is generally considered as ability of pollen grains to germinate and deliver the sperm cells to the embryo sac to accomplish the compatible fertilization (Shivanna *et al*., 1991). Pollen can preserve its value for long time if it is kept dry. Similarly Nebel & Rulty (1937) reported that pollen is capable of compatible fertilization even after long periods of storage. Albert (1930) observed pollen stored at 3.3°C and recorded higher germination percentage as compared to pollen stored at room temperature. One of the most optimal conditions to maintain long term pollen viability is freeze drying (Boughediri *et al*., 1995). Furr & Ream (1968) determined the clear responses of in vitro germination of date palm under the different combinations of temperature and time tested. The response of date palm pollen tube growth after the storage period of 3, 7 and 12 months for both pollen grains stored either freeze dried or unfreeze dried at 4, -20°C and -80°C was studied.

The objectives of this investigation were (1) optimization of an in vitro germination protocol to effectively determine the viability of date palm pollen of commercial cultivars (2) to compare the effect of different storage conditions and incubation temperature on date pollen longevity.

Materials and Methods

Plant material: Three mature spathes were collected from each male tree of three cultivars, i.e. Dhakki, Khadrawy and Hillawi (Plants develop from seedlings) at the Experimental Fruit Orchard Square No.9, University of Agriculture, Faisalabad, Pakistan. For anther dehiscence, the flower strands were cut off and spread on a paper sheet to dry at room temperature for 2 days. Then the pollen grains were separated from the flowers by using fine sieves (40 mesh).

Pollen storage: The air dried pollen grains of date palm cvs. Hillawi, Dhakki and Khadrawy were put in sealed capsules and stored for different storage periods of 1, 3, 6, and 12 months. The sealed capsules were stored 4, 20 and -80°C.

Pollen viability: Pollen viability of fresh and stored pollens was determined by three methods i.e., *In vitro* germination; Staining with acetocarmine and Absolute pollen viability.

***In vitro* germination:** The media used for germination was consisted of $Ca(NO_3)_2.2H_2O$ (0.417 g), H_3BO_3 (0.200 g), KNO_3 (0.101g), $MgSO_4.7H_2O$ (0.217 g), agar (10 g) and sugar (200g)) per litre. The pH of the media was adjusted at 5.7 with pH meter prior to addition of agar. Media was sterilized in Hirayama HVE-50 autoclave at 121°C and 15 psi for 20 minutes. After sterilization media was poured in Petri plates in laminar air flow cabinet and placed at room temperature to settle down. Stored pollen grains were thawed by swirling tubes in a 45°C water bath until melting of ice. After thawing stored pollens were cultured in Petri plates containing pollen germination media under the laminar air flow cabinet. These Petri plates were incubated at 20, 25 and 30°C for a period of 3, 6, 12 and 24 hours. Fresh as well as stored pollen grains germination and pollen tube length was assessed.

The germinated pollens were counted under Nikon fluorescent microscope at the magnification of 200X after 3, 6, 12 and 24 hours of culture using 6 replications in a single treatment. Then pollen germination % age was calculated by using the following formula:

$$\text{Germination \%} = \frac{\text{Germinated pollen} \times 100}{\text{Total pollen}}$$

Staining with acetocarmine: Pollen viability was also determined by staining fresh and stored pollen grains with 1% acetocarmine (Moreira & Gurgel, 1941). Fresh and stored pollens 50 each were counted per slide and 9 slides per cultivar. The pollen grains looking normal and stained red were considered viable, whereas poorly stained or colourless were recorded as non viable.

Absolute pollen viability (APV):

Absolute pollen viability or the effective germination capacity was calculated using the formula of Visser *et al*. (1977).

$$\text{APV} = \frac{\text{Staining \%} \times \text{Germination \%}}{100}$$

Statistical analysis: The experiment was laid out according to completely randomized design (CRD) with four factor factorial. Each treatment unit was repeated six times. The experimental data was subjected to analysis of variance (ANOVA) using statistics 8.1. Within the analysis of variance, the effects of different treatment and their interactions were assessed. Least significant differences (Fisher's protected LSD) were calculated following significant F test (p=0.05).

