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Occurrence of *Tomato chlorosis virus* on tomato in Réunion Island

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Pronounced yellowing symptoms on the lower and middle leaves of tomato plants, similar to those caused by magnesium deficiencies, were observed in 2004 and 2005 in farmers' greenhouses in Réunion Island, situated east of Madagascar in the Indian Ocean. Yellow-leaf symptoms included irregular chlorotic mottling, and the interveinal areas of leaves developed red or brown necrotic flecks. Fruit size was reduced on plants with symptoms and yields were indirectly affected. The flame-like pattern of the discoloured leaves, and the abundance of whiteflies on affected plants, suggested the possible involvement of *Tomato chlorosis virus* (ToCV) or *Tomato infectious chlorosis virus* (TICV) (*Closteroviridae, Crinivirus*) (Wisler *et al*., 1998).

Twenty leaf samples with symptoms were collected from tomato plants in March 2005, and total RNA was extracted from these samples using the Qiagen RNeasy Plant Mini kit. For the detection of a potential crinivirus, a nested PCR was performed (Dovas *et al*., 2002). The method consisted of a one-step RT–PCR using primers HS-11 and HS-12, followed by nested PCR with primers TIC-3/TIC-4 and ToC-5/ToC-6, for detecting TICV or ToCV, respectively. These primers were designed to amplify the highly conserved region of the heat-shock protein 70 gene. A PCR product of the expected size was observed with ToCV primers for 14 of the 20 leaf samples. No PCR product was observed for the PCR performed with TICV primers. Three PCR products were cloned using pGEM-T easy vector system II (Promega), and sequenced (Genome Express) (EMBL accession nos AJ968394, AJ968395 and AJ968396). Sequences obtained from the three samples had 99·5% nucleotide identity when aligned (DNAMAN, Lynnon BioSoft, Quebec, Canada). The most significant sequence alignments (NCBI, BLASTn) were 98% with ToCV isolates from the USA (GenBank accession no. AF024630), Spain (GenBank accession nos AF233435, AF215818 and AF215817) and Italy (GenBank accession nos AF234029 and AY048854). This is the first report of the presence of ToCV in Réunion Island.

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First report of *Tomato leaf curl New Delhi virus* infecting chilli in India

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Chilli (*Capsicum annuum*), a member of the Solanaceae, is an important spice crop cultivated in tropical and subtropical countries. The crop is severely affected by leaf curl disease in most of the chilli-cultivating areas of India. Symptoms consisting of leaf curling, shortening of internodes and petioles, crowding of leaves and stunting of whole plant were observed in chilli fields around Lucknow. The disease was experimentally transmitted from infected to healthy chilli and tomato seedlings by the whitefly *Bemisia tabaci*. Inoculated chilli plants developed typical symptoms of the disease. However, the inoculated tomato plants developed severe leaf curl symptoms similar to those of leaf curl disease of tomato caused by *Tomato leaf curl New Delhi virus* (ToLCNDV).

To confirm the association of a begomovirus with the disease, polymerase chain reaction (PCR) was performed using the total DNA isolated from naturally infected chilli leaves and a set of primers designed to amplify the coat protein region of ToLCNDV (previously called *Indian tomato leaf curl virus*; Hallan, 1998). An amplification product of the expected size (~800 bp) was produced. The amplicon was cloned and sequenced (AY883570). A Blast search analysis of the nucleotide sequence showed 89–93% identity with various ToLCNDV isolates (X78956, AY428769, TLU15016 and AF448058). However, the isolate under study was found to be only distantly related to *Pepper leaf curl Bangladesh virus* (AF314531) and *Chilli leaf curl virus*-Multan] (AF336806), to which it shows 86 and 81% identity, respectively.

Based on these findings the virus infecting chilli has been tentatively identified as an isolate of ToLCNDV. This is the first report of ToLCNDV affecting chilli crops in India. Taken together with the recent report of ToLCNDV affecting chilli cultivation in Pakistan (Hussain *et al*., 2004), this indicates that, on the Indian subcontinent, chilli is a major alternative host of ToLCNDV.

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