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Localization of *Sweet potato chlorotic stunt virus* (SPCSV) in synergic infection with Potyviruses in sweet potato

Claudia Fernanda Nome[1], Sergio Fernando Nome[2], Fabiana Guzmán[1], Luis Conci[1] and Irma Graciela Laguna[1,2]

1. Instituto de Fitopatología y Fisiología Vegetal. IFFIVE-Instituto Nacional de Tecnología Agropecuaria (INTA). Córdoba. Argentina.
2. CONICET.

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ABSTRACT: Among diseases reported worldwidely for sweet potato (*Ipomoea batatas* (L) Lam) crop, one of the most frequent is the Sweet potato virus disease (SPVD), caused by sweet potato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus (SPFMV) co-infection. In Argentina, there exists the sweet potato chlorotic dwarf (SPCD), a sweet potato disease caused by triple co-infection with SPCSV, SPFMV and sweet potato mild speckling virus (SPMSV). Both diseases cause a synergism between the potyviruses (SPFMV and SPMSV) and the crinivirus (SPCSV). Up to date, studies carried out on the interaction among these three viruses have not described their localization in the infected tissues. In single infections, virions of the crinivirus genus are limited to the phloem while potyviral virions are found in most tissues of the infected plant.

The purpose of this work was to localize the heat shock protein 70 homolog (HSP70h), a movement protein for genus crinivirus, of an Argentinean SPCSV isolate in its single infection and in its double and triple co-infection with SPFMV and SPMSV. The localization was made by *in situ* hybridization (ISH) for electron microscopy (EM) on ultrathin sections of sweet potato cv. Morada INTA infected tissues.

The results demonstrated that viral RNA coding HSP70h is restricted to phloem cells during crinivirus single infection, while it was detected outside the phloem in infections combined with the potyviruses involved in chlorotic dwarf disease.

Introduction

Fifteen viral entities have been reported to affect sweet potato (*Ipomoea batatas* (L) Lam) crops around the world (Fauquet *et al.*, 2005a). One of the most frequent and economically important diseases in sweet potato is the sweet potato virus disease (SPVD) (Gutiérrez *et al.,* 2003; Tairo *et al.,* 2004), caused by the synergism between a potyvirus, sweet potato feathery mottle virus (SPFMV), and the crinivirus sweet potato chlorotic stunt virus (SPCSV) (Gibson *et al.,* 1998; Di Feo *et al.,* 2000; Mwanga *et al.*, 2002; Mukasa *et al.*, 2006). The yield reduction reported for this disease is of 65.25 to 72.24% on different sweet potato cultivars (Gutiérrez *et al.,* 2003).

In Argentina, the following viruses have been reported in sweet potato crops: sweet potato vein mosaic virus (SPVMV) (Nome, 1973), SPFMV (Nome *et al.*, 1980) sweet potato mild speckling virus (SPMSV) (Alvarez *et al.,* 1997) and SPCSV (Di Feo *et al.,* 2000).

Address correspondence to: Dra. Claudia F. Nome. Instituto de Fitovirología y Fisiología Vegetal (IFFIVE-INTA). Camino 60 Cuadras Km 5 1/2. (X5020ICA) Córdoba, ARGENTINA.
E-mail: cnome@correo.inta.gov.ar
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In 1984, Argentinean sweet potato cv Morada INTA plants, known to be tolerant to SPFMV, presented a serious viral disease named chlorotic dwarf, characterized by symptoms of chlorotic mosaic, leaf deformation and area reduction, and plant dwarfism (Di Feo and Nome, 1990). Following studies showed that there are three viruses involved in this disease: SPFMV, SPMSV and SPCSV, with a synergistic effect between SPCSV and the two potyviruses (SPFMV and SPMSV). The combined action of the three virus produced more severe symptoms on cv Morada INTA (Di Feo *et al.,* 2000). Resent investigations in SPVD have shown that the crinivirus suppresses the RNA silencing (Kreuze *et al.*, 2005), the concentration of potyviruses in the plant increases (Karyeija *et al.,* 2000), therefore the synergistic effects occur. The yield reduction by SPCD may reach an 80% (Di Feo *et al.*, 1995).

SPVMV, SPFMV and SPMSV viruses belong to the family *Potyviridae*, genus *Potyvirus*, while SPCSV belongs to the family *Closteroviridae,* genus *Crinivirus.*

The synergism among not related viruses is a known event that has been and is being studied (Shi *et al.,* 1995, 1997; Pruss *et al.,* 1997; Fondong *et al.,* 2000; Karyeija *et al.,* 2000; Wang *et al.,* 2002; Wintermantel, 2005; Mukasa *et al.*, 2006); precise mechanisms involved in this process are still uncertain (Wang *et al.,* 2002; Kreuze *et al.*, 2005).

The potyvirus family does not posses one movement protein but a group of proteins related to this function (coat protein, CP; helper component protein, HC-Pro; cylindrical inclusions proteins, CI; and genome linked viral protein, VPg). Potyviral HC-Pro suppresses gene silencing and consequent plant responses, allowing an increase in viral replication (Savenkov *et al.,* 2001). Potyviral CI are necessary for viral replication, they display RNA helicase activity and are involved in cell-to-cell movement. The conic deposits of CI may translocate the virus within the cell (already encapsulated), and the viral CP may alter the SEL (size exclusion limit) of the plasmodesms to allow the passing to the adjacent cell. HC-Pro is considered to act in long distance movement, to enter and exit the vascular system. Once in the phloem, the CP + VPg are thought to bind plant factors facilitating their distribution through the plant (Revers *et al.,* 1999).

