

Abstract

Diseases caused by viruses are responsible for poor crop yield in several vegetable crops. Several million rupees are lost worldwide due to infection of our crops by viruses. *Begomoviruses* are the most devastating plant pathogens that causes high amount of crop loss every year in India. Several vegetable crops are cultivated in sub-Himalayan West Bengal and Assam. Due to yearlong conducive atmosphere production of the vegetable crops are also popular to the local farmers.

The present work entitled “Studies on genetic variability and distribution of begomoviruses affecting economically important crops of Sub-Himalayan plains of North-east India” consists of the following objectives such as i) Identification of begomoviruses infecting economically important crops of sub-Himalayan plains of North-east India using Polymerase chain reaction (PCR) and Rolling circle amplification. ii) Sequence analysis and determination of variability among begomoviruses and their strains using bioinformatics tools. iii) Eco-friendly management of important *Begomovirus* diseases using botanicals.

To know the begomoviral diseases of the present study area, a survey was carried. Around 20-75% begomoviral disease incidences have been found in some vegetable crops. Ten important crops of the present study area are tomato, cucumber, potato, kenaf, pointed gourd, papaya, loofah, pumpkin, chilli and ladies finger. Begomoviral symptoms were found in all the ten crops, resulting to significant crop loss. Major symptoms observed were mild to severe yellow mosaic, upward leaf curling, leaf rolling, stunted growth, leaf deformation, networking of yellow veins, followed by thickening of veins and vein lets, puckering, stunting of whole plant and reduced fruit yield.

Altogether 55 samples were tested through PCR using different primer sets. Among the 55 samples, 17 samples were positive for *begomoviruses*.

Out of the 17 samples eight were from tomato, seven were from cucumber; one was from kenaf and one was from pumpkin plants.

Out of the 16 *Begomovirus* isolates of the present study, nine were identified as *Tomato leaf curl New Delhi virus* (ToLCNDV), two were identified as *Radish leaf curl virus* (RaLCV), two were identified as *Papaya leaf curl virus* (PaLCuV), one was identified as *Tomato leaf curl Karnataka virus* (ToLCKV), one was identified as *Tobacco curly shoot virus* (TbCSV) and one was identified as *Ageratum enation virus* (AEV). Among the ToLCNDV isolates, three were detected in tomato (Accession Nos. KX108860, KX817297 and MZ516898), five in cucumber (Accession Nos. KY783746, KY807530, MG721011, MG721012 and MG721013) and one in pumpkin (Accession No. MG721010).

Two isolates of tomato of the present study were found to be *Radish leaf curl virus* [Accession no. MK333456] as they showed 97% sequence similarity with other RaLCV sequences previously submitted in GenBank.

Based on 94-96% sequence similarities following results were found: i) *Papaya leaf curl virus* detected from tomato (Accession No. MZ516896) and cucumber plants (Accession No. MK333457). ii) *Tobacco curly shoot virus* was also detected from cucumber (Accession No. MG721014) plants iii) one tomato sample was found to contain *Tomato leaf curl Karnataka virus* (Accession No. KX108859) iv) One *Begomovirus* species *Ageratum enation virus* [Accession no. MZ516897] infecting tomato was identified. That AEV showed 95% sequence similarity with the other AEV of the GenBank.

In this study, infected leaves of selected plants like tomato, cucumber and kenaf were used for *Begomovirus* detection through Rolling Circle Amplification (RCA). High molecular weight DNA obtained by RCA of the infected sample was visible in 1% agarose gel under UV-transilluminator, indicating the amplification of circular DNA. RCA product (5 µl) was digested with a number of different restriction enzymes to select an enzyme with a single restriction site in the DNA components. XbaI and BamHI were found to have a single site in both DNA-A and DNA-B component. In the HindIII

digested sample two bands of 2.7kb and 1.4 kb were found. In the Pst digested product, three bands of 2.74kb, 1.4kb and 1.45kb were found. In the EcoRI digested sample, two bands of 2.74 kb and 1.4kb were found. The band 2.7kb probably indicate the presence of whole genome of Begomovirus either DNA- A or DNA-B, and the band of about 1.45 kb probably indicate the presence of beta-satellite or alpha-satellite until sequenced. The amplified fragment of 2.74kb were purified and ligated in to pGEM-3Z vector which was also digested by the same enzyme XbaI and cloned according to manufactures instruction. After transformation in to DH5a strain of *E.coli*, white colonies were screened for the gene of interest by PCR and restriction digestion with XbaI. Positive PCR white colony was selected for sequencing. Three isolates (To/Guw, Cu/Khr and K/Blg) were found positive following RCA and RFLP. Those three isolates produced distinct bands of ~2.7kb and ~1.4kb.

