Cryopreservation *of Solanum tuberosum* germplasm

At the NCGRP, cryopreservation of *S. tuberosum* (potato) shoot tips is based on a method developed by Kim, et al. (2006). All cryopreserved accessions represent voucher germplasm of PVP cultivars.

In vitro* culture of *S. tuberosum

Plants were multiplied on Murashige & Skoog (MS) multiplication medium in Magenta® GA7* culture vessels (Magenta Corp., Chicago, IL*) and subcultured every 4-6 weeks. All *in vitro* cultures were kept in a growth room (see supplemental information).

***In vitro* culture of nodal sections**

Nodal sections of *Solanum* were harvested from 4-6 week old *in vitro*-grown plants and plated on MS multiplication medium. Nodal sections were grown in culture until they reached a height of 6-10 cm (3-5 weeks).

Procedure for cryopreservation of *S. tuberosum*: Vitrification

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

I. Isolation and preculture of nodal-derived shoot tips (day 1)

A. Shoot tip isolation

Excise shoot tips from plated nodes, grown out to a height of 6-10 cm, and place in liquid MS+0.3 M sucrose medium. A shoot tip consists of the apical dome plus 2-3 leaf primordia (0.8-1.3 mm in length).

B. Preculture (day 1)

Allow shoot tips to soak in MS+0.3 M sucrose medium sucrose medium for 16-24 hours/25°C.

C. Preculture (day 2)

After 16-24 hours, replace the MS+0.3 M sucrose with MS+0.7 M sucrose medium and soak for 7-8 hours/25°C.

II. Dehydration and vitrification (day 2)

A. Dehydration

1. After 7-8 hours, replace the MS+0.7 M sucrose with PVS2 and soak for 20 minutes.

2. With a few minutes remaining, using a glass Pasteur pipette, transfer shoot tips plus ~50 µl PVS2 to a sterile 1.5 x 0.5 mm foil strip (10 shoot tips/foil strip).

B. Vitrification

1. When the remaining time has elapsed plunge the foil strip with shoot tips into a shallow vessel of liquid nitrogen (LN).

2. Using pre-cooled forceps, insert the frozen foil strip into a LN-cooled, 1.2 ml cryovial. Cap the cryovial making sure it contains enough LN to cover the enclosed foil strip.

III. Rewarming and recovery of vitrified shoot tips

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

A. Rewarming

1. Quickly remove the foil strip from the cryovial and transfer to MS+0.8 M sucrose medium. Remove the foil strip from the medium once the rewarmed shoot tips become detached. Allow shoot tips to soak for 30 minutes.
Note: Wear safety glasses; cryovials may burst due to a sudden increase in pressure.

2. After 30 minutes, remove some of the sucrose medium with a pipette and drain the remaining liquid shoot tips onto sterile filter paper.

B. Recovery

1. Transfer the shoot tips from the filter paper to a Petri dish containing solid Kim potato recovery medium. Seal the dish with Parafilm®* to inhibit desiccation.

2. Move the sealed plate to a growth room (see supplemental information) for 2 days and cover loosely with a paper towel to reduce light intensity.

3. After 7 days transfer shoot tips to MS multiplication medium.

4. Viable shoot tips will develop into plantlets approximately 5-8 weeks post-thaw.

Supplemental information

In vitro cultures of *Solanum* 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}.

References

Kim H-H, Yoon J-W, Park Y-E, Cho E-G, Sohn J-K, Kim T-S, Engelman F. 2006. Cryopreservation of potato cultivated varieties and wild species: critical factors in droplet vitrification. CryoLetters 27(4):223-234.

*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.