3. Sweetpotato Germplasm Health Testing

Protocol for health testing of sweetpotato material has been modified to increase the range of viruses tested and to reduce the risk of any disease escape. The primary diagnostic tool is indexing by *Ipomoea setosa*. In-vitro diagnosis is carried out by NCM-ELISA for 10 viruses: SPFMV, SPMMV, SPVG, SPLV, SPMSV, SPCFV, C-6, SPCSV, SPCaLV, CMV. Some grafted *Ipomoea setosa* show symptoms caused by virus infection but they do not react to any of the above antisera. Symptoms observed in *I. setosa* resemble to those caused by begomoviruses (confirmed by PCR), potyviruses, and caulimoviruses. The procedure for Sweetpotato is given in Annex 2.

During 2006-2007 , a total of 737 sweetpotato accessions were made virus-tested (Table 7).

A further 197 accessions are in the process of virus testing during 2007.

A validation process is being carried out for the methods applied to determine the health status of the material.

3.1 Methods

A total of 268 accessions were submitted to the health status testing procedure (Annex 2) for producing HS2 materials for national and international distribution during 2007. Accessions resulting in virus-infected were transferred to the virus elimination process and tested to verify the efficiency of the cleaning method.

3.2 Results and discussion

Initial health status testing applied in 2006/2007 to sweetpotato accessions, prior to enter the cleaning process, shows that 65% (479 accessions) sweetpotato were found pathogen negative at HS2. They were transferred to the pathogen-tested collection and are ready for national and international distribution.

Virus-infected accessions were submitted to virus cleaning procedure (thermotherapy and meristem culture). 110 sweetpotato accessions have completed the procedure, 275 are still on-going, and 41 accessions remained infected with one or two viruses; a second attempt is being performed to clean up these accessions.

Validating health status testing applied to 737 sweetpotato accessions confirmed the highest status (HS2) of 479 sweetpotato accessions (65%); testing for further 197 accessions is still in process for 2007. (Table 7).

Table 7. Sweetpotato accessions processed for health testing and virus elimination (2006 -2007)

Crop	Total processed	Virus tested (HS2)	Virus infected	In process
Sweetpotato	737	479	258	197

Out of the validated accessions that were found to be virus-infected analysis showed that SPFMV and SPVG were in higher frequency of infection (see Table 8).

Table 8. Incidence of virus infection in potato accessions detected during the validation process

	Accessions infected	
Virus detected by ELISA	**Number**	**Percentage %**
SPFMV	56	20
SPVG	30	10
SPCSV	5	2
SPCFV	3	1
SPCaLV	3	1
Not determined- +ve index –ve diagnosis	192	66
Total		**100**

Diagnosis is done by NCM-ELISA for 10 viruses: SPFMV, SPMMV, SPVG, SPLV, SPMSV, SPCFV, C-6, SPCSV, SPCaLV, CMV. Analysis of the relationship between detection by Index and in-vitro diagnosis (see Table 9) indicates that some grafted *Ipomoea setosa* show symptoms caused by virus infection but they do not react to any of the above antisera. Typical symptoms observed in *I. setosa* resemble to those caused by begomoviruses (possible confirmed by PCR), potyviruses, and caulimoviruses.

Table 9. Number of accessions detected by indexing and diagnostic method applied during the health testing of sweetpotato accessions.

