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Papaya Ringspot Virus Isolates From Papaya in Bangladesh: Detection, Characterization, and Distribution

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Abstract

Papaya ringspot virus (PRSV) is the major constraint to papaya (*Carica papaya*) production in Bangladesh. Disease symptoms occurred in 90 to 100% of the plants surveyed. Full-length genomes of PRSV strains from severely infected papaya plants were determined using the Illumina NextSeq 500 platform, followed by Sanger DNA sequencing of viral genomes obtained by reverse-transcription PCR(RT-PCR). The genome sequences of two distinct PRSV strains, PRSV BD-1 (10,300 bp) and PRSV BD-2 (10,325 bp) were 74 and 83% identical to each other, respectively, at the nucleotide and amino acid levels. PRSV BD-1 and PRSV BD-2 were 74 to 75% and 79 to 88% identical, respectively, to other full-length PRSV sequences at the nucleotide level. Based on phylogenetic analysis, PRSV BD-2 was most closely related to PRSV-Meghalaya (MF356497) from papaya in India. PRSV BD-1 formed a branch distinct from the other PRSV sequences based on nucleotide and amino acid sequence comparisons. Comparisons of the genome sequences of these two strains with other sequenced PRSV genomes indicated two putative recombination events in PRSV BD-2. One recombinant event contained a 2,766-nucleotide fragment highly identical to PRSV-Meghalaya (MF356497). The other recombinant event contained a 5,105-nucleotide fragment highly identical to PRSV-China (KY933061). The occurrence rates of PRSV BD-1 and PRSV BD-2 in the sampled areas of Bangladesh were approximately 19 and 69%, respectively. Plants infected with both strains (11%) exhibited more severe symptoms than plants infected with either strain alone. The full-length genome sequences of these new PRSV strains and their distribution provide important information regarding the dynamics of papaya ringspot virus infections in papaya in Bangladesh.

Keyword: virus diversity

Papaya (*Carica papaya* L.) is a highly nutritious, economically valuable fruit crop extensively cultivated in Bangladesh (Akhter et al. 2013; Hamim et al. 2014). Native to the Americas, the fruit is consumed fresh, in salads, or with curry, and it is widely used in the leather, pharmaceutical, cosmetic, juice processing, and food industries (Rodríguez et al. 2013). Several pests and diseases affect papaya and can significantly impact production (Hamim et al. 2018a). Plant viruses from diverse genera have been reported to occur in papaya. These include the following: the potyviruses, papaya ringspot virus (PRSV), and papaya leaf distortion mosaic virus (PLDMV); the potexvirus, papaya mosaic virus (PapMV); sobemoviruses, papaya lethal yellowing virus (PLYV); begomoviruses, papaya leaf curl virus (PLCV), and tomato leaf curl virus (ToLCV); and the tospovirus, tomato spotted wilt virus (TSWV) (Hamim et al. 2018a; Raj et al. 2008). Of these, papaya ringspot disease caused by PRSV is the primary constraint to papaya production in Bangladesh (Jain et al. 2004). PRSV outbreaks in papaya were first reported in Bangladesh in 2004 (Jain et al. 2004); since then, disease incidences as high as 100% have been reported (Hamim et al. 2018c; Hamim et al. 2019).

Aphids spread PRSV in a nonpersistent manner, although seed transmission and mechanical transmission also occur (Bayot et al. 1990; Gonsalves et al. 2010). PRSV also has an extended host range that makes disease management challenging, because alternate hosts can act as reservoirs and drive evolution of this virus (Alabi et al. 2017).

The PRSV genome encodes a single large polyprotein. This polyprotein is cleaved by virus-encoded proteases into eight smaller functionally active proteins: P1, HC-Pro, P3, CI, 6K, NIa-Pro, Nib, and CP (Yeh et al. 1992). An additional protein, 'P3N-PIPO', is derived from a different small open reading frame (ORF) that overlaps with the P3 coding region (Chung et al. 2008; Fermin and Randle 2015). The complete genome sequences of 31 PRSV isolates have been reported in Taiwan, Brazil, India, China, Thailand, South Korea, Venezuela, Hawaii, and Mexico (Mishra and Patil 2018; Ortiz-Rojas and Chaves-Bedoya 2017). To date, all full-length genome sequences of PRSV isolates from the Indian subcontinent have been reported in India. Preliminary characterization of PRSV isolates in Bangladesh using sequence comparisons of the partial coat protein (CP) gene indicated that these isolates shared amino acid (aa) sequences 86 to 95% identical to Asian isolates and 83 to 93% identical to American isolates. Amino acid sequence divergence based on the partial CP of PRSV isolates from Bangladesh was up to 14% (Akhter et al. 2013).

