VIABILITY METHOD—SHOOT TIPS

NLGRP CLONAL MS VITIS SHOOT-TIP-REGROWTH 1

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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
Cryogenic storage (vapor phase)

Plant Material Description
Shoot tips on foil strips inside of cryo vials

Warming
Remove foil strips from vials and warm foil strips in room temperature unloading solution until melted and shoot tips are floating in unloading solution; incubate for 20 minutes

Regrowth/Viability Conditions
Plate shoot tips onto recovery medium #1 (.6M sucrose, -NH4) overnight at 25°C in darkness. Transfer shoot tips to recovery medium #2 (30 g L^{-1} sucrose, -NH4) and culture for two weeks at 25°C in darkness. Transfer shoot tips to recovery medium #3 (30 g L^{-1} sucrose, +NH4) and expose to light (~40 umol/m²/s or ~3150 lux) at 25°C

Regrowth/Viability Assessment
Evaluate for shoot regrowth after 8 weeks. Shoot tips are plated in 60X15mm petri plates with 12 mL of media and 5 shoot tips per plate wrapped with 2 layers of plastic sealing film (Phytotech Labs A003)

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

1.2 M Sucrose + ½ MS (unloading solution): 1L

- M & S Basal Medium with Vitamins (Phytotechnology Labs M519) = 2.22g
- Sucrose= 410.76g
- M519= 2.22g
- Bring to volume
- pH= 5.8 final
- Dispense 25 mL/glass culture test tubes

Shoot Tip Recovery Medium #1 (.6M sucrose –NH4): 500mL

- M & S Modified Basal Salt Mixture (Phytotechnology Labs M571) = .67g
- M & S Microelements (Phytotechnology Labs M554) = .025g
- Vitis Vitamins (100X) = 5 mL
- Sucrose (.6M) = 102.7g
- Bring to Volume
- Agar = 4g
- pH = 5.7
- Dispense into 60X15mm petri plates after autoclaving (12 mL/plate)

Shoot Tip Recovery Medium #2 (3% sucrose w/v –NH4): 500mL

- M & S Modified Basal Salt Mixture (Phytotechnology Labs M571) = .67g
- M & S Microelements (Phytotechnology Labs M554) = .025g
- Vitis Vitamins (100X) = 5mL
- Sucrose (.1M) = 15g
- BAP (.1mg/mL stock) = 1mL
- Bring to Volume
- Agar = 4g
- pH = 5.7
- Dispense into 60X15mm petri plates after autoclaving (12 mL/plate)

Shoot Tip Recovery Medium #3 (3% sucrose w/v +NH4): 1L

- M & S Macroelements (Phytotechnology Labs M502) = 2.12g
- M & S Microelements (Phytotechnology Labs M554) = .1g
- Vitis Vitamins (100X) = 10mL
- Sucrose (.1M) = 30g
- BAP (.1mg/mL stock) = 2mL
- Bring to Volume
- Agar = 8g
- pH = 5.7
- Dispense into 60X15mm petri plates after autoclaving (12 mL/plate)

***Vitis* Vitamins Stock Solution (100X): 200mL**

- Myo inositol = 2g
- Thiamine HCl = 200mg
- Nicotinic acid = 20mg
- Pyridoxine HCl = 20mg
- Ca pantothenate = 20mg
- Biotin = .2mg

- Glycine = 40mg
- Dispense into 10 mL aliquots and freeze, **use 10 mL per liter of medium**

*(this calculates to the following final concentrations in the medium: myo inositol 100 mg L^{-1}; thiamine HCl 10 mg L^{-1}, nicotinic acid 1 mg L^{-1}, pyridoxine HCl 1 mg L^{-1}, Ca pantothenate 1 mg L^{-1}, biotin .01 mg L^{-1}, glycine 2 mg L^{-1})