Work in progress

Currently, NCGRP has 68 accessions of *Ipomoea batatas*, sweet potato, in tissue culture. All accessions were obtained through a collaboration with the USDA, Plant Genetic Resources Conservation Unit in Griffin, GA.

The protocol used, at NCGRP, for the cryopreservation of sweet potato shoot tips is based on one developed by Hirai & Sakai (2003) utilizing encapsulation-dehydration and vitrification. After having limited success with the original protocol, the following modifications were implemented: (1) a dark induction period (8 hours) of nodal sections prior to shoot tip excision; (2) a gradual transition, of rewarmed alginate-encapsulated shoot tips, to light exposure; and (3) minor alterations to the Hirai-Sakai media recipes. These newly implemented changes improved our programs success in the cryostorage of sweet potato.

*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).

*Nodulation of* *Ipomoea*

*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).

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.
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 NH₄ 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 µmol m⁻² s⁻²) in growth room.

Supplemental information

Cultures are maintained in a controlled environment, a growth room, at 25±3 °C under a 16-hour light (55 µmol m⁻² s⁻²)/8-hour dark photoperiod.

Dim light (~27 µmol m⁻² 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 of rewarming.
References and supplemental reading


