PRESERVATION METHOD—SHOOT TIPS

NLGRP CLONAL MS VITIS DROPLET-VITRIFICATION 1

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Study Reason
Preservation

Introduction
It is important to have reliable cryopreservation technologies available for the safe, long-term conservation of Vitis genetic resources in ex situ genebanks. This would allow Vitis field collections to be conserved as vegetative explants at liquid nitrogen (LN) temperatures for extended lengths of time. Despite the reports of successful Vitis cryopreservation, to our knowledge it has not been routinely implemented in plant genebanks. In fact, there are several reports where researchers experienced low levels of survival and/or regrowth after cryoexposure using previous methods. Herein, we focus on improving the explant physiological state, pre-treatment conditions, and regrowth medium to achieve successful regrowth after the cryopreservation of diverse Vitis species. We combined several previously published Vitis cryopreservation methods and optimized preculture and regrowth conditions to attain a method that is widely applicable and can be easily adopted by genebanks.

Source of Plant Material
In-vitro

Plant Material Description
8-12 week-old in-vitro shoot cultures

Plant Material Culture Conditions
Cultures are initiated using nodal sections (~2cm) from actively-growing vines in the field surfaced sterilized with 70% isopropanol for 1 minute, followed by 10% bleach +.1% Tween 20 for 10 minutes, followed by 3 sterile water rinses, then inserted into 25 mL of medium in 25X150mm test tubes. In-vitro cultures are kept at 25C with 16-hour photoperiod under cool white fluorescent lighting (~90 umol/m²/s or ~7100 lux) during establishment and multiplication using Vitis shoot maintenance medium in 946mL glass culture vessels (Phytotechnology Labs C607) with 180mL of medium per vessel.

Plant Material Cleanliness
Ideally plant material should be free of microbial or other contaminants but generally has not been tested to verify. Cultures are observed for signs of microbial contaminants and treated appropriately (antimicrobial agents or antibiotics) for removal of contaminants or re-established from fresh source plants.

Pretreatment
Cut nodal sections (~1-1.5 cm) and remove leaves from 2-3 month old *in-vitro* shoot cultures and plate on pretreatment medium in 100X25mm petri plates (50 mL of media per plate with 40 nodes per plate). Place plates in growth room at 25°C with a 16 h photoperiod (~90 umol/m²/s or ~7100 lux) and culture for 2-3 weeks until shoots are 1-2 cm tall.

Shoot Tip Excision and Preculture
Excise apical shoot tips (~1 mm), plate on preculture medium then incubate for 3 days at 25°C in darkness.

Cryopreservation Method
Remove shoot tips from preculture medium and place in loading solution for 20 minutes at room temperature. Remove from loading solution and cryoprotect with half-strength PVS2 at room temperature for 30 minutes and then with full-strength PVS2 at 0°C for 90 minutes. Place shoot tips into very thin layer of PVS2 on foil strips and plunge 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 et al. 2018. Successful Cryopreservation of Vitis Shoot Tips: Novel Pre-Treatment Combinations Applied to Nine Species, CryoLetters 39 (5), 322-330

Appendices
Shoot Maintenance Medium: 1L

- M & S Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43g
- Sucrose = 30g
- IAA (.1 mg/mL stock) = 1.75mL
- Bring to Volume
- Gellan Gum = 2.5g
- pH = 5.7

Vitis Shooting medium w/ BAP: 1L

- M & S Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43g
- Sucrose = 30g
- BAP (.1 mg/mL stock) = 2mL
- Salicylic acid (.1mM) (1 mg/mL stock) = 13.8mL
- Ascorbic acid (.25mM) = 44.28mg (.0443g)
- Glutathione-reduced (.25mM) (Phytotech G3399) = 76.88mg (.0769g)
- Bring to volume
- Gellan gum = 3g
- pH = 5.7

- Dispense into 100 X 25mm petri plates after autoclaving (50 mL/plate)

Vitis Preculture Medium: 250mL

- M & S Basal Medium with Vitamins (Phytotechnology Labs M519) = .55g
- Sucrose (.3M) = 25.7g
- Salicylic acid (.1mM) (1 mg/mL stock) = 3.45mL
- Ascorbic acid (.25mM) = 11.07mg (.0111g)
- Glutathione-reduced (.25mM) (Phytotech G3399) = 19.22mg (.0192g)
- Bring to volume
- Agar = 2g
- pH = 5.8
- Dispense into 60X15mm petri plates after autoclaving (12 mL/plate)

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

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

½ Strength PVS2 Cryoprotectant (.4M sucrose + ½ MS): 250mL

- Glycerol (15% w/v) = 37.5g
- Ethylene glycol (7.5% w/v) = 16.9mL
- Sucrose (.4M)= 34.25g
- DMSO (7.5% w/v) = 17.1mL
- M & S Basal Medium with Vitamins (Phytotechnology Labs M519) = .55g
- Bring to volume
- pH = 5.8
- Filter sterilize using .45 micron syringe filter or Stericup filter units; put in 50 ml centrifuge tubes

PVS2 Cryoprotectant (.4M sucrose + ½ MS): 250mL

- Glycerol (30% w/v) = 75g ***weigh this first in flask***
- Ethylene glycol (15% w/v) = 33.8mL
- Sucrose (.4M) = 34.25g
- DMSO(methyl sulfoxide) (15% w/v) = 34.1mL
- M & S Basal Medium with Vitamins (Phytotechnology Labs M519) = .55g
- Bring to volume
- pH = 5.8

- Filter sterilize using .45 micron syringe filter or Stericup filter units; put in 50 ml centrifuge tubes