Germplasm Virus Indexing: Supporting ISO/IEC 17025 Accreditation

J. Arellano, A. Perez, M. Meza, R. Lara, B. Zea, J. Cruzado, J. Kreuze and S. Fuentes

April 12, 2012

Outline

- **Introduction**
- **ISO/IEC 17025 accreditation**
- **Virus indexing procedure**
- **Analysis of indexing results**
- **Research derived from indexing**
- **Maintaining ISO accreditation**
- **New molecular tests (validation)**

Outline

- **Introduction**

Fact:

CIP is the custodian of world's largest in vitro germplasm

Number of accessions maintained in CIP's *in vitro* germplasm (April 04, 2012)

Sweetpotato: 5,757	**Potato:** 8,557

Data provided by B. Zea

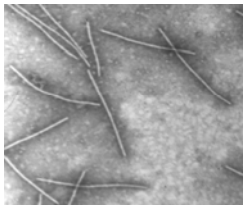
Number of accessions distributed internationally

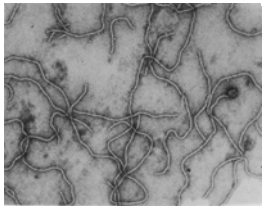
	Year						
Crop	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**Average**
Sweetpotato	291	939	394	632	206	899	**560**
Potato	413	172	999	512	723	611	**571**
TOTAL	704	1,096	1,341	1,122	886	1,510	**1, 131**

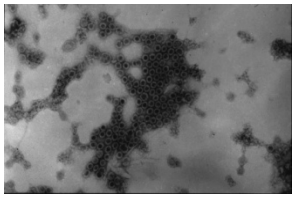
Data provided by R. Vollmer, E. Grande and E. Rojas

Virus-free *in vitro* germplasm (HS2)

Viruses affecting sweetpotato





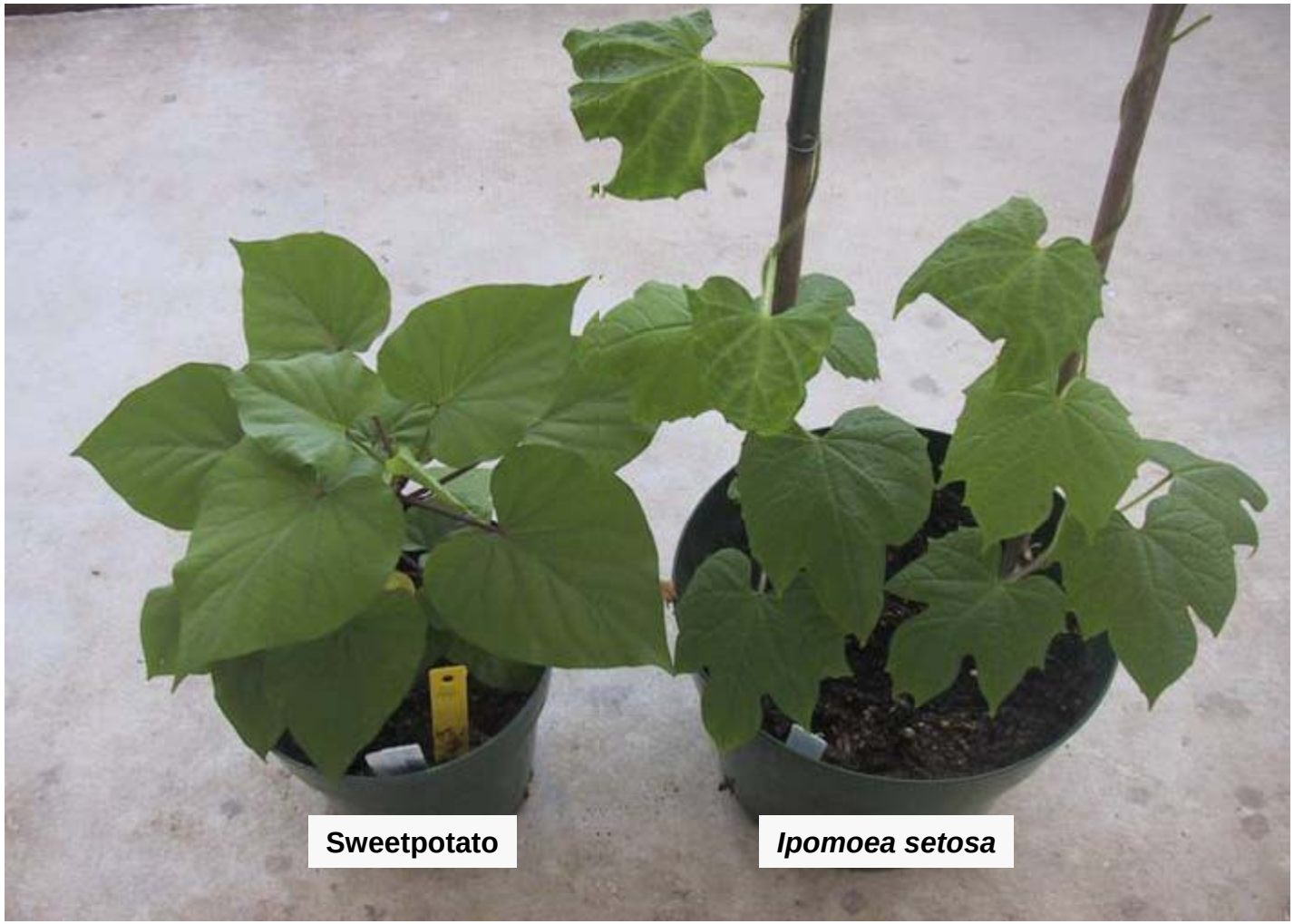


> 30 viruses

Symptoms induced by virus infection

Symptoms induced by virus infection

Symptomless infection



Virus Indexing

- **It combines the knowledge of the viruses with the methodologies available for their detection (to ensure effective safe movement of germplasm)**

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- **Guidelines from the "Safe Movement of Sweet Potato Germplasm" (1989)**

It can be revised and modified over time

Virus Indexing

- **It combines the knowledge of the viruses with the methodologies available for their detection (to ensure effective safe movement of germplasm)**

- **Guidelines from the "Safe Movement of Sweet Potato Germplasm" (1989)**

- **Guideline on our SP virus indexing includes:**

  - Observation of symptoms on SP plants

  - Grafting to *Ipomoea setosa*

  - Serological test (NCM-ELISA) from grafted plants

  - Molecular test (PCR)

Outline

- Introduction
- **ISO/IEC 17025 accreditation**

Facts about CIP's ISO Accreditation:

- **CIP awarded the ISO/IEC 17025 accreditation on February 2008 and applies this ISO Standard for the management and distribution of CIPs germplasm.**

ISO/IEC 17025

UKAS
UNITED KINGDOM ACCREDITATION SERVICE

Testing laboratory
No. 4229

For safe and secure germplasm movement.

Source: CIP's ISO Accreditation Website

Facts about CIP's ISO Accreditation:

- **CIP awarded the ISO/IEC 17025 accreditation on February 2008 and applies this ISO Standard for the management and distribution of CIPs germplasm.**

- **ISO/IEC 17025 is a worldwide Quality Standard that sets down requirements for the competence of testing laboratory.**

Management Requirements

- Complaints
- Control of records
- Corrective and preventive actions
- Document control
- General Requirements
- Improvement
- Internal audits
- Management review
- Non-conforming Work
- Purchasing of services and Supplies
- Review of requests for germplasm material
- Service to clients
- Subcontracting
- Technical Management and Quality Manager

Technical Requirements

- Accommodation and environment
- Copy of Quality Control procedures
- Germplasm Health Statement
- Handling of plant materials
- Measurement traceability
- Personnel and training
- Quality Control procedures
- Selection and validation of diagnostic methodology

Source: CIP's ISO Accreditation Website

Facts about CIP's ISO Accreditation:

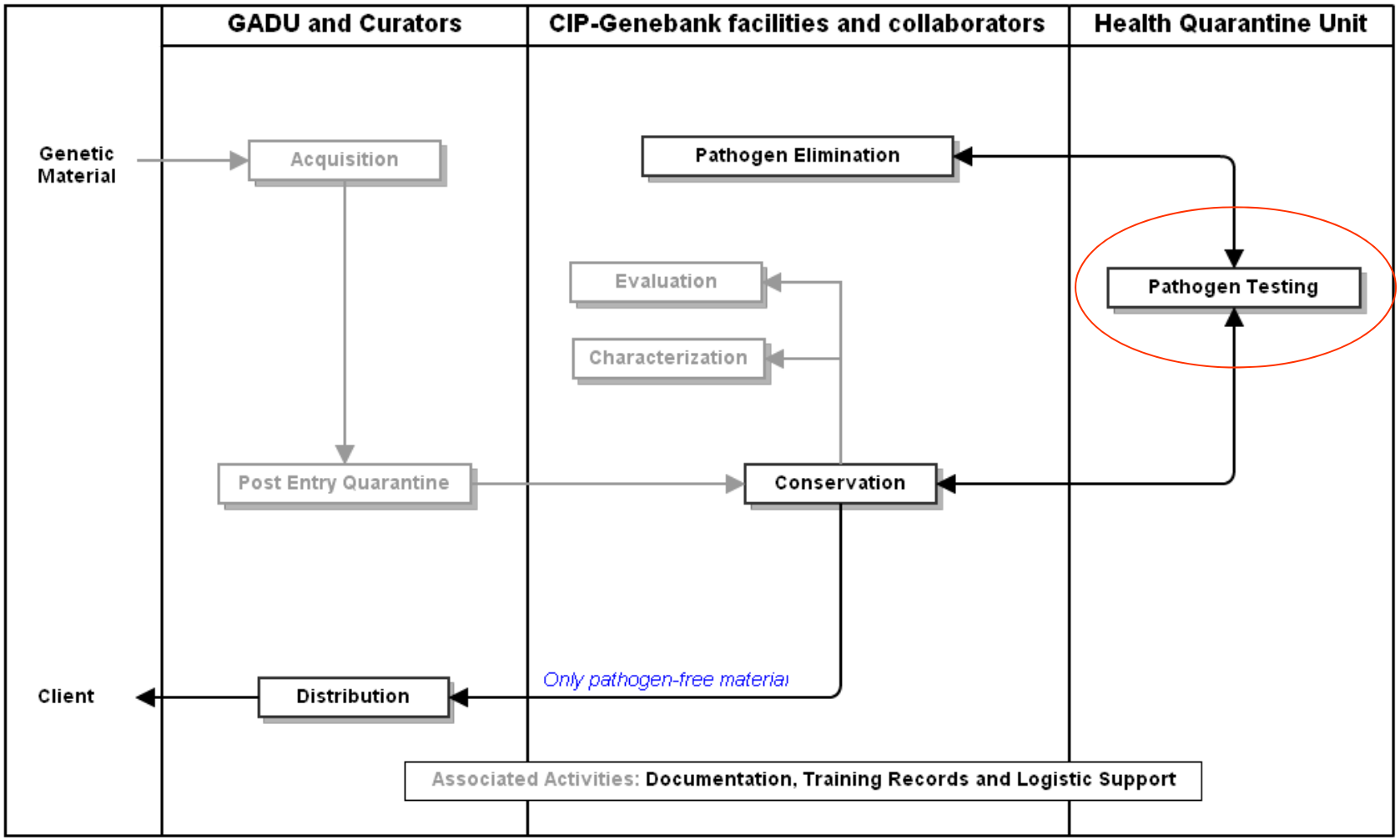
- **ISO/IEC 17025 is a worldwide Quality Standard that sets down requirements for the technical competency of testing laboratory.**

- **CIP awarded the ISO accreditation on 22 February 2008 and applies this ISO Standard the for management and distribution of CIP-HQ's germplasm.**

- **With ISO accreditation, CIP gives the users of the Genebank a visible assurance of the quality of the germplasm being distributed.**

Source: CIP's ISO Accreditation Website

WORKFLOW 1 (WF1) : Acquisition, Post Entry Quarantine, Consevation, Pathogen Testing, Pathogen Elimination and Distribution of materials in CIP-Genebank: Overview



In gray color: non-accredited process

Source: CIP's ISO Accreditation Website

List of Pathogen Detection In-Vivo Diagnostic Methodology

1. Test Covered by the UKAS Scope of Accreditation

Methodology	Pathogens Detected
Potato	
DAS-ELISA	PVX, PVY, PLRV, PVS, APMoV, APLV, PYV, AVB-O
NASH	PVT, PSTVd
Sweetpotato	
NCM-ELISA	SPFMV, SPLV, SPVG, SPMSV, SPMMV, SPCFV, C-6 virus, SPCSV, SPCV (former as SPCaLV), CMV

2. Other Tests (to be incorporated into the Accreditation scope with time)

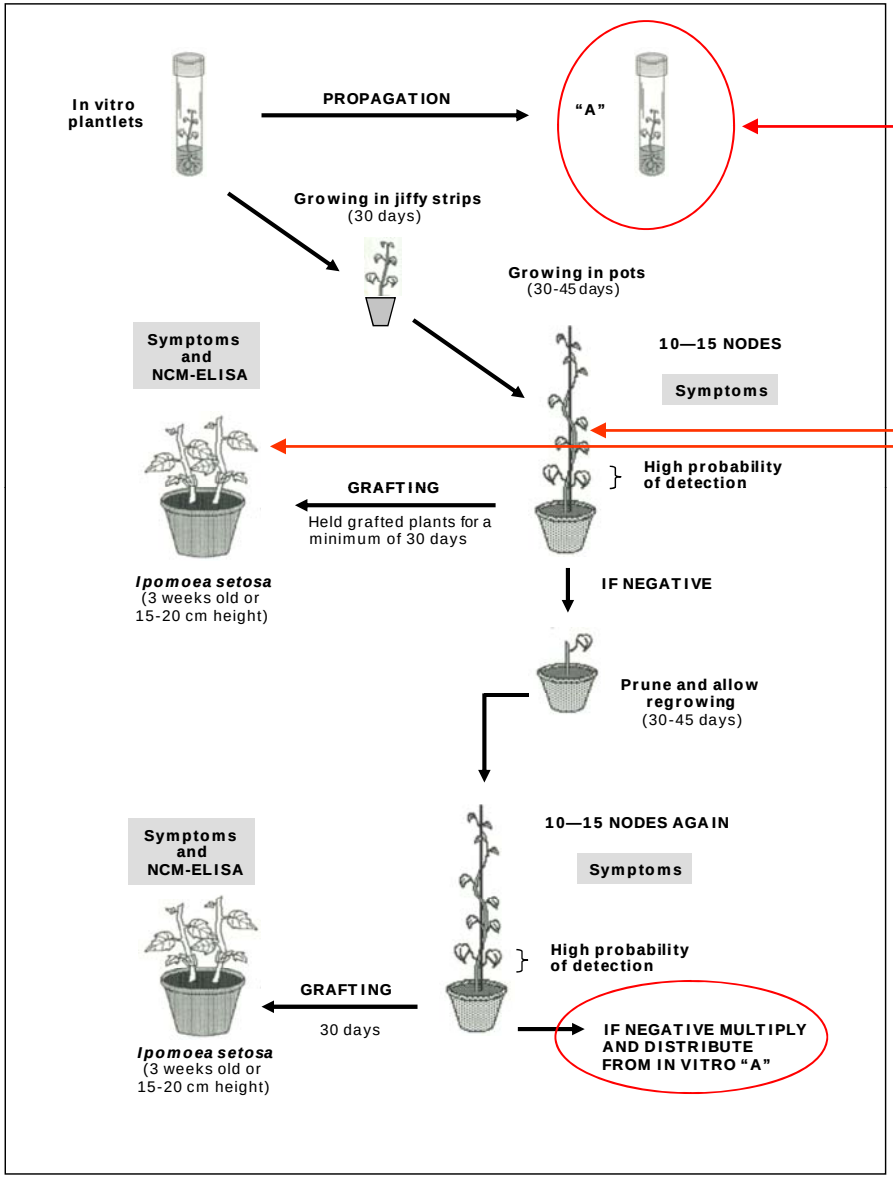
Methodology	Pathogens Detected
Potato	
PCR	Phytoplasmas, Begomoviruses transmitted by whiteflies, PYVV
Sweetpotato	
PCR	Begomoviruses including SPLCV, Phytoplasmas

