

VIABILITY METHOD—SHOOT TIPS
NLGRP CLONAL MS MENTHA SHOOT TIP REGROWTH 1
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Introduction

Shoot tips of *Mentha* spp. are cryopreserved using droplet-vitrification. They are recovered through shoot tip regrowth.

Source of Plant Material

Mentha shoot tips are stored in the vapor phase of liquid nitrogen.

Plant Material Description

Shoot tips were cut to 1 mm in size and preserved in droplets of PVS2 on foil strips. There are approximately 10 shoot tips per foil strip, and 1 foil strip per 1.2 mL internally threaded cryovial.

Warming

Remove cryovial from LN, quickly uncap and remove the foil strip containing shoot tips. Immediately submerge the foil and shoot tips in room temperature Unloading Solution (MS + 1.2 M sucrose). Allow shoot tips to soak for 20 minutes. Plate onto 60 mm x 15 mm petri plates containing about 12 mL solid Recovery Medium (MS + BA + IBA).

Regrowth/Viability Conditions

Place Petri dish in the dark for two weeks followed by dim light (~ 32 $\mu\text{mol}/\text{m}^2/\text{s}$ or ~ 2500 lux with 16-hour photoperiod) for 1 week, then transfer to full light (~ 63 $\mu\text{mol}/\text{m}^2/\text{s}$ or ~ 5000 lux with 16-hour photoperiod) for approximately 3 weeks, until viability is evaluated. Grow *in vitro* cultures of *Mentha* in an environmentally controlled growth room at 25 °C.

Regrowth/Viability Assessment

Plantlets are grown for several weeks to allow time for healthy shoot, leaf, and root production. Plantlets are considered viable with at least 4 mm shoot growth.

Comments

N/A

References

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Appendices

Unloading Solution (MS + 1.2 M sucrose): 1 L

- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43 g
- Sucrose = 411.0 g
- Bring to volume
- pH = 5.7
- Dispense into desired vessels
- Sterilize in autoclave

Recovery Medium (MS + BA + IBA): 1 L

- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43 g
- Sucrose = 30.0 g
- BA (6-benzylaminopurine) = 0.5 mg
- IBA (indole-3-butyric acid) = 0.1 mg
- Bring to volume
- Agar = 7.0 g
- pH = 5.7
- Sterilize in autoclave
- In laminar flow hood, dispense liquid medium into sterile Petri dishes