The protein HSP70h of the closterovirus family is related to a large family of chaperons involved in folding and transport of animal proteins. It has been observed that binding of HSP70h to the virus is required for the infectivity of the viral particles (Agronovsky *et al*., 1998); HSP70h may be responsible for carrying the virus from the cytoplasm to and through the plasmodesmata (Peremyslov *et al.,* 1999). Family *Closteroviridae* codes two CPs: CP and minor CP (CPm). CP covers most of the viral particle, while CPm is found on one extreme of the particle. CPm is considered to be involved in cell-to-cell and long distance movements, as well as in vectors interaction (Agranovsky *et al.,* 1995).

In the infected plants with chlorotic dwarf, the crinivirus is the only virus containing a protein with sequence homolog to HSP70, called HSP70 homolog (HSP70 h) (Yeh *et al.,* 2000), HSP-like (Agranovsky *et al.,* 1998), or HSP70 related protein homologue (Fauquet *et al.*, 2005b). The sequence coding for HSP70h is highly conserved within the criniviruses and considered characteristic of the family *Closteroviridae*; it was, therefore, chosen as a target for this study.

The purpose of this work was to determine if the crinivirus SPCSV is localized in cells others than the phloem during synergistic infection with potyviruses SPFMV and SPMSV in chlorotic dwarf disease.

Material and Methods

1. Virus isolates and detection

All experiments were performed on sweet potato plants cv Morada INTA. Plants were experimentally inoculated with the following combinations of viruses: SPMSV + SPCSV, SPFMV + SPCSV, SPMSV + SPCSV and SPCSV.

Healthy plants were propagated agamically. The virus isolates were obtained from symptomatic sweet potato plants from Cordoba (Argentina) crop fields (material involved in previous investigations, Di Feo *et al.*, 2000), and maintained by grafting and viral transmission using the corresponding vectors, by semi-persistent transmission with whiteflies *Bemisia tabaci* (order *Hemiptera*, family *Aleyrodidae*) for SPCSV and by non-persistent transmission with aphids *Myzus persicae* (order *Hemiptera*, family *Aphididae*) for both potyviruses. SPCSV was also isolated in *Nicotiana benthamiana* with whitefly transmition.

Greenhouse plants were treated with acaricides ovacides (Clofentezine; Acaristop 50 SC, from AgrEvo, Hoesch and Schering), aphicide (Deltamethin, Decis, Bayer) and /or insecticides against white flies (Buprofezin, Applaud, S. Ando & Co.) after vectors transmission. Healthy plants were kept in separated compartments and treated similarly as those infected ones. Viral isolates were kept in growth chambers under con-

trolled conditions of temperature (26-28ºC), humidity (65-75%) and light (14 hs).

Serological analyses to control the different viral isolates, using immuno electro microscopy with decoration (IEM-D), were performed with the following antisera: antiserum against EC triple viral co-infection (Di feo *et al.*, 2000), antiserum for SPFMV (Di feo *et al*., 2000), antiserum for SPMSV (Di feo *et al*., 2000), antiserum for SPSVV (=SPCSV) (Cohen *et al*., 1992), monoclonal antiserum for SPCSV and purified immunoglobulin anti SPCSV (Dr J. Cohen, Agricultural Research Organization The Volcani Centre, Dept of Virology, Bet Dagan, Israel, and Dr J. Vetten, Inst Biochemie und Pflanzenvirologie, Braunschweig, Germany).

2. Viral localization

RT-PCR, Cloning and sequencing of HSP70h of SPCSV isolate

To amplify a partial sequence of the heat shock protein 70 homologue gene of SPCSV isolate from Argentina, RT-PCR was performed using specific primers (Winter *et al*., 1997) with total RNA purified from infected tissue. Access RT-PCR System kit (Promega Biotech, Madison WI, USA) was used.

The viral extraction was prepared by partial purification (clarified and filtrated on 25% sucrose cushion) of *Nicotiana benthamiana* plants infected with the SPCSV isolate. The RT-PCR product was purified with MicroSpin™ S-400 HR Columns (Amersham Pharmacia; Amersham Biosciences, AB; Uppsala; Sweden) and cloned into PCR-4-TOPO vector (Invitrogen, California, USA) following the manufactures´s instructions. Competent *Escherichia coli* TOP 10 cells were transformed according to Sambrook *et al.* (1989). Clones were analyzed with the restriction enzyme Eco RI and a clone selected was sequenced in both directions, using M13 Forward and M13 Reverse universal primers (Cornell University, USA).

Computer analysis of the sequenced data

Sequence data were assembled and analyzed by EditSeq, SeqMan, MegAlign and Protean (Lasergene software, DNASTAR ver. 5, 2001) and manual adjustment were performed when necessary. GeneBank database was searched using BLAST program (Altschul *et al.,* 1990). Multiple sequence alignment was performed using ClustalW version 1.82, MegAlign option (Lasergene software, DNASTAR ver. 5, 2001). Phylogenetic tree was constructed using PHYLIP software Package version 3.57c (Felsenstein, 1993) by the parsimony method, bootstrapped (n=100) and visualized with the TreeView program (versión 1.6.5) (Page, 1996).

