5. Discussion

Virus infections in plant need to be detected early and fast using inexpensive diagnostic methods in order to successfully combat these ubiquitous pathogenic agents. This area of virology has really seen massive progress with the use of simple serological and PCR based assays that resulted in improved capacity for reliable diagnosis of viral diseases of plants (Lopez *et al.,* 2008; Massart *et al.,* 2014). Recently, high throughput techniques such as next generation sequencing and bioinformatic analysis of sequence data has created a situation where any viral agent may be detected in a host for both known and unknown viruses (Prabha *et al.,* 2013). This has led to major advances not only in identifying the agents and their characterization, but also in the field of viral ecology as well as epidemiology (Massart *et al.,* 2014). However, bioinformatic analysis of viral metagenomes by reference-based approach is still difficult because less than 1% of the extent of viral diversity has been suggested to be explored till date (Pirovano *et al.,* 2015). Besides, phylogenetic distances between viral populations are substantial and viral communities are highly heterozygous because their polymerases particularly in those of RNA viruses are low in fidelity.

In the beginning of the present study, a survey on occurrences of RNA viral diseases in crop plants was conducted in some areas of north-east India including sub-Himalayan West Bengal. The entire eastern Himalayas have been identified a priority Global 200 Ecoregion by WWF (http://web.worldbank.org/archive/website01062/WEB/IMAGES/PAPER_13.PDF, Website reference 5,). The Indian Council of Agricultural Research has identified the region as a centre of rice germplasm. Apart from rice, there are other 35 crops that are cultivated locally some of which are of major economic importance. Although all crops recorded losses due to viral diseases (Pennazio *et al.*, 1999; Savary *et al.*, 2016), not much research has been undertaken on the etiology and control of the diseases of this region. Results of the initial survey comprising of locations in West Bengal, Assam

and Tripura revealed around 20-85% incidence of RNA viral disease in 11 different crops. Symptoms such as leaf mosaic, leaf curling, leaf deformation, shoe string, blistering on leaves, vein yellowing, yellow mosaic, leaf puckering, lamina distortion, 'persistent red pigmentation on elder leaves' and 'ring spot on fruits' were commonly observed.

Papaya ringspot virus (PRSV) infection with characteristic mild mottling, yellow mosaic, vein clearing, leaf blistering, leaf distortion and shoe stringing symptoms on papaya leaves were reported from West Bengal and Sikkim (Jain *et al.,* 2004). Leaf mosaic, leaf blistering and filiformy symptoms on PRSV infected papaya plants were observed by Sharma *et al.* (2005). Association of *Potyvirus* with papaya showing leaf mosaic, leaf deformation and ringspot on fruits was reported by Chittarath *et al.* (2017). Symptoms like leaf mosaic, leaf curling, leaf deformation, shoe string, blistering on leaves, vein yellowing, yellow mosaic, leaf puckering, lamina distortion on papaya plants (Mansilla *et al.*, 2013; Barbosa *et al.*, 2016), tomato (Mathioudakis *et al.*, 2012; Ambros *et al.*, 2017), common bean (Green *et al.*, 2017) and cucurbits (Gonzalez *et al.*, 2002; Singh *et al.,* 2003; Piche *et al.*, 2004; Babu *et al.,* 2012; Mohammed *et al.*, 2012; Lobin *et al.*, 2015) were reported by several researchers worldwide. *Potexvirus* was also reported to be associated with leaf mosaic and mottling symptoms of bottle gourd in Myanmar (Kim *et al.*, 2010). Mild mosaic, blistering and malformation on leaves have been reported to be caused by *Potexvirus* (Purcifull *et al.*, 1988; 1999; Adams *et al.*, 2004; Verchot-Lubicz, 2005; Verchot-Lubicz *et al.*, 2007; Massumi *et al.*, 2014). Rose rosette disease with symptoms like leaf curling, leaf puckering, deformation of flower, lamina distortion, 'persistent red pigmentation on older leaves' have been observed in number of cases (Conners, 1941; Thomas and Scott, 1953; Laney *et al.*, 2011; Babu *et al.*, 2014; Baker *et al.*, 2014; Di Bello *et al.*, 2015).

