VIABILITY METHOD—SHOOT TIPS

NLGRP CLONAL MS FRAGARIA SHOOT-TIP-REGROWTH 1

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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
Cryogenic storage (vapor/liquid phase)

Plant Material Description
Shoot tips on foil strips inside of cryo vials

Warming
Remove foil strips from vials and warm foil strips in room temperature unloading solution until melted and shoot tips are floating in unloading solution; incubate for 20 minutes

Regrowth/Viability Conditions
Transfer shoot tips to recovery medium in 60 X 15mm petri plates (~12mL medium with 5 shoot tips per plate) and culture for two weeks at 25ºC in darkness. Transfer plates to low light intensity (~55 umol/m²/s or ~4500 lux with 16-hour photoperiod) at 25ºC.

Regrowth/Viability Assessment
Evaluate for shoot regrowth after ~4 weeks

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
- **Unloading solution:** MS salts/vitamins + .8M sucrose at pH 5.8 (pH 6.45 prior to autoclaving)

- **Recovery medium:** MS salts/vitamins + 25g/L sucrose + .2mg/L BAP + 1g/L PVP + 8g/L agar at pH 5.8 (pH 6.45 prior to autoclaving)