

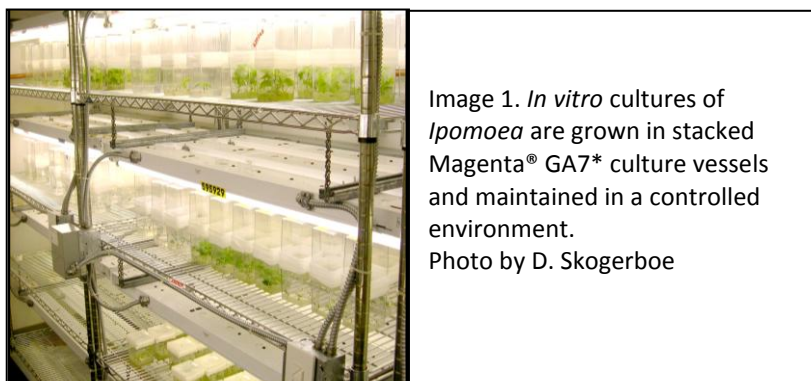
Cryopreservation of *Ipomoea* germplasm

At the NCGRP, cryopreservation of *Ipomoea* (sweet potato) shoot tips is based on encapsulation-dehydration and vitrification method developed by Hirai & Sakai (2003) with a few modifications. The modifications were introduced after a limited success with the original cryopreservation method described in the 2003 report. These changes improved the post cryo survival of sweet potato at the Center.

Plant material in the form of tissue culture used at the NCGRP was obtained through collaboration with the Plant Genetic Resource Conservation Unit, Griffin, Georgia.

In vitro* culture of *Ipomoea

Plants were grown in stacked Magenta® GA7* culture vessels (Magenta Corp., Chicago, IL*) on MS multiplication medium 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 *Ipomoea* were harvested from *in vitro*-grown plants and plated on MS+BA nodal medium 5-10 days prior to excision. Each section (0.5 – 0.75 cm) consisted of a single axillary node. Nodes were harvested from 4-6 week old cultures.

Procedure for cryopreservation of *Ipomoea* shoot tips: Encapsulation-dehydration and vitrification

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

I. Shoot tip isolation, encapsulation and preincubation (day 1)

A. Shoot tip isolation

1. Excise shoot tips from plated nodes immediately following 8 hours of darkness and place in liquid MS preincubation medium. A shoot tip consists of the apical dome plus 2-3 leaf primordia (0.5 – 1.0 mm).

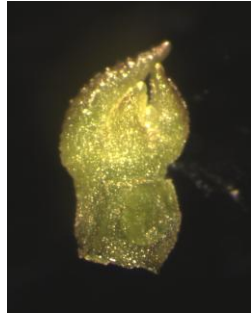
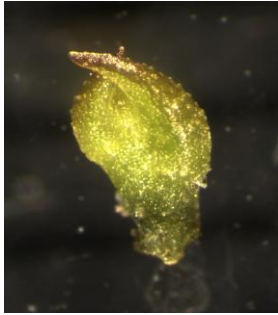


Image 2. Sweet potato shoot tips are harvested from nodal segments immediately following 8 hours of darkness. Shoot tips are ~0.5-1mm long and consist of an apical dome plus 3-4 leaf primordia
Photos by D. Ellis

B. Encapsulation

1. Once all shoot tips have been isolated replace the medium they are suspended in with Ca-free MS+3% Na-alginate medium.
2. Using a modified plastic pipette, with an opening of ~2 mm, transfer shoot tips, one per drop, into 0.1 M calcium chloride+MS encapsulation medium (25 beads/50 ml liquid). If successful, each drop will form a bead. Allow the alginate beads to remain in the encapsulation liquid for at least 30 minutes. As they polymerize the beads will change in appearance from transparent to whitish-opaque.

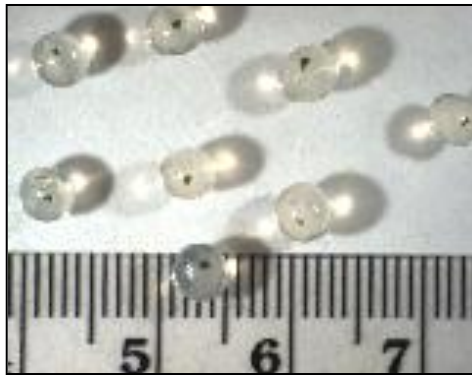


Image 3. Alginate-encased shoot tips
Photo by G. Holman

C. Preincubation

1. Transfer beads to a flask containing MS preincubation medium (25 beads/50 ml liquid). Cover each flask completely with sterile foil and place on a rotary shaker (90 rpm) for 24 hours.

