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First Report of *Capsicum chlorosis virus* in Tomato in India

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During field monitoring studies conducted in Northern India during the 2007 post-rainy season (December through May) for the occurrence of tospoviruses (family: *Bunyaviridae*, genus: *Tospovirus*) in vegetables, it was observed that tomato (*Lycopersicon esculentum*) plants in three fields showed the symptoms mimicking those caused by *Peanut bud necrosis virus* (PBNV). The symptoms included mild chlorosis of leaves, chlorotic and necrotic spots and rings on the leaves, and leaflet necrosis (Fig. 1a). Occasionally, the infected plants showed necrosis of growing tips leading to bud necrosis. Fruits produced on the infected plants showed discolorations with concentric rings on the surface (Fig 1b). Mechanical inoculation of sap from symptomatic tomato leaves collected from farmers field produced local chlorotic spots on the primary leaves of cowpea cv. C152 (*Vigna unguiculata*) 3 to 4 days after inoculation (Fig. 1c). Tomato seedlings inoculated with the same sap produced symptoms similar to those described in Fig 1. In direct antigen coating ELISA, both field samples as well as mechanically inoculated plant samples gave positive reaction with a polyclonal antiserum produced against the nucleocapsid (N) protein of PBNV. Total RNA isolated from tomato samples using RNeasy Mini Plant kit (Qiagen Inc., Chatsworth, CA) was used in RT-PCR to amplify approximately 850 bp fragment (Fig. 2) using degenerate primer pair (MH-F: 5'-CATGCCATGGCAATGTCTAMCGTYAAGCAACT-3' and MH-R: 5'-CCGCTCGAGCAMTTCCARMGAAGKRCYAG-3') that can amplify the N gene of members of *Watermelon silver mottle* (WSMoV) serogroup of tospoviruses. The amplicons were cloned into pGEM-T Easy vector (Promega, Madison, WI) and plasmid DNA isolated from two individual colonies was used for sequencing in both directions. A comparative analysis of these sequences with corresponding N gene sequences of other tospoviruses in the GenBank revealed highest sequence identity with CaCV (AY036057) from Australia with 95.8 and 96.7 percent at the nucleotide and amino acid level, respectively. The N gene sequences of the Indian isolate of CaCV (EU095940) showed 85.5 and 91.6 percent identity at the nucleotide and amino acid level, respectively, with CaCV isolate from China (DQ 0355974) and 84.2 and 92 percent identity at the nucleotide and amino acid level, respectively, with an isolate from Thailand (AY 256123). In contrast, the Indian isolate of CaCV shared only 79 to 85.1 and 77.2 to 81.5 percent identity with the N gene sequences of other tospoviruses [PBNV, U27809 and *Watermelon bud necrosis virus* (WBNV), AF045067] previously reported from vegetables in India (2). To our knowledge, this is the first report of the occurrence of CaCV in tomato in India.



A



B



C

Fig. 1. Symptoms of *Capsicum chlorosis virus* (CaCV) on tomato: **(A)** leaf chlorosis and necrosis (inset) 8 days post inoculation (dpi); **(B)** field-infected fruit showing chlorotic rings and spots on the surface; and **(C)** CaCV inoculated cowpea cv. C-152 with chlorotic spots 4 dpi.

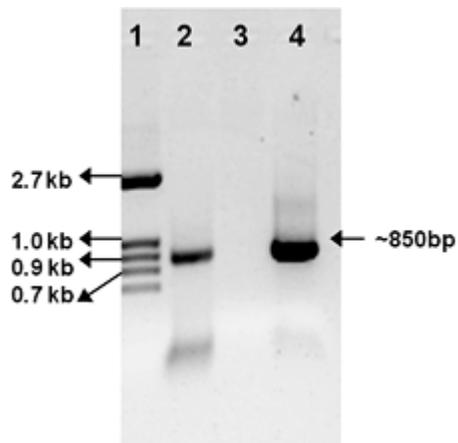


Fig. 2. RT-PCR analysis of *Capsicum chlorosis virus* (CaCV) N gene using degenerate primers: DNA marker (Lane 1); ~850bp fragment amplified from total RNA isolated from symptomatic field-infected tomato fruit sample of CaCV-Indian isolate (Lane 2); healthy tomato fruits. (Lane 3); and *Peanut bud necrosis virus*-infected tomato fruits as positive control (Lane 4).

CaCV is recognized as an economically important virus infecting vegetables like tomato and pepper in Australia and Thailand (3,4) and recently peanut (*Arachis hypogaea*) in China (1). Although the distribution and economic impact of CaCV in India is yet to be realized, the observation that both PBNV and CaCV can produce similar, though not identical, symptoms in tomato warrants further studies for developing crop improvement strategies against these two tospoviruses in vegetable and legume crops in India. In addition, our report highlights the need for continuous monitoring on the prevalence of CaCV in India and other countries in the Asia-Pacific region.

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