Careful observations of the visual symptoms of diseases in plants provide some indications on the type of viruses that have potentially caused the diseases. Therefore, relying on visual observation of

characteristic symptoms of RNA viral attack, primer sets were selected for RT-PCR based detection of virus occurrences by targeting specific regions of the genomes of *Potyvirus*, *Potexvirus* and RRV. PCR or RT-PCR is a technique for quick synthesis of specific DNA sequence to produce millions of copies (amplicons) based on DNA (PCR) or RNA template (RT-PCR) that is used extensively for diagnostic purposes. RT-PCR method is followed for detecting RNA viruses. Here, reverse transcriptase (RT) enzyme is used to synthesize a cDNA strand complementary to the virus. Subsequently, a targeted sequence from this cDNA is amplified with oligonucleotide primers complementary to flanking part, which is then extended by a thermostable DNA polymerase following a series of denaturation and extension steps that causes exponential increase in the target DNA. Presence of the specific target sequence is normally detected by fluorescent labeling following gel electrophoresis. Size of amplicon is measured in order to eliminate any probable non-specific amplification. (Webster *et al.,* 2004).

In this study, altogether 52 samples were screened through RT-PCR using three different primer sets *viz.,* 'CPuP and P9502' (potyviruses), 'Potex5 and Potex2RC' (potexviruses), and RRV For' and 'RRV Rev' (*Rose rosette virus*, RRV). Results showed 16 samples to be positive for *Potyvirus*, 4 samples to be positive for *Potexvirus* and two samples to be positive for RRV which was evident from the characteristic amplicon sizes of 650 bp (Singh *et al.*, 2007; van der Vlugt *et al.*, 1999), 577 bp (Kim *et al.*, 2010; van der Vlugt and Berendsen, 2002) and 375 bp (Di Bello *et al.*, 2015; Babu *et al.*, 2014; Baker *et al.*, 2014; Laney *et al.*, 2011) respectively. The north-east Indian plains exhibit a rich diversity in the different types of crops grown here. A thorough literature study was undertaken to find reports of viral diseases of crops grown in this region. However, almost no disease incidence has been recorded till date. One report from adjoining West Bengal described the detection of *Potyvirus* in papaya through RT-PCR using primer sets amplifying nuclear inclusion b and coat protein genes (Jain *et al.* 2004).

In the current study, artificial inoculation of healthy papaya plants with *Potyvirus* infested sap obtained from naturally affected plants showed typical yellow mosaic symptoms on leaves after 15 days. Presence of virus could be confirmed by RT-PCR. Several other authors have also reported successful mechanical sap transmission of different potyviruses (Li *et al.*, 2016; Singh *et al.,* 2003; Reddy, 2000). Singh *et al.* (2017) reported that mechanical sap transmission of PRSV in papaya and *Chenopodium* showed 100% infection. In another study, PRSV inoculation to zucchini, watermelon, rockmelon and honeydew melon gave positive result with variable symptom severity (Maina *et al.*, 2017). Similarly, leaf curl symptoms were found to develop on healthy bottle gourd plants that were inoculated mechanically with *Potexvirus* infested sap prepared from natural disease samples during this study. Initial symptoms gradually developed into stunted growth, blistering and leaf distortion and presence of virus was confirmed by RT-PCR. Kim *et al.* (2010) also reported *Potexvirus* transmission from infected bottle gourd plants through sap and they also reported development of mosaic and yellowing in healthy bottle gourd and ash gourd plants. In contrast, mechanical sap inoculation with RRV infested samples did not induce any disease symptoms in experimental plants during the present study. Confirmatory RT-PCR also yielded negative result. This showed that the current RRV isolates were not transmissible mechanically. This was expected as there are no reports of successful artificial sap transmission of RRV in the literature. According to Di Bello *et al.* (2015), RRV could be transmitted by its vector as is the case for other members of the genus *Emaravirus*.