II. Preculture (day 2)

1. After 24 hours, replace the MS preincubation medium, with 0.3 M sucrose+MS preculture medium (25 beads/50 ml liquid). Cover each flask completely with sterile foil and place on a rotary shaker (90 rpm) for 16 hours.

III. Loading, dehydration and vitrification (day 3)

A. Loading

1. After 16 hours, replace the 0.3 M MS incubation medium with 1.6 M sucrose+2 M glycerol+MS loading medium (25 beads/10 ml liquid). Cover each flask completely with sterile foil and place on a rotary shaker (60 rpm) for 3 hours replacing the medium twice, every hour, with fresh solution.

B. Dehydration

1. After 3 hours, transfer beads to PVS2+½ strength MS (25 beads/10 ml liquid) for 1 hour replacing the liquid once, after 30 minutes, with fresh PVS2. In the interim, add PVS2 to cryovials (0.5 ml PVS2/1.2 ml cryovial).

C. Vitrification

1. With a few minutes remaining, move beads from PVS2 to the prepared cryovials (10 beads/cryovial). When the remaining time has elapsed quickly plunge vials in liquid nitrogen.

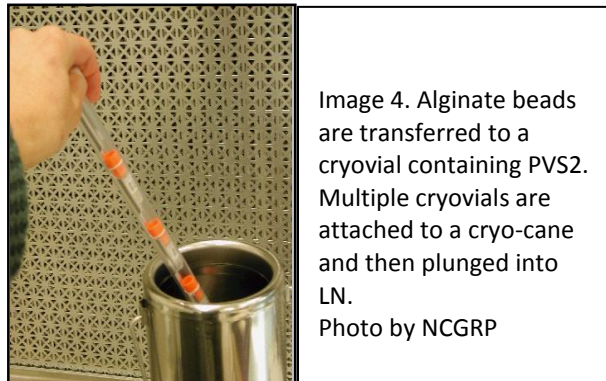


Image 4. Alginate beads are transferred to a cryovial containing PVS2. Multiple cryovials are attached to a cryo-cane and then plunged into LN.

Photo by NCGRP

IV. Rewarming and recovery

Cryopreserved shoot tips should remain in liquid nitrogen for at least 1 hour prior to rewarming. Conduct the remaining steps in dimmed light.

A. Rewarming

1. Submerge LN-treated cryovials in a 38°C water bath for 1.5 minutes.
2. Pipette PVS2 from each cryovial and replace with 1.2 M sucrose+MS minus NH_4 medium. Allow beads to soak for 20 minutes replacing the liquid once, after 10 minutes, with fresh medium.

B. Recovery

1. Remove beads from cryovials and place them in a Petri dish containing solid Ipomoea recovery I medium. Exclude light by wrapping the Petri dish completely in aluminum foil and move to a growth room for 2 days.
2. After 2 days, remove shoot tips from the alginate beads and return them to the same medium. Return Petri dish to the growth room, in dim light (see supplemental information), for an additional 3 days.
3. Five days after rewarming, move shoot tips to a Petri dish containing Ipomoea recovery II medium. Place in full light ($55 \mu\text{mol m}^{-2} \text{s}^{-2}$) in growth room.

Supplemental information

Cultures are maintained in a controlled environment, a growth room, at $25 \pm 3^\circ\text{C}$ under a 16-hour light ($55 \mu\text{mol m}^{-2} \text{s}^{-2}$)/8-hour dark photoperiod.

Dim light ($\sim 27 \mu\text{mol m}^{-2} \text{s}^{-2}$) is accomplished by placing a paper towel under and loosely over, the Petri dish. Paper towels obscure the full intensity of the growth room lighting.

Shoot growth can be confirmed 2-4 weeks post thaw; Survival within 5-14 days. If the shoot tips are damaged regrowth will consist of callus only.

If present, cryptic contamination will be apparent within 2-3 days after rewarming.

References and supplemental reading

Hirai D, Sakai A. 2003. Simplified cryopreservation of sweet potato [*Ipomoea batatas* (L.) Lam.] by optimizing conditions for osmoprotection. *Plant Cell Rep.* 21(10):961-96.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plantarum* 15:473-497.

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Towill LE, Jarrett RL. 1992. Cryopreservation of sweet potato [*Ipomoea batatas* (L.) Lam.] shoot tips by vitrification. *Plant Cell Rep.* 11:175-178.

*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.