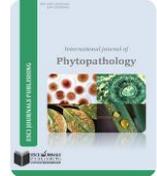




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GENOMIC VARIABILITY OF CORCHORUS GOLDEN MOSAIC VIRUS ORIGINATING FROM BANGLADESH

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ABSTRACT

Yellow mosaic disease of jute (*Corchorus capsularis*) was observed on several plants in different jute growing region (Bangladesh) in April 2013. PCR assays and BLAST analysis of the DNA sequences were performed. We have cloned and sequenced eleven isolates of *Corchorus golden mosaic virus* (CoGMV) collected from eleven different regions in Bangladesh. DNA A sequence of CoGMV Bangladeshi (BD) isolates shared highest identity 97.3-99.1% with the Indian isolates and 93.9-94.3% with the Vietnamese isolate of CoGMV, whereas DNA B shared sequence identity 93.9-94.3% with the CoGMV isolates reported from Vietnam and India. Genetic analysis revealed that CoGMV isolates originating from Bangladesh possessed greater genetic variability than the Indian and Vietnamese isolates. This is thought to be the first report of *Corchorus golden mosaic virus* (CoGMV) associated with yellow mosaic disease of jute from Bangladesh.

Keywords: Genome, Variability, *Corchorus golden mosaic virus* (CoGMV).

INTRODUCTION

Jute (*Corchorus capsularis* L.) is an important natural fibre in the world fibre industry and ranks second among the fibre producing crops in terms of global production. Reduced fiber production has been observed, although it has high demand in textile industries because it is used as raw material for various value added products (Samanta *et al.*, 2011). The most important factor contributing to this declining production of fibre is wide range of diseases of biological origin. Viral diseases are very destructive for fibre production because they lead to overall stunting of the jute plant. *Corchorus yellow vein virus* (CoYVV) and *Corchorus golden mosaic virus* (CoGMV) have been reported in jute from India and Vietnam (Ha *et al.*, 2006; Gosh *et al.*, 2008).

Corchorus golden mosaic virus (CoGMV) was first reported and characterized in jute (*Corchorus capsularis*) from Vietnam in 2008 (Ha *et al.*, 2008), and it is a member of the family *Geminiviridae* and genus *Begomovirus* of plant viruses, can cause significant yield

losses and reductions in jute fibre quality. In 2008, natural occurrence of CoGMV in field grown jute and insect transmission in greenhouse were reported in India (Ghosh *et al.*, 2008). Begomovirus produced symptoms on jute which generally include crinkled, leathery and malformed leaves with a yellow mosaic of varying intensity. The most challenging symptom for grower is stunted plant, that is, the infected plant became dwarf due to remarkable alteration of biochemical components of the infected jute plants (Ghosh *et al.*, 2011) and produced less fibre.

During the initial infection stage, the disease is characterized by symptoms on the leaf lamina such as small yellow flakes that gradually increase in size to form green and chlorotic intermingled patches producing a yellow mosaic appearance (Gosh *et al.*, 2008). The CoGMV causes crinkled, leathery leaves with yellow mosaic symptom and stunted plants. Several CoGMV isolates reported from India and Vietnam are bipartite with DNA A and DNA B genomes of about 2.7 kb each, encoding seven ORFs (Ha *et al.*, 2008). The DNA A component of the bipartite begomoviruses is involved in replication and production of virions, but requires the DNA B component for nuclear localization, systemic

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infection, host range determination and symptom expression (Lazarowitz, 1992). Both DNA A and DNA B components have a non-coding common region (CR) sequence of approximately 200 bp that contains sequence motifs required for the control of gene expression and replication (Bowdoin *et al.*, 2000).

The occurrence of yellow mosaic disease, molecular characterization of CoGMV and biochemical alteration of the infected plants has been reported from India and Vietnam and the reports are limited in number (Ghosh *et al.*, 2011). Considering the importance of the jute fiber and the economic impact of the yellow mosaic virus disease of jute, we designed our research work on molecular characterization of the CoGMV from Bangladesh.