Transmission electron microscopic (TEM) studies were undertaken to detect characteristic virus particles in infected leaf samples and study their morphology. Results revealed the presence of around 700 nm long filamentous rod-shaped particles in the preparation from infected papaya leaf. These particles were found to be comparable with the particles reported by Jiang *et al.* (2017) and Rezende *et al.* (2016). Thus, our particles were identified as *Potyvirus* particles. TEM studies with infected bottle gourd leaf samples showed presence of 500-700 nm long filamentous

rod-shaped particles which were identified as *Potexvirus*. Similar particles of *Potexvirus* have also been reported by several researchers (Alvarez-Quinto *et al.,* 2017; Arkhipenko *et al.,* 2017; Montero-Astúa *et al.,* 2017; Park *et al.,* 2017; Therien *et al.,* 2017 and Kim *et al.,* 2010). Thus our findings are in good agreement with that of earlier workers. TEM studies with the infected rose leaf tissue samples showed the presence of round membrane-bound structures of 120-150 nm diameters, known as double membrane bodies (DMBs). The presence of DMBs was observed to be a characteristic feature of several *Emaravirus* infected samples (Mielke-Ehret and Muhlbach, 2012; Castellano *et al.,* 2007; Appiano *et al.*, 1995; Martelli *et al.,* 1993; Plavsic and Milicic, 1980; Bradfute *et al.,* 1970). Thus, presence of characteristic DMBs further confirmed the presence of RRV in our samples.

The accurate identification of viral phytopathogens is essential for virtually all aspects of plant pathology from fundamental research on the biology of the pathogens to the control of the diseases they cause. RT-PCR is a popular diagnostic tool for RNA viral diseases in plants. Once sequenced, the PCR amplicons are analyzed for similarity to other known sequences, and the identity of the organism can be suggested based on phylogenetic relatedness (Amann *et al.*, 1995; Blanchard *et al.*, 2009; Verdu *et al.*, 2012). In the current study, RT-PCR was used at first to detect specific viruses in symptomatic plant tissue samples by analyzing the PCR products on agarose gels. These amplicons were then purified, cloned, transformed into *E. coli* and finally sequenced. Altogether 20 sequences were obtained which were then subjected to phylogenetic analysis. Two *Potyvirus* amplicons that were initially sampled from very close locations of same host were not sequenced. Sequence data for virus identification depends on the identification of homologies with already discovered viral sequences. Presently the amount of public databases of genomic sequences is growing at a rapid pace which has paved the way for a smooth and successful diagnostic (Massart *et al.,* 2014).

Out of the fourteen isolates of the genus *Potyvirus* identified in the current study, eight were identified as *Papaya ringspot virus* (PRSV), five were identified as *Potato virus Y* (PVY) and one was identified as *Soyabean mosaic virus* (SMV). Among the PRSV isolates, six were detected in papaya, one in bottle gourd and one in ash gourd. This finding was based on the fact that the sequences showed 95-99% sequence similarity with other PRSV sequences upon BLASTn analysis. PRSV has been detected worldwide through RT-PCR followed by sequencing in many crops by several workers such as in bitter gourd by Zhu *et al.* (2016), in cucumber, pumpkin, rockmelon, honeydew melon and watermelon by Baek *et al.* (2017) and Maina *et al.* (2017). Mederos *et al.* (2017) identified PRSV from sponge gourd. Several other workers (Chittarath *et al.*, 2017; Singh *et al.*, 2017; Rodriguez-Martinez *et al.*, 2015) have also identified PRSV from papaya following RT-PCR technique.