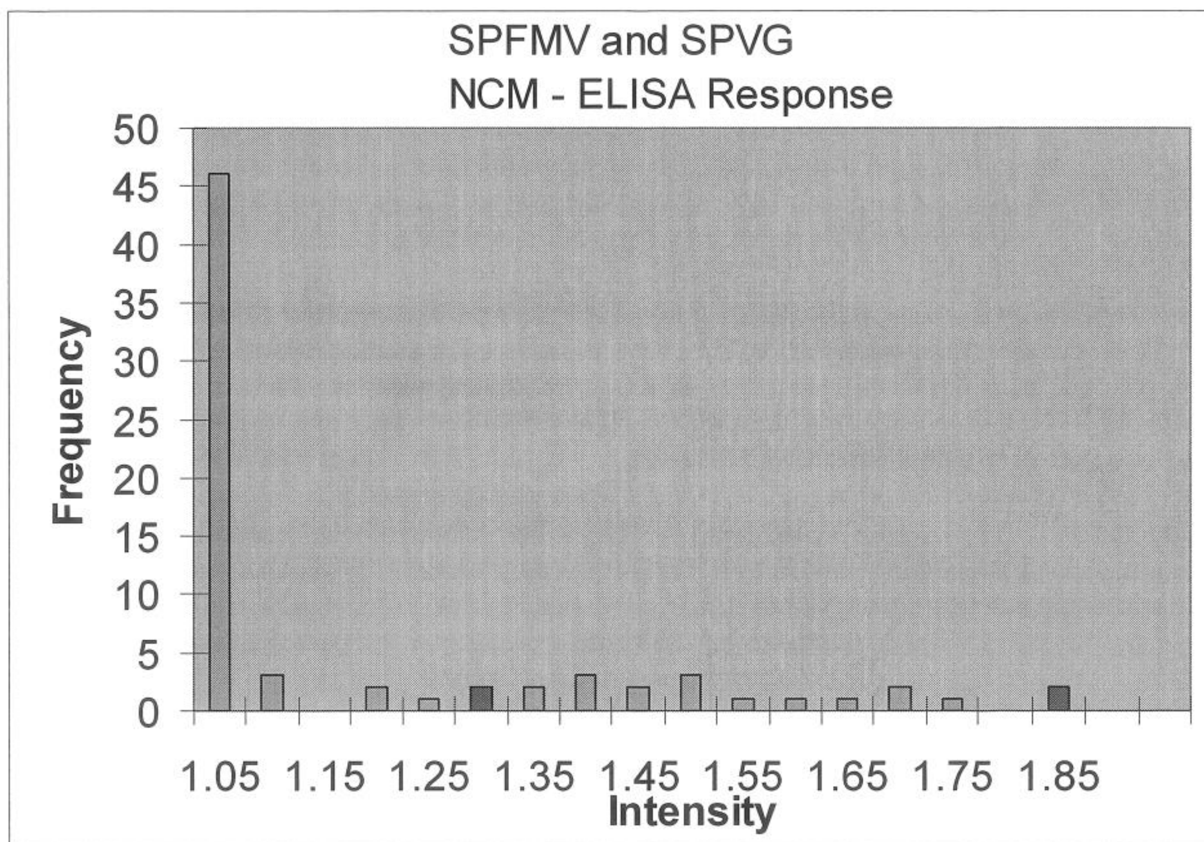
	+ve Diagnosis	-ve Diagnosis	TOTAL
+ve Index	66	192	258
-ve Index	2	479	481

