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Cryopreservation (droplet vitrification) of *Malus* using apical shoot tips derived from plants grown *in vitro*

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Plant Material Culture Conditions

Malus in vitro stock cultures are maintained in an actively growing state on multiplication medium in growth room at 25°C with a 16 h photoperiod (40 μM m^{-2}·s^{-1}) and subcultured once every 4 weeks .

Shoot Tip Excision and Preculture

Harvest apical shoot tips (1.5 mm) from 4-week-old *in vitro* stock cultures and plate on basal medium overnight at 25°C in darkness. Remove shoot tips from basal medium and place on preculture medium; preculture for 1 days at 25°C in darkness.

Cryopreservation Method

Remove shoot tips from preculture medium and cryoprotect with full-strength PVS2 (Sakai et al. 1990) at room temperature for 20-40 minutes (40 minutes has been the most effective time for most species/cultivars). Place shoot tips into very thin layer of PVS2 on foil strips and plunge into liquid nitrogen.

Warming

Warm foil strips in room temperature unloading solution and hold for 20 minutes. Plate shoot tips onto basal medium overnight at 25°C in darkness. Transfer shoot tips to fresh basal medium and culture for 7 days at 25°C in darkness and then expose to light at 25°C. Evaluate for survival/recovery after 8 weeks, transferring to fresh media if necessary.

References

Li B, Feng C, Wang M, Hu L, Volk G, Wang Q. 2015. Recovery patterns, histological observations and genetic integrity in *Malus* shoot tips cryopreserved using droplet-vitrification and encapsulation-dehydration procedures. Journal of Biotechnology 214:182-191.

Sakai A, Kobayashi S, Oiyama I. 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. Var. Brasiliensis Tanaka) by vitrification. Plant Cell Rep. 9(1):30-33. doi: 10.1007/BF00232130

Wang M, Chen L, Teixeira da Silva JA, Volk GM, Wang Q. 2018. Cryobiotechnology of apple (*Malus* spp.): development, progress and future prospects. Plant Cell Reports 37:689-709.

Media and Solutions

- **Multiplication medium:** MS + 30 g L^{-1} sucrose + 0.1 g L^{-1} myo-inositol, 1 mg L^{-1} 6-benzyl aminopurine (BAP), 0.3 mg L^{-1} indole-3-butyric acid (IBA), 0.2 mg L^{-1} gibberellic acid and 7.5 agar at pH 5.5 (pH 5.8 prior to autoclaving)
- **Basal medium:** MS + 30 g L^{-1} sucrose + 0.25 mg L^{-1} BAP + .01 mg L^{-1} IBA + 2.6 g L^{-1} gellan gum at pH 5.7 (pH 6.0 prior to autoclaving)
- **Preculture medium:** MS + 2M glycerol + 0.8M sucrose at pH 5.8 (pH 6.4 prior to autoclaving)
- **PVS2 solution**: 1/2 MS + 30% (w/v) glycerol + 15% (w/v) ethylene glycol + 15% (w/v) dimethyl sulfoxide + 0.4 M sucrose at pH 5.8 (filter sterilized solution)
- **Unloading solution:** MS + 1.2M sucrose at pH 5.7 (pH 6.6 prior to autoclaving)
- **Other parameters:** wrap all cultures with two layers of plastic wrap (not Parafilm); after pouring plates allow to cool for thirty minutes before covering/packaging