Cryopreservation of *Fragaria* germplasm

At the NCGRP, cryopreservation of *Fragaria* (strawberry) shoot tips is based on a vitrification with cold acclimation technique developed by Niino et al., 2003. Several accessions were cryopreserved at the National Clonal Germplasm Repository at Corvallis, Oregon and placed in the NCGRP cryotanks for long term storage. The cryostored material includes three species: *F. x ananassa, F. virginiana* and *F. chilonesis*. Plant material, in the form of tissue culture, used for cryopreservation at the NCGRP was obtained through collaboration with the Corvallis repository.

In Vitro* Culture of *Fragaria

Fragaria plants are multiplied in Magenta® GA7* culture vessels (Magenta Corp., Chicago, IL*) on *Fragaria* growth medium for 2 weeks in a controlled growth chamber (see supplemental information). After 2 weeks, plants are subcultured on fresh *Fragaria* growth medium in LifeGuard®*culture vessels (Sigma-Aldrich Dallas, TX*) and allowed to grow for an additional 2 weeks. A subcultured section consists of two axillary nodes.

Image 1. *In vitro*-grown *Fragaria* plants established in Magenta® GA7 culture vessels.
Photo by K. Oles

Image 2. *Fragaria* plants in LifeGuard®* culture vessels.
Photo by K. Oles

Cold acclimation of *Fragaria*

After 2 weeks of *in vitro* culture, *Fragaria* plants are moved to a cold acclimation chamber (see supplemental information) for 7 days

Cryopreservation of *Fragaria* shoot tips (Vitrification with cold acclimation):

All steps take place under aseptic conditions and at room temperature (21 °C) unless noted otherwise.

I. Shoot tip isolation and preculture (Day 1)

A. Shoot tip isolation

1. Excise shoot tips from of cold acclimated plants. Shoot tips consist of the apical dome and 2-3 leaf primordia (1.5-2.0 mm). Place shoot tips in a Petri dish containing **MS+2.0 M glycerol+0.3 M sucrose medium** until the desired number has been obtained.
   Note: the Petri dish and the LifeGuard® vessel should be kept on ice during the entire shoot tip isolation procedure.

B. Pre-culture

1. Move Petri dish with shoot tips to the cold acclimation chamber for 24 hours.

II. Loading, dehydration and vitrification (Day 2)

A. Loading

1. After 24 hours, transfer shoot tips to a 1.2 ml cryovial containing MS+2.0 M glycerol+0.4 M sucrose medium (1 ml medium/ cryovial) and soak for 20 minutes.

B. Dehydration

1. After 20 minutes, remove sucrose medium from the cryovial and replace with PVS2 (1 ml solution/cryovial). Allow shoot tips to soak for 50 minutes; refresh the PVS2 once during that time.

2. In the last minute, remove 0.5 ml of PVS2 from the cryovial.

C. Vitrification

1. Immediately plunge cryovial into LN.

III. Rewarming and recovery (Day 2)

Keep cryopreserved shoot tips in liquid nitrogen for at least one hour prior to rewarming.

A. Rewarming

1. Submerge the LN-cooled cryovial in a 35°C water bath for 2 minutes.
2. Uncap cryovial and add 1 ml MS+.8 M sucrose medium. Allow shoot tips to soak for 20 minutes; refresh the sucrose medium twice during that time.
3. After 20 minutes, pour off sucrose medium and shoot tips onto sterile filter paper to drain.

B. Recovery

1. Transfer shoot tips to a Petri dish containing solid *Fragaria* recovery medium. Place Petri dish in the dark for 48 hours followed by dim light for 72 hours and after, transfer to full light. Viability can be assessed 4-6 weeks post thaw.

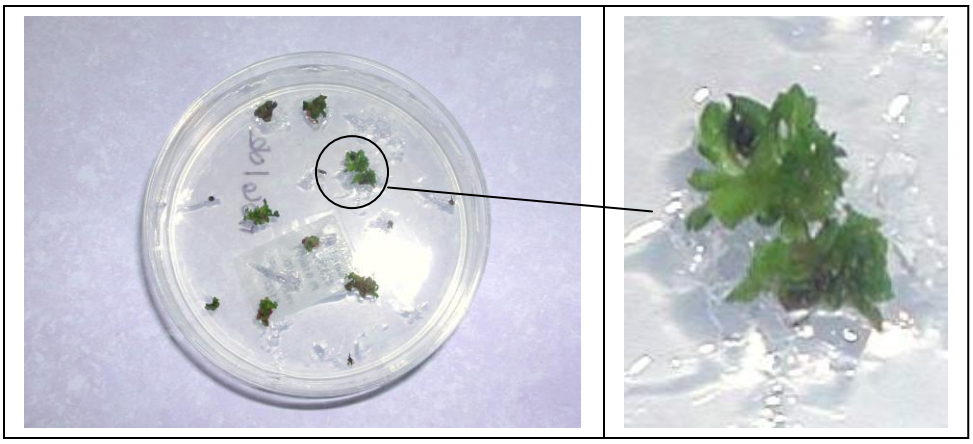

Image 3. *Fragaria virginiana* plantlets ~4 weeks post-thaw.
Photo by K. Oles

Supplemental Information

In vitro cultures of *Fragaria* were grown in an environmentally controlled growth room set at 25±3 °C with a 16-hour light/8-hour dark photoperiod. Light intensity was 55 µmol m^{-2} s^{-2}.

The cold acclimation chamber was programmed for 16 hours of dark at 20°C, with a light intensity of 27 µmol m^{-2} s^{-1}, followed by 8 hours of light at -1.0°C.

References and supplemental reading

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*Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.