Short communication

An artificial chimeric derivative of *Citrus viroid V* involves the terminal left domain in pathogenicityPEDRO SERRA¹, SEYED MEHDI BANI HASHEMIAN¹, GIOVANNI PENSABENE-BELLAVIA¹, SELMA GAGO² AND NÚRIA DURAN-VILA^{1,*}¹Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias, Apartado Oficial, 46113-Moncada, Valencia, Spain²Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia, 46022 Valencia, Spain**SUMMARY**

The recently described *Citrus viroid V* (CVD-V) induces, in Etrog citron, mild stunting and very small necrotic lesions and cracks, sometimes filled with gum. As Etrog citron plants co-infected with *Citrus dwarfing viroid* (CDVd) and CVD-V show synergistic interactions, these host–viroid combinations provide a convenient model to identify the pathogenicity determinant(s). The biological effects of replacing limited portions of the rod-like structure of CVD-V with the corresponding portions of CDVd are reported. Chimeric constructs were synthesized using a novel polymerase chain reaction-based approach, much more flexible than those based on restriction enzymes used in previous studies. Of the seven chimeras (Ch) tested, only one (Ch5) proved to be infectious. Plants infected with Ch5 showed no symptoms and, although this novel chimera was able to replicate to relatively high titres in singly infected plants, it was rapidly displaced by either CVD-V or CDVd in doubly infected plants. The results demonstrate that direct interaction(s) between structural elements in the viroid RNA (in this case, the terminal left domain) and as yet unidentified host factors play an important role in modulating viroid pathogenicity. This is the first pathogenic determinant mapped in species of the genus *Apscaviroid*.

Viroids are infectious, small, single-stranded, circular RNAs (246–401 nucleotides) that fold into compact secondary structures as a result of the high degree of self-complementarity of their sequences. In spite of their apparent simplicity, the sequence of the viroid genome ensures maintenance of the secondary and tertiary structures, and provides specific motifs for interaction with the host. Species of the family *Pospiviroidae* have a central conserved region (CCR) and lack hammerhead ribozymes. Citrus are natural hosts of several viroid species, all of

which belong to the family *Pospiviroidae* (Duran-Vila *et al.*, 1988; Flores *et al.*, 2004) and adopt *in vitro* rod-like or quasi-rod-like secondary structures of minimal free energy. On denaturation, the viroid RNA may acquire metastable branched structures resulting from base pairing of inverted repeats that lead to the formation of stem loop structures (hairpins I and II) (Loss *et al.*, 1991; Riesner *et al.*, 1979). Following the model proposed by Keese and Symons (1985), the rod-like secondary structure is divided into five structural–functional domains: P (pathogenicity), C (central), V (variable), T_L (terminal left) and T_R (terminal right). Although members of the genus *Pospiviroid* appear to be well adjusted to this model, evidence shows that the P domain is not the sole determinant of symptom development, and suggests a more complex relation between different structural domains and pathogenicity. Studies with artificial chimeras derived from variants of the same viroid (Góra *et al.* 1996; Reanwarakorn and Semancik, 1998; Visvader and Symons, 1986), from closely related viroids of the same genus (Sano *et al.*, 1992) and, to a lesser extent, from viroids of different genera (Sano and Ishiguro, 1998) have provided relevant information on the relationship between specific regions of the viroid molecule and symptom expression.

In members of the genus *Apscaviroid*, all restricted to woody species, studies to determine the relationship between different portions of the RNA secondary structure and the biological properties are very limited. Although a CCR and terminal conserved region (TCR) have been clearly identified, the presence and role of the P, V, T_L and T_R domains remain unknown. *Citrus viroid III* (CVD-III), recently renamed *Citrus dwarfing viroid* (CDVd) (<http://www.ictvonline.org/virusTaxonomy.asp>), is a member of the genus *Apscaviroid* that induces, in Etrog citron (*Citrus medica* L.), stunting and a characteristic ‘leaf dropping pattern’ caused by the moderate epinasty resulting from petiole and mid-vein necrosis (Rakowski *et al.*, 1994; Semancik *et al.*, 1997). The recently described *Citrus viroid V* (CVD-V) also has a rod-like conformation and induces, in Etrog citron, mild stunting and very small necrotic lesions and cracks, sometimes filled with gum (Serra *et al.*, 2008a, b). Moreover, as Etrog citron plants

