In vitro conservation of sweetpotato - OP26

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46	Sep 14, 2010 11:49	Luis Rojas	Changed page structure for bilingual format
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43	Feb 15, 2010 17:42	Luis Rojas	Changes given by Ana Panta
42	Feb 05, 2010 11:34	Alison Light	Accessions that do not sucessfully produce six stock tubes (two planlets per tube), will continue subculturing until six stock tubes are achieved.
41	Feb 04, 2010 16:59	Ana Panta	

INTRODUCTION

In vitro sweetpotato germplasm conservation utilizes tissue culture techniques reducing plantlets growth. This is done applying incubation at low temperature. The methods described below allow the medium-term conservation of about 5,500 sweetpotato genotypes, following subculture procedures approximately every one year .

GENERAL RULES

- Conservation can be long term or for a transitory term according to scientists' requests and material type. Germplasm and pathogen tested materials, as well as some research material accessions, are conserved long-term. However, most research material is conserved for a transitory-term. The name of the scientist responsible and conservation period data must be recorded in the tissue culture laboratory data base (TCL-DB).
- Accessions are grouped by health status: HS0, HS1, or HS2. Each group comprises three material types:
1) 'germoplasm', containing native cultivars, and wild genotypes from accessions requiring clonal propagation (non-producing botanical seed); these accessions are identified by the letter "G" in their corresponding laboratory code (Labcode).
2) 'pathogen tested', containing improved varieties and cultivars representing the crop diversity (core collection), made pathogen tested until 1999; they are identified with the letters "PT" in the Labcode.
3) 'research material' comprises all material in trust not corresponding to any group described before; they are mainly breeding materials and are identified by the letter "R" in their Labcode.

SCOPE

The scope of this procedure covers the conservation of all the *in vitro* sweetpotato germplasm in the genebank.

SAFETY

No specific safety requirements above the normal laboratory practices

MATERIALS

Equipment	
Autoclave	Medium dispenser
PHmeter	Analytical balance
Laminar flow chamber	Shakers
Refrigerator	Cultivation growth chamber
Oven	Microwave oven
Other materials	
Glass test tube	Cotton
Petri dishes	Alcohol
Forceps	Burner
Blades	Sterilizer
Saran wrap or Parafilm	

See Table 2 for media composition

See Preparation of cultivation media

PROCEDURE

1. Storage
1.1. *In vitro* cultures are stored in a chamber under controled conditions: 19-21º C, 2500 Lux* of illuminance with a photo-period 16 h of light and 8 h of dark (Fluorescent lamp COOL DAYLIGHT, 36W). Under these conditions the cultures can be viable for a period of 8-14 months approximately.
1.2. Six tubes per accession are conserved, two plantlets per tube. Each tube must be labeled with bar-coding and passport identifications: CIP's accession number, Labcode, cultivar name, health status (HS0, HS1, or HS2), and subculture date.
1.3. Fifteen accessions are stored per tube rack, 3-4 tube racks per shelf rack. Each tube rack should be labeled indicating its location. Each shelf rack has a number. Accession location must be recorded in the TCL-DB.
2. Viability Evaluation

