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Natural infection of petunia by chrysanthemum stunt viroid

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Abstract

A viroid that behaved similar to chrysanthemum stunt viroid (CSVd) during return-polyacrylamide gel electrophoresis has been detected in petunia. Subsequent transmission studies as well as reverse transcription-polymerase chain reaction and sequence analysis showed that the viroid is indeed a strain of CSVd. As long as the viroid is absent from plants used for vegetative propagation, it appears not to pose a serious threat to petunia cultivation.

Over the past few years, there has been increasing interest in growing petunia plants that are propagated vegetatively. Such plants are often registered under different names, e.g. Cascadias, Fortunia and Surfinia. The vegetative propagation however, has led to a dramatic increase in the number of virus infections in this crop (Lesemann, 1996).

In 1995, the Dutch Inspection Service for Floriculture and Arboriculture (NAKB) detected a viroid by return-polyacrylamide gel electrophoresis (R-PAGE) analysis of nucleic acids extracted from a plant of *Petunia hybida* Surfinia® 'Purple' obtained from a commercial garden centre. This infected plant was later sent to the Plant Protection Service in Wageningen for definitive identification of the viroid. As the particular petunia plant showed mosaic on the malformed leaves and a generalized reduction in growth, it was first tested for virus infection. Mechanical inoculation of several test plant species and serological tests (DAS-ELISA) revealed the plant to be infected by both tobacco mosaic virus (TMV) and potato virus Y (PVY). For comparison, the plant was again tested for the presence of viroid(s) by R-PAGE (Huttinga et al., 1987), together with samples from chrysanthemum infected with chrysanthemum stunt viroid (CSVd) and tomato infected with potato spindle tuber viroid (PSTVd). After silver staining, the single band from the petunia sample was located at the same position as CSVd, and slightly below PSTVd (Figure 1).

For further characterization of the viroid, nucleic acids extracted from symptomatic petunia leaves were tested in a reverse transcription-polymerase chain reaction (RT-PCR) system designed to detect CSVd (Hooftman et al., 1996). In this system, both CSVd and PSTVd primer sets were used: CSV-C (5′-CCC TGA AGG ACT T, CT TCG CC-3′) complementary to CSVd nucleotides 85-66 in combination with CSV-H (5′-ATC CCC GGG GAA ACC TGG AGG AAG T-3′) homologous to CSVd nucleotides 86-110 (Hooftman et al., 1996), and (5′-CCC TGA AGC GCT CCT CCG AG-3′) complementary to PSTVd nucleotides 69-88 in combination with (5′-ATC CCC GGG GAA ACC TGG AGC GAA C-3′) homologous to PSTVd nucleotides 89-113 (Levy et al., 1994). A CSVd-infected chrysanthemum plant and a PSTVd-infected tomato plant were used as positive controls. The RT-PCR amplified products were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide. With the CSVd specific primers, the viroid from petunia yielded a product of the same size as the CSVd positive control, while with the PSTVd specific primers no product was obtained (Figure 2). With healthy petunia plants no products were obtained with either primer set (results not shown).

To compare their biological properties, the viroid from petunia and CSVd from chrysanthemum (Hooftman et al., 1996) were mechanically inoculated to three different hosts. Nucleic acids were extracted from two

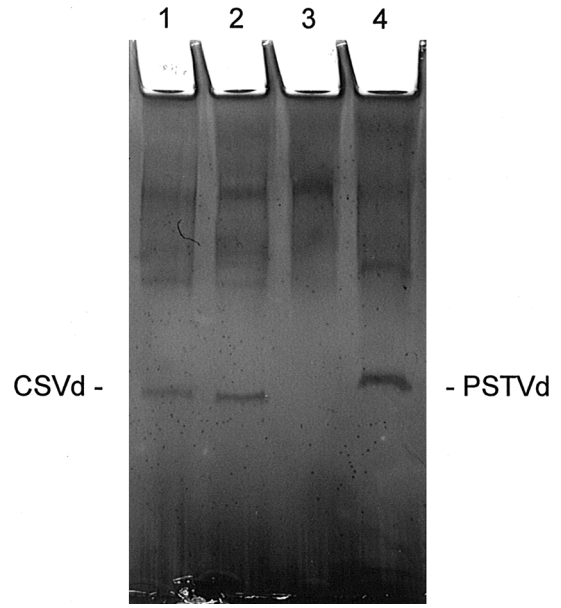

Figure 1. Silver-stained poly-acrylamide gel after return-electrophoresis showing nucleic acid extracts from the following samples: CSVd-infected chrysanthemum (lane 1), viroid-infected petunia (lane 2), healthy petunia (lane 3) and PSTVd-infected tomato (lane 4).

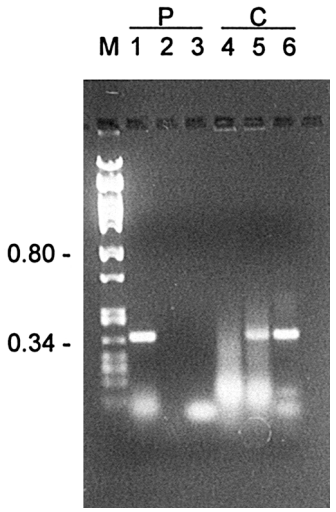

Figure 2. Ethidium-bromide stained agarose gel showing RT-PCR products obtained by the following samples and primer sets: PSTVd-infected tomato (lanes 1, 4), viroid-infected petunia (lanes 2, 5) and CSVd-infected chrysanthemum (lanes 3, 6); PSTVd specific primers (P: lanes 1, 2, 3), CSVd specific primers (C: lanes 4, 5, 6). Marker: λ x PstI (M).

