First Report of Apple Mosaic Virus Infecting Apple Trees in Ethiopia

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Apple (*Malus domestica*) is an important fruit crop in Ethiopia. Among the viruses reported to infect apple trees, apple chlorotic leaf spot virus (ACLSV), apple mosaic virus (ApMV), apple stem grooving virus (ASGV), and apple stem pitting virus (ASPV) play considerable roles in constraining fruit yield and quality (Hadidi and Barba 2011). ACLSV, ASGV, and ASPV have been reported from 10 different apple cultivars without visible disease symptoms in Ethiopia, whereas no samples tested positive for ApMV (Lemma et al. 2016). To verify whether ApMV is present in Ethiopia, a total of 14 leaf samples were collected in the southwest part of the country in May 2017 from 14 different apple trees and five different cultivars (BR-64 = 2, CP92 = 2, Fuji = 4, Gala = 3, Granny Smith = 3) showing chlorosis, necrosis, or mosaic. The mosaic patterns included yellow or cream-colored vein banding on the foliage, as well as irregular spots and/or line patterns. Total RNA was extracted using an RNAprep Pure Plant Kit (TianGen, China) following the manufacturer's instructions. The expected 608-bp amplicon was obtained by RT-PCR with ApMV-specific primers (Noda et al. 2017) only from the four cultivar Fuji plants showing mosaic symptoms. All 14 samples tested negative for *Apple necrotic mosaic virus* and *Prunus necrotic ringspot virus*, two ilarviruses that are also known causal agents of apple mosaic disease (Hu et al. 2016; Noda et al. 2017; Xing et al. 2018). The presence of ApMV in the RT-PCR positive samples was confirmed by ELISA with commercial ApMV-specific antisera according to the product instructions (Dogesce, China). To

further validate the presence of ApMV in the cultivar Fuji samples, an 1,879-bp fragment from RNA3 was amplified from one sample using primer pairs ApMV-Fr (5'-CCTCTCAAGATGACAACACTG-3') and ApMV-Rr123 (5'-GGGCATCAATTTCTTTACAGA-3'). *Escherichia coli* polymerase (NEB, England) was used to add 3' poly A tails to viral RNAs. Amplification of 5' and 3' cDNA ends of RNA3 were carried out by the SMARTer RACE 5'/3' Kit (Clontech, U.S.A.) following the manufacturer's instructions using the primer sets of ApMV3-5race1 (5'-CCATTAACCTCGCCAACTAC-3') and ApMV3-5race2 (5'-CAT TATCACCTTCGCTTCAC-3'), and ApMV3-3race1 (5'-CCTCTAATG GATGGATTGG-3') and ApMV3-3race2 (5'-TTGTGATGGAGCGATTAGC-3'), respectively. The PCR reactions were conducted with Phusion High-Fidelity DNA Polymerase (Thermo Scientific, U.S.A.). The obtained PCR products were cloned into pTOPO-blunt vector (Aidlab, China), transformed into *E. coli* cells, and sequenced by Sangon Biotech (Shanghai, China). The assembled genome of ApMV RNA3 was 2,056 nt in length (GenBank accession no. MT303163). BLASTn analysis revealed that it shared the lowest nucleotide (nt) identity (85.4%) with isolate Negret 7 infecting hazel (*Corylus avellana*) from Poland (HG328282) and the highest nt identity (99.3%) with isolate 13TF151 infecting *Malus* sp. from Canada (KY971019). The RNA3 contained two open reading frames (ORFs). The ORF at the 5'-proximal end encoded the movement protein (MP, 861 nt, 286 amino acids [aa]) with 87.9 to 99.7% nt identity with ApMV MP sequences in GenBank, and the ORF at the 3'-proximal end encoded the coat protein (CP, 672 nt, 223 aa) with 87.3 to 99.3% nt identity with reported ApMV CP isolates. To our knowledge, this is the first report of ApMV isolated from apple trees in Ethiopia. Additional studies on the distribution and transmission route of ApMV will help in the management of this virus in Ethiopia.

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