On the basis of 93-98% sequence similarity, *Potato virus Y* (PVY) was detected in five disease samples. These were obtained from tomato, potato, bottle gourd (two samples) and cucumber. Sequences from PVY infecting potato (Funke *et al.*, 2017, Chikh-Ali *et al.*, 2016a; Chang *et al.*, 2015; Schubert *et al.*, 2015), cape gooseberry (Green *et al.*, 2017), and tomato (Chikh-Ali *et al.*, 2016b; Hasiow-Jaroszewska *et al.*, 2014) have been reported from several continents of the world. Thus our studies are in agreement with the previous workers.

One particular bottle gourd sample in the present study was found to be infected by *Soybean mosaic virus* (SMV). The sequence obtained showed 94% similarity with other SMV sequences submitted in GenBank. SMV was first described infecting *Glycine max* in the United States (Gardener and Kendrick, 1921). SMV was also reported to infect *Nicotiana benthamiana, Vigna unguiculata, Cucumis sativus, Dolichos lablab, Phaseolus vulgaris, Datura stramonium* and *Chenopodium amaranticolor* (Jiang *et al.*, 2017; Nandakishor *et al.*, 2017). However, to the best of our knowledge, this is the first report of SMV infection in bottle gourd in the world.

Four *Potexvirus* positive samples (bottle gourd) in the present study contained the partial RNA dependent RNA polymerase (RdRP) gene of *Lagenaria mild mosaic virus* (LaMMoV). The sequences obtained were found to be 78-79% identical with LaMMoV during BLASTn analysis. In 2010, Kim *et al.* reported LaMMoV as a new member of *Potexvirus* infecting bottle gourd from Myanmar. *Potexvirus* consists of a positive-sense RNA encoding five ORFs (one RdRP gene, three TGB genes and a CP gene (Adams *et al.*, 2004; Verchot-Lubicz *et al.*, 2007; Kim *et al.*, 2010). RdRP is the only protein in the non-defective positive strand RNA viruses which is universally encoded. Therefore, in order to resolve the evolutionary framework in this huge virus class, phylogeny of RdRP is the main determinant (Martelli *et al.*, 2007).

Partial RdRP gene sequences obtained from two RRV positive leaf samples of infected garden rose plants in the present study showed 98% sequence similarity with other RRV RdRP sequences submitted in public databases. RRV belongs to the genus *Emaravirus* which was established comparatively recently. RdRP genes from this genus show significant conserved sections due to functional domains. Therefore, amino acid identities of 45-68% and sequence similarities of 56-83% were observed among the different viruses belonging to this genus such as EMARaV, FMV, RLBV and RRV (Mielke-Ehret and Muhlbach, 2012). Laney *et al.* (2011) was the first to sequence four segments of RRV genome infecting roses of eastern USA. Babu *et al.* (2014) also sequenced four RRV isolates infecting roses in gardens of Florida.

Viruses with RNA genome are known to evolve rapidly. This is thought to be due to high mutation rates, rapid replication and large population sizes. In addition, recombination plays a significant role in influencing genetic diversity in at least some of the RNA viruses infecting plants including many of the different positive sense RNA viruses. However, the rates of recombination have been found to vary widely among taxa which may be due to natural selection. The benefits of recombination due to selection are obvious. This leads to rapid formation of advantageous

genotypes and efficient removal of deleterious mutations through recombination with error-free regions of co-infecting genomes (Chare and Holmes, 2006).

