

1 **Synergistic interactions of begomoviruses with sweet potato**
2 **chlorotic stunt virus (genus *Crinivirus*) in sweetpotato**
3 **(*Ipomoea batatas* L.)**

4
5 **WILMER J. CUELLAR^{1†Δ}; MARCO GALVEZ^{1,2Ä}; SEGUNDO FUENTES¹; JOAB**
6 **TUGUME^{1,3}; JAN KREUZE^{1*}**

7 ¹*The Virology Laboratory, International Potato Center (CIP), Av. La Molina 1895, L12,*
8 *Lima, Peru. ²Facultad de Agronomía, Programa de Fitopatología, Universidad Nacional*
9 *Agraria La Molina, Lima12, Peru. ³Department of Agriculture, College of Agricultural*
10 *and Environmental Sciences, Makerere University, Kampala, Uganda*

11 ^Δ First two authors contributed equally to the study

12 **Corresponding author:** *Jan Kreuze; Address: International Potato Center (CIP), Av.
13 La Molina 1895, L12, Lima, Peru; Tel: +51 1 349 6017 ext 3054; Fax: +51 1 317 5326;
14 e-mail: j.kreuze@cgiar.org

15 **Current Address:** [†] Virology Laboratory. International Center for Tropical Agriculture
16 (CIAT). Km 17, Recta Cali-Palmira, Colombia

17

18 **Running title:** sweetpotato begomovirus synergism with SPCSV

19

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mpp.12200

1 **Keywords:** sweetpotato, synergism, begomovirus, sweet potato chlorotic stunt virus,

2 RNase3

3

4 **Word count:** 6778

1 SUMMARY

2

3 Three hundred ninety four sweetpotato accessions from Latin America and East Africa
4 were screened by PCR for presence of begomoviruses and forty six were found positive.

5 All were symptomless in sweetpotato and generated leaf-curling and/or chlorosis in
6 *Ipomoea setosa*. The five most divergent isolates, based on complete genome sequences,

7 were used to study interactions with sweet potato chlorotic stunt virus (SPCSV), known
8 to cause synergistic diseases with other viruses. Co-infections led to increased titres of

9 begomoviruses and decreased titres of SPCSV in all cases, although the extent of the
10 changes varied notably between begomovirus isolates. Symptoms of leaf curling only

11 developed temporary in combination with isolate StV1 and coincided with presence of
12 highest begomovirus concentrations in the plant. Small interfering RNA (siRNA)

13 sequence analysis revealed co-infection of SPCSV with isolate StV1 lead to relatively
14 increased siRNA targeting of central part of the SPCSV genome and a reduction in

15 targeting of the genomic ends, but no changes to targeting of StV1 as compared to single
16 infection of either virus. These changes were not observed in the interaction between

17 SPCSV and the RNA virus sweet potato feathery mottle virus (genus *Potyvirus*),
18 implying specific effects of begomoviruses on RNA silencing of SPCSV in dually

19 infected plants. Infection in RNase3 expressing transgenic plants showed this protein was
20 sufficient to mediate this synergistic interaction with DNA viruses, similar to RNA

21 viruses, but exposed distinct effects on RNA silencing when RNase3 is expressed from
22 its native virus, or constitutively from a transgene, despite a similar pathogenic outcome.

23

1 INTRODUCTION

2 Sweetpotato (*Ipomoea batatas* (L.) Lam) is a perennial plant and the sixth most important
3 crop which the world depends on for their food security (FAO, 2013). Because
4 sweetpotato is vegetatively propagated by taking cuttings from a previous crop (directly
5 or from sprouted tubers), build-up of virus infections over generations is a major
6 constraint and contributes to severe losses and cultivar decline (Clark *et al.*, 2012). More
7 than 30 viruses have been reported to infect sweetpotato and half of them are recently
8 described DNA viruses belonging to the families *Geminiviridae* and *Caulimoviridae*
9 (Clark *et al.*, 2012). Geminiviruses (family *Geminiviridae*) are plant viruses that have a
10 circular, single-stranded DNA genome encapsidated within twinned isometric particles
11 (Fauquet & Stanley, 2003). They are grouped into four genera based on insect vector,
12 host range, and genome organization (Fauquet & Stanley, 2003). Members of the genus
13 *Begomovirus* are transmitted by whiteflies, have single or bipartite component genomes,
14 and infect dicotyledonous plants. Within the genus *Begomovirus* the sweetpotato
15 infecting viruses are monopartite but are distinct from other monopartite begomoviruses,
16 forming a phylogenetically unique lineage, and are referred to as sweepoviruses as a
17 group (Fauquet & Stanley, 2003, Wasswa *et al.*, 2011, Esterhuizen *et al.*, 2012,
18 Albuquerque *et al.*, 2012). Sweepovirus taxonomy is complex and the currently
19 sequenced sweepoviruses have been suggested to correspond to up to 17 different species
20 based on nt sequence identities (Albuquerque *et al.*, 2012). However the occurrence of
21 frequent recombinants and the lack of any complementing biological differences between
22 suggested species currently render these classifications of little practical use and in this
23 paper we will refer to them simply as sweepoviruses.

1 Despite the lack of obvious symptoms associated with sweepovirus infections, yield
2 reduction of between 10-80% have been reported in infected sweetpotato plants (Clark &
3 Hoy, 2006, Ling *et al.*, 2010). Since they can occur at relatively high incidences in crops
4 they may thus be responsible for considerable crop loss on a global scale.

5 Studies with several other sweetpotato infecting viruses have shown that most of them
6 can cause severe synergistic disease complexes when co infected with sweet potato
7 chlorotic stunt virus (SPCSV; species: *Sweet potato chlorotic stunt virus*, Genus
8 *Crinivirus*, family *Closteroviridae*) leading to increased yield losses. These include RNA
9 viruses of the genera *Potyvirus*, *Ipomovirus*, *Carlavirus* and *Cucumovirus* as well as
10 otherwise symptomless DNA viruses of the genera *Cavemovirus* and *Solendovirus*
11 (Karyeija *et al.*, 2000, Cuellar *et al.*, 2011b, Mukasa *et al.*, 2006, Untiveros *et al.*, 2007).

12 The best studied of these synergistic diseases is the one between SPCSV and sweet potato
13 feathery mottle virus (SPFMV; species: *Sweet potato feathery mottle virus*, genus
14 *Potyvirus*, family *Potyviridae*) and has been shown to be mediated by the SPCSV
15 encoded RNase3 protein, which can also mediate synergistic disease with two other
16 unrelated RNA viruses (Cuellar *et al.*, 2009). RNase3 is a double-stranded RNA
17 (dsRNA)-specific class 1 RNA endoribonuclease III that can digest long as well as short
18 dsRNA and functions as an RNA silencing suppressor (RSS)(Cuellar *et al.*, 2009,
19 Weinheimer *et al.*, 2014). RNase3 catalytic activity is required for its RSS activity
20 (Cuellar *et al.*, 2009), implicating RNA cleavage in the process of RSS suppression as
21 well as synergistic disease induction. Whereas the exact mechanism of RNase3 action has
22 not yet been elucidated it is clear that it is able to mediate increased susceptibility of
23 sweetpotato to a wide range of viruses (Cuellar *et al.*, 2009).

1 Synergism between SPCSV and sweepoviruses has however not yet been reported.
2 Wasswa *et al.* (2011) reported that a Ugandan sweepovirus isolate was not obviously
3 synergized by co-infection with SPCSV, however our own observations with our
4 reference isolate StV1 seemed to indicate this is not the case for all strains. Therefore, in
5 the current study we screened a selection of sweetpotato germplasm for presence and
6 variability of sweepoviruses and selected the six most diverse isolates for complete
7 sequencing and co-infection experiments with SPCSV to determine if synergism with
8 SPCSV is a general phenomenon for sweepoviruses as it is with other viruses. siRNA
9 profiles in single and mixed infections were also determined with reference isolate StV1
10 and compared to those of the well-studied interaction between SPCSV and SPFMV.

