Diseases Caused by Viruses

Gynura japonica: A New Host of Apple Stem Grooving Virus and Chrysanthemum Virus B in China

Yuchao Lai,¹ Xinyang Wu,^{1,2} Lanqing Lv,¹ Jiajia Weng,¹ Kelei Han,¹ Ziqiang Chen,^{1,3} Jianping Chen,^{1,2} Fei Yan,^{1,†} and Hongying Zheng^{1,†}

¹ State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Key Laboratory of Biotechnology in Plant Protection of Ministry of Agriculture and Zhejiang Province, Institute of Plant Virology, Ningbo University, Ningbo, 315211, China

² College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, 310058, China

³ College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, 100098, China

Funding: This work was financially supported by the China Agriculture Research System (CARS-24-C-04), Ningbo Science and Technology Public Welfare Project (202002N3063), National key research and development program of China (2019YFD1001800) and sponsored by K. C. Wong Magna Fund in Ningbo University. Plant Dis. 105:3770, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2512-PDN>. Accepted for publication 26 April 2021.

Gynura japonica (Thunb.) Juel (Asteraceae; syn: *G. segetum* [Lour.] Merr.) is an important perennial medicinal herb used in China for topical treatment of trauma injuries (Lin et al. 2003). It grows naturally in the southern provinces of China and is also sometimes cultivated. During 2018 to 2020, wild *G. japonica* plants exhibiting chlorotic spots and mosaic symptoms were observed in Zhejiang province, China. To identify the possible causal agents of the disease, a single symptomatic leaf sample was collected in August 2019 and sent to Zhejiang Academy of Agricultural Sciences (Hangzhou, China) for next-generation sequencing (NGS). Total RNA extracted with TRIzol (Invitrogen, Carlsbad, CA) were subjected to high-throughput sequencing on the Illumina NovaSeq 6000 platform with PE150bp, and data analysis was performed by CLC Genomic Workbench 11 with default parameters (Qiagen, Hilden, Germany). A total of 37,314,080 paired-end reads were obtained, and 11,785 contigs (961 to 10,964 bp) were generated and compared with sequences in GenBank using BLASTn or BLASTx. Of the total of 12 viral-related contigs obtained, one with a length of 6,442 nt mapped to the genomic RNA of apple stem grooving virus (ASGV) (MN495979), seven contigs with lengths ranging from 1,034 to 2,901 nt mapped to chrysanthemum virus B (CVB), and four

mapped to broad bean wilt virus 2 (BBWV2), a virus that is known to infect *G. procumbens* (Kwak et al. 2017). To further confirm the presence of ASGV and CVB, primers were designed, and the complete nucleotide sequences of both viruses were amplified from the original NGS sample using reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends according to the manufacturer's instructions (Tiosbio, Beijing, China). BLASTn analysis revealed that the complete 6,451-nt sequence of ASGV (GenBank accession no. MW259059) shared the highest identity (81.2%) with a Chinese isolate of ASGV from citrus (MN495979). The two isolates grouped with another Chinese isolate (from pear) in phylogenetic analysis. The predicted coat protein of the virus had the highest nucleotide sequence identity of 93.7% (96.2% amino acid sequence identity) with that of the Chinese ASGV isolate XY from apple (KX686100). The complete genomes of two distinct molecular variants of CVB (both 8,987 nt in length) were also obtained from this sample (GenBank accession nos. MW269552 and MW269553). They shared 86.8% nt identity with each other and had 81.1 and 82.1% identity to the only known complete sequence of CVB from chrysanthemum (AB245142). Ten additional wild *G. japonica* plants with mosaic symptoms were collected randomly during 2019 to 2020 from Hangzhou ($n = 6$) and Ningbo ($n = 4$) in Zhejiang province and tested by RT-PCR with specific primer pairs to detect BBWV2, ASGV, and CVB. RT-PCR and subsequent sequencing revealed that these three viruses were present in all the samples tested, indicating that coinfection of *G. japonica* by ASGV, CVB, and BBWV2 is common. CVB mainly infects chrysanthemum (Singh et al. 2012), whereas ASGV is known as a pathogen of various fruit trees, especially in the family Rosaceae, although there are recent reports that it can also infect some plants in Gramineae, Asparagaceae, and Nelumbonaceae (Bhardwaj et al. 2017; Chen et al. 2019; He et al. 2019). Our results provide the first report that *Gynura* is a natural host of CVB and ASGV. Further surveys and biological studies are underway to evaluate the importance of *Gynura* as a virus reservoir for epidemics among the various hosts.

References:

- Bhardwaj, P., et al. 2017. Trees (Berl.) 31:367.
- Chen, Z. L., et al. 2019. Plant Dis. 103:1803.
- He, Z., et al. 2019. Plant Dis. 103:782.
- Kwak, H., et al. 2017. Plant Dis. 101:514.
- Lin, W. Y., et al. 2003. Planta Med. 69:757.
- Singh, L., et al. 2012. Arch. Virol. 157:531.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: apple stem grooving virus, chrysanthemum virus B, broad bean wilt virus 2, *Gynura japonica*

[†]Indicates the corresponding authors.

F. Yan; yanfei@nbu.edu.cn, and H. Y. Zheng; zhenghongying@nbu.edu.cn