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REVIEW
Chrysanthemum Stunt Viroid

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Abstract

The *chrysanthemum stunt viroid* (CSVd) is a small, single-stranded, infectious RNA forming a circular secondary structure, which belongs to the Pospiviroidae family and mainly infects certain species and cultivars of Compositae and Solanaceae. The effects on cultivated chrysanthemum are severe, including stunting of plant height, reduced flower size, and flower color bleaching. However, the expression of the symptoms depends on the chrysanthemum cultivars. CSVd is known to be readily transmitted by sap, but the infection rate and incubation period were observed to differ according to the varieties. Some CSVd-resistant cultivars have also been reported and the resistance is heritable in crosses between a CSVd-resistant chrysanthemum cultivar and CSVd-susceptible cultivars.

Discipline: Plant disease
Additional key words: *Chrysanthemum morifolium*, resistant, transmission, symptom, strain

Introduction

Viroids are the smallest and simplest form of plant pathogens. Each consists of a naked, single-stranded circular RNA genome, which is 246 to 401 nucleotides in length and lacks protein coding sequences[7,8,9]. Worldwide, approximately 30 viroids have been identified and classified into two families, Pospiviroidae and Avsunviroidae[9]. Members of the Pospiviroidae family, the type species for which is *Potato spindle tuber viroid* (PSTVd), have highly conserved regions in their rod-shaped secondary structure, replicate in the nuclei of infected cells, and lack ribozyme activity.

Chrysanthemum stunt viroid (CSVd; Fig. 1) belongs to the Pospiviroidae family[42]. Chrysanthemum stunt was first described by Dimock[6] and was a casual organism thought to be a virus until Diener and Lawson[5] reported that the chrysanthemum stunt pathogen had biochemical properties resembling those described for the PSTVd. By the 1950s, the disease had rapidly spread worldwide[2], causing undesirable symptoms in whole plants and flowers of different chrysanthemum cultivars (the florist chrysanthemum, *Chrysanthemum morifolium*, formerly *Dendranthema grandiflora*). Chrysanthemum is one of the most popular ornamentals worldwide. Its abundance diversity in flower type, color and plant architecture means it occupies a considerable proportion of the flower industry in South-East Asia and Euro-countries. In this review, I present basic information about CSVd on the host plants, strain, symptoms, environmental effects, transmission and control.

Host plants and strains

CSVd can experimentally infect certain species and cultivars of Compositae, Cucurbitaceae and Solanaceae[3,46]. Natural infections were reported for *Ageratum* sp., *Dahlia*

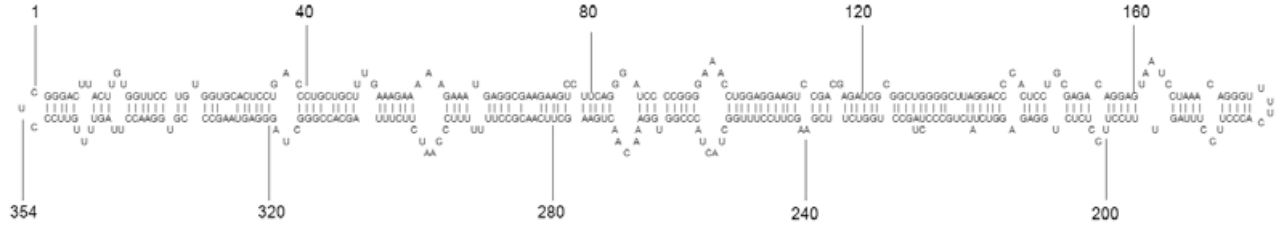

Fig. 1. *Chrysanthemum stunt viroid* (CSVd; GenBank/EMBL/DDBJ accession no. X16408) RNA sequence and predicted secondary structures

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spp.[38], *Petunia hybrida*[55], marguerite (*Argyranthemum frutescens*)[26,36], *Solanum jasminoides*[56], Vinca sp.[39,57] and 8 wild chrysanthemum species[29], *C. crassum*, *C. indicum* var. indicum, *C. japonense* var. japonense, C. makinoi, *C. wakasaense*, C. weyrichii, *C. yoshinaganthum* and *C. zawadskii*.

PSTVd variants that vary in RNA length from 341 nt (GenBank/EMBL/DDBJ accession no. Z34272.1) to 364nt (No. DQ308555, AY372395.1) have been isolated and described in different hosts. It was reported that the PSTVd isolate KF 440-2 is infectious to potato and tomato plants upon mechanical inoculation, but not to *Nicotiana tabacum* plants[58]. However, PSTVd-NT, which has a single base substitution (C259→ U259 substitution), was converted from a non-infectious PSTVd to an infectious RNA that is stable in both hosts (tomato and tobacco)[58]. Some studies on the accumulation profile of nucleotide substitutions and their effect on PSTVd structure, replication, movement and pathogenesis have been reported[41,43,59].

