

AMCRYO

Cryopreservation of endangered American plants

ABSTRACT

This project aimed at developing cryopreservation protocols for in vitro shoot tips of vanilla and *Clinopodium odorum* using new, vitrification-based techniques. In case of *Clinopodium*, we applied the vitrification (V) cryo-plate protocol for cryopreservation of in vitro-derived shoot tips. We studied the recovery of shoot tips after modification of various parameters of the V cryo-plate protocol, including sucrose concentration in the preconditioning medium, composition of the loading solution (LS) and vitrification solution (VS), and duration of treatment with PVS2 VS, which contained (w/v) 30% glycerol, 15% dimethylsulfoxide, 15% ethylene glycol, and 13.7% sucrose. We also compared the efficiency of the V cryo-plate protocol with the dehydration (D) cryo-plate protocol. The optimal conditions determined for the V cryo-plate protocol included a 24-h preconditioning treatment on medium with 0.3 M sucrose; treatment for 20 min at room temperature with LS containing 2.0 M glycerol and 0.4 M sucrose; and dehydration with PVS2 for 60 min at 0°C. Under these conditions, 71.0% recovery of cryopreserved shoot tips was achieved. Only 29.2% regeneration was noted with the D cryo-plate protocol.

- As regards vanilla, we compared the droplet-vitrification technique and the V cryo-plate protocol. Recovery of a few cryopreserved apices could be achieved using the V cryo-plate protocol. However, due to the very slow growth of the apices, a sufficient number of replications could not be made to allow determining optimal experimental conditions.

Keywords : Developing the plant of the future

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Project leader : Florent Engelmann

Project leader's institution : IRD

Project leader's RU : DIADE

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Funding : Labex

PERSPECTIVES

Cryopreservation is ready to be applied to *Clinopodium* shoot tips.