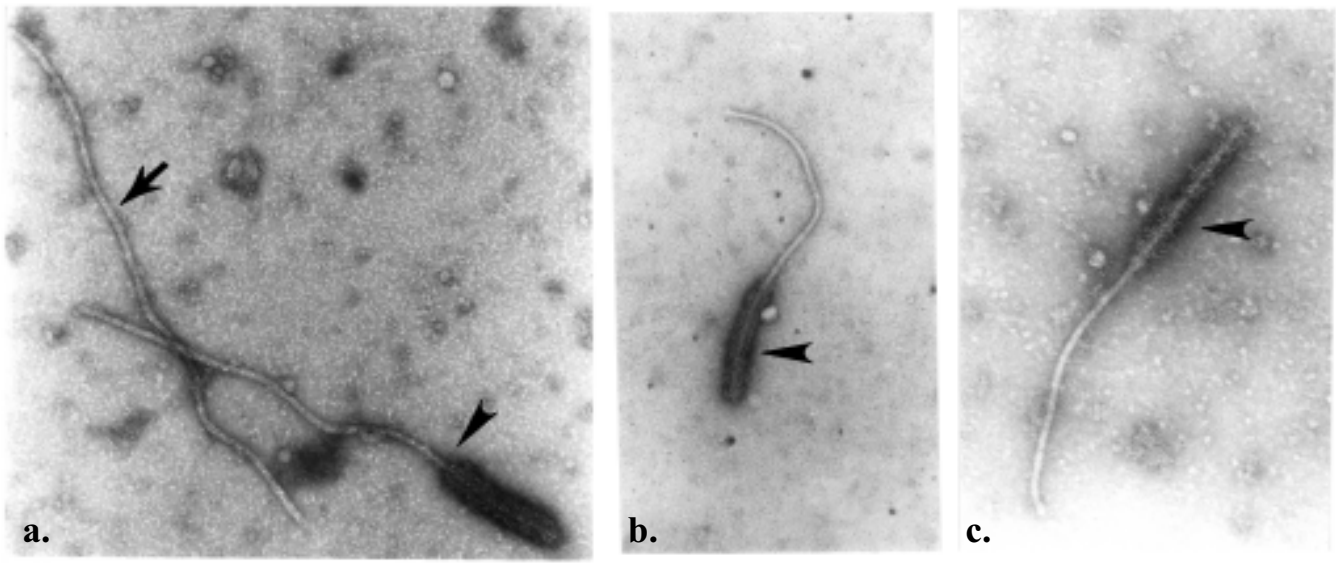

FIGURE 1. a. Viral particle partially decorated (arrow head) with ant-SPFMV serum, and viral particle without decoration (arrow) from sweet potato plants extract triply co-infected (SPFMV+SPCSV+SPMSV). X 72,860. **b.** Viral particle partially decorated (arrow head) with ant-SPFMV serum from of sweet potato extract infected with SPFMV + SPCSV (same plant as Fig. 1c) X 59,260. **c.** Viral particle partially decorated (arrow head) with ant-SPCSV serum from of sweet potato extract infected with SPFMV + SPCSV (same plant as Fig. 1b) X 71,430.

Sequence accession number

The nucleotide sequence reported in this paper has been deposited in GeneBank database as accession number AY729021.

Preparation of riboprobe for SPCSV HSP70h for in situ hybridization

Once the orientation of the insert in the clone was confirmed, the plasmid was purified, linearized with PvuII (Promega, USA) and used as a template for the synthesis of riboprobe. This one, for SPCSV isolate (complementary to the HSP70h gene viral sense sequence) included the T7 promoter present in the transcription vector and was labeled with digoxigenin (DIG)-11-UTP, following provider´s specifications and protocols (Eisel *et al.*, 2000). The evaluation of riboprobe labeling was estimated according to manufacturer´s recommendations (CDP-Star-ready to use DIG Luminescent Detection Kit, Roche Molecular Biochemicals, Mannheim, Germany).

In situ hybridization (ISH) and dot blot assay

This technique was applied to sweet potato plants, either healthy or infected with SPCSV, SPFMV +SPCSV, SPMSV +SPCSV and triple inoculated (SPCSV +SPMSV + SPCSV), young, middle aged and old leaves.

Ultra thin sections of the plant material included in LR-Gold resin were performed. These sections were used for *in situ* hybridization (ISH) (MacFadden, 1991), with 10 ng/ml of probe during 16 hs, at 45ºC, antidigoxigenin 1/40 (Roche Molecular Diagnostics,

