PRESERVATION METHOD—POLLEN

NLGRP POLLEN PRUNUS CRYOPRESERVATION 1

06/10/2024

File Name
NLGRP_POLLEN_PRUNUS_CRYOPRESERVATION_1

Authors
Gayle Volk[1*] (Gayle.Volk@usda.gov), Ashley Shepherd[1] (Ashley.Shepherd@usda.gov), Carolyn DeBuse[2] (Carolyn.Debuse@usda.gov)

*Method PDF contact person

[1]National Laboratory for Genetic Resources Preservation, 1111 S. Mason St., Fort Collins, CO 80521

[2]National Clonal Repository for Tree Fruit and Nut Crops and Grapes, One Shields Avenue, Davis, CA 95616

Abstract
Flowers are harvested at the "popcorn" stage and rubbed through a wire mesh to separate flower parts, then sifted through a finer sieve so that anthers and filaments filter through and left to dry overnight then shipped to NLGRP. Once at NLGRP, begin the moisture equilibration process using a vacuum desiccator containing a calcium nitrate saturated salt solution. Let pollen sit in desiccator at room temperature overnight. Package pollen into 1.2mL vials and plunge into vapor phase of LN.

Study Reason
Preservation

Introduction
This is the standard method of cryopreserving *Prunus* pollen germplasm at NLGRP.

Source of Plant Material
Pollen is collected from the flowers of field grown *Prunus* trees at the National Clonal Germplasm Repository in Davis, California in early spring.

Plant Material Description
The flowers are harvested at popcorn stage (flower is ready to bloom but petals are still closed) into a paper bag. The pollen arrives combined with gently crushed anther and filament fragments.

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
On the harvest day, flowers are rubbed through a wire mesh to separate the flower parts. They are then sifted through a finer sieve so that only the anthers and filaments filter through on to a clean paper. The anthers are dried overnight at ambient room temperature. The next day (8-12 hours) the dried anthers have released the pollen. The pollen and remaining dried flower parts (anthers and filaments) are weighed and packaged in falcon

centrifuge tubes and shipped overnight in a Styrofoam cooler with an ice pack to NLGRP. 8 mL per accession is the desired amount to be shipped in order to maximize the amount in long-term storage. 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, 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 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 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 1.2 mL cryovials containing approximately 0.5 mL pollen each. The goal is to half-fill 15 vials per accession placing 5 vials on a cane, achieving 3 canes in total. Canes are inserted into labeled plastic sleeves.

Cryopreservation Method
Remove one Petri dish of pollen from the desiccation chamber at a time and quickly package pollen into sterile, 1.2 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 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
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