11

12 **RESULTS**

13

14 *Virus detection and host symptoms*

15 During standard virus indexing of germplasm material at CIP generally between 10- 20%
16 of samples are found infected with begomoviruses (data not shown). We took advantage
17 of one of the batches of Latin American sweetpotato accessions (329 genotypes; Table
18 S1) going through virus indexing to characterize the amplified begomovirus nucleotide
19 (nt) sequences identified in 39 accessions. In addition we screened by PCR a collection of
20 65 (symptomless; Table S1) sweetpotato plants collected in East Africa, of which seven
21 were found positive for begomoviruses. Symptomatology was recorded among the
22 sweetpotato accessions from the Americas in the indicator plant *Ipomoea setosa*. No
23 large variation in symptoms was observed among plants infected with begomovirus, but

1 they could be broadly characterized into three categories: typical upwards leaf curling,
2 chlorosis, or both were observed in indicator plants with all isolates that were positive to
3 sweepovirus by PCR (Table 1); in several cases plants were co-infected by other
4 viruses, nevertheless, the infected sweetpotato plants themselves were all symptomless.
5 None of the samples used in this work were found co-infected with SPCSV.

6

7 *Sweepovirus sequence variability and characterization*

8 The sequences of the PCR fragment obtained using the universal sweepovirus primers
9 SPG1 and SPG2 (Li *et al.*, 2004) of forty six isolates identified in this study were
10 determined and compared to those available in the GenBank by PASC and phylogenetic
11 analysis. Phylogenetic analysis using our sequences and others available in the genebank
12 showed that the isolates sequenced in this study spanned the variability found in
13 sweepoviruses except for the cluster corresponding to *Ipomoea yellow vein virus* and
14 *Sweet potato leaf curl canary virus* (data not shown).

15

16 The complete genomes of the most divergent sweepovirus based on the analysis of partial
17 sequences described above, and found free of any other virus detectable by index grafting
18 to *I.setosa*, were selected for complete genome sequencing together with our reference
19 isolate. These were isolates StV1 (Saint Vincent), Mex31 (Mexico), Cub5 (Cuba), Jam12
20 (Jamaica), Per6 and Per10 (Peru). These isolates were then also used in double infection
21 studies with SPCSV-m2-47 as described below. Complete genome comparison confirmed
22 that all six viruses were quite different from each other with <89% nt identity over their
23 genome except for Jam12 and Cub5 (91.7%) and StV1 and Per10 (93.1%). Isolates StV1,

1 Per-6 and Per-10 are most similar to the ICTV approved species *Sweet potato leaf curl*
2 *virus* (93, 93 and 98% identity to type isolate respectively), whereas Jam-12 and Cub-5
3 are most similar to *Sweet potato leaf curl Georgia virus* (91 and 95% similarity to type
4 isolated respectively) and Mex-31 is most similar to *Sweet potato leaf curl South*
5 *Carolina virus* (93% identity to type isolate). Alignment and phylogenetic analysis using
6 108 complete sweepovirus genome sequences available from the genebank confirmed the
7 sequenced isolates were positioned well distributed among the known sweepovirus
8 variability (Fig. 1). All sequences were deposited in the GenBank database (see Table 1
9 for Genbank accession numbers)

10

11 *Synergistic interaction of sweepviruses with SPCSV in sweetpotato*

12 Sweepovirus isolates StV1, Mex31, Cub5, Jam12, Per6 and Per10 were used as inoculum
13 for synergistic interaction studies in sweetpotato cultivar 'Huachano'. Co-infection with
14 SPCSV led to symptoms of upward leaf curling transiently around 3-4 weeks post
15 inoculation only in case of the isolate StV1. This was repeated when plants were cut back
16 and symptoms occurred again in the re-growth after about 3-4 weeks. None of the other
17 five isolates caused any visible symptoms at any time-point. No increase in the severity
18 of SPCSV symptoms was observed (i.e. purpling/yellowing of older leaves or stunting) in
19 combination with any of the sweepovirus isolates. Signal intensity analysis of DNA dot-
20 blot hybridization (Fig. S1) from plants infected with the six sweepovirus isolates showed
21 a significantly higher accumulation of the viruses in plants co-infected with SPCSV (Fig.
22 2) in all cases except isolate Cub5 for which the difference was not significant ($p < 0.05$).
23 The extent and time-point of manifestation of this difference varied considerably between

1 the different isolates, but could be generally divided into three categories. Hybridization
2 signals of isolates StV1 and Mex31 were significantly different from the remaining
3 isolates and showed the overall highest virus titres and a distinct titre development:
4 sweepovirus titres initially increased much faster in mixed infected plants than singly
5 infected plants during the first three weeks, reaching a peak between 4 to 5 weeks post
6 inoculation and another peak at 10 weeks post inoculation. On the other hand, isolates
7 Per6 and Per10 co-infected with SPCSV showed an increase in titres during the first 6
8 weeks (primary infection) as compared to singly infected plants and after cutting back at
9 6 weeks the re-grown sweetpotato plants (secondary infection) reached a peak in titers
10 after one week, followed by a reduction. Finally isolate Cub5 and Jam12, although
11 significantly different from each other, showed much lower titres and smaller differences
12 between single and mixed infected plants during primary infection, whereas difference in
13 secondary infection were more pronounced, especially in Jam12. In general the severity
14 of symptoms caused in the indicator plant *I.setosa* correlated positively the titres
15 determined in sweetpotato, with StV1 and Mex31 showing the most rapidly developing
16 and severe symptoms in *I.setosa* and Cub5 the mildest symptoms. Isolate StV1 showed
17 the strongest signals of all isolates tested and except for the first week showed the biggest
18 differences in titer between singly and doubly infected plants throughout the experiment.
19 It was also the only isolate to induce symptoms typical of begomovirus infection, upward
20 leaf curling (Fig. 3a), although this only happened transiently during the 3rd and 4th week
21 after infection and again 4 weeks after cutting back the plants. This happened in all plants
22 co-infected with SPCSV and coincided with the time of maximum virus accumulation in
23 the plants (Fig. 2).

1 Whereas the dot-blot indicated increased titres of sweepviruses in dual infections, qRT-
2 PCR analysis of the SPCSV Hsp70 gene region (RNA2) in the same plants, indicated that
3 average SPCSV RNA titres were significantly reduced ($p < 0.01$) compared to those in
4 SPCSV single infection in all combinations (Fig. 2, Table S2) and this was confirmed by
5 TAS-ELISA tests (detecting the coat protein) with the isolate StV1 where a decrease in
6 SPCSV antigen was observed (data not shown).

7 To test if the observed increases in sweepvirus titres could be mediated by RNase3
8 alone as has been shown for RNA viruses, transgenic plants expressing RNase3 (Cuellar
9 *et al.*, 2009) were inoculated with StV1. The infected plants developed typical leaf-curl
10 symptoms in the same temporary fashion as seen for non-transgenic plants co-inoculated
11 with SPCSV and StV1 and a similar increase in virus titres (Fig. 3). Similarly, infection
12 with two unrelated DNA viruses, sweet potato collusive virus (SPCV; species: *Sweet*
13 *potato collusive virus*, genus: *Cavemovirus*, family: *Caulimoviridae*) and sweet potato
14 vein clearing virus (SPVCV; species: *Sweet potato vein clearing virus*, genus:
15 *Solendovirus*, family: *Caulimoviridae*) also reproduced symptoms similar to those caused
16 by co-infection with SPCSV and resulted in increased viral titres (Fig. S2)

17
18 *Analysis of virus-derived siRNA from single and double-infected, and RNase3 transgenic*
19 *sweetpotato plants*

20 Raw sRNA sequence reads of the seven different samples analysed in this study are
21 available at <https://research.cip.cgiar.org/confluence/display/cpx/CIP.sweetpotato.2014>.