Outline

- Introduction
- ISO/IEC 17025
- **Virus indexing procedure**

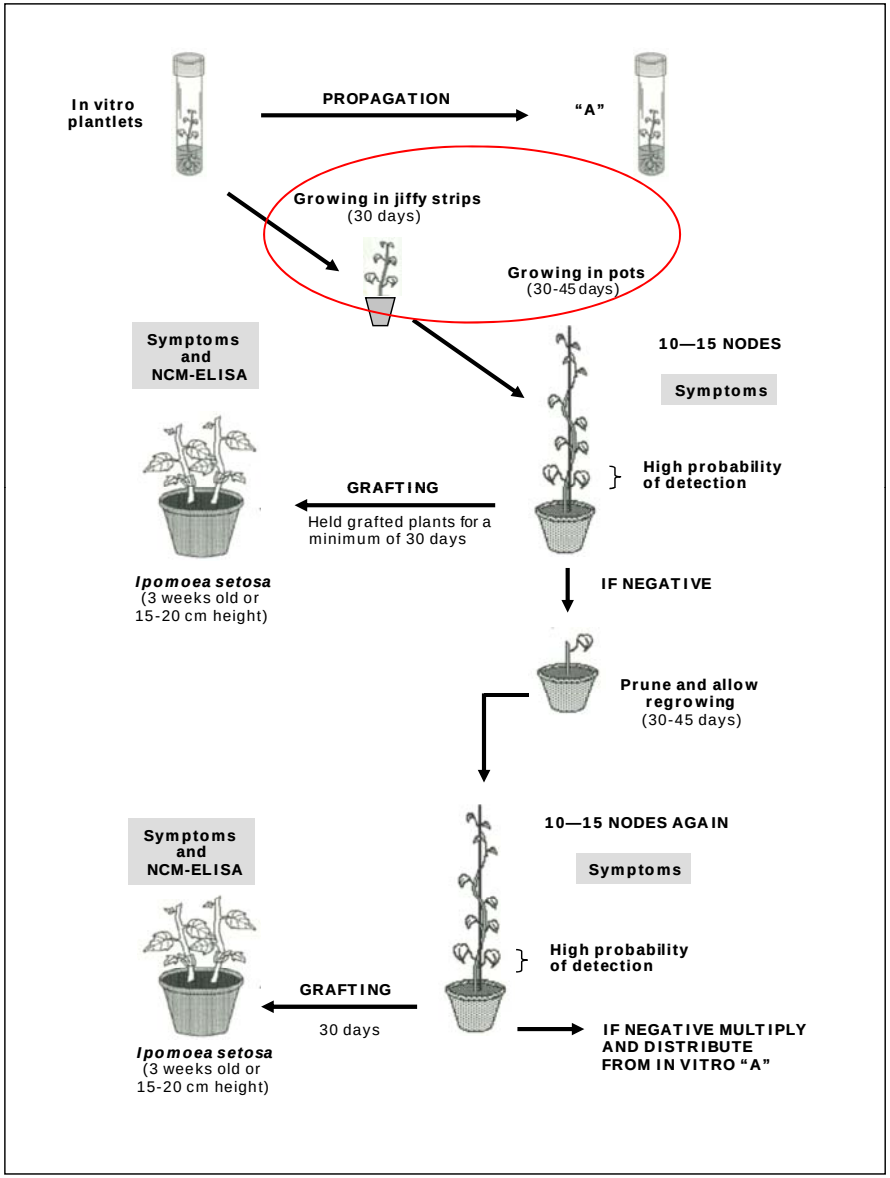
Sweetpotato virus indexing procedure

Duration: 5-6 months



Indexing procedure for sweet potato viruses. NCM-ELISA is performed for 10 viruses (SPFMV, SPLV, SPVG, SPMSV, SPMMV, SPCSV, SPCFV, C-6, SPCV, and CMV). PCR is performed to detect begomoviruses.

Sweetpotato virus indexing procedure



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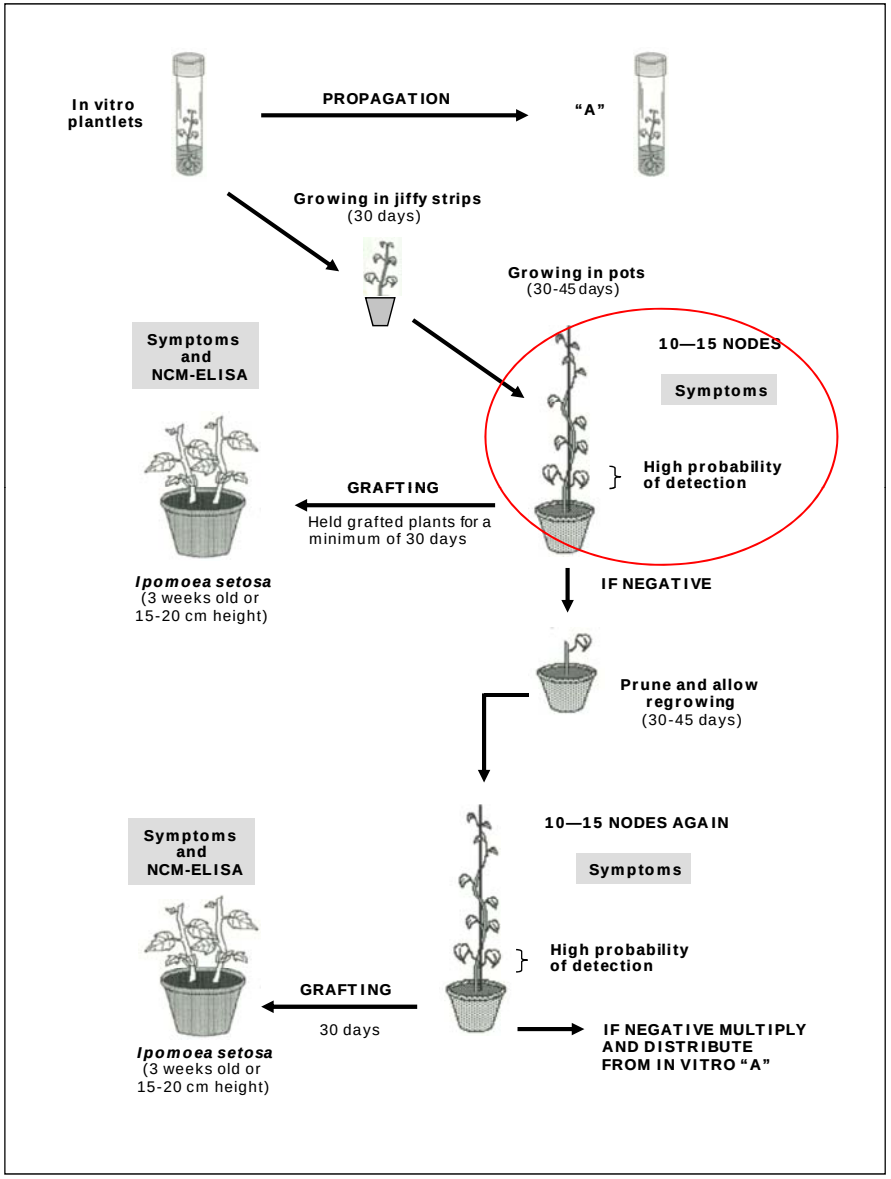
Growing plantlets in a greenhouse

Sweetpotato virus indexing procedure



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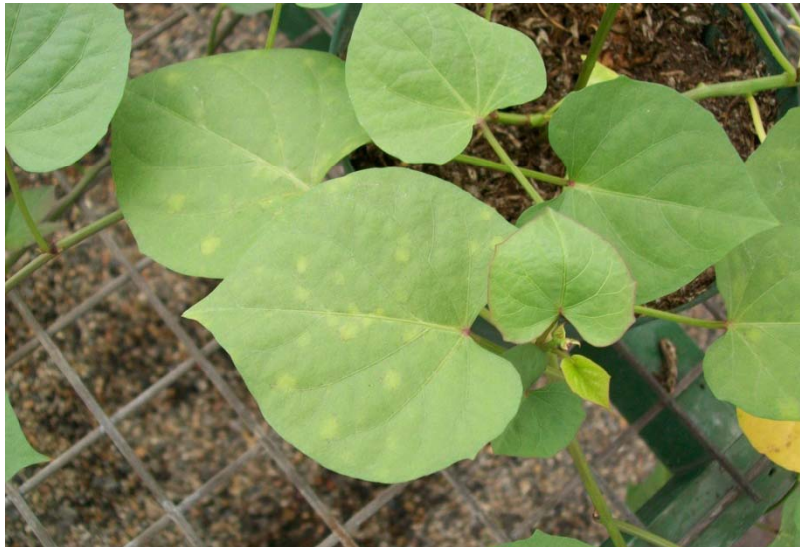
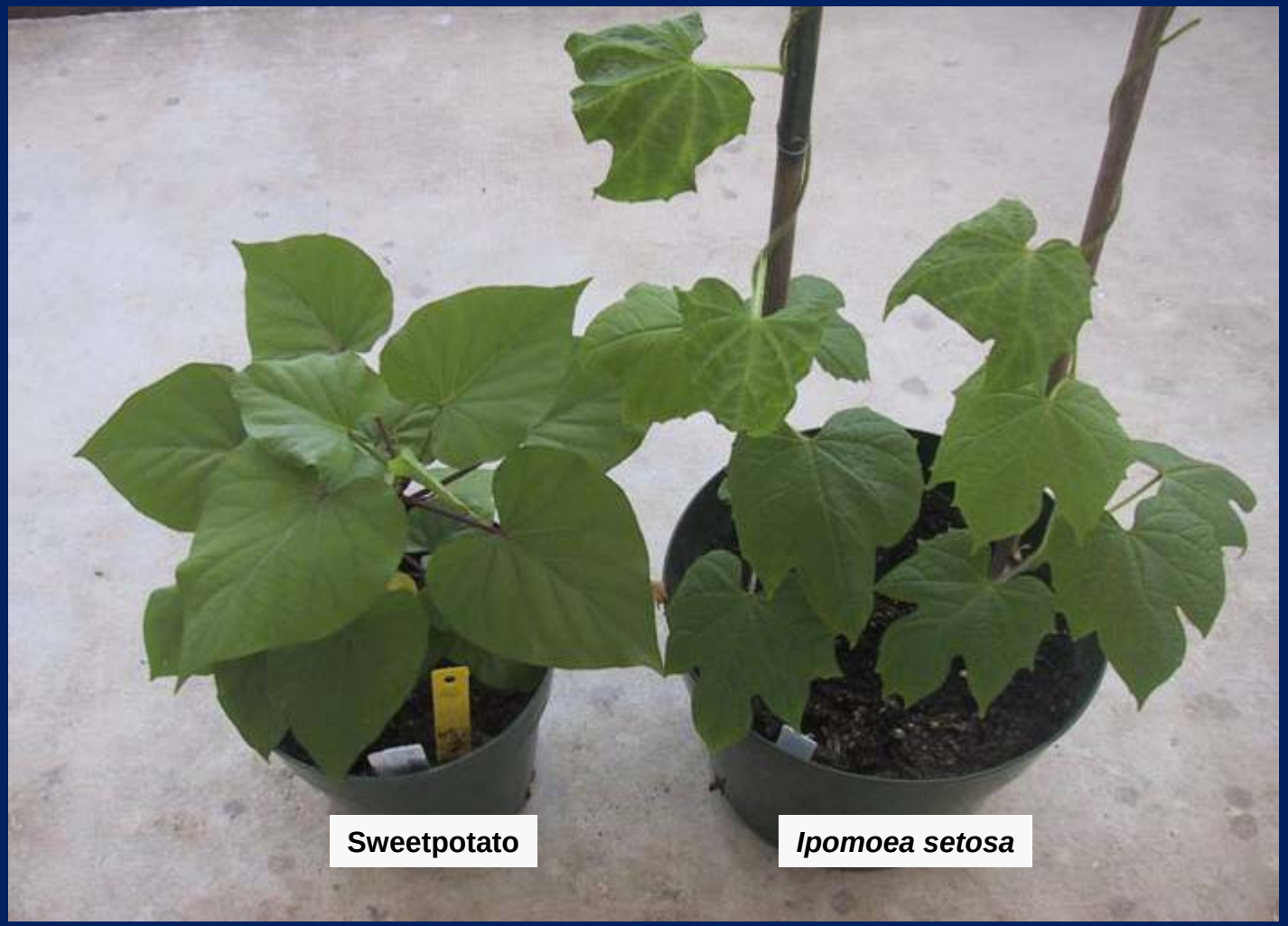
Symptoms observed in grown sweetpotato plants