MATERIALS AND METHODS

Viral sample sources: Naturally infected jute plants showing typical symptoms of begomoviruses infection with yellow mosaic, leaf curling, stunting etc. were collected from eleven jute-producing districts of Bangladesh in April 2013 (Fig. 1). Sampling was conducted at the vegetative growing stage of jute plants. At least two samples were taken for each location and the number of samples collected was 24. Samples were placed in plastic box together with silica gel for 24 hrs to make the samples dry.

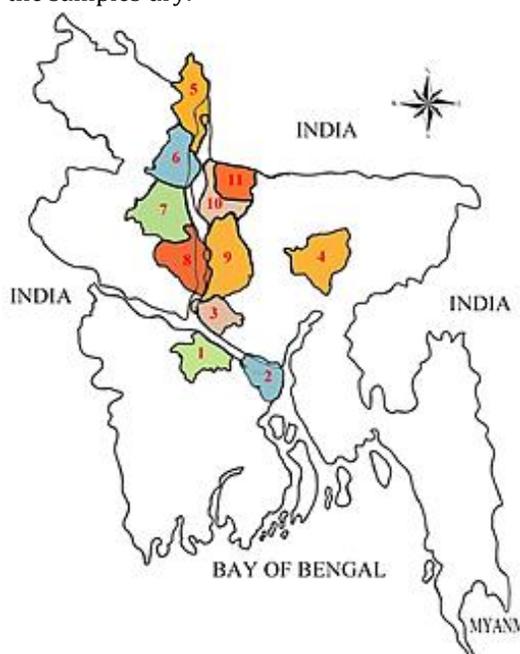


Figure 1. Geographical map of Bangladesh showing the locations from where jute samples were collected: 1 : Isolate 1→Faridpur, 2 : Isolate 2→Shariatpur, 3 : Isolate

3→Manikganj, 4 : Isolate 4→Kishoreganj, 5 : Isolate 5→Kurigram, 6 : Isolate 6→Gaibangha, 7 : Isolate 7→Bogra, 8 : Isolate 8→Sirajganj, 9 : Isolate 9→Tangail, 10 : Isolate 10→Jamalpur, 11 : Isolate 11→Sherpur.

The dry samples were carried to the plant virology laboratory, Graduate School of Science and Technology, Niigata University, Japan, for DNA extraction and analysis of the viruses.

DNA extraction: Nucleic acids were extracted from symptomatic leaves, dry seeds and seedlings by a modified cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Leaf samples (20 mg) were ground in 50 vols (V/W) of 100 mM Tris-HCl, pH 8, containing 10 mM EDTA pH 8, 1.4 M NaCl, 2% CTAB and 0.2% β -mercaptoethanol. The samples were then incubated at 60° C for 30 min and mixed three to four times by inverting tubes. The homogenates were mixed with 0.6 vols of chloroform and centrifuged at 16,500 *g* for 10 min. The upper aqueous phase was mixed with 0.6 vols of iso-propanol followed by centrifugation at 14,000 *g* for 10 min to obtain nucleic acid precipitate. The crude nucleic acid pellet was dissolved in 200 μ l of distilled water and mixed with 2 vols of alkaline solution (1% SDS and 0.2 M NaOH mixture) and then with 1.5 vols of potassium acetate. After incubation at -20°C for 1 h, the mixture was centrifuged at 14,000 *g* for 10 min. The supernatant was again extracted once with phenol-chloroform (1:1) followed by chloroform. To the aqueous phase, an equal volume of iso-propanol was added and the mixture was centrifuged at 20,000 *g* for 10 min. The pellet was washed with 70% ethanol, dried and dissolved in 0.5 ml of 1 M NaCl containing 10 μ g/ml RNase A (Pharmacia), and incubated at 37°C for 30 min. Again, the aqueous phase was precipitated with an equal volume of iso-propanol by centrifugation at 20,000 *g* for 10 min and then washed with 70% ethanol and dried. Finally, the pellet was dissolved in 15 μ l of sterile distilled water.

