International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(5): 2640-2642

Received: 21-07-2018
Accepted: 24-08-2018

Anushma PL
ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post Bengaluru, Karnataka, India

Linta Vincent
ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post Bengaluru, Karnataka, India

Rajesekharan PE
ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post Bengaluru, Karnataka, India

Rajesekharan PE
ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post Bengaluru, Karnataka, India

Correspondence
Anushma PL
ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post Bengaluru, Karnataka, India

Pollen storage studies in date palm (*Phoenix dactylifera* L.)

Anushma PL, Linta Vincent, Rajesekharan PE and Ganeshan S

Abstract
Date palm cultivation has been gaining popularity in the dry tracts of India. To optimize the storage conditions for long term storage of date palm pollen. Air dried pollen was sealed airtight in aluminium pouches and stored at 4 and -20 and -196℃. Viability of fresh and stored pollen was tested *in vitro* using hanging drop technique for different durations such as 2, 3, 6 and 12 months. Among the different storage conditions, the pollen retrieved from cryopreservation recorded highest viability throughout the experiment period. Significant and gradual reduction in the germinability of pollen stored under 4℃ was observed throughout the period of investigation**.** Cryogenic storage is a promising method to store date palm pollen for commercial date production, breeding programs and conservation of elite pollen parents.

Keywords: date palm, pollen, cryopreservation

Introduction
Date palm (*Phoenix dactylifera* L.) has been an important subsistence crop throughout the arid regions of the world, especially in South-west Asia and North Africa. Cultivated date palms are dioecious in nature wherein male and female inflorescences are produced on separate trees in the leaf axils of previous year's growth. Though fair fruit set by natural pollination by wind and bees is observed in various areas of date growing countries such as Morocco, Spain, Peru etc., female flowers fail to fertilize in the absence of natural pollination, resulting into the development of parthenocarpic fruits of no commercial value (Zaid and De Wet, 1999) [13]. Thus the demand for artificial pollination in the commercial date fruit production is well recognized to overcome the barriers of dichogamy and reducing the number of male palms. The male to female trees ratio in a modern plantation is 1:50 (Enaimi and Jafar, 1980) [5].
Mainly three pollination techniques are being adopted in date fruit production; using fresh male strands, pollen suspensions and use of dried pollen. Placing 2-3 strands of freshly opened male flowers between the strands of female inflorescence is the common technique (Dowson, 1982) [4]. Spraying pollen grain suspension containing 10 percent sucrose and 20 ppm GA_3 was also found to be good for fruit set (Ahmed and Jahjah, 1985) [1]. Application of dried pollen by placing after dusting it on cotton pieces between strands of female inflorescence or by mechanical pollination is also widely practiced.
Emergence of many early inflorescences in the female palms well before the opening of adequate number of male spathes on available male palms results in scarcity of pollen (Zaid and De Wet, 1999) [13], producing dates with kernels necessitates conserving pollen from one year to the next (Boughediri, 1995) [3]. Also, due to the metaxenic effect present in the date palms, pollen parents largely influence on the morphology and biochemical parameters of the fruits (Haider *et al*., 2014) [7] and accordingly, pollen conservation is a necessity for conservation of germplasm as well as crop improvement. Pollen is capable of compatible fertilization even after long periods of storage (Nebel and Ruttle, 1937) [11]. As reported by (Boughediri, 1995) [3], freeze drying is one of the optimal conditions for maintaining pollen viability for longer durations. Since date palm cultivation has been gaining popularity in the dry tracts of the country during recent past and efforts to preserve date palm germplasm in the form of pollen needs to be intensified, this study was taken up to optimize storage conditions for long term storage of date palm pollen.

Materials and Methods
Plant Material
The pollen collected from the mature spathes of palms maintained at Central Institute of Arid

Horticulture (CIAH), Bikaner, Rajasthan was brought to the pollen cryobank, Indian Institute of Horticultural Research, Bengaluru by air to initiate the storage studies.