22 Comparison of siRNA sequences determined from uninfected, StV1, SPCSV, and
23 SPCSV and StV1 infected plants showed notable changes in the relative amounts siRNAs

1 corresponding to each virus as well as the regions to which they mapped, particularly in
2 the case of SPCSV (Fig. 4-5 & S5). The relative number of siRNA reads corresponding
3 to SPCSV increased by more than threefold from 7,500/million reads to 23,708/million
4 reads, with most of the increase corresponding to RNA1 (Fig. 4). Relative amounts of
5 begomovirus specific siRNA did not change, beyond the variation found between
6 individual samples of the same treatment (+/- 0.1%), in double infected plants as
7 compared to singly infected plants (1.1 fold; from 82032 to 90275/million reads). StV1
8 specific siRNAs also mapped to similar positions in the genome, and no obvious
9 differences could be observed (Fig. 5a). This was in stark contrast to the mapping of
10 reads to the SPCSV genome, where a dramatic change could be observed in the relative
11 amounts and positions to which the siRNAs mapped on the genome (Fig. 5b). This was
12 characterized by a several fold increase in siRNAs corresponding to SPCSV (Fig. 4), a
13 reduction in 21nt siRNA (reduced from 37% to 15.8% of all siRNAs corresponding to
14 SPCSV) and corresponding increase in 22 and 23nt siRNA (increased from 39.6% to
15 47.1% and 12.8% to 26.5% respectively, of all siRNAs corresponding to SPCSV), as well
16 as a near disappearance of siRNAs matching to the 5' regions of SPCSV (Fig. 5b) in
17 plants co-infected with isolate StV1 as relative to single SPCSV infection. To determine
18 if the effect of change in siRNA mapping to SPCSV was specific to the synergism
19 between SPCSV and StV1 or a general response found in synergistic interactions with
20 other viruses, we sequenced siRNAs from plants infected with SPCSV and SPFMV. No
21 reduction in the mapping of siRNAs to the 5' region was observed in these plants (Fig.
22 5b), nor was there a change in the total amount of siRNAs corresponding to SPCSV (Fig.
23 4).

1 When comparing distribution of StV1 specific siRNAs between StV1 and SPCSV co-
2 infected plants and StV1 infected RNase3-expressing plants on the other hand a
3 surprising difference could be noted, in that a several-fold reduction in 21 and 22 nt
4 siRNAs could be observed in RNase3 expressing plants as compared to SPCSV co-
5 infected plants and also StV1 singly infected plants (Fig. 5b).

6

7

8 **DISCUSSION**

9

10 Until the beginning of this century, most surveys of sweetpotato viruses did not mention
11 begomoviruses (Valverde *et al.*, 2007). Nevertheless closely related begomoviruses have
12 now been reported from all over the world including North America (Lotrakul *et al.*,
13 2002, Lotrakul *et al.*, 1998, Lotrakul, 2003), the Mediterranean (Lozano *et al.*, 2009,
14 Cohen *et al.*, 1997, Banks *et al.*, 1999, Briddon *et al.*, 2006), Asia (Luan *et al.*, 2007,
15 Onuki *et al.*, 2000, Bi & Zhang, 2012), South America (Fuentes & Salazar, 2003,
16 Rodríguez-Pardina *et al.*, 2012, Albuquerque *et al.*, 2012, Paprotka *et al.*, 2010) and
17 Africa (Miano *et al.*, 2006, Wasswa *et al.*, 2011). Our report corroborates the common
18 occurrence of sweepoviruses and reveals clear synergistic interactions of sweetpotato
19 begomoviruses with SPCSV, another widely distributed virus and mediator of several
20 synergistic interactions (Cuellar *et al.*, 2011b, Karyeija *et al.*, 2000, Untiveros *et al.*, 2007,
21 Mukasa *et al.*, 2006).

22

1 Few reports exist on synergistic interactions between RNA and DNA viruses. We
2 previously showed that SPCSV can interact with members of the *Caulimoviridae* family
3 of reverse transcribing viruses (Cuellar *et al.*, 2011b), and here we show that this is also
4 the case for sweepoviruses. However, our data also show that the extent of this synergism
5 varies considerably between different sweepovirus isolates and that, in the majority of
6 cases, it was not associated with clear symptoms. This observation may explain why such
7 interactions have not been noticed before. Still, the fact that 5 out of 6 diverse viruses
8 tested showed clear and significant increase in titers in co-infection with SPCSV
9 indicates that this may be a common phenomenon among sweepoviruses. As has been
10 shown for other synergistic interactions with SPCSV (Cuellar *et al.*, 2011b, Mukasa *et al.*,
11 2006, Cuellar *et al.*, 2008) we also found that an increase in titre of the synergized virus
12 is associated with a corresponding decrease in titre of SPCSV compared to single
13 infection (Fig. 2). It is not yet clear why SPCSV titres are reduced in synergistic
14 interactions, but it may simply be a result of competition for limited resources of the two
15 co-infecting viruses in infected cells, where the association is favoring one over the other.
16 Nevertheless, when we analyzed siRNA targeting of SPCSV in single as compared to
17 dual infection with sweepovirus isolate StV1 we were able to detect a striking difference
18 in the relative amount and distribution of siRNA reads. Total siRNAs corresponding to
19 SPCSV increased several fold (Fig. 4), and 22 and 23nt siRNA increased relatively as
20 compared to 21nt siRNA, whereas siRNAs matching to the 5' regions of SPCSV nearly
21 disappeared (Fig. 5b) in plants co-infected with isolate StV1 relative to single SPCSV
22 infection. This effect was apparently specific to the interaction of SPCSV with StV1, as

1 similar changes were not observed in the interaction between SPCSV and SPFMV (Fig. 4
2 and 5b).

3 The specific changes in distribution and relative quantity of SPCSV specific siRNAs as a
4 result of co-infection with StV1 suggests a modified and increased targeting of SPCSV
5 by the plants RNA silencing system. A plausible explanation is that this is the result of
6 interference by StV1 with the hosts RNA silencing machinery. Although this may seem
7 counterintuitive, it has become clear in recent years that different branches of the RNA
8 silencing system in plants compete with each other for access to cellular machinery
9 (Jauvion *et al.*, 2012). RSS suppression by sweepoviruses is more likely to affect
10 pathways, inhibiting replication of DNA viruses in the nucleus, and this may not
11 necessarily benefit, or even be detrimental to, replicating RNA viruses in the cytoplasm.
12 Two RNA viruses with similar replication strategies on the other hand are less likely to
13 compromise each-others silencing suppression. This alone may explain why similar
14 changes are not seen in the interaction between SPCSV and SPFMV as compared to the
15 interaction between SPCSV and SvT1. Also, although the tissue tropism of
16 sweepoviruses has not yet been determined, many begomoviruses are phloem limited,
17 similar to SPCSV, and if this is the case also for sweepoviruses the effects the two
18 viruses may have on each other's replication may be expected to be more evident than in
19 the case where tissue tropism is distinct such as SPFMV and SPCSV (Karyeija *et al.*,
20 2000).

21
22 Although RNA silencing suppressors (RSS) have not yet been reported for sweepoviruses,
23 a number of studies have reported up to three RSS proteins encoded by single and

1 multipartite begomoviruses, including the (homologs of) V2, C2 and C4 proteins (Amin
2 *et al.*, 2011, Chellappan *et al.*, 2005, Vanitharani *et al.*, 2004, Buchmann *et al.*, 2009,
3 Zrachya *et al.*, 2007, Zhang *et al.*, 2011). These RSS have been found to suppress
4 silencing both at the transcriptional and post-transcriptional level, but not all homologous
5 proteins from different viruses have RSS activity or necessarily function in similar ways
6 (Raja *et al.*, 2010, Amin *et al.*, 2011). Thus although it can be expected that
7 sweepoviruses encode RSS proteins, it is not possible to deduce which they will be or
8 how they will function based on knowledge from other begomoviruses. On the other hand
9 we can use our observations regarding relative changes in siRNA distributions to
10 speculate which components of the RNA silencing machinery might be affected. The
11 phenotype of reduced siRNA targeting of the 5' region and increased targeting towards
12 the 3' of SPCSV is reminiscent to that found in CMV infected *RDR1* defective
13 Arabidopsis (Wang *et al.*, 2010). This may indicate that StV1 interferes with RDR1
14 function. RDR proteins could be expected to fulfill an important role in antiviral silencing
15 against geminiviruses, since they do not normally produce dsRNA. Nevertheless one
16 must be careful to extrapolate conclusions from the one specific model system to
17 sweetpotato because marked differences have been found between different geminivirus
18 host combinations (Akbergenov *et al.*, 2006, Rodríguez-Negrete *et al.*, 2009, Miozzi *et*
19 *al.*, 2013).