PCR amplification of DNA A and DNA B components:

Extracted DNAs from twenty four samples were used in polymerase chain reaction (PCR) for amplification using the following primers: Co-V1, -V2, -R1 and -R2 (Supplementary file table 1). These primers were designed based on the alignment of DNA A sequences CoYVV and CoGMV, and PCR products with the expected sizes (2320, 2190, 260 and 138 bp for Co-V1/R1, Co-V1/R2, Co-V2/R1 and Co-V2/R2 primer pairs,

respectively) were amplified from twenty two samples and sequenced all 88 amplified products. According to the determined sequences, two abutting primer pairs, CoGMVAF/CoGMVAR and CoGMVBF/CoGMVBR, were designed and used to amplify the complete viral DNA A and DNA B genomes of CoGMV. The primers used in this study are listed in Table 1 and all amplifications were performed in a reaction mixture of 20 μ l using PrimeStar Max enzyme according to the manufacturer's instructions (Takara Bio Inc., Japan). The PCR cycles were programmed as 20 s for initial denaturation at 98°C, and 15 s each for denaturation at 98°C, annealing at 58°C and chain extension at 72°C. To confirm the completion of amplification of all the target templates, a final extension cycle was carried out at 72°C for 7 min.

Cloning and sequencing: The PCR products of 11 samples were cloned into T-vector pMD20 and used to transform *Escherichia coli* (JM109) following the manufacturer's instructions (Takara Bio Inc., Japan). DNA sequencing was carried out by a commercial company (SolGent Co., Ltd., Korea). The sequence data were assembled and analyzed using GENETYX Win. software package (GENETYX, Tokyo, Japan) ver. 12. Viral genomic sequences were aligned and pairwise comparisons were carried out with the help of ClustalW software (Thompson *et al.*, 1994). Phylogenetic trees were constructed using the neighbor-joining method calculated with Molecular Evolutionary Genetics Analysis (MEGA) software, version 6 (Tamura *et al.*, 2013).

Genetic variability of CoGMV isolates from Bangladesh, India and Vietnam: Genomic sequences were grouped by their identity as CoGMV (BD), (IND) and (VIET) variants from Bangladesh, India and Vietnam for determining the effects of geography on the genetic variation of CoGMV isolates. The analyses were carried out using the DnaSP v.5 program (Rozas *et al.*, 2003). The genetic variability determinants were the total number of polymorphic sites (S), mutations (Eta), nucleotide diversity (p), number of haplotypes (h), average number of nucleotide differences (k), and haplotype diversity (Hd).

RESULTS

Symptoms and detection: The symptoms on young leaves of CoGMV-infected plants consisted of yellow flakes on the lamina, which gradually increased in size to form green and chlorotic intermingled patches (Fig. 2). Using the primer pairs CoGMVAF & CoGMVAR and CoGMVBF & CoGMVBR, the full-length DNA A and B

clones of eleven CoGMV isolates, namely, BD1 (Faridpur, Accession No. AB971842-43), BD2 (Shariatpur, Accession No. AB971844-45), BD3 (Manikganj, Accession No. AB971846-47), BD4 (Kishoreganj, Accession No. AB971848-49), BD5 (Kurigram, Accession No. AB971850-51) BD6 (Gaibangha, Accession No. AB971852-53), BD7 (Bogra, Accession No. AB971854-55) BD8 (Sirajganj, Accession No. AB971856-57), BD9 (Tangail, Accession No. AB971858-59) BD10 (Jamalpur, Accession No. AB971860-61) and BD11 (Sherpur, Accession No. AB971862-63) were amplified and fully sequenced. The primer pairs used in the initial screening were targeted to amplify genomic DNAs of CoYV and CoGMV; however, we did not find any CoYV sequence in the amplified DNA fragments. In addition, our attempts to amplify sub-genomic components from the jute samples using universal primer pairs targeting the alphasatellite (Bull *et al.*, 2003) or betasatellite (Bridson *et al.*, 2002) were unsuccessful.

