Diseases Caused by Viruses

First Report of Vicia Cryptic Virus M Infecting Cowpea (*Vigna unguiculata*) in China

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Cowpea (*Vigna unguiculata*) is a crop grown worldwide as a protein source for food and feed (Lonardi et al. 2019). During the summer of 2019, noticeable virus-like symptoms such as mosaic, leaf narrowing, stunt, and chlorosis were observed on 'Xianfeng' cowpeas planted in Yangzhou city and its suburbs, Jiangsu Province, East China. The total RNA was extracted from both symptomatic and asymptomatic plants by RNAiso Plus (TaKaRa, Dalian, China), and sRNAs were separated and recovered by gel purification. The NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB, U.K.) was used for sRNA library construction. The library was sequenced with the paired-end method on the Illumina HiSeq 2000 platform (Sangon, Shanghai, China). The obtained sequencing files were treated with Illumina's CASAVA pipeline (version 1.8). The reads resulting from sequencing were further processed with adaptor removing, and the most abundant sRNAs were distributed from 21 to 24 nt. The de novo assembly was performed with Velvet software 0.7.31 ($k = 17$), and the obtained contigs (~12,000, contigs >500 bp) were used to perform a BLAST search against the GenBank viral reference database. Fifteen contigs with high similarities of 98.61 to 99.64% and coverage of 94% to the reported Vicia cryptic virus M (VCV-M) genomic sequence (GenBank accession no. EU371896) were identified. Other common viruses, such as cowpea mosaic virus (CPMV), cowpea aphid-borne mosaic virus (CABMV), and cucumber mosaic virus (CMV), were also included (unpublished). VCV-M belongs to the genus *Amalgavirus*, family *Amalgaviridae* (Nibert et al. 2016). Amalgaviruses are efficiently transmitted through seeds but not mechanically or by grafting (Sabanadzovic et al. 2009). To confirm the presence of VCV-M in the collected plants, total RNA was isolated, and the first-strand cDNA was prepared by M-MLV reverse transcription (TaKaRa) using specific primers. Primers were designed according to the assembled contigs. Polymerase chain reaction (PCR) was performed to amplify the targeted genomic fragment of VCV-M, and the predicted 3,434-bp

amplicon was obtained from five cowpea plants. A randomly selected amplicon was purified with the TIANGel Midi Purification Kit (Tiagen, Beijing, China) and cloned to pMD19-T (TaKaRa) for sequencing (Sangon). The obtained consensus sequence (GenBank accession no. MN015673) was analyzed and showed 99.39% similarity with the reported VCV-M genome (GenBank accession no. EU371896). To confirm the occurrence and distribution of VCV-M infection, 17 cowpea samples of different cultivars (four with yellowing and stunt symptoms and 13 without visible symptoms) were collected from different regions of Jiangsu Province and tested using reverse transcription PCR (RT-PCR) with specific primers. They were further tested by western blot (WB) detection as described previously (Zhang et al. 2017). Specific CP^{VCV-M} antiserum was obtained by immunizing New Zealand white rabbits with the prokaryotic expressed recombinant His-CP^{VCV-M} protein (HuaBio, Hangzhou, China). WB results and RT-PCR resulted in five samples that were positive out of a total of 17 samples, suggesting the VCV-M infection is common in cowpea plants. To determine whether the VCV-M was the causal agent or contributor to the observed symptoms, we investigated the presence of other cowpea-infecting viruses (CPMV, CABMV, and CMV) in these samples through RT-PCR with specific primers for each virus and ELISA with commercial kits. RT-PCR and ELISA detection results showed mixed infection by VCV-M/CPMV ($n = 1$), VCV-M/CABMV ($n = 1$), VCV-M/CMV ($n = 1$), or VCV-M/CPMV/CABMV/CMV ($n = 2$). The VCV-M/CABMV coinfecting sample was asymptomatic. Taken together, the symptoms on cowpea could not be attributed to one particular viral infection. To further confirm VCV-M infection, we selected four samples (two positive and two negative, as determined by RT-PCR and WB) for northern blot assay. The probe was prepared with the DIG Random Labeling and Detection Kit I (POD) for color detection with DAB (BOSTER, Wuhan, China). The northern blot assay was performed as previously described with minor modifications (Prośniak et al. 2001). The results confirmed the accuracy of previous RT-PCR and WB analyses. To our knowledge, this is the first report of VCV-M infection of cowpea plants in China. Although it is commonly accepted that VCV-M causes no symptoms, the roles of such viruses in affecting their hosts' biological characteristics, which are often influenced by coinfection conditions, remains unclear.

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