VIABILITY METHOD—POLLEN

NLGRP POLLEN CARYA IN VITRO GERMINATION 1

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File Name
NLGRP_POLLEN_CARYA_IN-VITRO-GERMINATION_1

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Abstract
Remove a vial from LN vapor and let sit for 10 minutes. Rehydrate pollen in a desiccator containing a copper sulfate saturated salt solution for 4 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
Carya pollen is shipped from the National Collection of Genetic Resources for Pecans and Hickories in College Station, Texas and Brownwood, Texas to NLGRP in Fort Collins, CO for long-term storage in liquid nitrogen vapor. The optimal viability method for *Carya* pollen at NLGRP is an in vitro germination assay.

Source of Plant Material
Pollen is collected in the spring from field grown trees in College Station, Texas or Brownwood, Texas. 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
Prepare a Pyrex vacuum desiccator (250 mm diameter) with a saturated salt solution of copper sulfate. The saturated salt solution is made by adding copper sulfate 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.

Remove 1 vial (or portion thereof) from liquid nitrogen vapor and let sit at room temperature (~25°C) for 10 minutes. Then pour the 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 the Petri dish containing pollen, uncovered, into the prepared desiccator and ensure the lid of the desiccator is sealed shut. Let sit at room temperature for 4 hours.

Regrowth/Viability Conditions
After the 4 hours of rehydration, pollen is carefully plated onto Marquard pollen germination medium (with 15% sucrose) in a small Petri dish (35 x 10 mm). Use a small, soft-bristled watercolor paint brush to 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
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

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