Health status testing and virus elimination in sweetpotato - OP18

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INTERNATIONAL POTATO CENTER - CIP

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INTRODUCTION

Virus elimination will be carried out in all materials maintained or generated at CIP, and prior to its distribution. The virus elimination technique is based on thermotherapy and meristem culture. Clones, which have tested negative to all known pathogens, using enzyme-linked immunosorbent assay on nitrocellulose membranes (NCM-ELISA) and the indicator plant *Ipomoea setosa* test, can be distributed internationally.

SCOPE

This procedure covers the health testing and virus elimination of sweetpotato germplasm maintained in the genebank or generated at CIP prior to its distribution.

SAFETY

No specific requirements above the normal laboratory safety procedures.

See the following link for details of media preparation: Preparation of cultivation media

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	

PROCEDURE

Material
1. Starting material can came from *in vitro* plantlets from the CIP genebank, *in vitro* plantlets from outside CIP or from roots or cuttings.
2. In vitro plantlets from outside CIP must pass an incubation period of 'quarantine' before the initial health status testing.
3. Roots or cuttings are planted in pots under quarantine conditions.
Introduction to *in vitro* culture and multiplication
4. Introduce *greenhouse* material into *in vitro* according to the protocol for introduction to *in vitro* culture.
5. Culture one explant containing two buds for 5 weeks in an 18x150 mm test tube with MPB media (Table 1). This plantlet is considered the mother plant.
6. Multiply the mother plantlet into three test tubes. Place one explant containing two buds in an 18x150 mm test tube with MMB media. Two test tubes will be conserved as the stock HS0, and one test tube will be used for health testing.
Initial health status testing
7. Health status testing includes: NCM-ELISA, symptomatology observation and grafting stem cuttings into the indicator plant *Ipomoea setosa*. NASH test and PCR are optional to confirm the presence of some viruses for which antisera are not available.
8. One month-old *in vitro* plantlet is grown in jiffy for 30 days and then transferred to a pot for 30-45 days under greenhouse conditions.
9. Make grafts of two nodes from the basal part of each sweetpotato plant to two separated *I. setosa* plants three-weeks-old (both growing together in a pot).

10. Hold grafted- *I. setosa* plants for a minimum of 30 days for observation and recording of symptoms expression if any.
11. Assay the *I. setosa* plants by NCM-ELISA test with available antisera (SPFMV, SPLV, SPMMV, SPVG, SPMSV, SPCFV, C-6 virus, SPCSV, SPCaLV, and CMV). See below for definition of virus
12. Prune negative sweetpotato plants and allow them to grow to at least 10-15 nodes before doing a second round of grafting, NCM-ELISA test, and recording symptoms to confirm results.
13. After the initial virus testing, virus positive accessions are submitted to the virus elimination process.
14. Pathogen-free clones are multiplied and included in the *in vitro* genebank as HS2.
Virus elimination: thermotherapy, meristem isolation and culture
15. Multiply the stock HS0 plantlet into four 25x150 mm test tubes with MPB medium, placing two explants in each tube.
16. 10 -15 days old *in vitro* plantlets are submitted to thermotherapy at 35-37°C during 1 month.
17. Eight meristems, 0.2-0.35 mm long, are excised and cultivated in 13x100 mm test tubes containing meristem medium 1 (MM1) (Table 1).
18. Meristems are sub-cultivated with MM1 medium at 3 and 6 days after meristem excision, then they are sub-cultivated every 15 days with MM3 medium (Table 1), until a rooted plantlet with at least three nodes is obtained
Health testing
19. After plantlets are obtained from meristem culture, they are propagated and tested as described above to detect any remaining virus infection.
20. Accessions that result pathogen-free are multiplied and included in the *in vitro* genebank as HS2. Identity verification must be conducted on these materials before their distribution.
21. Accessions that result virus positive must enter the cleaning process again (thermotherapy and meristem culture).

Table 1. Multiplication and meristem media composition for *in vitro* culture of sweetpotato

	MPB	MM1	MM3
MS salts (g/L)	4.3	4.3	4.3
Ascorbic acid (g/L)	0.2	0.1	0.1
Calcium nitrate (g/L)	0.1	0.1	0.1
Calcium panthotenate (mg/L)	2	2	2
Gibberellic acid (mg/L)	10	20	10
L-Arginine (g/L)	0.1	0.1	0.1
Putrescine-HCl (mg/L)	20	20	20
Sucrose (g/L)	30	30	30
Coconut milk (mL/L)	—	10	10
Agar (g/L)	—	6	6
Phytagel (g/L)	3	—	—
pH	5.7	5.7	5.7

Virus names

SPFMV: *Sweetpotato feathery mottle virus*; SPLV: *Sweetpotato latent virus*; SPMMV: *Sweetpotato mild mottle virus*; SPVG: *Sweetpotato virus G*; SPMSV: Sweetpotato mild speckling virus; SPCFV: Sweetpotato chlorotic fleck virus; C-6 virus; SPCSV: *Sweetpotato chlorotic stunt virus*; SPCaLV: Sweetpotato caulimo-like virus; and CMV: *Cucumber mosaic virus*.