Results and Discussion

In vitro germination

a. Germination time: Germination percentage of pollen of three date palm cultivars significantly differed when observed at four germination times (Fig. 1). The highest mean pollen germination was observed in cv. Khadrawy (74.78%) followed by the cvs. Hillawi (60%) and Dhakki (53%). Pollen germination percentage at different germination times was highly significant and showed the highest value (74.78%) after 24 hours and minimum value (14.44%) after 3 hours of culturing (Fig. 6A).

b. Incubation temperature (°C): Incubation temperature had the significant effect on pollen germination in date palm cultivars. Germination percentage increased with the rise in incubation temperature after culture. Pollens incubated at 30°C had higher germination percentage (59.08%) followed by the 25°C (42.23%) and 20°C (40.21%) (Fig. 2). The best combination of interaction was with Khadrawy at 30°C (59.08%) and poorest was of Hillawi at 20°C (22.17%).

c. Pollen storage temperature and duration: Pollen germination percentage of three cultivars was significantly affected by storage temperature and storage time duration (Fig. 3). Germination percentage was the highest in cv. Khadrawy (32.24%) while Dhakki and Hillawi were close to each other (17.38% and 18.86%, respectively) at -80°C after 12 months of pollen storage. The results indicated that pollen germination percentage decreased with increase in storage duration. Maximum germination percentage was observed after 1 of month storage (43.25%) and minimum after 12 months (32.24%). Pollen stored at three storage temperatures, i.e. 4°C, -20°C and -80°C was significantly different in Khadrawy and Hillawi. Maximum mean germination was obtained at -20°C (27.40%) followed by -80°C (24.64%) and the lowest by 4°C (24.39%).

Pollen viability by stain ability: Mean viability percentage of fresh pollen showed that viable pollen percentage was significantly higher in Khadrawy (92.83%) followed by Hillawi (87.83%) and Dhakki (85.17%) (Fig. 4). Storage duration had significant effect on pollen viability of three cultivars of date palm. Interaction effect of storage duration with cultivars gave the maximum viability in Khadrawy (90.28%) after 1 month of pollen storage (Fig. 6B) and minimum in Dhakki (62.78%) after 12 months of storage.

Absolute pollen viability: The effect of storage duration on different date palm cultivars pollen was significantly different (Fig. 5). Maximum absolute pollen viability was recorded in cultivar Khadrawy (28.66%) followed by Hillawi (17.74%) and Dhakki (16.63%). It was also noticed that absolute pollen viability decreased as storage duration increased. The maximum absolute pollen viability in Khadrawy (35.85%) was examined in one month of stored pollen.

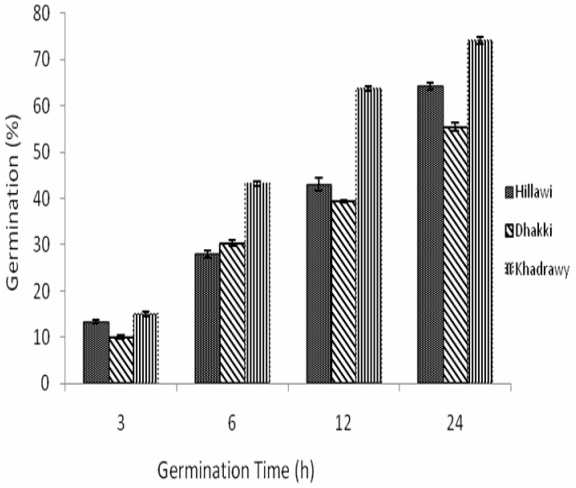

Fig. 1. Effect of time period on pollen germination (%) in date palm cvs. Hillawi, Dhakki and Khadrawy.

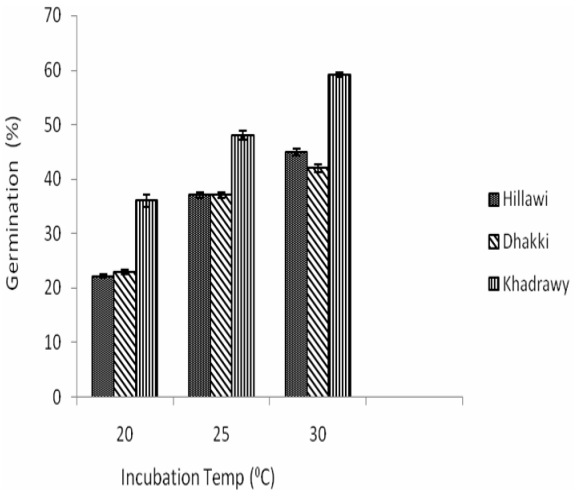

Fig. 2. Effect of incubation temperature over pollen germination (%) in date palm cvs. Hallawi, Dhakki and Khudrawy.

