First record of *Pepper cryptic virus 2* in chilli (*Capsicum annuum*) in India

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Abstract The occurrence of *Pepper cryptic virus 2* was confirmed by RT-PCR and partial sequencing of coat protein gene in chilli cultivars Jwalamukhi and Jwalasakhi grown in the experimental farm at IARI, New Delhi. To the best of our knowledge this is the first report of occurrence of cryptovirus in chilli in India.

Keywords *Pepper cryptic virus 2* · *Capsicum* · India

During a survey in experimental farm of Indian Agricultural Research Institute (IARI), symptoms suspected to be caused by viruses were observed in different accessions of chilli genotypes. Among them, Arka Suphal, Jwalamukhi, Jwalasakhi and DKC-8 showed prominent yellowing while the genotype Chilli Black Megha (CBM) was apparently symptomless. RNA from one symptomatic samples of each of the above genotypes and one asymptomatic sample of CBM were isolated and pooled to conduct a microarray analysis using a chip developed at Plant Virology Unit, IARI [7], to identify the associated viruses. This chip was designed to detect all the known plant viruses and viroids for which sequences were available till 2012. Initial analysis with microarray indicated the presence of a cryptovirus along with other viruses known to infect chilli in India. Cryptoviruses are symptomless viruses belonging to the family *Partitiviridae* [3]. They are not transmissible from

plant to plant and are present at very low titers [2]. In pepper, two cryptoviruses, *Pepper cryptic virus 1* (PCV1) [1, 6] and *Pepper cryptic virus 2* (PCV2) have been reported [4], however, there is no record of occurrence of cryptovirus on chilli from India (Fig. 1).

In order to confirm the presence of cryptovirus, as well as to identify the genotypes harboring it, reverse transcription PCR (RT-PCR) using primers specific to partial coat protein gene of PCV1 (PCV1F-ACATCATCGAG-GAGTTCACC/PCV1R-GCTGTCCTAGAATTGTCTTC) and PCV2 (PCV-2F 5'TCATCCGTCAGCTAACGTA3'/PCV-2R 5'CGTCTCTTTTCTGAGCGGTA3') [4] was performed. Total RNA was isolated from leaves of each of these five lines using RNeasy mini kit (Qiagen). cDNA was prepared from total RNA using Improm II Reverse transcription system, Promega as per manufacturer's instructions. PCR conditions were programmed as follows: 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 50 °C for 10 s, and 72 °C for 1 s [4]. Even after repeated attempts no amplification could be obtained using primers specific to PCV1. An expected ~400 bp was amplified in the RT-PCR using primers specific to PCV2 from CBM, Jwalamukhi and Jwalasakhi while there was no amplification in Arka Suphal and DKC-8 using these primers. All genotypes except CBM also had mosaic symptoms which were later diagnosed to be associated with *Cucumber mosaic virus* by ELISA (data not shown). Percent sequence identity was analyzed using Bioedit Sequence Alignment Editor [5]. The amplified fragment from CBM was cloned in RBC TA cloning vector and sequenced. The fragment was 387nt (Accession Number: KM464727) and showed very high sequence identity with partial coat protein gene sequence of other PCV2 isolates. Maximum identity (98.9 %) was observed with HW-01 isolate from the USA (JN117279.1) and the South Korean isolate (KR676354).

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Fig. 1 Agarose gel electrophoresis of RT-PCR products for detection of PCV2. M: 100 bp DNA ladder; 1 Cultivar Jwalamukhi, 2 Cultivar Jwalasakhi, 3 negative control

Another South Korean isolate (KR676354) shared 98.7 % identity with the Indian isolate.

Our findings contribute the first sequence based evidence of presence of cryptoviruses in Indian genotypes of chilli. Even though they do not have any direct economic

significance due to symptomless infection, their role in aggravating or alleviating symptoms caused by other viruses still needs to be examined.

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