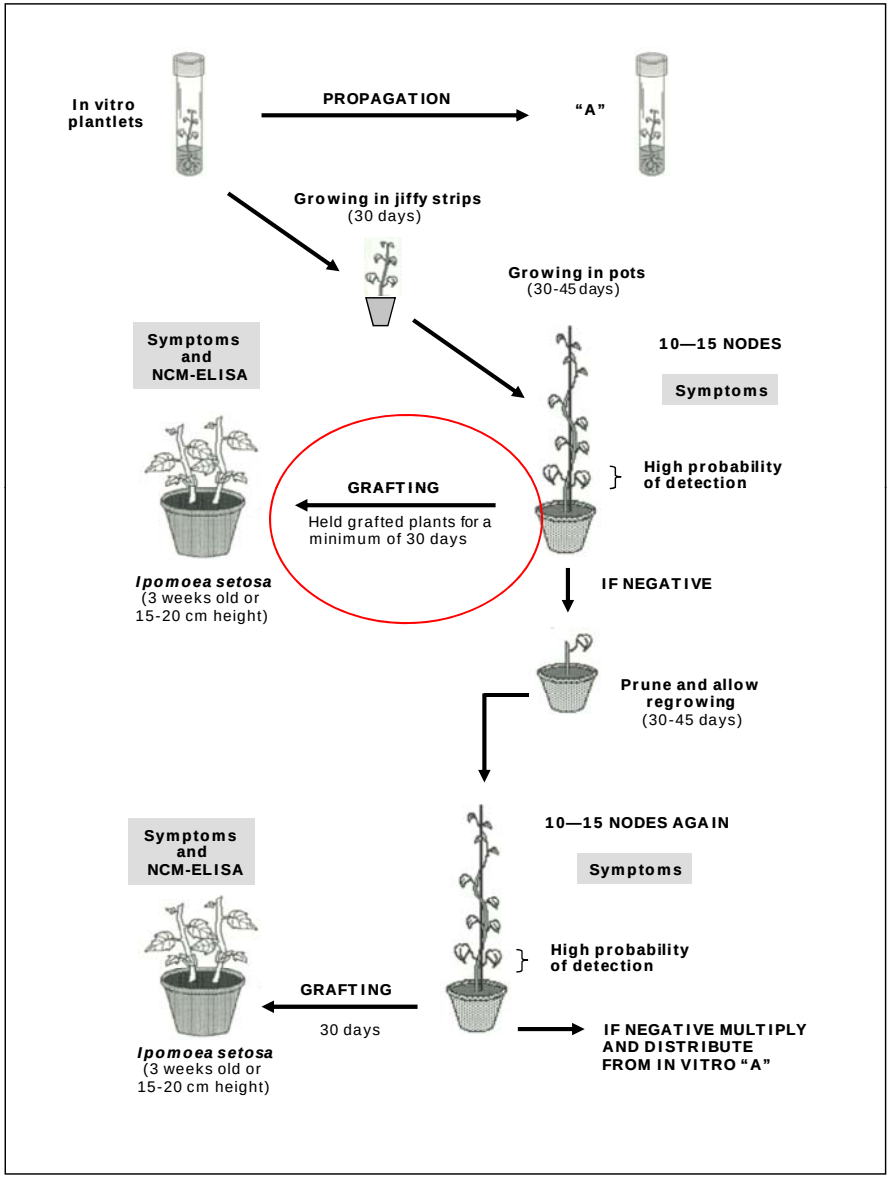


Symptomless infection



• **Low virus concentration in sweetpotato tissues (virus is not detected by serology from the SP plant)**

Sweetpotato virus indexing procedure



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Transmission of plant viruses

I. Mechanical and by contact

II. Grafting ← (universal method for transmitting viruses) — **Sweetpotato**

III. Vectors

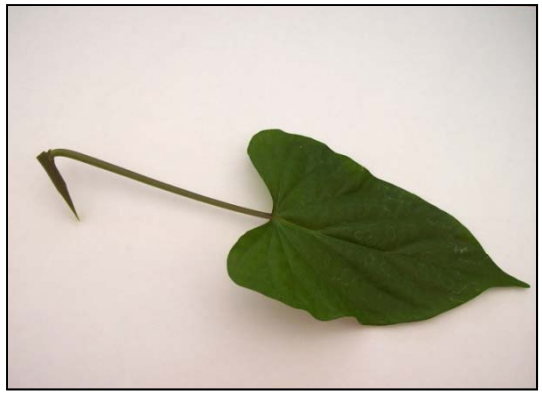
Insects (aphids, whiteflies)

IV. Pollen and sexual seed

Ipomoea setosa

Universal indicator plant for viruses that infect sweetpotato

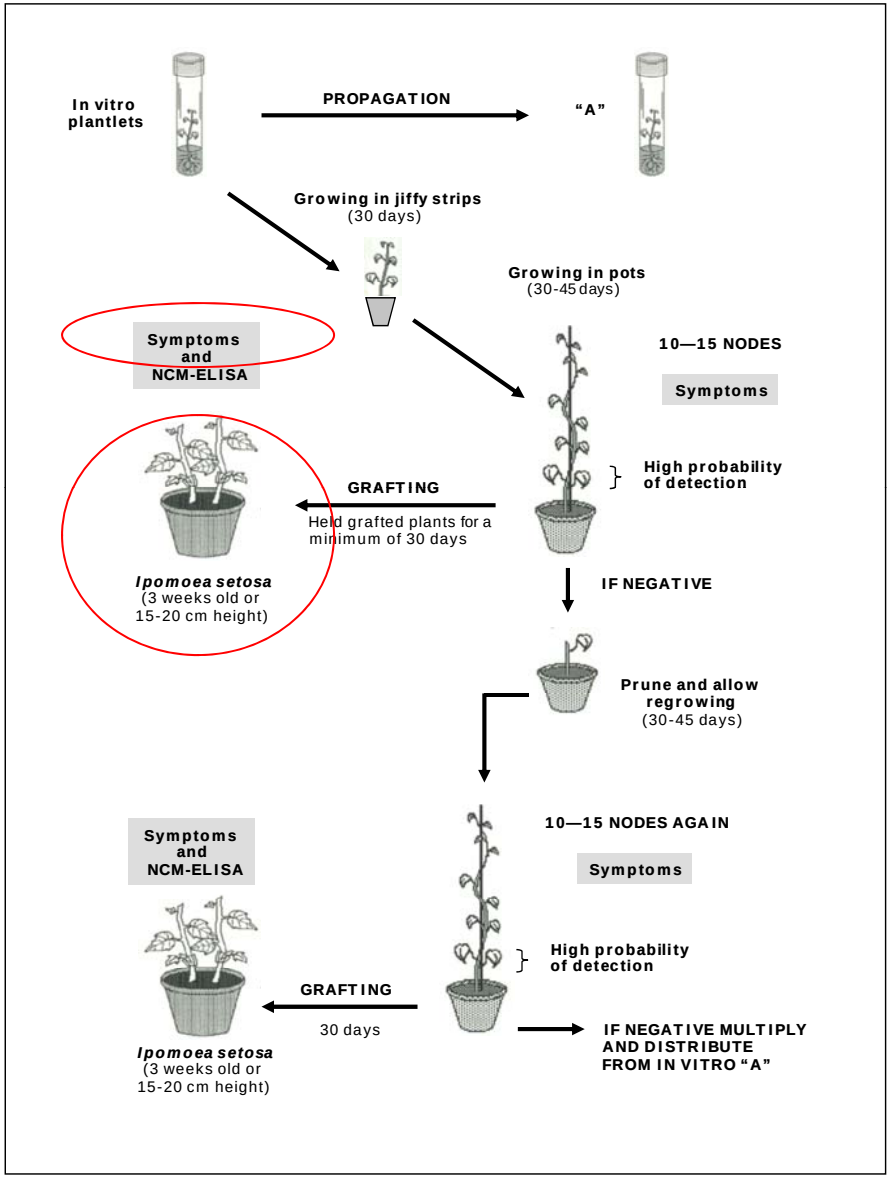
Wedge-grafting to *Ipomoea setosa*






Grafting: universal method for virus transmission

Sweetpotato virus indexing procedure



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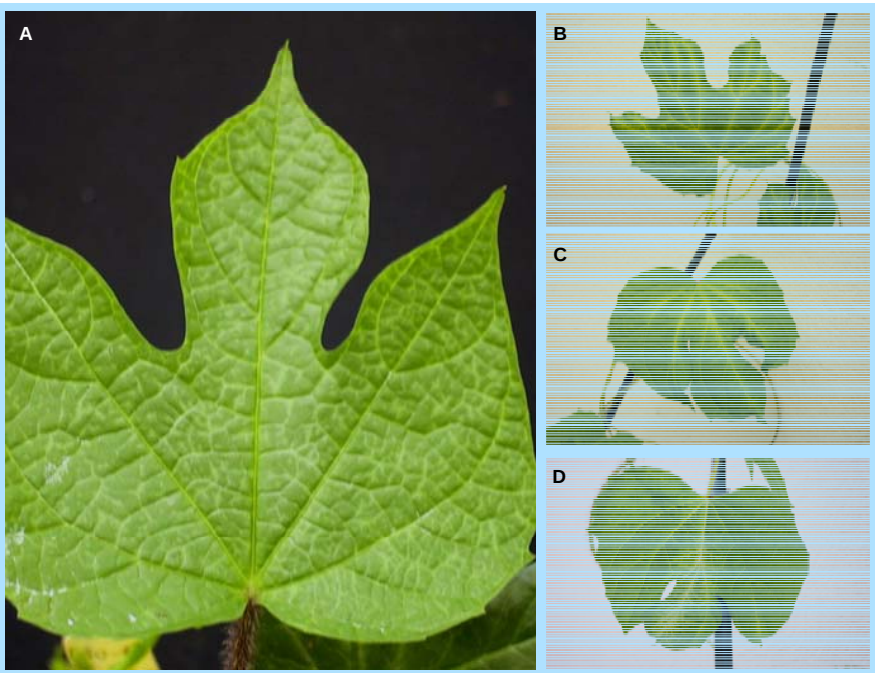
Symptoms recording and NCM-ELISA test

Preventive spraying of insecticides (controlling of whiteflies and aphids)

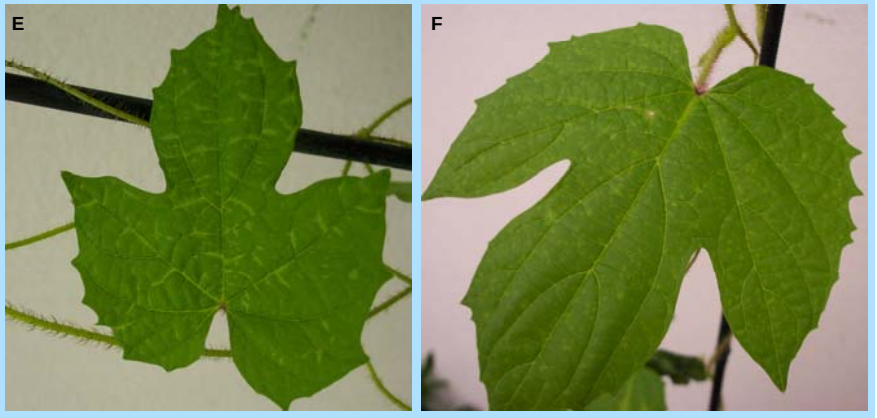
Symptoms1: Vein clearing

(Potyvirus)

SPFMV



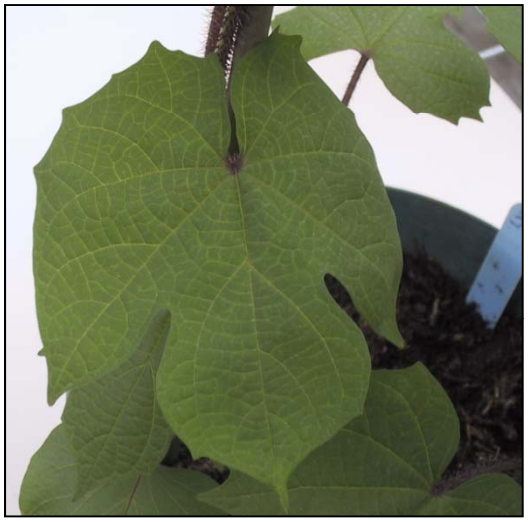
SPVG

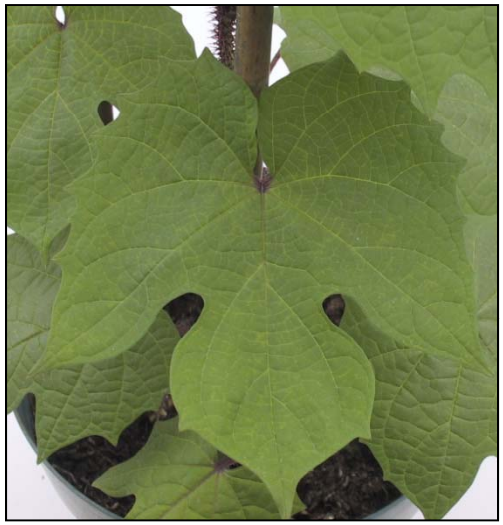


Unknown
(Negative in NCM-ELISA)

Symptoms 2: Chlorotic points

(Carlavirus)





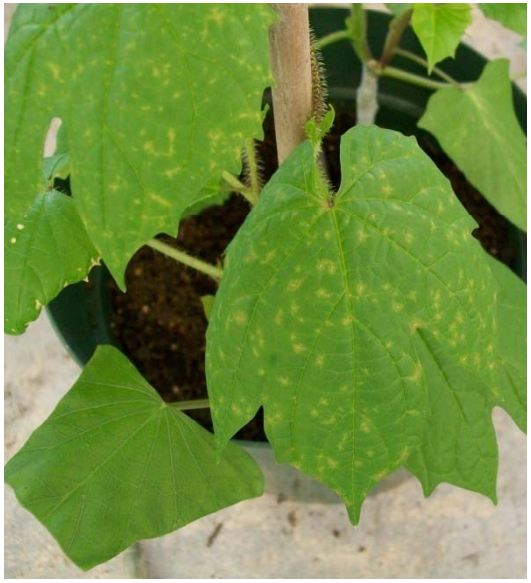
Symptoms 3: Vein clearing / rugosity

Symtoms 4: Vein necrosis/ necrotic points or spots

(Cavemovirus/ Solendovirus)






Symptoms 5: Chlorosis/ interveinal chlorosis/ roll up/ roll down

(Begomovirus)

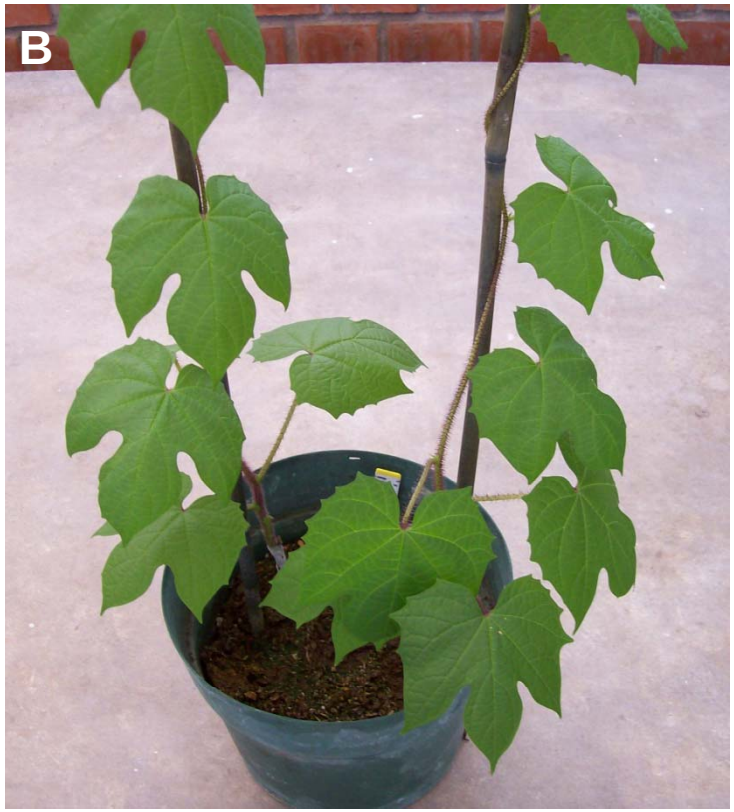






Somatic mutation

Clone CIP 401430 (Morada) showing somatic mutation (symptom of genetic distortion of leaves).

