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Effect of stored pollen grains on quality and yield of date palm in Western Rajasthan

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Abstract

Phoenix dactylifera L. belongs to the family Palmae or Arecaceae. Date palm require artificially pollination for fertilization and fruit set due to dioecious condition. Storage of pollen is an important concept to make availability of pollen at the time of pollination. Pollen viability is important for species dispersal, fitness, and survival of the next plant generation. The experiment conducted at Date palm Research Centre, SKRAU, Bikaner from March 2020 to July 2021. There were twelve treatment combination made for stored pollen application viz., glass bottle stored at room temperature (T₁), plastic bottle stored at room temperature (T₂), polythene bag stored at room temperature (T₃), paper bag stored at room temperature (T₄), glass bottle stored at refrigerator 5 °C temperature (T₅), plastic bottle stored at refrigerator 5 °C temperature (T₆), polythene bag stored at refrigerator 5 °C temperature (T₇), paper bag stored at refrigerator 5 °C temperature (T₈), glass bottle stored at deep freeze (-15 °C) (T₉), plastic bottle stored at deep freeze (-15 °C) (T₁₀), polythene bag stored at deep freeze (-15 °C) (T₁₁), paper bag stored at deep freeze (-15 °C) (T₁₂). Performance of treatment T₉ found superlative followed by T₁₀ in respect of fruit set, quality and yield attributes.

Keywords: Pollen viability, pollen storage, fruit set, quality attributes and yield

Introduction

Phoenix dactylifera L. commonly known as date palm belongs to the genus Phoenix. Only *Phoenix dactylifera* cultivated for its fruits among 14 species, other species found ornamental or wild type (Al-Khalifah and Askari, 2011) [3]. It contain chromosomes number 2n = 36. Date palm is a dioecious fruit palm which have pistillate and staminate flower on different palms. Its inflorescences, also known as racemes, grow among the axils of the leaves next to the trunk of the palm, inside a hard cover called the spathe, which opens when the inflorescence is mature (Zaid and Arias-Jimenez, 2002) [20]. Date palm flowers are small in size and white in color, richly branched, the spadix surrounded by a large single spathe. Three-toothed and cup-shaped calyx and petals are found in date palm flowers (El Hadrami and Al-Khayri, 2012) [8]. Flowers consist of three ovaries out of which only one develops into a fruit. Six stamens have a linear dorsifixed anther arrangement. Mode of pollination is anemophilous and artificial pollination is recommended to enhance productivity of date palm. Male plant produce pollen grain, which is applied to female flowers, this process called pollination (Bekheet and Hanafy, 2011) [6]. In the process of fertilization pollen grain settle on stigma of female flower and pollen tube formation starting. The two synergid cells attract pollen tube to grow down the length of the style towards the ovule (egg cell) for fertilization to form seed (Higashiyama *et al.*, 2001). In order to achieve a successful fertilization, the tip growth of the pollen tube, is precisely guided by female cues (Higashiyama and Takeuchi, 2015) [11].

The male flower generally emerge earlier from female flowers. Sometimes, male flowers does not open at the time of pollination due to unfavorable climatic condition (Hegland *et al.*, 2009) [10]. At the time of early appearance of female spathes absence of adequate number of male spathes creates a problem for farmers in pollination of date palm. In such a condition, growers use pollen with unknown features, since the source of pollen influence the quality and quantity of fruits (Rezazadeh *et al.*, 2013, Shafique *et al.*, 2011) [17]. Sometime temperature fluctuation create some kind of stress on reproductive organs, thereby asynchrony seen in male and female reproductive part development (Mommott *et al.*, 2007; Zinn *et al.*, 2010) [13, 21]. To overcome from this obstacle, storage of pollen is necessary. Viability of pollen declines with the time of storages. Therefore, optimization of pollen storage for long time is required for date palm cultivation.

A number of investigators have developed experimental techniques for date palm pollen storage, such as cryopreservation (Anushma *et al.*, 2018)^[4], freezing of pollen grain in liquid nitrogen and lyophilization (Babahani and Bouguedoura, 2009)^[5]. But, these techniques can be, often, difficult to implement by date palm growers, since they require easy and cheap storage technique by which they store pollen for longer duration (El Kadri and Mimoun, 2020)^[9]. This study conducted for observe the effect of stored pollen while applied on female spath in field condition.

Material and Method

The experiment was conducted at Date Palm Research Centre, Agricultural Research Station, S. K. Rajasthan Agricultural University, Bikaner during March 2019 to July 2021. In the month of February – March, male spath which was ready to burst, cut off from palm. Male strands were dried at room temperature. After complete drying pollen shaded from strands. Pollen grains were sieved and checks the pollen viability before store. Pollen grains were packed in different packing containers *via.* glass bottle (C₁), plastic bottle (C₂), polythene bag (C₃) and paper bag (C₄). These containers were place at room temperature (S₁), refrigerator 5 °C (S₂) and deep freeze (-15 °C) (S₃) for twelve months.

