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SHORT COMMUNICATION

# Genetic Diversity Based on Coat Protein of *Papaya ringspot virus* (Pathotype P) Isolates from Bangladesh

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**Abstract** The coat protein (CP) sequences of twelve *Papaya ringspot virus* (PRSV) (pathotype-P) isolates from six major papaya growing areas were determined and compared with those of published PRSV. The CP coding region varied in size from 846–852 nucleotides, encoding a protein of 282–284 amino acids. Comparative CP sequence analysis revealed that the PRSV-P isolates originating from Bangladesh were divergent up to 14 % at amino acids level. Further, the isolates from Bangladesh shared 86–95 % amino acid sequence identity with those reported from rest 21 of the Asia and 83–93 % amino acid sequence identity with isolates from the other parts of the world. A number of KE repeats were observed in the N terminus of the CP coding region of all Bangladesh isolates. Phylogenetic branching pattern revealed that the PRSV-P isolates originating from Bangladesh formed a distinct clade from those from the rest of the world. This forms the first report on the genetic diversity of PRSV-P isolates from Bangladesh.

**Keywords** *Papaya ringspot virus* · Pathotype-P · Bangladesh isolates · Coat protein

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Papaya (*Carica papaya* L.) is an important crop in Bangladesh and widely cultivated due to its high nutritional value as fruits and vegetables. *Papaya ringspot virus* (PRSV), currently ascribed to the genus *Potyvirus* of the family *Potyviridae*, is one of the most important limiting factors in production in Bangladesh [2]. The virus induces diverse symptoms that include mosaic, mottling, leaf distortion, vein clearing, leaf shoe-string, ring spotting and streaking on fruits and water soaked streaks on stems and petioles. The virus is vectored by several aphid species in a non-persistent manner. Based on host specificity, the virus is classified into pathotypes P and W. While both pathotypes naturally infect cucurbitaceous plants, pathotype P only infects papaya. In Bangladesh, PRSV was first identified in cucurbitaceous plants [1] and subsequently in papaya [4]. PRSV is a serious problem in northern part of Bangladesh, the major commercial papaya producing region of the country. Attempts to manage the virus in Bangladesh have been made by using cross protection strategy with mild strains of local PRSV-P [5]. For the successful implementation of management strategies either based on cross protection or transgenic resistance, understanding of the genetic diversity of the virus is critical [6]. The present study was thus undertaken to characterize the genetic diversity of PRSV-P isolates occurring in the major papaya producing regions in Bangladesh.

A field survey was conducted during December 2010 to February 2011 in four different districts in the northern and central parts of Bangladesh—Rajshahi, Chapai Nowabgong, Pabna and Gazipur (Fig. 1). In Gazipur district, survey was conducted on three locations, Bangladesh Agricultural Development Corporation (BADC) farm, Kashimpur; Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) farm, Salna; and farmer papaya orchards at Mirzapur. Percent disease incidence and

reduction in plant height and number of fruits/plant in comparison to the healthy plants were calculated using standard statistical tools. Suspected PRSV affected papaya samples (95) from six different locations were collected and lyophilized for further study. Presence of the virus in lyophilized samples was confirmed by direct antigen-coated enzyme-linked immuno-sorbent assay (DAC-ELISA) using polyclonal antibodies (PAb) to PRSV developed at IARI, New Delhi as described by Clark and Bar-Joseph [3]. Leaf tissue (500 mg) was ground in 1 ml extraction buffer and 200 µl samples were used in duplicates for ELISA. The PAb to PRSV was used at 1:2000 dilutions. The reaction was recorded 1 h after adding substrate by an ELISA reader (BIO-TEK Instruments, USA). ELISA positive samples (12, two from each location) were subjected to reverse transcription and polymerase chain reaction (RT-PCR) [4].

Total RNA was isolated from lyophilized samples (100 mg) using RNASure® Plant Kit (Genetix, New Delhi, India) according to the manufacturer's instructions. The resulting RNA preparation was used as a template in RT-PCR. The first strand cDNA was synthesized with 15.0 µl RNA and 10 pM reverse primer RKJ3 (RKJ3 5′GTTGCGCATA CTCAGAG3′) using 2.0 units of IMPROM II reverse transcriptase (Promega, Madison, USA) and 23.0 µl sterile distilled water to make up the volume to 50.0 µl. The 3′-terminal region of PRSV genome comprising of a part of small nuclear inclusion protein (NIb) region + complete CP coding region was amplified by polymerase chain reaction (PCR) using 20.0 µl cDNA template, 2.5 units of GoTaq Flexi DNA polymerase (Promega, Madison, USA) with 10 pM each of forward (HRP50: 5′ATGATAGAGTCATGGGG3′) and reverse (RKJ3) primers. Thirty cycles of PCR were performed with the following thermal profile: denaturation for 30 s at 94 °C, annealing for 1 min at 52 °C, synthesis for 1 min at 72 °C and extension for 10 min at 72 °C. The resultant PCR amplicons (~800 bp) were further gel purified using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA)


