VIABILITY METHOD—POLLEN

NLGRP POLLEN JUGLANS IN VITRO GERMINATION 1

6/10/2024

File Name
NLGRP_POLLEN_JUGLANS_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 3 hours. Plate pollen on Marquard medium with 15% 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
Juglans pollen is shipped from the National Clonal Germplasm Repository for Tree Fruit and Nut Crops in Davis California to NLGRP in Fort Collins, CO for long-term storage in liquid nitrogen vapor. The optimal viability method for *Juglans* pollen at NLGRP is an in vitro germination assay.

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

Plant Material Description
The sifted pollen is cryopreserved in 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 3 hours.

Regrowth/Viability Conditions
After 3 hours of rehydration, pollen is carefully plated onto Marquard pollen germination medium (with 15% sucrose) in a small Petri dish (35 x 10 mm). Using a small, soft-bristled watercolor paint brush, gather a small amount of pollen onto the tips of the bristles. Carefully and gently tap the brush over the germination medium to lightly dust the surface

of the medium. Cover the Petri dish with a lid, then place it 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
Additional information

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

Appendices
Marquard pollen germination medium: 15% sucrose + 0.01% boric acid + 0.03% calcium nitrate + 0.02% magnesium sulfate + 0.01% potassium nitrate + 2% agarose