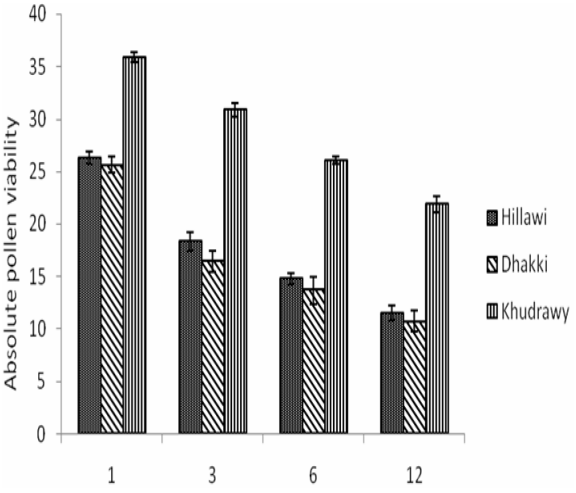

Fig. 5. Effect of storage duration on absolute pollen viability (%) in date palm cvs. Hillawi, Dhakki and Khudrawy.

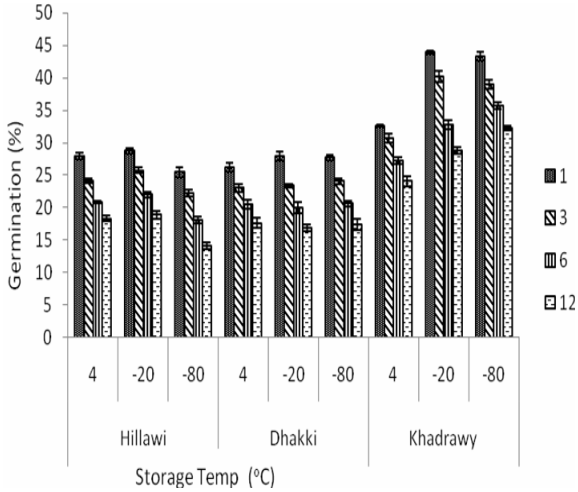

Fig. 3. Effect of storage temperature and duration on pollen germination (%) in date palm cvs. Hillawi, Dhakki and Khadrawy.

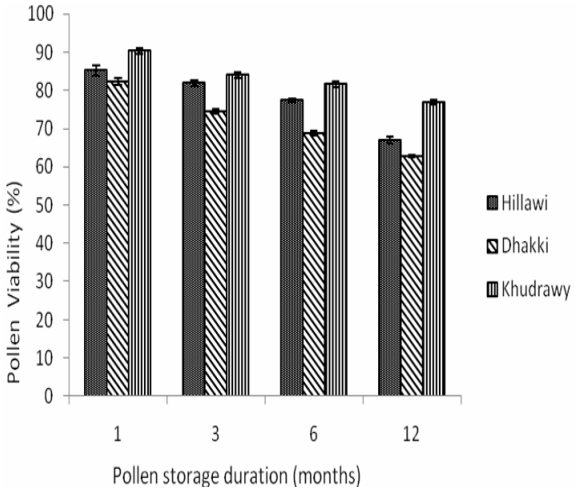

Fig. 4. Effect of storage duration on pollen viability (%) by staining in date palm cvs. Hillawi , Dhakki and Khudrawy.

Discussion and Conclusion

Melagarejo *et al*. (2000) stated that the germination percentage was slightly higher at 27°C although temperature factor did not produce the significant differences. They found that after 48 hours of incubation germination percentage was higher than after 24 hours. Whitehead (1963) reported that pollen germination in coconut was superior at 30°C than other temperatures. At 15°C or 43°C no germination occurred. The pollen germination is also genotype dependent as Barigioni (1980) reported 22-25°C as appropriate temperature for pollen germination in sweet cherry. Melagarejo *et al*. (2000) stated that incubation temperature gave maximum germination percentage in pomegranate pollen as observed at 28°C rather than 10°C. It was reported that fresh and frozen pollen in date palm had greater pollen viability rather than pollen stored at room temperature or refrigerated (Hussein *et al*., 1986). Contradictory results were produced by Aldrich & Crawford (1941), Rahim (1975), Elsabraut (1979) and Abo-Hassan *et al*. (1982)