Phylogenetic analysis was done during the current study by comparing the generated coat protein sequences of the PRSV infecting different host plants with the other similar PRSV sequences obtained from GenBank. The sequences from papaya showed close relationship among themselves and they clustered together with other papaya infecting PRSV sequenecs. This host specific clustering was observed in bottle gourd also. However, the ash gourd infecting PRSV sequence was positioned alone when compared with the other ash gourd infecting genomes but showed close relationship with the bottle gourd infecting sequence generated in this study. Prucifull *et al.* (1984) proposed that PRSV formed two major biotypes: biotype P and biotype W. Biotype P was able to infect both papaya and cucurbits, where as biotype W can infect only cucurbits but not papaya (Prucifull *et al.*, 1984). In this study, the PRSV CP isolates infecting papaya and cucurbits formed different clusters (except in one sub-cluster where papaya-infecting PRSV clustered with *C. pepo*-infecting PRSV). This indicated indicated a significant difference between papaya-infecting and cucurbit-infecting PRSV groups and they presumably belonged to the different biotypes. The phylogenetic tree further revealed that papaya-infecting PRSV (PRSV-P) presumably arose from cucurbit-infecting PRSV (PRSV-W). Evolution of PRSV-P from PRSV-W has been reported by several researchers (Bateson *et al.*, 1994; 2002; Gibbs *et al.*, 2008; Olarte Castillo *et al.*, 2011; Maina *et al.*, 2016). Further, Jain *et al.* (1998; 2004) proposed that the Indian PRSV isolates were the most divergent virus group worldwide and PRSV originated in the Indian subcontinent. During phylogenetic analysis, formation of several small clusters within the papaya-infecting PRSVs and cucurbit-infecting PRSVs might be due to the diversified nature of the PRSV-CP.

Phylogenetic analysis of the present five PVY sequences along with other PVY sequences obtained from public database revealed close

relationship among them and clustered together. Potato- and tobacco-infecting PVY clustered together, whereas different PVY isolates infecting different other hosts formed separate clusters. Although chili-infecting PVY isolates formed two distantly related clusters in the phylogenetic tree, one of such clusters showed close relationship with some *Physalis*-infecting PVY. Quenouille *et al.* (2013) suggested that PVY evolution was influenced by increasing host range in a way that involved interaction with new hosts. High recombination frequency between different strains of PVY also caused diversified clusters as evidenced from several recombinant PVY strains (Green *et al.*, 2018; Chikh-Ali *et al.*, 2016a; Schubert *et al.*, 2015). 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. International trade of infected planting material has impeded the effect of continental origin of PVY in Europe, Japan, South Africa and North America (Quenouille *et al.,* 2013). But, still the effect of geographical origin had a major influence in shaping PVY diversity (Cuevas *et al.*, 2012; Quenouille *et al.,* 2013). The relatively less international trade of crops in present study area may be a probable reason behind genetic relatedness among our PVY isolates.

Phylogenetic tree of the SMV isolates showed that soybean-infecting SMVs formed several small clades or sub-clusters. Frequent recombination events in PVY caused diverse phylogeny of the virus even among the soybean-infecting SMVs (Zhou *et al.*, 2015). *P. edulis*-infecting SMVs and *V. unguiculata*-infecting SMVs formed separate clusters in the phylogenetic tree and both the clusters have originated from soybean-infecting SMVs. The isolate of the present study also originated from soybean-infecting SMVs. According to Gibbs *et al.* (2008) SMV originated in China within its host soybean and BCMV acted as minor parent for recombination in SMVs. From the present study it seemed that this recombination might contribute to increased host range of the virus.

Phylogenetic analysis of *Potexvirus* isolates showed that all the current LaMMoV isolates clustered together whereas different *Potexvirus*

groups formed separate clusters. LaMMoV was established as a distinct species of *Potexvirus* (Kim *et al.,* 2010). Although complete genome sequence of LaMMoV showed close relatedness with *Papaya mosaic virus* (PapMV) and *Alternanthera mosaic virus* (AltMV) (Kim *et al.,* 2010), but in the present study, LaMMoV showed close relationship with *Pepino mosaic virus* (PepMV) among the *Potexvirus* group when compared using RdRP gene sequence.

During phylogenetic analysis of RRV isolates in comparison with other viruses of the genus *Emaravirus*, the isolates of the present study clustered with the group comprising of five RRV isolates worldwide, whereas other viruses of the genus produced separate clusters. In 2011, Laney *et al.* sequenced RRV genome and it was established as a member of *Emaravirus* group that was closely associated with EMARaV.

