DEVELOPMENT OF A PVS2 DROPLET VITRIFICATION METHOD FOR YACON (*SMALLANTHUS SONCHIFOLIUS*) CRYOPRESERVATION

Stacy Denise Hammond Hammond[1], Bart Panis[2], Iva Viehmannová[1], Jiri Zamecnik[3]

[1]Czech University of Life Sciences Prague, Faculty of Tropical AgriSciences, Dept. of Crop Sciences and Agroforestry, Czech Republic

[2]Bioversity International, c/o KU Leuven, Belgium

[3]Crop Research Institute, Plant Physiology and Cryobiology Laboratory, Czech Republic

Yacon [*Smallanthus sonchifolius* (Poepp. and Endl.) Robinson] is a perennial root crop belonging to the Asteraceae family and originating from the Andean region. It is cultivated for its edible tuberous roots high in inulin-type fructooligosaccharides of low caloric value. This study aims at developing an efficient cryopreservation protocol for the long-term preservation of yacon using the PVS2 droplet vitrification method, which is considered to be an emerging generic method for cryopreservation of plant tissues. Until now, no studies have been reported of this method being applied to this species. Our results showed that yacon can survive after cryopreservation using PVS2 droplet vitrification.

MATERIALS AND METHODS

Apical shoot tips (1.8-2.5 mm) were excised from 3-4 weeks old in vitro cultures of an octoploid yacon landrace obtained from Ecuador, classified as ECU 41 and maintained at the Czech University of Life Sciences Prague, Czech Republic (Fig. 1 a-d). Twenty individual shoot tips were excised per PVS2 treatment (10 shoot tips as PVS2 control and 10 for LN). An additional 10 shoot tips were dissected per treatment to use as control (0 minutes exposure to PVS2 and LN).