Some strains of CSVd have been also reported to date, which are 354, 355 and 356nt in length (Table 1). Matsushita et al.[30] determined whether the CSVd–infected tomato plants could transmit the viroids to other plants and establish host-specific infectious CSVd RNA. They grafted petunia plants with CSVd-infected tomato plants (infected with the CSVd X16408 strain) and then gained the strain with a single G residue insertion for one year after grafting. The G base insertion was common to both the natural petunia isolate (No. U82445; 55) and the gained strain, suggesting that the insertion of a G residue is essential for establishing the CSVd infection in petunia. However, details of nucleotide substitutions of effect on CSVd structure, replication, movement and pathogenesis remain unclear.

Effects

The effects on the chrysanthemum plant are known to be severe, including stunting of plant height, poor root

Table 1. Strains of Chrysanthemum stunt viroid

Hosts	Length	Accession number in DDBJ (Reference)	Reference
–	356	V01107	18
Cineraria	354	M19506	15
Petunia hybrida	355	U82445	56
Chrysanthemum morifolium	354	X16408	49
C. morifolium	354	AB006737	24
C. morifolium	354	D88895	22
Solanum jasminoides	354	DQ406591	26
C. morifolium *C. japonense var. japonense,* *C. weyrichii*	354	X16408	29
C. japonense var. japonense, *C. indicum var. indicum,* *C. makinoi,* *C. zawadskii*	354	M19506	29
C. yoshinaganthum	354	AB279770	29
C. morifolium	354	AB279771	
Vinca major	355	DQ094298	39
Dahlia spp	354	AB255880	38
Dahlia spp	354	AB255879	
C. morifolium	354	AB279769	35
Argyranthemum frutescens	355	JF938538	26
C. morifolium	354	AB679211	37
C. morifolium	354	AB279769	

development, reduced flower size, and flower color bleaching (Figs. 2 and 3), resulting in a decline in the quality and yield of cut flowers[10,20,29,54]. However, the effects on plants infected with CSVd varies according to the chrysanthemum cultivar and environmental conditions. *C. morifolium* 'Bonnie Jean' and 'Mistletoe' infected with CSVd express chlorotic flecking and spotting on the upper and lower leaves[1] (Fig. 4). 'Mistletoe' has been used as the indicator, and the test is carried out by grafting, although the results depend on environmental conditions, especially light and temperature[1]. They reported that special growing conditions combining high temperatures of 25-28°C and high light intensity (20000 lux) allowed stunt to be detected 20 days after inoculation. Generally, viroid replication is enhanced as the temperature exceeds 20°C, at least up to 35°C[50,51]. In general, the effects on chrysanthemum are known to intensify at high temperatures.

Conversely, certain chrysanthemum cultivars do not express symptoms of CSVd, such as stunting[10]. It is therefore possible that infected seedlings of asymptomatic cultivars spread CSVd to other susceptible cultivars in nurseries.

Doi and Kato[10] reported no correlation between the severity of stunting and concentration of CSVd was found

Fig. 2. Plant infected with *Chrysanthemum stunt viroid* (right) and healthy control (left)

Fig. 3. Symptoms induced by *Chrysanthemum stunt viroid* on chrysanthemum showing poor root development (left) compared to healthy control (right)

Fig. 4. Symptoms induced by *Chrysanthemum stunt viroid* on chrysanthemum cultivar 'Mistletoe' showing chlorotic flecking and spotting on leaf

when they inoculated 10 chrysanthemum cultivars with CSVd and then compared the concentration of CSVd using dot-plot hybridization. Several groups have described a positive correlation between symptom severity and the accumulation of small RNAs derived from PSTVd[21,27,28]. However, in general, symptom severity was not correlated with accumulated levels of viroid RNAs[14,16,17,21,44,48] and no small RNA derived from CSVd has been reported to date. It seems difficult to evaluate the severity of effects on chrysanthemum because it takes so many days for the CSVd infection to emerge and effects to be seen.

