Cryopreservation of *Pyrus* sp. germplasm

At the NCGRP, cryopreservation of *Pyrus* (pear) shoot tips is based on a protocol developed by Reed (2004). Several accessions were cryoprocessed at the National Clonal Germplasm Repository at Corvallis, Oregon and placed in the NCGRP cryotanks for long-term storage. The cryostored material includes 18 *Pyrus* species and hybrids: *P. amygdaliformis, P. betulifolia, P. calleryana, P. communis, P. communis subsp. pyraster, P. cordata, P. cossonii, P. elaeagrifolia, P. gharbiana, P. hondoensis, P. x hybrid, P. koehnei, P. mamorensis, P. pashia, P. pyrifolia, P. syriaca, P. ussuriensis,* and *P. x phaeocarpa*.

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 *Pyrus

Plants were multiplied on pear multiplication medium in Magenta® GA7* culture vessels (Magenta Corp., Chicago, IL*) and subcultured at four to six week intervals.

All *in vitro* cultures were kept in a growth room (see supplemental information).

Image 1. *In vitro* cultures of *Pyrus* are multiplied in Magenta® GA7* culture vessels. Subculturing occurs at 4 to 6 week intervals.

Procedure for cryopreservation of *Pyrus* shoot tips: Slow-cooling

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

I. Cold acclimation

Transfer three to four week old in vitro-grown cultures to a cold acclimation chamber (see supplemental information) for 28 days.

II. Shoot tip isolation and preculture (day 1 and 2)

a. Shoot tip isolation

1. Excise shoot tips from cold acclimated plants. Shoot tips consist of 2-3 leaf primordia plus the apical dome (0.8–1.0 mm). Partially embed shoot tips in solid

pear pretreatment medium with 5% DMSO (40 shoot tips/Petri dish). Seal each dish with Parafilm®*.

b. Pre-culture

1. Move Petri dishes containing excised shoot tips to a cold acclimation chamber (see supplemental information) for 48 hours.

III. Slow (Two-step) Cooling Protocol

1. Place 50ml PGD (see recipes) in freezer (-15°C) 30 minutes before using.
2. Place 1.5ml cryovials in ice (we use Nalgene Labtop Cooler Jr.*)
3. Add two drops of liquid MS (no PGR 3% sucrose) per vial and add 10 shoot tips to each vial.
4. Add two drops of PGD at time 0, 2, 4, and 6 minutes.
5. Add four drops of PGD every two minutes for the remainder of 30 minutes.
6. Place the vials in slow cooler at 0°C and hold at 0°C for 30 minutes.

Slow cooling program is as follows:

a. 50°C/m C to 0°C
b. Wait at 0°C
c. 0.1°C/m S to -9°C
d. 99°C/m C to -50°C (exotherm should happen here)
e. 20°C/m C to -15°C
f. 0.1°C/m S to -40°C
g. Wait at 0°C
h. End
7. Plunge vials containing shoot tips into liquid nitrogen (LN).

IV. Rewarming and recovery

1. Wait at least one hour and remove 2 vials from the LN for viability testing.
2. Warm for one minute in a 45°C water bath then move the vials to 25°C for another 2 minutes.
3. Pipette off PGD.
4. Add room temperature liquid MS (no PGR and 3% sucrose) to each vial.
5. After five minutes drain the tips on sterile filter paper.

6. Transfer tips to pear recovery medium in 60x15mm Petri dishes and seal with Parafilm*.

7. Place recovering shoot tips in a growth chamber set at 25°C, 12h photoperiod.

8. Cover with a white paper towel for two days.

9. Evaluate viability after four to six weeks.

Image 2. Viable *Pyrus* shoot tips will develop into plantlets approximately six weeks post-thaw (here shown in a Petri dish).

Supplemental Information

In vitro cultures of *Pyrus* 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^{-2}, 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.