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Fig. 1 (A) Proposed secondary structures of minimum free energy of *Citrus viroid V* (CVd-V) and *Citrus dwarfing viroid* (CDVd). Broken lines divide the secondary structures into four segments (S1, S2, S3 and S4). Conserved regions [terminal conserved region (TCR) within S1 and central conserved region (CCR) within S3] of members of the genus *Apscaviroid* are shaded. Boxed nucleotides show the putative motifs involved in the formation of hairpin II-like. (B) Schematic representation of the seven chimeric viroids (Ch1 to Ch7) and their parental viroids (CVd-V and CDVd). Shaded and non-shaded regions show the segments from CDVd and CVd-V, respectively. Boxed and non-boxed numbers indicate the first and last nucleotides of the segments from CDVd and CVd-V, respectively. (C) Proposed secondary structure of minimum free energy of Ch5. Shaded region shows the S1 segment from CDVd that replaces the S1 segment of CVd-V. Nucleotide changes between CVd-V and Ch5 are indicated by asterisks. The change C → U found in the progeny of Ch5 is shown boxed.

co-infected with CDVd and CVd-V show synergistic interactions manifested in enhanced leaf symptoms and very pronounced dwarfing (Serra *et al.*, 2008a), these host–viroid combinations provide a convenient model to identify the pathogenicity determinant(s) in members of the genus *Apscaviroid*. The aim of this study was to evaluate the effect on symptom expression of exchanging discrete portions of the CVd-V rod-like structure for their corresponding counterparts of CDVd.

The compositions of the seven chimeras designed by the interchange of discrete portions of the secondary structure of CVd-V and CDVd are illustrated in Fig. 1. Unlike the strategy followed previously for the construction of chimeric viroids (Góra *et al.*,

1996; Owens *et al.*, 1990; Reanwarakorn and Semancik, 1998; Sano *et al.*, 1992; Sano and Ishiguro, 1998; Spieker, 1996; Visvader and Symons, 1986), a polymerase chain reaction (PCR)-based novel approach, which does not rely on the availability of shared restriction sites and permits the construction of any choice of chimeric viroids, was developed. Briefly, the segments of CDVd used to replace the corresponding segments of CVd-V were obtained by PCR, employing a cDNA insert cloned into a recombinant plasmid (Bluescript KS+, Promega®, Madison, WI, USA), pCDVd, containing the full-length CDVd sequence (GENBANK accession number EU934004) as a template and different primer sets phosphorylated at their 5' termini (Table S1,

see Supporting Information). The CVD-V sequences were also obtained by PCR using the recombinant Bluescript KS+ plasmid, pCVD-V, containing the full-length CVD-V sequence (GENBANK accession number NC010165) as a template and different primer sets (Table S2, see Supporting Information). This PCR results in the amplification of the full plasmid sequence plus the CVD-V insert devoid of the segment to be replaced. Ligation of CVD-V and CDVd segments generated the designed chimeric variants.

Considering that structural–functional domains have not been clearly defined in members of the genus *Apscaviroid*, the segments to be replaced in the present work were chosen to preserve the CCR and the flanking inverted repeats responsible for the formation of hairpin I and the TCR, both located in the upper strand of the viroid secondary structure. Four discrete segments were then defined in the secondary structure of the two viroids (Fig. 1A): (S1) segment containing the T_L domain and delimited in the upper strand by the 3' end of the TCR (positions 28 and 27 of CVD-V and CDVd, respectively), and the corresponding fragment of the lower strand; (S2) segment delimited by the 3' end of the TCR (positions 29 and 28 of CVD-V and CDVd, respectively) and the left flanking repeat of the CCR (position 73 of CVD-V and CDVd, respectively), and the corresponding fragment of the lower strand; (S3) segment containing the CCR and both flanking repeats, and the corresponding fragment of the lower strand; (S4) segment containing the T_R domain and delimited in the upper strand by the end of the right flanking repeat of the CCR, and the corresponding fragment of the lower strand.

