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# Cryopreservation of coconut (*Cocos nucifera* .L) pollen

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**Abstract**

Pollen cryopreservation is an additional technique for the conservation of genetic resources, which supplements seed or clonal preservation. Present paper explains the protocol for cryopreservation of coconut pollen, comparing the germinability and pollen tube growth of cryopreserved pollen with fresh as well as incubator-dried pollen. Germination medium for coconut pollen consisted of 8% sucrose, 1% gelatin, 1% agar and 0.01% boric acid. The germination percentage of cryopreserved pollen was 38.54 %, which is significantly lesser than that of fresh pollen (46.65%). However, more vigorous pollen tube growth was observed in cryopreserved pollen. There was also significant palm-to-palm variation in germination and pollen tube growth.

*Key words* : Cryopreservation, Coconut, pollen viability, pollen germination, pollen tube growth

## Introduction

Pollen cryopreservation supplements conventional conservation of germplasm and offers a safe and reliable technology for germplasm collection, conservation and sustained maintenance . Pollen is cryopreserved in pollen banks, where large quantity of pollen can be compactly preserved in a relatively limited area, so that it can be made available to breeders for use in crop improvement programmes. The detailed study of coconut pollen was done by various workers (Nair and Sharma, 1963; Gangolly *et al.*, 1961). Nair and Sharma (1963) reported the occurrence of coconut pollen variations comprising the trichotomocolpate, porate and operculate forms, apart from the I-furrowed ones. Patel (1938) observed about 25 per cent of infertile pollen grains in coconut under Indian conditions, while Aldaba Victor (1921) has observed 3 to 33 per cent of infertile pollen grains in the Philippines. Pollen yield, quality and viability depend upon environmental conditions prevailing during flowering and microgametophyte differentiation and development. The viability, tube growth and morphological homogeneity related to pollen quality are the most important properties of palms that are useful for plant breeders.

Germination medium for coconut pollen has been standardized by Varkey and Davis (1960). Nampoothiri (1970) germinated pollen grains on media containing 8% sucrose, 2% gelatin and 2% agar dissolved in distilled water. T.T.C. (2,3,5-triphenyl tetrazolium chloride) and acetocarmine were used for determining pollen-sterility of coconut pollen.

As on today, there is no report on cryopreservation of coconut pollen. The current paper aims to evaluate the feasibility of cryopreserving the coconut pollen collected from nine pollen parents used for regular hybridization programme of Central Plantation Crops Research Institute, Kasaragod.

## Material and Methods

The pollen used for this study was extracted from male flowers of nine West Coast Tall (WCT) palms Nos. 9,20,44,68,79,96,99,133 and 149 of CPCRI, Kasaragod during the months of May-June, 2006.

Pollen was collected on sunny days between 8-10 am. The spikes with male flowers were obtained from the above numbered palms after the spathe has just opened or after anthesis. The spikes containing male flowers were brought to the laboratory and the male flowers stripped off and anthers removed and fresh pollen extracted by gentle tapping of the anthers. In case of incubator drying, the male flowers were striped off from spike and placed in a Petri dish on an aluminium foil

and kept inside the B.O.D incubator, set at 40 °C for 24 hrs. Pollen was extracted by sieving the dried flowers.

Cryopreservation method: Incubator dried pollen were enclosed in an aluminium foil pouch and kept inside the cryovials (1.5 ml) and directly immersed in a Dewar flask containing liquid nitrogen (-196°C). The minimum time of storage was 24 hrs. After cryopreservation of pollen, the cryovials were removed from liquid nitrogen and kept in ambient condition (30-32°C) for fifteen minutes for thawing.

**Viability test**

This was done by *in vitro* germination of pollen and the percent germination and average pollen tube length in μm (vigour) estimated. The viabilities of the fresh pollen, incubator dried pollen and cryopreserved pollen were calculated. The germination medium used consisted of 8%sucrose, 1% agar, 1% gelatin, and 0.01% boric acid. Each time, freshly prepared medium was used for testing the germinability These pre treated pollen were dispersed on a micro-slide already smeared with germination medium and placed in a Petri plate lined with moist filter paper and incubated at ambient condition (30 ±1° C and RH at 80 %)in a dark chamber place for 1 to 2 hrs.

The slides with the germinated pollen, random 10 fields in each were photographed 10 X. with the help of automatic Leica Qwin image analyzer connected to Leica Diaplan microscope.

Average pollen tube length in μm and percentage germination was calculated using Qwin software. For each field, number of germinated pollen and the percentage was recorded. Ten fields/palm were selected and within the field, 20 randomly selected pollen were utilized for the study. For each field, pollen tube length was also recorded after 1 ½ to 2 hours. An average, of ten fields was estimated using the software.