20
21 Previously we demonstrate that RSS encoded by SPCSV (RNase3) is responsible for the
22 enhanced accumulation of co-infecting RNA viruses in synergistic interactions mediated
23 by SPCSV (Cuellar *et al.*, 2009). Although the exact mechanism of RNase3 function is

1 not fully elucidated its dsRNase activity is essential for silencing suppression as well as
2 enhanced accumulation of viruses in transgenic plants (Cuellar et al., 2009, Kreuze *et al.*,
3 2005). RNase3 has little substrate specificity in vitro, processing both long and short
4 dsRNA including siRNAs and pre-miRNAs (Cuellar et al., 2009, Kreuze et al., 2005,
5 Weinheimer et al., 2014). Nevertheless, its target in plants must be specific, since
6 transgenic plants are phenotypically completely normal except for their extreme
7 susceptibility to viruses (Cuellar *et al.*, 2009). We hypothesize that the same mechanism
8 is involved in SPCSV synergisms with RNA and DNA viruses. Indeed infection of
9 RNase3 transgenic sweetpotato plants with StV1 (Fig. 3) as well as SPCV and SPVCV
10 (Fig. S2) provoked characteristic symptoms as seen in plants co-infected with SPCSV.
11 Surprisingly however siRNA distribution patterns of StV1 were perceptibly different in
12 plants constitutively expressing RNase3 as compared to those from plants co-infected
13 with SPCSV, in that 21 and 22 nt siRNAs were strongly reduced in RNase3 plants. This
14 suggests that despite the biologically similar outcomes of enhanced StV1 viral titres and
15 symptom induction, clear differences occur in how RNA silencing is affected in either
16 situation. We offer two possible explanations for this discrepancy: i) constitutive
17 overexpression of RNase3 in all plant cells results in a distinct effect of RNase3 on the
18 silencing pathway as compared to phloem specific expression, or ii) RNase3 function is
19 modulated by other SPCSV encoded proteins to limit its effect to certain sites in the
20 silencing pathway. It is intriguing that in spite of its constitutive expression in all plant
21 cells, RNase3 will not cause visible collateral effects on sweetpotato. Future analysis of
22 siRNA sequences in RNase3 plants infected with different types of viruses may shed

1 more light on the exact target and mechanism of RNase3 provoked susceptibility to
2 viruses.

3
4 Although we did not analyze the potential effect on yield of the different virus
5 combinations in the current study, the strong increase in sweepovirus titres found in some
6 interactions suggests that yield impacts could be expected and this should be a priority for
7 future studies. Indeed, other studies have already shown significant impacts of
8 sweepovirus infection on yield of sweetpotato, despite being largely symptomless (Ling
9 et al., 2010, Clark & Hoy, 2006). In addition, increases in sweepovirus titres in plant
10 tissues could lead to an increased rate of transmission of the virus by its vector
11 contributing to more rapid virus spread.

12 13 **EXPERIMENTAL PROCEDURES**

14 15 *Virus isolates*

16 The 39 begomovirus isolates described in this study (Table 1 & S1) were identified in
17 sweetpotato accessions from the Central and South America (Mexico, Guatemala, Cuba,
18 Jamaica, Nicaragua, Dominican Republic, Saint Vincent and the Grenadines, Colombia,
19 Ecuador, Peru, Argentina, Paraguay, Panama and Puerto Rico), after indexing by grafting
20 onto the indicator plant *Ipomoea setosa* followed by PCR (see below), during routine
21 virus indexing performed at CIP (329 accessions). These accessions were either collected
22 by CIP or acquired from other collections between 1986 and 1994. Accessions collected
23 by CIP were established under an insect proof screenhouse before being transferred to in-

1 vitro, where they were maintained as part of CIPs global sweetpotato collection. Samples
2 acquired from other sources were obtained as in-vitro, vine cuttings or roots and in the
3 latter cases established and introduced to in-vitro as described for the CIP collected
4 materials. An additional 7 virus isolates were identified by PCR screening from 65
5 sweetpotato genotypes collected from different regions of East Africa (Uganda, Kenya,
6 Tanzania) and originally maintained under field conditions for breeding purposes and
7 subsequently transferred to in vitro for transfer to CIPs sweetpotato collection at CIP-
8 Lima. Metadata of the accessions in which sweepoviruses were identified are provided in
9 supplementary Table S1. For synergism experiments SPCSV isolate m2-47, lacking the
10 p22 gene (Cuellar *et al.*, 2011a, Cuellar *et al.*, 2008) and maintained in *I. setosa* was used.
11 The SPCV and SPVCV isolates used are described in Cuellar *et al.* (2011b).

12

13 *DNA amplification, cloning and sequence analyses*

14 The Saint Vincent and the Grenadines isolate (StV1) was isolated from sweetpotato
15 accession CIP400025. The accession has been tested for 10 viruses by ELISA, and
16 grafting onto *I. setosa*. To amplify begomovirus specific fragments from different
17 sweetpotato accessions (Table 1) a simple and quick method of DNA extraction using
18 sodium hydroxide was used to prepare template DNA for PCR (Wang *et al.*, 1993).
19 Shoots were collected from *in vitro* plantlets and homogenized in 0.5M NaOH buffer in
20 ratio of 1/5 (tissue: Buffer). The samples were centrifuged at 12000 g for 10 min to spin
21 down the debris. After a spin down samples were diluted 100 times with Tris-HCl
22 100mM (pH8) and 1 ul of leaf extract was used directly for PCR in a 25ul reaction using
23 the 2X phusion polymerase readymade master mix (Finnzymes, Finland) and

1 sweepovirus specific primers SPG1: 5'-CCC CKG TGC GWR AAT CCA T-3' and
2 SPG2: 5'-ATC CVA AYW TYC AGG GAG CTA A-3' (Li *et al.*, 2004), designed to
3 amplify a 901 bp region encompassing partial AC1 and AC2 ORFs.

4 For cloning of the selected begomovirus genomes total DNA was extracted using a
5 modified CTAB protocol (see below) followed by separation of small molecular weight
6 DNA using a plasmid isolation protocol (Alkaline lysis) and the Wizard miniprep kit
7 (PROMEGA, USA). The quality and amount of DNA was checked by agarose gel
8 electrophoresis and by espectrophotometry using a nanodrop analyzer (ND-1000, Thermo
9 Fisher Scientific, USA), respectively. In the case of isolates Per10 and Jam12, 5 ug of
10 small molecular weight DNA was used for amplification of circular DNA using Phi29
11 polymerase (New England Biolabs, USA) reaction with a 5X excess of random hexamer
12 primers according to the manufacturer's instructions. The amplified DNA was then
13 linearized using *SmaI* for Per10 and *StuI* for Jam12, resulting in the expected 2.7kb
14 fragment. Isolate Per6, Mex31, Cub5 and StV1 were amplified by inverse PCR using a
15 set of degenerate primers designed based on previously amplified and sequenced region
16 (Bego-F: 5' CTG RCC TCC TCT AGC AGA TCK CC -3'; Bego-R: 5' - GAR CCT GCK
17 CCT GGA TTG CAG AGR -3') resulting in the expected 2.3kb fragment.

18 The PCR and digested Phi29 amplified products were separated by agarose gel
19 electrophoresis and then excised and purified using a gel extraction kit (Promega-WI-
20 Madison). The fragments were cloned into pGEM-T easy vector (Promega).
21 Transformation of *E.coli* DH5 α was done by heat shock at 42°C for 90seconds. Using
22 blue white screening putative transformants were screened and confirmed by restriction
23 analysis using *EcoRI* enzyme prior to sequencing. The samples were then prepared for

1 sequencing (Macrogen, Korea) using SP6 and T7 primers and a set of specifically
2 designed internal primers.

3 Sequence alignments and phylogenetic analysis were performed using MEGA5.1
4 (Tamura *et al.*, 2011). Alignments were performed using Muscle and phylogenetic trees
5 were generated after calculating the best fitting model: Maximum-Likelihood method
6 with the General time-reversible model using Gamma distributed rates (with 5 discrete
7 gamma categories) with invariant sites.