In this report, both high-throughput sequencing (HTS) of total RNAs isolated from PRSV-infected papaya and Sanger sequencing of reverse-transcription PCR(RT-PCR) products were used to determine the full-length coding sequences of two PRSV isolates from infected papaya plants in Bangladesh. Specific RT-PCR assays were also used to determine the distribution of two newly identified PRSV strains in Bangladesh.

Materials and Methods

Source of virus material/isolate collection. We collected leaf samples from 118 papaya plants with virus-like symptoms from December 2016 to January 2017. We surveyed locations in eight

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districts of Bangladesh: Pabna (24.070032°N, 89.113417°E); Rajshahi (24.366346°N, 88.642614°E); Chapai Nawabganj (24.607635°N, 88.290519°E); Chandpur (23.348015°N, 90.708153°E); Munshiganj (23.550215°N, 90.534806°E); Gazipur (23.998997°N, 90.419802°E); Tangail (24.643032°N, 90.055994°E); and Mymensingh (24.746026°N, 90.373906°E) (Supplementary Fig. S1). Disease incidence was visually assessed in each of these locations (Hamim et al. 2014). The collected leaves were immediately stored in RNA*later* solution (Qiagen, Valencia, CA) and brought to the plant virology laboratory at the University of Hawaii at Manoa under USDA PPQ 526 permits P526-160413-009 and P526P-16-03662. After arrival at the plant virology laboratory, all samples were stored at −80°C until total RNA extractions were conducted.

Preliminary detection of PRSV. The 118 papaya samples with virus-like symptoms were tested with PRSV-specific single-tube nested PCR (STNP) and enzyme-linked immunosorbent assay (ELISA) (Agdia, Inc.), as previously described (Hamim et al. 2018b). Total RNAs were extracted from leaf samples using the RNeasyPlant Mini Kit (Qiagen, Valencia, CA), and cDNAs were synthesized according to the method described previously (Hamim et al. 2018c). RNA purity as assessed by A260/A280 ratios ranged from 1.9 to 2.2. Previously identified PRSV-infected and PRSV-free leaves were included as positive and negative controls, respectively.

HTS of total RNAs from PRSV-infected composite samples. Eleven leaf samples with virus-like symptoms were randomly selected and divided into two separate composite samples for HTS conducted at Foundation Plant Services (Davis, CA). Composite sample pap-1 consisted of six different papaya samples with virus-like symptoms: CD-141, Gaz-30, Gaz-52, Tang-62, Tang-66, and Tang-72. Composite sample pap-2 consisted of five different papaya samples with virus-like symptoms: CD-112, CD-125, CD-129, Gaz 34, and Gaz-39. The composite samples were prepared using 10-μg aliquots of total RNA from each sample that were then combined. The composite RNA samples were subjected to ribosomal RNA (rRNA) depletion, and a cDNA library was constructed from the RNA template using TruSeq Stranded Total RNA with a Ribo-Zero Plant Kit (Illumina). Sequencing was performed at Foundation Plant Services using the Illumina NextSeq 500 platform, and raw HTS reads were analyzed as described by Al Rwahnih et al. (2018).