Although hairpin II has only been observed in *Potato spindle tuber viroid* (PSTVd) and closely related viroids (Schröder and Riesner, 2002), CVD-V and CDVd contain, in the lower strand of their secondary structures, two stretches of 7 bp (CVD-V) or 11 bp (CDVd) (boxed in Fig. 1A) that could form a stable hairpin II-like structure with a GC-rich stem (Serra *et al.*, 2008a). With the purpose of preserving this putative structure, an additional segment (S3') resembling S3, but containing the CCR without the flanking repeats and the corresponding segment of the lower strand, was also considered. The utilization of S3' required concurrent modifications of the adjacent segments S2 and S4 into S2' (S2 plus the left inverted repeat of the CCR and the corresponding fragment of the lower strand) and S4' (S4 plus the right inverted repeat of the CCR and the corresponding fragment of the lower strand), respectively.

The resulting chimeric viroids generated using these criteria were as follows (Fig. 1B): Ch1 (CVD-V containing segments 1 and 2 of CDVd); Ch2 (CVD-V containing segment 4 of CDVd); Ch3 (CVD-V containing segments 1, 2 and 4 of CDVd); Ch4 (CVD-V containing segments 1, 2' and 4' of CDVd); Ch5 (CVD-V containing segment 1 of CDVd); Ch6 (CVD-V containing segment 2 of CDVd); and Ch7 (CVD-V containing segments 2 and 4 of CDVd).

To obtain infectious preparations of the seven chimeric viroids, head-to-tail dimeric cDNAs were generated and used as

templates to produce the corresponding *in vitro* transcripts homologous to the seven chimeric constructions employing T7 RNA Polymerase (Roche Applied Science®, Basel, Switzerland). These preparations were inoculated mechanically (50 ng of transcript per plant) into three citron seedling plants each. Inoculated plants were kept in the glasshouse at 28–32 °C for 9 months. Dimeric *in vitro* transcripts of CVD-V and CDVd were synthesized and inoculated as positive controls, and three non-inoculated plants were maintained as negative controls.

Three months post-inoculation, plants inoculated with CVD-V or CDVd developed symptoms, whereas plants inoculated with chimeric variants remained symptomless and indistinguishable from the negative controls over a 9-month period. However, analysis by northern blot hybridization with viroid-specific DNA probes (Serra *et al.*, 2008a) and reverse transcriptase-polymerase chain reaction (RT-PCR) (Bernad and Duran-Vila, 2006) showed that a single plant from the three inoculated with the chimeric variant Ch5 was infected. RT-PCR analysis using P1 and P2 primers (Table S1, see Supporting Information) and amplicon sequencing confirmed that the chimeric variant Ch5 was stable, except for a single 42C → U substitution in the S2 segment (Fig. 1C).

In contrast with the success achieved with chimeras between *Citrus exocortis viroid* (CEVd) and *Tomato apical stunt viroid* (TASVd), two closely related viroids of the same genus (Sano *et al.*, 1992), and, to a lesser extent, between CEVd and *Hop stunt viroid* (HSVd), belonging to different genera (Sano and Ishiguro, 1998), only one (Ch5) of the seven chimeric constructs between CVD-V and CDVd was infectious. Unexpectedly, Ch4, which differs from CDVd in only four nucleotides in the lower strand of CCR, was not infectious. These changes that appear to annul Ch4 infectivity were also present in all the other chimeric constructs, which, with the exception of Ch5, were not infectious. The infectivity of Ch5 is probably because it is essentially identical to CVD-V, except in T_L, which was replaced with that of CDVd. It should be noted that replication of Ch5 in only one of the three inoculated plants was associated with a 42C → U substitution in the S2 segment, which seems essential for viability. Thus, this is the first report of an artificial chimeric viroid within the genus *Apscaviroid* that replicates, becomes systemic and is readily transmissible. Overall, Ch5 differs with regard to 11 changes with respect to CVD-V, leading to a secondary structure of T_L identical to that of CDVd. In contrast with previous studies conducted with PSTVd, in which mutations in the loops of the T_L domain resulted in low replication and impaired or defective trafficking (Zhong *et al.*, 2008), the replication of Ch5 was comparable with that of CVD-V and was trafficking competent.