Before testing the viability of pollen, twelfth month store pollen grain thawed for 2 hours and after that, slide prepared. Pollen viability was determined by staining stored pollen grains with 1% acetocarmine (Moreira & Gurgel, 1941). For preparing slide a clean slide was taken and a pin dip in stored pollen and pollen place on slide. One drop of acetocarmine pour out on that slide and cover it with covering slip. After pollen staining observation taken with the help of electronic microscope. The pollen grains appear round and stained red were considered viable, whereas, poorly stained or colourless pollen were recorded as non viable.

Experiment was conducted on Halawy variety of date palm which was 8 year old and stand at 6 × 6 m² spacing. Uniform 36 palms were selected for investigation, each treatment replicate three times. There were twelve treatment combination for stored pollen application *viz.*, glass bottle stored at room temperature (T₁), plastic bottle stored at room temperature (T₂), polythene bag stored at room temperature (T₃), paper bag stored at room temperature (T₄), glass bottle stored at refrigerator 5 °C temperature (T₅), plastic bottle stored at refrigerator 5 °C temperature (T₆), polythene bag stored at refrigerator 5 °C temperature (T₇), paper bag stored at refrigerator 5 °C temperature (T₈), glass bottle stored at deep freeze (-15 °C) (T₉), plastic bottle stored at deep freeze (-15 °C) (T₁₀), polythene bag stored at deep freeze (-15 °C) (T₁₁), paper bag stored at deep freeze (-15 °C) (T₁₂). Pollen were applied after one day of opening of female spath. Cotton ball impregnated with thawed pollen grains were insert in between female spath for pollination. Pollination done from first week of March to first week of April in the year 2020 and 2021.

Fruit setting percentage was calculated after one month of pollination. For fruit setting, drop and retention percentage three bunches/palm were selected and at each bunch, five strands were selected. Fruit setting percent was calculated by dividing total no of fruit set with total flower number and multiply by hundred. Fruit drop percentage was calculated by number of fruit remain at harvesting subtract from total fruit set and divided by total fruit set and multiply with hundred. Fruit retention was calculated by number of fruit present at

the time of harvesting divided by total fruit set and multiply by hundred. Quality parameters taken at the time of harvesting of fruits. Pulp percentage calculated by stone weight subtract from fruit weight and divided with fruit weight and multiply by hundred. Stone percentage was calculated by pulp weight subtract from fruit weight and divided by fruit weight and multiply with hundred. Fruit weight, pulp weight, stone weight, yield of fruit/bunch and yield of fruit/palm were taken by electrical balance in gram or kilogram. Fruit length and fruit diameter were calculated by Vernier caliper in centimeter. Number of fruits/strand and number of fruit/kg was counted manually. Fruits were immersed in water filled measuring cylinder and increased level of water noted as volume of fruits.

The experiment was laid out in randomized block design. Data obtained on various characters were analyzed statistically according to the analysis of variance techniques as suggested by Panse and Sukhatme, (1985); Chandel, (1999). The critical difference (CD) was calculated to access the significance or non-significance of difference between treatment means at 5 per cent level of significance.

Result and Discussion

Treatments were significantly affected the fruit set, fruit retention, number of fruit/strand and fruit drop percentage. Table 1 shows that the highest fruit set, fruit retention, number of fruit per strand and minimum fruit drop (70.65%, 55.46%, 17.19 and 35.64%, respectively) were exhibited in T₉ followed by T₁₀ (68.76%, 54.06%, 16.48 and 36.63%, respectively). Whereas lowest fruit set, fruit retention, number of fruit per strand and maximum fruit drop (26.89%, 23.71%, 5.04 and 71.16%, respectively) were registered in T₄ followed by T₃ (28.02%, 24.75%, 5.98 and 69.35%). Temperature and storage material of pollen grains were significantly affect the quality of date palm fruit. Highest weight, length, diameter and volume of fruit (7.91g, 3.42 cm, 2.06 cm and 8.79 cm³ respectively) were shown by treatment T₉ followed by T₁₀ (7.71 g, 3.38 cm, 1.90 cm, 8.31 cm³). Whereas, lowest fruit weight, length, diameter and volume (5.01 g, 2.17 cm, 1.22 cm and 4.76 cm³ respectively) were registered in T₄ followed by T₃ (5.39 g, 2.25 cm, 1.28 cm and 5.09 cm³, respectively). Data presented in the table 3 exhibit the significant difference in between treatments regarding pulp weight, pulp % and stone %, whereas stone weight was shown non significant difference. Supreme pulp weight, pulp % and subordinate stone % (7.07 g, 89.33% and 10.67%, respectively) was revealed by T₉ and it was found at par with T₁₀ (6.88 g, 89.24% and 10.76%, respectively). Lowest pulp weight, pulp % and stone %, (4.23 g, 84.43% and 15.57%, respectively) was exhibited in T₄ followed by T₃ (4.60 g, 85.35% and 14.65%, respectively). Minimum number of fruit/kg and maximum yield/bunch and yield/palm (138.33, 5.88 kg and 29.38 kg) was exhibited in the T₉ and maximum number of fruit/kg and minimum yield/bunch and yield/palm (219, 1.97 kg and 9.83 kg) was exhibited by T₄.