RT-PCR sequencing to validate the HTS results. To validate the results of HTS, specific primers (Supplementary Tables S1 and S2) were designed to amplify DNA fragments of the virus genome by RT-PCR. cDNA templates were produced from the isolated total RNA from the HTS samples using Qiagen RNeasy Kits (Qiagen, Germany). Total RNAs extracted from each sample were quantified as described, and cDNAs were synthesized as described previously (Hamim et al. 2018b). The conditions for PCR amplification were as follows: 4 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 50 to 65°C depending on the primers, and 2 min at 72°C; and final elongation at 72°C for 7 min. The PCR products were resolved on 1% agarose gel in 1X TAE buffer for 60 min at 60 V. Gels were stained with ethidium bromide and visualized in a UVP transilluminator. PCR products of the predicted sizes were purified, cloned into the PGEM-T Easy cloning vector (Promega, USA), and sequenced at the University of Hawaii's Advanced Genomic and Sequencing Services and at Genwiz in California (Wang et al. 2017). Sequences were assembled with the Cap3 sequence genome assembly program (http://doua.prabi.fr/software/cap3). Complete genome sequences of all PRSV strains were deposited in GenBank.

Analysis and characterization of the genome sequences of PRSV strains in Bangladesh. We used BLASTn and BLASTx analyses to compare all sequences with those available in GenBank. The ORFs and their predicted aa sequences were analyzed with the ORF finder (https://www.ncbi.nlm.nih.gov/projects/gorf/). Then, we used the sequence demarcation tool (SDT) with default parameters to obtain the specific nucleotide sequence identity of the PRSV strains and compared their sequence to PRSV strains from different countries and hosts (Muhire et al. 2014). Multiple sequence alignment was performed using Clustal W (https://www.ebi.ac.uk/), followed by phylogenetic analysis with MEGA 7.0 (https://www.megasoftware.net/). Phylogenetic trees were constructed by the neighbor-joining method (NJ) and bootstrapped with 1,000 replicates (Kumar et al. 2016; Thompson et al. 1994). Further recombination analyses were performed to identify potential recombinants and likely parental sequences and recombination break points using the RDP4 package (version 4.72) with RDP, GENECOV, Bootscan, Max Chi, Chimara, Siscan, and 3Seq methods (Martin et al. 2010). A multiple comparison corrected *P* value cutoff of 0.05 and default settings were used, and only events detectable by at least four different methods were considered for further analysis (Martin et al. 2010).

RT-PCR screening of field samples. The distribution and frequency of occurrence of the two PRSV strains were determined by RT-PCR with purified RNAs isolated from leaves of symptomatic PRSV-infected papaya plants. Specific diagnostic primers were designed based on the sequence of PRSV strains found in Bangladesh and used for routine diagnosis. Total RNAs were extracted using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA) and the synthesis of cDNAs performed as described. To detect PRSV strains, the thermal cycler program was performed as follows: 94°C for 5 min; 35 cycles of amplification at 94°C for 30 s, 57°C (PRSV BD-1) or 58°C (PRSV BD-2) for 30 s, and 72°C for 30 s; and final extension at 72°C for 10 min.

Results

Preliminary detection of PRSV in papaya plants with virus-like symptoms. During the December 2016 to January 2017 survey in Bangladesh, 90 to 100% of the papaya trees evaluated were severely infected with papaya ring spot disease (Fig. 1). Infected trees had striking symptoms of leaf mosaic, distortion, vein clearing, yellow mottling, brittleness, and water-soaked streaking on petioles and trunks. Papaya trees with severe virus infections also exhibited shoestring-like leaves, occasional systemic necrosis, and wilting. In some orchards, trees were stunted, bushy, and bore no fruit because of extreme disease pressure during early growth stages. Many mature infected plants produced poor-quality fruit with ring-like spots characteristic of disease. The occurrence rates of PRSV-like symptoms by region were as follows: 100% (345/345) in Pabna; 91% (10/11) in Rajshahi; 99% (165/167) in Chandpur; 97% (192/168) in Gazipur; 94% (15/16) in Chapai Nawabgonj; 95% (164/172) in Tangail; 90% (124/137) in Mymensingh; and 94% (89/95) in Munshigonj. For preliminary confirmation of PRSV in papaya, PRSV-specific STNP and ELISA were used (Hamim et al. 2018b; Hamim et al. 2018c). All 118 samples with virus-like symptoms were positive for PRSV according to both tests.

