



Pollen storage of coconut dwarf accession Chowghat Orange Dwarf at low temperature

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Coconut (*Cocos nucifera* L), the signal palm of the tropical world, plays a vital role in the socio-economic life of people and provides livelihood security to millions of people. It is a highly cross-pollinated crop of tropics and propagation is possible only via seed. Production of quality seedling material had utmost importance in perennial plantation crops like coconut and there is a huge gap exists between the planting material production and demand, especially in coconut. There was a great demand among the farmers for of hybrid material due to their higher yields along with intermediate plant growth characteristics. In coconut, hybrid material is produced by crossing the Tall and Dwarf types using artificial crossing procedures. Pollen required for crosses is usually obtained from the inflorescences of selected male parental lines using standard extraction procedures and the quality of the pollen influences the success rate of crosses made. Improving the availability of quality pollen round the year using low temperature storage techniques provides uninterrupted supply of coconut pollen for breeding and production of quality planting materials, which solves the growing needs quality coconut material among the growers. Under natural condition, viability of coconut pollen is lost within 48 h. Hence, development of standardized storage methods for coconut pollen is necessary for enhancing duration of pollen viability there by making it available for quality seedling material production. In the present study, trails were taken up using different low temperature conditions to ensure the better storage of coconut pollen.

Pollen collection and extraction: Pollen required for experimentation was collected from selected representative palms of Chowghat Orange Dwarf (COD) based on

standardized extraction procedures (Karun *et al.* 2014) during 2017–18.

Treatments and storage conditions: Extracted pollen was tested for initial germination percentage and was used for experimentation. Pollen grains collected from each palm were wrapped in aluminium foil (20 mg each) and inserted into 2 ml cryovials. These cryovials were maintained under different low-temperature regimes, i.e. T1: refrigerator (4°C), T2: freezer (-20°C), T3: ultra-freezer (-45°C) and T4-liquid nitrogen (-196°C) along with control (Room temperature). A total of three replications were made for each treatment (i.e. three replications from each representative COD palm). Stored pollen was retrieved sequentially on 7th day, 14th day, 30th day and 60th day of storage and tested for germination percentage after pre-conditioning was done at 40°C for two minutes.

In vitro pollen germination assay: For testing pollen germination, artificial medium comprising 8% sucrose, 1% agar, 1% gelatine and 0.01% boric acid was used (Karun *et al.* 2006). Prepared media was evenly poured over the glass slide and pollen from different treatments was uniformly dusted on media using a brush. Three slides were prepared for each palm for each treatment and these prepared slides were incubated in a Petri dish lined with moist filter paper at room temperature (27±2°C) for germination. Germination percentage and pollen tube lengths were observed under microscope after 90 min of incubation. Pollen grains were considered to have germinated when pollen tube length (PTL) was at least equal to or greater than pollen grain diameter. A total of 50 pollen grains per slide were counted in 10 randomly selected microscopic fields for measuring germination percentage and pollen tube length. Images of each observed fields were captured using a Leica camera (DFC 250) and pollen tube lengths (PTL) were measured using Leica Q Win software. These observations were recorded at four different intervals (7th day, 14th day, 30th day and 60th day).

Statistical analysis: Experiment was designed in two factorial Randomized Complete Block Design (RCBD).

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Data were analyzed as per corresponding ANOVA. Analysis was performed using SAS Ver. 9.3. Differences between individual treatments were tested using Tukey's HSD. The date on germination percentage was transformed (arc sine) before analysis.