Table 1. Mechanical transmission of the viroid isolate from petunia and CSVd from chrysanthemum

Plant species	Transmission rate*	
	Viroid from petunia	CSVd
Dendranthema morifolium 'Sunny Reagan'	5 / 10	5 / 10
Lycopersion esculentum 'Moneymaker'	7 / 12	4 / 4
L. esculentum 'Trust'	12 / 12	4 / 4
Petunia hybrida 'Polo Pink'	0 / 26	0 / 23

* All data expressed as numbers of infected plants over number of inoculated plants.

grams of symptomatic leaf tissue from petunia and chrysanthemum, respectively, as described by Huttinga et al. (1987). Precipitated nucleic acids were resuspended in 1.2 ml of sterile demineralized water and used to inoculate carborundum-dusted leaves of chrysanthemum, tomato and petunia. Inoculated plants were grown at temperatures of at least 23 °C under conditions described earlier (Verhoeven et al., 1995). Six weeks after inoculation plants were inspected for symptoms and individually indexed for the presence of viroid by R-PAGE. Infected chrysanthemum plants showed no symptoms, although both the viroid from petunia and CSVd were detected by R-PAGE in half of the inoculated plants (Table 1). Infected tomato plants from both cultivar 'Moneymaker' and 'Trust' were all severly stunted by CSVd. The viroid from petunia induced stunting that varied from very mild to severe. Mechanical inoculation to petunia was not successful however, and additional tip-graft inoculations were performed in efforts to transmit the viroid. Eight weeks after grafting, the viroid indeed could be detected by R-PAGE in all three graft-inoculated petunia plants.

Finally, the identity of the viroid isolated from petunia was definitively established by direct sequence analysis of the RT-PCR product resulting from amplifications containing the CSVd-specific primers. Analysis of uncloned double-stranded PCR products was carried out using a Model 373 automated DNA sequencer and DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). Careful inspection of the chromatogram generated using primer CSV-C revealed that the viroid isolated from petunia was a mixture of at least two CSVd sequence variants. As shown in Figure 3, the longer variant contained 355 nucleotides and differed from the shorter (i.e. 354 nt) variant only in the number of G residues near position 130. The presence of five (rather than four) G residues at positions 128–132 in more than half of the viroid population resulted

CSVd-Petunia GenBank Accession #U82445

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  1 cgggacttac  ttgtggttcc  tgtggtgcac  tcctgaccct  gctgctttga  50
 51 aagaaaaaga  aatagggcga  agaagtcctt  cagggatccc  cggggaaacc 100
101 tggaggaagt  ccgacgagat  cgcggttggg  ggcttaggac  cccactcctg 150
151 cgagacagga  gtaatcctaa  acagggtttt  cacccttcct  ttagtttcct 200
201 tcctctcctg  gagaggtctt  ctgccctagc  ccggtcttcg  aagcttcctt 250
251 tggctactac  ccggtggaaa  caactgaagc  ttcaacgcct  ttttttccta 300
301 tcttctttag  caccgggcta  gggagtaagc  ccgtggaacc  ttagttttgt 350
351 tccct 355
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Figure 3. Nucleotide sequence of a CSVd sequence variant isolated from petunia. The complete sequence of the longer of two variants detected by automated sequence analysis of uncloned RT-PCR amplification products is shown. In the shorter (i.e. 354 nt) variant, the oligo(G) sequence between positions 128-132 contains only four residues. Note that the sequence of the primer binding sites (i.e. positions 66-110) was not directly determined.

in overlapping signals for positions 128–86. No other obvious signs of sequence heterogeneity were detected elsewhere in the molecule. Pairwise comparisons with 11 other CSVd sequences in GenBank Release 104.0 revealed a high degree of sequence homology (96.4 – 99.4%).

Based on a combination of results obtained by the R-PAGE, RT-PCR and sequence analysis, and transmission studies, it was concluded that the viroid isolated from petunia resembles CSVd in all essential aspects and can, therefore, be considered as an isolate of this viroid. Previously, natural infections of CSVd have only been reported for *Ageratum*, (Henkel and Sänger, 1995) and *Dendranthema* (chrysanthemum) (Dimmock, 1947; Diener and Lawson, 1973). Although several other plant species including *Senecio* (cineraria) (Gross et al., 1982) and petunia (Brierley, 1953; Runia and Peters, 1980) can be infected by CSVd experimentally, this is the first time that the viroid has been isolated from a naturally infected petunia plant.

Most probably, the viroid is not responsible for the symptoms observed in the original infected petunia plant, neither for those observed in the graft-inoculated petunia plants, as Runia and Peters (1980) did not observe any symptoms in CSVd infected petunia plants after mechanical inoculation. Based on earlier observations (Roenhorst and Verhoeven, 1995; 1996), either the presence of TMV, PVY or a synergistic interaction between the two viruses might be responsible for the mosaic, malformations and growth reduction observed.

The origin of the infection for this particular *Petunia hybrida* Surfinia® 'Purple' plant could not be traced. Possible sources include plant species grown in the same garden centre, but transmission of CSVd to petunia via contact does not seem very efficient. Runia and Peters (1980) were only able to infect one out of ten plants via mechanical inoculation, and mechanical transmission was not successful in the experiments described above. Alternatively, infection of petunia may have taken place sometime in the past and has been spread via vegetative propagation.

Thus far, these observations suggest that CSVd does not pose a significant threat to petunia cultivation as long as the viroid is absent in plants used for vegetative propagation. Because of the symptomless nature of the infection however, testing of individual mother plants is required to ensure the absence of the viroid.

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