Introduction of sweetpotato to in vitro culture - OP58

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30	Feb 16, 2010 09:47	Brenda Zea	
29	Feb 04, 2010 15:10	Alison Light	Procedure 1. At least one dose should be applied 7 days before initiating the process. Annex 1 no 6. There is a word missing at the end of the sentence - ", and a negative control test tube containing only ?"
28	Jan 07, 2010 16:04	Luis Rojas	
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INTRODUCTION

The establishment of tuber crops into *in vitro* culture requires the isolation of vegetative structures (explants) containing buds. Explants should be inoculated in vessels containing a nutritive substrate (culture medium) where buds will grow to became whole plantlets. For the case of sweetpotato, the preferred explant for *in vitro* culture is the apical or axilar bud of the stem. Because plant material may carry superficial contaminants, a surface sterilization process to eliminate microorganisms is conducted. Fungi and bacteria that are not adequately eliminated can grow and contaminate the culture media damaging the explants in culture. The procedure detailed below has been found to reduce contamination to less than 5% on plant material samples taken from potted plants.

SCOPE

The procedure covers the introduction of sweetpotato crops

SAFETY

No specific requirements above normal good laboratory safety practices

MATERIALS

PROCEDURE

1. Select healthy mother plants grown in pots for 2 months under greenhouse conditions. Previously, plants should be treated with a fungicide chemical, 1 g/L benomil (Farmathe), by spraying. At least one fungicide treatment should be applied 7 days before initiating the process.

2. Remove the leaves from a stem containing at least six buds. Cut stem segments containing one bud each and place them in a paper bag. Avoid the explants wilting after removal.

3. Move the samples to the laboratory and place them in a container with 150 mL of disinfecting solution (1 ml/L fenpyroximate (Kenyo), 20 ul/L sppiromesifen (Oberon-240SC), 1 g/L benomil (Farmathe), and 1 mL/L of Tween 20) for 10 minutes; this will control mites and fungi. Rinse with running water three times and wash with 150 mL of 70% alcohol for 30-60 seconds.

4. Discard the alcohol and add 150 mL of 2.5% sodium hypochlorite solution; wait 15 min.

5. In a laminar flow chamber, remove the samples from the hypochlorite solution and rinse with 150 mL sterilized water five times, shaking the container between each cycle. The first and second rinse are done immediately after discarding the hypochlorite solution, the third and fourth rinse are done after 5 minutes and the fifth rinse is done 10 minutes later. In the last cycle do not discard the water.

6. Remove the explants from water, draw off the excess water and place in a sterilized petri dish. Using a blade, remove tissue bleached by the hypochlorite treatment (the tissue turns white).

7. Place the explant on MIB culture medium (Table 1) in an 18x150 mm test tube. One explant is cultured per vessel. Cultures are incubated in a controlled climate chamber at 23-25°C with a photoperiod of 16 h of light and 8 h dark.

8. After 1 week, cultures are visually evaluated in order to detect bacteria and fungi contamination. All vessels contaminated with bacteria or fungi are incinerated.

9. After 4 to 5 weeks of culture, clones are submitted to the bacteria detection test (Annex 1) using nutritive broth (NB) and are subcultured to one 18x150 mm test tube with conservation medium (MPB) (Table 1).

10. When results of the NB test are obtained, select a vigorous and bacteria negative clone and discard the other tubes.

11. Selected plantlet is subcultured into six 18x150 mm test tubes with MPB culture medium and included in the *in vitro* genebank collection.

Table 1. Media composition for sweetpotato introduction to *in vitro* culture and micropropagation

	MM3	MCB
MS salts (g/L)	4.3	4.3
Ascorbic acid (g/L)	0.1	0.2

Calcium nitrate (g/L)	0.1	0.1
Calcium panthotenate (mg/L)	2	2
Gibberellic acid (mg/L)	10	-
L-Arginine (g/L)	0.1	0.1
Putrescine-HCl (mg/L)	20	20
Sucrose (g/L)	30	30
Coconut milk (mL/L)	10	
Agar (g/L)	---	
Phytagel (g/L)	2.8	3
pH	5.7	5.7

Annex 1

***In vitro* culture bacteria testing**

1. After 30 days of culture, test possible bacterial contamination, culturing plant segments into Nutritive Broth (NB) (Table A) for detecting bacteria growth.
2. Select one test tube per accession and under aseptic conditions, take off plantlet and place on a petri dish.
3. Remove the leaves and roots and cut the stem in 2-3 nodal segments.
4. Isolated explants are transferred to 18 x 150 mm test tubes containing MPB.
5. Residual material (basal part of plantlet) is cut finely until obtaining a mince of plantlet leaves, stems and roots.
6. This mince of residual material and a little portion of culture medium is inoculated into a 16 x 150 mm test tubes containing 3 ml of NB. Include a positive control test tube containing original bacteria-contaminated accession, and a negative control test tube containing only.
7. Incubate the cultures at 30^{o}C for 72 hours. Evaluate bacteria presence at 24, 48 and 72 hours. Negative cultures follow additional culture at room temperature.
8. Cultures showing bacteria growing (cloudiness) in NB indicates that the clone is bacteria positive. Bacteria negative clones must be returned to *in vitro* genebank
9. When tested clone results bacteria positive, testing is repeated with another clone of the same accession.

Table A. Nutritive broth composition for detection of bacterial contaminants in sweetpotato *in vitro* culture

Peptone (g/L)	5
Beef extract (g/L)	1
Yeast extract (g/L)	2
Glucose (g/L)	10
Sodium chloride (g/L)	5
pH	7.0

Preparation of media

1. Mix the ingredients in 1 liter of distilled water
2. Stir until dissolution is complete
3. Measure the pH
4. Distribute 3 ml in 16x150mm test tubes

5. Sterilize in autoclave at 121^{o}C for 15 minutes
6. Store at 4^{o}C