PRESERVATION METHOD—SHOOT TIPS

NLGRP CLONAL MS MENTHA DROPLET-VITRIFICATION 1

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File Name
NLGRP_CLONAL_MS_MENTHA_DROPLET-VITRIFICATION_1

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Study Reason
Preservation

Introduction
Shoot tips can be excised from *in vitro* cultures of *Mentha* spp. and cryopreserved using droplet-vitrification.

Source of Plant Material
Mentha plantlets are kept as *in vitro* cultures at the National Clonal Germplasm Repository in Corvallis, OR. Target material is shipped overnight to the National Laboratory for Genetic Resources Preservation in Fort Collins, CO.

Plant Material Description
3- to 8-week-old *in vitro* cultures.

Plant Material Culture Conditions
Mentha tissue culture is grown on solid Murashige and Skoog (MS) Shoot Growth Medium in GA-7 Magenta vessels. Cultures are grown in an environmentally controlled growth room (~90 umol/m²/s or ~7100 lux with 16-hour photoperiod at 25 °C). Axillary nodes are subcultured every 3 to 12 weeks, or as needed.

Plant Material Cleanliness
Ideally plant material should be free of microbial or other contaminants but generally has not been tested to verify. Cultures are observed for signs of microbial contaminants and treated appropriately (antimicrobial agents or antibiotics) for removal of contaminants or re-established from fresh source plants.

Pretreatment
Harvest nodal sections from plantlets 3 to 8 weeks old. Nodal sections are 5-15 mm in length, each containing two axillary nodes. Plate onto 100 mm x 25 mm Petri plates containing about 40 mL solid MS Shoot Growth Medium until axillary nodes slightly elongate (2 to 6 days).

Shoot Tip Excision and Preculture
Using standard aseptic techniques, excise shoot tips and trim to approximately 1.0 mm. Immediately transfer shoot tips to Preculture Solution 1 (MS + 0.06 M sucrose), 22 °C, and

place in the dark for 24 hours. Use a pipette to replace Preculture Solution 1 with Preculture Solution 2 (MS + 0.3 M sucrose), 22 °C, and place in the dark for an additional 24 hours. Use approximately 1 mL of each solution for every 10 shoot tips.

Cryopreservation Method
[MENTHA_DROPLET-VITRIFICATION_1] After the preculture treatments, use a pipette to replace Preculture Solution 2 with the Loading Solution (MS + 2 M glycerol + 0.4 M sucrose), 22 °C. The Loading Solution is removed after 20 minutes, and replaced with PVS2, 0 °C. Allow shoot tips to soak in PVS2 for 30 minutes, then quickly transfer shoot tips to a sterile foil strip (approx. 5 x 15 mm). Arrange 10 shoot tips on each foil strip. Pipette off any excess PVS2, then immediately plunge the strip into liquid nitrogen.

Cooling Vessel and Method
Affix labels to 1.2 mL cryovials (Corning 430487) and submerge in liquid nitrogen. When ready to load, hold a vial just above the liquid nitrogen, open, pour out any liquid, place one foil strip inside, close, and quickly submerge. In a large container containing liquid nitrogen, load cryovials onto cryocanes and affix appropriately labeled sleeves.

Storage Conditions
Quickly transfer sleeved canes into the appropriate boxes in liquid nitrogen storage (either liquid phase or vapor phase).

Comments
N/A

References
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Appendices
<u>MS Shoot Growth Solid Medium (MS + 3% sucrose): 1 L</u>
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43 g
- Sucrose = 30.0 g
- Bring to volume
- Agar = 8.0 g
- pH = 5.8

- Dispense into desired vessels
- Sterilize in autoclave

Preculture Solution 1 (MS + 0.06 M sucrose): 1 L
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43 g
- Sucrose = 20.0 g
- Bring to volume
- pH = 5.7
- Dispense into desired vessels
- Sterilize in autoclave

Preculture Solution 2 (MS + 0.3 M sucrose): 1 L
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43 g
- Sucrose = 102.69 g
- Bring to volume
- pH = 5.7
- Dispense into desired vessels
- Sterilize in autoclave

Loading Solution (MS + 2 M glycerol + 0.4 M sucrose): 1 L
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43 g
- Sucrose = 136.92 g
- Glycerol = 184.18 g (w/v)
- Bring to volume
- pH = 5.7
- Dispense into desired vessels
- Sterilize in autoclave

Plant Vitrification Solution 2 (PVS2): 250 mL
- Glycerol (30% w/v) = 75 g
- Ethylene glycol (15% w/v) = 33.8 mL
- DMSO (dimethyl sulfoxide) (15% w/v) = 34.1 mL
- Sucrose (0.4 M) = 34.25 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 0.55 g
- Bring to volume
- pH = 5.8
- Filter sterilize using 0.45 micron syringe filter or Stericup filter units
- Dispense into sterile glass or plastic tubes, seal and refrigerate