Identification of two distinct PRSV strains: BD-1 and BD-2. HTS performed on an Illumina HiSeq platform generated a total of 80,648,570 and 61,307,471 reads from composite samples pap-1 and pap-2, respectively (Table 1). Following de novo assembly using CLC Bio Genomic Workstation v8.5.1 (Qiagen, Valencia, CA), 134 plant virus contigs were generated from pap-1 and 79 contigs were generated from pap-2. Composite samples pap-1 and pap-2 generated 127 and 74 PRSV specific contigs, respectively. The size of the contigs varied from 200 to 10,282 bp in pap-1 and from 200 to 3,484 bp in

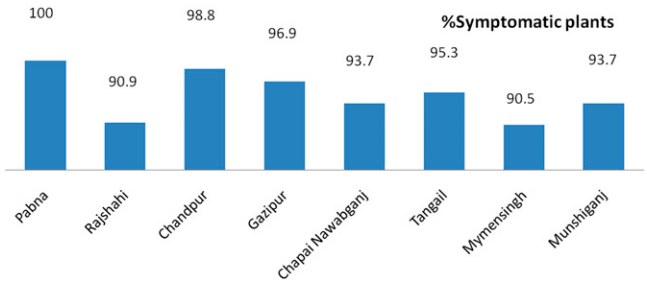

Fig. 1. Incidence of papaya plants with virus symptoms in the surveyed districts of Bangladesh in December 2016 to January 2017.

pap-2. The contigs in pap-1 and pap-2 were, respectively, 52 to 100% and 64 to 100% identical to PRSV isolates for which sequence information was available in GenBank. Distinct strains of PRSV were identified from pap-1 (PRSV BD-1) and pap-2 (PRSV BD-2). The full-length coding sequences of PRSV genomes BD-1 and BD-2 sequences were further verified by primer-walking of PCR-amplified overlapping DNA fragments amplified by virus-specific primers designed from the HTS-derived sequences and Sanger DNA sequencing.

The complete genome sequence of PRSV BD-1 (MH444652) was amplified from papaya sample GAZ-52, and that of PRSV BD-2 (MH397222) was amplified from papaya sample CD-129 (Table 1). PRSV BD-1 (10,300 nt) and PRSV BD-2 (10,325 nt) had polyproteins of 3,339 and 3,342 aa residues, respectively. The cleavage sites were identical in both strains, except for the cleavage sites between P1 and HC-pro and between CI and 6K2 (Fig. 2) (Alabi et al. 2017; Ortiz-Rojas and Chaves-Bedoya, 2017).

Sequence alignment and phylogenetic analyses. Identities between the PRSV BD-1 and BD-2 strains were 74 and 83% at the nt and aa levels, respectively. The genome sequence of PRSV BD-1 were 74 to 75% identical to the complete genome sequence of PRSV strains available in GenBank (Supplementary Table S3). PRSV BD-1 exhibited the lowest identity (82%) with PRSV-Meghalaya (MF356497) at the aa level, whereas the highest aa identity (86%) occurred with PRSV-SK (KY996464). The genome sequence of PRSV BD-2 were 79 to 88% identical at the nt level with sequences of PRSV strains in GenBank (Supplementary Table S4). At the aa level, PRSV BD-2 exhibited the lowest identity (87%) with PRSV-SK (KY996464) and the highest identity (90%) with PRSV-Meghalaya (MF356497). The P1 protein was the most variable in both strains (Supplementary Table S5), whereas 3′-UTR regions of PRSV BD-1 and PRSV BD-2 were highly conserved, with sequences identical to 90 to 96% of other PRSV isolates.

