PRESERVATION METHOD—POLLEN

NLGRP POLLEN PISTACIA CRYOPRESERVATION 1

02/22/2025

File Name
NLGRP_POLLEN_PISTACIA_CRYOPRESERVATION_1

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Study Reason
Preservation

Introduction
This is the standard method of cryo-preserving *Pistacia* pollen germplasm at NLGRP.

Source of Plant Material
In mid-spring, pollen is collected from the catkin flowers of field grown *Pistacia* trees at the National Clonal Germplasm Repository for Tree Fruit and Nut Crops in Davis, California.

Plant Material Description
Pistacia male catkins are collected and dried in Davis, California in mid-spring. Male catkins, at the ideal stage of development for collection, vary in size and color depending on the species. Typically, they first appear as green and change color to either yellow or red as the catkins mature. The size can range from 1-7 cm long. Routine monitoring is very important since pollen dehiscence occurs within a short period of time, particularly during warm, dry or windy conditions. Typically, the anthers will release pollen for approximately four days. Rain and high relative humidity (>85%) delay pollen dehiscence. Monitor and collect catkins in the early morning before pollen dehiscence, which begins mid-morning. Mature catkins appear swollen with pollen and are often splitting. They will release a cloud of yellow pollen when lightly tapped. At this stage (or slightly before), catkins should be carefully removed from the tree and placed in a paper bag labeled with the inventory identifier, location, and collection date. Ideally two paper bags with a volume of approximately 2500-3000 cubic centimeters per bag should be collected per tree to yield sufficient pollen for reaching the desired amount of pollen needed to maximize the amount of pollen in long-term storage.

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
Spread the collected catkins in a single layer on either unprinted paper or in clean plastic trays in a still, dry room. The temperature should be kept between 15°C (60°F) and 21°C (70°F). Pollen will shed within 24 hours of collection and be sufficiently dried for storage 48

hours after collection. Gently lift and shake the dried catkins or use a clean paint brush to release the remainder of the pollen, then discard the dehisced catkins. Separate the pollen from the larger plant debris using a medium #60 mesh sieve (#60=250 µm). Then, use a #100 mesh sieve (#100 =150 µm) to separate the smaller plant debris from the pollen onto a single layer of unprinted paper. The dry pollen will pour like fine flour. It should be placed into a screw-cap 50 mL conical centrifuge tube. 30 mL of pollen per accession is the desired amount to maximize the amount of pollen in long-term storage. Properly label the tube with the inventory identifier number, location, and collection date, and place it in a refrigerator until shipped overnight to NLGRP in a Styrofoam cooler with an ice pack. 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 to approximately 6-10% MC on a fresh weight basis (FWB). To begin this moisture equilibration process, have a room temperature (~25˚C) Pyrex vacuum desiccator (250 mm diameter) prepared with a saturated salt solution of calcium nitrate, which has critical relative humidity of approximately 47%. The saturated salt solution is made by adding calcium nitrate salt to a large Petri dish (140 x 20 mm), with enough water to make a salt slurry; fill the dish but leave a few millimeters of space at the top in to make handling easier. Place the uncovered Petri dish containing the saturated salt solution in the bottom of the desiccation chamber. Check daily to ensure that undissolved salts are present in the slurry. 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 the prepared desiccator and ensure the lid of the desiccator is sealed shut. Let sit at room temperature overnight.

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 boats 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 eCyocard and GRIN-Global.

Packaging

Package the moisture equilibrated pollen into 2 mL cryovials containing approximately 2 mL pollen each. The goal is to fill 15 vials per accession, placing 5 vials on a cane, achieving 3 canes in total. Canes are inserted into plastic sleeves.

Cryopreservation Method
Remove one Petri dish of pollen from the desiccation chamber at a time and quickly package pollen into 2 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 cryoboxes 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
Connor KF, Towill LE. 1993. Pollen-handling protocol and hydration/dehydration characteristics of pollen for application to long-term storage. Euphytica 68:77–84

Appendices
N/A