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Host range and properties of *Tomato chlorotic dwarf viroid*

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Abstract We characterised the host range and physical properties of *Tomato chlorotic dwarf viroid*. Among the 46 plant species inoculated with the viroid, two in the family Compositae and 23 in the family Solanaceae were found to be systemic hosts. The viroids in the crude sap from diseased tomato plants were thermally inactivated by heating to 100°C for at least 40 min. These viroids also lost their infectivity when diluted in phosphate buffer to at least 10^{-6}, or after 3 days of incubation at room temperature. However, the infectivity of the viroids in dried crude sap from the plants persisted throughout the 50-day test period.

Keywords Disease · Infection · Pospiviroid · TCDVd · Thermal inactivation · Tomato

In 2006, in Hiroshima Prefecture, Japan, a disease occurred to tomato plants, which showed typical symptoms of viroid infection, such as leaf chlorosis, leaf yellowing and dwarfing (Matsushita et al. 2008). The causal pathogen was identified as *Tomato chlorotic dwarf viroid* (TCDVd), based on its nucleotide sequence. TCDVd is a small, single-stranded, infectious RNA, which is 359–363 nucleotides in length, and forms a circular secondary structure. It belongs to the genus *Pospiviroid* and the family Pospiviroidae, the type species of which is the *Potato spindle tuber viroid* (PSTVd). TCDVd was first discovered in tomatoes cultivated in Canada (Singh et al. 1999), and was subsequently reported in tomatoes (Verhoeven et al. 2004) and petunias (*Petunia* × *hybrida*) in the USA (Verhoeven et al. 2007), in *Verbena* × *hybrida* in India (Singh et al. 2006), and in petunias in the UK (James et al. 2008).

Tomato plants infected with TCDVd are severely stunted, and their fruits are reduced in size and split or cracked, leading to economic losses. However, it is difficult to control TCDVd disease, as little is known about its biology. Here, we investigated the host range and physical properties of TCDVd, with the aim of developing further protective procedures against infection by this viroid.

The plants reported to be susceptible to TCDVd include *Nicandra physaloides*, *Nicotiana debneyi*, *Nicotiana glutinosa*, *Nicotiana physaloides*, *Physalis angulata, Physalis floridana, Scopolia sinensis, Solanum demissum, Solanum lycopersicum, Solanum tuberosum* (Singh et al. 1999), and *Vinca minor* (Singh and Dilworth 2008). Pospiviroids tend to have broad host ranges (Singh et al. 2003). For example, PSTVd infects numerous species within the families Solanaceae and Compositae, as well as species belonging to the families Boraginaceae, Campanulaceae, Caryophyllaceae, Convolvulaceae, Dipsaceae, Sapindaceae, Scrophulariaceae and Valerianaceae (Singh et al. 2003). Hence, TCDVd might also have a wide host range.

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Table 1 Host range of the *Tomato chlorotic dwarf viroid*

Plant species	Infection	Plant species	Infection
Aizoaceae		Pedaliaceae	
Tetragonia tetragonoides	-	*Sesamum indicum*	-
Amaranthaceae		Scrophulariaceae	
Gomphrena globosa	-[a]	*Antirrhium majus*	-
Apocynaceae		Solanaceae	
Catharanthus roseus	-	*Capsicum annuum*	+
Vinca major	+[b]	*Datura metal*	-[a]
Brassicaceae		*D. stramonium*	+
Brassica oleracea var. *capitata*	-	*Nicandra physaloides*	+[a]
B. rapa var. *peruviridis*	-	*Nicotiana benthamiana*	+
Chenopodiaceae		*N. clevelandii*	+
Spinacia oleracea	-	*N. debneyi*	+[a]
Chenopodium amaranticolor	-	*N. glutinosa*	+
C. quinoa	-	*N. occidentalis*	+
Compositae		*N. physaloides*	+[a]
Ageratum houstonianum	-	*N. rustica*	+
Arctium lappa	-	*N. tabacum* cv. *Samsun*	+
Chrysanthemum coronarium	+	*N. tabacum Xanthi-nc*	+
C. morifolium	-	*Physalis angulata*	+[a]
Helianthus annuus	-	*P. floridana*	+
Lactuca sativa	-	*Petunia x hybrida*	+
Leucanthemum paludosum'North Pole'	+	*Scopolia sinensis*	+[a]
Cucurbitaceae		*Solanum carolinense*	+
Cucumis melo	-	*S. demissum*	+[a]
C. sativus	-	*S. lycopersicum*	+[a]
Fabaceae		*S. melongena*	+
Crotalaria juncea	-	*S. mammosum*	+
Vigna unguiculata	-	*S. nigrum*	+
Gentianaceae		*S. tuberosum*	+[a]
Eustoma grandiflorum	-	Verbenaceae	
		Verbena× hybrida	+[c]

[a] Data from Singh et al. (1999)

[b] Data from Singh and Dilworth (2008)

[c] Singh et al. (2006)

To determine the host range of TCDVd, plants of 46 species in the following 13 families were mechanically inoculated with sap from an infected tomato plant: Aizoaceae, Amaranthaceae, Apocynaceae, Brassicaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Fabaceae, Gentianaceae, Pedaliaceae, Scrophulariaceae, Solanaceae and Verbenaceae. At least three young plants from each species were inoculated.

