SUMMARY

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Leaf curl disease of tomato (ToLCD) caused by begomoviruses (family-*Geminiviridae*), is one of the major constraints for successful cultivation of tomato in the tropics and sub-tropics including India. The prevalence and severity of tomato infecting begomoviruses have increased to epidemic proportions in the recent years. In India, ToLCD is the most devastating disease of tomato, wherever this crop is grown resulting in 100% losses under epidemic conditions. Variation of symptoms of ToLCD have suggested occurrence of strains/species of tomato-infecting begomoviruses in different geographical region of the country. Further, natural spread of vector whitefly (*Bemisia tabaci*) from one region to other have aggravated the problem often resulting in mixed infection of distinct begomoviruses, leading to severe leaf curl disease. Tomato-infecting begomoviruses include diversified groups like monopartite as well as bipartite viruses. Indian tomato-infecting begomoviruses known to be associated with ToLCD identified so far are *Tomato leaf curl Gujarat virus* (ToLCGV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Bangalore virus* (ToLCBV), *Tomato leaf curl leaf curl Karnataka virus* (ToLCKV), *Tomato leaf curl Kerala virus*, *Tomato leaf curl Rajasthan virus* (ToLCBV-[Raj]), *Tomato leaf curl Pune virus* (ToLCBV-[Pun]) and *Tomato leaf curl Palampur virus* (ToLCPMV). Although, there were indications on the occurrence of ToLCD in several areas within states like Bihar, Jharkhand, Rajasthan, Karnataka, Jabalpur and Jammu & Kashmir, very little or no information was available on detailed genomic characterization of the causal viruse(es). Keeping in view the losses caused by the tomato-infecting begomoviruses in these areas and works done so far, the present investigation was carried out on, "Molecular characterization of virulent isolates of Tomato leaf curl virus".

During the present study, a survey of tomato-infecting begomoviruses was conducted in several states of northern India. Several new isolates of reported species were obtained from these still unexplored vegetable growing areas of India. Survey of these areas from where no earlier report for characterization of tomato-infecting begomovirus was available, and detection of different known species from these geographical regions of country revealed the panorama of their distribution and genetic diversity.

ToLCD associated plant samples collected from six different tomato growing states (Jharkhand, Bihar, Jammu & Kashmir, Madhya Pradesh, Rajasthan and Karnataka)

of India, indicated presence of begomovirus infection. Detailed analysis of these samples established existence of a previously undescribed begomovirus species in India. In addition, this study also revealed that mixed infection is a common feature among Indian Tomato leaf curl viruses (ToLCVs). Association of ToLCNDV DNA-A along with DNA-B of ToLCNDV and ToLCGV both was detected, whereas ToLCGV DNA-A could not be detected in sample collected from Jammu. ToLCGV was found to be one of the most predominant species of northern India occurring as both bipartite as well as monopartite species. Therefore, it can be concluded that although distribution of Indian ToLCVs are not restricted to limited territory but particularly some species are more prevalent in specific regions than others resulting in severe ToLCD.

Recombination analysis of ToLCVs from India with other closely related species suggested the possible interaction among these species and also with the other species originating from other countries. However, species from southern India appeared more prone to recombination. ToLCV species which are known to be prevalent in southern India, *Tomato leaf curl Bangalore virus* (ToLCBV) and *Tomato leaf curl Karnataka virus* (ToLCBV), shared similar fragment inside the virion sense coding region, which appeared to be of recombinant nature and related with *Tomato leaf curl Bangladesh virus* (ToLCBDV).

A novel begomovirus species have been isolated and characterized, from ToLCD infected field of Patna, and it was named as *Tomato leaf curl Patna virus* (ToLCPaV). From the same sample the cognate DNA-β molecule was also cloned and identified as a new species of betasatellite, thus named as *Tomato leaf curl Patna betasatellite* (ToLCPaB). Phylogenetic analysis placed ToLCPaV in a separate clade, distant from all the characterized ToLCVs from India. The most closely related virus species for ToLCPaV, were *Tomato leaf curl Laos virus* and *Tomato leaf curl Malyasia virus,* sharing more than 88% identity between each other. Full-length sequence of ToLCPaV when compared with these begomovirus species it is found to have maximum identity (85.8%) with Tomato leaf curl Laos virus - [Laos];AF195782 and 85.1% with Tomato leaf curl Malaysia virus-[Malaysia:Klang:1997];AF327436. Phylogenetic analysis of DNA-β revealed that ToLCPaB is related with other betasatellites isolated from Joydebpur and Pakistan associated with ToLCD. A comparison of the complete sequence of ToLCPaB to

DNA-β sequences available within GenBank databases, indicates that the isolated satellite DNA-β shares maximum sequence identity (only 75.8% nucleotide sequence identity) with ToLCJoB-[BD: Gaz: 05]; AJ966244, and do not have close sequence identity with other betasatellite species reported so far. While analysed with different options of RDP, like GENECOV, Bootscan, Maxchi, and manual and automated RDP, using the default parameters, it was noticed that all the detected recombination events were not supported by every method, for the ToLCPaV genome. It appeared that ToLCPaV evolved separately after the earlier recombination event and reached upto present genome organization. ToLCPaB is found to have one clear evidence of recombination breakpoint in the βC1 ORF sharing sequences with ToLCJoB-[BD: Gaz: 05]; AJ966244. The length of fragment undergone recombination must had one end in the SCR region so could not be detected. Hence, it is concluded that this new species of tomato-infecting begomovirus along with its satellitebeta have comparatively early evidence of recombination in their genomes, and possess sufficient sequence dissimilarity with any known species.

