First report of cucumber mosaic virus subgroup IB and II isolates infecting tomatoes in Pakistan

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Received: 23 November 2018 / Accepted: 14 January 2019 / Published online: 19 February 2019
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Keywords CMV · Subgroup · Tomatoes

Cucumber mosaic virus (CMV) is one of the most prevalent plant viruses infecting a variety of plant species worldwide (Akhtar et al. 2010). During 2015–17, leaf samples were collected from field grown tomatoes at NIAB and UAF showing symptoms reminiscent of CMV. Sixteen samples were found CMV-positive using Immunostrips (Agdia, USA). The presence of CMV was confirmed in these samples by TAS-ELISA using polyclonal antibodies to CMV subgroup I and II (PSA 44700 and 44,800, Agdia, USA). CMV subgroup I was detected in 12 samples, subgroup II in three samples whereas one sample was positive for both subgroups. For RT-PCR confirmation, total RNA extracted from these ELISA positive samples was reverse transcribed to cDNA and used in PCR with primers CMVFnyF (TTGAGTCGAGTCATGGACAAATC) and CMVFnyR (AACACGGAATCAGACTGGGAG) for subgroup I, and CMVQF (TCTCCCAATGCTAGTAGAACCT) and CMVQR (TTTAACGTCTTCGGACGCCG) for subgroup II. Twelve samples were found positive for CMV-I (ca. 678 bp), three for CMV-II (ca. 675 bp) while one was positive for both subgroups. The purified RT-PCR amplified products of five representative samples was directly sequenced in both directions (Eurofins Genomics, USA) and submitted to NCBI GenBank (accession numbers MF351527, MF351523, MF351524, MF351525, MF351526). In BLASTn analysis, CMV isolates MF351523, MF351524 and MF351527 shared 89.10–

99.54% identity with various isolates of CMV subgroup I while MF351525 and MF351526 had nucleotide sequence identity of 97.83–99.84% with CMV isolates of subgroup II. Similarly, in phylogenetic tree MF351523, MF351524 and MF351527 clustered with CMV subgroup-IB while, MF351525 and MF351526 clustered with subgroup II. In the present study, all Pakistani CMV isolates shared a common *MspI* restriction site at nt position 445–448 and CMV-II isolates (MF351525, MF351526) contained *EcoRI* and *BsuRI* restriction sites at nt position 237–240 and 483–386, respectively (Eyvazi et al. 2015). To our knowledge, this is the first report of CMV subgroup IB and II isolates naturally infecting tomatoes in Pakistan.

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