Genebank - *in vitro* propagation of potato and sweetpotato

CIP – SOP056

Standard operational procedures

February 2020

Reference: Vollmer, R.; Panta, A.; Solis, R; Manrique, N. and Anglin, N.L. 2020. Genebank - *in vitro* propagation of potato and sweetpotato. CIP-SOP056 V 3.0 - 14 p.

February 6, 2020

Not for general distribution.
For most current version, please contact Rainer Vollmer (r.vollmer@cgiar.org)

CIP thanks all donors and organizations that globally support its work through their contributions to the CGIAR Trust Fund: www.cgiar.org/funders

This publication is copyrighted by the International Potato Center (CIP). It is licensed for use under the Creative Commons Attribution 4.0 International License

Contents

Introduction 4

Scope 4

Safety 4

Materials 5

Procedure 6

Internal quality control 12

History of revision 13

References 13

Introduction

The genebank at the International Potato Center (CIP) conserves the diversity of potato, sweetpotato, and nine Andean Root and Tuber Crop (ARTC) species. Most of these species are generally allogamous which means the unique traits of each accession can only be maintained if the genebank conserves a clone rather than seeds. The process of eliminating pathogens from plant material is quite laborious and expensive; therefore, *in vitro* conservation allows the genebank to ensure that once the material is clean of diseases, it is maintained in that state long term. Because we distribute germplasm globally, it is critical for the genebank to only distribute plant material free of diseases. Tissue culture offers the only safe alternative for conservation of clonal material. Using tissue culture techniques, the plants are grown under sterile and controlled conditions. The following protocol explains the *in vitro* plant propagation technique also known as micropropagation which is based on the principle that appropriate culture conditions induce the growth of pre-existent terminal or axillar buds, resulting in a new plantlet. The nutritional and hormonal conditions of the culture medium break the bud's dormancy and promote its rapid growth. Callus formation, followed by shoot regeneration, must be avoided because it can affect the genetic integrity of the clone by producing somaclonal mutations. Following the standard operational protocol (SOP056) described below, plantlets in active growth are multiplied approximately every 3-6 weeks. This protocol is applied in different areas of the CIP genebank such as, distribution of *in vitro* material, *in vitro* conservation, cryopreservation, and phytosanitary activities.

Scope

This procedure describes the *in vitro* multiplication of potato and sweetpotato germplasm for international and national germplasm distribution, as well as, *in vitro* conservation, phytosanitary, and cryopreservation activities.

Safety

1. Follow good laboratory practices during *in vitro* activities, including preparation and sterilization of material, cleaning, and disinfecting the work area, as well as, other control actions (as described in **RD017**).

2. Follow standard Health & Safety guidelines.

3. A clean laboratory coat must be worn during all *in vitro* lab activities.

4. Wear safety glasses, hair net, and a surgical mask when working in the laminar flow chamber.

5. Sterilize the tools for 20-30 seconds in the glass bead sterilizer to avoid burns on fingers.

6. Staff need to take care to avoid being cut. The edge of the scalpel blade should be orientated in the opposite direction of the person during installation and removal of the blade. Handling should be done smoothly without using much force or pressure.

Materials

Equipment	
Autoclave	Heat sealing machine
Barcode printer	Laboratory cart
Barcode scanner	Laminar flow chamber
Data logger for monitoring environmental conditions (HOBO®)	Max / Min thermometer
Desktop computer	Plant growth rooms: (Potato: 20±2 °C, Sweetpotato: 24±2 °C)
Environmental control system (SITRAD®)	Pocket Personal Computer (Pocket PC)
Glass bead sterilizer	Refrigerator
Other materials	
Alcohol gel	Scalpel holder
Antibacterial wipes	Sterilized paper sheets (size: A5)
Barcode labels	Sterilized tool holder
Blades No.10	Sterilization sleeves (width: 30 cm)
GA7 Magenta vessels	Surgical mask
Glass test tubes (13x100 mm, 16x125 mm, 18x150 mm and 25x150 mm)	Test tube caps
Hair cover/net	Test tube/GA7 racks
Lab coat	Tissue Culture Forceps (fine point, 23 cm)
Safety glasses	Wash bottle with alcohol (70%)
Saran wrap	
Chemicals	
Alcohol (70%)	Culture media (MSA, MPB, MRB) (**Tables 1a, b**)