The phylogenetic tree (Fig. 3) was based on nt sequences of the newly identified PRSV BD strains and sequences of other PRSV strains reported worldwide and available in GenBank. The PRSV strains were clustered into two major phylogroups, A and B. PRSV BD-1 was in phylogroup B, but phylogroup A included PRSV BD-2 and several other reported PRSV isolates. Phylogroup A was divided into six minor phylogroups (i to vi). PRSV BD-2 was in the same phylogroup (iv) that contained the Indian PRSV isolate, PRSV-Meghalaya (MF356497). However, two other Indian isolates, PRSV-HYD (KP743981) and PRSVP-DEL (EF017707), clustered in phylogroup iii and were closely related to American and Australian PRSV isolates found in phylogroups i and ii, respectively. All Chinese isolates formed two distinct phylogroups (v and vi) with the Taiwanese and Korean strains. A second phylogenetic tree was constructed (Supplementary Fig. S2) from aa sequences of PRSV strains and other potyviruses. Results of this analysis also supported the phylogenetic relationship of PRSV BD-1 and PRSV BD-2 with other PRSV strains and potyviruses. As in the phylogenetic tree based on nucleotide sequence comparisons, PRSV BD-1 was in a distinct major phylogroup (B), and PRSV BD-2 formed a minor phylogroup (v) with PRSV-Meghalaya (MF356497). Therefore, the phylogenetic analysis of PRSV populations revealed that PRSV BD-1 and PRSV BD-2 were located in two distinct major phylogroups.

Recombination analysis. We used the recombination analysis program RDP4 to screen for possible recombination events within the genome sequences of PRSV BD-1 and PRSV BD-2 (Supplementary Table S6 and Fig. 2) using sequences of other PRSV isolates from GenBank. PRSV BD-1 was not a recombinant strain, but an analysis revealed that PRSV BD-2 was a recombinant that contained two unique recombination events, E1 and E2. Recombination event E1 spanned the region from 1,302 to 4,068 nt, and E2 spanned the region from 4,095 nt to 9,200 nt (Fig. 2). BD-2 was a recombinant of a minor parent, PRSV-XM (KY933061), and a major parent, PRSV-Meghalaya (MF356497). PRSV-Meghalaya (MF356497) contributed the larger region of the sequence to PRSV BD-2 in both recombination events, whereas PRSV-XM (KY933061) contributed a smaller region of sequences in E1 and/or E2.

Distribution of PRSV BD-1 and PRSV BD-2. Two pairs of specific primers, BD-1F (1,779 to 1,802 nt)/BD-1R (2,201 to 2,225 nt) and BD-2 F (1,779 to 1,804 nt)/BD-2R (2,200 to 2,225 nt), were used to develop RT-PCR detection assays for PRSV BD-1 and BD-2, respectively (Supplementary Table S7). PCR assays specific for strains PRSV BD-1 and PRSV BD-2 produced amplicons of the expected size of 446 bp. Sequence analysis of the amplicons from each strain-specific assay confirmed the specificity of the primers for PRSV BD-1 and PRSV BD-2, because all amplicon sequences were identical to the corresponding region of the assembled strain genomes. The incidence rates of PRSV BD-1 among the 118 papaya samples with typical virus symptoms collected from Pabna, Rajshahi, Chapai Nawabganj, Chandpur, Munshiganj, Gazipur, Tangail, and Mymensingh were 6%(1/17), 0 (0/5), 0 (0/4), 33 (11/33), 22 (2/9), 36 (8/22), 0 (0/13), and 0% (0/15), respectively (Table 2). The incidence rates of PRSV BD-2 among the 118 papaya samples with typical virus symptoms collected from Pabna, Rajshahi, Chapai Nawabganj, Chandpur, Munshiganj, Gazipur, Tangail, and Mymensingh were 100 (17/17), 60 (3/5), 75 (3/4), 73 (24/33), 56 (5/9), 68 (15/22), 77 (10/13), and 73% (11/15), respectively (Table 2). In the surveyed districts, the overall percentage of samples that tested positive for PRSV BD-1 was 19% (22/118), and that for PRSV BD-2 was 75% (88/118). PRSV BD-1 had a mean incidence of 12% in all districts sampled, whereas PRSV BD-2 occurred in all districts with an overall average incidence of 73%. In the surveyed districts, 11% (13/118) of the symptomatic papaya plants were infected by both PRSV BD-1 and PRSV BD-2.