8

9 *Synergistic interaction of sweepviruses with SPCSV in sweetpotato*

10 Sweetpotato cultivar ‘Huachano’ (accession CIP420065) obtained from the germplasm
11 collection of the International Potato Center (CIP) was used as a rootstock for graft
12 inoculation of isolates StV1, Per-6, Per-10, Jam-12, Cub-5 and Mex-31 with or without
13 SPCSV. SPCSV isolate m2-47 (Untiveros *et al.*, 2007) was maintained in sweetpotato cv.
14 Huachano through cuttings, and all sweepvirus isolates were maintained in *I. setosa*
15 plants by serial grafting. Nodes from the middle part of virus-infected source plants were
16 used as scions to graft inoculate sweetpotato. Four weeks old cuttings of sweetpotato cv
17 “Huachano” were side grafted in the middle of the plant with SPCSV or healthy
18 sweetpotato scions. Two weeks later, two nodes above the initial graft, plants were
19 grafted with healthy or sweepvirus infected *I.setosa* scions, thus generating plants
20 infected with SPCSV alone, SPCSV plus sweepvirus, sweepvirus alone, and mock
21 inoculated. Three plants per treatment were inoculated and formation of graft union
22 confirmed. Plants were cut back below the graft unions 6 weeks after the last graft
23 inoculation and left to re-grow for 4 more weeks. Development of symptoms was

1 recorded every week after inoculation and total nucleic acid was extracted (see below for
2 methods) from 10 mm leaf disks from a combination of three leaves collected from the
3 apex, middle and bottom part of each plant at 1 to 10 weeks post inoculation for dot-blot
4 detection of begomovirus and quantitative real-time RT-PCR detection of SPCSV. Leaf
5 samples were stored at -20°C . Triple antibody sandwich enzyme-linked immunosorbent
6 assay (TAS-ELISA) for SPCSV were carried out as described previously (Karyeija *et al.*,
7 2000), 4-weeks after cutting back infected plants, only on plants with single SPCSV
8 infection and mixed infection of SPCSV and isolate StV1. In a separate experiment three
9 replicates of the RNase3 transgenic sweetpotato ‘Huachano’ event R3 (Cuellar *et al.*,
10 2009) and three non-transgenic events were also infected with StV1 and tested by DNA
11 dot blot together with non-transgenic plants infected with SPCSV and StV1 four weeks
12 after cutting back infected plants.

13

14 *Dot-Blot hybridization and signal quantification*

15 For detection by Dot-blot hybridization, total DNA was purified using the CTAB method
16 (Doyle & Doyle, 1987). Frozen leaf tissues (250 mg) were processed immediately by
17 grinding in 2 ml of CTAB buffer (2% CTAB 100 mM Tris-HCl, pH8.0, 20mM EDTA,
18 1.4M NaCl, 1.0% Na sulphite and 2.0% PVP-40) using polypropylene sack. The
19 homogenate was centrifuged at $10,000 \times g$ for 10 min and the supernatant (750 μl) was
20 transferred to a 1.5-ml microcentrifuge tube and mixed with an equal volume of
21 chloroform:isoamyl alcohol (24:1). The mixture was centrifuged at $12,000 \times g$ for 10 min
22 and the aqueous phase (500 μl) was transferred to a new 1.5-ml microcentrifuge tube
23 before mixing in 550 μl of isopropanol. The mixture was incubated on ice for 10 min and

1 centrifuged at $12,000 \times g$ for 10 min at 4°C . The pellet was washed with 70% ethanol,
2 centrifuged at $12,000 \times g$ for 5 min, air dried and dissolved in 100 μl of NFW. 5 μg of
3 total DNA in a total volume of 200 μl were used for hybridization. Standards of 125, 50
4 and 25 ng of plasmid DNA containing the region corresponding to the probe used were
5 also added in duplicate to each membrane tested, to normalize and quantify results.
6 Dioxygenin-labeled probes encompassing the Rep gene region (AC1) of StV1 were
7 synthesized by PCR using primers SPG1 and SPG2 (Li *et al.*, 2004), Taq polymerase
8 (Promega) and Dioxygenin-labeled deoxynucleotides (Roche, UK). Total DNA from
9 infected plants were transferred to a nylon membrane (Hybond-N; Amersham
10 Biosciences AB) using Bio-Dot SF Cell (BIO-RAD), cross-linked by UV-irradiation
11 (50mJ) in a cross-linking oven (Stratagene), prehybridized for 90 min at 65°C in 0.02%
12 SDS, 5X SSC (750 mM NaCl, 75mM sodium citrate), 50% formamide, 2% (w v⁻¹) N-
13 lauroylsarcosine and then hybridized in the same solution at 65°C for the 16h after
14 adding the DIG-labeled probe. After hybridization, membranes were washed twice in 2X
15 SSC and 1% SDS at room temperature for 15 min, incubated for 30 min with anti-DIG
16 antibodies conjugated with alkaline phosphatase, and washed twice with maleate buffer
17 with 0.3% Tween-20. The reaction was developed using CSPD chemiluminescent
18 substrate (Roche) and Omat-S film (Kodak). Signal intensities of the hybridized spots
19 was measured from developed films using the Gel Doc equipment in conjunction with the
20 Quantity One software (Biorad) under white light. Signal intensity was determined using
21 volume circle tool, ensuring circles were all the same size and covered each spot exactly,
22 with global background subtraction and avoiding overexposed pixels. The concentration
23 of viral DNA inside each circle was then determined using a regression curve based on

1 the volumes of the plasmid standards within each membrane using the Volume Analysis
2 Report and Volume Regression Curve within Quantity One. The estimated viral
3 concentrations (in ng) were then used for statistical analysis using the SAS statistical
4 package. Membranes were stripped and hybridized using rDNA specific probes
5 (amplified using primers Ribosomal F: 5'- ACA GCA GAA CGA CCA GAG AAC GC -
6 3', and Ribosomal R: 5'- GCA CGC TAG GTA CGA CCA CCA CT -3') to confirm
7 equal loading of DNA between samples. First a Repeated Measures Analysis of Variance
8 was performed revealing highly significant (<0.0001) probability of interactions between
9 timepoint, isolate and treatment. Subsequently an analysis of variance was performed for
10 each time point to determine the effect of treatments and isolates at each timepoint. A full
11 analysis of variance considering time and isolate as factors was also performed to
12 determine the global effect of single vs. dual infection for each isolate.

13 14 *Real-time quantitative RT-PCR for detection of SPCSV*

15 The same samples described for dot-blot hybridizations above were used to extract RNA
16 using the CTAB method described previously, but modified to precipitate total RNA by
17 adding an equal volume of 4M LiCl rather than isopropanol, and overnight incubation at
18 4°C followed by centrifugation at 10,000 × g for 20 min. The pellet was washed with
19 70% ethanol as described above and re-dissolved in 100 µl of NFW.

20 A TaqMan real-time RT-PCR assay was then used for detecting SPCSV. One-step real-
21 time RT-PCR assays were performed using the TaqMan Universal PCR Master Mix
22 (Applied Biosystems, Foster City, CA) in a 25-µl final reaction volume containing 2U of
23 MMLV, 300 nM of each primer, 100 nM of the probe, and 2 µl of template RNA. The

1 following thermal cycling conditions were used: 42°C for 30 min (cDNA synthesis),
2 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and
3 annealing/extension at 60°C for 1 min. Primers and probe for SPCSV were SPCSV-Uni-
4 E-F(5'-CGG AGT TTA TTC CCA CYT GTY T-3') and SPCSV-Uni-E-R (5'-GGG CAG
5 CCY CAC CAA-3') and probe SPCSV-Uni-E-P (5'-[FAM]-TCT GTC ACG GCT ACA
6 GGC GAC GTG-[TAMRA]-3'), corresponding to the Hsp70h region on RNA2.
7 Cytochrome oxidase (COX) was used as internal reference gene using the primers COX-
8 F (5'-CGT CGC ATT CCA GAT TAT CCA-3'), COX-R (5'-CAA CTA CGG ATA TAT
9 AAG AGC CAA AAC TG-3' and probe COX-P: 5'-[VIC]-TGC TTA CGC TGG ATG
10 GAA TGC CCT-[TAMRA]-3'.

11 Results were analyzed with MxPro QPCR Software and Statistical differences between
12 single and mixed infections were determined for each timepoint/virus combination as
13 well as each virus combination over all time-points using the Relative Expression
14 Software Tool (REST) v2.0.12 (QIAGEN GmbH, Germany).

