PRESERVATION METHOD—POLLEN

NLGRP POLLEN PHOENIX CRYOPRESERVATION 1

06/18/2025

File Name
NLGRP_POLLEN_PHOENIX_CRYOPRESERVATION_1

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Abstract
Flower spathes are harvested when they are fully elongated and just prior to spathe crack. They are hung and dried at ambient temperature for about 3 weeks until pollen is released and collected then shipped to NLGRP. Once at NLGRP, begin the moisture equilibration process in a cold room set at 5°C and 23% relative humidity for overnight. The next day, package the pollen into 4mL vials and plunge into the vapor phase of LN.

Study Reason
Preservation

Introduction
This is the standard method of cryopreserving Date Palm (*Phoenix dactylifera*) pollen germplasm at NLGRP.

Source of Plant Material
Pollen is collected from the flowers of field grown date palm trees at the National Clonal Germplasm Repository for Dates in Thermal, California starting in early spring (January – February).

Plant Material Description
Flower spathes are collected and dried in Thermal, California. Flower spathes are harvested when they are fully elongated and just prior to spathe crack. Spathes on each tree emerge and elongate sequentially, so multiple harvests from each tree may be necessary.

Plant Material Cleanliness
The material should be free of insects or other contaminants. Upon opening a new shipment, material is carefully inspected for insects. Otherwise, the material is not tested for pathogens.

Plant Material Preparation/Pretreatment
To prevent cross-contamination, flower spathes from each separate accession and inventory item are kept separate within individual wardrobe boxes (L X W X H approximately 20 in X 20 in X 36 in), being either hung from the cross bar or placed in the bottom of the box. The wardrobe boxes are kept in the shop area at ambient temperature for approximately three

weeks after the last spathe from the accession/inventory item is hung. When flowers are dry, the pollen is released. The pollen is harvested, sifted, weighed, and then packaged in 50 mL centrifuge tubes (or similar container). 60 mL per accession is the desired amount to maximize the amount of pollen in long-term storage. These are placed in a Styrofoam cooler with an ice pack and shipped overnight to NLGRP. The pollen arrives at NLGRP ready to begin the moisture equilibration process immediately. It is important to process the pollen very quickly.

Moisture Equilibration
At NLGRP, the pollen moisture content (MC) must be reduced from overnight dried moisture contents to approximately 6-10% MC on a fresh weight basis (FWB). To begin this moisture equilibration process, pour pollen into an appropriately sized Petri dish, making sure the pollen is evenly distributed with a depth of no more than a few millimeters. Place uncovered Petri dishes containing pollen into a cold room that is set at 5°C and 23% relative humidity for overnight.

In order to determine moisture contents, a tiny amount of pollen (~15 mg at most) must be sacrificed for taking weights. The following process will be done twice, the first time with fresh pollen as soon as it arrives at the lab, the second time it will be done with the pollen after it has been moisture equilibrated overnight.

For each accession, three weigh boats are prepared and weighed for tare weights. Weigh boats are made using a small square of aluminum foil and an unsharpened pencil. Wrap the foil around the base of the unsharpened pencil and then slide the foil off to create what looks like a tiny bucket. Weigh the empty weigh boat to get the tare weight. Place a small amount of fresh pollen into the weigh boat, then gently close the weigh boat using forceps and weigh it again for the tare+FW (fresh weight) amount. The fresh weight (FW) is the tare weight subtracted from the tare+FW amount. These samples are then placed in a 90°C oven for 2 days. After 2 days, the oven-dried pollen in the weigh boats is weighed again for the tare+DW (dry weight) amount. The dry weight (DW) is the tare weight subtracted from the tare+DW amount. The following equation is used to calculate the moisture content on a fresh weight basis: MC=((FW-DW)/FW), and on a dry weight basis: MC=((FW-DW)/DW). Moisture content is determined for the pollen before and after moisture adjustment. Only the moisture content after equilibration is recorded in the eCryocard and GRIN-Global.

Packaging
Package the moisture equilibrated pollen into 4 mL cryovials containing approximately 4 mL pollen each. The goal is to fill 15 vials per accession placing 3 vials on a cane, achieving 5 canes in total. Canes are inserted into labeled plastic sleeves.

Cryopreservation Method
Remove one Petri dish of pollen from the 5°C cold room and quickly package pollen into 4 mL cryovials. Plunge vials into the vapor phase of liquid nitrogen for a very fast cooling rate.

Storage Conditions
The loaded canes in sleeves are transferred to boxes and stored in the vapor phase of liquid nitrogen. Viability of cryopreserved pollen is assessed and samples with greater than 5% viable pollen are placed into cryotanks designated for long-term storage.

Comments
N/A

References
Araujo De Oliveira, A., Ledo, A., Polek, M., Krueger, R., & Volk, G.M. (2021). Optimization of in vitro germination and cryopreservation conditions for preserving date palm pollen in

the USDA National Plant Germplasm System. *Plant Cell, Tissue and Organ Culture,* **144**, 223-232.

Appendices
N/A