

Cryopreservation of *Vitis* Shoot Tips from Diverse Species



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Abstract

Cryopreservation, long term storage of materials in liquid nitrogen (LN), is an effective method for backing-up national fruit collections. When efficient methods are available, cryopreservation has become a routine addition to the genbanking process and provides off-site security for collections. We have identified methods that successfully cryopreserve *Vitis* shoot tips from diverse species. Traditionally, plants from field collections have been introduced into tissue culture for a multiplication step prior to shoot tip excision. We have compared recovery of shoot tips that were excised from greenhouse materials to those that were excised from in vitro plants. Our efforts focus on improving the efficiency of *Vitis* cryopreservation by optimizing the selection of shoot tip source material, cryoprotectant exposure times, desiccation timecourses, and freezing rates to ensure high survival post liquid-nitrogen exposure.

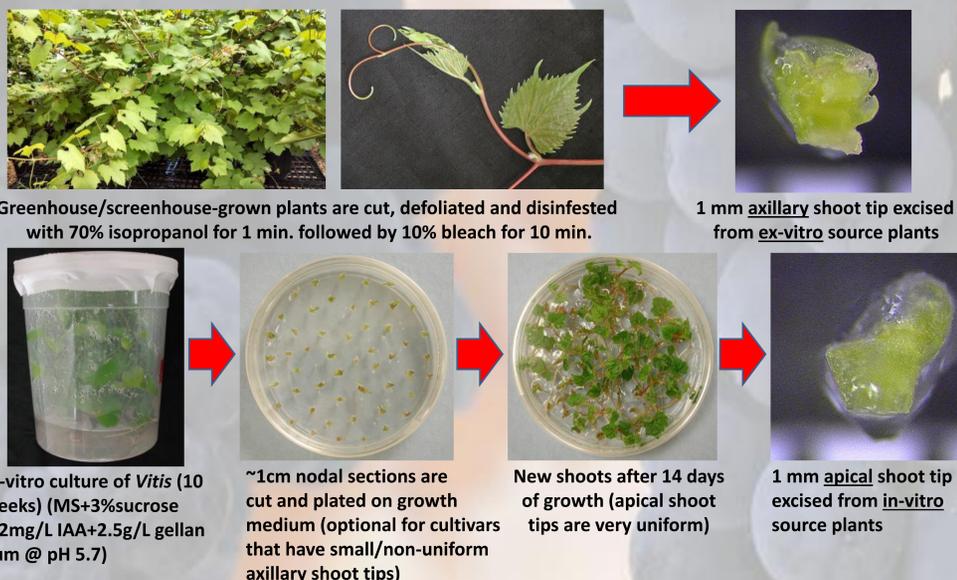
Introduction

Grape (*Vitis spp.*) is one of the most economically important fruit crops worldwide for the production of fresh and dried fruit, juice and wine. Collections of grape germplasm are most commonly stored as field collections but they are vulnerable to severe weather, pests/diseases and climate change. In-vitro back-up (both conventional and slow-growth) is possible but is very labor intensive and expensive to maintain. Cryopreservation of *Vitis* shoot tips has been shown to be feasible using both vitrification and encapsulation-dehydration techniques, however shoot tip source material has always been from in-vitro shoot cultures. Using explant material directly from already established field/greenhouse collections provides a low-cost and readily available source of shoot tips for cryopreservation, provided that microbial surface contaminants can be effectively disinfested while causing minimal injury to explants. When using shoot tips directly from ex-vitro material, ideally they will have a similar response to the cryopreservation process than those from in-vitro material.

Materials & Methods

Our on-going research has been investigating and optimizing cryopreservation methods for *Vitis* shoot tips from both in-vitro and ex-vitro sources using vitrification. The following diagram describes the basic process:

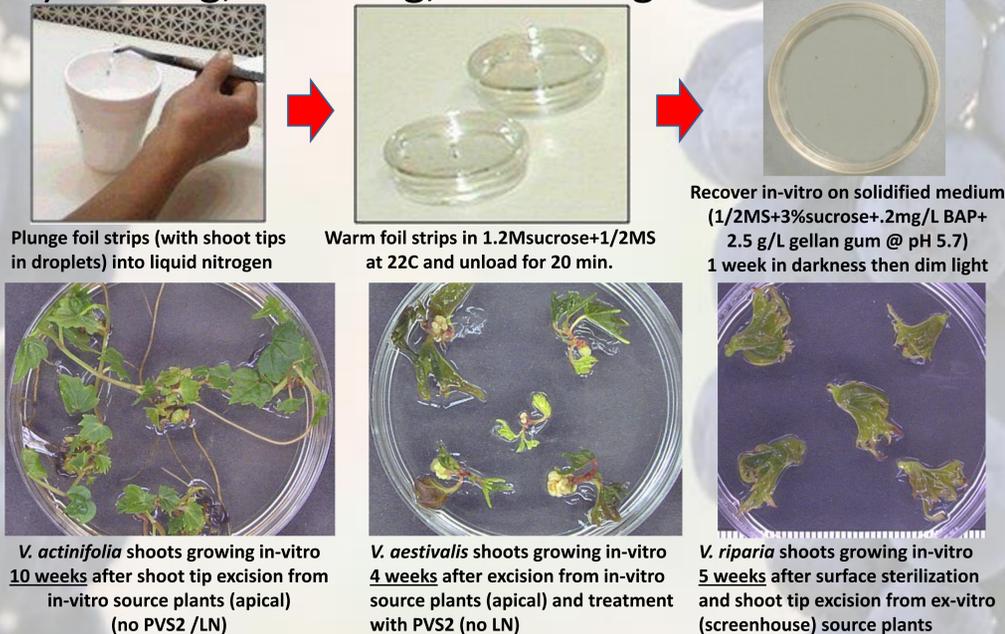
1) Harvest shoot tips from source plants:



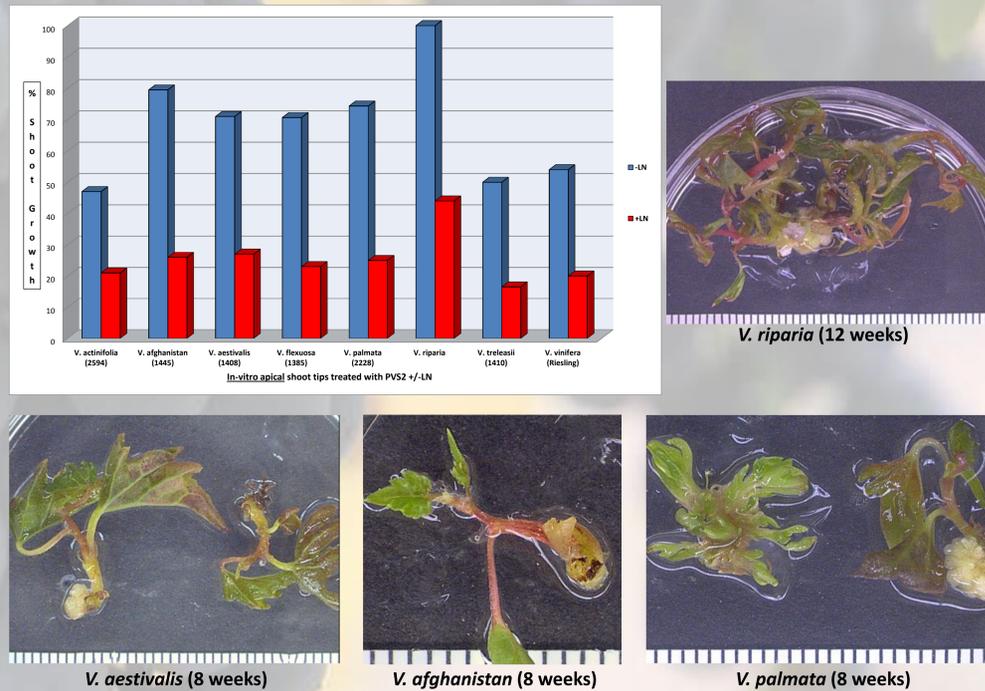
2) Preculture, loading and osmoprotection