**Fig. 1** Bangladesh *map* showing commercial papaya cultivated regions from where the Papaya ringspot disease symptomatic samples have been collected. *Triangle* and *square* shapes indicates ELISA positive and negative PRSV-P samples respectively, while *dimond* shape indicates ELISA positive samples studied for coat protein coding region sequence

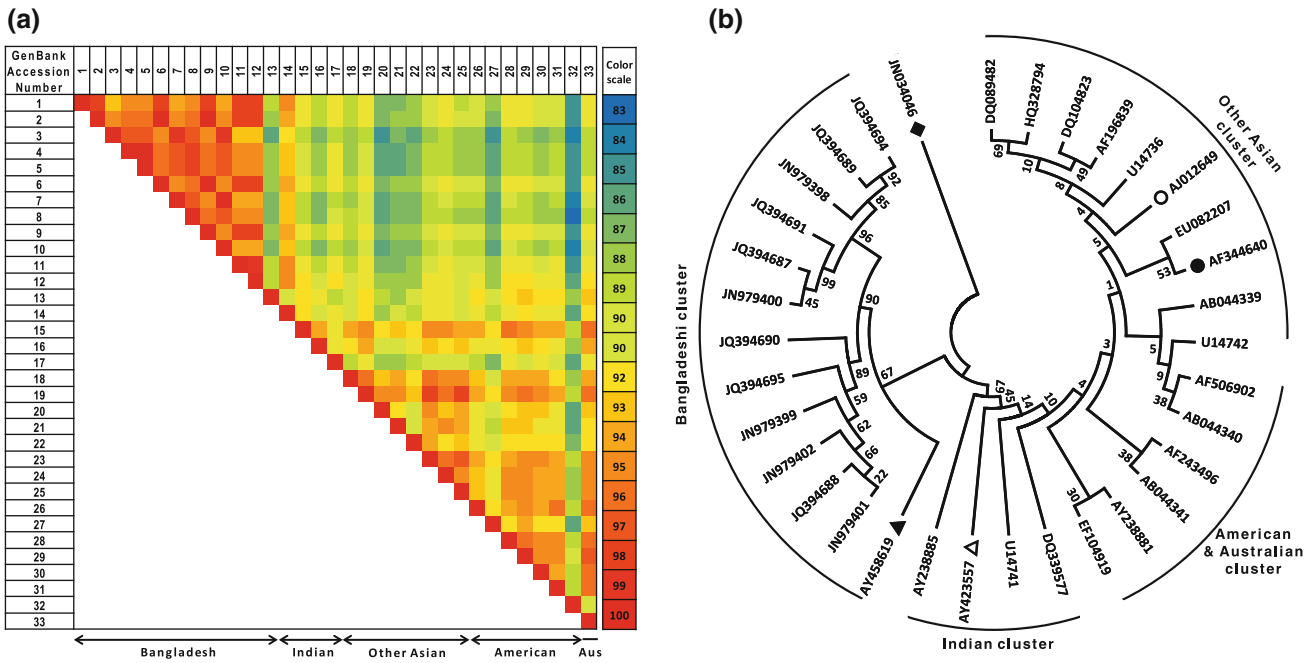


**Fig. 2** Diversity of *Papaya ring spot virus* (PRSV) in Bangladesh based on coat protein gene sequence. **a** Two-dimensional colour coded graphical representation of pairwise percent sequence identities among the PRSV isolates. The amino acid sequences compared in this study are: *Bangladesh isolates (1–13)* 1.JQ394695; 2.JN979402; 3.JN979400; 4.JQ394689; 5.JQ394694; 6.JN979401; 7.JQ394691; 8.JN979398; 9.JQ394688; 10.JQ 394687; 11.JN979399; 12.JQ394690; 13.AY423557; *Indian isolates (14–17)* 14.AY458619; 15.AY238881; 16.EF104919; 17.AY238885; *other Asian isolates (18–25)* 18.AF243496; 19.AB044339; 20.EU082207; 21.AF506902; 22.U14741; 23.AB044341; 24.AB044340; 25.U14742; *American isolates (26–32)* 26.DQ089482; 27.DQ104823; 28.AJ012649; 29.AF196839; 30.AF 344640; 31.HQ328794; 32.DQ339577; and *Australian isolates (33)* 33.U14736. **b** Phylogenetic tree (Neighbor-Joining method with bootstrap consensus from 1000 replicates) based on amino acid sequence of coat protein coding region of the PRSV-P isolates showing evolutionary clustering of Bangladesh isolates with globally distributed isolates. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analyses were conducted in MEGA5. PVY GeneBank Accession No. JN034046 (*filled diamond*) as an out group. Exceptional isolates are marked with *filled circle* and *filled inverted triangles* and *open inverted triangles*