Pollen storage

The pollen grains were air dried by spreading on clean petri dishes for 2 hours at 25±2℃. The air dried pollen was put in empty gelatin capsules and were sealed air tight in laminated poly aluminium pouches. The packed pollen was stored at 4 and -20 and -196℃. For cryopreservation, the pollen samples were stacked and lowered gradually into the canisters of a liquid nitrogen biological system (MVE, USA). Pollen samples were preserved for different durations such as 2, 3, 6 and 12 months.

Pollen viability

Viability of fresh and stored pollen was tested as a function of pollen germinability *in vitro* germination by hanging drop technique, using nutrient medium containing Sucrose (15%), $Ca(NO_3)_2.2H_2O$ (300ppm), H_3BO_3 (100ppm), KNO_3 (100ppm) and $MgSO_4$. $7H_2O$ (200ppm). The prepared slides were incubated at high RH conditions at 25±2℃ for 4-6 hours, after which the pollen were stained using a drop of versatile stain (2). Three replicates of fresh and stored pollen were prepared. Pollen samples were thawed by rapid thawing process, after retrieval from cryobiological system. In each preparation, more than 400 pollen grains were scored for their germinability under light microscope and expressed as percent pollen germination. Pollen grains having tube lengths longer than their diameter were considered as germinated or viable. Analysis of variance (ANOVA) was performed on arc sine conversions of percent germination data using a completely randomized design (CRD).

Results

Cryopreserved pollen samples of date palm retained their viability even after one year of storage (90.29%), which were comparable with that of fresh pollen (94.37%). There was significant and gradual reduction in the germinability of pollen stored under 4℃throughout the period of investigation. Pollen kept at 4℃ recorded 61.64 and 59.58 percent germination at 2 and 3 months after storage which had further reduced to 25.96 percent after 6 months. Though there was significant reduction in germinability, pollen kept under -20℃ retained viability throughout the storage period and germinated after one year of storage (73.61%). At 6 moths of storage, pollen kept in deep freezer and liquid nitrogen recorded viability while those stored at 4℃ did not germinate. Thus among the different storage conditions, the pollen retrieved from cryopreservation recorded higher germinability throughout the experiment period.

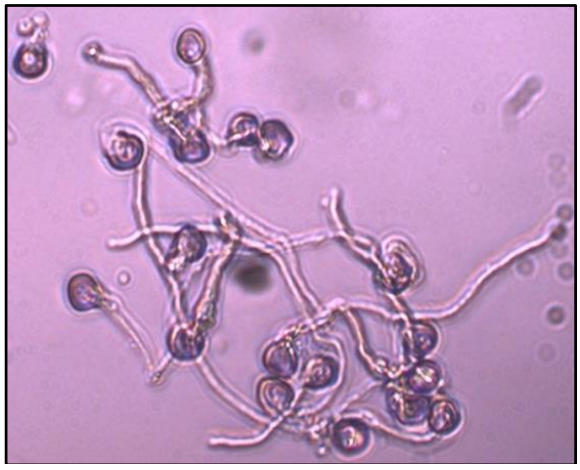

Plate: Germination in cryopreserved pollen after one year of storage under 40x magnification

Table 1: Viability of date palm pollen at different storage conditions

Treatment	Pollen germination (%)
T1: Fresh pollen	94.37 (76.44)
T2: 4℃; 2 months	61.64 (51.74)
T3: -20℃;2 months	78.45 (62.38)
T4: -196℃;2 months	79.40 (63.11)
T5: 4℃;3 months	59.58 (50.55)
T6: -20℃; 3 months	81.17 (64.31)
T7: -196℃; 3 months	89.42 (71.25)
T8: 4℃; 6 months	25.96 (30.38)
T9: -20℃; 6 months	71.25 (57.60)
T10: -196℃; 6 months	89.88 (71.63)
T11: 4℃;12 months	0.00 (0.286)
T12: -20℃; 12 months	73.61 (59.09)
T13: -196℃; 12 months	90.29 (71.86)
CD (0.01)	6.676
CD (0.05)	4.939
CV (%)	5.235
Values in brackets are arcsine transformed data	

Discussion

The present study was taken up to test the feasibility of pollen storage of date palm in view of establishing a pollen cryobank. The demand for date fruit has been growing day by day not only in India, but also globally, which has intensified the research activities on enriching the germplasm wealth and development of improved varieties and production technologies. Pollen grains carry enormous amount of genetic diversity which calls for conserving and utilizing this biological resource in crop breeding, gene conservation and commercial production (Ganeshan and Rajasekharan, 2005) [6]. For commercial production of date fruits, dried pollen are widely used. Scarcity of pollen during pollination periods urges its storage to overcome the barriers of asynchronous flowering. Also for effective exchange and supply of date palm pollen for both commercial production and conservation and for shuttle breeding programs, information on feasibility on low temperature storage is very much required.

