Diseases Caused by Viruses

First Report of Turnip Mosaic Virus in Peanut (*Arachis hypogaea*) in China

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Peanut (*Arachis hypogaea* L.) is an important source of edible oil in China, but its yield and quality in agricultural production are affected by a number of diseases including those caused by viruses. The four viruses most commonly reported to affect the production of peanut worldwide are peanut stripe virus, cucumber mosaic virus, peanut stunt virus, and peanut bud necrosis virus (Srinivasan et al. 2017; Xu et al. 2017). During a disease survey in June 2020, virus-like disease symptoms including mosaic and necrotic spots were observed in field peanut plants in Yuyao county, Zhejiang Province, China. These symptoms differed from those caused by the four major peanut viruses (Dunoyer et al. 2020; Srinivasan et al. 2017; Takahashi et al. 2018; Xu et al. 2017). To identify the putative viral agent(s) associated with the virus-like disease in these plants, leaves from six plants in the same field were collected, pooled, and subjected to high throughput RNA-Seq sequencing (HTS). The TruSeq RNA Sample Preparation Kit (Illumina) was used to construct cDNA library according to the manufacturer's instructions. An Illumina NovaSeq 6000 platform with PE150 bp and CLC Genomic Workbench 11 (Qiagen) was used for sequencing and data analysis. After data collection and analysis, a total of 18,592 contigs were generated from de novo assembly of the clean paired-end reads (35,935,936). After comparing with sequences deposited in GenBank using BLASTn, four assembled contigs (ranging from

4,969 to 8,937 nt in length) were found to share 94.9 to 95.9% identity to the turnip mosaic virus (TuMV, genus *Potyvirus*). No other virus sequences were detected in the data. To confirm the presence of TuMV and to obtain its full-length sequence, total RNA was extracted from a single plant selected from the initial sample pool using the plant RNA extraction KIT (Aidlab). Five primer pairs, which were anticipated to result in overlapping amplicons covering all but the 5'-end of the genome, were designed based on the TuMV contig sequences and the complete nucleotide sequence of TuMV was subsequently amplified by the reverse transcription (RT)-PCR and rapid amplification of cDNA ends (RACE) using the commercial SUPER-SWITCH 5'RACE cDNA Kit (Tiosbio). All the PCR products were subsequently cloned into pEASY-T5 Zero (TransGen Biotech), and three clones of each fragment were randomly selected and sequenced by Sanger sequencing at Ykang. The complete sequence of the TuMV isolate (designated Ningbo) was deposited in GenBank under accession no. MZ062212. BLASTn analysis showed that TuMV-Ningbo shared a sequence identity of 96.0% with a Brassica isolate of TuMV in China (HQ446216) and 95.9% with a Brassica isolate in the Czech Republic (LC537547). Phylogenetic analysis grouped the three into a cluster, suggesting Ningbo isolate as a member of the world-B Group (Kawakubo et al. 2021). A western blot analysis of leaf sap using a TuMV CP antibody prepared by our laboratory (unpublished data) confirmed the presence of TuMV in all of the samples used for the HTS analysis. Peanut and *Nicotiana benthamiana* plants growing in the greenhouse were also mechanically inoculated with peanut leaf sap obtained from one of samples. Seven days after inoculation, mosaic and leaf curling symptoms were observed on inoculated plants and the infection of TuMV was subsequently confirmed by RT-PCR (primers TuMV-CP: 5'-GCAGGTGAAACGCTTGATGC-3' and 5'-CAACCCCTGAACGCC AGTA-3') and western blot assay. In contrast, no symptoms nor TuMV were detected in the mock-inoculated plants. TuMV is an important pathogen of brassica crops and is known to have a worldwide host range. However, to our knowledge, this is the first report of TuMV infection in peanut in China and the finding suggests that the threat of TuMV should be considered when interplanting peanuts and cruciferous vegetables, as is common in this region of China.

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