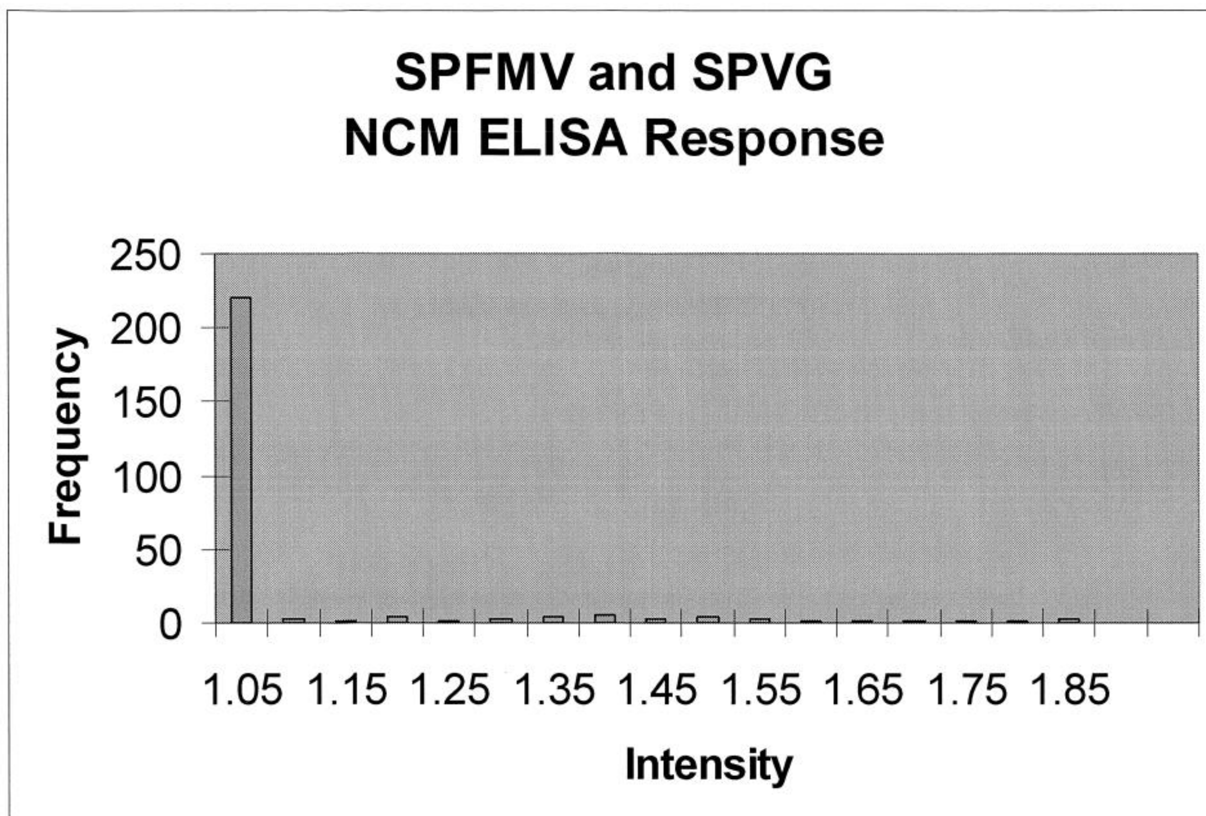
3.3 In-vitro Diagnostic Test Validation

Sweetpotato Feathery Mottle Virus (SPFMV)

Occurrence :	World-wide
Economic importance :	Over 50% (as a component of SPVD)
Method of detection	NCM-ELISA
	Indexing *I. setosa*
	Symptoms on grown on plant
Source of Antibodies :	CIP polyclonal antibody

ELISA Response data for SPFMV and SPVG





Sweetpotato Mild MottleVirus (SPMMV)

Occurrence :	Asia, Africa, PNG, India, Egypt, New Zealand
Economic importance :	Around 40% (in co-infection with SPCSV)
Method of detection	NCM-ELISA
	Indexing *I. setosa*
	Symptoms on grown on plant
Source of Antibodies :	CIP polyclonal antibody

Sweetpotato Virus G (SPVG)

Occurrence : Asia, Africa, Egypt, Barbados, USA, Peru

Economic importance :	
Method of detection	NCM-ELISA
	Indexing *I. setosa*
	Symptoms on grown on plant
Source of Antibodies :	CIP polyclonal antibody

Sweetpotato Latent Virus (SPLV)

Occurrence :	Asia, Africa, India, Egypt
Economic importance :	

Method of detection NCM-ELISA
Indexing *I. setosa*
Symptoms on grown on plant
Source of Antibodies : CIP polyclonal antibody

Sweetpotato Mild Speckling Virus (SPMSV)

Occurrence : Asia, Africa, New Zealand, S. America
Economic importance :
Method of detection NCM-ELISA
Indexing *I. setosa*
Symptoms on grown on plant
Source of Antibodies : CIP polyclonal antibody

Sweetpotato Chlorotic Fleck Virus (SPCFV)

Occurrence : Asia, Africa, New Zealand, S. America Cuba
Economic importance : Over 50% (SPVD), 30% (single infection)
Method of detection NCM-ELISA
Indexing *I. setosa*
Symptoms on grown on plant
Source of Antibodies : CIP polyclonal antibody

C-6 Virus

Occurrence : USA, Peru, Dom.Rep, Indonesia, Cuba
Economic importance : Around 50% (in co-infection with SPCSV)
Method of detection NCM-ELISA
Indexing *I. setosa*
Symptoms on grown on plant
Source of Antibodies : CIP polyclonal antibody

Sweetpotato Chlorotic Stunt Virus (SPCSV)

Occurrence : Worldwide
Economic importance : Over 50% (as component of SPVD), 30% (single infection)
Method of detection NCM-ELISA
Indexing *I. setosa*
Symptoms on grown on plant
Source of Antibodies : CIP polyclonal antibody

Sweetpotato Caulimo-like Virus (SPCaLV)

Occurrence : South Pacific Region, Madeira, China, Egypt, Puerto Rico, Uganda, Kenya, Nigeria
Economic importance :

Method of detection	NCM-ELISA Indexing *I. setosa* Symptoms on grown on plant
Source of Antibodies :	CIP polyclonal antibody