Low temperature promote the longevity of pollen by slower down metabolic process. Containers also effect the longevity of pollen. Air tight container maintain the survival of pollen by preventing air and moisture while paper bag permit the moisture inside bag, result of that pollen loss there viability. Pollen viability directly affect the fruit set and fruit drop. Pollen viability also responsible for fruit quality and fruit yield. It was observed that pollen stored at -20 °C were able to set 100% fruits, pollen stored at 0 °C could set 36% fruits,

while there was no fruit setting with pollen stored at room temperature and 4 °C. Artificial pollination with stored pollen can circumvent several uncertainties of natural pollination and guarantee adequate pollination in kiwifruit. In conclusion, the results indicate that -20 °C was the best temperature at which maximum viability of kiwifruit pollen can be retained up to one year and this pollen can be further used for artificial pollination (Naik, 2013) [15]. Pollen stored at low temperature showed better germination percentage as compared to pollen stored at +4 °C and in fresh pollen. Freeze dried pollen (-60 °C) showed the highest germination percentage (Perveen and Khan, 2008) [16]. Cryopreserved pollen under -80 °C gave the best results, and refrigerated and frozen pollen gave convenient results. Under room conditions, pollen of the two cultivars has lost its germinability and viability gradually, and after 52 weeks the pollen was nonviable (Ahmad, 2009) [1].

Storage of pollen at -20 °C for pollination among cultivars having non-synchronized flowering in a season. However, for long term storage cryo method proved to be the best for pollen storage in mango (Duttaa *et al.*, 2013) [7]. Glass bottle (34.12%) given maximum pollen viable percentage followed by PET bottles (33.81%) and poly beg (28.18%) after 12 month of storage (Sharma *et al.*, 2018) [19]. Mesnoua *et al.*, 2018 [14] were observed maximum percentage of fruit set with pollen stored at -20 °C than 4 °C temperature storage. While, pollen stored at room temperature was unable to fertilize. Fruit weight and pulp weight were more influenced by storage treatment while, fruit length and diameter, seed length and diameter were less affected. Pollen stored at 4 °C gave the lower fruit quality. This may be due to low pollen viability recorded in 4 °C compared to fresh pollen and pollen stored at -20 °C. Akond *et al.*, 2012 [2] also found similar results.

Table 1: Effect of temperature and storage condition on fruit set %, fruit drop %, fruit retention % and no. of fruit/strand of date palm.

Treatments	Fruit set %	Fruit retention %	No. of fruit/strand	Fruit drop %
T ₁	30.85	27.99	7.64	66.79
T ₂	29.67	26.19	7.04	67.93
T ₃	28.02	24.75	5.98	69.35
T ₄	26.89	23.71	5.04	71.16
T ₅	55.82	44.74	12.66	52.18
T ₆	54.62	43.66	11.95	53.40
T ₇	52.76	41.51	11.24	55.01
T ₈	50.26	40.55	11.09	56.20
T ₉	70.65	55.46	17.19	35.64
T ₁₀	68.76	54.06	16.48	36.63
T ₁₁	66.95	52.71	15.25	38.91
T ₁₂	65.51	52.09	14.60	39.95
SE(m)	0.36	0.36	0.15	0.32
CD 5%	1.06	1.05	0.45	0.95

Table 2: Effect of temperature and storage condition on fruit weight (g), length, diameter, volume of fruit of date palm

