



## First report of natural infection of *Pepper chat fruit viroid* in tomato plants in Thailand

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At the end of 2009, 60 leaf samples of tomato plants (*Solanum lycopersicum*) were collected from the field in Lampang province, Thailand. The plant samples were stunted and had leaf symptoms including necrosis, distortion and discoloration. After initial examination by electron microscopy, that revealed no virus particles, the samples were tested for viroids. Total RNA was extracted using a CTAB method (Tangkanchanapas *et al.*, 2005) and then tested for viroids by reverse transcription-polymerase chain reaction (RT-PCR; Tangkanchanapas *et al.*, 2005). Three different primer sets were employed (Table 1), with the PC2 primer set designed to detect six *pospiviroids*: *Columnea latent viroid* (CLVd), *Tomato apical stunt viroid* (TASVd), *Tomato planta macho viroid* (TPMVd), *Tomato chlorotic dwarf viroid* (TCDVd), *Potato spindle tuber viroid* (PSTVd) and *Mexican papita viroid* (MPVd). Of the 60 samples collected, three tested positive using the PC2 primer set. The amplified DNA bands from these three positive samples were purified, ligated to pGEM®-T Easy vector (Promega, Madison, USA) and sent for automated sequencing. Identical 348 bp sequences were obtained from all three isolates and these were deposited in GenBank (Accession Nos. JF446891, JF446892 and JF446893). These sequences shared 99% identity with a *Pepper chat fruit viroid* (PCFVd) isolate (FJ409044), previously reported from Netherlands (Verhoeven *et al.*, 2009).

One of the isolates (PCFVd-Thai; JF446893) was further characterised by mechanical transmission onto tomato seedlings (cv. Rutgers). Four weeks after inoculation, the infected plants showed necrosis on leaf veins, petioles and stems, leaf distortion and stunting (Fig. 1), closely matching the symptoms seen on the original tomato plants found in the field. Viroid diseases have previously been reported in Thailand: *Citrus exocortis viroid* (CEVd) in citrus and tomato, *Hop stunt viroid* (HSVd) and *Grapevine yellow speckle viroid* (GYSVd) in grapevine and CLVd in tomato. This is

the first report of PCFVd occurring in the country.

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Figure 1

Table 1. Details of primers used to detect tomato viroids.

Primer	Sequences	Target viroid	References
CE2	cCE2: 5' CCG GGG ATC CCT GAA GGA C 3' hCE2: 5' GGA AAC CTG GAG GAA GTC GAG 3'	CEVd	Gross <i>et al.</i> , 1982
HS2	hHS2: 5' TCG GAA GAG CCA GAA GG 3' hHS2: 5' TGA GAC GCGACC GGT GGC ATC ACC T 3'	HSVd	Shamloul <i>et al.</i> , 2002
PC2	cPC2 : 5' TGT TTC WRC DGG GAT TAC TCC TG 3' hPC2 : 5' GGG TTT TCA CCC TTC CTT TC 3' (W = A and T) (R = A and G) (D = A and G and T)	CLVd, MPVd TASVd PSTVd TCDVd TPMVd	Tangkanchanapas <i>et al.</i> , 2005

Figure 2

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