15 16 *siRNA sequencing and analysis*

17 Total RNAs were extracted from three leaves each of healthy, SPCSV infected, StV1
18 infected, SPCSV+StV1 infected, SPFMV infected and SPCSV+SPFMV infected
19 'Huachano' plants, as well as StV1 infected RNase3 transgenic 'Huachano' plants
20 (Cuellar *et al.*, 2009) at several months after inoculations using TRIZOL reagent. siRNAs
21 were purified from 4% agarose gel and sent for library preparation and Illumina
22 sequencing (Provider: Fasteris Life Sciences SA, Plan-les-Ouates, Switzerland).

1 Reads were mapped to the corresponding genomes using the MAQ software, and results
2 were visualized using a custom script (Fuentes *et al.*, 2012) and Microsoft Excel (bar-
3 charts).

4

5 **ACKNOWLEDGMENTS**

6 We are grateful to all members of the Virology Unit at CIP. Thanks to Silver
7 Tumwegamire and Willmer Perez for providing access to sweetpotato samples from East
8 Africa, and Neil Boonham and Ian Adams for design of real-time PCR assays. Financial
9 support from BBSRC/DFID (grant BBF0040281) and ICGEB-TWAS-UNESCO/IBSP
10 Joint Programme on Capacity Building in Basic Molecular Biology (Contract No.:
11 CRP/101005) is acknowledged.

12

1 REFERENCES

2

3 Akbergenov, R., Si-Ammour, A., Blevins, T., Amin, I., Kutter, C., Vanderschuren,
4 H., Zhang, P., Grisse, W., Meins Jr, F., Hohn, T. and Pooggin, M. M.
5 (2006) Molecular characterization of geminivirus-derived small RNAs in different
6 plant species. *Nucleic Acids Res.*, **34**, 462-471.

7 Albuquerque, L. C., Inoue-Nagata, A. K., Pinheiro, B., Resende, R. O., Moriones, E.
8 and Navas-Castillo, J. (2012) Genetic diversity and recombination analysis of
9 sweepoviruses from Brazil. *Virol. J.*, **9**, 241.

10 Amin, I., Hussain, K., Akbergenov, R., Yadav, J. S., Qazi, J., Mansoor, S., Hohn, T.,
11 Fauquet, C. M. and Briddon, R. W. (2011) Suppressors of RNA Silencing
12 Encoded by the Components of the Cotton Leaf Curl Begomovirus-BetaSatellite
13 Complex. *Mol. Plant-Microbe In.*, **24**, 973-983.

14 Banks, G. K., Bedford, I. D., Beitia, F. J., Rodriguez-Cerezo, E. and Markham, P. G.
15 (1999) A Novel Geminivirus of *Ipomoea indica* (Convolvulaceae) from Southern
16 Spain. *Plant Dis.*, **83**, 486.

17 Bi, H. and Zhang, P. (2012) Molecular characterization of two sweepoviruses from
18 China and evaluation of the infectivity of cloned SPLCV-JS in *Nicotiana*
19 *benthamiana*. *Arch. Virol.*, **157**, 441-454.

20 Briddon, R. W., Bull, S. E. and Bedford, I. D. (2006) Occurrence of Sweet potato leaf
21 curl virus in Sicily. *Plant Pathol.*, **55**, 286.

22 Buchmann, R. C., Asad, S., Wolf, J. N., Mohannath, G. and Bisaro, D. M. (2009)
23 Geminivirus AL2 and L2 proteins suppress transcriptional gene silencing and
24 cause genome-wide reductions in cytosine methylation. *J. Virol.*, **83**, 5005-5013.

25 Chellappan, P., Vanitharani, R. and Fauquet, C. M. (2005) MicroRNA-binding viral
26 protein interferes with Arabidopsis development. *Proc. Natl. Acad. Sci. U S A*,
27 **102**, 10381-10386.

28 Clark, C. A., Davis, J. A., Abad, J. A., Cuellar, W. J., Fuentes, S., Kreuze, J. F.,
29 Gibson, R. W., Mukasa, S. B., Tugume, A. K., Tairo, F. D. and Valkonen, J.
30 P. T. (2012) Sweetpotato Viruses: 15 Years of Progress on Understanding and
31 Managing Complex Diseases. *Plant Dis.*, **96**, 168-185.

32 Clark, C. A. and Hoy, M. W. (2006) Effects of common viruses on yield and quality of
33 Beauregard sweetpotato in Louisiana. *Plant Dis.*, **90**, 83-88.

34 Cohen, J., Milgram, M., Antignus, Y., Pearlsman, M., Lachman, O. and Loebenstein,
35 G. (1997) *Ipomoea* crinkle leaf curl caused by a whitefly-transmitted gemini-like
36 virus. *Ann. Appl. Biol.*, **131**, 273-282.

37 Cuellar, W. J., Cruzado, R. K., Fuentes, S., Untiveros, M., Soto, M. and Kreuze, J. F.
38 (2011a) Sequence characterization of a Peruvian isolate of Sweet potato chlorotic
39 stunt virus: Further variability and a model for p22 acquisition. *Virus Res.*, **157**,
40 111-115.

41 Cuellar, W. J., De Souza, J., Barrantes, I., Fuentes, S. and Kreuze, J. F. (2011b)
42 Distinct cavemoviruses interact synergistically with sweet potato chlorotic stunt
43 virus (genus *Crinivirus*) in cultivated sweet potato. *J. of Gen. Virol.*, **92**, 1233-
44 1243.

- 1 **Cuellar, W. J., Kreuze, J. F., Rajamäki, M.-L., Cruzado, K. R., Untiveros, M. and**
2 **Valkonen, J. P. T.** (2009) Elimination of antiviral defense by viral RNase III.
3 *Proc. Natl. Acad. Sci. U S A*, **106**, 10354-10358.
- 4 **Cuellar, W. J., Tairo, F., Kreuze, J. F. and Valkonen, J. P. T.** (2008) Analysis of gene
5 content in Sweet potato chlorotic stunt virus RNA1 reveals the presence of p22
6 RNA silencing suppressor in only few isolates: implications to viral evolution and
7 synergism. *J. Gen. Virol.*, **89**, 573-582.
- 8 **Doyle, J. J. and Doyle, J. L.** (1987) A rapid DNA isolation procedure for small
9 quantities of fresh leaf tissue. *Phytochem. Bulletin*, **19**, 11-19.
- 10 **Esterhuizen, L. L., Heerden, S. W., Rey, M. E. C. and Heerden, H.** (2012) Genetic
11 identification of two sweet-potato-infecting begomoviruses in South Africa. *Arch.*
12 *Virol.*, **157**, 2241-2245.
- 13 **FAO** (2013) FAOSTAT.
- 14 **Fauquet, C. M. and Stanley, J.** (2003) Geminivirus classification and nomenclature:
15 progress and problems. *Ann. Appl. Biol.*, **142**, 165-189.
- 16 **Fuentes, S., Heider, B., Tasso, R. C., Romero, E., Zum Felde, T. and Kreuze, J. F.**
17 (2012) Complete genome sequence of a potyvirus infecting yam beans
18 (*Pachyrhizus* spp.) in Peru. *Arch. Virol.*, **157**, 773-776.
- 19 **Fuentes, S. and Salazar, L. F.** (2003) First Report of Sweet potato leaf curl virus in Peru.
20 *Plant Dis.*, **87**, 98-98.
- 21 **Jauvion, V., Rivard, M., Bouteiller, N., Elmayan, T. and Vaucheret, H.** (2012) RDR2
22 Partially Antagonizes the Production of RDR6-Dependent siRNA in Sense
23 Transgene-Mediated PTGS. *PLoS ONE*, **7**, e29785.
- 24 **Karyeija, R. F., Kreuze, J. F., Gibson, R. W. and Valkonen, J. P. T.** (2000)
25 Synergistic Interactions of a Potyvirus and a Phloem-Limited Crinivirus in Sweet
26 Potato Plants. *Virology*, **269**, 26-36.
- 27 **Kreuze, J. F., Savenkov, E. I., Cuellar, W., Li, X. and Valkonen, J. P. T.** (2005) Viral
28 Class 1 RNase III involved in suppression of RNA silencing. *J. Virol.*, **79**, 7227-
29 7238.
- 30 **Li, R. H., Salih, S. and Hurtt, S.** (2004) Detection of geminiviruses in sweetpotato by
31 polymerase chain reaction. *Plant Dis.*, **88**, 1347-1351.
- 32 **Ling, K.-S., Jackson, D. M., Harrison, H., Simmons, A. M. and Pesic-VanEsbroeck,**
33 **Z.** (2010) Field evaluation of yield effects on the U.S.A. heirloom sweetpotato
34 cultivars infected by Sweet potato leaf curl virus. *Crop Prot.*, **29**, 757-765.
- 35 **Lotrakul, P., Valverde, R. A., Clark, C. A., Hurtt, S. and Hoy, M. W.** (2002)
36 Sweetpotato leaf curl virus and related geminiviruses in Sweetpotato. *Acta Hort.*
37 (*ISHS*), **583**, 135-141.
- 38 **Lotrakul, P., Valverde, R. A., Clark, C. A., Sim, J. and De La Torre, R.** (1998)
39 Detection of a Geminivirus Infecting Sweet Potato in the United States. *Plant Dis.*,
40 **82**, 1253-1257.
- 41 **Lotrakul, P., Valverde, R. A., Clark, C. A.** (2003) Properties of a Begomovirus isolated
42 from Sweet potato [*Ipomoea batatas* (L.) Lam.] infected with Sweet potato leaf
43 curl virus. *Revista Mexicana de Fitopatologia* **21**, 128-136.
- 44 **Lozano, G., Trenado, H. P., Valverde, R. A. and Navas-Castillo, J.** (2009) Novel
45 begomovirus species of recombinant nature in sweet potato (*Ipomoea batatas*) and