Through the present investigation, it is evident that cryogenic

storage is a promising method to store date palm pollen as the pollen grains retained viability similar to that of fresh pollen after a year of storage. Higher germinability of pollen maintained at subzero temperatures viz., -20 and -196℃may due to the minimal metabolic activities taking place, while loss of pollen viability at 4℃could be due to higher metabolic rates. According to (Honda *et al*., 2002) [8], pollen grains with more than 30 percent germination after storage at ultra-low temperatures could induce fruit setting similar to fresh pollen. Similar results of lesser pollen viability when stored in refrigerator (4℃) were already reported (Maryam *et al*., 2017; Shaheen, 1986) [9, 12]. The advantage of conserving pollen in liquid nitrogen is by maintenance of temperature throughout the storage period which does not require any power supply. However, in the event of non-availability of cryobiological systems, pollen grains can be stored in the freezers without considerable reduction in viability (Mortazavi *et al*., 2010) [10]. The results of the study confirms the relevance of pollen storage conditions in date palms where artificial pollination has been the widely adopted technique for production as well as for conserving the germplasm in the form of haploid genetic diversity.

Acknowledgements
We wish to express our sincere gratitude to Director, Indian Institute of Horticultural Research for the financial and technical support to take up the work.

References

1. Ahmed HS, Jahjah MA. The pollination of date palm with pollen grains suspension. Date Palm Journal (FAO/NENADATES), 1985.
2. Alexander MP. A versatile stain for pollen fungi, yeast and bacteria. Stain technology. 1980; 55(1):13-8.
3. Boughediri L, Cerceau-Larrival MT, Doré JC. Significance of freeze-drying in long term storage of date palm pollen. Grana. 1995; 34(6):408-12.
4. Dowson VH. Date Production and Protection (Plant Production and Protection Paper No. 35). FAO Food and Agriculture Organization of the United Nations, Rome, Italy, 1982.
5. Enaimi JH, Jafar A. La physiologie ET la morphologie du palmier dattier. Phoenix dactylifera, 1980.
6. Ganeshan S, Rajasekharan PE. Conservation and management of haploid genetic diversity through pollen cryopreservation. J Palynol. 2005; 41:39-48.
7. Haider MS, Khan IA, Jaskani MJ, Naqvi SA, Khan MM. Biochemical attributes of dates at three maturation stages. Emir. J. Food. Agric. 2014; 15:953-62.
8. Honda K, Watanabe HT. sutsui K. Cryopreservation of Delphinium pollen at–30 °C. Euphytica. 2002; 126(3):315-20.
9. Maryam M, Fatima BI, Haider MS, Abbas S, Naqvi M, Ahmad R *et al*. Evaluation of pollen viability in date palm cultivars under different storage temperatures. Pak. J Bot. 2015; 47(1):377-81.
10. Mortazavi SM, Arzani K, Moini A. Optimizing storage and *in vitro* germination of date palm (Phoenix dactylifera) pollen. J Agric. Sci. Tech. 2010; 12:181-9.
11. Nebel BR, Ruttle ML. Storage experiments with pollen of cultivated fruit trees. Journal of Pomology and Horticultural Science. 1937; 14(4):347-59.
12. Shaheen MA. Pistil receptivity in three cultivars of date palm (*Phoenix dactylifera* L.). In Proc. 1st Hort Sci. Conf. Tanta Univ. Egypt Sep. 1986; 2:489-499.
13. Zaid A, De Wet PF. Chapter II Origin, Geographical Distribution and Nutritional Values of Date palm. FAO Plant Production and Protection Papers, 1999, 29-44.