Procedure

1. Turn on the laminar flow chamber and the glass bead sterilizer. Wait 15-30 minutes until the sterilizer has reached its correct operating temperature of 250 °C. Using an antibacterial wipe with alcohol (70 %), clean the work surface and the side/back walls of the flow chamber (see **RD017**). Set up the following materials inside the flow chamber: sterilized tool holder (*), sterilized paper sheets (size: A5) (*), scalpels with No.10 blades, long tissue culture forceps (23 cm), and a wash bottle with alcohol (70%). Sterilize forceps and scalpel holders. Install blades onto holders.

(*) Tool holders and paper sheets are autoclaved within sterilization sleeves (that were sealed prior to autoclaving). Select the dry-heat sterilization program of the autoclave for sterilizing this material.

2. Using a laboratory cart, transfer the rack with the accessions to be multiplied from the active growth room to the transfer room. Using a desktop computer, obtain the list of accessions to be processed from the tissue culture database, and print out the required number of labels with the barcode printer. (The purpose of the propagation dictates the number of vessels which are needed to be propagated). The information to be recorded in the database and contained on the barcode label (**) varies between activities. For recording information into the database, first read the accession label with a barcode scanner or pocket PC.

(**) E.g.: accession identifiers (CIP number or labcode), propagation date, health status, name of person who has propagated the plants, digital object identifier of the accession (DOI), cultivar name, etc.

3. Process *in vitro* accessions separately, propagating only one single accession at a time. Using sterilized forceps, take out the plantlets from the *in vitro* vessel and place them onto a stack of 3 to 5 sterilized paper sheets (size A5) [**Fig. 1a, b**].

4. Using a sterilized scalpel with a No. 10 blade, remove the tip, leaves, and roots of the plantlets **(Fig. 1c)** and cut the stem into several segments (or explants) of approximately 1-1.5 cm with one to two buds per segment **(Fig. 1d)**. If the distance between the nodes is very short or if faster growth is desired, it is recommendable to cut stem segments with two buds. The internodal distance varies depending on the genotype and plant age.

5. The number of stem segments placed into each vessel depends on the plant species, and the type and size of the vessel **(Fig. 2).**

- Potato: Place two explants per 13x100 mm test tube (for national and international distribution), four explants per 16x125 mm tube (for *in vitro* conservation sub-culture), four to six explants per 25x150 mm test tube and 16-25 explants per GA7 magenta vessel (for *in vitro* multiplication). Place explants on sterilized MSA propagation medium (**Table 1a**), maintaining the bud facing upwards **(Fig. 1e, f)**.
- Sweetpotato: Place one single explant per 13x100 mm tube (for national and international distribution), two explants per 18x150 mm tube (for *in vitro* conservation sub-culture), one to two explants per 25x150 mm tube, and five to six explants per GA7 magenta vessel (for *in vitro* multiplication). Place explants on sterilized MPB or MRB propagation medium (**Table 1b**).

For details about culture media preparation, please see **RD008 - Preparation of Culture Media**.

6. Cap and seal the tubes or GA7 magenta vessels with Saran wrap® and label correctly (***). If accelerated *in vitro* plant growth is required, seal all vessels with only one-single layer of Saran wrap (for better gas exchange) or don't seal at all. Discard the stack of paper sheets and remaining plant tissue after the multiplication of each accession. Use a new stack of sterile paper sheets for each accession. After the propagation of each accession, sterilize forceps and scalpels for 20-30 seconds in the glass bead sterilizer. Clean hands periodically with alcohol gel. After processing each accession, clean work surface of the laminar flow chamber with an antibacterial wipe containing alcohol (70 %).

(***) To label test tubes, first stick the label onto a piece of Saran wrap and then wrap the Saran wrap with the label on the container. This prevents the label from sticking directly onto the vessel and its subsequent removal is simple. Stick the label that was on the original vessel onto the test tube where the propagated material is transferred to, as a control label along with the newly printed label. This helps to track correct labeling in each multiplication cycle.