2.1. Evaluate the cultures every 2-3 months.
2.2. Transfer the cultures from the conservation chamber (at 19-21°C) to the active collection chamber (at 23-25°C). The tube racks containing the accessions should be transported using a laboratory car.
2.3. Using a pocket PC, record the tube rack accessions following the Manual for Data Monitoring or Protocol for data monitoring - Item 3.
2.4. Verify that the accession's location recorded in the TCL-DB is correct.
2.5. Verify that absent accessions and those with one tube in stock are in subculturing. The cultures from these accessions should be in the active collection chamber. Using a pocket PC, find the accession's location, following the Monitoring Data Manual or Monitoring Data Protocol-Item 2.
2.6. Evaluate accessions in subculture. These should be in: a) routine renewal, b) treatments for increasing vigor, and c) microbial contaminant elimination treatments (Annex 1). The evaluation is visual using indicators specified in Table 1. When the cultures continue to have low viability and/or microbial contamination, repeat specific treatments.
2.7. Continue evaluating all other accessions present in the tube rack. Accessions found to have the category 'regular', as well as those with 'medium' category, should be subcultured for renewal. For this, four tubes should be transferred to another tube rack in a transitory location. For all cases, using a pocket PC, record evaluation results and the new transitory location.
2.8. Return the tube rack to the conservation chamber. Using the pocket PC, verify that its location is correctly recorded in the TCL-DB.
2.9. Place the transitory tube rack in the active collection chamber, in the corresponding site according to its Health Status (HS0, HS1 o HS2). Identify the tube rack with a plaque indicating the corresponding rack and shelf number.
2.10. Record stock and location of all accessions contained in the transitory tube rack in the data base.
2.11. Subculture in fresh medium no longer than one week after evaluation.
3. Cultures renewal
3.1. List and labeling
3.1.1. Each tube rack for processing is assigned to one operator. Transfer the tube rack with accessions to be processed to the transfer area.
3.1.2. Generate the accessions list following the Data Monitoring Manual.
3.1.3. Record the medium to be utilized (ie. MCB) in the TCL-DB.
3.1.4. Generate labels with barcoding only for accessions to be processed on that working day. DO NOT print additional labels for any reason. The operator is obligated to process all accessions for which labels were printed on the same day . Print six labels per accession. Prepare labels following the Manual for Data Monitoring or Protocol for data monitoring- Item 6.
3.2. Sub-culture
3.2.1. At least 30 minutes before starting subculture, turn on and disinfect the laminar flow chamber, turn on the tools sterilizer, and prepare all required materials and tools. Follow the "Best practices for in vitro culture laboratory" procedures.
3.2.2. Under aseptic conditions process the accessions one by one. Take tubes from one accession. Separate the six corresponding labels and leave ready for use after the accession subculturing.
3.2.3. Using a scalpel remove the sealing tube tape (ie. parafilm). Keep one label beside the new labels. This label will be placed on one new tube as a control.
3.2.4. Culture the plantlets following the Sweetpotato in vitro multiplication protocol. Use tubes (18 x 150 mm) containing MCB medium (Table 2). Place two stem segments, each comprising two buds, in each tube. Six tubes per accession are cultured.
3.2.5. Label the tubes. Verify that the label from mother plants has the same identificators as new labels.
3.2.6. Attach a saran wrap tape to each tube. Attach the labels onto the saran wrap tape. The old label should be attached to one of the six tubes. Seal tubes with permeable tape, parafilm.
3.2.7. After processing all accessions from one tube rack, place the rack in the active collection chamber. The incubation conditions are: 23-25º C, 3,000 - 4,000 Lux* of illuminance (Fluorescent lamp COOL DAYLIGHT, 36W) with a photoperiod of 16 h light and 8 h of dark. Record the location in the TCL-DB.
3.2.8. Contamination monitoring: - Five days after the sub-culture, evaluate the cultures for signs of microbial contamination (fungi, bacteria). - Using the pocket PC, generate the list of accessions. Evaluate visually and, following the procedures indicated in the Manual of Data Monitoring (or Protocol for data monitoring-Item 5), record the results in the TCL-DB. - Contaminated cultures are removed from the rack and placed inside a bag for autoclaving. The bag is transferred to the washing and preparation area for sterilization by autoclave. - If all tubes from one accession are contaminated, repeat the subculture using another stock tube from the conservation chamber. - Culture with no signs of contamination will continue incubation in the active collection chamber.

3.2.9. Viability monitoring: - After 15-20 more days of culture, evaluate viability indicators according to Table 1. First, generate the accessions list using the pocket PC and the Data Monitoring Manual. - Accessions found to be in the 'medium' or 'good' category should be transferred to another tube rack for transfering to the conservation chamber.
3.2.10. Accessions that do not sucessfully produce six stock tubes (two planlets per tube), will continue subculturing until six stock tubes are achieved.
3.2.11. After recording viability data, record cultures transferring to conservation chamber. - Using the pocket PC, select the option "Save" (down site) - Select option "Move accessions to CF (Cold chamber)"
3.2.12. Transfer the cultures to conservation chamber and locate in their corresponding place. Using the PC, verify that the transfer, stocks number, and location have been correctly recorded in the TCL-DB.
3.2.13. Evaluate viability every 2 months as indicated in item 2.

Table 1. Indicators used for viability evaluation of the *in vitro* sweetpotato collection

Viability Categories	Shoot / Stem Necrosis	Defoliation
Good	0-10%	0-20%
Medium	10-30%	20-50%
Bad	30-70%	50- 70%
Lost (died)	100%	100%

Table 2. Medium composition for sweetpotato *in vitro* conservation (MCB)

	MCB
MS salts (g/L)	4.3
Ascorbic acid (g/L)	0.2
Calcium nitrate (g/L)	0.1
Calcium panthotenate (mg/L)	2
L-Arginine (g/L)	0.1
Putrescine-HCl (mg/L)	20
Sucrose (g/L)	30
Phytagel (g/L)	3
pH	5.7
Temperature (°C)	19-23

Annex 1

Treatments for increasing vigor and eliminating microbial contaminants.

From each accession that is to be processed, the stock in better health status is transferred to one transitory tube rack, where all accessions requiring specific treatments are placed. The treatments are: a) accessions showing poor growth and rooting are transferred to highly nutritive media (ie. MPB supplemented with 10ml/L coconut milk); b) accessions showing fungi with or without bacterial contaminants are disinfected using calcium hypochloride (2.5%) and transferred to MPB fresh medium; c) accessions showing bacterial contaminants are transferred to MPB medium containing antibiotic (Claforan, 200mg/l); d) accessions showing bacterial contaminants resistant to Claforan are disinfected with calcium hypochloride (2.5%) and transferred to MPB medium containing antibiotic Rocephin (300mg/l).

Treatments for controlling contamination should be applied the same day as contaminants are detected. Transferring to highly nutritive media (i.e. MPB supplemented with 10ml/L coconut milk) should be done as soon as possible.

Composition for sweetpotato *in vitro* propagation medium (MPB)

MPB components	Quantity
Sales MS *	4.3 gr
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