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Development of a PVS2 Droplet Vitrification Method for Yacon (*Smallanthus sonchifolius*) Cryopreservation

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Abstract

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 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. To carry out the experiment, 1.8–2.5 mm apical shoot tips were excised from 3–4 weeks old *in vitro* cultures of an octoploid yacon landrace, originated from Ecuador and maintained at Czech University of Life Sciences Prague. Excised shoot tips were exposed to loading solution and three different time intervals for PVS2 dehydration at 0°C were tested. Shoot tips were then exposed to ultra-rapid cooling on aluminum foil strips (0.5×2 cm) in liquid nitrogen (LN) and were then rewarmed in 1.2 M sucrose MS (Murashige and Skoog, 1962) liquid medium. Post-cryo cultures were placed on recovery MS media containing 6-Benzylaminopurine (BA) and on MS without BA to determine which media was more efficient for survival and recovery after PVS2 droplet method. Ten shoot tips per treatment were used and 3 repetitions were carried out to ensure the reliability of the results. Callus formation, shoot plus callus formation, full growth normal, hyperhydration and colour of shoot tips were evaluated. Preliminary results showed that yacon can survive after cryopreservation using PVS2 droplet vitrification. Survival of shoot tips exposed to short time (15 min) PVS2 was higher; the optimal recovery media was MS without BA as BA induced a higher level of hyperhydration and callus formation in post-cryo cultures. However, further evaluation will be carried out on post-cryo cultures to ensure the regeneration of well-rooted plants that can survive *ex vitro* transfer. Cryopreservation of crop species contributes to the world food security. The long-term conservation of this species is important, as it ensures the safe storage of the species for future generations.

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Keywords: Long-term preservation, PVS2, *Smallanthus sonchifolius*

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