New Disease Reports

First report of *Tomato leaf curl virus* and Croton yellow vein mosaic betasatellite infecting chilli plants in Pakistan

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Leaf curl disease poses a major constraint to chilli (*Capsicum annuum*) production in Pakistan. In October 2014, chilli plants exhibiting geminivirus-like symptoms, including leaf curling and stunting were observed at the National Agriculture Research Centre (Islamabad, Pakistan), with an incidence of 50%. Leaf samples were collected from ten diseased chilli plants. The samples were tested by TAS-ELISA using a panel of ten monoclonal antibodies (Harrison *et al.*, 1997). A total of eight monoclonal antibodies indicated the presence of begomoviruses in five field samples.

Total plant DNA was purified from leaf tissue using the DNeasy Plant Minikit (Qiagen, USA). A degenerate primer pair (Yasmin *et al*., 2017), designed to amplify a fragment of approx. 650 bp from the coat protein gene of the genome or DNA-A component of begomoviruses, was used in PCR. Five amplified fragments were cloned and sequenced. A BLASTn search indicated the presence of a begomovirus most closely related to *Tomato leaf curl virus* (ToLCV-[NP:Pan:00]; GenBank Accession No. AY234383), with 99.0% nucleotide sequence identity.

To enrich circular viral DNA, rolling circle amplification was done using total nucleic acid extract as template with an IllustraTempliPhi 500 amplification kit (GE Healthcare, USA). The product from one plant sample was digested with *Hind*III, cloned into the plasmid vector pGEM3Z (Promega, USA) and sequenced. Pairwise nucleotide sequence comparisons using the Sequence Demarcation Tool (SDT v1.2) (Muhire *et al*., 2014) indicated that the chilli isolate was most closely related to ToLCV-[NP:Pan:00], with 99.2% nt identity. This indicates that the virus in chilli is an isolate of ToLCV for which the isolate descriptor ToLCV-[Pakistan: Islamabad: chilli; 2014]-(KY799159) is proposed. The presence of a betasatellite in the plant from which the virus was cloned, was confirmed by PCR amplification using the betasatellite-specific primer pair F-5/R-5 (Yasmin *et al*., 2017). One amplified betasatellite genome was cloned in pGEM3Z and sequenced. SDT analysis showed the highest level of identity (93.4%) with Croton yellow vein mosaic betasatellite (CroYVMB; HM143903). This showed the betasatellite associated with ToLCV to be an isolate of CroYVMB, for which the isolate descriptor CroYVMB-[Pakistan: Islamabad: chilli; 2014](KY769276) is proposed.

In India ToLCV is a widespread and important pathogen of tomato (Jyothsna *et al*., 2013; Ranjan *et al*., 2014).This is the first report of the association of ToLCV and CroYVMB with chilli plants exhibiting leaf curl symptoms in Pakistan.

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Figure 1

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