Treatments	Fruit Weight (g)	Length of fruit (cm)	Diameter of fruit (cm)	Volume of fruit (cm ³)
T ₁	5.99	2.53	1.35	5.64
T ₂	5.75	2.34	1.33	5.30
T ₃	5.39	2.25	1.28	5.09
T ₄	5.01	2.17	1.22	4.76
T ₅	6.96	3.02	1.55	6.92
T ₆	6.54	2.88	1.50	6.56
T ₇	6.23	2.73	1.43	6.33
T ₈	6.07	2.63	1.37	6.04
T ₉	7.91	3.42	2.06	8.79
T ₁₀	7.71	3.38	1.90	8.31
T ₁₁	7.18	3.30	1.78	7.96
T ₁₂	7.11	3.19	1.68	7.14
SE(m)	0.10	0.04	0.03	0.09
CD 5%	0.29	0.10	0.08	0.27

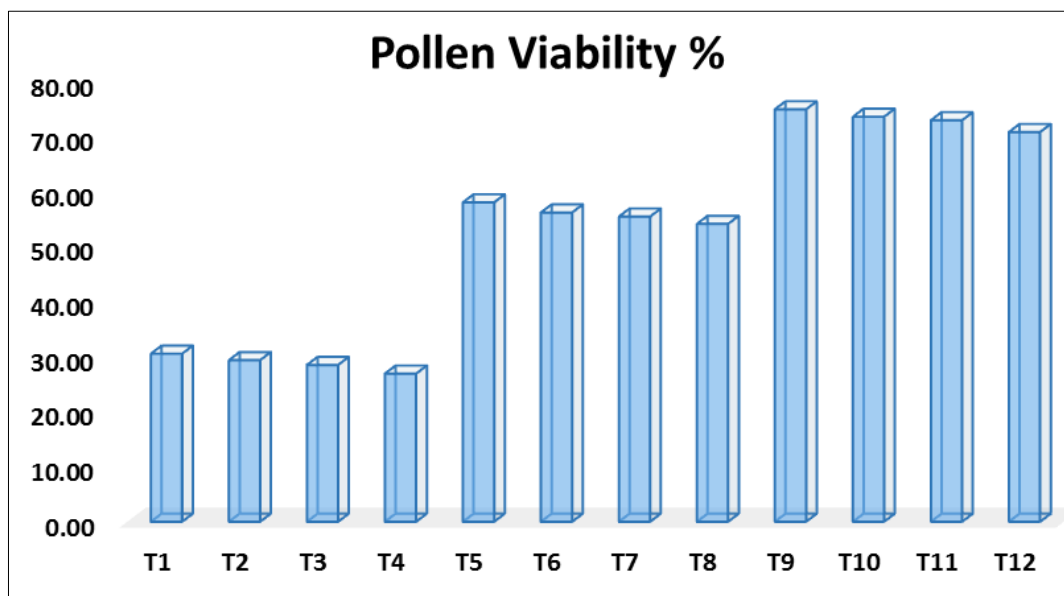
Table 3: Effect of temperature and storage condition on pulp weight, pulp %, stone weight and stone % of date palm

Treatments	Pulp weight	Pulp %	Stone weight	Stone %
T ₁	5.20	86.81	0.79	13.19
T ₂	4.97	86.44	0.78	13.56
T ₃	4.60	85.35	0.79	14.65
T ₄	4.23	84.43	0.78	15.57
T ₅	6.15	88.36	0.81	11.64
T ₆	5.74	87.67	0.81	12.33
T ₇	5.43	87.21	0.80	12.79
T ₈	5.26	86.76	0.80	13.24
T ₉	7.07	89.33	0.84	10.67
T ₁₀	6.88	89.24	0.83	10.76
T ₁₁	6.36	88.58	0.82	11.42
T ₁₂	6.30	88.60	0.81	11.40

SE(m)	0.10	0.32	0.02	0.32
CD 5%	0.29	0.93	NS	0.93

Table 4: Effect of temperature and storage condition on no. of fruit/kg, yield/bunch and yield/palm of date palm

Treatments	No. of fruit/kg	Yield/bunch	Yield/palm
T ₁	204.33	2.61	13.03
T ₂	208.00	2.55	12.73
T ₃	213.67	2.23	11.15
T ₄	219.00	1.97	9.83
T ₅	174.67	4.33	21.63
T ₆	181.00	3.65	18.27
T ₇	185.67	3.34	16.68
T ₈	187.00	3.23	16.17
T ₉	138.33	5.88	29.38
T ₁₀	143.33	5.61	28.03
T ₁₁	150.33	5.13	25.65
T ₁₂	158.33	4.97	24.87
SE(m)	2.95	0.13	0.65
CD 5%	8.66	0.38	1.91

**Fig 1:** Pollen viability percentage after twelfth month of storage

Conclusion

Pollen storage at -15 °C temperature in glass bottle T₉ gives best result in respect of fruit set, quality and yield of date palm. Farmer can store the pollen at this temperature and resolve the problem of availability of pollen in early season of pollination.

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