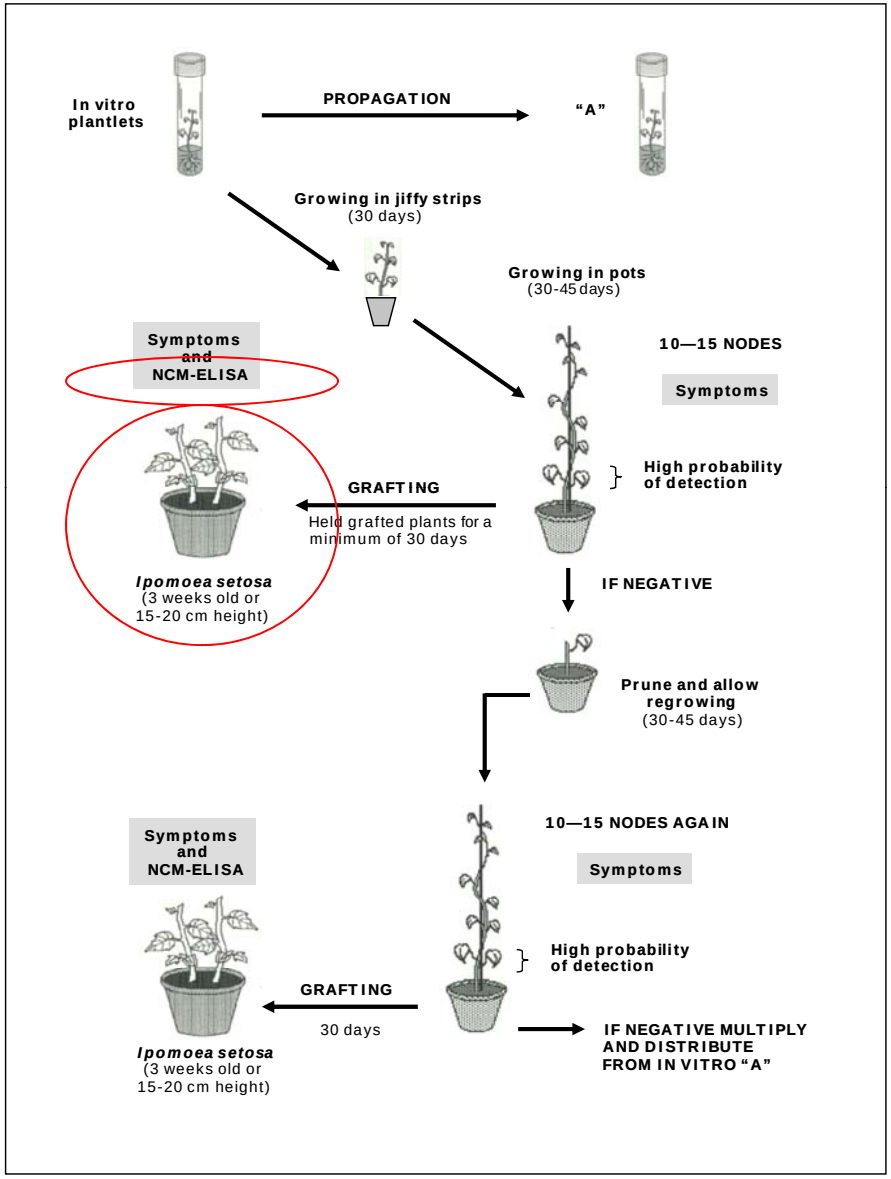


Indicator plant *I. seotsa* grafted with tissue scion from clone CIP 401430. No symptom is observed.

Final indexing report: Group 26 (G-26) 2010

Record	Other code	Accession number	Labcode	Collecting number	Accession name	SP symptoms	*I. setosa* symptoms	PCR Serology	Results	Observations
48	2	441443	SG-4412	I01091	Red Sweet Potato				APROVED	
49	2	441129	SG-4523	I01570	So 10		C,RU	Begomo	REJECTED	
50	2	420275	SG-5417	DLP 3311	Zapallo		Cp,Vc,Net,Vb	SPFMV,SPVG	REJECTED	
51	3	102081.28	SR-0190				C,RU	Begomo	REJECTED	
52	3	189121.15	SR-0192						APROVED	SP (Variegation)
53	1	189121.2	SR-0193						APROVED	
54	5	189123.57	SR-0199						APROVED	
55	5	189123.66	SR-0200						APROVED	
56	5	189123.73	SR-0202						APROVED	
57	1	189151.34	SR-0214						APROVED	
58	5	194582.24	SR-0253						APROVED	
59	4	194579.1	SR-0255						APROVED	
60		421081	G-0001	RCB IN- 1	Huachano Chico				APROVED	
61		421089	G-0021	RCB IN- 21	Quince Dias				APROVED	
62		420173	G-0263	DLP 318	unknown				APROVED	
63		441741	G-0343	RCB IF- 48	CAL-257				APROVED	
64		422638	G-0454	UNPRG 25	Morado De Huaura				APROVED	
65		400035	G-0505	RCB IF- 5	IAC-85-17				APROVED	
66		401493	G-0604	DLP 426			C,Cp,RU,Vc	Begomo,SPFMV,SPVG	REJECTED	
67		401538	G-0785	DLP 1340	Unknown				APROVED	
68		400460	G-0874	DLP 1792	Blanca				APROVED	
69		400480	G-0888	DLP 1873	unknown		C,RU	Begomo	REJECTED	
70		400165	G-1293	DLP 1360	unknown				APROVED	SP (Variegation)
71		400262	G-1405	DPW 2586	Paulista				APROVED	SP (Variegation)
72		400414	G-1768	DLP 1734	Leonarda				APROVED	

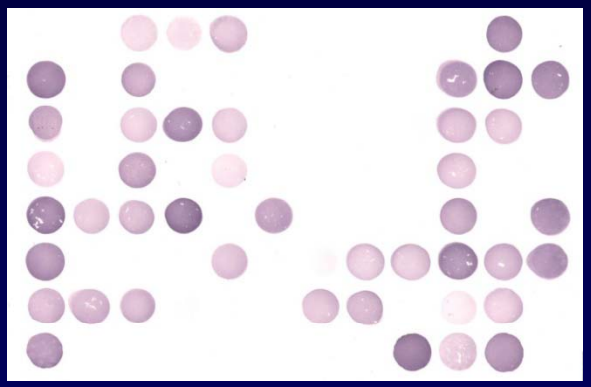
Sweetpotato virus indexing procedure



Indexing procedure for sweet potato viruses. NCM-ELISA is performed for 10 viruses (SPFMV, SPLV, SPVG, SPMSV, SPMMV, SPCSV, SPCFV, C-6, SPCV, and CMV). PCR is performed to detect begomoviruses.

Serological test

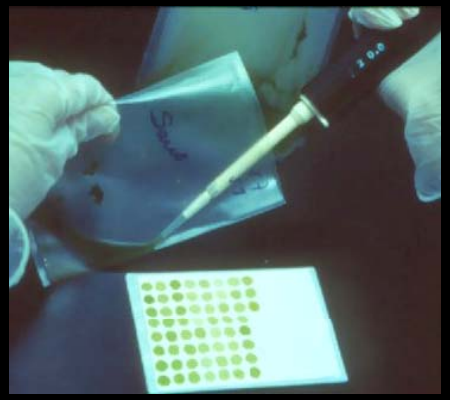
NCM-ELISA test



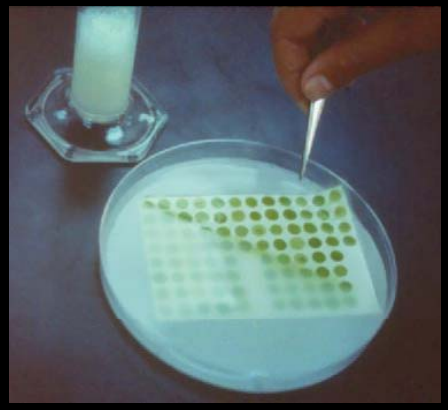
For 10 viruses

NCM-ELISA

(A) Processing samples

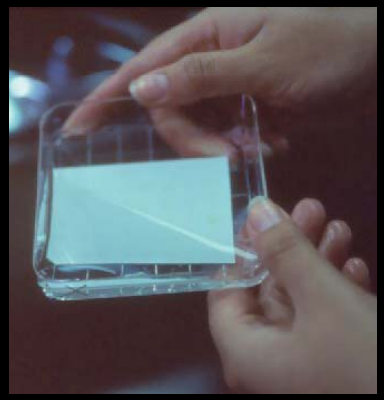


(B) Blocking



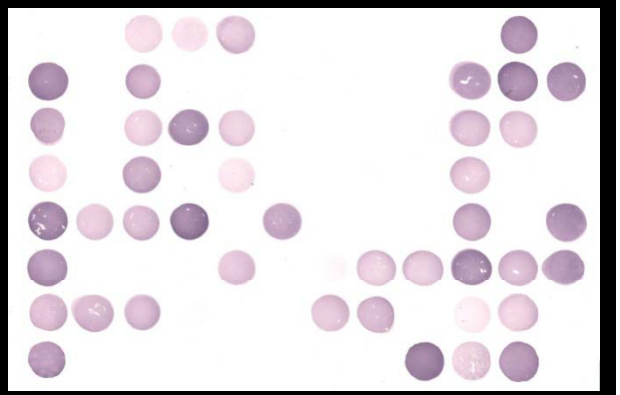
(C) 1st antibody

Washing

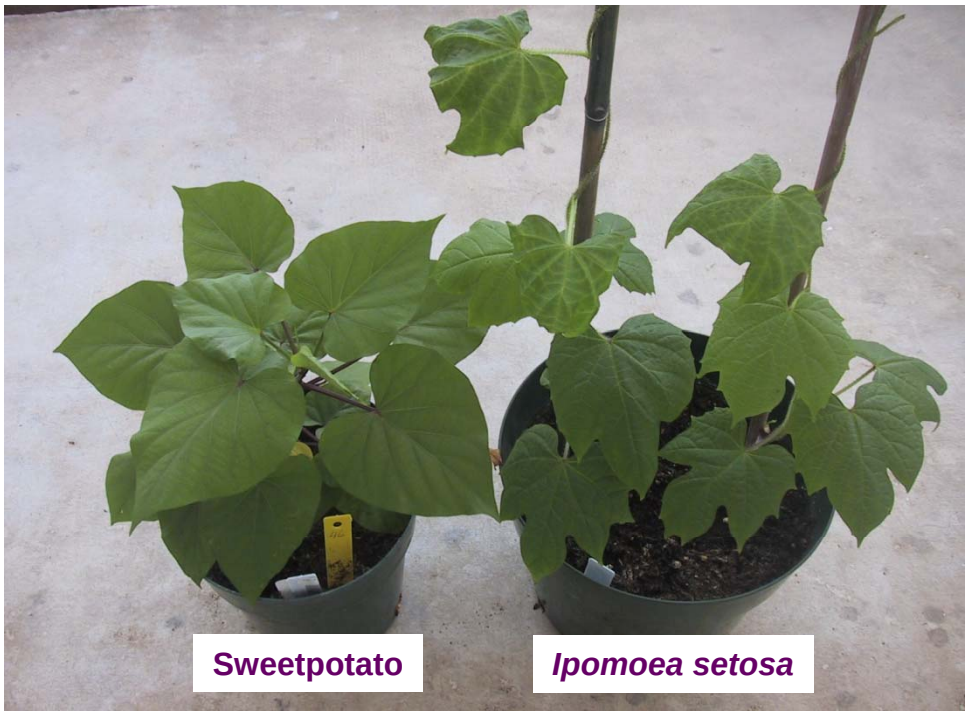


(D) 2nd antibodies (conjugate)