7. Check that all tubes are correctly labelled immediately after the propagation of each accession. Tubes of the same accession must be labelled with identical accession number (for *in vitro* conservation: update the number of propagated vessels in the database). Transfer propagated *in vitro* cultures to the active growth room. Environmental conditions differ between growth rooms depending on the species:

- Potato: temperature of 20±2°C [(****)], photoperiod of 16h/8h (light/darkness), light intensity of 85±20 $\mu mol.m^{-2}.seg^{-1}$, provided by cool daylight fluorescent tubes.
- Sweetpotato: temperature of 24±2°C [(****)], photoperiod of 16h/8h (light/darkness), light intensity of 85±20 $\mu mol.m^{-2}.seg^{-1}$, provided by cool daylight fluorescent tubes.

(****) Temperature of the growth rooms is monitored using three independent methods: (a) Environmental monitoring System (SITRAD®) [automatically recorded], (b) datalogger (HOBO®) [automatically recorded], (c) Max/Min thermometer (manually recorded). For details on environmental conditions monitoring, please see **RD003 - Environment Monitoring**.

8. Five to eight days after propagation, visually evaluate the plantlets. Record and/or update the evaluation data in the database. If an accession shows signs of contamination, it is discarded, sterilized by autoclaving, and re-processed.

9. 12-16 days after propagation, visually evaluate rooting and shoot growth of the plantlets, and record the results in the database. Accessions with bad or poor growth and/or rooting are discarded by autoclaving and processed again.

10. Germplasm Distribution: on the day of packaging the material for delivery to a client, perform a visual quality check of the plants. Record the evaluation data in the database. The potato and sweetpotato *in vitro* plantlets showing "good" growth are sent to the distribution office (for national and international distribution) or directly to the client (CIP internal distribution). Potato and sweetpotato plantlets are packaged for delivery three to five and four to six weeks after their propagation, respectively. Occasionally, six- to eight-week-old *in vitro* plants are sent to requestors. For *in vitro* conservation, follow the workflows of the standard operational procedures **CIP – SOP025** (potato) and **CIP – SOP026** (sweetpotato). For details about the distribution of *in vitro* material, please see **SOP072 - Distribution of CIP Genetic Resources** and **SOP068 - Distribution of *in vitro* material**.