Discussion

Virus diseases are a major threat to papaya production worldwide, and several different viruses have been reported. Among these PRSV, which is a dominant and widespread virus, was first reported in Bangladesh in 2004 (Jain et al. 2004). PRSV-like disease

Table 1. Assembly of HTS data from PRSV-infected composite papaya samples in Bangladesh[a]

Composite sample ID	pap-1	pap-2
Total reads	80,648,570	61,307,471
No. of nonvirus contigs	906	1043
No. of plant virus contigs	134	79
No. of PRSV-specific contigs	127	74
Sequence length of PRSV contigs	200–10,282 bp	200–3,484 bp
Percentage of identical matches with reported isolates	52–100%	64–100%
Identified strains	PRSB BD-1 (10,300 bp)	PRSV BD-2 (10,325 bp)

[a] PRSV = papaya ringspot virus, pap-1 = composite sample of papaya samples CD-141, Gaz-30, Gaz-52, Tang-62, Tang-66, and Tang-72; pap-2 = composite sample of papaya samples CD-112, CD-125, CD-129, Gaz-34, and Gaz-39; bp = base pair.

Table 2. Occurrence and distribution of strains PRSV BD-1 and PRSV BD-2 of PRSV sampled from papaya in eight districts of Bangladesh[a]

Districts	No. of plants tested	PRSV BD-1–positive	PRSV BD-2–positive	Combined infection of PRSV BD-1 and PRSV BD-2
Pabna	17	1	17	1
Rajshahi	5	0	3	0
Chapai Nawabganj	4	0	3	0
Chandpur	33	11	24	8
Munshiganj	9	2	5	1
Gazipur	22	8	15	3
Tangail	13	0	6	0
Mymensingh	15	0	8	0
Total	118	22	81	13

[a] PRSV = papaya ringspot virus.

symptoms occurred in 90 to 100% of the plants surveyed in this study. Most of the symptomatic plants were severely affected, with stunted growth and severe reductions in fruit yield and quality.

To detect the presence of different strains of PRSV in Bangladesh and obtain their full-length sequences, we used total RNAs extracted from infected papaya tissue to construct cDNA libraries, followed by HTS and conventional Sanger sequencing (Hamim et al. 2018c). The sequences of two distinct PRSV strains, PRSV BD-1 (10,300 bp) and PRSV BD-2 (10,325 bp), represent the first complete genomes of PRSV strains from Bangladesh. These strains were 74 and 83% identical at the nt and deduced aa levels, respectively. PRSV BD-1 was 74 to 75% and PRSV BD-2 was 79 to 88% identical to other full-length PRSV isolates from around the world. Alignment of the coding regions of the PRSV strains from Bangladesh with the available PRSV genomes in GenBank indicated that the CP regions and 3′-UTRs were relatively conserved and that the P1 regions were the most variable. Based on phylogenetic sequence analyses, PRSV BD-2 was most closely related to PRSV-Meghalaya (MF356497) from papaya in India. However, PRSV BD-1, was on a distinct branch away from the other PRSV sequences based on nt and aa sequence comparisons. PRSV BD-1 was only distantly related to PRSV populations from other countries, thereby supporting the hypothesis that PRSV BD-1 emerged in Bangladesh instead of being introduced from other countries. PRSV BD-2 was phylogenetically close to a PRSV isolate from Meghalay, in northeast India, with which it formed a minor phylogroup based on both nt and aa sequences.

According to the current International Committee on Taxonomy of Viruses (ICTV), the species demarcation criteria for different species within the genus *Potyvirus* requires complete genomic nt sequence identity <76% and aa sequence identity <82% for a distinct species (Wylie et al. 2017). Based on the sequences obtained in this study, PRSV BD-1 and PRSV BD-2 are divergent strains of PRSV at the nucleotide level with identities of 74 to 75% and 79 to 88%, respectively, of the complete viral genome compared with other complete genomes of PRSV worldwide. At the aa level, PRSV BD-1 exhibited 82 to 86% identities and PRSV BD-2 exhibited 87 to 90% identities of other full-length coding regions of PRSV strains reported globally.

