



Dahlia latent viroid and Potato spindle tuber viroid in dahlia plants in the UK

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Dahlia latent viroid (DLVd) (*Hostuviroid*, *Pospiviroidae*) is a recently discovered viroid of dahlia (family *Asteraceae*) (Verhoeven *et al.*, 2013). Surveys in both The Netherlands and Japan found the viroid to be widespread in both field- and pot-grown dahlia. No other natural host of this viroid has been found and attempts to inoculate the viroid to other plant species have failed (Verhoeven *et al.*, 2013; Tsushima *et al.*, 2015). *Potato spindle tuber viroid* (PSTVd) (*Pospiviroidae*), is the only viroid to naturally infect cultivated species of potato (*Solanum tuberosum*) and is a quarantine pest in the European Union. Recently PSTVd has been found in dahlia in The Netherlands (Verhoeven *et al.*, 2016) and in mixed infections with DLVd in Japan (Tsushima *et al.*, 2015).

In 2017 routine viroid testing at a tissue culture facility in the UK, included four dahlia lines from three cultivars and found DLVd and PSTVd in different cultivars. The initial investigation for viroid was performed using a digoxigenin (DIG) labelled full-length PSTVd RNA probe (Agdia, USA) that can detect all known pospiviroids (Monger & Jeffries, 2015). Two dahlia lines, both cv. Tiger Eye, tested positive. Generic pospiviroid primer sets 3H1-F/2H1-R; Posp11-FW/Posp11-RE and Vid-FW/Vid-RE (International Plant Protection Convention, 2016) were used in RT-PCR reactions and the products sequenced to identify PSTVd. The full-length genomes (360 nt) from both Tiger Eye lines were constructed from the overlapping PCR products (GenBank Accession Nos. MG450357 and MG450358). The two Tiger Eye lines had been separated in tissue culture for approximately five years and differed in sequence by three nucleotide substitutions. Figure 1 shows the phylogenetic relationship of these isolates with related PSTVd isolates from different host plants. The Tiger Eye isolates form a separate clade with the two known PSTVd isolates from dahlia (The Netherlands and Japan).

In addition, the four dahlia lines were tested for DLVd using the specific primer set DLVd-P1 and DLVd-P2 (Verhoeven *et al.*, 2013), in a one-step RT-PCR reaction. Dahlia cv. Fire Mountain gave a strong PCR product that when sequenced confirmed the presence of DLVd. A set of internal primers were designed, DLVd int F (5'-GAC TAC CGC CCT TTT GCT TC-3') and DLVd int R (5'-AGC TAC AAG GAG CGG AGC T-3'), to give a full-length contig (MG214159). The genome was 342 nt and was 100% identical to the DLVd sequences from The Netherlands (JX263426) and Japan (LC036322). The identical sequence of DLVd isolates from

different countries and cultivars of dahlia was surprising and can in part be explained by the specific host/viroid relationship. However this level of sequence conservation has not been reported with other viroids.

The infected dahlia cultivars showed no obvious symptoms with either viroid; although the growth rate in tissue culture was observed to be slower than other dahlia cultivars, particularly the PSTVd-infected lines. The symptom of leaf curl has been reported in dahlia when these two viroids are in a mixed infection (Tsushima *et al.*, 2015) and other subtle symptoms may become apparent with further study. No infected dahlia plants had left the facility and all lines were subsequently removed or destroyed. This report represents the first finding of DLVd in the UK and PSTVd in dahlia in the UK.

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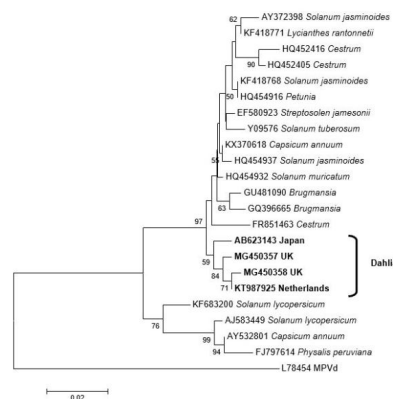


Figure 1

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