that there was no significant difference between fresh and pollen stored in freezer. Pfeiffer (1955) made improved room temperature storage but no improvement appeared in low temperature storage in lilium pollen. The results are in line with Shaheen *et al*. (1986) that 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. The viability of the pollen grains was more than 68% stored even for 12 months. These results are similar with Al-Tahir & Asif (1981).Storage temperature did not significantly affect the viability of stored pollen grains as Shaheen *et al*. (1986) observed that pollen storage of date palm either in refrigeration (3-4°C) or in a deep freezer (-20°C) and storage in (-80°C) showed non-significant differences for pollen grains viability tested by acetocarmine. Similarly higher viability percentages were observed from acetocarmine testing technique compared to germination on culture media (Shaheen, 2004). However, various methods have been reported which show variable pollen viability (Furr & Enriquez, 1966; Asif *et al*., 1983). Many investigators observed the pollen viability by using acetocarmine technique (Randhawa & Nagi, 1965; Prasade, 1969) in some fruit tree species. They found more than 90% pollen viability in the grape cultivars. Very high pollen variability was determined by Randhawa & Ramakishnan (1960) in plum, Soost (1963) in citrus, Marnedov (1984) and Sharma & Gaur (1984) in Pomogranate, and Pearson & Harney (1984) and Roberts (1977) in rose. Absolute pollen viability was also determined by Mahmoud *et al*. (1998) who reported that absolute pollen viability percentage ranged from 41.75 to 89.74% in various cultivars of pomegranate.

Encouraging progress has been made regarding the date palm pollen storage. It was observed that storage temperature, storage duration, incubation temperature and germination time had significant effect on long term date palm pollen viability. As three cultivars responded variably so there is need to exploit most suitable cultivar, Khadrawy as a male parent, having long term pollen storage ability for successful pollination and commercial production of date palm.

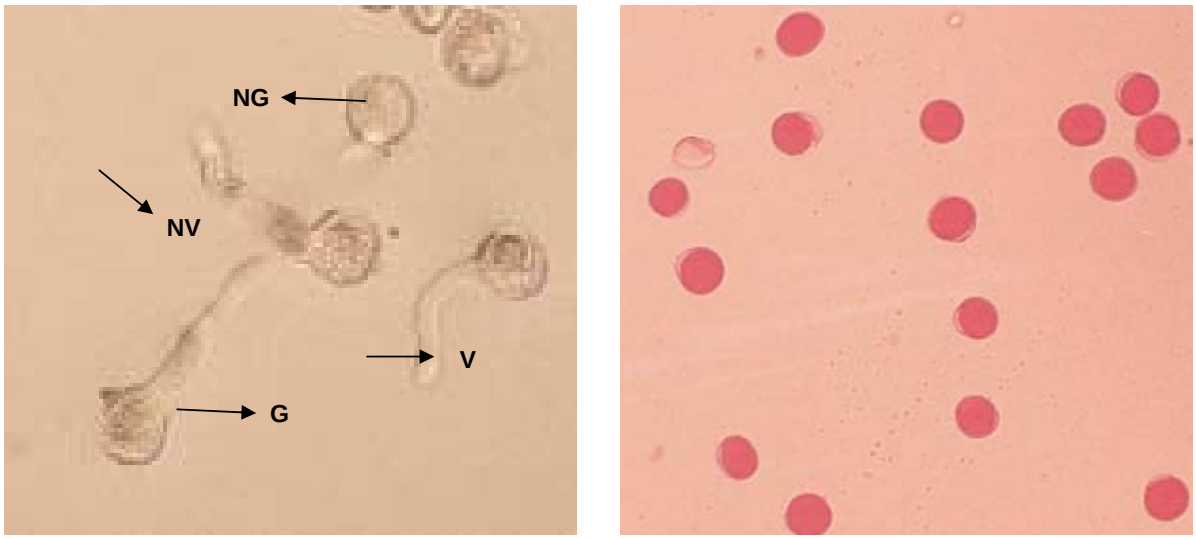

Fig. 6. A. *In vitro* pollen germination of date palm variety Hillawi incubated at 30°C (200X) [NG= Non germinated, G= Germinated]; B. Viability of twelve months stored pollen grains tested by acetocarmine staining in cv. Khadrawy (200X) [NV= Non viable, V= Viable].

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