Several factors such as t-RNA abundance (Kanaya *et al.*, 2001); mRNA and protein structure (Knight *et al.*, 2001); random genetic drift (Bulmer, 1991; Sharp and Li, 1986a, 1986b); replicational, transcriptional, and translational bias (Hershberg and Petrov, 2008); and other environmental factors (Behura *et al.*, 2013) play vital roles in shaping codon usage pattern in different organisms. According to Wright (1990) and several other scientists (Su *et al.*, 2017; Belalov and Lukashev, 2013; Zhang *et al.*, 2011; Xu *et al.*, 2008; Adams and Antoniw, 2004), if the GC3 content of the gene falls on the continuous curve of ENC, the codon usage pattern is constrained only by mutational bias. However, if the codon choice was influenced by other factors also, *viz.*, translational selection, gene length and gene function, along with mutational bias, the values would lie below the standard curve (Adams and Antoniw, 2004; Wright, 1990). It seemed that codon usage pattern of all the viruses of the present study was also influenced by other factors along with mutational bias because the GC3 values were found to lie below the continuous curve of ENC.

In neutrality plot analysis the slope of regression close to 1 (both positive and negative value) indicated strong contribution of mutational

pressure whereas, the same close to 0 (both positive and negative value) indicated strong contribution of natural selection in shaping codon usage pattern (Zhao *et al.*, 2016; Wei *et al.*, 2014; Xu *et al.*, 2008). In the present study, the CP gene of PVY and RdRP gene of LaMMoV were more influenced by mutational pressure (40% and 33.3% respectively), whereas, the CP gene of PRSV and RdRP gene of RRV were less affected by mutational pressure (5.4% and 5.7% respectively). 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 (94.6%) followed by RdRP gene of RRV (94.3%), CP gene of PVY (60%) and RdRP gene of LaMMoV (67.7%). According to Su *et al.* (2017) and Zhao *et al.* (2016) significant correlation between GC3 and GC1/2 (R^2 close to 1) indicates the similar force of selection on each codon position (1^{st}, 2^{nd} and 3^{rd}). From the results it appears 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.

Xu *et al.* (2008) proposed that the selection pressure has a major role in shaping mutational bias, as the direct change in the nucleotide sequences may be harmful for the virus itself. Stoletzki and Eyre-Walker (2007) also proposed that the conserved genes and coding sequences show higher codon bias. A relationship between the codon preferences of viruses in the same family or genus, regardless of host or genomic nucleotide content is reported previously by Cardinale *et al.* (2013). In this study, from the correspondence analysis it was obvious that most of the genes were distributed along the first axis *i.e.*, axis 1, indicating a common codon usage pattern (Sharp *et al.*, 1988).

Being obligate parasite, codon adaptation of the viruses to different hosts is one of the important factors for host-pathogen co-evolution. Cost effective expression in the hosts often leads to modification of genes in certain organisms, which include modification of translation initiation region of the genome, redesigning of mRNA structural elements and biased