To confirm the absence of symptoms in plants infected with Ch5, buds from sensitive selection 861-S1 of Etrog citron were graft propagated onto rough lemon seedlings and, concurrently, graft inoculated (three plants each) with bark from Etrog citron seedlings infected with the chimeric variant Ch5 or with CVD-V

or CDVd. Three additional non-inoculated plants were also maintained as negative controls. The growing material of the grafted plants was observed over a 4-month period at 28–32 °C, and infection was confirmed by sequential polyacrylamide gel electrophoresis (sPAGE) (Fig. 2A) and RT-PCR (data not shown). CVd-V-infected citron plants were stunted and presented very small necrotic lesions with gum exudates in the stem and mild leaf epinasty (Fig. 3A, B). CDVd-infected citron plants were also stunted and displayed the characteristic 'drooping leaf pattern' caused by moderate epinasty resulting from petiole and mid-vein necrosis (Fig. 3A, C). By contrast, Ch5-infected citron plants were symptomless (Fig. 3A, D). The titres of the three viroids were comparable (Fig. 2A) and RT-PCR analysis and amplicon sequencing confirmed the stability of the inoculated sequences. Unlike plants infected with CVd-V or CDVd, plants infected with Ch5 were slightly taller than the non-infected controls and did not display any of the symptoms induced by either of these two viroids: necrotic lesions and cracks, sometimes filled with gum, characteristic of CVd-V, and stunting and moderate epinasty resulting from petiole and mid-vein necrosis, characteristic of CDVd. This observation suggests that the T₁ domain is involved in blocking symptom expression. A different situation was found by Sano *et al.* (1992) with chimeras between CEVd and TASVd, both belonging to the genus *Pospiviroid*, in which the T₁ domain did not block, but modulated, pathogenesis.

As CDVd and CVd-V co-infecting the same plant show synergistic interactions, specifically enhanced leaf epinasty and very pronounced dwarfing (Serra *et al.*, 2008a), two additional treatments were carried out to examine the effect of co-infections of Ch5 with CDVd or CVd-V. Six buds from Ch5-infected citron were each graft propagated onto rough lemon seedlings; concurrently, three of these plants were graft inoculated with bark from CDVd-infected citron, and the other three with bark from CVd-V-infected citron. Three additional buds from CVd-V-infected citron were also graft propagated on rough lemon seedlings and, concurrently, graft-inoculated with bark from CDVd-infected citron as a synergistic control. Three buds each from CDVd-, CVd-V- and Ch5-infected plants, and three from non-inoculated plants, were graft propagated as controls. Inoculated plants were kept in the glasshouse at 28–32 °C. Data on plant height and number of internodes collected 4 months after inoculation were analysed using one-way analysis of variance (ANOVA) and the Student–Newman–Keuls post-test, which compares all pairs of means (Table 1). The corresponding results showed that: (i) there were significant differences among treatments; (ii) no significant differences were found among plants singly infected with CDVd or CVd-V, or co-inoculated with Ch5, which, however, were significantly smaller than non-infected controls; (iii) plants co-inoculated with CDVd and CVd-V were significantly smaller than those of the other treatments; and (iv) plants infected with Ch5 were significantly taller than the non-infected controls.

Fig. 2 (A) Analysis by sequential polyacrylamide gel electrophoresis (sPAGE) and silver staining of citron plants infected with *Citrus viroid V* (CVd-V), chimera 5 (Ch5) or *Citrus dwarfing viroid* (CDVd). (B) Analysis by sPAGE and northern hybridization of citron plants singly infected with CVd-V or CDVd, or co-infected with both viroids. (C) Analysis by sPAGE and northern hybridization of citron plants singly infected with Ch5 or CDVd, or co-infected with both. (D) Analysis by sPAGE of citron plants singly infected with CVd-V or Ch5, or co-infected with both. Silver staining of the second denaturing gel of sPAGE and northern hybridization show the viroid circular forms (CF). H (mock-inoculated control) is shown in all the analyses. Ethidium bromide staining of the first non-denaturing gel of sPAGE shows that RNA levels (6S and 5S RNAs) in all preparations are comparable.

Fig. 3 Symptoms of viroid infection in Etrog citron plants at 4 months post-inoculation. (A) General aspect of plants non-inoculated (healthy), infected with a single viroid [*Citrus viroid V* (CVd-V), *Citrus dwarfing viroid* (CDVd) or chimera 5 (Ch5)] or co-infected with two viroids (Ch5 and CVd-V, Ch5 and CDVd or CVd-V and CDVd). (B) Leaf epinasty and cracks and gum exudate in the stem of CVd-V-infected plants. (C) Petiole necrosis and leaf epinasty of CDVd-infected plants. (D) Symptomless plant infected with Ch5. (E) Leaf epinasty and cracks and gum exudate in the stem of plants co-infected with Ch5 and CVd-V. (F) Petiole necrosis and leaf epinasty of plants co-infected with Ch5 and CDVd. (G) Severe leaf epinasty in plants co-infected with CVd-V and CDVd.

