



# First report of *Pepper mild mottle virus* infecting chilli pepper in Pakistan

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Chilli pepper (*Capsicum annuum*) is an important cash crop in Pakistan which is cultivated on an area of 62,500 ha with an annual yield of 145,100 tonnes (Farooq, 2014). *Pepper mild mottle virus* (PMMoV) is a single-stranded RNA virus belonging to the genus *Tobamovirus*, family *Virgaviridae* (King *et al.*, 2011). The virus possesses a serious threat to pepper cultivation worldwide due to its long-term survival and highly efficient transmission through seeds, plant debris, and on contaminated worker's hands and tools.

During the summers of 2013 and 2014, surveys were done to identify the major viruses infecting chilli crops in the Pothwar region of Pakistan. A total of 25 chilli pepper leaf samples showing symptoms of puckering and yellow or light green mottling were collected from stunted plants from different locations. Total RNA was extracted from these samples using TRIzol<sup>®</sup> Reagent (Life Technologies, Carlsbad, USA). Six of the 25 samples were positive by one step RT-PCR using PMMoV-specific primers MP/for (5'-TAAAATTGGGCAGAAGCTCGGAG-3' and 3'UTR/rev (5'-ACGACAACCCCTTCGATTTAAGT-3'), designed from an alignment of PMMoV sequences. After purification using the Amicon<sup>®</sup>Ultra kit (EMD Millipore, Billerica, USA) the amplicons were directly sequenced in both directions (BioFab, Rome, Italy). A phylogenetic tree was obtained using the neighbour-joining method based on the Kimura 2-parameter model in MEGA6 (Tamura *et al.*, 2013). The sequences of the 629 bp amplicon from all six isolates, including the complete coat protein gene (474 bp), were identical. The sequence of one isolate (AAR-PK) was submitted to GenBank (Accession No. KT853037). BLASTn analyses showed >99% sequence identity of the Pothwar-Pakistan isolate of PMMoV with PMMoV isolates from Asia, including China (KP345899), India (KJ631123) and Japan (KJ631123). Phylogenetic analysis (Fig. 1) supported the grouping of the Pothwar-Pakistan isolate within the

pathotype P12 cluster (Cağlar *et al.*, 2013; Rialch *et al.*, 2015), although validation of the pathotype would require phenotyping on differential hosts including alleles with *L*<sup>2</sup> gene-mediated resistance.

To the best of our knowledge, this is the first report of PMMoV infecting chilli pepper in Pakistan. Therefore, there is a need to strictly control seed quality and to extend surveillance to assess PMMoV incidence in other chilli-growing areas of Pakistan.

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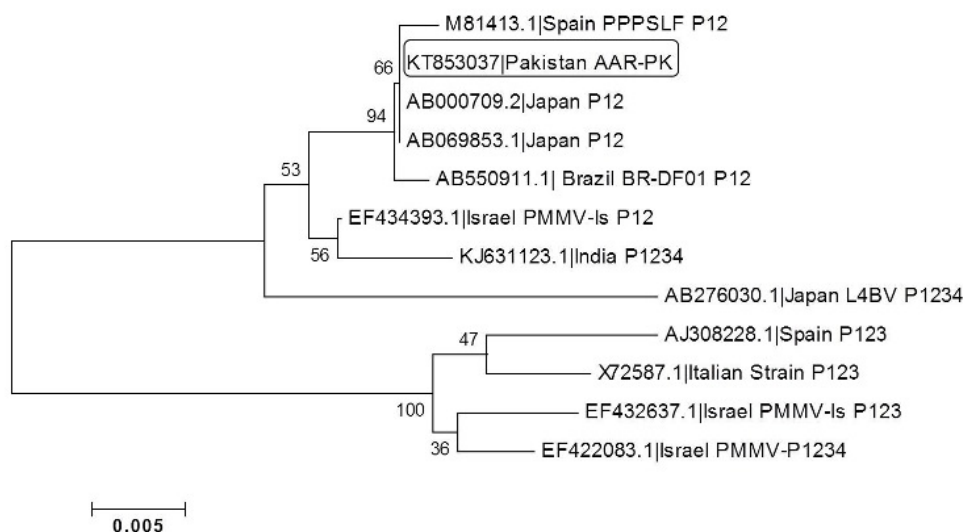


Figure 1

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