Sweetpotato virus indexing procedure - OP23

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INTRODUCTION

Virus indexing combines knowledge of viruses with methodologies for their detection to assure the effective safe movement of sweetpotato germplasm.

Due to low virus titers and the absence of symptoms from single infections in sweetpotato by most viruses, grafting onto indicator plants is often required to boost titers and detect viruses reliably. The commonly used indicator plant is *Ipomoea setosa,* which is susceptible to most viruses infecting sweetpotato.

This standard procedure has been established based on current information for virus testing of sweetpotato germplasm, with the recognition that it will need to be revised as new information becomes available. The procedure includes symptomatology in sweetpotato plants grown in greenhouse (pots), as well as in *Ipomoea setosa* plants grafted with scions of the basal part of sweetpotato. Virus detection and identification is confirmed by serology with antisera available to known viruses. A second round of the process is usually carried out to confirm results.

SCOPE

Grafting allows transmission of all viruses. Most sweetpotato viruses infect *Ipomoea setosa* causing visible symptoms and reaching higher concentrations, thus facilitating their detection by serological (NCM-ELISA) or molecular (PCR, RT-PCR) tests. Scions for grafting taken from the lower part of the sweetpotato plant have the highest probability of containing virus for testing. Factors that could affect reliability for virus detection in sweetpotato

SAFETY

A laboratory coat should be worn at all times.
Both source and indicator plants should be grown in as near optimal conditions as possible to stimulate rapid and luxuriant growth.

New razor blades should be used when grafting per accession to minimize contamination.
Good control of insect vectors (e.g whiteflies, aphis) of viruses into the greenhouse.
Wear dry and clean gloves and/or use forceps when manipulating the nitrocellulose membrane during serological tests.

MATERIALS

See lists of materials in the procedures for Indicator plant diagnostic procedure - OP22 and NCM-ELISA procedure - OP21

