Cryopreservation of oil palm pollen

Long-term storage of pollen grains is a useful means of conserving haploid gene pool[1,2] and also of overcoming temporal and spatial isolation of the parent species in the breeding programmes. Cryopreservation (preservation at ultra-low temperatures of –150 to –196°C) has been one of the effective methods of long-term pollen storage[3,4]. Under cryogenic conditions all metabolic processes in the biological systems, including pollen are virtually arrested, thus permitting maintenance of viability. Although theoretically, cryogenic conditions should permit maintenance of pollen viability for several decades, reports of long-term storage beyond five years are limited to a few species[4].

Oil palm (*Elaeis guineensis* Jacq.) is the highest oil-yielding crop in the world[5]. The total oil yield depends upon production of fruits, which yield palm oil from the mesocarp and palm kernel oil from the seeds. Oil palm has been introduced to India as a commercial crop under irrigated conditions. Plants are monoecious and flower throughout the year. On each plant, the male and female inflorescences are produced alternately. Plants are known to be pollinated by wind and weevils[6]. The ratio of male to female phases in a plantation of oil palm keeps changing. The good and bad seasons in yield may thus depend upon the number of plants in male phase and their ratio to female plants in a population. When there are very few plants in male phase in the plantation and also during the monsoon season when insect activity as well as pollen density in the air is reduced, pollination becomes a constraint and results in low yield[7]. Consistent yield can be sustained through assisted pollination during unfavourable conditions. Under ambient temperature conditions oil palm pollen grains completely lose their viability within seven days[8]. Pollen grains could be effectively stored under sub-zero conditions (–20°C) for as long as 12 months (our unpublished data). So far there are reports of successful oil palm pollen storage for up to 1 year at temperatures of –15 (ref. 9) and –10°C after vacuum-drying[10]. *In vitro* pollen germination protocol established for oil palm pollen has been found to be suitable for monitoring germinability of stored pollen grains before using them for assisted pollination[8].

We had stored nearly 5 g of oil palm pollen in liquid nitrogen in March 1998, to check the feasibility of long-term storage without loss of viability and the ability to germinate. We assessed the status of stored pollen samples in April 2006 (over 8 years of storage). To our knowledge this is the first report on cryopreservation and its assessment in oil palm pollen.

Pollen samples for storage were obtained from the plantation at Karnataka Oswal Oil Palm Limited located near Bhadravati, Karnataka in March 1998. Pollen grains were randomly collected from freshly opened male inflorescences and pooled, air-dried for 1 h and brought to Delhi in sealed bottles within 32 h. The viability of pollen sample at the time of cryopreservation was assessed using fluorescein diacetate (FDA) test[11] and *in vitro* germination test using the standardized pollen germination medium [sucrose (2.5%) + boric acid (100 ppm) + polyethylene glycol (10%, MW 10,000)] for oil-palm pollen[7]. Sitting drop pollen cultures in triplicates were raised in the improvised humidity chamber (IHC)[12]. The incubation period was uniformly kept for 150 min. Scoring was done for percentage pollen germination (N = 200 pollen grains per culture) and tube length (N = 50 germinated pollen grains per culture). Pollen viability at the time of storage was 62% through FDA test and 52% through germination test, and the moisture content was 23.3% on fresh weight basis. After transporting the pollen sample to the cryogenebank, it was distributed in screw-cap polypropylene cryovials (2 ml capacity, Nunc) and directly immersed in liquid nitrogen (at –196°C) in Dewar flasks. After 1 h, the vials were transferred to vapour phase of liquid nitrogen (–170 to –180°C temperature) in large capacity (960 l) cryotanks in the cryogenebank located at the National Bureau of Plant Genetic Resources, New Delhi.

For assessing the viability and germinability of 8-year cryostored pollen, four cryovials were thawed in a water bath at + 38°C for 5 min. Pollen grains were taken out and pooled on a clean slide. The pollen sample was prehydrated on a clean glass microslide for 1 h in IHC[12]. Pollen viability and germinability were assessed with the same protocols used for fresh pollen. The results were statistically analysed for significance in variation in the viability and germinability of pollen at the time of storage and after 8 years of cryostorage using one-way ANOVA at probability level of 0.05. Confocal images were recorded using Carl Zeiss LSM 5 Pascal Confocal Microscope.

Our results clearly showed that pollen grains of oil palm cryopreserved for up to 8 years retained as high as 54 ± 1.72% viability, as assessed by FDA test (Figure 1 *a*). The generative and the vegetative nuclei showed normal association in the prehydrated pollen (Figure 1 *a*). The difference in viability at the time of storage (62 ± 4.33%) and after 8 years was not significant ($P \leq 0.05$). Cryopreserved pollen grains showed 49 ± 1.2% *in vitro* germinability (Figure 1 *b*). This was

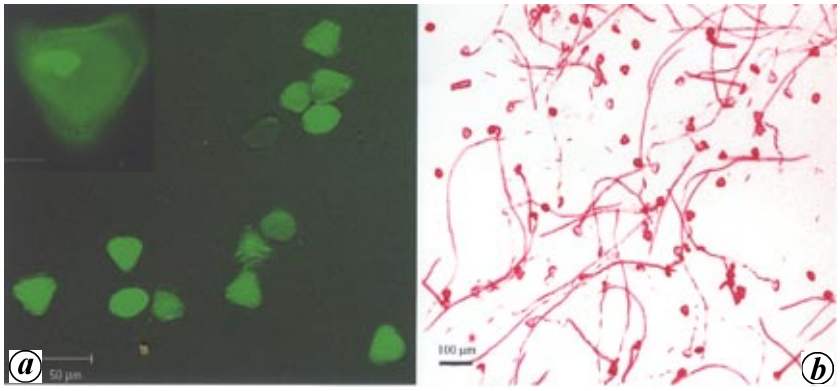

Figure 1. A confocal image of 8-year cryostored pollen grains stained with FDA; pollen grains showing bright fluorescence are viable. (Inset) Ethidium bromide-stained pollen showing vegetative cell nucleus and generative cell nucleus. ***b***, *In vitro* germinated cryostored pollen grains after 150 min culture. Note that the pollen tubes are uniformly smooth with round tip.

comparable to 52 ± 2.08% germination before storage. This difference in percentage germination was insignificant ($P \leq 0.05$). Marginal loss in viability could be due to transit of pollen samples to and from the cryopreservation facility at NBPGR and the University of Delhi at the time of storage and after retrieval. The pollen tubes did not show any structural abnormality or aberration in tube growth. The generative and vegetative nuclei entered the pollen tube after it had attained a length of ~ 100–150 μm. The generative nucleus did not divide until the pollen tubes grew for about 3 h and formed 2 or 3 callose plugs.

Pollen grains stored at ultra-low temperature conditions with more than 30% germination have been reported to be satisfactory to induce fruit set comparable to fresh pollen[13]. Marginally lower *in vitro* germination when compared to FDA test is in general agreement with that recorded earlier for most other pollen grains[14]. Our results clearly show that it is feasible to cryogenically store pollen grains of oil palm for longer periods beyond 8 years without any significant loss in their viability and germinability, and they may be used effectively for assisted pollination.

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