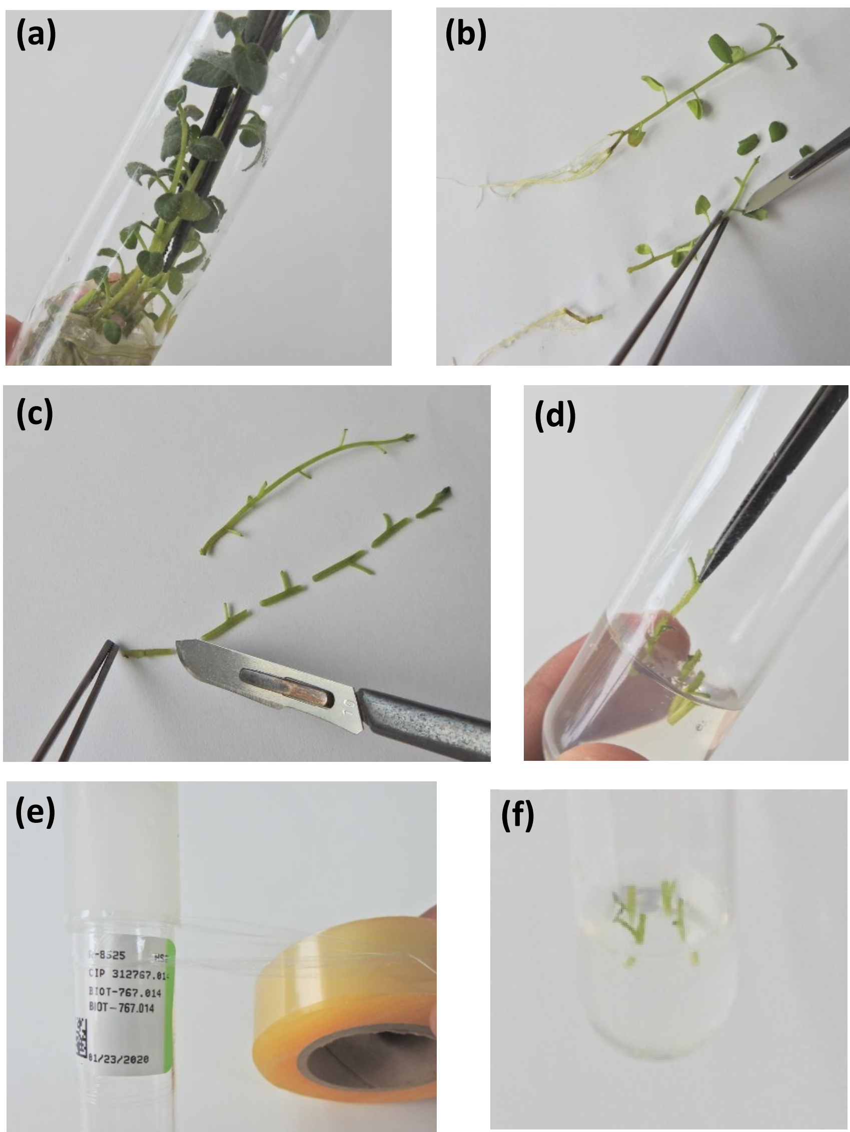


Fig. 1 *In vitro* propagation of potato and sweetpotato. **(a)** Remove mother plants from test tube (using long tissue culture forceps); **(b)** remove tip, leaves and roots using scalpel No.10 and forceps (working on a stack of three to five sterile paper sheets) **(c)** cut stem into segments (with one to two buds per stem segment [1]); **(d)** place segments on sterile culture medium [potato: MSA medium, sweetpotato: MPB (for distribution) or MRB medium (for *in vitro* conservation)]; for distribution place two (sweetpotato) or four to six segments (potato) per 25x150 mm test; for *in vitro* conservation place two explants per 18x150 mm test tube (sweetpotato) and four explants per 16x125 mm test tube (potato); **(e)** cap test tube, seal with Saran wrap, and label using both original and new barcode label, **(f)** recently propagated 25x150 mm test tube culture, containing four explants (potato).

[1] If the distance between nodes is very short, or if faster growth is desired, it is recommendable to cut stem segments with two buds.

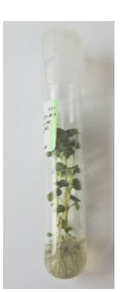
	Vessel type	Number of explants per vessel	
		Potato	Sweetpoato
	25x150 mm test tube (distribution)	4-6	1-2
	13x100 mm test tube (distribution)	2	1
	16x125 mm test tube (*in vitro* conservation)	4	-
	18x150 mm test tube (*in vitro* conservation)	-	2
	GA7 Magenta® vessel (distribution)	16-25	5-6

Fig 2. *In vitro* propagation of potato and sweetpotato. Vessel types and number of plantlets per vessel.

Table 1a. Composition of MSA medium for *in vitro* propagation of potato.

Component	Supplier	Catalog number	Quantity	Storage conditions [2]
MS salts [1]	CAISSON	MSP01	4.33 g/l	Store in a refrigerator at 5±3 °C. Hygroscopic.
Gibberellic acid	SIGMA	G7645	0.1 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place.
Glycine-HCl	SIGMA	G2879	2 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place.
Myo-inositol	SIGMA	I5125	100 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place.
Nicotinic acid	SIGMA	N0765	0.5 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place. Light sensitive.
Pyridoxine-HCl	SIGMA	P8666	0.5 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place
Thiamine-HCl	SIGMA	T4625	0.1 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place. Hygroscopic. Light sensitive.
Sucrose	SIGMA	S8501	25 g/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place.
Agar	SIGMA	A7002	6.5 g/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place. Moisture sensitive.
pH = 5.60 ± 0.02				

[1] Murashige y Skoog basal salts (1962)

[2] According to Material Safety Data Sheet (MSDS)

Table 1b. Composition of *in vitro* propagation media of sweetpotato (distribution: MPB medium; *in vitro* conservation: MRB medium).