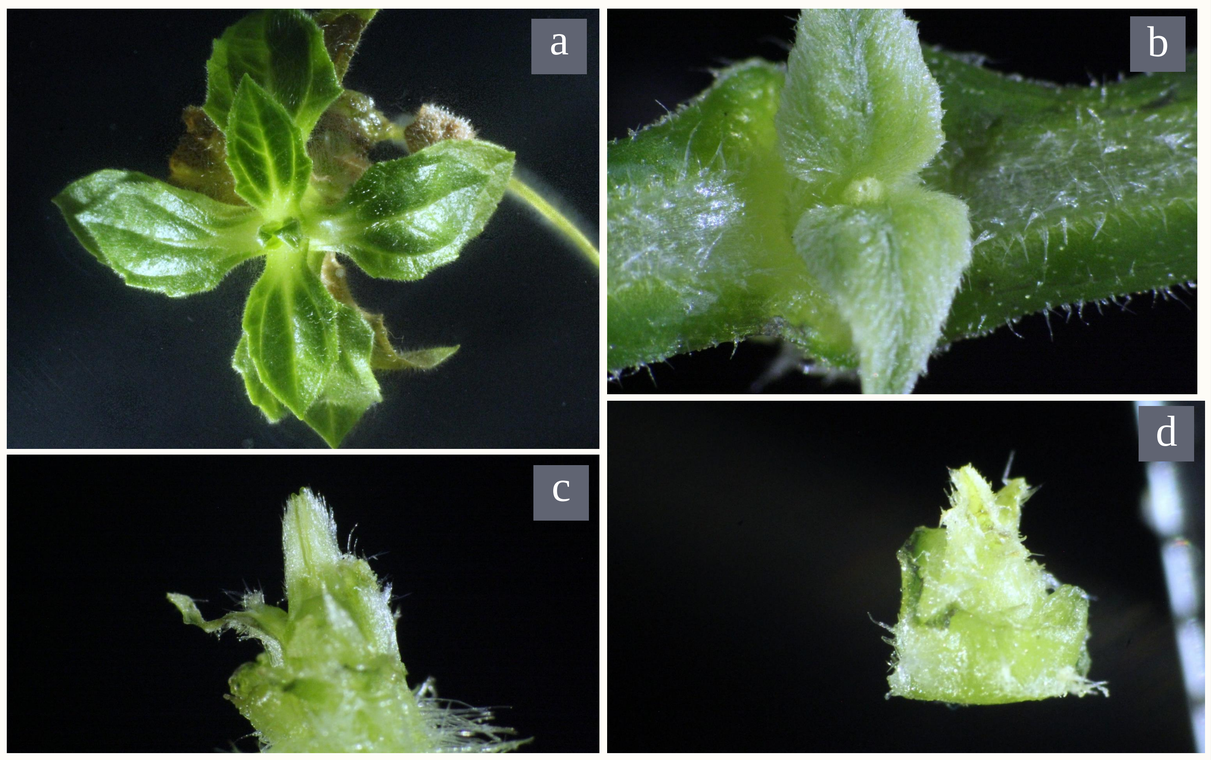

Figure 1. Yacon plantlet (1a), birds view of apical meristem placement on the plant apex (1b), exposed apical meristem (1c), and excised (1.8-2.5 mm) apical shoot tip (1d) under a binocular microscope.

Droplet-vitrification with PVS2 and cooling in liquid nitrogen

Excised shoot tips were placed in loading solution (LS) for 20 min at room temperature and were then immersed in ice-cold PVS2 at 0 °C. Three different time intervals of PVS2 dehydration (15 min; 30 min and 60 min) were tested. Thereafter, out of the 20 shoot tips excised per treatment, 10 were placed in recovery solution (RS) after which 5 pieces were placed on MS+ 1 mg/l BA and 5 on MS medium free of plant growth regulators as recovery media as control, the remaining 10 explants were placed in a droplet of PVS2 solution (25-30 µl) on a sterile strip of aluminum foil (5 mm × 20 mm) kept at 0 °C and where then plunged in liquid nitrogen (LN).

Rewarming and unloading

After PVS2 dehydration and exposure to LN, the explants were placed in the unloading solution (US) that consisted of 1.2 M sucrose dissolved in MS medium for 15 min. Five shoot tips were placed on MS+BA and five on MS without BA as regrowth media. The survival rate was evaluated every 2 weeks after thawing for a period of 8 weeks (Figure 2). Callus formation (C), shoot plus callus formation (S+C), full growth normal (FGN), hyperhydration (HH) and survival (white as dead/black as live) were measured. The experiment was repeated three times to ensure the reliability of the results.