Environmental effects

It is generally accepted that viroid replication is enhanced with increasing temperature beyond 20°C, at least up to 35°C[50,51]. The concentration of PSTVd in tubers stored at room temperature or in diffused light storage is sufficient for direct viroid detection in tuber flesh, eyes, or sprouts. However, storage of infected tubers at low temperature (4°C) reduced the PSTVd concentration in sprouts. Moreover, viroid concentration usually declines after 3-4 months and reaches undetectable levels at 5 or 6 months[47]. PSTVd can be easily detected in the sprouts of infected tubers maintained at 4°C if the tubers are transferred to 17°C but not 10°C. CSVd was not detected from the winter rosette sucker of infected chrysanthemum plants[54]. Subsequent assays indicated that by vegetative propagation during a low-temperature period, CSVd could be eliminated. The concentration of CSVd in chrysanthemum plants through the winter decreased to an undetectable level by dot-blot hybridization[49]. In addition, CSVd was eliminated from infected plants using meristem-tips cut from infected plants kept in the growth chamber at 5°C for 16 h daily for 6 months. Actually, CSVd was not detected by *in situ* hybridization in the meristem of infected chrysanthemum plants due to the low temperature of winter, while the meristem of the infected chrysanthemum plants maintained in a greenhouse was infected with CSVd (Matsushita et al., unpublished data).

Transmission

Mechanical transmission has been reported for most viroids in both families, Pospiviroidae and Avsunviroidae, by contaminated machinery and equipment[25]. PSTVd and *Tomato chlorotic dwarf viroid* (TCDVd), which shows similar physical stability, can be easily transmitted mechanically by contaminated knives and other equipment or by contact between infected and healthy plants[25,31]. CSVd is also known to be readily transmitted by sap[2]. However, differences in the infection rate and incubation period were observed among the chrysanthemum varieties[53]. For example, the 'Shuho no chikara' chrysanthemum cultivar was infected with CSVd 290 days after mechanical inoculation with carborundum, suggesting that it is resistant to CSVd attack and had a long incubation period, while 'Seikoogon' was infected with CSVd 85 days after inoculation.

Some reports exist concerning the contact transmission of CSVd through roots[12,53]. However, no infection was found to occur through the soil due to the dried residues plowed in it, while infection readily occurred through the contact with the root part of diseased cuttings and fresh root residues[53]. When healthy chrysanthemum plants were cultured for three months in contact with the root of chrysanthemum plants infected with CSVd, CSVd was detected at a rate ranging from 4.2-8.3%[12].

Chung and Pak[4] also reported CSVd in highly transmittable form by seed and pollen on chrysanthemums. PSTVd is known to be transmitted at high frequency by contaminated seeds[11]. PSTVd was able to invade the outer integument around the embryo sac, suggesting that such specific distribution might reflect the frequent occurrence of PSTVd seed transmission[32]. The high rates of CSVd seed transmission suggest that CSVd can invade the tissue around and/or in ovules, such as the embryo sac.

There is no evidence that CSVd can be transmitted by insect vectors[2].

Control

Since chrysanthemum is, in general, produced by vegetative propagation, it is important to maintain CSVd-free mother plants and prevent CSVd infection in the field. Accordingly, control of the disease basically involves preventing transmission by hands and equipment. To date, several chemical agents, including sodium hypochlorite (NaOCl) and sodium hydroxide plus formaldehyde, have reportedly been effective in disinfecting viroid-contaminated tools[13,34,45,52]. Low-pH sodium hypochlorite solution, which has powerful oxidizing activity, was found to degrade TCDVd dramatically, even at low concentrations (below 0.1%). However, the effectiveness of such low-pH solutions in disinfecting TCDVd decreased to a level resembling that of high-pH solutions in the presence of plant tissue residues on tool surfaces, probably due to a reduction in cleaning activity[34]. Matsuura et al.[34] found that 5% trisodium phosphate solution was effective against TCDVd. However, 2.5% was insufficiently effective against TCDVd. A 2% Trisodium phosphate did not reduce the infectivity of CSVd[19]. Since the effectiveness of disinfection to viroid depends on the pH and concentration of the chemical solution and host plant components, it is necessary to investigate the effectiveness of disinfection against CSVd on chrysanthemums.

Some chrysanthemum cultivars do not express symp-

toms such as stunting[10]. It is therefore possible that infected seedlings of asymptomatic cultivars spread CSVd to other susceptible cultivars in nurseries. Breeding of a CSVd-resistant cultivar for disease control is clearly crucial. Although it has been asserted that no chrysanthemum cultivar is resistant to CSVd[23], recent studies showed the potential to obtain highly resistant cultivars[33,40]. CSVd was absent from the shoot apical meristems and leaf primordia of resistant plants following CSVd inoculation[40]. Nabeshima et al.[37] screened CSVd-resistant cultivars using this characteristic as a phenotype marker. F_1 progeny produced by crossing this resistant cultivar with two other susceptible cultivars were not infected with CSVd following inoculation, suggesting that CSVd resistance was expressed in the first hybrid generation[33]. Since chemical and cultural approaches to control CSVd epidemics are difficult, breeding for CSVd resistance provides a promising alternative.

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