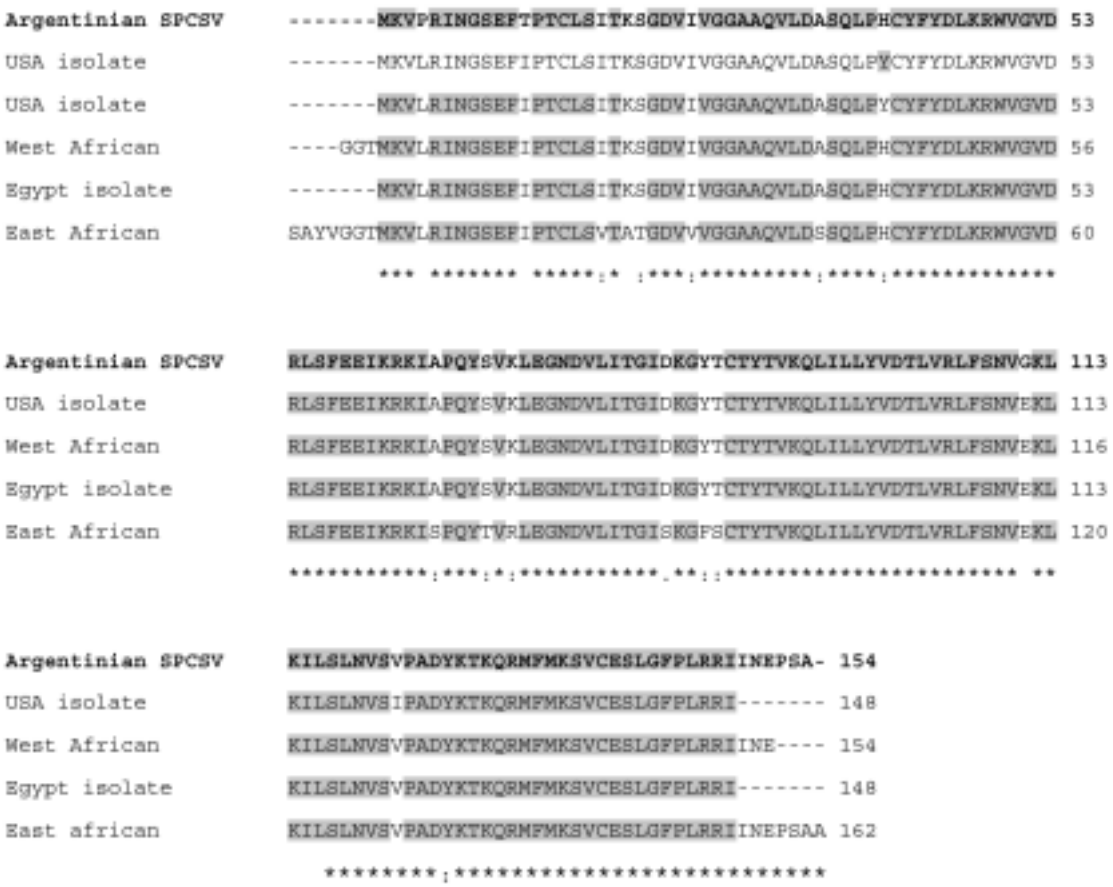

FIGURE 2. Multiple sequence alignment of Argentinean SPCSV isolate, West African isolate clone 2, Egypt isolate, USA isolate and East African SIEA-19b isolate-encoded proteins, constructed using the program ClustalW version 1.83. Grey regions represent conserved amino acids among the isolates.

Mannheim, Germany) for 2 hs, and Protein A 5nm gold conjugate 1/70 (Pelco, California, USA) for 1:30 hs Observations were made on a transmission electron microscope Jeol 1220 EXII (Jeol, Tokyo, Japan).

The riboprobe reaction against healthy tissue was evaluated by dot-blot assay, using crude extracts of healthy and infected sweet potato plants with single (SPMSV) and triple co-infection (SPFMV + SPCSV + SPMSV), total RNA purified from same plants, and plasmid DNA as positive control. Plant tissues were macerated in 1/10 of extraction buffer (PBS + 0.5 ml of Tween20 + 20% PVP + sodium sulfite 1%) and centrifuged for 5 min at 5000 g. Total RNA was extracted directly from infected sweet potato tissues according the protocol of Conci *et al.* (1999), which was modified with the addition of 30 mg/ml polyvinylpolypyrrolidone (PVPP) to prevent the precipitation of polysaccharides and 1% sodium phosphate as antioxidant. The hybridization was made with an estimated riboprobe concentration of 100 µg/ml at 45ºC. The reaction was developed with chemiluminescent kit (CDP-Star-ready to use DIG Luminescent Detection Kit, Roche Molecular Biochemicals, Mannheim, Germany).

Results

1. Virus isolates and detection

Plants with the different isolates developed the characteristic symptomatology (chlorotic mosaic, dwarfism and leaf deformation) to the given temperature, light and humidity conditions used.

The presence of SPCSV, SPFMV and SPMSV was confirmed by IEM-D assays in sweet potato plants infected with SPCSV isolate, SPCSV + SPMSV, SPCSV + SPFMV and SPCSV + SPFMV + SPMFV. Intensive specific decoration was observed on virions of single infections: SPFMV, SPMSV and SPCSV with each corresponding antisera.

In plants co-infected with SPFMV + SPCSV, SPMSV + SPCSV or triply inoculated, partially decorated particles were observed with anti-SPFMV, anti-SPCSV and anti-SPMSV and serum (Figs. 1a, b and c). This particles were in low proportions.

2. Viral localization

RT-PCR, Cloning, sequencing and analysis of HSP70h of SPCSV isolate

The RT-PCR amplified product of HSP70h of SPCSV isolate showed a fragment of approximately 485 nucleotides, corresponding to the size of the *Crinivirus* HSP70h.

