Molecular evidence of Chili vein mottle virus and Chilli leaf curl virus simultaneously from naturally infected chilli plants (Capsicum annuum L.)

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The occurrence of Chilli leaf curl virus (ChLCV) and Chilli vein mottle virus (CVMV) were detected by using the duplex PCR in the mixed infected Chilli plants (Capsicum annuum L.). The duplex PCR was done by using the specific primer Pot 1 and Pot 2 for CVMV and AVF28 and AV29R for ChLCV. The amplicon and the sequence analysis confirmed the presence of potyvirus and begomovirus in the mixed infection. This combinations exhibited synergistic symptoms and large numbers of cells were doubly infected. This paper reports highly specific conventional PCR assays for detection of two independent viruses infecting chilli plants simultaneously.

Keywords: Chilli leaf curl virus, Chilli vein mottle virus, Duplex PCR, begomovirus, potyvirus

Chilli is an important and widely cultivated spice and vegetable crop. From the economic point of view, chilli is no longer considered a minor vegetable crop. It is one of the most susceptible crops and has been found to be affected by various viral pathogens, which cause heavy losses to chilli production. Natural occurrence of several viruses have been reported on chilli by various workers and among them Chilli leaf curl virus (ChLCV), Cucumber mosaic virus (CMV) and Chilli vein mottle virus (CVMV) have been reported as most destructive viruses affecting chilli cultivation in terms of incidence and yield loss. The literature survey revealed that in last few years, the leaf curl disease of chilli has become the major constraint for chilli production in most of the chilli growing areas. In India, the most important disease caused by viruses in chilli is yellow mosaic disease.

Incidence of the disease is reported to be 12-95% resulting yield losses as high 65-75%.

The mixed infection by the two or more plant viruses results in the complete loss of crop. Biological diversity of the plant virus increases the mixed infection in nature, which leads the interference and cross-protection. The cross-protection strategies may control the various plant viruses and increase the possibility of disease management. Although detection of mixed infection through serological methods is available, it fails at the time of low titer of the viruses. In the present study, we have used the duplex PCR for the detection of CVMV and ChLCV infecting the chilli simultaneously.

During the survey in year 2012-13, about twenty Chilli leaves showing the mosaic and leaves curling were collected from the Shekhawati region of Rajasthan, India. Both the viruses were present alone and in mixed fashion in the samples tested. Dual infection of CVMV and ChLCV was the most prevalent mixed virus infection in the fields. Most of the viruses infecting samples showed similar symptoms, i.e., mosaic, leaf curling and leaf distortion. Two sets of the universal primer as per the Table 1 were used for the detection and the presence of CVMV and ChLCV, respectively. Total RNA was extracted from mixed infection as well as from infected chilli samples using TRI regent as per the manufactures protocol. DNA was also extracted from mixed as well as leaf curl samples using CTAB method. The duplex PCR was carried out by using the two sets primers in a 50 µL reaction mixture, which contained 3 µL DNA, 5 µL cDNA, 10× buffer 5 µL, MgCl2 2.5 µL, dNTP 4 µL, each primers 1 µL and Taq polymerase 0.6 µL. The PCR was performed for 35 cycles at 94°C for 30 sec, 56-58°C for 45 sec and 72°C for 30 sec, followed by 72°C extensions for 10 min.

The amplified products were analyzed electrophoretically in 0.8% agarose gels (Fig. 1). Further, the amplicon was sent for the sequencing and submitted to GenBank, USA [JN000700 for ChLCV and KF220408 for CVMV (Fig. 1; lane 3)]. Sequence analysis confirmed that the viruses in the mixed infection belong to potyvirus and geminivirus family. Phylogenetic tree was constructed by
Fig. 1—Detection of potyvirus and begomovirus in the mixed infected chili sample. [Lane M: 1 kbp DNA marker; Lane 1: Sample with ChLCV begomovirus infection; Lane 2: Mixed infection sample: Lane 3: Sample with CVMV infection; & Lane 4: Healthy control.]

using MEGA 4.0.2 software. Phylogenetic tree was obtained with bootstrap values in cluster algorithm, phylip format and topological algorithm (Figs 2a & b). Further, the betasatellite molecules associated with ChLCV were also detected by using the specific primer (Acc. No. KC684927).

The present study clearly shows that infection of CVMV and ChLCV prevailed in localities with different percentages and a large number of screened samples had confirmed a mixed virus infection. Both the viruses had been found to systemically infecting the host plant by using hybridization (data not shown). We also raised the seeds from infected chilli plants, but they did not show any symptoms during the seedling stage under controlled conditions. This confirms that the CVMV and ChLCV are not transmitted by seeds\(^\text{10}\). Although, chilli leaf curl diseases are prevalent in Rajasthan but, in the present study, we found that the infection severity increased due to the presence of both the viruses. We have to make continuous effort to develop the multiple resistance varieties against the plant viruses. The concept of transgenic plant or pathogen-derived resistance should also be applied for effective virus control.

Table 1—Primer used for the presence of CVMV and ChLCV

<table>
<thead>
<tr>
<th>Primer</th>
<th>Viruses</th>
<th>Gene</th>
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<tbody>
<tr>
<td>Pot1 F: 5’ GACGAATTCTGAYGAYGCBGATGGYTC 3’</td>
<td>Potyvirus (CVMV)</td>
<td>CP &amp; Nib</td>
</tr>
<tr>
<td>Pot2 R: 5’ GACTGGATCCATTTBTDATRCAACA 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVF28: 5’GCCACATGYTC TTYCNNGT-3’</td>
<td>Begomovirus (ChLCV)</td>
<td>CP</td>
</tr>
<tr>
<td>AV29R: 5’GGCTTYCT RT ACATRGG-3’</td>
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Fig. 2 (a & b)—Neighbour-joining phylogenetic dendograms based on pair-wise and multiple alignments of nucleotide sequences of CVMV and ChLCV: (a) Predicted Nib and CP gene sequences with other reference potyvirus sequences from GenBank database; & (b) Predicted CP sequence with other begomovirus CP sequences from GenBank database. [The tree was constructed using MEGA 4.0.2 software.]

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Reference

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