PROCEDURE

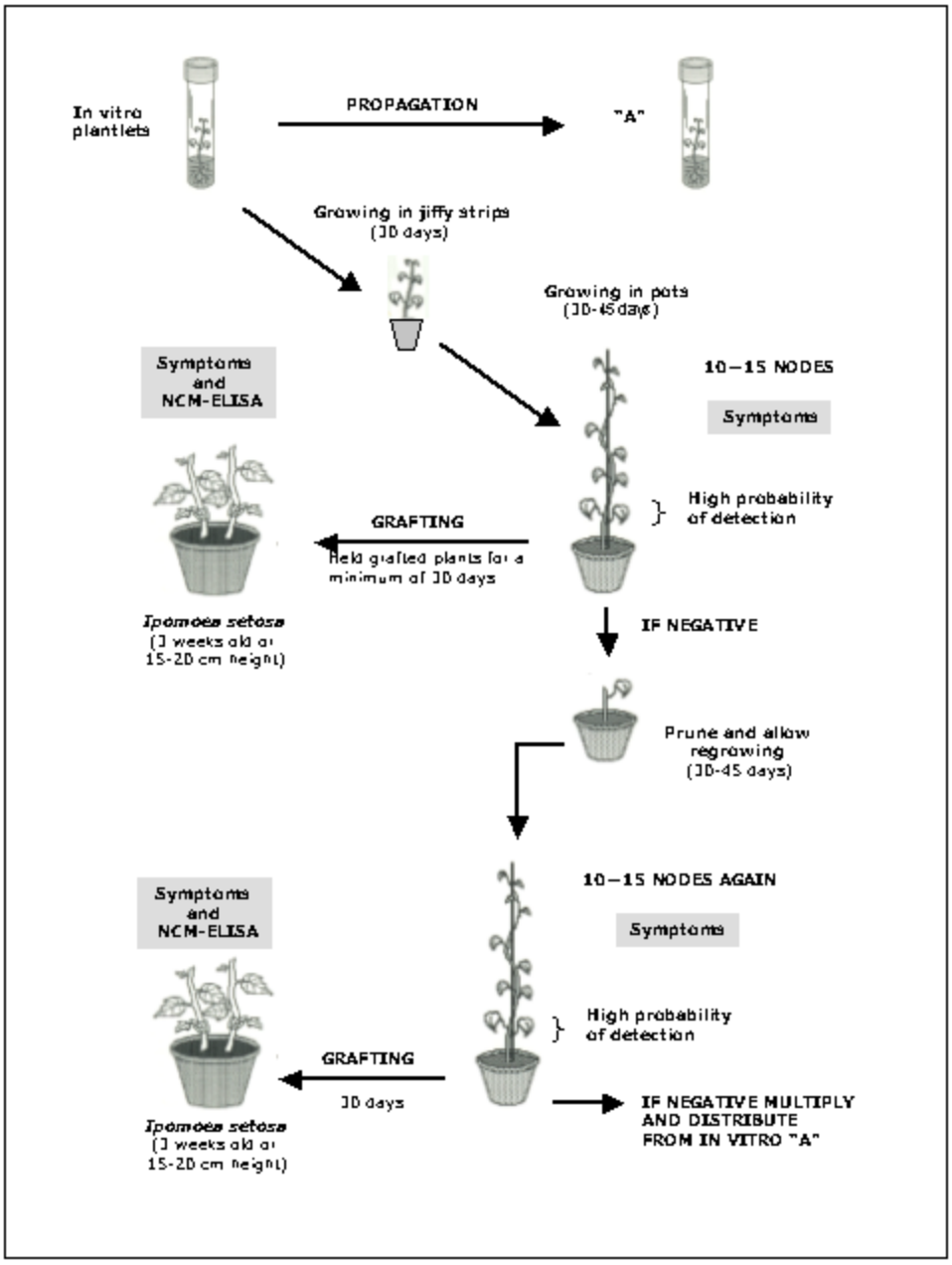

Figure 1. Indexing procedure for sweet potato viruses. NCM-ELISA is performed for 10 viruses (SPFMV, SPLV, SPVG, SPMSV, SPMMV, SPCSV, SPCFV, C-6, SPCaLV, and CMV).

Grow the *in vitro* plantlets in jiffy strips for 30 days, and then in a pot for 30-45 days in an insect-free greenhouse until their stem has at least 10-15 nodes.

During the growing period, observe the sweetpotato plant twice for any symptoms (see FAO guidelines or CIP Sweetpotato virus symptom guidance material for sweetpotato symptoms). Symptoms are checked when the plant is grafted and 1 month after grafting. Record results of symptoms expression on the sweetpotato summary record sheet. Transfer any recorded symptom and its digital image to the corporative server through wireless LAN using a hand held computer.

Make grafts of two nodes from the basal part of each sweet potato plant to two separated three-week-old *I. setosa* plants (both growing together in a pot).

Hold grafted *I. setosa* plants for a minimum of 30 days for observation of symptom expression (see FAO guidelines or CIP Sweetpotato virus symptom guidance material for sweetpotato symptoms) and the recording of symtpoms, if any, on the Sweetpotato summary record sheet.

Transfer any recorded symptom and its digital image to the corporative server through wireless LAN using a hand held computer. The plants are observed three times during this period at 10, 20 and 30 days of growth, and results of observations recorded on the Sweetpotato summary record sheets.

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). Record results on the NCM-ELISA record sheetand on the Sweetpotato summary record sheets. Transfer the NCM-ELISA results to the corporative server through wireless LAN using a hand held computer. 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. Record of symptoms expression and NCM-ELISA results are done as previously on the NCM-ELISA record sheet and on the Sweetpotato summary record sheets. Transfer the NCM-ELISA results to the corporative server through wireless LAN using a hand held computer.

If all tests are negative, plantlets originated from the same meristems are approved for distribution.

Internal quality control

To confirm success on grafting, some stems from one healthy *I. setosa* and scions from a SPFMV-infected sweet potato plant are grafted on to *I. setosa* plants as negative and positive controls, respectively. Both controls are used for each batch of grafts.

For NCM-ELISA, the last six spaces for dots on the membranes are used for controls (two for buffer, two for negative and two for positive controls).

Further information on quality control is given in Indicator plant diagnostic procedure - OP22 and NCM-ELISA procedure - OP21.