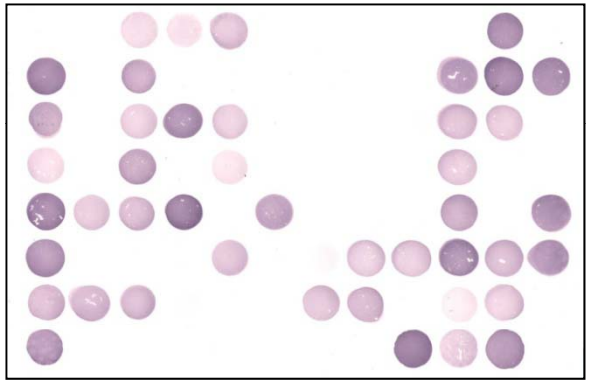
(E) Color development



Sweetpotato versus *I. setosa* versus serology



NCM-ELISA

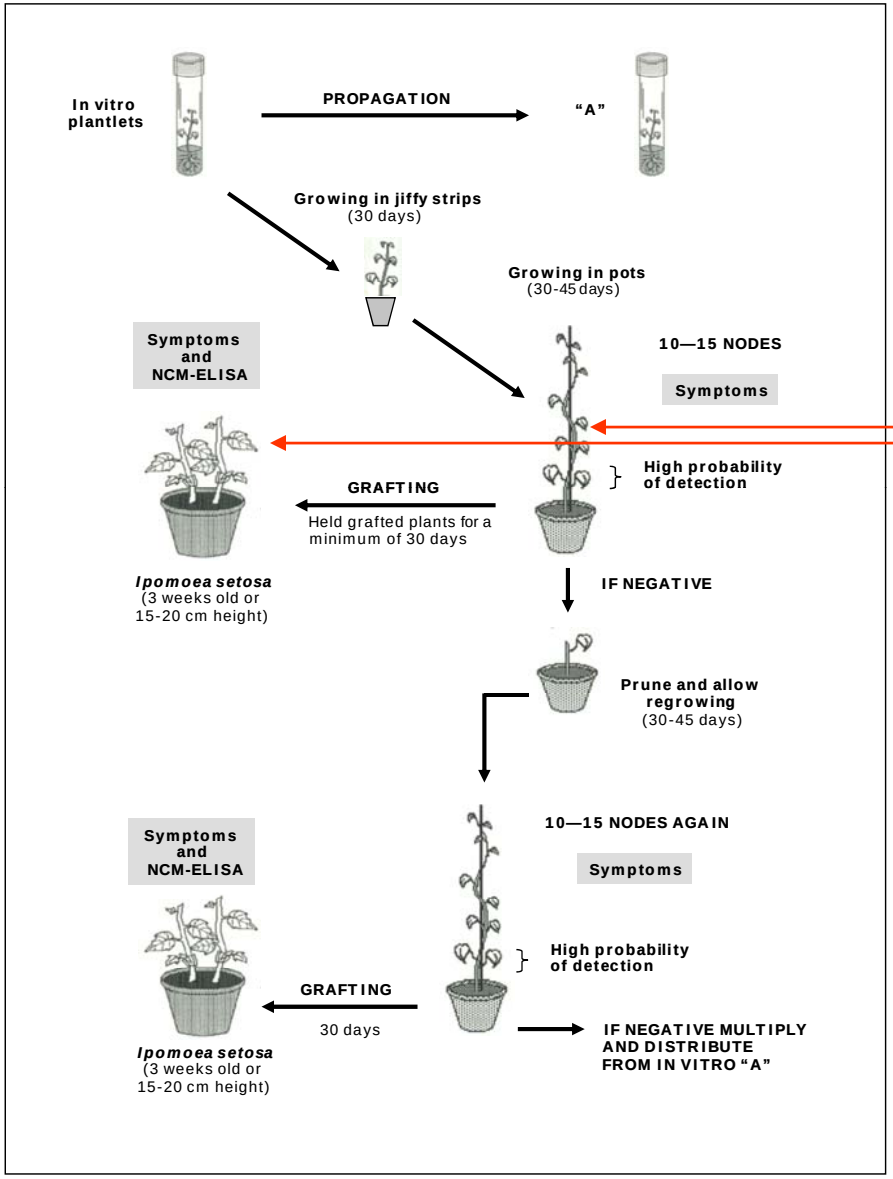


- **Symptomless**
- **Low virus concentration**

Indicator plant + **Serology (ELISA)**

Reliable detection of viruses

Sweetpotato virus indexing procedure



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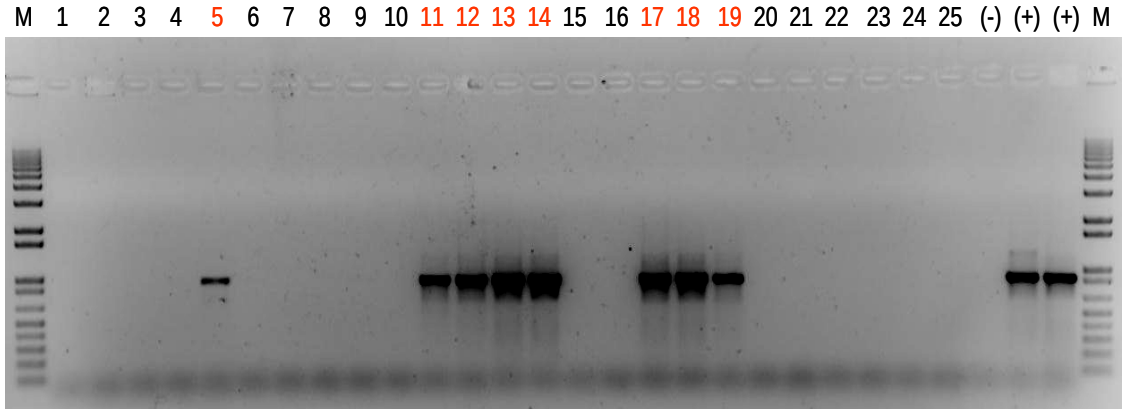
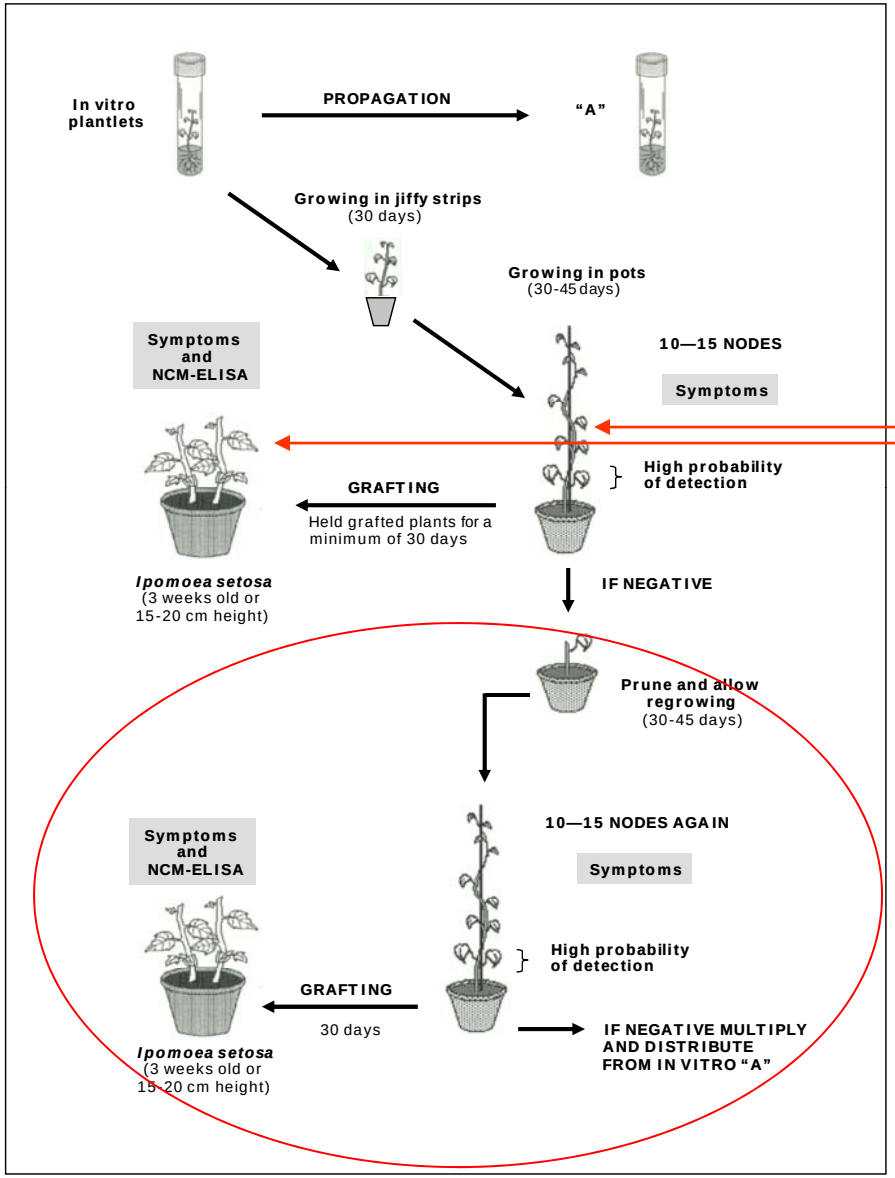
Sweetpotato vs *Ipomoea setosa* vs PCR (begomovirus)



Li, R., Salih, S., and Hurtt, S. 2004. Detection of geminiviruses in sweetpotato by polymerase chain reaction. Plant Dis. 88:1347-1351.

Sweetpotato virus indexing procedure



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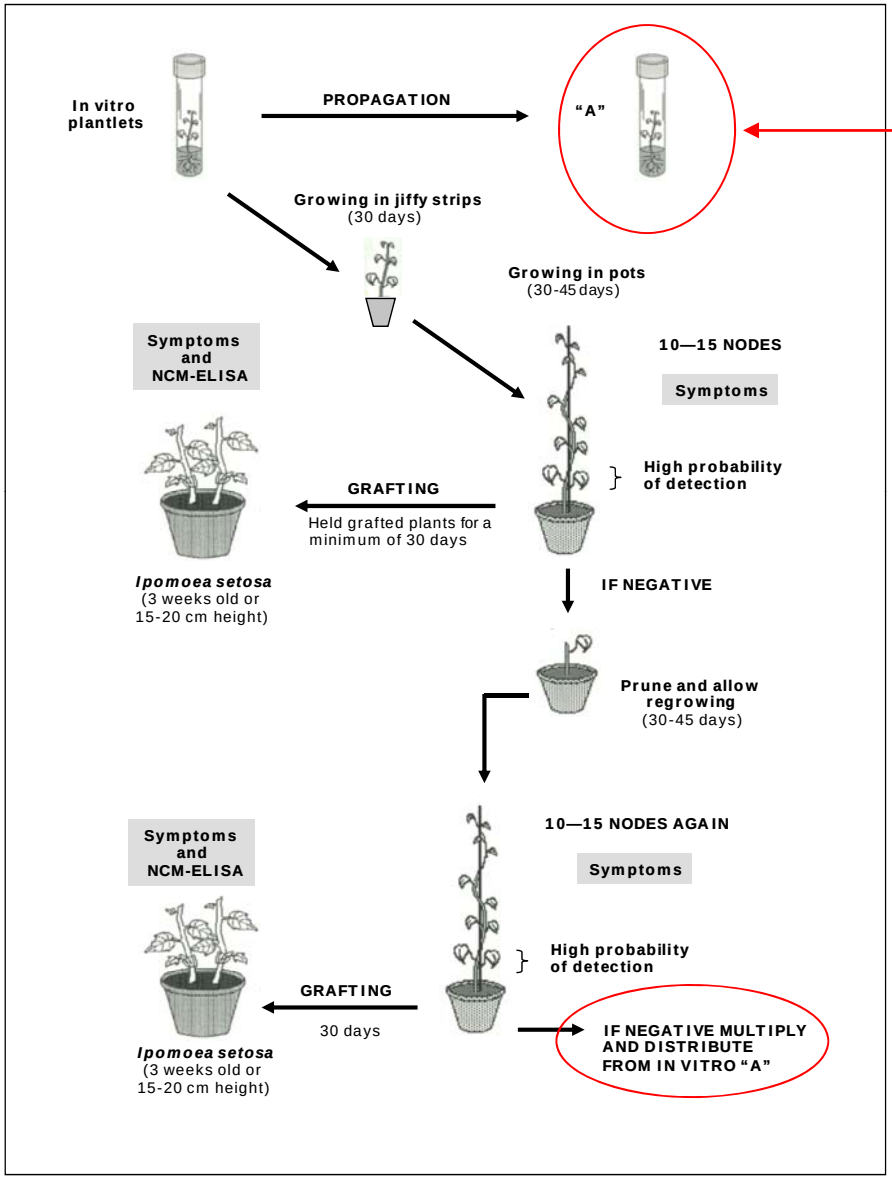

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Final Results of Virus Indexing (Group 25, 2009)

3	Record	Other code	Accession number	Labcode	Collecting number	Accession name	SP symptoms	*L setosa* symptoms	Serology	PCR	Results	Observations
38	35	1	CIP 401066	SG-1477	GTM 392	Camote Morado		Vc,Vn			REJECTED	
39	36	2	CIP 401448	SG-1524	DLP 2888	Morada					APROVED	
40	37	2	CIP 403380	SG-1558	INIVIT 9	Blanco Especial					APROVED	
41	38	2	CIP 402666	SG-1588	INIVIT1049			RU,D,IVC		Begomo	REJECTED	
42	39	5	CIP 402691	SG-1592	INIVIT1078			C,RU		Begomo	REJECTED	
43	40	5	CIP 402721	SG-1604	INIVIT1216			RU		Begomo	REJECTED	
44	41	6	CIP 401130	SG-1681	GTM 1109	Camote Morado		IVC		Begomo	REJECTED	
45	42	7	CIP 401375	SG-1688	SVG 27	Six Weeks		D,IVC		Begomo	REJECTED	
46	43	4	CIP 402619	SG-1702	INIVIT 217			RU		Begomo	REJECTED	
47	44	3	CIP 402655	SG-1717	INIVIT1038			D,Vc,Vn,Ln	SPCaLV		REJECTED	SPCaLV (PCR)
48	45	5	CIP 402696	SG-1731	INIVIT1084			C,IVC		Begomo	REJECTED	
49	46	1	CIP 403435	SG-1786	CESDA861607	86-1607					APROVED	
50	47	5	CIP 400789	SG-1790	CENDA 20	Jacobina 5		RD,D,Vc,Vn,Ln	SPCaLV	Begomo	REJECTED	Virus C-9 (PCR)
51	48	2	CIP 401120	SG-1816	GTM 949	Camote Blanco					APROVED	
52	49	5	CIP 401277	SG-1827	DLP 3898	Rojo-kinit					APROVED	
53	50	1	CIP 420565	SG-1852	DLP 66	Espelma		D,Vc	SPFMV,SPVG		REJECTED	
54	51	6	CIP 420163	SG-1859	DLP 123	Morado Morado					APROVED	
55	52	2	CIP 420181	SG-1907	DLP 887	unknown		Cp	SPFMV,SPVG		REJECTED	
56	53	6	CIP 420715	SG-1935	DLP 1312	Zapallona		RD,Vc	SPFMV,SPVG	Begomo	REJECTED	
57	54	6	CIP 420291	SG-2089	DLP 3549	unknown					APROVED	
58	55	5	CIP 420113	SG-2154	ARB 323	Camote Papa		Cp	SPFMV,SPVG		REJECTED	
59	56	1	CIP 420114	SG-2156	ARB 347	Camote					APROVED	
60	57	6	CIP 420488	SG-2172	ARB 425	Espelma					APROVED	
61	58	3	CIP 442115	SG-2283	IITA-CL 57			C,RU,D		Begomo	REJECTED	
62	59	2	CIP 443083	SG-2384	IITA-CL 249	TIS 2180		C,B		Begomo	REJECTED	
63	60	3	CIP 442858	SG-2452	IITA-CL 377	TIS 8030					APROVED	

SP G-25 (Oct. 2009)

G-32B

http://sol/appdb/research/Div4ICM/CIPVIR/diagnosticIndicatorPlant.aspx?strYear=2011&strNumber=5

http://intranet.cip.cgiar.org/appdb/research/Div4ICM/CIPVIR/diagnosticIndicatorPlant.aspx?strYear=2011&strNumber=5

http://sol/appdb/research/Div4ICM/CIPVIR/diagnosticNCMElisa.aspx?strYear=2011&strNumber=8

http://intranet.cip.cgiar.org/appdb/research/Div4ICM/CIPVIR/diagnosticNCMElisa.aspx?strYear=2011&strNumber=8

Outline

- Introduction
- ISO/IEC 17025
- Virus indexing procedure
- **Analysis of indexing results**

Number of sweetpotato accessions indexed for viruses between years 2006 and 2011

Year	Group	Analyzed accessions	Positive accessions	By grafting (*I. setosa*)	By serology (NCM- ELISA)	By PCR (Begomovirus)	
2011	34	232	85	81	43	49	25%
	33	11	9	9	5	7	
	32	163	82	72	45	49	
	31	244	92	89	47	58	
2010	30	131	57	55	38	24	17%
	29	220	86	86	44	26	
	28	66	2	1	1	0	
	27	243	123	122	103	62	
2009	26	329	141	141	46	103	29%
	25	231	103	103	91	59	
2008	24	201	77	74	31		
	23	72	15	10	15		
	22	272	111	111	43		
2007	21	197	100	100	9		
	20	10	0	0	0		
	19	151	44	44	9	2	
2006	18	193	60	60	4		
	17	65	40	40	32		
	16	318	114	112	21		
TOTAL		**3,349**	**1,341 (40%)**	**1,310**	**627** **SPFMV= 361** **SPVG= 270** **SPCV= 72** **SPCSV= 52** **SPCFV= 35** **SPLV= 9** **SPMSV= 4** **C-6= 1** **CMV= 1**	**Begomovirus = 439**	

Country of origin of accessions which tested positively to viruses during indexing

