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Leaf curl virus in chilli and its characterization

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ABSTRACT : Twelve symptomatic chilli samples showing variable leaf curl symptoms resembling to those caused by begomovirus were collected from chilli fields of Vegetable Research Farm, Department of Vegetable Sciences, PAU, Ludhiana. Type of symptoms exhibited by these samples was recorded. To prove the association of begomovirus with these samples, PCR based detection method was used. Once the samples were positive for begomovirus presence, the same set of samples were further used as template using virus species tomato leaf curl Palampur Virus (ToLCPV), tomato leaf curl New Delhi Virus (ToLCNDV), tomato leaf curl Karnatka Virus (ToLCKV), specific primers as well DNA-B and *DNA-β primers* were used. The samples (C1 and C4) which were not amplified by universal degenerated primers as well as species specific primers were subjected to RCA (Rolling circle amplification) followed by PCR using begomovirus specific AV494/AC1048 primers for partial characterization. In sample no. C3 and C9, where the plants were exhibiting cupping of leaves, thickening of veins and slight puckering kind of symptoms, mixed infection of two virus species i.eToLCPV and ToLCNDV were found to be present. In six samples viz., C2, C8 and C11 ToLCKV and in sample viz., C7, C10 and C12, ToLCPV along with beta satellite molecules were found to be associated. Similarly, in sample C6 a bipartite ToLCNDV with beta satellite was detected. However, the ToLCPV and ToLCNDV are bipartite viruses and association of beta satellite is unusual but this could be due to the fact that the set of primers used were unable to detect the monopartite virus, which is also associated in the form of mixed infection. In sample, C5 ToLCPV alone was detected. In both chilli samples, the expected size amplicon (~575 bp) from core CP region was observed, eluted and cloned in plasmid vector pTZ57R/T vector. The recombinant plasmids checked for the presence of insert using M13 F and M13 R primers. An expected size amplicon of ~800 bpwas observed in positive clones. Phylogenetic analysis, based on partial genomic DNA sequences, revealed that the two viruses clustered within different clades. Chilliclone1.1 shares a close common ancestor with papaya leaf crumple virus, forming a subclade that is distantly related to other begomoviruses used for analysis. Similarly, Chilliclone4.1 shared a close common ancestor with recently described tomato leaf curl Joydebpurvirus and forms a separate subclade. Further attempts made to clone the full-length genome of both the samples C1 and C4.The results further confirmed with AV494/AC1048 primers showing amplification of 575bp fragment from same clone.

Key Words : Begomoviruses, monopartite, phylogenetic analysis, amplicon, chilli.