## Results and Discussions

At ultra- low temperature, coconut pollen undergo negligible metabolic changes in terms of physiological and biochemical processes. Fresh coconut pollen, do not survive immersion in liquid nitrogen at ultra low temperatures, because of intracellular ice crystal formation. In jojoba (Lee *et al*, 1985) and hops (Haunold and Stanwood, 1985) pollen retained viability over a 2-year test period in cryogenic storage. Gladiolus pollen stored cryogenically for 10 years retained high levels of *in vitro* germination and seed set (Rajasekharan *et al*, 1994). This method has also been recommended for convenient economical storage and transport of germplasm. At -196°C the pollen of *Secale cereale, Tritium aestivum* and *Zea mays* could be maintained in viable state for up to 10 years (Kovacs and Barnabas, 1993).

The viability of the pollen, pollen tube growth and morphological homogeneity are directly related to pollen quality/fertility. In the present study, viability test was conducted on germination of fresh pollen, incubated dried pollen and cryopreserved (after incubated dried) pollen of nine West Coast Tall palms. Maximum germination was obtained with fresh pollen (46.6%), which was not statistically significant with the germination of incubator dried pollen (42.3%). However, the germination of cryopreseved pollen (38.5%) was significantly less compared to fresh pollen, but on par with incubator dried. It was observed that palm to palm variation is a factor to be reckoned with and hence the data was analyzed by taking palm (genotype) as a factor. .

Analysis of variance revealed that there was significant difference among genotypes for pollen germination and also the interaction between genotype and pollen processing was significant. Per cent germination of pollen from the 9 palms are shown in Table 1. The germination of fresh pollen was very low (less than 25%) in palms 44, 79 and 9. The per cent germination in these palms was improved when pollen was either incubator dried or cryopreserved. No significant difference in germination was noticed among these two methods in these three palms. Maximum germination for fresh pollen was observed in palm number 99 (67.2%), but significantly reduced to 39.3% and 47.1% respectively when incubator dried and cryopreserved. The range of per cent germination of incubated dried pollen (31.4) and cryopreserved pollen (26.8) among genotypes was less compared to that of fresh pollen (49.6). In other words, variation among genotypes was reduced considerably when pollen were cryopreserved. It is interesting to note that except in 3

**Table 1. Per cent germination of fresh pollen, incubator dried and cryopreserved pollen among palms**

| Palm Number | Fresh pollen | Incubator (40° C dried pollen (24 hrs) | Cryopreserved pollen (24 hrs) |
|---|---|---|---|
| 9 | 24.5 | 47.0 | 44.9 |
| 20 | 66.9 | 46.2 | 39.6 |
| 44 | 17.5 | 43.3 | 35.6 |
| 68 | 45.5 | 62.7 | 48.7 |
| 79 | 21.2 | 38.9 | 40.9 |
| 96 | 61.4 | 31.2 | 43.9 |
| 99 | 67.2 | 39.3 | 47.1 |
| 133 | 56.6 | 34.9 | 21.9 |
| 149 | 59.0 | 37.4 | 24.4 |

CD (5%) for comparison among means is 9.7

palms (149, 133, and 44), the per cent germination of cryopreserved pollen was on par (between 39.6 to 48.7).

In pollen germination medium, addition of optimum boric acid (0.01%) resulted in good germination and pollen tube growth. This is supported by the fact that pollen of coconut are deficient in boron content, the deficiency being made up by comparatively high levels of boron in the stigma and styles. Boron reduces bursting of pollen tubes and also enhances percentage germination and pollen tube growth. By increasing the concentration from 0.01 to 0.1%, the percentage of germination and pollen tube growth was less.

The overall pollen tube growth was significantly more when pollen were incubated dried (190.8$\mu$m) or cryopreserved (192.5$\mu$m) compared with fresh pollen (140.8$\mu$m). As in the case of pollen germination, significant palm to palm variation was observed for pollen tube growth. (Table 2). The pollen from palm number 96 had maximum pollen tube growth (200.7$\mu$m) before processing (i.e., fresh pollen), but became the lowest (78.4$\mu$m) on cryopreservation. Against the reduction in palm to palm variation for germination on pollen processing, the variation for pollen tube growth was observed to be increased on pollen processing. However, cryopreservation of pollen resulted in increased pollen tube growth except in the case of palms 79 and 96. Extent of pollen tube growth indicates the vigour of the pollen. Perry (1978) opined that assessment of vigor is a better indication of quality than viability. The increase in vigour of pollen on cryopresevation could be further noticed from the fact that except in two palms, the pollen tube growth was below 142$\mu$m with respect to fresh pollen, while it was more than 142$\mu$m when cryopreserved in all the palms except the two mentioned earlier. Maximum pollen tube growth (319.7$\mu$m) was noticed in palm number 44 after cryopreservation ( Table 2 and Fig 1).