- 1 Ipomoea indica: taxonomic and phylogenetic implications. *J. Gen. Virol.*, **90**,
2 2550-2562.
- 3 **Luan, Y. S., Zhang, J., Liu, D. M. and Li, W. L.** (2007) Molecular characterization of
4 sweet potato leaf curl virus isolate from China (SPLCV-CN) and its phylogenetic
5 relationship with other members of the Geminiviridae. *Virus Genes*, **35**, 379-385.
- 6 **Miano, D. W., LaBonte, D. R., Clark, C. A., Valverde, R. A., Hoy, M. W., Hurtt, S.**
7 **and Li, R.** (2006) First Report of a Begomovirus Infecting Sweetpotato in Kenya.
8 *Plant Dis.*, **90**, 832-832.
- 9 **Miozzi, L., Pantaleo, V., Burgyan, J., Accotto, G. P. and Noris, E.** (2013) Analysis of
10 small RNAs derived from tomato yellow leaf curl Sardinia virus reveals a cross
11 reaction between the major viral hotspot and the plant host genome. *Virus Res.*,
12 **178**, 287-296.
- 13 **Mukasa, S. B., Rubaihayo, P. R. and Valkonen, J. P. T.** (2006) Interactions between a
14 crinivirus, an ipomovirus and a potyvirus in coinfecting sweetpotato plants. *Plant*
15 *Pathol.*, **55**, 458-467.
- 16 **Onuki, M., Honda, Y. and Hanada, K.** (2000) Geminiate Particle Morphology of Sweet
17 Potato Leaf Curl Virus in Partially Purified Preparation and Its Serological
18 Relationship to Two Begomoviruses by Western Blotting. *J. Gen. Plant Pathol.*,
19 **66**, 182-184.
- 20 **Paprotka, T., Boiteux, L. S., Fonseca, M. E. N., Resende, R. O., Jeske, H., Faria, J. C.**
21 **and Ribeiro, S. G.** (2010) Genomic diversity of sweet potato geminiviruses in a
22 Brazilian germplasm bank. *Virus Res.*, **149**, 224-233.
- 23 **Raja, P., Wolf, J. N. and Bisaro, D. M.** (2010) RNA silencing directed against
24 geminiviruses: Post-transcriptional and epigenetic components. *Biochimica et*
25 *Biophysica Acta*, **1799**, 337-351.
- 26 **Rodríguez-Negrete, E. A., Carrillo-Tripp, J. and Rivera-Bustamante, R. F.** (2009)
27 RNA Silencing against Geminivirus: Complementary Action of
28 Posttranscriptional Gene Silencing and Transcriptional Gene Silencing in Host
29 Recovery. *J. Virol.*, **83**, 1332-1340.
- 30 **Rodríguez-Pardina, P., Luque, A., Nome, C., López-Colomba, E., Fuentes-Delgado,**
31 **S. and Feo, L.** (2012) First report of Sweet potato leaf curl virus infecting sweet
32 potato in Argentina. *Australasian Plant Dis. Notes*, **7**, 157-160.
- 33 **Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S.** (2011)
34 MEGA5: molecular evolutionary genetics analysis using maximum likelihood,
35 evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, **28**,
36 2731-2739.
- 37 **Untiveros, M., Fuentes, S. and Salazar, L. F.** (2007) Synergistic Interaction of Sweet
38 potato chlorotic stunt virus (Crinivirus) with Carla-, Cucumo-, Ipomo-, and
39 Potyviruses Infecting Sweet Potato. *Plant Dis.*, **91**, 669-676.
- 40 **Valverde, R. A., Clark, C. A. and Valkonen, J. P. T.** (2007) Viruses and Virus Disease
41 Complexes of Sweetpotato. *Plant Viruses*, **1**, 116-126.
- 42 **Vanitharani, R., Chellappan, P., Pita, J. S. and Fauquet, C. M.** (2004) Differential
43 Roles of AC2 and AC4 of Cassava Geminiviruses in Mediating Synergism and
44 Suppression of Posttranscriptional Gene Silencing. *J. Virol.*, **78**, 9487-9498.
- 45 **Wang, H., Qi, M. Q. and Cutler, A. J.** (1993) A SIMPLE METHOD OF PREPARING
46 PLANT-SAMPLES FOR PCR. *Nucleic Acids Res.*, **21**, 4153-4154.

- 1 **Wang, X. B., Wu, Q., Ito, T., Cillo, F., Li, W. X., Chen, X., Yu, J. L. and Ding, S. W.**
2 (2010) RNAi-mediated viral immunity requires amplification of virus-derived
3 siRNAs in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U S A*, **107**, 484-489.
- 4 **Wasswa, P., Otto, B., Maruthi, M. N., Mukasa, S. B., Monger, W. and Gibson, R. W.**
5 (2011) First identification of a sweet potato begomovirus (sweepovirus) in
6 Uganda: Characterization, detection and distribution. *Plant Pathol.*, **60**, 1030-
7 1039.
- 8 **Weinheimer, I., Boonrod, K., Moser, M., Wassenegger, M., Krczal, G., Butcher, S. J.**
9 **and Valkonen, J. P.** (2014) Binding and processing of small dsRNA molecules
10 by the class 1 RNase III protein encoded by sweet potato chlorotic stunt virus. *J.*
11 *Gen. Virol.*, **95**, 486-495.
- 12 **Zhang, Z., Chen, H., Huang, X., Xia, R., Zhao, Q., Lai, J., Teng, K., Li, Y., Liang, L.,**
13 **Du, Q., Zhou, X., Guo, H. and Xie, Q.** (2011) BSCTV C2 attenuates the
14 degradation of SAMDC1 to suppress DNA methylation-mediated gene silencing
15 in *Arabidopsis*. *Plant Cell*, **23**, 273-288.
- 16 **Zrachya, A., Glick, E., Levy, Y., Arazi, T., Citovsky, V. and Gafni, Y.** (2007)
17 Suppressor of RNA silencing encoded by Tomato yellow leaf curl virus-Israel.
18 *Virology*, **358**, 159-165.
19

1 SUPPORTING INFORMATION LEGENDS

2 **Table S1.** Origin of plants, collection date, genotype name and germplasm accession
3 numbers of plants screened for sweepviruses in this study.

4
5 **Table S2.** Relative expression of SPCSV in mixed infection with 6 sweepvirus isolates
6 as compared to single infection over all time-points based on REST analysis of qRT-PCR
7 results.