The RCA product was subjected to PCR by β satellite specific primer and the amplicons were found to the betasatellite (after cloning and sequencing). Out of the two positive samples, one was identified as *Tomato leaf curl betasatellite* (ToLCB) infecting tomato (Accession No. MK333455) as it showed 96% sequence similarity with ToLCB. Another one was identified as *Cotton leaf curl Multan betasatellite* (CLCuMB) infecting Kenaf plants (Accession No. MK358823), which showed 98% sequence similarity with CLCuMB upon BLASTn analysis.

The recombination analysis by RDP4 showed that the isolate GUW-01 (*Tomato leaf curl betasatellite*; Accession No. MK333455) was a recombinant having major parent *Tomato yellow leaf curl Thailand betasatellite* (TYLCTHB, Accession No. GU058327), and minor parent TYLCTHB (Accession No. GU058324) infecting tomato in China. This suggests that the ToLCB isolate GUW-01 (Accession No. MK333455) may have derived from recombination and it contains sequences derived from TYLCTHB (Accession No. GU058324).

The sequences of AEV infecting tomato were clustered together with other tomato infecting AEV isolates in the phylogenetic analysis. PaLCV isolates of the present study clustered with PaLCV infecting *Carica papaya* whereas PaLCV from other hosts showed separate clusters. RaLCV isolates infecting tomato of the present study showed close relationship among them and clustered with tobacco infecting RaLCV isolate. Phylogenetic analysis of TbCSV isolate infecting cucumber showed that the isolate formed separate small cluster with *Phaseolus vulgaris* infecting TbCSV whereas the isolates infecting other host formed different cluster.

Tomato infecting ToLCKV of the present study clustered with tomato infecting ToLCKV of GenBank and showed close relationship with tomato infecting isolates found worldwide. Phylogenetic analysis of the present nine ToLCNDV sequences along with other ToLCNDV sequences obtained from public database revealed close relationship among them and clustered together. Pumpkin-infecting isolate of the present study showed close relationship with other pumpkin-infecting ToLCNDV isolates. Cucumber-infecting isolates was also clustered together with other cucumber-infecting ToLCNDV isolates except one isolate which positioned alone. But tomato infecting isolates clustered with cucumber-infecting

In phylogenetic analysis *Cotton leaf curl Multan betasatellite* (CLCuMuB) isolate showed close relationship and clustered together with other CLCuMuB isolates whereas *Tomato leaf curl betasatellite* (ToLCB) isolate showed close relationship with *Tobacco curly shoot betasatellite* (Accession No. KX857135) and also clustered together with that isolate.

In the present study, four enzymes (peroxidase, β -1,3-glucanase, Polyphenol oxidase and PAL) activity was studied following application of the chemical inducers. BABA treated and BABA treated-inoculated plants showed highest peroxidase activities followed by AABA treated and AABA treated-inoculated plants. Highest increase in β -1,3-glucanase activity was found after 12 days of treatment in the GABA treated-ToLCNDV inoculated plants followed by BTH treated-ToLCNDV inoculated plants as compared to the control plants. Polyphenol oxidase activity was also increased in all

treated plants except BABA treated plants. Among the inducer treated plants, BABA treated plants showed maximum PAL activity. GABA and BTH treated plants showed comparatively less PAL activity.

Among the 'plant extract-treated' plants, *P. betel* treated plants showed Maximum peroxidase enzyme activity followed by *A. indica* treated plants. Treated-inoculated plants also showed significant increase of enzyme activity. Among the experimental plants, *A. indica* leaf extracts treated-inoculated plants showed highest enzyme activity followed by *C. sinensis* treated-inoculated plants. Botanical plant extracts also increased the level of β -1,3-glucanase activity in treated plants. Among the treated and treated-inoculated plants, *C. sinensis* treated and treated-inoculated plants showed maximum enzyme activity followed by *P. betel* treated and treated-inoculated plants. PAL enzyme activity was also studied after exogenous application of the plant leaf extracts. Here in this study *A. indica* leaf extract treated and treated-inoculated plants showed highest enzyme activity than the control. *Piper betel* treated-inoculated plants also showed an increased level of PAL activity. PPO activity was also significantly increased in the plant extract treated and treated-inoculated experimental plants of this study. *B. diffusa* treated and treated-inoculated plants showed highest enzyme activity followed by *P. betel* treated and treated-inoculated plants.

Some of the new findings are (i) New host (tomato) infected by *Radish leaf curl virus*, (ii) New host (Cucumber) infected by *Papaya leaf curl virus* and *Tobacco curly shoot virus* (iii) *Tomato leaf curl New Delhi virus* infecting cucumber is being reported for the first time from this region and (iv) betasatellites of begomovirus isolate like *Tomato leaf curl betasatellite* infecting tomato plants along with their recombination events. Genetic variability of the isolates was also studied. Finally management of the begomoviral disease caused by *Tomato leaf curl New Delhi virus* has been done by four chemical inducers and five botanical leaf extracts. Some of the chemical and botanical inducers showed significant disease reduction.