Identification of Alfalfa dwarf virus in Xinjiang Province, China

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Alfalfa (*Medicago sativa* L.) is a primary leguminous forage crop with high biomass production and nutritional value for livestock. Alfalfa is naturally infected by many viruses, some of which were identified in high-throughput sequencing recently (Bejerman et al. 2020; Guo et al. 2020). In 2011, alfalfa dwarf virus (ADV), a novel cytorhabdovirus, was first detected in alfalfa plants showing dwarfism, shortened internodes, and a bushy appearance in Argentina (Bejerman et al. 2011). In 2019, several transcriptome sequencing contigs of ADV were identified from diseased alfalfa plants in Henan Province, China, suggesting the presence of ADV in China (Guo et al. 2020). In August 2019, dwarf and bushy symptoms were observed in alfalfa plants in four fields of Hutubi County (44.2°N, 86.9°E), Xinjiang Province, China. To unveil whether the disease was associated with ADV infection, 15 plant samples were collected for detection by reverse transcription PCR (RT-PCR) and western blotting. Total RNA was extracted from the symptomatic alfalfa plants with using TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.) following the manufacturer's guidelines. The resulting RNA samples were subjected to RT-PCR using ADV-specific primers (ADV-1F, 5'-gcaagtatgc-aaaaataagaaaaagtagg-3' and ADV-NR, 5'-tcacccacgatgtctttccag-3' for the nucleoprotein [N] gene; ADV-MF, 5'-atgactaccttagttacgacc-3'; and ADV-MR, 5'-tcatttcttcgggactcg-3' for the matrix protein [M] gene). Of the 15 samples, six tested positive with ADV by both the N- and M-gene based

RT-PCR assays. To confirm the identity of the amplicons, the amplified products of the N and M gene were purified using M5 Hiper Gel Extraction Kit (Mei5 Biotechnology, Beijing, China) and subjected to direct Sanger sequencing. The obtained sequence of N and M genes of ADV Xinjiang isolate were deposited in GenBank (MW591994 and MW591995), and shared a sequence identity at the nucleotide level of 81.4% and 77.1% with the ADV reference isolate Manfredi (KP205452). The deduced N and M proteins of isolate Xinjiang shared an amino acid sequence identity at the levels of 92.7% and 90.0% with those of the isolate Manfredi. To further detect ADV using western blotting analyses with anti-ADV-M polyclonal antibodies, the open reading frame of ADV M was amplified using specific primers of ADV-MF2, 5'-tatacatatgatgactaccttagttacgacc-3' and ADV-MR2, 5'-gtgtgtcgtcgagtttcccttcgggactcgct-3', and then cloned into the pET-30a (+) vector (Novagen, Madison, WI, U.S.A.). The resulting plasmids were transformed into the BL21 strain of *Escherichia coli* (Invitrogen) and protein expression was induced by IPTG. Thereafter, the recombinant M protein of ADV was purified with Ni-NTA resin according to manufacturer's procedure (Qiagen, Hilden, Germany) and used to immunize rabbits for preparing polyclonal antibodies as described previously (Leenaars et al. 1999). Anti-ADV-M polyclonal antibodies were diluted (1:3,000) to detect accumulation of the ADV M protein. Western blotting analysis with the newly generated antibody showed the accumulation of the M protein of ADV in the two RT-PCR-positive samples but not in the asymptomatic sample, thus confirming the RT-PCR results. Using RT-PCR and 3'/5' RACE, we also obtained the full-length genome sequence (GenBank: NW700334) of the isolate Xinjiang. BLASTn analysis demonstrated that it shared 78.1% nucleotide identity with the isolate Manfredi. These results clearly indicate that ADV was present in Xinjiang Province, China. Future studies are required to determine the incidence and effect of ADV in alfalfa plants in Xinjiang Province, China.

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