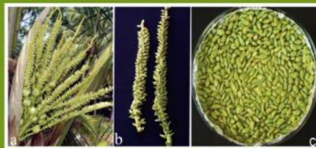

Fig. 1. Collection of male flowers from coconut inflorescence.

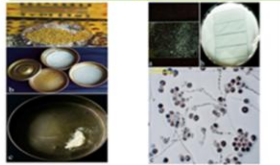

Fig. 2. Pollen extraction from desiccated male flowers

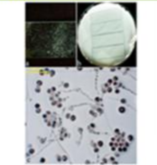

Fig. 3. Viability check in desiccated pollen of coconut through *in vitro* germination assay.

Fig. 4. Cryo-conservation of pollen in coconut

Fig.5. Fertility test of cryo-conserved pollen in coconut

Table.1 Percent pollen germination and vigour (pollen tube length in µm) of WCT and COD pollen at various durations of storage in liquid nitrogen

Variety and particulars	Germination (%)	Pollen tube length (µm)
WCT		
Initial	32.07	221.40
After 1 year	32.16	421.47
2 years	40.05	292.79
3 years	34.32	250.62
COD		
Initial	46.34	205.60
After 1 year	32.69	213.36
2 years	44.14	213.76
3 years	32.40	224.36

Application of pollen cryopreservation protocol to exotic and indigenous accessions

Exotic accessions:

MYD, Cochin China, CRD, PHOT, JYT, FJT, MOD, STVT

Indigenous accessions:

LCT, KTOB, GBGD, CGD, AGT, ADOT, GBGD, LMT, TPT, BENT, CALT, SKGT

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Cryopreservation of coconut pollen

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Introduction

Cryopreservation is a technique for long-term conservation of genetic diversity. Studies conducted at ICAR-CPCRI have shown that coconut pollen could be successfully cryopreserved (Karun et al., 2014). Normal nut set have been observed in Chowghat Orange Dwarf (COD) and West Coast Tall (WCT) accessions of coconut pollinated with WCT and COD pollen respectively. The factors like germinability and vigour of pollen (pollen tube growth) are to be observed before cryopreserving the pollen.

Check list for pollen cryopreservation

a). Extraction of spikelets:
i. Pollination bag
ii. Sharp knife or scissors
iii. Polythene bag
iv. Marking pen

b). Drying male flowers
i. Petri dish
ii. Aluminium foil
iii. B.O.D incubator
Sieve for collecting pollen from dried male flowers

c). Cryopreservation
i. Aluminium foil
ii. Cryovials
iii. Canisters
iv. Cryotank with liquid nitrogen

d). Pollen germination
i. Micro slides
ii. Beaker
iii. Measuring cylinder
iv. Cotton
v. Petri dish
vi. Germination medium
vii. Microscope (Binocular)

Male flower extraction and pollen collection

In coconut, once in 20-25 days a fresh male inflorescence emerges out from the leaf axil. Male flowers start opening from the top (basipetal) of each spikelet and sheds the pollen continuously for 24 days depending upon the variety and season

Procedure for extraction and collection of pollen for storage

I. Bag the inflorescence to prevent the contamination from foreign pollen once it completely emerges out of the leaf axil and just before the natural opening of the male flowers starts. Normally it is 6-7 days before the collection of spikelets
II. When male flowers are about to open from the tip, collect the spikelets in a polythene bag on bright sunny day, between 8-10 AM and label it.
III. Strip off the male flowers from the spikelets to an aluminium foil placed on petri dish

Dehydration of pollen

I. Keep the Petri dish with male flowers in an incubator set at 35°C-38°C for 24 hours
II. Extract pollen by sieving the dried male flowers

Assessment of pollen viability before storage in liquid nitrogen

Preparation of artificial medium for germination of pollen:

I. Dissolve 8% sucrose, 1% agar, 1% gelatin and 0.01% boric acid in distilled water
II. Boil it until the agar and gelatin gets fully dissolved
III. Allow it to cool for a while
IV. Take a clear slide and smear the medium uniformly over the slide
V. Allow the medium to solidify

Procedure for pollen germination

I. With the help of a small cotton ball disperse the pollen grains evenly over the medium by gently tapping it by holding between the fingers
II. Place the slide in a Petri dish lined with moist filter paper and cover it to maintain humidity inside the chamber
III. Incubate at ambient condition in dark for 90 minutes, use an incubator with a temperature set to 30°C

Note: if the ambient temperature is very low (below 25°C)

Scoring of pollen for germinability

I. Observe the slide under compound microscope
II. Score the germinated pollen in 10 randomly selected microscopic fields
III. Calculate the germination percentage using the formula

Percentage germination= [No. of germinated pollen in the field / Total number of pollen in the field] × 100

Note: the accepted quality norm for pollen germination was found to be above 25%. The pollen with poor viability may be discarded

Cryopreservation procedure

I. Keep the desiccated pollen in aluminum foil pouch and insert it in to the cryo vial
II. Plunge the canister containing cryovials in liquid nitrogen container

Retrieval of cryostored pollen

I. Lift the canisters from the liquid nitrogen
II. Allow it to thaw for 15 minutes at ambient temperature

Post storage viability assessment: the same procedure may be followed as in case of desiccated pollen

Assessment of nut set after pollination with cryopreserved pollen

It is necessary to test the potential of cryopreserved pollen for seed set under field condition through controlled pollination. Cryopreserved pollen samples were taken out from the cryotank and kept at room temperature for thawing for an hour. The pollen is subsequently mixed with talcum powder (1:9) to protect it from the heat and becoming too moist

It was observed that there is no appreciable change in pollen viability after 3 years of storage under liquid nitrogen