Significant differences were noticed in pollen for tube length and germination percentage in response to low temperature storage (Table 1, Fig 1). Under room temperature, a sharp decline in pollen germination was noticed within two weeks. After second week, germination percentage of pollen was lost permanently at room temperature. On the other hand, sustained viability was observed in pollen stored under low temperatures (4°C, -20°C, -45°C and -196°C) up to 60th day. Longevity of pollen is primarily determined by the genetic material and influenced by environmental conditions (Kozaki 1975). Pollen viability in different plant species varies from few minutes to several years depending upon their taxonomic status (Harrington 1970) and prevailed storage conditions. Studies have shown that longevity of pollen can be extended in low-temperature conditions for different horticultural as well as field crops under regulated humidity (Akond *et al.* 2012). The percent germination obtained was more in coconut pollen stored at -196°C (39%) followed by -45°C (32%) and -20°C (31%) (Table 1) after eight weeks of pollen storage. Similarly, Machado *et al.* (2014) reported the extended pollen viability and germination percentage under low temperatures as well as in liquid nitrogen for some of the tall and dwarf accessions of coconut up to 60 days. Prolonged viability of date palm pollen was also noticed under storage temperature of -20°C over 4°C (Mesnoua *et al.* 2018) and these results were comparable with present experimental results where sub-zero temperatures were (-196°C, -45°C and -20°C) found to be superior to refrigerated storage at 4°C for storage of coconut pollen. Temperatures at or below 0°C were found to be more effective for maintenance of pollen viability in several other crops (Du *et al.* 2018).

With storage duration, a slight reduction for germination percentage of coconut pollen was noticed in almost all low-temperature treatments and the observed variability between different treatments were significant (Table 1). The overall percentage of reduction over initial value of germination percentage observed was 31.14%, 16.61% and 14.04% at storage temperatures 4°C, -20°C and -45°C successively. But a slight increase (5.03%) for *in vitro* germination percentage was noticed with pollen kept under liquid nitrogen (-196°C). Storage temperatures above 0°C reduces metabolic activity of pollen leading to gradual decrease and then total loss of viability, whereas sub-zero temperatures further slowdown physical and chemical processes taking place in pollen causing loss of pollen viability or death (Akond *et al.* 2012). Increased viability of coconut pollen, post cryopreservation in present study corroborates with the previous results (Karun *et al.* 2014). Germination of cryopreserved pollen is expected to be stable during storage but there are inconsistent results observed with pollen viability during cryostorage in

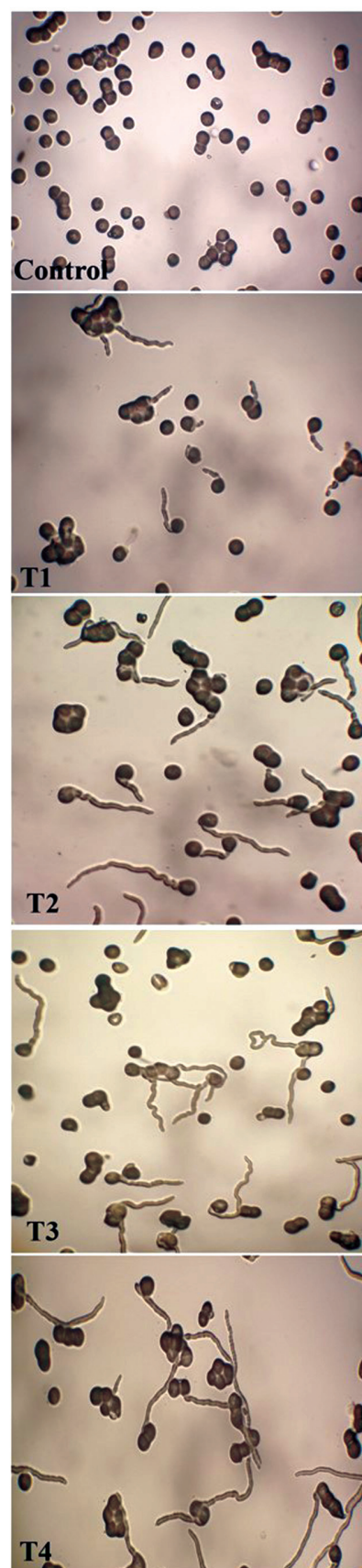


Fig 1 Pollen germination percentage and tube lengths at different low temperature treatments: Control, (T1) 4°C, (T2) -20°C, (T3) -45°C and (T4) -196°C after 60 days of storage.

