



First report of *Dahlia latent viroid* and *Potato spindle tuber viroid* mixed-infection in commercial ornamental dahlia in Japan

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Dahlia (Asteraceae) has been cultivated since 1841 in Japan. Two viroids belonging to the family *Pospiviroidae*, *Potato spindle tuber viroid* (PSTVd) and *Dahlia latent viroid* (DLVd), were identified separately from dahlia plants in Japan and in the Netherlands, respectively (Tsushima *et al.*, 2011; Verhoeven *et al.*, 2013). Because DLVd was characterised as a novel species, the findings drew attention towards the study of DLVd infection in dahlia and its potential risk in domestic dahlia cultivation. A total of four dahlia samples infected with PSTVd-dahlia (Tsushima *et al.*, 2011) were collected, and total RNA was extracted from approximately 200 mg of leaf tissue using TRIzol[®] reagent (Life Technologies, Carlsbad, USA) according to manufacturer's instructions. RNA was separated in 2-dimensional polyacrylamide gel (2D-PAGE) (Schumacher *et al.*, 1983) and two specific bands appeared, one corresponding to PSTVd infection and the other being unknown (Fig. 1). The unknown circular RNA samples were gel-excised and first tested with PSTVd-specific primers using a two-step reverse-transcription-polymerase chain reaction (RT-PCR) (Tsushima *et al.*, 2011), with, however, negative results. Therefore, a DLVd-specific primer set, DLVd-P1 (5'-GGGGCTCCTCAGAGAGTCTC-3') and DLVd-P2 (5'-GGGGCAACTCCGAGAGTGCTG-3') (Verhoeven *et al.*, 2013), was then used in two-step RT-PCR for the unknown viroid-like circular RNAs. The amplified DNA bands of the expected size (~340 bp) from these positive samples were purified, ligated to pGEM[®]-T easy vector (Promega, Madison, USA) and sequenced. Identical 342 bp sequences were obtained from all four dahlia isolates (GenBank Accession No. LC036322), corresponding with DLVd reported from the Netherlands (JX263426). Furthermore, a total of 78 dahlia leaf samples were collected from fields around Japan, irrespective of disease symptoms, and verified by two-step RT-PCR using the same DLVd-specific and pospiviroid-specific primers (Tsushima *et al.*, 2011). Of these samples, DLVd infection was detected in 38, suggesting that DLVd is widespread in Japan. New PSTVd infection was not found. To evaluate the pathogenicity of DLVd and PSTVd, these

viroids were co-inoculated into wild hop (*Humulus lupulus* var. *cordifolius*) and dahlia seedlings. DLVd alone was inoculated into members of the Asteraceae family, *Chrysanthemum pacificum*, *Tithonia rotundifolia*, *Helianthus annuus* and *Glebionis coronaria*. Inoculated dahlia seedlings were positive for both DLVd and PSTVd, but wild hop and the remaining members of Asteraceae were negative. Dahlia seedlings that were positive for DLVd and PSTVd showed mild leaf curling in the early stage of growth (Fig. 2). To the best of our knowledge, this is the first report of DLVd infecting commercial dahlia in Japan and also of the mixed-infection of DLVd and PSTVd in commercial dahlia plants. A possible negative synergistic interaction of DLVd and PSTVd during dahlia cultivation must be considered.

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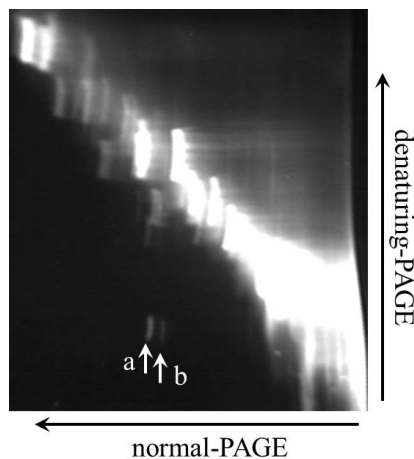


Figure 1



Figure 2

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