Comparison of the nucleotide sequence with strains from other areas of the world, revealed an overall 88.6% identity with West African isolate clone 2 (in 465 nt, accession # AJ278653), 88.3% with Egypt isolate (in 446 nt, accession # AJ515381), 87.9% with USA isolate (446 nt, accession # AF260321) and 71.3% with East African S1EA-19b isolate (in 486 nt, accession # AJ010929). The deduced 154 amino acids sequence of HSP70h Argentinean SPCSV isolate showed significant identities with the proteins encoded by West African isolate clone 2 (98% in 154 aa), Egypt isolate (97% in 148 aa), USA isolate (96% in 148 aa) and East African S1EA-19b isolate (90% in 162 aa).

Multiple sequence alignment of these homologous proteins were carried out by using ClustalW (Fig. 2).

The analysis of the phylogenetic relationships of HSP70h from Argentinean SPCSV isolate with other crinivirus isolates showed a closer relationship with West African isolate clone 2; next, Egypt and USA isolates; and last, to East African S1EA-19b isolate. These results are consistent with the topology of the tree obtained by the maximum parsimony method using the amino acids sequence of HSP70h from Argentinean SPCSV isolate (Fig. 3).

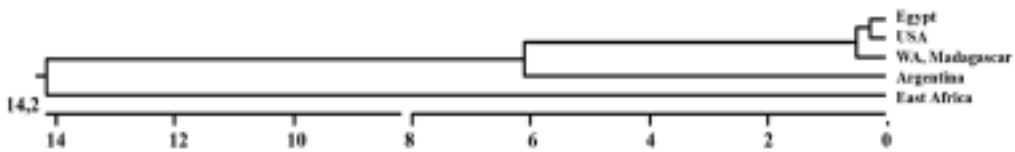

FIGURE 3. Phylogenetic tree constructed with HSP70 homologue from Argentinean SPCSV isolate and related sequences using the Parsimony method analysis (100 replicates).

Preparation of riboprobe for SPCSV HSP70h for in situ hybridization

The yield for 50 µl of labeled probe was of 250 ng/ µl.

In situ hybridization (ISH) and dot-blot assay

A positive reaction was observed in sweet potato plants infected with the Argentinean SPCSV isolate, within cells nucleus of young sieve tubes and phloem parenchyma. In sweet potatoes triply co-infected, SPFMV + SPCSV, and SPMSV + SPCSV, signal was detected in the nucleus of phloem cells and xylem parenchyma, and also extended towards mesophyll cells. Reaction was always negative in healthy plant tissues (Figs. 4a, b, c, d and e). The specific reaction was observed as small black dots, corresponding to the 5nm gold particles; bigger black dots were considered background.

Table 1 shows electron microscopy observations of *in situ* hybridization on ultra thin sections from healthy plants infected with SPCSV, SPMSV + SPCSV, SPFMV + SPCSV, and triply co-infected, in young, average and old leaves.

The dot-blot assay detected viral RNA on crude extracts of infected plants and total RNA purified from single infected plants (SPCSV), triply co-infected plants (SPFMV + SPCSV + SPMSV) and plasmid DNA2. There were no hybridization signals with extraction buffer (mock) and crude extracts from healthy plants, confirming the results obtained by *in situ* hybridization assay (Fig. 5).

By *in situ* hybridization and dot-blot assays, it was demonstrated that the riboprobe for HSP70h of Argentinean SPCSV isolate detected specifically its mRNA on infected sweet potato tissues, no signal was observed when healthy tissues were tested.

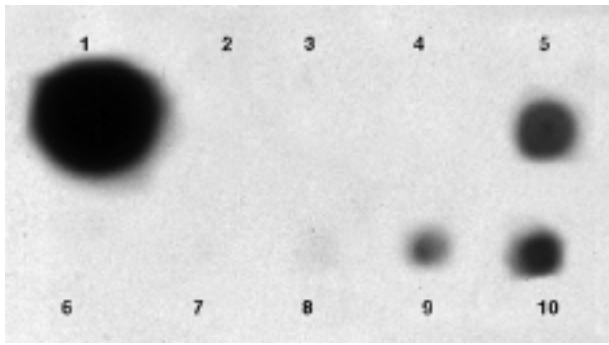

FIGURE 5. Dot-blot hybridization for evaluation of HSP70h of SPCSV isolate. 1: plasmid DNA, 2: healthy crude tissue extract (1/10), 3: total RNA from healthy sweet potato tissue (1/10), 4: crude extract from triply co-infected plants (1/10), 5: total RNA from triply co-infected sweet potato plants (1/10), 6: Mock, 7: healthy sweet potato plant crude extracts, 8: total RNA from healthy sweet potato plant, 9: crude extract of triply co-infected sweet potato plants, 10: total RNA from triply co-infected sweet potato plant.

TABLE 1.

Electron microscopy observations of young, average and old leaves of healthy and infected plants with SPCSV, SPMSV + SPCSV, SPFMV + SPCSV, and triple co-infection used for *in situ* hybridization using *Crinivirus* HSP70h riboprobe.