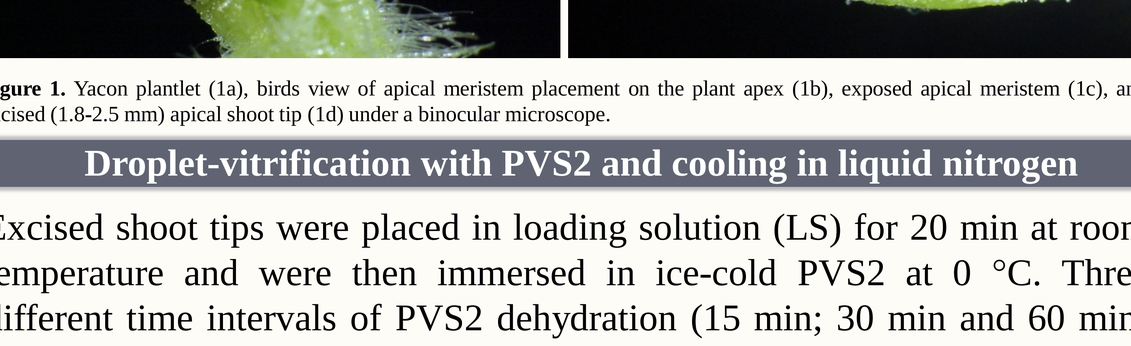

Figure 2. Shoot tips on recovery mediamedia eight weeks after thawing

RESULTS

The experiments showed that survival rate of yacon shoot tips after ultra-rapid cooling in LN is correlated to the time of exposure to PVS2 and type of regrowth medium. Shoot tip survival rate proved to be the highest (90%) on MS+BA and decreases at the extremes if, PVS2 exposure is too low as in the case of 15 min or too high as shown at 1 hr (Fig. 3). However, the formation of callus, hyperhydricity, and shoot plus callus was highest for surviving shoot tips on MS+BA (Fig. 5) suggesting that BA may have an adverse effect on shoot tips after cryopreservation. Therefore the optimal media for recovery is considered to be BA free MS and a PVS2 exposure time of 15 or 30 minutes (Fig. 4) with 15 minutes considered as the overall best treatment for recover showing no signs of formation of callus, hyperhydricity, and shoot plus callus formation (Fig. 6).

