Diseases Caused by Viruses

Cucurbit Chlorotic Yellows Virus Infecting *Rehmannia glutinosa* Was Detected in China

Kun Zhang,¹ Xinjian Zhuang,¹ Xiao Guo,¹ Hongmei Xu,¹ Zhen He,¹ and Jiahuan Chen^{2,†}

¹College of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, Jiangsu Province, P.R. China

²Department of Pharmacy, Affiliated Hospital of Yangzhou University, Yangzhou University, Yangzhou 225009, Jiangsu Province, P.R. China

Funding: This study was supported by the National Natural Science Foundation of China (31801699 and 81903764), Natural Science Foundation of the Jiangsu Province (BK20180904), Natural Science Foundation of the Jiangsu Higher Education Institutions (18KJB210012), the Project of Science and Technology Development Plan for Traditional Chinese Medicine of Jiangsu Province (YB201993), and the Key Project at Central Government Level: The Ability Establishment of Sustainable Use for Valuable Chinese Medicine Resources (no. 2060302-1904-11). Plant Dis. 105:3310, 2021; published online as <https://doi.org/10.1094/PDIS-02-21-0292-PDN>. Accepted for publication 27 April 2021.

Rehmannia glutinosa Libosch. is a perennial herbaceous plant of the family Scrophulariaceae. Its roots can be used as a traditional Chinese medicine. The asexual reproduction by vegetative organ of *R. glutinosa* leads to an increased viral disease that seriously affects its yield and quality (Kwak et al. 2018, 2020; Ling and Liu 2009). Leaves of *R. glutinosa* in Wenxian County, Henan Province, China, showed symptoms of chlorosis, mosaic, and irregular yellow in August 2019. In general, the older leaves at the base or middle of the plant (samples 2 and 5) first became irregular yellow, followed by gradual extending to the leaves at the top. Six plants (nos. 2, 3, 5, 7, 8, and 9) with these symptoms were collected. The total RNA was extracted, and its siRNAs were obtained. High-throughput siRNA sequencing (Sangon, Shanghai, China) was performed on an Illumina HiSeq 2000 platform with the paired-end method after siRNA library construction (NEBNext Ultra II RNA Library Prep Kit, NEB, U.K.). Sequencing files were treated with Illumina's CASA-VA pipeline (version 1.8). The length of the resulting reads with adaptor removed mostly ranged from 21 to 24 nt. Velvet software 0.7.31 (k = 17) was used for de novo assembling, and the contigs (~13,000, contigs > 300 bp)

were used to perform BLASTN against the GenBank database. Two viruses, *Rehmannia mosaic virus* (ReMV) and *cucurbit chlorotic yellows virus* (CCYV), frequently appeared in the six symptomatic samples analyzed. To further identify the infection by CCYV of *R. glutinosa*, 10 samples with virus-infected symptoms were randomly collected. Total protein and RNAs were extracted for RT-PCR and ELISA (Haling, Shanghai, China). A specific pair of primers was designed to amplify the 753-bp coat protein (CP) gene of CCYV. The result showed that two samples produced a specific band of the expected size on the agarose gel, which indicated that they were infected by CCYV. The same result was obtained by ELISA. The amplified CP fragment of CCYV was recycled and purified by a TIANgel Midi Purification Kit (Tiangen, Beijing, China), followed by cloning into pMD19-T (TaKaRa, Dalian, China) and transforming into *E. coli* DH5a. Ten separate clones were selected and sequenced (Sangon) after PCR verification. The obtained sequences (GenBank accession nos. MW521380 and MW521381) were analyzed by BLASTN and bioEdit software (version 7.2.3). The results showed 100% identity with the CCYV CP sequences that mainly derived from infected cucurbit. To confirm the occurrence and distribution of CCYV and ReMV in the planting area, the other 24 samples (20 with chlorosis and stunt symptoms and four with invisible symptoms) were randomly collected for RT-PCR in different regions of Henan Province. The results showed that the CCYV and ReMV infection rates were 20.5 and 61.7%, respectively. Coinfection by CCYV and ReMV was 5.8% in fields. In sum, these results indicated that CCYV can naturally infect *R. glutinosa* in China. CCYV is transmitted by whitefly in a semipersistent manner and mainly damages cucurbits (Orfanidou et al. 2017). CCYV has been discovered in many places (Huang et al. 2010). To date, there is no report about CCYV infecting *R. glutinosa* in nature. This is the first report of CCYV naturally infecting *R. glutinosa* in China.

References:

- Huang, L. H., et al. 2010. Plant Dis. 94:1168.
- Kwak, H. R., et al. 2018. Plant Dis. 102:1046.
- Kwak, H. R., et al. 2020. J. Gen. Plant Pathol. 86:143.
- Ling, H., and Liu, R. R. 2009. Russ. J. Plant Physiol. 56:591.
- Orfanidou, C., et al. 2017. Plant Dis. 101:2053.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Rehmannia glutinosa*, cucurbit chlorotic yellows virus, coat protein, new host

[†]Indicates the corresponding author.
J. H. Chen; 092015@yzu.edu.cn