Diseases Caused by Viruses

First Report of Broad Bean Wilt Virus 2 in Marigold (*Tagetes erecta*) in China

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Broad bean wilt virus 2 (BBWV-2), a member of the genus *Fabavirus* in the family *Secoviridae* mainly transmitted by aphids, has been recognized as a severe pathogen affecting the production of horticultural and ornamental plants worldwide (Xia et al. 2020). The virus was reported to infect many plant species mostly belonging to the family Fabaceae in China (Wang et al. 2017). In August 2018, marigold plants with the symptom of mosaic were observed in the field of Huairou, Beijing. Total RNA was extracted from symptomatic leaf samples from a single plant with TRIzol reagent (Invitrogen, Carlsbad, CA) with a standard procedure following the manufacturer's instructions, and small RNAs were isolated for deep sequencing library construction with an Illumina TruSeq Small RNA Sample Prep Kit (Illumina, San Diego, CA). High-throughput sequencing (HTS) was carried out on an Illumina HiSeq2500 platform. After raw data processing, 8,911,917 clean reads were gained and further de novo assembled into contigs with CLC Genomics Workbench software. BLASTN and BLASTX analysis against the GenBank database showed that 81 of the 9,495 assembled contigs shared high nucleotide (nt) sequence identity with the bipartite genome of BBWV-2 isolate Gyp (KX686589 and KX686590 for RNA1 and RNA2, 89% of the genome coverage and 90% nt identity), and 34 of the assembled contigs shared high nt sequence identity with cucumber mosaic virus (CMV) from

Tagetes erecta L. (EU665000 to EU665002 for RNA1 to RNA3, 89% of the genome coverage and 96% nt sequence identity), with sequence coverage ranging from 24-fold to 8,078-fold at different genome positions. To further confirm the presence of BBWV-2, a RNA2-specific primer pair targeting the coat protein (CP) region (F1423-1448, 5-CTGACAGAGGAATACTATTC CAAAG-3'; R2692-2719, 5-CCTGTAAAATTGATATCTCCGGACAAAC-3') was designed from the obtained HTS sequence, and reverse transcription polymerase chain reaction (RT-PCR) was conducted. The 1.3-kb amplicon was ligated to pMD19-T vector (TaKaRa, Dalian, China) and sequenced. Sequence analysis showed it (BBWV-2-marigold, MW322809) shared 99% nt sequence identity with the Gyp isolate infecting *Gynura procumbens* from South Korea (LC497425.1). Phylogenetic analysis constructed with MEGA6 with the CP nt sequence of other reported BBWV-2 isolates showed BBWV-2-marigold clustered closely with the isolates from South Korea infecting *G. procumbens*, in accordance with the sequence identity analysis. Further RT-PCR with a primer pair targeting the RNA1 (F3025-3050, 5-GACA GAGTGATATTCCTAATCGAGAT-3'; R4035-4062, CACTCAATGCAA TAAAGGTCTGGCACCT) was conducted, and specific bands with the expected size of 1.0 kb were obtained in the agarose gel (data not shown), which further confirmed the existence of BBWV-2. A total of 16 marigold leaf samples (seven from Huairou and nine from Yanqing) with mosaic symptom were collected and tested by RT-PCR with the abovementioned primer pair, and four from Huairou were BBWV-2 positive. Sequence analysis showed that these four isolates shared 100% nt sequence identity with the former sequenced isolate BBWV-2-marigold. Furthermore, a CMV-specific primer pair targeting the CP (F, 5-ATGGACAAATCTGGATCTCCCAAT-3'; R, 5-CTAAGTCGGGAGCATCCGTGAGAT-3') was designed to detect the existence of CMV in these samples, and results showed that all these 16 samples were positive for CMV. To the best of our knowledge, this is the first report of BBWV-2 in marigold in China. These findings will assist investigations on the epidemiology of diseases caused by BBWV-2 in China.

References:

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