Component	Supplier	Catalog number	Quantity (MPB [1])	Quantity (MRB [2])	Storage conditions [3]
MS salts [4]	CAISSON	MSP01	4.33 g/l	4.33 g/l	Store in a refrigerator at 5±3 °C. Hygroscopic.
Gibberellic acid	SIGMA	G7645	10 mg/l	-	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place.
Ascorbic acid	SIGMA	A7506	200 mg/l	200 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place. Light sensitive.
Calcium nitrate	FISHER	C109	100 mg/l	100 mg/l	Store at room temperature (22±3 °C). Do not store near combustible materials. Keep containers tightly closed in a dry, cool and well-ventilated place. Highly moisture sensitive.
Calcium pantothenate	SIGMA	P5155	2 mg/l	2 mg/l	Store in a refrigerator at 5±3 °C. Keep container tightly closed. Air, light, and moisture sensitive. Heat sensitive.
L-Arginine	SIGMA	A8094	100 mg/l	100 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place
Putrescine-HCl	SIGMA	P5780	20 mg/l	20 mg/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place. Hygroscopic. Store under inert gas.
Sucrose	SIGMA	S8501	30 g/l	30 g/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place.
Agar	SIGMA	A7002	6.0 g/l	3.5 g/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place. Moisture sensitive.
Phytagel	SIGMA	P8169	-	1.5 g/l	Store at room temperature (22±3 °C). Keep container tightly closed in a dry and well-ventilated place. Moisture sensitive.
pH = 5.70 ± 0.02					

[1] For distribution

[2] For *in vitro* conservation

[3] According to Material Safety Data Sheet (MSDS)

[4] Murashige y Skoog basal salts (1962)

Internal quality control

1. For each national / international / CIP internal distribution, prior to delivery, a quality check list is filled out. The document contains information regarding correct identification and labelling of the accessions, growth, rooting, and general aspect of the plants, and lack of contamination.
2. Test tubes are identified with barcoded labels. To ensure that test tubes of the same accession are labelled identically, the original and new labels are validated for *in vitro* conservation using an application on the pocket PC (in each propagation cycle).
3. Propagated material is inspected visually for absence of signs of microbial contamination: one week after propagation and immediately before packaging and delivery (for distribution), and one and three weeks after propagation (for *in vitro* conservation).
4. Automatic notification by email, seven days before accessions are ready to be delivered to the distribution office or client (only CIP internal)
5. International and national distribution: preparation of a back-up tube for the requested accessions. The back-up tubes are stored for three to four months or until the accessions are requested in a new order.
6. For International distribution, *in vitro* plantlets are inspected by a representative of the National Service for Plant and Animal Health (SENASA) before its shipment, so that a phytosanitary certificate can be issued.
7. Expiration date of reagents is verified before preparing culture media.
8. To verify the ISO-17025 accreditation standard are accomplished, the protocol is audited at least two times per year, both by internal and external auditors (UKAS).

History of revision

Date	Version #	Description	Editor
2008	V1 for potato and V1 sweetpotato	First version, two separated SOPs (SOP056: potato, SOP059: sweetpotato) published on ISO-web for the first year of the ISO-17025 accreditation	
2015	V2	Second version, SOP056 and SOP059 were merged into one single procedure (SOP056). The new document was revised and approved by the head of genebank	
2020	V3	Current version. Quality of protocol was substantially enriched with additional information and photos.	
This document is reviewed and updated on a yearly basis.			

References

Murashige T & **Skoog** F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-97

CIP-Genebank internal related documents and standard operational procedures (currently no available for open publication) RD003, RD008, RD017, SOP025, SOP026, SOP068 and SOP072.

CIP is a research-for-development organization with a focus on potato, sweetpotato and Andean roots and tubers. It delivers innovative science-based solutions to enhance access to affordable nutritious food, foster inclusive sustainable business and employment growth, and drive the climate resilience of root and tuber agri-food systems. Headquartered in Lima, Peru, CIP has a research presence in more than 20 countries in Africa, Asia and Latin America.
www.cipotato.org

CIP is a CGIAR research center
CGIAR is a global research partnership for a food-secure future. Its science is carried out by 15 research centers in close collaboration with hundreds of partners across the globe.
www.cgiar.org

For more information, please contact CIP Headquarter. Av. La Molina 1895, La Molina. Apartado 1558, Lima 12, Peru.
5-11-3496017 *cip-cpad@cgiar.org* *www.cipotato.org* | *@cipotato* *@Cipotato* *@cip_cipotato*