8
9 **Figure S1.** a) DNA dot-blot results of sweepviruses used to generate graphs in figure 2.
10 b) Results of same blots with ribosomal DNA probe demonstrating equal loading

11
12 **Figure S2.** Symptoms of pararetroviruses (SPVCV and SPCV) in transgenic sweetpotato
13 cultivar 'Huachano' expressing the RNase3 gene of Sweet potato chlorotic stunt virus
14 (SPCSV). a) and c) = Non transgenic 'Huachano' infected with SPVCV and SPCV,
15 respectively. b) and d) = transgenic 'Huachano' plants expressing RNase3 and infected
16 with SPVCV and SPCV, respectively. e) = Dot-Blot detection of SPVCV in transgenic vs.
17 non-transgenic sweetpotato 'Huachano' plants. N.I.= Not infected plant. F = Detection of
18 SPCV by NCM-ELISA 1-2 = Not infected 'Huachano' plant, 3-4 = SPCV, 5-6=SPCV in
19 RNase3 transgenic plants, 7-8 = SPCV in co-infection with SPCSV.

20
21 **Figure S3.** Distribution of total small RNA size classes corresponding to SvT1 (white
22 bars), SPCSV (black bars), SPFMV (dark grey bars) and other sequences (light grey bars)
23 in a) wild-type plants singly and dually infected with StV1 and SPCSV or RNase3

1 transgenic plants infected with StV1, b) wild-type plants singly and dually infected with
2 SPFMV and SPCSV, or c) non-infected wild-type plants. Vertical axis shows total
3 number of small RNA reads, and horizontal axis indicates size class of siRNA, and the
4 exact numbers are tabulated below the graph including the sums and grand total.

5

6 **TABLES**

7 **Table 1.** Sweepovirus isolates used in this study

Accepted Article

1 **FIGURE LEGENDS**

2

3 **Figure 1.** Maximum likelihood phylogenetic tree based on complete genome sequences
4 of 123 isolates. Sequences are indicated with their accession numbers and country of
5 origin, sequences determined in this study are in bold and highlighted. Country codes:
6 CHN China, PRI Puerto Rico, JPN Japan, KOR Republic of Korea, ESP Spain, USA
7 United States of America, BRA Brazil, IND India, UGA Uganda, ITA Italy, Mex
8 Mexico, Jam Jamaica, Per Peru , Cub Cuba, StV Saint Vincent and the Grenadines.

9

10 **Figure 2.** Sweepovirus titres and relative SPCSV titres from single and dually infected
11 plants with different sweepovirus isolates. For each sweepovirus isolate the titres of
12 sweepovirus, determined by signal intensity analysis of DNA dot-blot, are shown in the
13 top graph in single (solid line) and mixed (dotted line) infection with SPCSV m2-47. The
14 bottom graph indicates the titers of SPCSV (grey line) in dual infection with sweepovirus
15 isolates relative to single SPCSV infection as determined by quantitative real-time PCR.
16 Horizontal axis indicates weeks post inoculation of sweepovirus and the dotted vertical
17 line through the graphs indicates the time point at which plants were cut back and left to
18 re-grow. Error bars indicate range of minimum and maximum values.

19

20 **Figure 3.** Symptoms and increased sweepovirus titres in SPCSV co-infected and RNase3
21 expressing transgenic sweetpotato plants 10 weeks after inoculation. a) Phenotype of
22 typical leaves from uninfected non-transgenic, uninfected RNase3 transgenic, and
23 SPCSV infected non-transgenic sweetpotato cultivar Huachano respectively (top), or

1 StV1 infected non-transgenic, StV1 infected RNase3 transgenic, and SPCSV and StV1
2 dual infected non-transgenic plants respectively of the same cultivar. b) DNA dot blot of
3 uninfected and StV1 infected non-transgenic, StV1 infected RNase3 transgenic, and
4 SPCSV and StV1 dual infected non-transgenic plants respectively.

5
6 **Figure 4.** Distribution of siRNA size classes corresponding to sweepovirus (white bars),
7 SPCSV (black bars) and SPFMV (grey bars) in single and dually infected plants. Vertical
8 axis shows number of siRNA reads per million total reads, and horizontal axis indicates
9 size class of siRNA. Numbers above 21 and 22 nt siRNAs in the right bottom graph
10 indicate the number of siRNAs corresponding to SPFMV, which exceed the scale of the
11 graph. Analysis of the total number of all small RNA reads are provided in
12 supplementary Fig. S3.

13
14 **Figure 5.** Coverage of viral genomes by different siRNA size classes a) coverage of
15 sweepovirus StV1 genome in single (top graph) or dual (middle graph) infection with
16 SPCSV or in RNase3 transgenic plants (bottom graph). b) Coverage of SPCSV genome
17 by different siRNA size classes in single (top graph) or dual infection with StV1 (middle
18 graph) or SPFMV (bottom graph). Vertical axis shows number of siRNA reads per
19 million total reads, and horizontal axis indicates nucleotide position on the genome. Red
20 green, black and blue lines indicate 21, 22, 23 and 24 nt RNAs respectively. A schematic
21 representation of the virus genome is provided on top with named box arrows
22 representing the locations of the respective encoded genes.

23

1
Table 1. Sweepovirus isolates used in this study

Isolate name	Symptoms in <i>I.setosa</i> ¹	Co-infected with	Country of origin	sequence accession number
Arg33	C,RU		Argentina	KC253260
Arg34	C,RU,D		Argentina	KC253261
Arg35	R,C,RD,RU,D,Vc	SPFMV	Argentina	KC253262
Arg36	C,RU,D		Argentina	KC253263
Arg37	RU		Argentina	KC253264
Arg38	R,C,Cp,RU,D,Vc,IVC	SPFMV	Argentina	KC253265
Col22	RU,Vc,Np,Ln		Colombia	KC253252
Col9	C,RU		Colombia	KC253242
Cub5*	C		Cuba	KC253236
Cub42	C,IVC		Cuba	KC288164
Dom13	C,RU,IVC		Dominican Republic	KC253244
Dom2	C,RU		Dominican Republic	KC253239
Ecu8	C,Cp,RU,Vc	SPFMV, SPVG	Ecuador	KC253241
Gtm16	C,RU		Guatamala	KC253247
Gtm17	C,RU	SPVVCV	Guatamala	KC253248
Gtm19	C,RU,D,IVC		Guatamala	KC253250
Gtm20	C,RU		Guatamala	KC253251
Jam12*	C,RU		Jamaica	KC253235
Jam23	C,RU	SPVVCV	Jamaica	KC253253
Jam24	C		Jamaica	KC253254
Mex31*	C,RU,D		Mexico	KC253237
Mex32	C,RU,D		Mexico	KC253259
Mex39	C,RU,D,Np	SPCV	Mexico	KC288161
Nic25	C,RU,D		Nicaragua	KC253255
Pam18	C,RU,D,IVC		Panama	KC253249
Pan14	C,RU,D		Panama	KC253245
Pan15	RD,RU,D,Ld		Panama	KC253246
Per10*	C,RU		Peru	KC253233
Per6*	C,RU		Peru	KC253234
Per7	C,RU		Peru	KC253240
Pri21	C,RU,D,IVC		Puerto Rico	KC288165
Pry11	C		Paraguay	KC253243
Pry26	Cp,RU,D,Vc	SPFMV, SPVG	Paraguay	KC253256
Pry27	C,RU		Paraguay	KC253257
Pry29	RU		Paraguay	KC253258
Pry30	C,RU		Paraguay	KC288166
Pry40	C,RU		Paraguay	KC288162
StV1*	RD,RU,D,Ld		St. Vincent and the Grenadines	KC253238
StV41	D, IVC		St. Vincent and the Grenadines	KC288163
Tza13	nt		Tanzania	KC288167
Tza16	nt	SPMMV	Tanzania	KC288169
Uga15	nt		Uganda	KC288168
Uga19	nt		Uganda	KC288170
Uga29	nt	SPFMV	Uganda	KC288171
Uga34	nt		Uganda	KC288172
Uga37	nt		Uganda	KC288173

2 * Genomes were fully sequenced and isolates used in synergism studies

3 ¹ Symptoms on leaves, C: Chlorosis; CP: Chlorotic point; D: Dwarfing; IVC: interveinal
4 chlorosis; Ld: Leaf deformation; Ln: leaf necrosis; Np; Necrotic point; R: rugosity; RD:
5 Roll down; RU: Roll up; Vc: vein clearing.; nt: not tested