Country	NCM-ELISA							PCR		*I. setosa* (symptoms)			
	Potyvirus			Carlavirus		Crinivirus	Cavemovirus	Begomovirus	Solendovirus	Begomovirus	Cavemovirus/ Solendovirus	Potyvirus	Other ?
	SPFMV	SPVG	SPMSV	SPCFV	C-6	SPCSV	SPCV		SPVCV				
Argentina								4		9			1
Bolivia										5		1	
Brazil								2		5		1	2
China										2			
Colombia			1					3		7			1
Costa Rica								2					1
Cuba	1						3*	15		34	7		2
Dominican Repub.							1	8	1	64	10	1	
Ecuador								3		5	1	2	
Guatemala							6	5	2	9	10		
Honduras										1			
India	10	6					3	14			2		
Jamaica								1			8	1	
Japan	1	1						2		2		1	2
Sri Lanka										1			
Mexico				3			1+1	2		5			
Malaysia													1
Nicaragua													1
New caledonia								1		1			
Nigeria	1	1						4		48			
Panama							1*	5		2			
Peru	135	99	2	12		5		12		50	3	4	4
Philippines										1			
Papua New Guinea	44	25		1			7	8		58	3	1	6
Puerto Rico	1	1						2		1	1	1	1
Paraguay	2	2								3			
Solomon Islands	2	1		1			1	4		6			1
Thailand						1				6			1
Tonga										2			
Taiwan	6	3				1	2	6		3			2
Uganda								1					
USA	1	1			1		3*	4		7	1		
Saint Vincent								1		5			
Venezuela								1		4	4	2	1

Outline

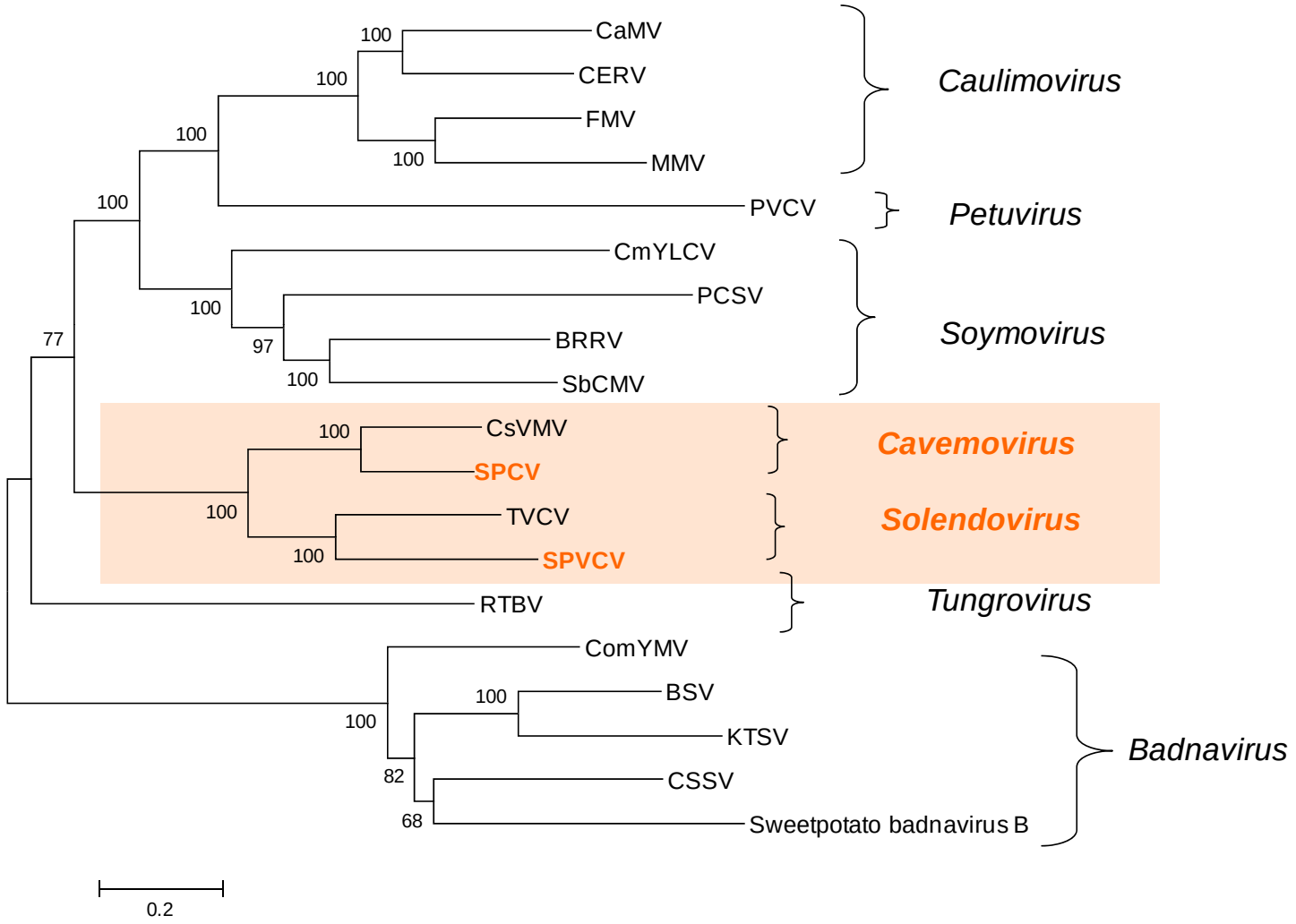
- Introduction
- ISO/IEC 17025
- Virus indexing procedure
- Analysis of indexing results
- **Research derived from indexing**

Isolate C-9 (CIP 400851)

Dominican Republic, Sosa 30, “Chambrita” cultivar

2002

Phylogenetic analysis (complete genome)



J. De Souza's Thesis

De Souza y Cuellar. 2011. Archives of Virology 156: 535-537.
Cuellar et al 2011. Journal of General Virology 92: 1233-1243.
Geering et al 2009. Archives of Virology 155: 123-131.

Geographic distribution

Based on serological and PCR test, respectively

J. De Souza's Thesis

Identification of begomovirus isolates

M. Galvez's Thesis

San Vicente (400025)

Perú-6 (421076)

Perú-10 (420993)

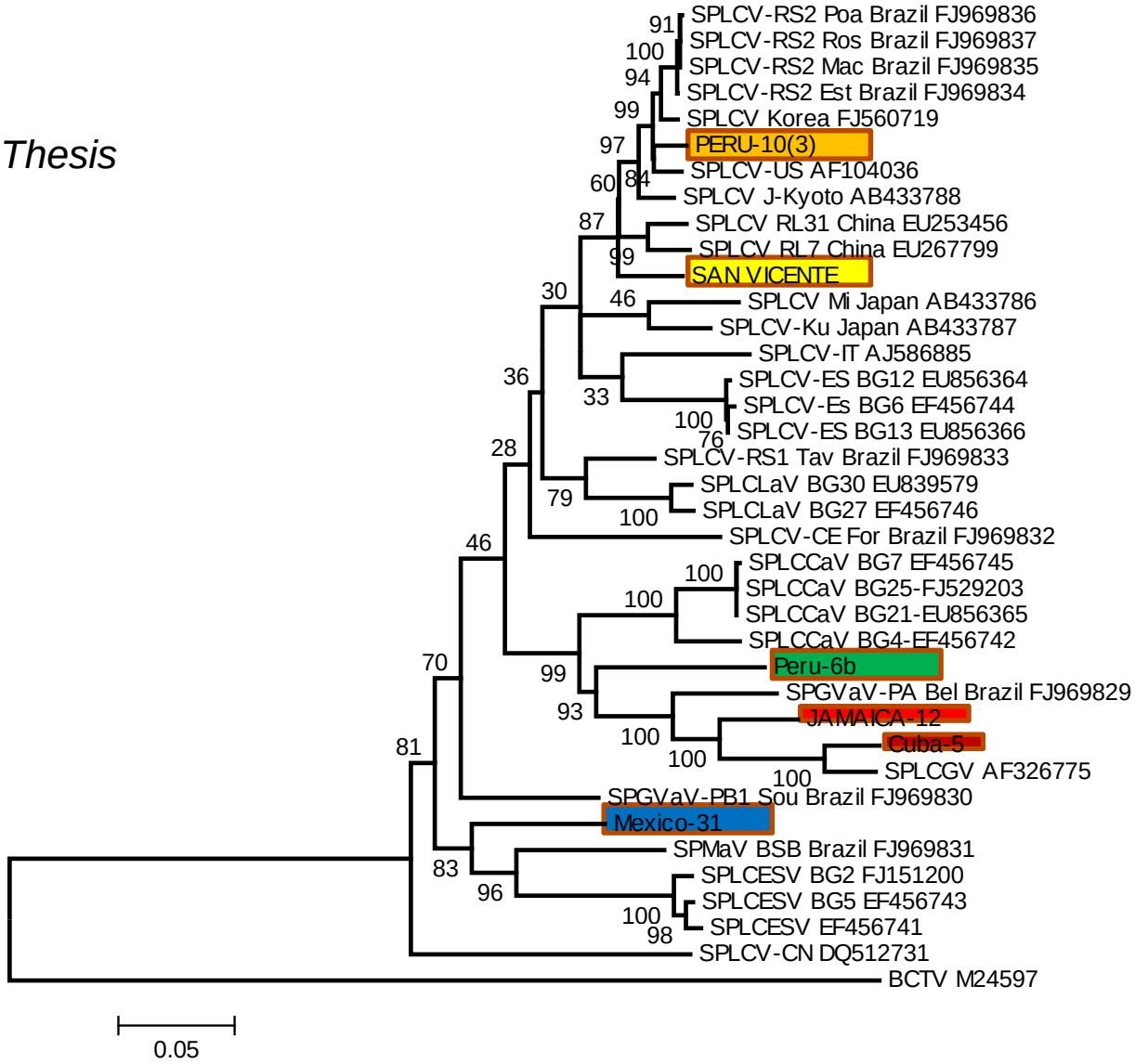
Jamaica-12 (401201)

Cuba-5 (402645)

México-31 (401468)

Swepovirus phylogenetic analysis (complete genomes)

M. Galvez's Thesis



New virus?

CIP 440962 (Papua New Guinea)

CIP 420883 (Peru)

New virus?

CIP 401580 (Guatemala)

New virus?

CIP 400789 (Dominican Rep.)

Outline

- Introduction
- ISO/IEC 17025
- Virus indexing (SP in vitro germplasm)
- Analysis of indexing results
- Research derived from indexing
- **Maintaining ISO accreditation**

External Audit conducted by UKAS (C. Jeffries), Octobre 2009

ISO 17025

UKAS
UNITED KINGDOM ACCREDITATION SERVICE

Testing laboratory
No. 4229

Equipments

Digital balance

Rotatory shaker

Freezer

pHmeter

Magnetic stirrer

Equipment calibration

Digital balance

Refrigerator

Monitoring temperature

Manual temperature recording

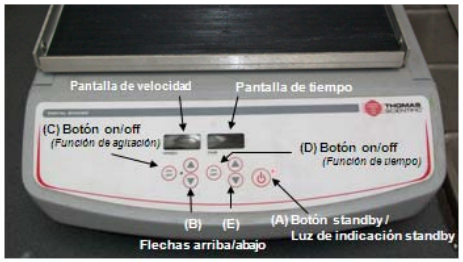
Data logger with thermocouple

Equipment operational instruction

IS - HQU - 0304010167

INSTRUCTIVO DE USO DE AGITADOR ORBITAL DIGITAL

MARCA: THOMAS SCIENTIFIC
MODELO: 989224
CODIGO CIP: 0304010167



Poniendo operativo el equipo

1. Presionar el botón standby **(A)** para mover la unidad del modo standby.

Seleccionando la velocidad de agitación

2. Presionar la flecha arriba/abajo **(B)** hasta alcanzar la velocidad deseada.
3. Presionar el botón on/off **(C)** para comenzar la función de agitación.
4. Ajustar la velocidad con las flechas arriba/abajo **(B)** sin interrumpir la agitación.
5. Parar la función de agitación presionando el botón on/off **(C)**.

Seleccionando el tiempo a cero (0:00) y en modo continuo (tiempo acumulado)

6. Presionar y mantener presionado el botón on/off **(D)**.
7. Presionar simultáneamente ambas flechas arriba y abajo **(E)** para colocar el tiempo a cero.
8. Presionar el botón on/off **(D)**. La pantalla de tiempo indicará el tiempo acumulado.
9. Para parar el tiempo, presionar nuevamente el botón on/off **(D)**.

Programando el tiempo

10. Presionar las flechas arriba/abajo **(E)** hasta alcanzar el tiempo deseado.
11. Comenzar esta función presionando el botón on/off **(D)**.
12. Para interrumpir el ciclo de tiempo automático antes que se complete, presionar el botón on/off **(D)**.

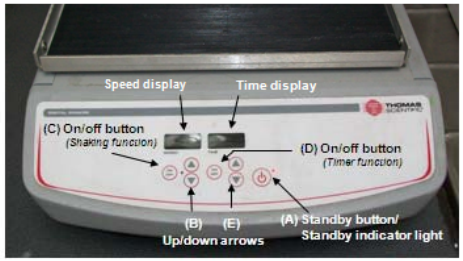
Apagando la unidad

13. Presionar el botón standby **(A)** para mover la unidad al modo standby.

IS - HQU - 0304010167

INSTRUCTION SHEET TO USE DIGITAL ORBITAL SHAKER

SUPPLIER: THOMAS SCIENTIFIC
MODEL: 980224
CIP CODE: 0304010167



Getting ready

1. Press the standby button **(A)** to move the unit from standby mode.

Setting speed

2. Press the up/down arrow **(B)** until you reach the desired speed.
3. Press the on/off button **(C)** to start the shaking function.
4. Adjust speed without interrupting shaking by using the up/down arrows **(B)**.
5. Stop the shaking function by pressing the on/off button **(C)**.

Setting time to zero (0:00) and continuous mode: Accumulated time

6. Press and hold the on/off button **(D)**.
7. Set time to zero by simultaneously pressing both the up and the down arrows **(E)**.
8. Press the on/off button **(D)**. The display will indicate accumulated time.
9. Stop timer by pressing the on/off button **(D)** again.

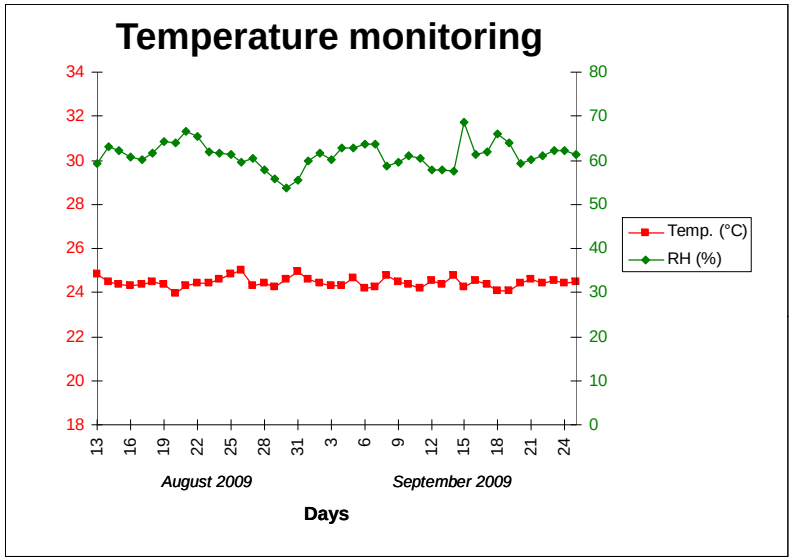
Setting timed mode: Programmed time

10. Press the up/down arrows **(E)** until you reach the desired time.
11. Start this function by pressing the on/off button **(D)**.
12. To interrupt an automatic timing cycle before it is completed, press the on/off button **(D)**.