From the infected tomato sample collected from Ranchi district of Jharkhand, a new recombinant DNA-A molecule was isolated, which shared 89.9% and 90.2% sequence identity with Tomato leaf curl Bangladesh virus (ToLCBDV-[BD:2]; AF188481) and Tobacco curly shoot virus (TbCSV-[CN:Yn35:01]; AJ420318), respectively. This DNA-A isolate of begomovirus was tentatively named as Tomato leaf curl Bangladesh virus- Ranchi isolate (ToLCBDV-[Ran]). ToLCBDV-[Ran] was found to be associated with a satellite DNA-β in field condition which shared 79.0% sequence identity with ToLCBDB-[BD:Gaz:01]; AJ542489, thus identified as *Tomato leaf curl Bangladesh betasatellite* (ToLCBDB), and named as Tomato leaf curl Bangladesh betasatellite - Ranchi isolate (ToLCBDB-[Ran]). Detailed sequence comparisons provided strong evidence of recombination in ToLCBDV-[Ran]; sharing sequence of putative recombinant origin with ToLCBDV-[BD] and TbCSV-[Yn35].

Two new isolates of ToLCGV were cloned from Dhanbad and Ramgadh districts of Jharkhand, and were named as Tomato leaf curl Gujarat virus- Dhanbad (ToLCGV-[Dhn]) and Tomato leaf curl Gujarat virus- Ramgadh (ToLCGV-[Ram]), respectively. These isolates shared more than 95% sequence identity among each other and with other earlier reported isolates of ToLCGV. Interestingly, both of these

isolates were found to be associated with *Tomato yellow leaf curl Thailand betasatellite* (TYLCTHB). Infectivity of cloned infectious construct of DNA-A component of ToLCGV-[Dhn], ToLCGV-[Ram] and ToLCGV-Varanasi isolate (ToLCGV-[IN:Var:07]; AY190290) was studied. Their pathogenicity on their natural host tomato, revealed that ToLCGV-[Ram] was a milder than the other two isolates of this species.

Infectivity study of infectious clone of ToLCPaV and its cognate DNA-β, showed that unlike many other DNA-β reported earlier, ToLCPaB was not involved in enhanced accumulation of DNA-A when co-inoculated together on either *Nicotiana benthamiana* or tomato plants. ToLCPaV, behaved like a typical monopartite and was alone capable to cause systemic disease on *N. benthamiana* and tomato. It was observed that ToLCPaB did not possess relaxed transreplication with the bipartite ToLCNDV but could be trans-replicated by mono-bipartite begomovirus like ToLCGV in natural host tomato. In addition, the well evolved ToLCPaV could not transreplicate the DNA-B molecule from ToLCGV and ToLCNDV possiblly due to difference in iteron sequences.

Infectivity of cloned DNA-A and DNA-β components of ToLCBDV-[BD] was also demonstrated. ToLCBDV-[BD] was found to be infectious on *N. benthamiana* and tomato when agroinoculated. ToLCBDV-[BD] was alone found to be infectious to both the plants, however when co-inoculated with its cognate DNA-β, symptom severity increased, and symptoms appeared earlier. The increase in symptom severity was not found in accordance with the viral DNA accumulation level in presence of DNA-β, rather in both the cases accumulation of DNA-A remain unchanged.

To understand transreplication of DNA-β, TYLCTHB and ToLCPaB were co-inoculated with DNA-A of either ToLCPaV, ToLCGV-[Var] and ToLCNDV on *N. benthamiana* and tomato. All the construct combinations could infect and produce discernible symptoms on *N. benthamiana* when inoculated as DNA-A alone or with cognate DNA-B/DNA-β or transcomplemented with DNA-β. However, variation and degree of symptoms were observed depending upon the construct combinations used. In the present study, none of the betasatellite was found to complement movement function of ToLCNDV DNA-A in tomato plants, which impaired systematic infection on tomato. Chilli leaf curl virus from Mograhat (ChLCV-[Mgr]) and its cognate DNA-β (ChLCV-[Mgr]), were when inoculated on tomato, produced no

symptoms, although they were infectious to *N. benthamiana.* Similarly, Reddish leaf curl virus (RLCV-[Var]) and associated betasatellite (RLCV-[Var]) were also not able to infect tomato in glass-house conditions. Thus, host specificity for tomato-infecting begomoviruses was observed. Also the tomato-infecting monopartite begomoviruses was able to form viable pseudorecombinant with more than one betasatellites but bipartite begomovirus (ToLCNDV) could not. Hence, it is suggested that host adaptation of begomoviruses play major role in determining host-range and assist in overcoming barrier to infect a new host.