PRESERVATION METHOD—SHOOT TIPS

NLGRP CLONAL MS CITRUS DROPLET-VITRIFICATION 1

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File Name

NLGRP_CLONAL_MS_CITRUS_DROPLET-VITRIFICATION_1

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Study Reason

Preservation

Introduction

World citrus collections provide researchers, breeders, and industry members access to diverse genetic resources. These collections are usually maintained in field, greenhouse, or screenhouse conditions, making them susceptible to both environmental and biotic threats. Several of the primary U.S. collections of Citrus are maintained in California. These include the Citrus Variety Collection (CVC), the Citrus Clonal Protection Program (CCPP), and the National Clonal Germplasm Repository for Citrus and Dates (NCGRCD). More than 865 accessions of Citrus and related genera are maintained in the CVC, which is based at the University of California Riverside. Although there are some built-in duplications among these collections, the threats of Huanglongbing, Citrus tristeza virus, Citrus tatter leaf virus, Citrus canker, Citrus black spot, and Sweet orange scab as well as extreme temperatures and stormy weather patterns have emphasized the vulnerability of these collections. Some Citrus shoot tip cryopreservation methods have been developed, but these methods have not been shown to have broad applicability across diverse citrus species. In 2012, we published a method for cryopreserving shoot tips excised from citrus vegetative buds derived from greenhouse or screenhouse-grown stock plants. This method has the advantage of not requiring plants to be propagated in vitro prior to cryopreservation treatments. The availability of this method has increased the likelihood of adoption of citrus shoot tip cryopreservation techniques in citrus collections worldwide.

Source of Plant Material

Potted screenhouse or greenhouse plants at the National Clonal Germplasm Repository for Citrus and Dates in Riverside, CA (shipped overnight to NLGRP)

Plant Material Description

Budwood should come from vegetative shoots at the triangular growth stage about 3-5mm in diameter. Collect material 2-6 weeks after new growth flushes have occurred; the material should not be actively growing and should be somewhat “hard.” Remove leaves and petioles. If needed, budwood may be stored up to 4 weeks at 4°C in sealed plastic bags. However, it is best to use within 2 to 3 days especially if material is tender or small diameter.

Plant Material Culture Conditions
Cut budwood into nodal sections. Surface disinfect sections in 70% isopropanol for 2 minutes. Drain the isopropanol and rinse three times with tap water, then add 10% bleach with Tween 20 (1 drop per 100 mL) and shake for 10 minutes. In a laminar flow hood, drain the bleach water and rinse nodal sections 3 times with sterile distilled water.

Plant Material Cleanliness
Potted screenhouse plants are screened annually for virus/viroid pathogens but NOT endophytes. Endophytes may be present but have caused little issues in the past for Citrus shoot tip cryopreservation.

Pretreatment
N/A

Shoot Tip Excision and Preculture
Excise axillary shoot tips from non-elongated buds. The final size should be about 1-2 mm. Make sure to leave some plant tissue (.5-1mm) as a base for the shoot tip because this will need to be trimmed in order to make a clean cut before micrografting.

Cryopreservation Method
Put excised shoot tips into Liquid Preculture Medium in a small Petri dish (35 x 10mm). Leave shoot tips overnight in the Liquid Preculture Medium at 25ºC in darkness. After the overnight preculture treatment, remove the preculture medium with a pipette and immerse shoot tips into Loading Solution (2M glycerol + 0.4M sucrose + ½x MS) for 20 minutes at 22ºC. Use a pipette to remove the Loading Solution from the shoot tips. Add the Plant Vitrification Solution 2 for 30 to 60 minutes at 0ºC (this step can be done on a tub of crushed ice). Use 30 minutes in PVS2 for smaller shoot tips (1mm) and 60 minutes for larger shoot tips (2mm). While shoot tips are being treated with PVS2, prepare foil strips with PVS2 droplets. Place autoclaved foil strips (about 2-3 cm in length and 0.5 cm wide) on a sterile Petri dish on the crushed ice. With a pipette, make 5 PVS2 droplets either individually or as one long channel on a foil strip. Begin moving shoot tips to the droplets/channel (2 shoot tips per droplet, 10 shoot tips total per strip) shortly before the 30 or 60 minutes is up. At 30 or 60 minutes, begin to plunge foil strips into liquid nitrogen.

Cooling Vessel and Method
Foil strips are transferred to 1.2mL cryo vials (Corning 430487) in liquid phase.

Storage Conditions
Cryo vials with foil strips inside are transferred to canes/boxes in vapor phase.

Comments
N/A

References
Volk, GM, Bonnart R, Krueger R, Lee R. 2012. Cryopreservation of Citrus shoot tips using micrografting for recovery. CryoLetters 33:418-426.

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Volk GM, Bonnart R, Shepherd A, Yin Z, Lee R, Polek ML, Krueger R. 2016. Citrus cryopreservation: Viability of diverse taxa and histological observations. Plant Cell Tissue and Organ Culture 128:327-334. DOI 10.1007/s11240-016-1112-4

Volk GM, Jenderek MM, Walters C, Bonnart R, Shepherd A, Skogerboe D, Hall BD, Moreland B, Krueger R, Polek ML. 2019. Implementation of Citrus shoot tip cryopreservation in the USDA-ARS National Plant Germplasm System. Acta Hort. 1234: 329-334.

Appendices

Shoot Tip Dip/Rinse Solution (3% sucrose w/v + MS solution): 1 L

- Sucrose = 30 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43 g
- Bring to volume
- pH = 5.8
- Dispense 25 mL per 150 X 25 mm glass culture tubes

Liquid Preculture Medium (0.3M Sucrose + ½ MS): 1 L

- Sucrose = 102.69 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 2.22 g
- Bring to volume
- pH = 5.8

Loading Solution (2M glycerol + 0.4M Sucrose + ½ MS): 1 L

- Glycerol = 184.2 g
- Sucrose = 136.9 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 2.22 g
- Bring to volume
- pH = 5.8

Plant Vitrification Solution 2 (PVS2): 250 mL

- Glycerol (30% w/v) = 75 g ***weigh this first in flask***
- Ethylene glycol (15% w/v) = 33.8 mL
- DMSO (dimethyl sulfoxide) (15% w/v) = 34.1 mL
- Sucrose (0.4 M) = 34.25 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 0.55 g
- Bring to volume
- pH = 5.8
- Filter sterilize using 0.45 micron syringe filter or Stericup filter units
- Dispense into sterile glass or plastic tubes, seal and refrigerate