preferences to some codons over other synonymous codons (Gustafsson *et al.*, 2004). According to Belalov and Lukashev (2013) the dinucleotide composition at 2nd and 3rd codon position also had an effect on codon usage pattern of the viruses. Comparison between *Zika virus* (ZIKV) and its hosts revealed that both the virus and the hosts followed similar codon usage pattern and host's selection pressure may affect the virus codon usage pattern to adapt and replicate efficiently within the hosts. The variation of host and virus codon usage might arise due to the host's defense mechanism (Wang *et al.*, 2016c). Virus codon usage was proposed to be positively correlated with tRNA abundance in the host (Ikemura, 1985; Kanaya *et al.*, 1999; Quax *et al.*, 2015). Quax *et al.* (2015) further proposed that codon usage pattern of the viruses were meant to be biased for increasing translational rate within its hosts. Viruses that infect bacteria were markedly biased towards their host in terms of codon usage pattern and were less related to the non-hosts (Bahir *et al.*, 2009). Codon usage comparison between prasinoviruses and its host, *Ostreococcus tauri* revealed that the bias in the viral gene was positively correlated with gene expression level within its host. Optimum codons in the viruses were optimized by the relative abundance of host t-RNA. Even the viral t-RNA genes complemented the host t-RNA pool (Michely *et al.*, 2013). Codon usage pattern in bacteriophages were high due to selection pressure and capsid coding genes along with the genes related to interaction with host, virion morphogenesis and DNA binding were mostly affected by adaptive pressure (Carbone, 2008). *Citrus tristeza virus* (CTV) evolution within citrus hosts was also reported to be driven by the host codon preferences to some extent along with recombination and gene flow (Cheng *et al.*, 2012). In the present study 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 are one of the sources of virus transmission. They have profound effect on creating epidemics; hence, their role in virus transmission is of much concern. According to Groen *et al.* (2017) one

vector might be able to transmit more than one type of viruses. A thorough survey was made in the fields of north-east Indian plains to study the major insect vectors associated with the RNA viral diseases in different crops. One aphid, *Myzus persicae* was found to be associated with several diseases in the present study area. Transmission of different potyviruses through the vector, *Myzus persicae* was reported by several workers (Reddy, 2000; Giampan and Rezende, 2001; Reddy *et al.*, 2007; Mondal and Gray, 2017). Potyviruses are transmitted mechanically and also by aphids in a non-persistent manner (Gibbs *et al.*, 2003; Poutaraud *et al.*, 2004; Fauquet *et al.*, 2005). More than 200 species of aphids spread potyviruses and most are from the subfamily Aphidinae (genera: *Macrosiphum* and *Myzus*). *Myzus persicae* (family: Aphididae), known as the green peach aphid, acts as a vector for the transmission of potyviruses and other mosaic viruses.

The presence of the virus within the vector was studied through RT-PCR. In the vector transmission study, symptoms were found to appear like those of naturally infected bottle gourd plants. The successful transmission of PRSV to *Chenopodium amaranticolor, Chenopodium quinoa, Cucumis sativus, Cucurbita pepo, Cucurbita maxima* and *Citrullus vulgaris* was confirmed by Makkou and Lesemann (1980) using *Myzus persicae* in stylet borne manner. Reddy (2000) reported that *Myzus persicae* showed 90% transmission of the virus from papaya to papaya. *M. persicae* successfully transmitted PVY^{O}, $PVY^{N:O}$ or PVY^{NTN} from infected potato leaves to healthy plants (Mondal and Gray, 2017).

Occurrence of plant diseases are a major threat to sustainable agriculture and food safety. Several disease control strategies have, therefore, been adapted to maintain crop-yield. A study on disease management is, therefore, an important part of any plant pathological studies. Nature has conferred upon plants several lines of defense against invading pathogens including preformed barriers and inducible systems. Inducible responses occurs as a result of recognition by plant cell receptors of elicitor molecules derived either from the pathogen or from exogenously

applied molecules and eventually triggers a signal-transduction cascade that causes synthesis of signalling compounds which spread systemically to tissues away from the infection site leading to development of overall resistance against pathogenic invaders (Hahlbrock *et al.,* 2003; Maffei *et al.*, 2012; Mishra *et al.*, 2012). The application of elicitor molecules is now an established mode of plant disease management which allows induction of systemic resistance in plants thereby providing protection against multiple pathogens.

In the present study three inducer chemicals *viz.* Benzothiadiazole (BTH), α-aminobutyric acid (AABA) and γ-aminobutyric acid (GABA) were tested for their ability to elicit systemic resistance in bottle gourd against PRSV. Results revealed that GABA was the best elicitor which reduced the disease index to considerable extent followed by BTH and AABA.

