Abstract

RNA viruses are the most diverged family among the plant viruses. Although every year high amount of crop loss have been experienced in India due to virus attack, the data from north-east Indian plains are scanty. The objectives of the present study mainly deal with a thorough survey followed by detection of some RNA viruses infecting economically important crops in the study area and development of eco-friendly management strategies. In the present study, different crop growing fields of north-east Indian plains (plains of northern West Bengal, Assam and Tripura) were surveyed for viral disease symptoms which showed occurrences of leaf mosaic, leaf curling, leaf deformation, shoe string, blistering on leaves, vein yellowing, yellow mosaic, leaf puckering, lamina distortion, persistent red pigmentation on older leaves and ringspot on fruits. About 20-85% disease incidence was observed in different fields. Altogether 52 diseased leaf samples were collected from 11 different crops (papaya, bottle gourd, ash gourd, cucumber, common bean, potato, field bean, coriander, chili, tomato and garden rose) for virus identification. All the samples were tested through reverse transcription-PCR (RT-PCR) using three different primer sets specific for the amplification of *Potyvirus* coat protein (CP) gene and the RNA dependent RNA polymerase (RdRP) gene from *Potexvirus* and *Rose rosette virus* (RRV). Among the 52 samples, 16 samples were found positive for the presence of *Potyvirus*, 4 samples were found positive for *Potexvirus* and 2 samples were positive for RRV.

Mechanical sap transmission studies with the virus samples in respective hosts showed that the *Potyvirus* (papaya) and *Potexvirus* (bottle gourd) was sap transmissible. However, RRV was not sap transmissible. Transmission electron microscopy (TEM) of the infected papaya samples showed the presence of ~700 nm long filamentous rod-shaped particle which was identified as *Potyvirus*. Similarly TEM of infected bottle gourd leaf samples revealed presence of *Potexvirus* which appeared as 500-700 nm long filamentous rod-shaped particles. Rose leaf tissues showed the

presence of round membrane-bound structures of 120-150 nm diameter, known as double membrane bodies (DMBs) that was identified as *Emaravirus*.

The amplified DNA obtained from diseased leaf samples by RT-PCR was cloned into pGEM-T vector in *E. coli* JM109, sequenced and the sequences were submitted to GenBank. Two *Potyvirus* amplicons that were initially sampled from very close locations of same host were not sequenced. The sequences were subjected to BLAST searches and matched with available data in the GenBank. Results showed that out of the 14 *Potyvirus*-positive samples, 8 samples were identified as *Papaya ringspot virus* (PRSV) infecting papaya (Accssion Nos. KF906262, KF918720, KF918721, KP238488, KP238489 and KP238490), bottle gourd (Accssion No. KF888662) and ash gourd (Accssion No. KC865305). Five samples were identified as *Potato virus Y* (PVY) infecting tomato (Accssion No. KJ941195), potato (Accssion No. KY751705), bottle gourd (Accssion Nos. MG752891 and MG752892) and cucumber (Accssion No. MG752893). One sample was identified as *Soybean mosaic virus* (SMV) infecting bottle gourd (Accssion No. KJ481802). Four *Potexvirus* positive samples were identified as *Lagenaria mild mosaic virus* (LaMMoV) infecting bottle gourd (Accssion Nos. MG752887, MG752888, MG752889 and MG752890). Two RRV positive samples were confirmed as *Rose rosette virus* (RRV) infecting garden roses (Accssion Nos. KT223518 and KX013764).

Two new viruses *viz.*, *Rose rosette virus* infecting garden roses and *Lagenaria mild mosaic virus* infecting bottle gourd have been reported for the first time from India. *Soybean mosaic virus* is being reported to infect bottle gourd. Phylogenetic analysis of the sequences in comparison to other sequences in database showed that papaya-infecting isolates of the present study showed close relationship among them and they clustered together with other papaya-infecting PRSV isolates. The cucurbit-infecting PRSV isolates of the present study also clustered together with other cucurbit-infecting PRSV isolates. In most of the cases papaya-infecting PRSV and cucurbit-infecting PRSV formed separate clusters and the papaya-infecting

PRSVs arose from cucurbit-infecting PRSVs. In the phylogenetic tree of PVY, potato- and tobacco-infecting PVY clustered together. However, the PVY isolates of the present study infecting different hosts showed close relationship among them and formed a separate cluster regardless of their hosts. Phylogenetic tree of SMV revealed that the isolate of the present study clustered with some soybean-infecting SMVs. All the LaMMoV isolates in the phylogenetic tree clustered together whereas different *Potexvirus* groups formed separate clusters. LaMMoV showed close relationship with *Pepino mosaic virus*. During phylogenetic analysis of RRV, the present isolate clustered with the RRV group comprising of five RRV isolates worldwide, whereas other viruses of the family produced separate clusters.

Further, a study on codon usage analysis showed that the codon usage pattern of all the viruses of the present study seemed to be influenced by mutational bias along with other factors like translational selection, gene length and gene function because the GC3 values were found to lie below the continuous curve of ENC. However, in all the four viruses the contribution of natural selection pressure was more than that of mutational pressure. The effect of natural selection pressure was highest in CP gene of PRSV followed by RdRP gene of RRV, CP gene of PVY and RdRP gene of LaMMoV. From the results it was also observed that different forces of selection pressure acted on each codon position of these two genes of the four viruses of the present study as the R^2 value of all the 4 viruses were close to 0. However, SMV was not included in this study because only one sequence of *L. siceraria* infecting SMV was available. The relative synonymous codon usage (RSCU) pattern of CP genes of three potyviruses *viz.*, PRSV, PVY, SMV and RdRP genes of LaMMoV, RRV were also calculated and were compared with their respective hosts. More than 68% more preferred codons of all the viruses matched with their respective hosts. This suggested host specific codon adaptation of the viruses for successful invasion, adaptation and evolutionary fitness towards their hosts.

Insect vectors present in the study area were also considered as one of the major factors for successful disease establishment. Thus, a thorough survey was also made in search of the major insect vector that was responsible for disease spread. One aphid, *Myzus persicae* was found to be associated with several diseases in the present study area. The presence of the *Potyvirus* within the vector was also detected through RT-PCR. Disease transmission studies with aphids in bottle gourd showed successful induction of leaf curl caused by PRSV in healthy plants.

Finally for the management of the virus disease caused by PRSV, some known chemical inducers (Benzothiadiazole, α-aminobutyric acid and γ-aminobutyric acid) as well as some plant leaf extracts (*Azadirachta indica*, *Bougainvillea spectabilis*, *Clerodendrum infortunatum*, *Lantana camara* and *Camellia sinensis*) were used as foliar sprays on bottle gourd. Significant disease reduction was evident in most cases. Thus, the present work reports the incidence of different RNA viral diseases in north-east Indian plains and the management of a major viral disease of north-east India.