**Table 2. Pollen tube length ($\mu$m) observed after 1 ½ to 2 hours culture in vitro cryopreserved pollen among palms**

| Palm Number | Fresh pollen | Incubator (40° C) dried pollen (24 hrs) | Cryopreserved pollen (24 hrs) |
|---|---|---|---|
| 9 | 116.0 | 125.3 | 221.0 |
| 20 | 121.5 | 318.1 | 161.7 |
| 44 | 198.6 | 153.2 | 319.7 |
| 68 | 142.5 | 267.9 | 232.4 |
| 79 | 115.3 | 223.8 | 109.0 |
| 96 | 200.7 | 160.3 | 78.4 |
| 99 | 132.2 | 156.3 | 142.7 |
| 133 | 112.4 | 191.1 | 281.5 |
| 149 | 128.5 | 137.0 | 171.4 |

CD(5%) for comparison among means is 39.1

Fig: 1 a. Cryopreserved pollen of WCT (Palm No. 44) germinated on semisolid media and observed after 1 hour 45 minutes 1b. Incubator dried pollen of WCT (Palm No. 20), germinated in semisolid media and observed after 1 hour 45 minutes.

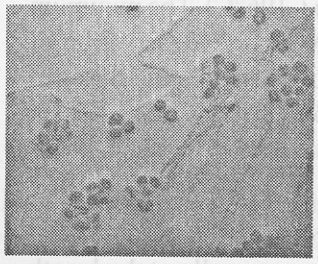

Fig. 1a

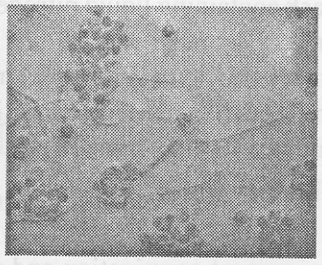

Fig. 1b

Coconut pollen is tricelled (Patel, 1938) and generally shed at relatively high moisture content and survives desiccation. Vasil (1961) opined that the life span of pollen is primarily determined by the plant genome and also influenced by external environmental conditions. The pollen longevity of family araceae is long-lived (6months to 1 year)(Barnabas and Kovacs, 1997). Whitehead (1963) reported that coconut pollen could be stored under 5° C with 40% RH for 1.5 years

Temperature and humidity also play an important role in germination of pollen grains. In case of coconut it was observed, that the maximum germination was occurred at 30 ° C with 80% RH. The same condition was followed in all treatments.

Cryogenic storage of pollen has been possible as shown by the numerous available reports. Experiments done on maize pollen show that 50% of the pollen grains could be kept viable for a year with seed-setting ability of 30% (Barnabas and Rajki, 1976) when pollen moisture content was reduced to 14%. Hanna (1990) reported that pearl millet pollen stored for several years showed 100% seed set when pollen moisture content was reduced below 7.2% and stored. Thus, coconut pollen could also be stored cryogenically as supported by this data although the longevity was tested only over a period of 24 hrs. Thus, further study has to be conducted on the longevity of the coconut pollen over a longer storage.

Temperature and relative humidity also play an important role in *in vitro* germination. From the above preliminary trial it was observed that the optimum temperature and relative humidity was 30°C and 80 % respectively for incubator- dried and cryopreserved coconut pollen. The effect of temperature on pollen germination and tube elongation of cotton pollen over a range of temperatures from 20 to 43°C was studied by Burke *et al* (2004) and germination was observed to be

high across the range of temperatures from 20 to 37°C.

Humidity also plays a vital role in pollen germination Burke *et al* (2004) assessed the effect of different levels of relative humidity 35, 50, 80 and 100% on cotton pollen germination. At 35% relative humidity resulted in short pollen tubes while those germinated in 50% RH gave increased pollen tube length. The best elongation was found at 80% RH. This is in agreement with the present study, since best tube growth for coconut pollen was observed in 80% RH.

It can be concluded from the present study that cryopreservation of coconut pollen is possible and viability maintained after the pollen was incubator- dried or cryopreserved. The best pollen germination medium for coconut comprised of 8% sucrose, 1% gelatin, 1% agar and 0.01% boric acid. There was significant palm-to-palm variation in germination and pollen tube growth of WCT pollen. Out of 9 WCT palms studied, pollen of palm No.68 was best for incubator drying (62.68% and 267.88$\mu$m ) as well as cryopreservation (48.65% and 232.39 $\mu$m).

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