		Epidermis	Mesophyll	Vascular tissue
SPCSV	Young leaves	(-)	(-)	(+)
SPMSV+SPCSV	Young and average leaves	(-)	(+)	(+)
SPFSV+SPCSV	Young and average leaves	(-)	(+) (+)	(+) (+)
Chlorotic dwarf	Young, average and old leaves	(-)	(+) (+)	(+) (+)
Healthy	Young and average leaves	(-)	(-)	(-)

(+) (+): positive reaction was observed. (+): light positive reaction was observed. (-): No reaction was observed.

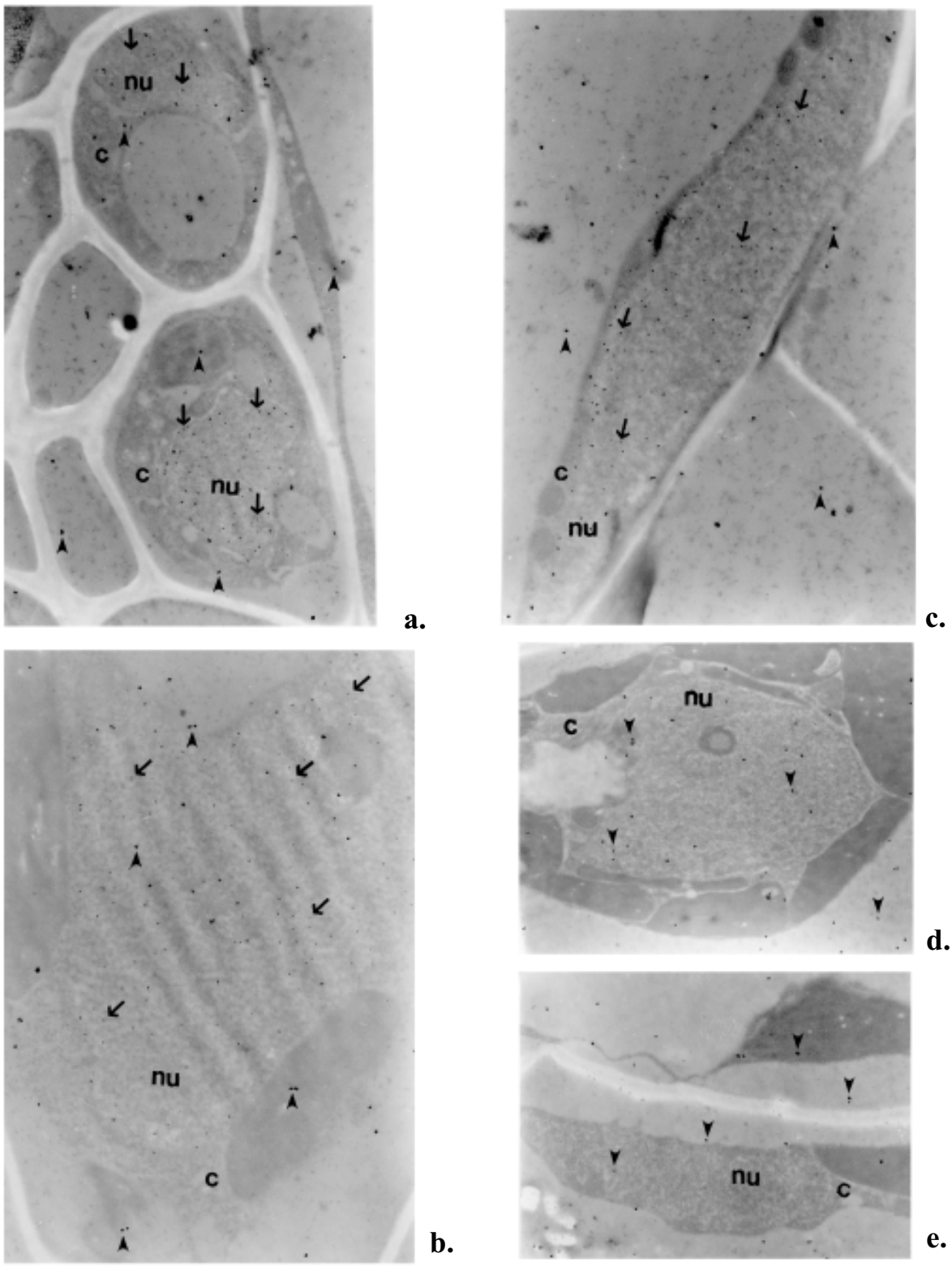

FIGURE 4. nu: nucleus, c: cytoplasm, arrows: small black dots gold particles, positive reaction, arrow heads: big black dots, background and stain artifacts.
a. Positive reaction within nucleus of phloem cells from sweet potato plants infected with SPCSV using HSP70h riboprobe. X 12,800. **b.** Positive reaction using HSP70h riboprobe within nucleus of xylem parenchyma cells of sweet potato infected with chlorotic dwarf disease. X 15,790. **c.** Positive reaction within nucleus of mesophyll cells of sweet potato infected with chlorotic dwarf disease. X 12,210. **d.** Negative reaction in phloem cells of healthy sweet potato using HSP70h riboprobe. X 67,000. **e.** Negative reaction of HSP70h probe with mesophyll cells of healthy sweet potato. X 9,690

Discussion

RNA silencing or post transcriptional genetic silencing (PTGS) is one of the defense mechanisms used by plants to resist viral infections; where viral genome is detected and removed by plant endonucleases (Dunoyer *et al.*, 2004).

