VIABILITY METHOD—POLLEN

NLGRP POLLEN PRUNUS IN VITRO GERMINATION 1

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File Name
NLGRP_POLLEN_PRUNUS_IN-VITRO-GERMINATION_1

Authors
Gayle Volk (Gayle.Volk@usda.gov)*, Ashley Shepherd (Ashley.Shepherd@usda.gov)

*Method PDF contact person

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

Abstract
Remove a vial from LN vapor and let sit for 10 minutes. Rehydrate over wet a paper towel for 2 hours. Plate pollen on Marquard medium with 20% sucrose overnight. Viability is ready to be assessed approximately 12-24 hours after plating onto medium. Three fields of 100 pollen grains are assessed using a microscope.

Introduction
Pollen is shipped from the National Clonal Germplasm unit in Davis, California to NLGRP in Fort Collins, CO for long-term storage in liquid nitrogen vapor. The optimal viability method for *Prunus* pollen at NLGRP is an in vitro germination assay.

Source of Plant Material
Pollen is collected in early spring from field grown trees in Davis, California. Pollen is sent to NLGRP, moisture equilibrated, packaged, then frozen and stored in the vapor phase of liquid nitrogen.

Plant Material Description
Approximately 0.5 mL pollen mixed with other crushed flower parts is cryopreserved in 1.2 mL vials.

Warming/Rehydration
Remove 1 vial (or portion thereof) from liquid nitrogen vapor and let sit at room temperature (~25°C) for 10 minutes. Prepare a large, glass Petri dish (140 x 20 mm) for rehydration; place a folded paper towel on the bottom of the dish and thoroughly saturate the paper towel with water. After the vial has warmed for 10 minutes, pour the contents of the vial into a small Petri dish (35 x 10 mm). Place the uncovered Petri dish with pollen on the wet paper towel within the large Petri dish. Place the lid onto the large Petri dish and place in the dark at room temperature for 2 hours.

Regrowth/Viability Conditions
After rehydration, pollen is carefully plated onto Marquard pollen germination medium (with 20% sucrose) in a small Petri dish (35 x 10 mm). Because this pollen has debris mixed in with it, dumping a scoop of pollen mixture onto the media and then tapping the plate upside down to remove the debris is a sufficient method of plating *Prunus* pollen. The amount of pollen contained within the debris will guide how big or little of a scoop should be used, therefore, this amount will vary. After plating, the Petri dish is placed in the dark (room temperature, ~25°C) for overnight or up to 24 hours.

Regrowth/Viability Assessment
Viability is ready to be assessed approximately 12-24 hours after plating onto Marquard medium. Three fields of 100 pollen grains are assessed using a microscope (200x). Pollen germination is considered successful when the pollen tube length exceeds the pollen grain diameter. Each of the three measurements for viability, as well as the average, is recorded on eCryocards and in GRIN-Global. Accessions with greater than 5% viable pollen are placed into 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

Marquard, Robert D. 1992. Pollen Tube Growth in Carya and Temporal Influence of Pollen Deposition on Fertilization Success in Pecan. Journal of American Society of Horticulture Science 117(2):328-331

Martínez-Gómez P, Gradiel TM, Ortega E, Dicenta F. 2002. Low temperature storage of almond pollen. HortScience 37:691–692

Appendices
Marquard pollen germination medium: 20% sucrose + 0.01% boric acid + 0.03% calcium nitrate + 0.02% magnesium sulfate + 0.01% potassium nitrate + 2% agarose