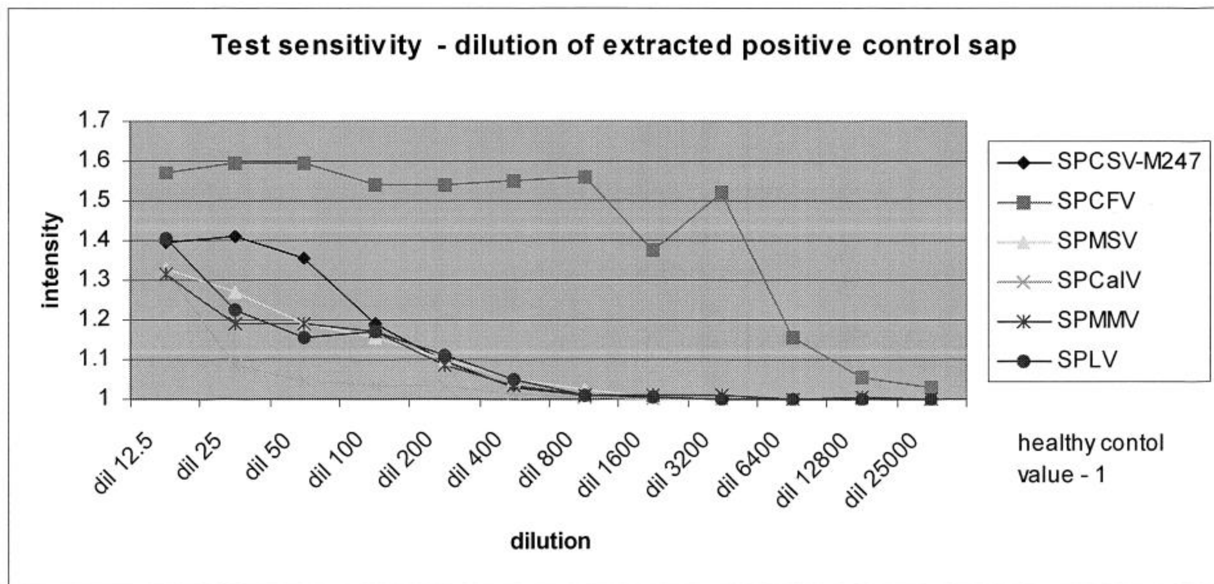
Cucumber Mosaic Virus – CMV

Occurrence :	Israel, Egypt, Kenya, Uganda, Japan,
Economic importance :	40% (in coinfection with SPCSV)
Method of detection	NCM-ELISA Indexing *I. setosa* Symptoms on grown on plant
Source of Antibodies :	CIP polyclonal antibody

Assessment of NCM-ELISA Method Sensitivity

Sap from positive control material was extracted and diluted to different levels of concentration. The response of the NCM-ELISA was measured for the different dilutions.

Results of the responses of different viruses and virus strains are given below.



Test sensitivity - dilution of extracted positive control sap

Intensity: 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6

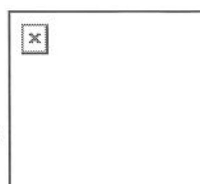
Dilution: dil 12.5, dil 25, dil 50, dil 100, dil 200, dil 400, dil 800, dil 1600, dil 3200, dil 6400, dil 12800, dil 25000

SPVD (sweetpotato 1)
SPFMV-RC (I.nil)
SPFMV-C (I.nil)
SPFMV-YV (I.nil)
SPFMV-C1 (I.nil)
SPVD (sweetpotato 2)
Healthy
Healthy (I.nil)

Annex 2

Health status testing and virus elimination in sweetpotato

International Potato Center, P.O. Box 1558. Lima, Peru.

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 (Figure 1). 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.

PROCEDURE

Material

1. Starting material can came from *in vitro* plantlets from 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 *in vivo* material into *in vitro* according to the protocol for introduction to *in vitro* culture.

5. Culture one explant containing 2 buds for 5 weeks in an 18x150 mm test tube with MMB media (Table 1). This plantlet is considered the mother plant.

6. Multiply the mother plantlet into three test tubes. Place one explant containing 2 buds in 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 presence of some viruses for which antisera are not available.

8. One month-old in vitro plantlet is growing 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 sweet potato plant to two separated *I. setosa* plants 3 weeks old (both growing together in a pot).

10. Held grafted- *I. setosa* plants for a minimum of 30 days for observation of symptoms expression and recording 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 sweet potato 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 accession 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 MMB medium, placing 2 explants in each tube.

16. 10 -15 days old in vitro plantlets are submitted to thermotherapy at 35-37°C during one month.

17. Eight meristems of 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 at MM1 medium at 3 and 6 days after meristem excision, then every 15 days are sub-cultivated at MM3 medium (Table 1), till obtain a rooted plantlet with at least 3 nodes

Health testing

19. After plantlets are obtained from meristem culture, they are propagated and tested as described above (2.3) to detect any remaining virus infection.

20. Accessions that resulted pathogen-free are multiplied and included in the *in vitro* genebank as HS2. Identity verification must be conducted to these materials before their distribution.

21. Accessions that resulted 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

	MMB	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	40	30
Coconut milk (mL/L)	—	10	10
Agar (g/L)	—	6	—
Phytagel (g/L)	3	—	2.8
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*.