Recombination within single-stranded RNA viruses is a major evolutionary means for viruses to adapt to new hosts and environments (Noa-Carrazana et al. 2007), and recombination has occurred frequently in the evolution of potyviruses (Hamim et al. 2018b). A computer analysis detected the likely occurrence of recombination within PRSV BD-2, but not in PRSV BD-1. Comparisons of the genome sequences from the newly identified PRSV strains to other sequenced PRSV genomes detected two putative recombination events

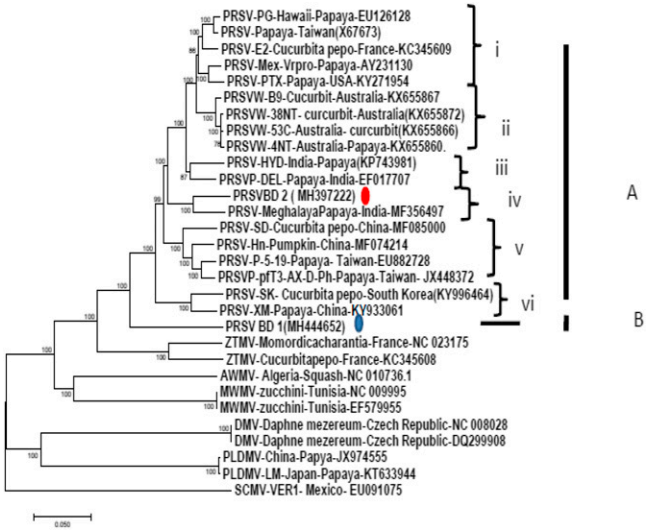

Fig. 3. Neighbor-joining phylogram obtained from the alignment of nucleotide (nt) sequences of PRSV BD-1 and PRSV BD-2 with sequences of other PRSV strains and potyviruses obtained from GenBank. The tree was constructed in MEGA 7.0.14 using Clustal W with 1,000 replicates. Bootstrap values are percentages; only values >75% are shown at the nodes. The tree was rooted with SCMV-Ver1-Mexico nucleotide nt sequence (EU091075). The symbols show two newly sequenced strains from Bangladesh, PRSV BD-1 (blue oval) and PRSV BD-2 (red oval).