Infact, SPCSV induces RNAi; the exact mechanism however, is not completely elucidated yet (Kreuze *et al.,* 2005). In chlorotic dwarf disease, there is a synergism between any of the involved potyvirus (SPFMV and SPMSV) and the crinivirus (SPCSV). The SPFMV increases its concentration in the phloem when accompanied by the crinivirus (Karyeija *et al.,* 2000), this is not observed among both of the potyvirus (Di feo, 1994, 1996). These results have been previously appreciated by the plant symptomatology. The *in situ* hybridization studies reported in the present work demonstrate that the crinivirus (SPCSV) is confined to the phloem in single infections, while in co-infections with the potyviruses (SPFMV and SPMSV) is found in the mesophyll to different extents. Therefore, somehow the interaction between them allows at least the subgenomic HSP70h RNA from the crinivirus to abandon the vascular tissue.

On the other hand, both potyviruses increases their concentration when accompanied by the crinivirus on sweet potato cv Morada INTA (observations by C.F. Nome, unpublished data). An attempt to explain these phenomena could be done using the previously mentioned mechanisms, suggesting that the movement protein (MP) from potyviruses allows the crinivirus to move towards tissues other than the phloem. Also it could be considered that the particles decorated by portions (Figs. 1a, b and c) might be involved in this process; relocation of the crinivirus outside of the phloem may be due to the potyvirus portion, with its ability to get to and trespass the plasmodesmata. At the same time, a mechanism of gene silencing suppression by crinivirus might be acting in the multiple infection allowing the SPFMV and SPMSV increase their concentrations (observations by C.F. Nome, unpublished data).

In conclusion, our data indicates that at least one of the subgenomic SPCSV RNAs (the one coding HSP70h protein) leaves out of the phloem in presence of the potyviruses involved in chlorotic dwarf disease.

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References

Agranovsky A, Folimonov AS, Folimonova SY, Morozov SY, Schiemann J, Lesemann DJ, Atabekov G (1998). *Beet yellows closterovirus* HSP70-like protein mediates the cell-to-cell movement of a *Potexvirus* transport-deficient mutant and a *Hordeivirus*-based chimeric virus. J Gen Virol. 79: 889-895.

Agranovsky AA, Lesseman DE, Maiss E, Hull R, Atabekov JG (1995). "Rattlesnake" structure of a filamentous plant RNA virus built of two capsid proteins. Proc Nat Acad Sci USA. 92: 2470-2473.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lippman DJ (1997). Gapped BLAST and PSI BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-3402.

Alvarez V, Ducase D, Biderbost E, Nome SF (1997). Sequencing and characterization of the coat protein and 3´ non-coding region of a new sweet potato virus. Arch Virol. 142: 1635-1644.

Cohen J, Franck A, Vetten HJ, Lesemann DE, Loebenstein G (1992). Purification and properties of *Closterovirus*-like particles associated with a whitefly-transmitted disease of sweet potato. Ann Appl Biol. 121: 257-268.

Conci LR, Guzman, FA (1999). Sondas de hibridación molecular para la detección del mal de Río Cuarto (MRCV). In: Métodos para detectar patógenos sistémicos. D.M. Docampo and S.L. Lenardón, eds. IFFIVE-INTA-JICA, Córdoba, pp. 95-98.

Di Feo LV, Nome SF (1990). Chlorotic Dwarf: a new sweet potato disease in Argentina. Newsl. Int. Working group Sweet Potato Viruses (IWGSPV), nº 3.

Di Feo LV, Nome SF, Ducasse D, Alvarez V, Biderbost EB (1994). Caracterización parcial de un nuevo virus en plantas de batata (*Ipomoea batatas* L.) cv. Morada INTA afectadas por "enanismo clorótico". In: Libro de Resumenes del XVII Congreso Argentino y VI Latino-americano de Horticultura. Soc. Arg. Horticultura. Huerta Grande, pp. 83.

Di Feo LV, Biderbost EB, Racca R, Nome SF, Mollinedo V, Lopez Lambertini P (1995). Effect of ontogeny and chlorotic dwarf, a viral disease, on the productivity of sweet potato (*Ipomoea batatas* (L) Lam.) cv. Morada INTA. Fitopatología 30: 96-99.

Di Feo LV, Castellanos P, Nome S, Biderbost E (1996). Comportamiento del cv. Morada INTA de batata frente a infecciones simples y dobles con SPFMV y SPSSV. In: Actas del XIX Congreso Argentino de Horticultura. Soc. Arg. Horticultura. San Juan. pp. 24.

Di Feo LV, Nome SF, Biderbost E, Fuentes S, Salazar LS (2000). Etiology of sweet potato chlorotic dwarf disease in Argentina. Plant Dis. 84: 35-39.

DNASTAR ver. 5. (2001). Lasergene Biocumputing Software for Windows. DNASTAR, Madison, WI.

Dunoyer P, Ledellier C-H, Abreu Parizotto E, Himber C and Voinnet O (2004). Probing the microRNA and small interfering RNA pathways with virus-encoded suppressors of RNA silencing. The Plant Cell 16: 1235-1250.