Table 1 *In vitro* germination assay of pollen stored at different temperature regimes: T1 (4°C), T2 (-20°C), T3 (-45°C) and T4 (-196°C) for a period of 60 days (Pollen tube lengths and mean germination (%) is represented with angular transformed values within parenthesis)

	Days after storage-germination percentage				Days after storage-pollen tube length (µm)			
	7 th day	14 th day	30 th day	60 th day	7 th day	14 th day	30 th day	60 th day
Control	19.96 (26.62)	9.82 (18.74)	0.00 (04.06)	0.00(04.06)	340.17	145.97	0.00	0.00
T1 (4°C)	40.75 (39.93)	35.63 (36.89)	30.05 (33.54)	25.45 (30.54)	539.32	361.91	311.97	172.93
T2 (-20°C)	39.30 (39.09)	42.27 (40.84)	31.81 (34.64)	30.82 (33.98)	580.35	470.67	409.58	282.89
T3 (-45°C)	36.57 (37.48)	33.64 (35.75)	29.74 (33.35)	31.77 (34.58)	624.33	551.49	459.51	400.73
T4(-196°C)	36.06 (36.06)	35.25 (35.25)	35.92 (35.92)	38.82 (28.82)	604.4	505.33	503.71	535.9
CD at 5%					CD at 5%			
Treatments (T): 2.50					Treatments (T): 17.18			
Days (D): 1.67					Days (D): 11.45			
T × D: 5.00					T × D: 34.36			

different crops. For example, reduced germination (20%) in *in vitro* tested cryopreserved pollen was observed in *Hevea* after preserving the sample for a month in liquid nitrogen (Tongkaemkaew and Kaewbunjong 2017); while improved germination percentage of cryostored pollen over fresh samples reported in coconut (Karun *et al.* 2014). The differences noticed for cryostorage of pollen in different plant samples may be due to their differences in the ability to sustain low-temperature stress. Pollen tube lengths varied from 172.93 µm (4°C) to 535.9 µm (-196°C) in different treatments on 60th day of storage but in control, value was zero due to loss of viability (Table 1). Reduction in pollen tube lengths was also noticed with storage duration and the trend was similar to pollen germination percentage. Decreased germination percentage and pollen tube growths were noticed in cold-stored *Lilium longiflorum* pollen due to rapid degradation of pre-synthesized proteins and mRNAs during cold storage (Wang *et al.* 2004).

Coconut pollen of cv. Chowghat Orange Dwarf collected for artificial pollination can be successfully stored up to one month at 4°C (refrigerator) and to two months at -20°C and -45°C (freezer) without much loss of viability and germinating capacity apart from long term storage in liquid nitrogen at -196°C.

SUMMARY

Pollen collection and storage assume importance in hybridization programs in coconut. Development of an effective short-term storage method for coconut pollen ensures availability of pollen for coconut breeding throughout the year. In the present study, pollen collected from dwarf coconut cultivar Chowghat Orange Dwarf (COD), using standardized extraction procedures, was utilized for studying their storage behaviour under four different low-temperature regimes, i.e. 4°C, -20°C, -45°C and -196°C along with control at room temperature. Data on pollen germination percentage and pollen tube lengths were collected based on *in vitro* procedures at four different storage intervals (1st day, 14th day, 30th day and 60th day) to identify best short-term storage temperature. Germination

percentage of pollen gradually reduced with duration of storage in all treatments but in case of pollen, which was stored at room temperature (control, i.e. 28±2°C), viability decreased drastically and became nil within two weeks. Results revealed that higher germination percentage (38.82%) and pollen tube length (535.9 µm) were observed at -196°C followed by -45°C (31.77%, 400.73 µm) and -20°C (30.82%, 282.89 µm) after a storage period of eight weeks. This study shows that desiccated pollen grains can be successfully stored at -45°C for its utilization in breeding programmes besides long term storage at -196°C.

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