Turning unit off

13. Press the standby button **(A)** to move the unit to standby mode.

Control of all factors affecting reliability on virus detection



- **Virus variability**
- **Low virus concentration on the SP plants**
- **Viruses no uniformly distributed on the SP plants**
- **Symptom expression on grafted *I. setosa* plants**

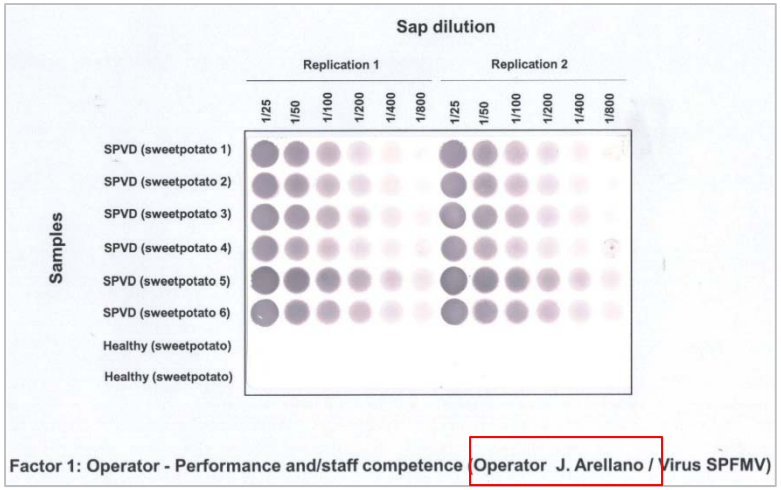
Personnel: competent / trained



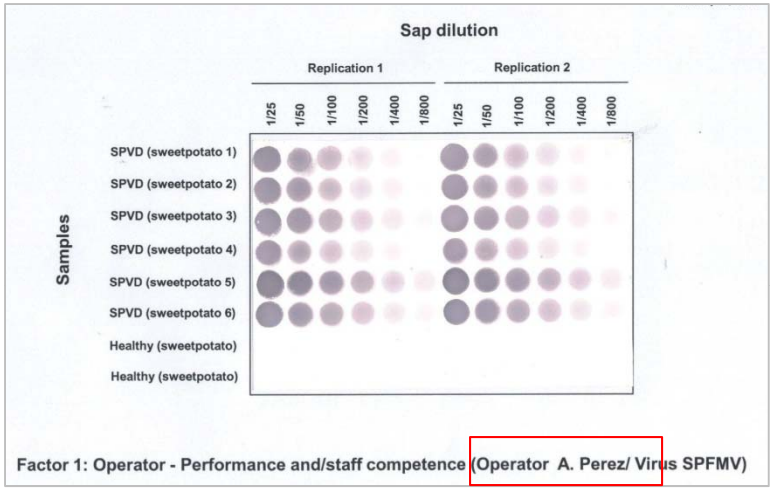



IQC / Uncertainty of measurement or NCM-ELISA

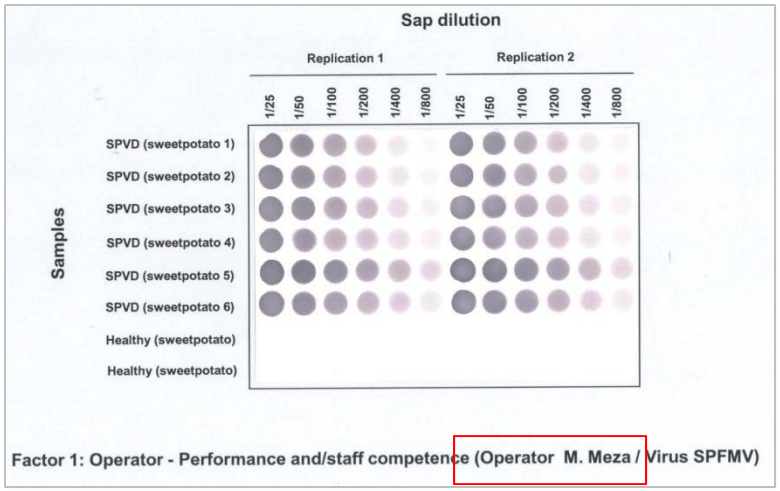
February 28, 2012



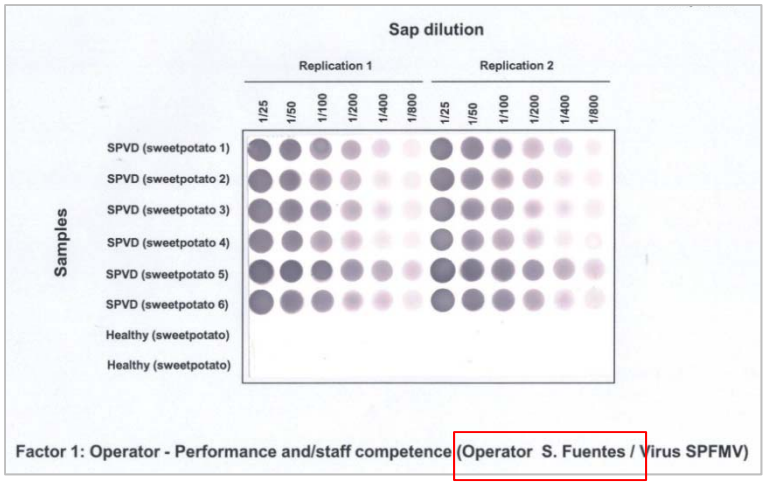
Factor 1: Operator - Performance and/staff competence (Operator J. Arellano / Virus SPFMV)



Factor 1: Operator - Performance and/staff competence (Operator A. Perez/ Virus SPFMV)



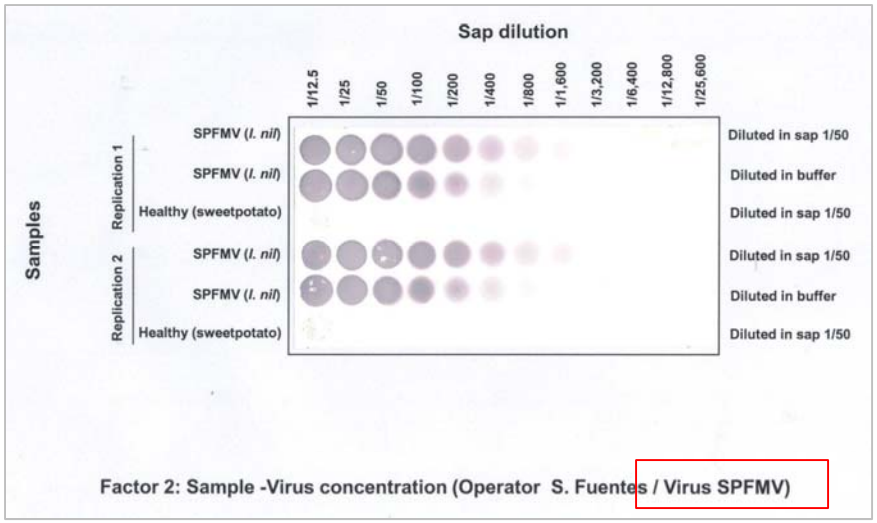
Factor 1: Operator - Performance and/staff competence (Operator M. Meza / Virus SPFMV)



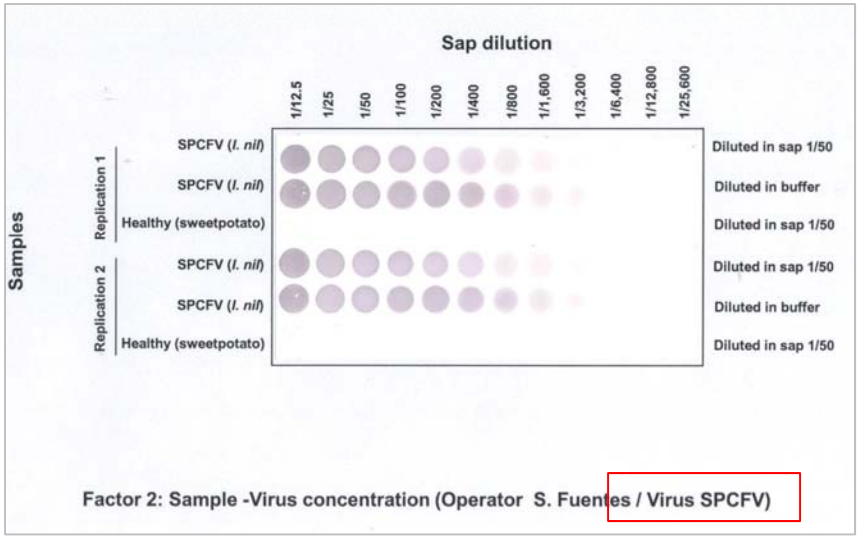
Factor 1: Operator - Performance and/staff competence (Operator S. Fuentes / Virus SPFMV)

IQC / Uncertainty of measurement or NCM-ELISA

February 28, 2012



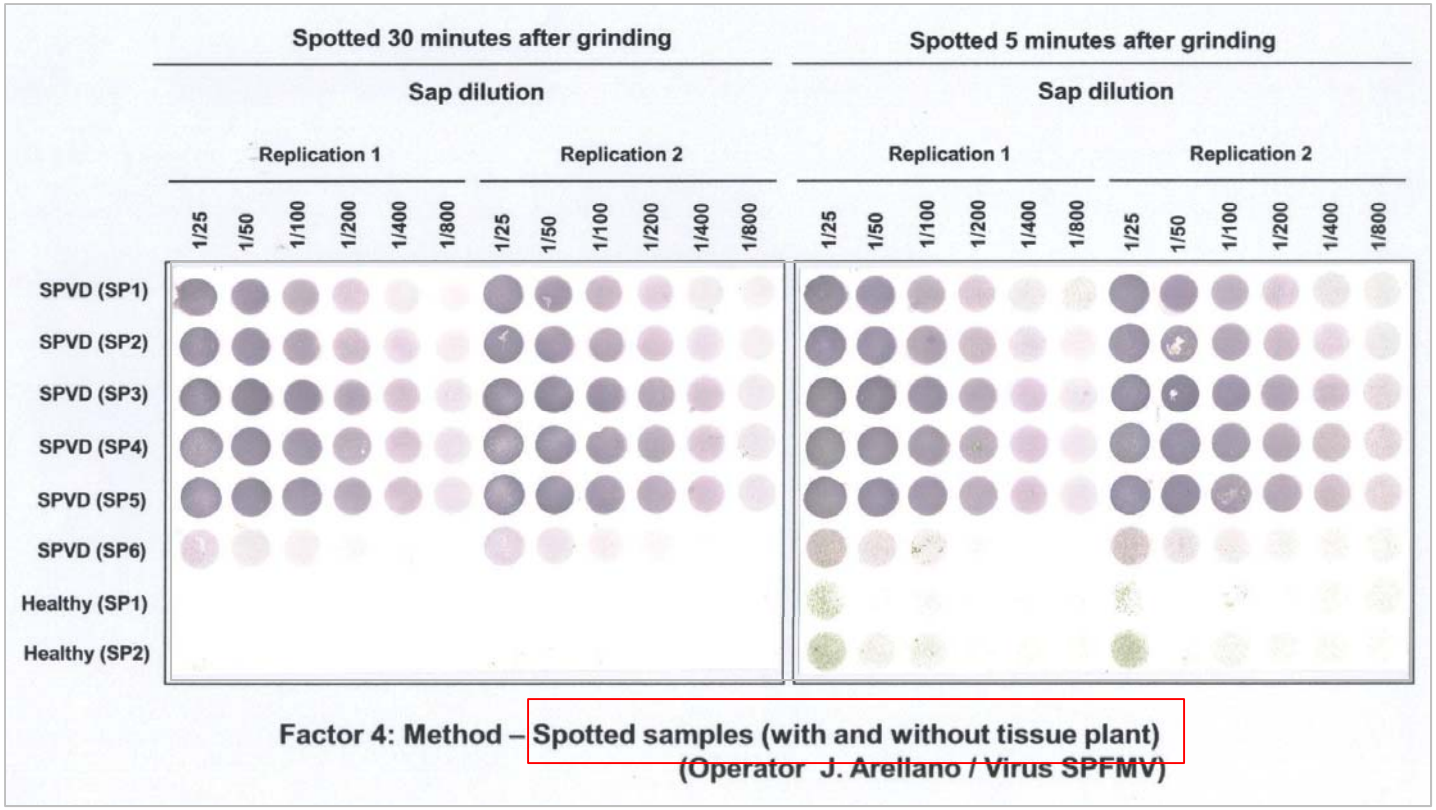
Factor 2: Sample -Virus concentration (Operator S. Fuentes / Virus SPFMV)



Factor 2: Sample -Virus concentration (Operator S. Fuentes / Virus SPCFV)

IQC / Uncertainty of measurement or NCM-ELISA

March 05, 2012



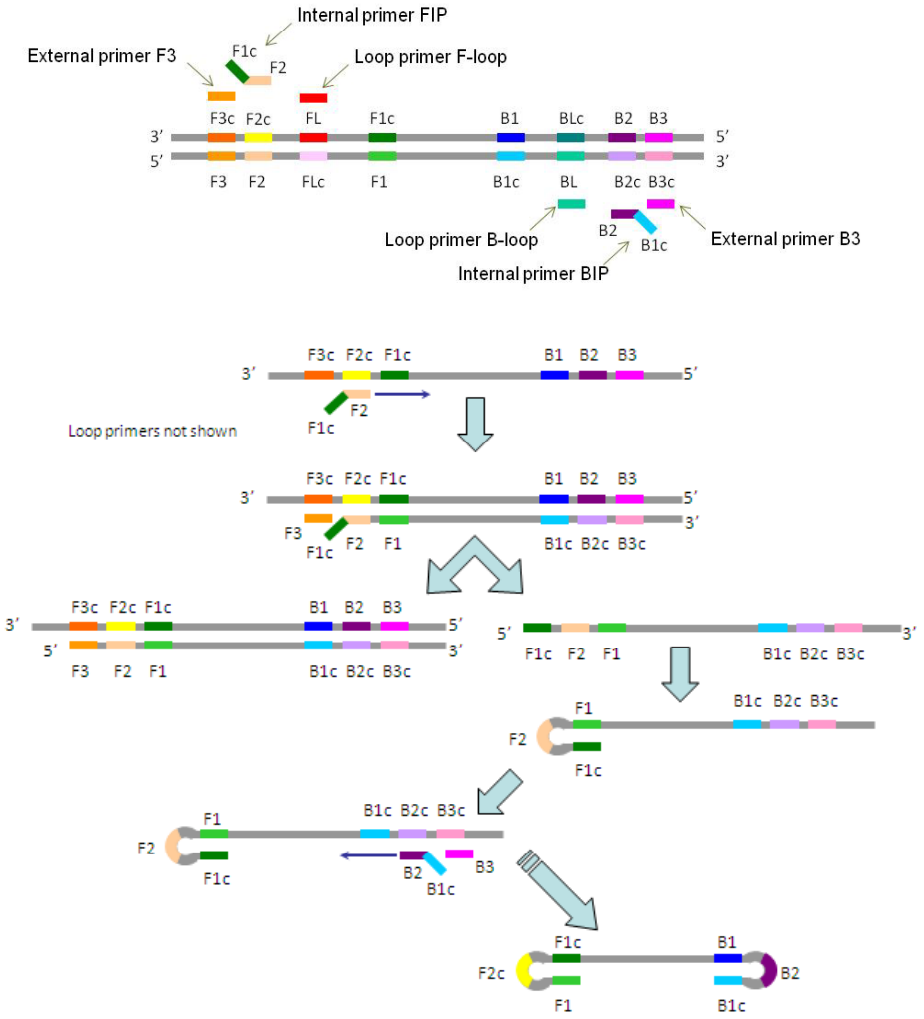
Factor 4: Method – Spotted samples (with and without tissue plant)
(Operator J. Arellano / Virus SPFMV)