and cloned in the pGEM-T Easy vector (Promega, Madison, WI, USA). Recombinant clones were identified by restriction endonuclease digestion and minimum of two selected clones were sequenced in both directions.

The CP sequences were assembled by using BioEdit software version 5.0.9 and were compared with the already available CP sequences of PRSV isolates from different locations around the world (http://www.ncbi.nlm.nih.gov/Genbank/index.html) (Fig. 2). Multiple alignment and identity matrix of the sequence were performed using CLUSTAL-W program. Phylogenetic analysis was conducted using MEGA version 5 using an isolate of *Potato virus Y* as the outgroup. The recombination analysis was performed using the Recombination Detection Program (RDP) software version 3.44 with the highest acceptable probability (*P* value).

During the survey conducted in major papaya growing areas of Bangladesh (Fig. 1), a single papaya cultivar, BARI—Papaya-1 locally named as Shahi, was predominant along with Red Lady and Gazi (F1 Hybrid). PRSV disease incidence ranged from 61 to 91 % in BADC farm Kashimpur, followed by BSMRAU farm (up to 76 %) of Gazipur District and Sador in Chapai Nowabgong (up to 61 %). The percent reduction in plant height with reference to the healthy plant ranged from 11 to 32 % in different locations, with maximum reduction at BADC farm (up to 32 %) at Kashimpur. The percent reduction in number of fruits per plant ranged from 31 to 79 %, with maximum reduction at BADC, Kashimpur (up to 79 %). Of 95 symptomatic papaya samples (Fig. 1), presence of PRSV was confirmed in 87 samples by DAC-ELISA, suggesting that the PRSV population occurring in Bangladesh is antigenically related to Indian PRSV population. The OD value (at 405 nm) ranged from 0.10 to 3.00. ELISA negative samples might be associated with virus serologically unrelated to PRSV.

The CP coding region of PRSV-P isolates under study showed heterogeneity in length and sequence identity. The CP length varied from 846–852 nucleotides, encoding proteins of 282–284 amino acids. Like other potyviruses the conserved motifs such as DAG and WCIEN were present in these isolates. Besides, N-terminal region of the CP is more variable and large numbers of KE repeats were present. For comparison of the twelve isolates originating

from different locations of Bangladesh, two dimensional color graph was constructed based on the pairwise percent amino acid sequence identity of coat protein of PRSV isolates using Microsoft Excel (version, 2007) which shared 93–100 % identity at amino acid levels (Fig. 2a). Interestingly, one PRSV isolate (AY423557) reported previously from Gazipur District seems to be more distinct, as it shared only 86–89 % identity at amino acid levels with the present isolates. This can be further confirmed by analyzing more PRSV affected samples from Gazipur district. Comparative sequence analysis revealed that the Bangladesh isolates shared 87–95 % amino acid sequence identity with Indian isolates, 86–91 % with other Asian isolates, 89–93 % with Australian isolate and 84–92 % identity with American isolates. Limited study suggests that PRSV population from Bangladesh is more closely related to Indian isolates. No recombination was detected among PRSV isolates from Bangladesh. However, one of the isolates (JQ394689) was identified as a major parent for the recombinant isolate (AY238885) occurring in an Indian state West Bengal, adjacent to Bangladesh.

The unrooted tree based on amino acid sequences of the coat protein of 31 PRSV-P isolates is shown in Fig. 2b. Clustering of isolates did not correlate well with their geographical region. PRSV-P isolates from Bangladesh with one exception (AY423557) formed one cluster. With some exceptions (AY458619-India; AJ012649 and AF344640-American), the Indian, other Asian and Australian and American isolates formed separate clusters.

The present study provides a partial profile of PRSV-P population in Bangladesh. More sequence data need to be generated. The existing sequence diversity will have significant impact on devising PRSV management strategy in Bangladesh through CP gene derived transgenic resistance.

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