PRESERVATION METHOD—SHOOT TIPS

NLGRP CLONAL MS FRAGARIA DROPLET-VITIFICATION 1

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File Name
NLGRP_CLONAL_MS_FRAGARIA_DROPLET-VITIFICATION_1

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Study Reason
Preservation

Introduction
(Excerpt from Niino et al., 2003)
Strawberry is an important economic and nutritious crop in many countries. Strawberry germplasm is commonly preserved as plants either in field or in insect proof screen houses. In these gene banks, the plants are subject to risks of losses caused by biological and natural hazards. For strawberry preservation, there are some disadvantages, such as periodic replanting, possibility of contamination by runners from other clones and naturally spread viruses or virus-like diseases. An alternative method is in vitro preservation of strawberry cultures at refrigerated temperature for medium term storage. Over 350 Fragaria accessions are stored in gas permeable tissue-culture bags at 4ºC at the National Clonal Germplasm Repository in Corvallis, OR.

Cryopreservation is one of the ideal and suitable options for long-term storage of plant germplasm. Cryopreservation techniques are now advanced at the stage where they can be implemented for useful storage of germplasm. Techniques of controlled freezing, vitrification, encapsulation-dehydration, encapsulation-vitrification, dormant bud preservation and combinations of these are now available for use. Although a few papers have been reported for strawberry cryopreservation, there has been no application on a large-scale to germplasm lines.

In Japan, about 700 accessions of strawberry germplasm (Fragaria species) have been maintained as field collections. The objective in this study was to establish a reliable protocol for the cryopreservation of strawberry shoot tips using vitrification. This method would then be used for a largescale cryopreservation project of Fragaria species.

Source of Plant Material
In-vitro

Plant Material Description
3-4 week-old in-vitro shoot cultures

Plant Material Culture Conditions
Divide small clusters or individual microplants (1-2cm) from 3 to 4-week-old stock cultures and plate in Magenta GA7 vessels with shoot maintenance medium (16 plants/each). Grow

microplants at stock culture conditions (~90 umol/m²/s or ~7100 lux with 16-hour photoperiod at 25ºC) for 1 week.

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
Transfer 1-week-old cultures to cold acclimation chamber (~90 umol/m²/s or ~7100 lux with 8-hour photoperiod at 4ºC) for 2 weeks

Shoot Tip Excision and Preculture
Excise shoot tips (~2mm) from cold-acclimated plants and plate on preculture medium; preculture overnight (~16 hours) at 4ºC in darkness

Cryopreservation Method
Remove shoot tips from preculture medium and place in loading solution for 20 minutes at room temperature. Remove from loading solution and cryoprotect with PVS2 at room temperature for 45 minutes. Place shoot tips into thin layer of PVS2 on foil strips and plunge into liquid nitrogen.

Cooling Vessel and Method
Foil strips are transferred to 1.2mL cryo vials (Corning 430487) in liquid phase.

Storage Conditions
Cryo vials with foil strips inside are transferred to canes/boxes in vapor/liquid phase.

Comments
N/A

References
Niino et al. 2003. Cryopreservation of In Vitro-Grown Apical Shoot Tips of Strawberry by Vitrification. Plant Biotechnology, 20 (1), 75-80.

Appendices
- **Shoot maintenance medium:** MS salts/vitamins + 25g/L sucrose + .2mg/L BAP + 3g/L agar + 1.3g/L gellan gum at pH 5.8
- **Preculture medium:** MS salts/vitamins + 2M glycerol + .3M sucrose + 8g/L agar at pH 5.8
- **Loading solution:** MS salts/vitamins + 2M glycerol + .4M sucrose at pH 5.8
- **PVS2 (250mL):** 1/2X MS salts/vitamins + 75g glycerol + 33.8mL ethylene glycol + 34.1mL DMSO + 34.25g sucrose + at pH 5.8 (FILTER STERILIZE with .45um filter)