BTH is a functional analog of salicylic acid (SA) and is a well known resistance inducer molecule (Wang *et al.*, 2015; Feliziani *et al.*, 2013; Wendehenne *et al.*, 1998). Besides BTH, AABA and GABA are the non-protein amino acids that are also well known inducer molecules (Li *et al.,* 2017; Fu *et al.*, 2016; Usha *et al.*, 2016). All these molecules were reported to be involved in inducing systemic acquired resistance (SAR) in plants (Sabir Tariq *et al.*, 2017; Fu *et al.*, 2016; Wang *et al.*, 2015; Trejo-Saavedra *et al.*, 2013). From the present study it was observed that PRSV was the major pathogen in the present study area and cucurbits were most susceptible to different RNA viral diseases. Hence, bottle gourd was selected for the management study against PRSV.

Effect of AABA and GABA in inducing resistance in plants against several fungal and bacterial pathogens have been reported so far (Li *et al.,* 2017; Fu *et al.*, 2016; Usha *et al.*, 2016; AL-Quraan *et al.,* 2015; Ramesh *et al.,* 2015). Exogenous application of SA and BTH on tomato against *Cucumber mosaic virus* (CMV) showed reduced disease severity and delayed symptom development and caused alteration in peroxidase, superoxide dismutase, catalase and ascorbate peroxidase level (Sabir Tariq *et al.*, 2017). Application of BTH on pepper plant showed resistance against

Pepper golden mosaic virus by inducing SA signaling pathway (Trejo-Saavedra *et al.*, 2013).

Sustainable solutions in agriculture have gained importance due to consumer pressures on environment-friendly alternatives and this has led to a boom in the organic farming sector. Considerable interest has focused on botanical sources for novel plant protection approaches. Plants are a big reservoir of a huge variety of active substances, thus providing a natural and benign alternative to hazardous synthetic chemicals (Slusarenko *et al.*, 2008). In the current study, extract preparations from five different plant species (*A. indica*, *B. spectabilis*, *C. infortunatum*, *L. camara* and *C. sinensis*) were tested for their efficacy in controlling leaf curling disease in bottle gourd caused by PRSV isolates. Results showed that all the tested extracts except *C. infortunatum* reduced disease incidence in comparison to untreated control. Among the different extracts tested, *A. indica* (neem) extract showed maximum efficacy.

Neem has been reported to possess antiviral properties by several researchers. Neem and *Bougainvillea* leaf extracts showed 50-60% disease reduction against *Tobacco mosaic tobamovirus* and *Tomato mosaic tobamovirus* in *Nicotinia glutinosa* (Madhusudhan *et al.*, 2011). Neem leaf extract was found effective in reducing leaf curl and bud necrosis diseases of tomato (Ruth *et al.*, 2016). *A. indica* leaf extract, *Boerhaavia diffusa* root extract, *Clerodendrum aculeatum* leaf extract and *Terminalia arjuna* bark extracts were also reported to reduce viral diseases in watermelon (Sharma *et al.*, 2017). Essential oil of tea plants showed antiviral activity against *Tobacco mosaic virus* in *N. glutinosa* upto 10 days (Bishop, 1995). Thus, our results are in agreement with other findings.

The present work deals with the detection of some RNA viruses causing diseases of some economically important crops in north-east Indian plains and also the eco-friendly management of the major disease in the present study area. These works extended the study of some previous researchers as well as highlighted some new findings such as (i) New host (bottle gourd) of *Soybean mosaic virus* is being reported for the first time,

(ii) *Rose rosette virus* infecting garden roses is being reported first from India and (iii) *Lagenaria mild mosaic virus* infecting bottle gourd is being reported first from India. Codon adaptation of the viruses for successful replication, transcription, translation and disease establishment have been proposed. Finally management of the major viral disease caused by *Papaya ringspot virus* has been done by three known chemical inducers and five plant leaf extracts. Significant disease reduction has been observed by some inducers and botanicals. 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.