Table 1 Size of Etrog citron plants infected with single viroids or co-infected with two viroids.

Viroid	Plant size*	
	Height (cm)	Internodes (number)
Uninfected	63.3 ± 1.8a	32.3 ± 1.5ab
CVd-V	37.0 ± 1.0b	23.5 ± 0.5bc
CDVd	33.0 ± 0.1b	20.0 ± 2.6bc
Ch5	76.0 ± 3.1c	39.0 ± 1.5a
Ch5 + CVd-V	49.0 ± 1.2b	27.0 ± 2.9b
Ch5 + CDVd	46.3 ± 7.2b	30.4 ± 3.8ab
CVd-V + CDVd	16.5 ± 3.5d	15.0 ± 1.0c

*Data show the mean and standard error of the height and internode number. Data followed by the same letter are not significantly different according to analysis by one-way analysis of variance (ANOVA) and the Student–Newman–Keuls post-test (95%).

CDVd, *Citrus dwarfing viroid*; Ch5, chimera 5; CVd-V, *Citrus viroid V*.

Plants co-infected by two viroids or two strains of the same viroid may display two distinct types of interaction, termed 'cross-protection' (Duran-Vila and Semancik, 1990; Pallás and Flores, 1989) and 'synergism' (Serra *et al.*, 2008a). Given the high sequence similarity between Ch5 and CVd-V, Ch5 could be regarded as a latent strain of CVd-V and a 'cross-protection' phenomenon should be expected in co-infected plants. On the other hand, as plants co-infected with CVd-V and CDVd displayed a synergistic interaction, a similar interaction was anticipated in plants co-infected with Ch5 and CDVd. Unexpectedly, the results of the present study showed that, in contrast with the synergistic effect observed in plants co-inoculated with CVd-V and CDVd (Fig. 3A, G), plants co-infected with Ch5 and CVd-V (Fig. 3A, E) or with Ch5 and CDVd (Fig. 3A, F) developed the same symptoms as plants singly infected with CVd-V (Fig. 3A, B) or CDVd (Fig. 3A, C), respectively. This suggests that no interaction exists between Ch5 and these two related viroids.

The synergistic effect in plants co-infected with CVd-V and CDVd is not associated with changes in the titre of these two viroids (Serra *et al.*, 2008a). Northern blot hybridization analysis using CVd-V- and CDVd-specific probes confirmed this observation (Fig. 2B) but, unexpectedly, analysis of plants co-infected with Ch5 and CDVd showed very low or even undetectable accumulation of the former (Fig. 2C). The low titre of Ch5 was corroborated using a more sensitive hybridization test (Murcia *et al.*, 2009) and by RT-PCR with the primer pair P1 and P2 (Fig. 4A). Analysis by sPAGE and silver staining showed that plants co-infected with Ch5 and CVd-V presented similar viroid titres as plants singly infected with Ch5 or CVd-V (Fig. 2D). Given the high sequence identity (96%) between CVd-V and Ch5, these viroids could not be differentiated by northern blot hybridization and required RT-PCR analysis with discriminating primer pairs C1 and V7 for CVd-V or C2 and V7 for Ch5 (Table S1, see Supporting Information) (Fig. 4B). To estimate the relative concentrations of

CVd-V and Ch5 in the co-infected plants, an RT-PCR approach was designed as follows: (i) the efficiency of reverse transcription in the generation of cDNAs from CVd-V and Ch5 with primers P1 and P2 was evaluated by PCR using, as templates, serially diluted (1 : 2, 1 : 20, 1 : 200) cDNAs generated from equalized preparations of CVd-V or Ch5 (Fig. 4C); (ii) the suitability of this approach was confirmed using mixtures (1 : 1, 1 : 9 and 9 : 1) of initially equalized preparations of CVd-V and Ch5 that were subjected to RT-PCR. The resulting amplicons were ligated into the pGem-T vector, and PCR analysis using discriminating primer pairs of 11 clones from each ligation resulted in the same ratios of CVd-V and Ch5 as in the original mixtures. Using this approach, preparations from each of the three plants co-infected with CVd-V and Ch5 were examined: PCR analysis of 15 clones from each preparation revealed the presence of CVd-V in all. These results show that, as in plants co-infected with CDVd and Ch5, the titre of Ch5 was also very low in plants co-infected with CVd-V and Ch5.