Eisel D, Grunewald-Janho S, Kruchen B (2000). Transcriptional labeling of RNA probes: Procedures for non radioactive labeling and detection. In: DIG application manual. Roche Diagnostics Corporation, Germany, pp. 65.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (2005a). Virus taxonomy. Eighth report of the international committee on taxonomy of viruses. Pp. 1251.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (2005b). Virus taxonomy. Eighth report of the international committee on taxonomy of viruses. Pp. 1084.

Felsenstein J (1993). PHYLIP (Phylogeny Inference Package) version 3.57c. Distribuited by the author. Department of Genetics, University of Washington, Seattle.

Fondong VN, Pita JS, Rey MEC, Kochko A, Beachy RN, Fauquet CM (2000). Evidence of synergism between African cassava mosaic virus and double-recombinant *Geminivirus* infecting cassava in Cameroon. J Gen Virol. 81: 287-297.

Gibson RW, Mpembe I, Alicai T, Carey EE, Mwanga, Seal S, Vetten HJ (1998). Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda Plant Pathology 47: 95-102.

Gutiérrez DL, Fuentes S, Salazar LF (2003). Sweet potato Virus Disease (SPVD): Distribution, Incidence, and Effect on Sweet potato Yield in Peru International Potato Center (CIP), Plant Disease 87: 297-302.

Karyeija RF, Kreuze JF, Gibson RW, Valkonen JPT (2000). Synergistic interaction of a potyvirus and a phloem-limited crinivirus in sweet potato plants. Virology 269: 26-36.

Kreuze JF, Savenkov EI, Cuellar W, Li X, Valkonen JPT (2005). Viral Class 1 RNase III Involved in Suppression of RNA Silencing. J Virol, 79: 7227-7238.

MacFadden GI (1991). *In situ* hibridization techniques: Molecular cytology goes ultrastructural. In: Electron Microscopy of Plant Cells. JL Hall and C Hawes, eds. Academic Press, New York, pp. 242-254.

Mukasa SB, Rubaihayo PR, Valkonen JPT (2006). Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfected sweet potato plants. Plant Pathology 55: 458-467.

Mwanga ROM, Moyer JW, Zhang DP, Carey EE, Yencho GC (2002). Nature of resistance of sweet potato to sweet potato virus disease. I International Conference on Sweetpotato. Food and Health for the Future. Acta Hort. (ISHS) 583: 113-119.

Nome SF (1973). *Sweet potato vein mosaic virus* in Argentina. Phytopathol Z. 77: 44-54.

Nome SF, Giorda LM, Vasquez A (1980). El virus del moteado plumoso de la batata (SPFMV), en Argentina. Rev Invest Agropec 15: 625-634.

Page RDM (1996). Treeview: an application to display phylogenetic trees on personal computers. Comput Appl Biosci. 12: 357-358.

Peremyslov VV, Hagiwara Y, Dolja VV (1999). HSP70 homolog functions in cell-to-cell movement of a plant virus. Biochemistry 96: 14771-14776.

Pruss G, Shi XM, Carrington JC, Vance VB (1997). Plant viral synergism: The potyviral genomes encodes broad-range pathogenisity enhancer that transactivates replication of heterologous viruses. Plant Cell. 9: 859-868.

Revers F, Le Gall O, Candresse T, Maule AJ (1999). New advances in understanding the Molecular Biology of plant/potyvirus interactions. Mol Plant Microbe Interact 12: 367-376.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning. A Laboratory Manual. Second Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. 1: 33-39.

Savenkov EI, Valkonen JPT (2001). Potyviral helper-component proteinase expressed in transgenic plants enhances titers of *Potato leaf roll virus* but does not alleviate its phloem limitation. Virology. 283: 285-293.

Shi XM, Pruss G, Xin G, Sriskanda V, Vance VB (1995). Identification of plant synergism genes. In: 7th Symposium on Environmental-Releases of Biotechnology Products: Risk Assessment Methods and Research Progress. Pensacola, Flo1rida. Chapter 3. pp 11-22.

Shi XM, Miller H, Verchot JM, Carrington JC, Vance VB (1997). Mutations in the region encoding the central domain of the helper component-proteinase (HC-Pro) eliminate potato virus X / potyviral synergism. Virology. 231: 35-42.

Tairo F, Kullaya A, Valkonen JPT (2004). Incidence of viruses infecting sweet potato in Tanzania. Plant Disease 88: 916-920.

Wang Y, Gaba V, Yang J, Palukaitis P, Gal-On A (2002). Characterization of synergism between *Cucumber mosaic virus* and potyviruses in cucurbit hosts. Phytopathology 92: 51-58.

Winter S, Korbler M, Kollner U (1997). Detection and differentiation of Sweet potato closteroviruses by RT-PCR and single-strand conformational polymorphism. In: Diagnosis and identification of plant pathogens. Proceedings of the 4th International Symposium of the European Foundation for Plant Pathology, Kluwer Academic Publishers, Dordoecht. pp 473-475.

Wintermantel WM (2005). Co-infection of Beet mosaic virus with Beet Yellowing Viruses Leads to Increased Symptom Expression on Sugar Beet. Plant Dis 89: 325-331.

Yeh HH, Tian T, Rubio L, Crawford B, Falk BW (2000). Asynchronous accumulation of *Lettuce infectious yellows virus* RNAs 1 and 2 and identification of an RNA 1 tRNAs enhancer of RNA2 accumulation. J Virol 74: 5762-5768.