Outline

- Introduction
- ISO/IEC 17025
- Virus indexing (SP in vitro germplasm)
- Analysis of indexing results
- Research derived from indexing
- Maintaining ISO accreditation
- **New molecular tests (validation)**

New molecular test (LAMP)

LAMP: Loop-mediated isothermal **AMP**lification



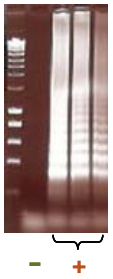
- Non-denatured target
- Isothermal reaction at 65ºC
- 20-60 minute reaction time

Overcoming many of the limitations of PCR-based methods (cost and complexity of thermal cycling equipment)

Detection of LAMP products

DNA/RNA extraction → LAMP

- agarose gel electrophoresis



method development / optimisation

- colour change/ precipitation

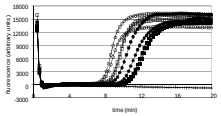
non-instrumented

- LFD detection

multiplex detection

end-point

- real-time: Genie



(semi)-quantitative real-time

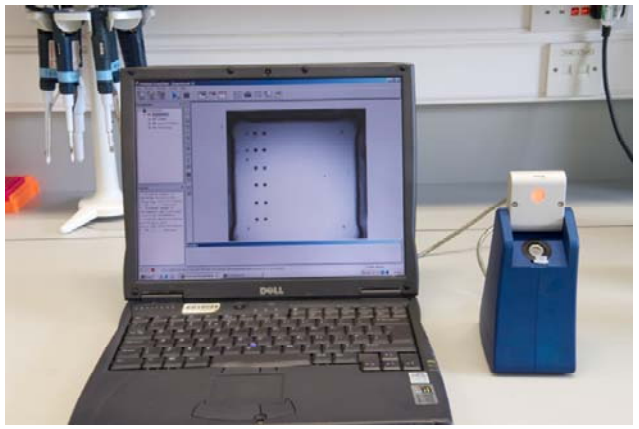
in the lab

in the field

New technology (ClonDiag array)

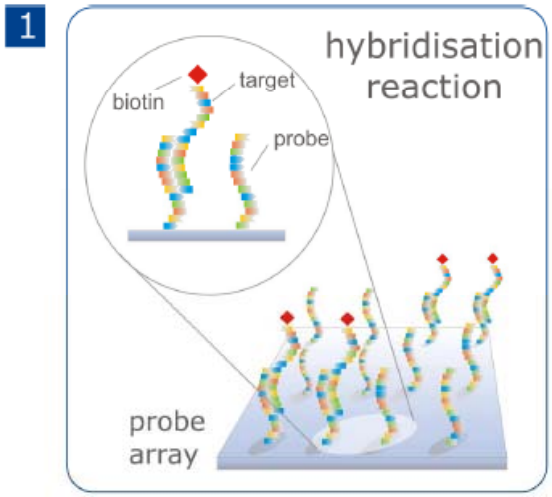
Array Tube

- Mini microarray embedded in tube
- Up to 80 features
- One step labelling
  (biotin amp)
- Cheap scanner
- Manipulations in tube

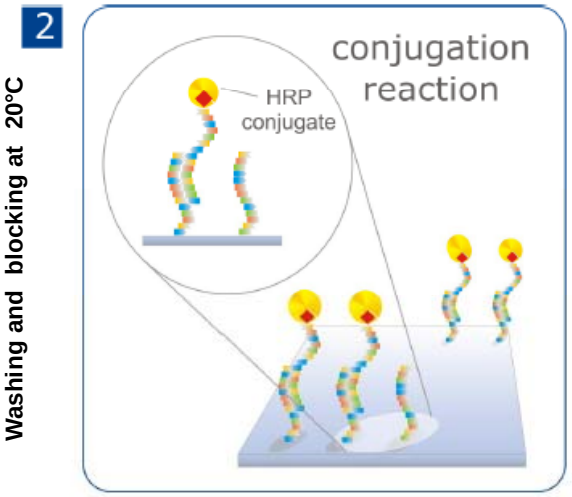


Clondiag Array Work Flow

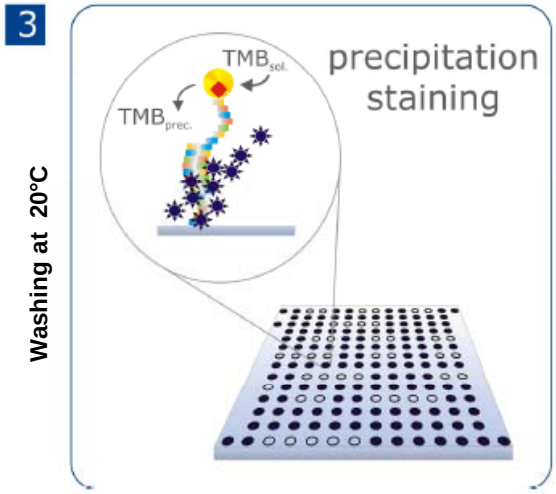
55°C /1h



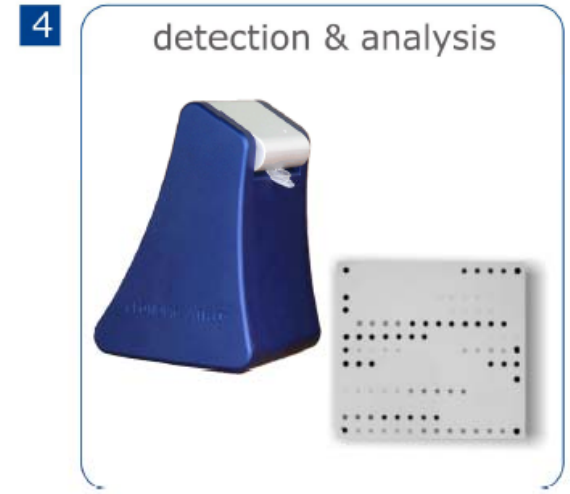
Anti-biotin, HRP-linked Antibody (20°C)



Washing and blocking at 20°C



Washing at 20°C



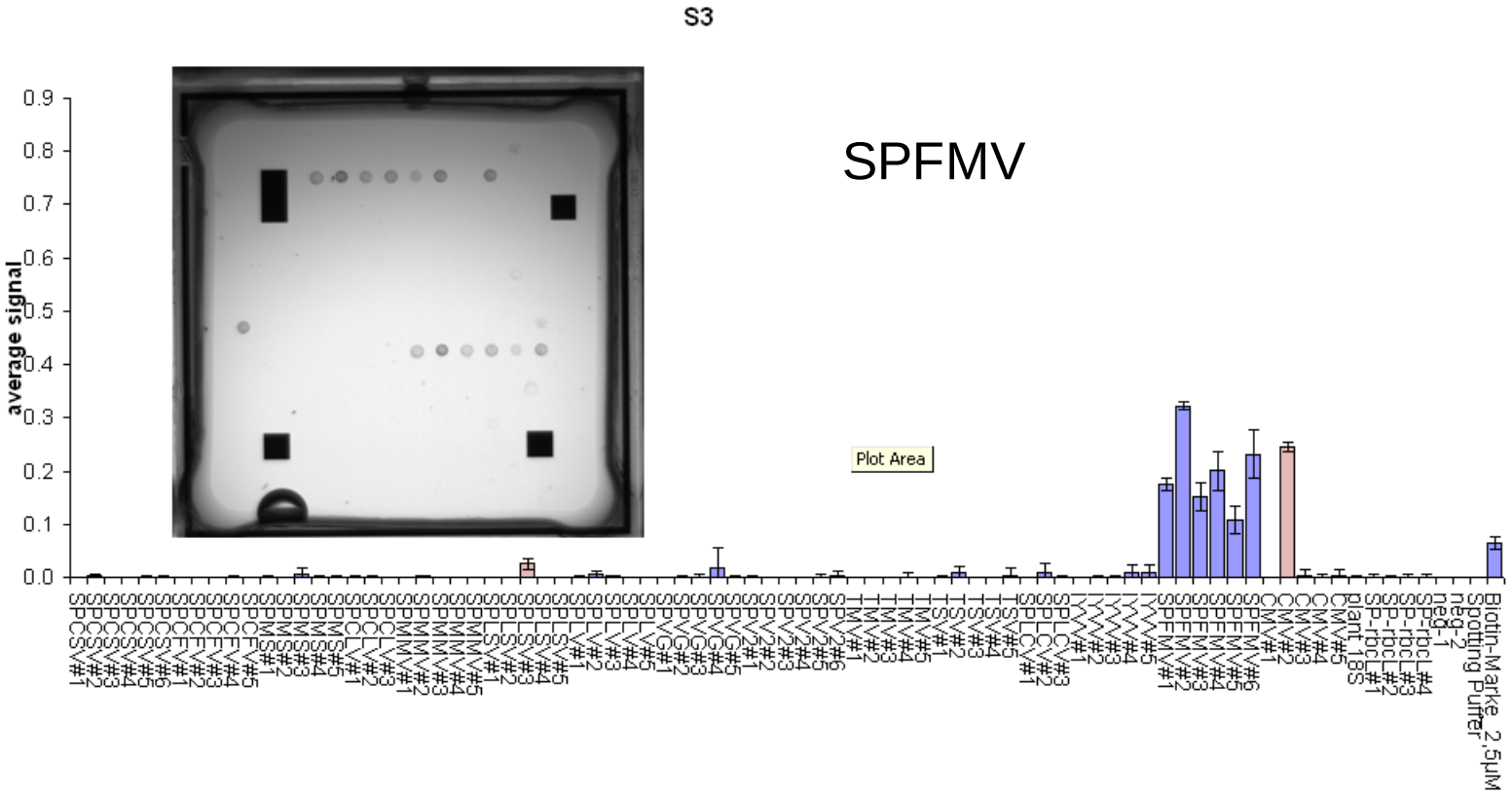
Tetramethyl bendzidine (TMB) or o-Dianisidine

When using array tube

- **Do not allow dry out**
- **Never spin**

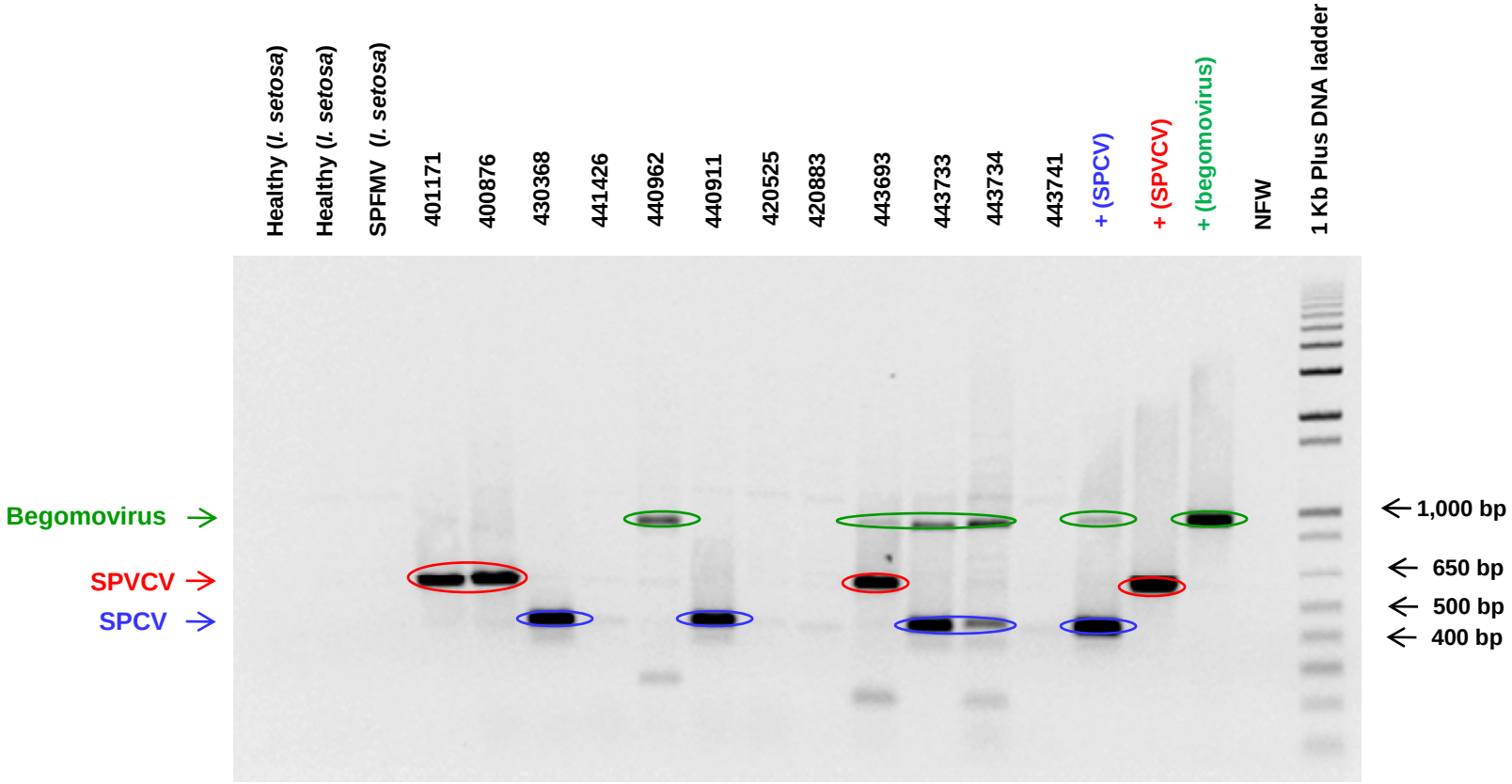
www.**clondiag**.com/

Sweetpotato Virus Array



Multiplex PCR (under validation)

For detecting DNA viruses: SPCV, SPVCV, begomovirus



A. Berrocal's Thesis

Staff involved in SP virus indexing

- **Segundo Fuentes**
- **Jaime Arellano**
- **Marco Meza**
- **Ana Pérez**
- **Raúl Lara**

HQU

- **Brenda Zea**
- **Juan Cruzado**
- **Miguel Velarde**

IVU

- **Edwin Rojas**
- **D. Hiraoka**

RIU

CIP's ISO Team 2011