



First report of *Coleus blumei* viroid 5 infection in vegetatively propagated clonal coleus cv. 'Aurora black cherry' in Japan

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Plectranthus scutellarioides (synonym: *Coleus blumei*) is a popular garden plant, cultivated worldwide and is known to be infected with several viroids of the genus *Coleviroid*, family *Pospiviroidae*. *Coleus blumei* viroid 1 (CbVd-1) is the most dominant species and has been widely reported worldwide. However, reports are limited on the other species: CbVd-2 and CbVd-3 in Germany and China; CbVd-5 in China, India and Indonesia; and CbVd-6 only in China (Hou *et al.*, 2009a, 2009b; Jiang *et al.*, 2013). Molecules of *Coleviroid* consist of natural chimeras, presumably emerging by RNA recombination events taking place among the members of *Coleviroid* co-infecting the same plants, implying that they are actively evolving. In addition, most of them are seed-transmissible at various rates (Jiang *et al.*, 2014), and some of them have alternative hosts in herb species (Ishiguro *et al.*, 1996). Viroids have expanded their known geographical range and host species in the past two decades through globalisation of agriculture in addition to development of new detection technology, thereby threatening world phytosanitary security, as is the case in *Coleviroid*. Infection with CbVd-1 has long been known in Japan (Ishiguro *et al.*, 1996).

Given that numbers of coleus cultivars sold in Japan are increasing, with some of them showing abnormal discoloration or growth retardation, we surveyed coleus cultivars with abnormal growth retardation available in domestic markets for viroid infection. Total RNAs were extracted for analysis by 2D-PAGE (Schumacher *et al.*, 1983). A circular RNA band corresponding to CbVd-1 was frequently detected in the samples examined. In addition, another circular RNA band with a size larger than that of CbVd-1 was also detected in three samples in the cultivar 'Aurora black cherry', a vegetatively propagated clonal coleus (Figs. 1, 2). The band was excised from the gel and was subjected to cDNA amplification by RT-PCR using CbVd group-specific primers: CbVd-UCCR-P (5'-TTGCAGCGCTGCAACGGAAT-3') and CbVd-UCCR-M (5'-TTGCAGCGCTGCCAGGAAC-3'). As a result, two specific bands were detected, a major band corresponding to CbVd-1 and a faint band with a size similar to that of CbVd-5 (Hou *et al.*, 2009b). Therefore, new CbVd-5 specific primers, CbVd5-182fw (5'-CGTCTTCCGGGTTCTGCT-3') and CbVd5-169rv (5'-AAGACGGCCGACCCACGAG-3'), were designed for amplifying the entire CbVd-5 genome. The major sequence variant (GenBank Accession No. LC068970; 14 of 26 cDNA clones sequenced) was completely matched to CbVd-5 reported in China (FJ151372; Hou *et al.*, 2009b).



Figure 1

Ocimum basilicum and *Mentha spicata*, which are experimental host plants for CbVd-1, were inoculated with 2D-PAGE purified CbVd-5. However, they were not infected, indicating that pathogenicity/host specificity of CbVd-5 is different from that of CbVd-1. *Coleus* 'Aurora black cherry' shows poor seed production due to its clonal nature and vegetative propagation is a key factor in the dissemination of CbVd-5 in this situation. A possible synergism of CbVd-1 and CbVd-5 causing growth retardation observed in some coleus cultivars should be further studied. This is the first report of CbVd-5 infection in commercial coleus in Japan.

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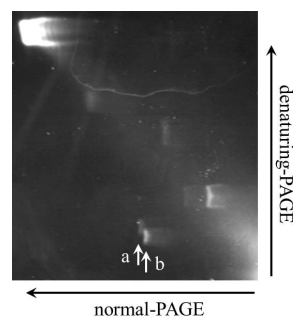


Figure 2

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