

Effects of ascorbic acid on PVS2 cryopreservation of dendrobium Bobby Messina's PLBs supported with SEM analysis.

ABSTRACT

Regrowth of the cryopreserved protocorm-like bodies (PLBs) of *Dendrobium Bobby Messina* was assessed based on the plant vitrification solution 2 (PVS2) optimisation conditions. The optimized protocol obtained based on TTC spectrophotometrical analysis and growth recovery were 3–4 mm of PLBs size precultured in 0.2 M sucrose for 1 day, treated with a mixture of 2 M glycerol and 0.4 M sucrose supplemented with half-strength liquid MS media at 25 °C for 20 min and subsequently dehydrated with PVS2 at 0 °C for 20 min prior to storage in liquid nitrogen. Following rapid warming in a water bath at 40 °C for 90 s, PLBs were treated with unloading solution containing half-strength liquid MS media supplemented with 1.2 M sucrose. Subsequently, the PLBs were cultured on half-strength semi-solid MS media supplemented with 2 % (w/v) sucrose without any growth regulators and resulted in 40 % growth recovery. In addition, ascorbic acid treatment was used to evaluate the regeneration process of cryopreserved PLBs. However, growth recovery rates of non-cryopreserved and cryopreserved PLBs were 30 and 10 % when 0.6 mM ascorbic acid was added. Scanning electron microscopy analysis indicates that there are not much damages observed on both cryopreserved and non-cryopreserved PLBs in comparison to PLBs stock culture.

Keyword: Cryopreservation; *Dendrobium Bobby Messina*; Regeneration rates; Ascorbic acid.