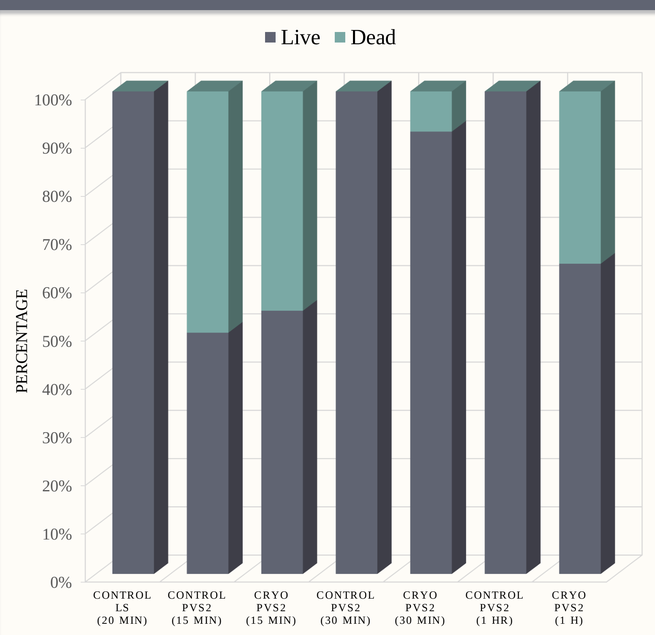

Figure 3. Survival rate after 8 weeks recovery from cryopreservation on MS+BA

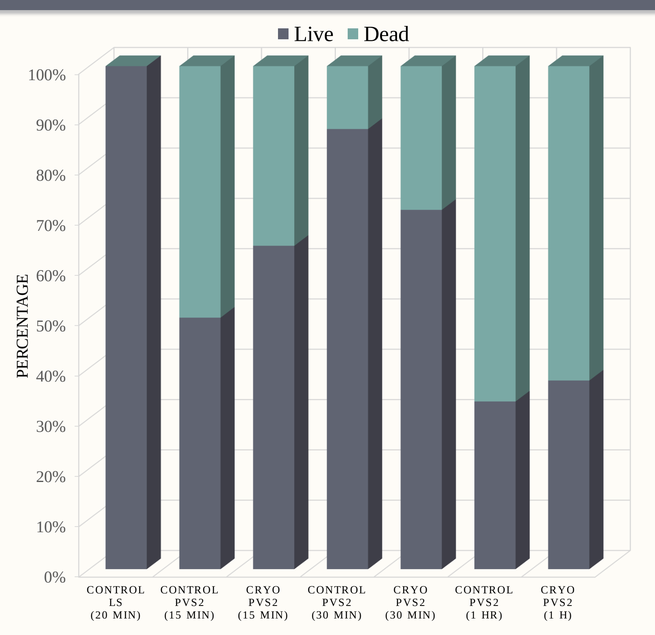

Figure 4. Survival rate after 8 weeks recovery from cryopreservation on MS

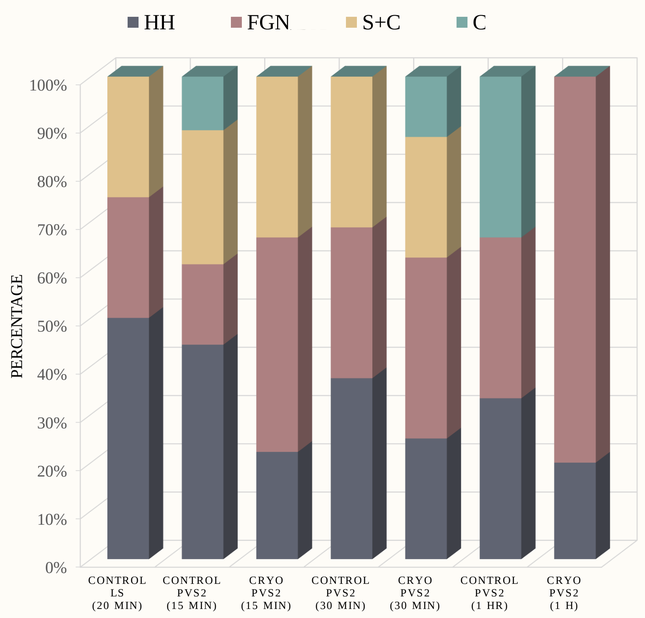

Figure 5. Formation of Callus (C), shoot+callus (S+C), full growth normal (FGN), and hyper hydration (HH) on surviving plants after 8 weeks recovery from cryopreservation on MS+BA

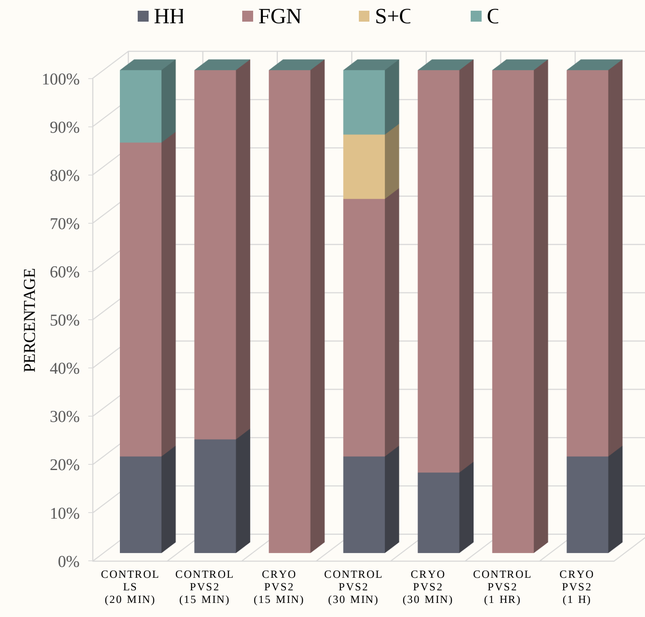

Figure 6. Formation of Callus (C), shoot+callus (S+C), full growth normal (FGN), and hyper hydration (HH) on surviving plants after 8 weeks recovery from cryopreservation on MS

CONCLUSION

Preliminary results show that yacon is able to survive the PVS2 droplet vitrification method. Fifteen minutes PVS2 solutions exposure time and MS without BA as regrowth media post ultra-rapid cooling in LN proved to be the most effective in terms of the survival and quality of shoot tips. Thirty minutes PVS2 solutions exposure time and MS+BA as recovery medium shows great potential in boosting the overall survival of shoot tips. However, further optimization is necessary to overcome the adverse effects of BA on shoot tips and to determine the regeneration rate of yacon after cryopreservation. The development of a cryopreservation protocol for this species will ensure the preservation of its genetic resources and constant availability for farmers, researchers and breeders globally.

ACKNOWLEDGEMENTS

This research was financially supported by the Internal Grant Agency of Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague IGA (Project No. 20185015) and the project QJ1630301 of the Czech Ministry of Agriculture. BP gratefully acknowledges the Directorate-General for Development, Belgium (DGD) for the financial support to the project 'Safeguarding vegetatively propagated crop diversity to nourish people now and in the future.'

Corresponding author: Stacy Hammond · hammondstacy9@gmail.com