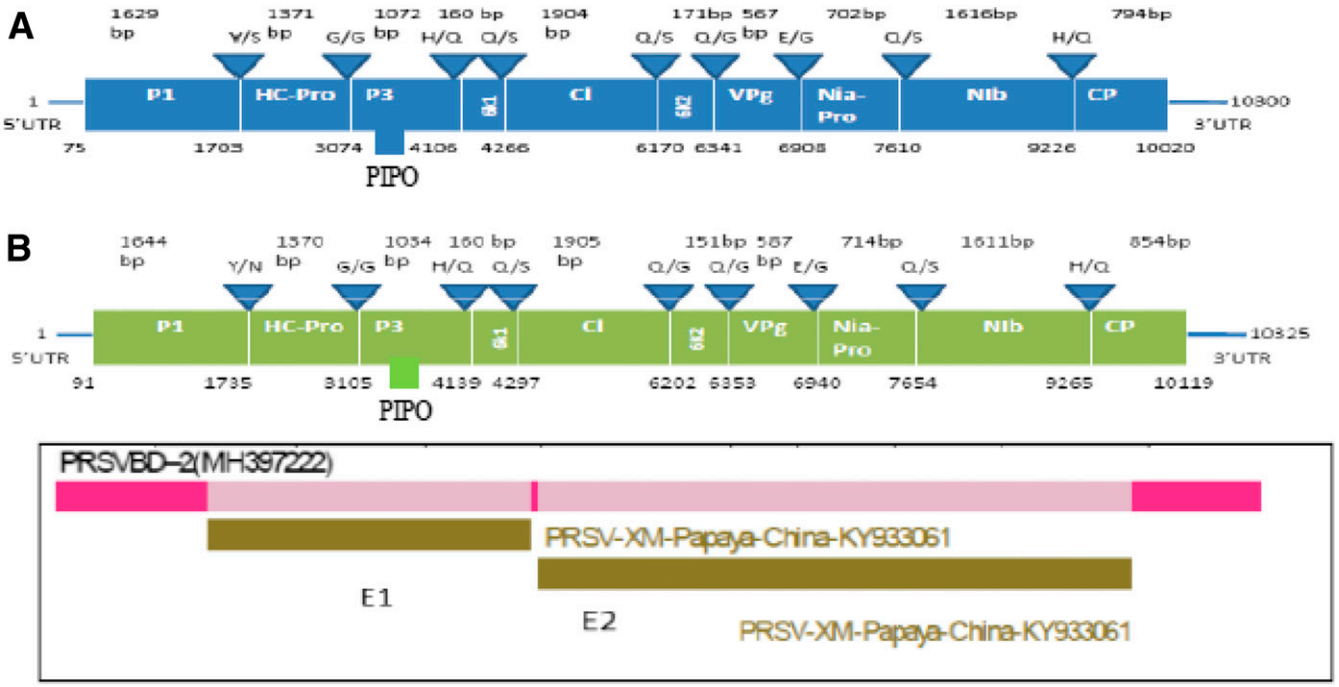

Fig. 2. Genome organization of PRSV BD-1 and PRSV BD-2. **A,** Genome organization of PRSV BD-1 determined from Sanger DNA sequencing of PCR DNA fragments by primer-walking. **B,** Genome organization of PRSV BD-2 determined from Sanger DNA sequencing of PCR fragments by primer-walking. Two recombination events detected in PRSV BD-2 by the RDP4 package: RDP, Maxchi, Chimaera, and 3Seq. Minor parental strain = PRSV-XM-Papaya-China-KY933061, and major parental strain = PRSV-Meghalaya–Papaya-India-MF356497.

in PRSV BD-2 based on the presence of a 2,766-nt fragment that was identical to PRSV-Meghalaya (MF356497) and a 5,105-nt fragment identical to PRSV-China (KY933061). This agrees with the results of a previous study that indicated that the C1 and CP regions in the PRSV genome are prone to recombination events (Noa-Carrazana et al. 2007). Furthermore, PRSV BD-2 was not found to be a parent of any other recombinants. The presence of two recombination events in PRSV BD-2 originating from PRSV strains from India and China may reflect the increasingly frequent exchanges of agricultural products between the two countries and Bangladesh. Our results contribute new knowledge regarding the PRSV genomes that occur in Bangladesh and will facilitate further study of PRSV genetic variations and the development of more accurate diagnostic assays to monitor the spread of this important virus.

The natural spread of PRSV could occur when the annual strong monsoonal winds blow across India toward Bangladesh. PRSV is transmitted nonpersistently by aphids, which may be spread by these strong winds (Coutts et al. 2011). The spread of other potyviruses by similar means has been documented (Coutts et al. 2011).

In this study, we developed specific RT-PCR assays capable of identifying two strains of PRSV in the same papaya plant. PRSV BD-1 and PRSV BD-2 in papaya tissue can be detected and distinguished from other isolates using our specific RT-PCR assays. To our knowledge, this is the first report of specific primers used to differentiate strains of PRSV occurring in the same papaya plant. The RT-PCR assays provided a way to detect and monitor PRSV BD-1 and PRSV BD-2 in papaya orchards that could assist with the management of this disease in the field. The relative occurrence of PRSV BD-1 (19%) and PRSV BD-2 (69%) in Bangladesh was based on 118 randomly sampled papaya plants with virus-like symptoms. Of the 118 samples with virus-like symptoms, 13 were simultaneously infected with both strains, and 28 samples tested negative for both strains. The latter results suggest that there are still unknown strains of PRSV, or other viruses, infecting papaya in Bangladesh.

Two distinct strains of PRSV could be found in the same plant, suggesting that genetic diversity exists in the PRSV population in Bangladesh. Because weeds and cucurbit hosts may contribute to the molecular diversity of PRSV, we plan to screen papaya plants and their associated weeds growing in other districts in Bangladesh for the presence of PRSV strains. Regarding future research, the sequencing of putative PRSV strains and further studies aimed at linking various symptoms with specific virus populations are important.

Transgene-derived resistance is currently the only method of successfully managing PRSV in papaya (Gonsalves 1998; Hamim et al. 2018a). However, PRSV-resistant transgenic papaya cultivars are highly specific to local virus strains (Hamim et al. 2018a). Our study showed that PRSV strains in Bangladesh are diverse compared with PRSV strains from other geographical locations, and they may not be easily controlled by the transgene approach. Therefore, characterization of local PRSV populations and identification of conserved regions of the Bangladesh strains are needed to develop transgene-derived, PRSV-resistant papaya for Bangladesh.

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