Table 2 Infectivity of crude sap after incubation at 100°C

Treatments[a] (min)	Test plants infected[b]
No treatment	9/9
10	4/9
20	1/9
30	1/9
40	0/9

[a] Preparations were heated at 100°C for the times stated

[b] Number of test plants infected with TCDVd/total number of plants.

Table 3 Infectivity of diluted crude sap

Dilution of inoculum[a]	Test plants infected[b]
10^{-1}	8/8
10^{-2}	7/8
10^{-3}	5/8
10^{-4}	5/8
10^{-5}	2/8
10^{-6}	0/8
10^{-7}	0/8

[a] Preparations were diluted with phosphate buffer (pH 7.0)

[b] Number of test plants infected with TCDVd/total number of plants.

Table 4 Infectivity of crude sap after incubation at room temperature

Storage period[a] (days)	Test plants infected[b]
0	8/8
1	3/8
2	1/8
3	0/8
4	0/8

[a] Preparations were maintained at room temperature for the number of days stated

[b] Number of test plants infected with TCDVd/total number of plants.

After inoculation, the plants were grown in a greenhouse at 23–25°C. Viroid infection was confirmed using reverse-transcription polymerase chain reaction (RT-PCR) 2 months after inoculation, as previously described by Matsushita et al. (2007). Back-inoculation to tomato plants was also conducted at the same time.

As shown in Table 1, some of the plants from the families Compositae and Solanaceae were susceptible to the viroid. RT-PCR analysis and back-inoculation tests identified 25 species in total from the two families as systemic host plants of TCDVd. Most of the infected plants were largely symptomless, with the exception of two species. All of the tomato cultivars tested ('Rutgers', 'Momotaro', 'Fukuju', 'Redall' and 'Ponderosa') developed leaf yellowing 3–4 weeks after inoculation, often followed by necrosis of the yellowing leaves and stem stunting, identical to the parental symptoms. The flowers of *N. glutinosa* plants infected with TCDVd showed colour-break symptoms, whereas those without flowers did not show any symptoms, as described previously by Singh et al. (1999). TCDVd and PSTVd share 85–89% nucleotide sequence identity (Singh et al. 1999), and tomato plants infected with either viroid show identical symptoms. Gomphrena globosa (Singh et al. 1999) and Datura metel (Table 1) were not susceptible to infection by TCDVd, although they are hosts for PSTVd (Singh and Bagnall 1968; Diener et al. 1972). Differences in the nucleotide sequences of the viroids could be responsible for the differences in host range. Most of the susceptible plant species were asymptomatic, as mentioned above. This raises the possibility that TCDVd hosts, especially asymptomatic ornamental and/or vegetable plants in international trade, could potentially spread the viroid to potato and tomato propagation fields through contaminated seeds or infected seedlings (Singh and Dilworth 2008).

TCDVd-infected sap samples were incubated in a water bath at 100°C for 10, 20, 30 or 40 min, and then immediately chilled on ice. The thermal inactivation time of TCDVd was 40 min at 100°C, as shown in Table 2. The dilution endpoint of the TCDVd-infected tomato sap in phosphate buffer was tested, and the results showed the infectivity was lost at a dilution of at least 10-6 (Table 3). In the in vitro longevity test of TCDVd in crude sap at room temperature, the infectivity of TCDVd was lost after 3 days of incubation (Table 4). When TCDVd-infected crude sap of tomato was completely dried, the infectivity still persisted throughout the 50-day test period (data not shown). Our data indicate that TCDVd has strong physical properties, such as thermal resistance, longevity and dry-resistance, similar to those of PSTVd (Diener and Raymer 1969).

TCDVd has infected many thousands of tomato plants grown in greenhouses in Japan (Matsushita et al. 2008). This might be attributable to mechanical transmission, because TCDVd is highly stable. Indeed, PSTVd, which shows similar physical stability, can be easily transmitted mechanically by contaminated knives and other equipment or by contact between infected and healthy plants (Manzer and Merriam 1961). In addition, transmission assays conducted under greenhouse conditions demonstrated that *Citrus exocortis viroid*, HSVd, *Citrus viroid* III and *Citrus viroid* IV can all be spread between citrons (*Citrus medica*) via a single slash made with a knife blade as a form of mechanical contact (Barbosa et al. 2005). The results of the current study suggest that TCDVd might easily be transmitted mechanically via contaminated tools, including the hands of labourers. Further investigation is thus required into sanitation processes for the equipment used by tomato farmers.

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