First Report of Broad Bean Wilt Virus 2 Infecting *Perilla frutescens* in China

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Plant Dis. 104:3085, 2020; published online as <https://doi.org/10.1094/PDIS-09-19-2017-PDN>. Accepted for publication 1 June 2020.

Broad bean wilt virus 2 (BBWV2) is a member of the genus *Fabavirus* in the family *Secoviridae*, which is transmitted mainly by aphids, and it has been recognized as a severe pathogen affecting the production of horticultural and ornamental plants worldwide (Ferrer et al. 2011). BBWV2 infection leads to severe mosaic, vein clearing, and stunting symptoms on the leaves of host plants (Kwak et al. 2013). In August 2018, leaf samples from 15 perilla plants with visual symptoms were randomly collected from the urban area of Shenyang, Liaoning province, China, where at least 90% of approximately 200 perilla plants exhibited mosaic and leaf curling symptoms, leading to over 50% yield losses of perilla production. The perilla leaf samples were pooled, and total RNA was extracted using TRIzol reagent from the mixed samples, followed by reverse transcription (RT) into cDNA (Cao et al. 2012). These cDNAs were amplified by PCR with a pair of primers specific for BBWV2 and five conserved primers for cucumber mosaic virus, tobamoviruses, potyviruses, potexviruses, and poleroviruses (Dong et al. 2017). A fragment with about 1,300 bp in length was amplified from symptomatic perilla plants using BBWV2 primers alone. Subsequently, two pairs of specific primers (data not shown) were designed to amplify partial RNA1 and RNA2 of BBWV2 genome, respectively. The amplified products were cloned into *pEASY-T1* vector

(Transgen, Beijing, China), and four clones per each PCR product were individually sequenced. Sequence analyses revealed that the RNA1 fragment (GenBank accession no. MN380789) with 1,476 nucleotides (nt) in length encodes the complete protease and a portion of the viral genome-linked protein and RNA-dependent RNA polymerase, and the 1,755-nt RNA2 fragment (no. MN380790) encodes the complete large and partial small coat protein. The nucleotide sequence of the 1,476-nt RNA1 fragment was homologous to BBWV2-LN isolate (no. MK116519) from *Sesamum indicum* with 85.23% identity, whereas the 1,755-nt RNA2 fragment shared the highest identity of 97.49% with BBWV2-BC isolate (no. FJ485685) from *Bupleurum chinense*. Additionally, a positive reaction in the pooled perilla leaf sample was obtained by ELISA assay using a BBWV2 monoclonal antibody provided by Prof. Xueping Zhou (Zhejiang University, China). Meanwhile, crude extracts were prepared by homogenizing the BBWV2-infected perilla leaf tissues in 0.01 M phosphate buffer (pH 7.0) at 1:10 (w/v) ratio. The crude extracts were mechanically inoculated onto five of healthy plants of *Nicotiana benthamiana* and *Capsicum annuum*. RT-PCR analysis with the primers specific to BBWV2 (Dong et al. 2017) at 7 days post inoculation showed that the 10 inoculated plants were infected by BBWV2. To the best of our knowledge, this is the first report of BBWV2 occurrence on perilla plants in China. These findings will assist further investigations on the epidemiological forecast and sustainable management of diseases caused by BBWV2 in China.

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The author(s) declare no conflict of interest.

Keywords: broad bean wilt virus 2, *Perilla frutescens*, first report, China

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