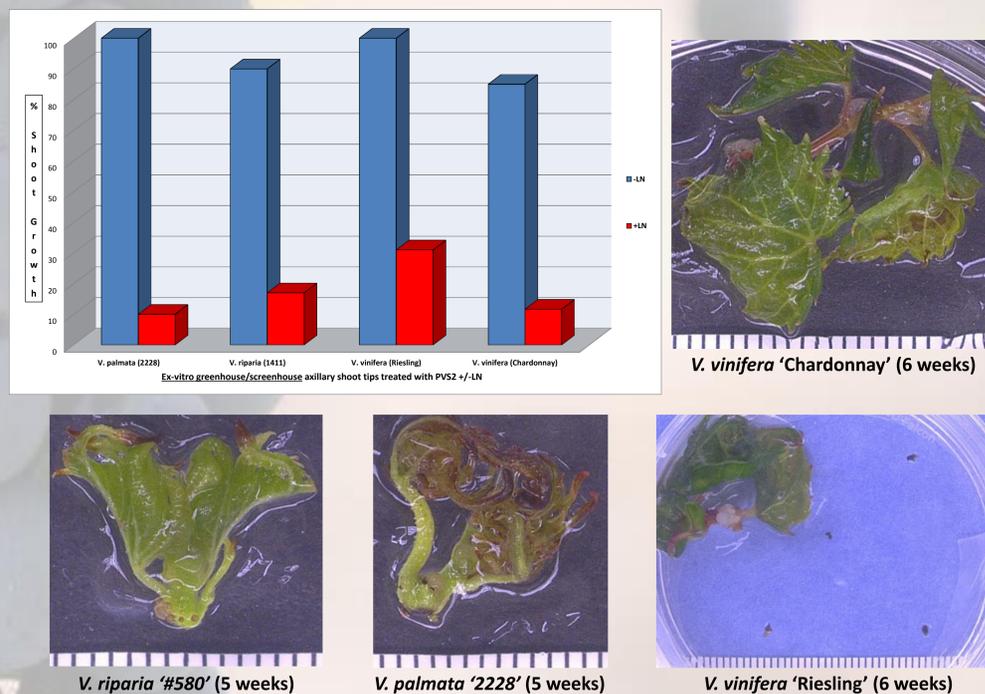
3) Cooling, warming, unloading and recovery



In-vitro apical shoot tips regenerating after cryopreservation



Ex-vitro (greenhouse/screenhouse) axillary shoot tips regenerating after cryopreservation



Discussion

On-going research in our laboratory suggests that cryopreservation of *Vitis* shoot tips from diverse species using the droplet vitrification method is possible using material from both in-vitro and ex-vitro source plants. Our method is an adaptation of the two-step protocol published by Matsumoto and Sakai in 2002. Preliminary results show shoot regeneration after cryopreservation ranging from 17-44% (mean=25%) using in-vitro shoot tips and 12-31% (mean=17%) using ex-vitro greenhouse/screenhouse shoot tips. Controls treated with PVS2 but no LN had shoot regeneration rates ranging from 47-100% (mean=67%) and 85-100% (mean=91%) for in-vitro and ex-vitro shoot tips, respectively.

We have found that several factors can increase survival and subsequent shoot regeneration of *Vitis* shoot tips during the cryopreservation process. The use of antioxidant/anti-stress compounds reduces oxidative browning of shoot tips during and after the cryopreservation process (data not shown), which is similar to the findings of Uchendu, et. al. (2010) using *Rubus* shoot tips. We are routinely using both glutathione and ascorbic acid in a solidified preculture medium prior to loading and osmoprotection.

Cooling rates during the cryopreservation process appear to have a profound impact on regeneration of *Vitis*. We have found that using 1.2mL plastic cryovials (cooling rate ~-3°C/sec.), with shoot tips suspended in cryoprotectant inside, results in little to no viability (data not shown). In contrast, using foil strips (cooling rate ~-106°C/sec.), with shoot tips suspended in droplets, results in much higher viability. This is most likely due to the higher thermal mass associated with cryoprotectant-filled vials versus that of droplets on a foil strip, which has a relatively low thermal mass resulting in significantly faster, almost instantaneous cooling.

Continuing research will focus on improving the viability and subsequent shoot regeneration of cryopreserved *Vitis* shoot tips using both in-vitro and ex-vitro source plants. The use of screenhouse/greenhouse-grown plants as sources for shoot tips is of particular interest mainly due to the fact that they are already established and available from germplasm collections. Our preliminary research has shown that shoots can be effectively sterilized which results in little to no contamination in cultures. Furthermore, this decreases or eliminates the need for in-vitro culture establishment and maintenance, which is very expensive, time-consuming and labor intensive.

References

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- Uchendu, E., M. Muminova, S. Gupta & B. M. Reed, 2010. Antioxidant and anti-stress compounds improve regrowth of cryopreserved *Rubus* shoot tips. *In Vitro Cell. Dev. Biol.—Plant* 46: 386–393.
- Wang, Q., E. Tanne, A. Arav & R. Gafny, 2000. Cryopreservation of in vitro-grown shoot tips of grapevine by encapsulation-dehydration. *Plant Cell, Tissue and Organ Culture* 63: 41–46.