EFFECTIVE CRYOPRESERVATION APPROACH FOR ANDEAN POTATO SHOOT TIP IN VITRO CULTURE

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A droplet vitrification protocol was employed to set up the long term conservation of the potato collection at the Potato Genebank (EEA Balcarce-INTA, Argentina). The study objective was to evaluate the effects of different pre and post-treatment conditions for shoot tips regeneration during cryopreservation. Plantlets of the Andean potato variety 'Chaquana' were cultivated on MS medium with 25 g.L⁻¹ sucrose, 4.5 g.L⁻¹ phytagel and vitamins. Nodal segments were precultured in two conditions, A: 22°C, 16h/8 h (control) and B: 22°C, 16 h and 10°C, 8h, both photoperiods with light intensity 45 mmol.m⁻².s⁻¹, for four weeks. Isolated apical shoot tips were cultivated overnight on MS medium containing 0.3 M sucrose, then transferred to loading solution for 20 min, and dehydrated with PVS2 for 20, 25 and 30 min. Shoot tips were placed in PVS2 droplets onto aluminium foil strips, then plunged into cryovials with liquid nitrogen. Therecovery was tested in five conditions (in dark; I: 5, II: 7, III: 14 and IV: 21 days, or V: 7 days dark + 7 days diffuse light) and tips with and without cryogenic exposure was assessed after 8 weeks. Shoot tips and cryoprotecting solutions were analysed by Differential Scanning Calorimetry to investigate their thermal behaviour and the eventual ice formation during the protocol. Also, the frozen and unfrozen water fraction was determined for all stages at each protocol. The B pre-treatment increased the samples quality after post-cryo recovery. The best regrowth results were for III and V conditions, with 40 and 58 %, respectively. Dehydration during 20 min was enough to vitrify the samples during cooling to liquid nitrogen temperatures, as detected by DSC. However, survival and re-growth results were lower than those obtained with 25 min in PVS2, attributed to traces of frozen water present in samples dehydrated at 20 min.

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