Although co-infected plants were produced by graft propagation of Ch5-infected buds, which were further inoculated with either CVd-V or CDVd, analysis of the co-infected plants showed that infection with CVd-V or CDVd reduced the concentration of Ch5 to extremely low titres. These results explain the lack of interaction regarding symptom expression. Similar results have been found in *Gynura aurantiaca* and tomato plants co-infected with PSTVd and CEVd, where CEVd displaced PSTVd in *G. aurantiaca*, whereas PSTVd displaced CEVd in tomato (Pallás and Flores, 1989). These observations suggest that a limiting host factor that was initially used by one of the viroids was later monopolized by the other because of its higher affinity. Similarly, Ch5 could be displaced by either CVd-V or CDVd. Ch5 is an artificial viroid with primary and secondary structures of T_L different from those of its CVd-V progenitor, which appears to be well adapted to the Etrog citron host. However, in contrast with the naturally occurring CDVd and CVd-V, which have been subjected for a long time to natural selection under different hosts and environmental pressures, Ch5 has not suffered similar evolutionary pressures.

The mechanisms involved in viroid-induced symptoms are poorly understood. As sequence variants with changes affecting specific domains of the viroid secondary structure are responsible for the induction of certain diseases (Malfitano *et al.*, 2003; Palacio *et al.*, 2000; Reanwarakorn and Semancik, 1998) or for pronounced changes in symptom severity (De la Peña *et al.*, 1999; Serra *et al.*, 2008c), the hypothesis that the viroid RNA is the primary effector has been entertained for many years. According to this hypothesis, the interaction of viroid RNA with a cell factor would interfere with its normal function and would lead to symptom expression. Undoubtedly, symptom development is a complex process that could involve additional viroid-

Fig. 4 (A) Top panel: northern hybridization analyses of samples electrotransferred from a non-denaturing gel using a digoxigenin (DIG)-labelled probe specific for chimera 5 (Ch5) of citron plants singly infected with Ch5 or co-infected with Ch5 and *Citrus dwarfing viroid* (CDVd). Ethidium bromide staining of the first non-denaturing gel of sequential polyacrylamide gel electrophoresis (sPAGE) shows that RNA levels (6S and 5S RNAs) in all preparations are comparable. Bottom panel: reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of citron plants singly infected with Ch5 or co-infected with Ch5 and CDVd using primers P1 and P2. (B) RT-PCR analysis of citron plants singly infected with Ch5 or co-infected with Ch5 and *Citrus viroid V* (CVd-V) using primers C2 and V7. (C) Efficiency of reverse transcription for the generation of cDNAs from CVd-V and Ch5 using primer P2 was evaluated by PCR using, as templates, serially diluted (1 : 2, 1 : 20, 1 : 200) cDNAs generated from equalized preparations of CVd-V or Ch5.

related RNA molecules, such as small interfering RNAs (siRNAs) and microRNAs (miRNAs) (Baulcombe, 2004; Carrington and Ambros, 2003). The replacement of the T_L domain of CVd-V by that of CDVd would alter the structure of the viroid RNA and its affinity for a host factor, or the generation of certain specific viroid-derived siRNAs. Recently, it has been shown that viroid infection can downregulate or upregulate gene expression (Itaya *et al.*, 2002; Tessitori *et al.*, 2007), ultimately leading to symptoms. This cascade of events must be mediated by specific elicitors. The range of symptoms associated with CVd-V, which are not induced by Ch5, suggests that the latter does not act as an appropriate elicitor.

ACKNOWLEDGMENTS

This research was supported by grants AGL2005-01468, AGL2008-01491 and AGL2008-00596 from the Ministerio de Educación y Ciencia. P. Serra, G. Pensabene-Bellavia and S. M. Bani Hashemian are recipients of fellowships from the Consellería de Agricultura – IVIA (PS and GPB) and from the Iran Citrus Research Institute (SMBH). Selma Gago has a contract (I3P 2005) from the CSIC. The authors would like to acknowledge R. Flores and J. Guerri for critical reading of the manuscript, L. Navarro for providing the plant materials used in this study and R. Carbó for technical assistance.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Primers used to amplify monomeric viroid sequences, specific viroid segments and plasmids containing partial viroid sequences.

Table S2 Strategies for construction of chimeric viroids.

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