

Development of an efficient cryoconservation protocol for Himalayan mulberry (*Morus laevigata* Wall. ex Brandis) using dormant axillary buds as explants

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The Himalayan mulberry, *Morus laevigata* Wall. ex Brandis, is a cold tolerant species found commonly in India from Himalayan foothill to Andaman islands, and it is known for its timber value, forage use, particularly as silkworm's feed. Preservation of germplasm, cryopreservation, particularly for long term storage, of such economically important plant species helps in breeding and development of new cultivars. In the present study, three cryotechniques viz., two-step freezing, encapsulation-dehydration and vitrification were attempted for cryopreservation of *M. laevigata* using winter dormant buds as explants. A two-step freezing cryo procedure preceded by desiccation to 15-25% moisture content was developed. Recovery conditions, including dark incubation and rehydration in sterile moist moss grass for different durations, after cryopreservation led to higher survival when compared to untreated controls. For encapsulation-dehydration, alginate beads containing descaled buds were dehydrated for 1-3 days in various sucrose concentrations (0.3, 0.5, 0.75 or 1.0 M). Bead desiccation was performed using laminar air flow for either 1-6 h. For vitrification, descaled buds were directly immersed for 20, 40, 60, 90 or 120 min in a vitrification solution (PVS₂). Following encapsulation-dehydration, treatment of alginate beads with 0.75 M sucrose was more effective in promoting regrowth of explants after immersion in liquid nitrogen than in the presence of 0.75 M sucrose for 48 h. Regrowth of explants was also observed following vitrification which reached 50% with increasing duration of the PVS₂ treatment for 20-90 min. Overall, the highest frequency of explant regrowth was obtained when explants were subjected to encapsulation-dehydration. This is possibly the first attempt for cryopreservation of Himalayan mulberry adopting these three new cryotechniques.

Keywords: Alginate beads, Breeding, Conservation, Cryopreservation, Encapsulation-dehydration, PVS₂, Sericulture, Sucrose, Vitrification