Characterization of CoGMV DNA A: The complete nucleotide sequences of CoGMV DNA A were determined to be of 2688 nt (BD1, 3), 2687 nt (BD2,4-9 and,11) and 2682 nt (BD10) in length. The smaller size of BD10 isolate was due to the 6 nucleotides deletion at the position just after stem loop region. The DNA A component of CoGMV had a typical New World bipartite begomovirus genome organization (Ha *et al.*, 2008), with the virion sense strand of the isolates encoding one open reading frame (ORF), named AV1, and the complementary sense strand encoding four ORFs, named AC1, AC2, AC3 and AC4, for all the isolates.

The DNA A component of all BD isolates lacked the AV2 ORF that is present in Old World begomoviruses. A PWRNAGT motif was identified at the N-terminus of the deduced CoGMV CP sequence encoded by AV1 (Fig. 3). This motif was first identified by Harrison and colleagues as 7-PWRsMaGT (Harrison *et al.*, 2002). The complete BD1 DNA A sequence shared the highest nucleotide sequence identity at 99.1% with CoGMV DNA A from India (Table 1). The DNA A sequences of BD isolates shared the highest nucleotide sequence identities at >97.3% with Indian isolate of CoGMV DNA A (Table 1). The percent nucleotide identities of ORFs with the respective sequences of different begomoviruses used for analysis are listed in supplementary file table 2.

Characterization of CoGMV DNA B: The complete nucleotide sequences of CoGMV DNA B were determined to be of 2665 nt (BD1,7,8), 2660 nt (BD2), 2666 nt

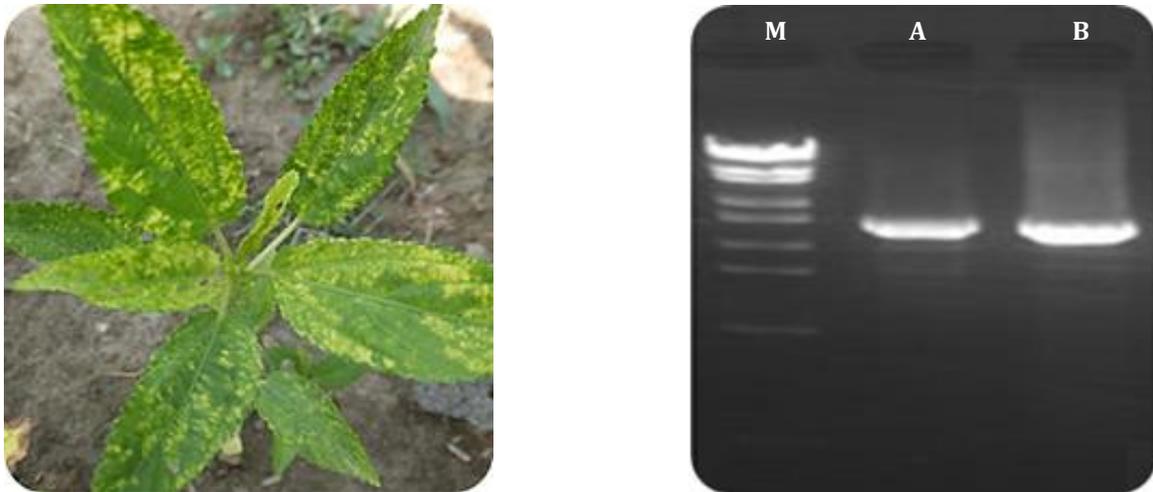


Figure 2. Yellow mosaic symptoms of jute plants infected with CoGMV and Full length CoGMV genome amplified by PCR (M-DNA marker (2.7 kb and Takara Bio Inc., Japan), A-DNA A and B- DNA B).

BD 1-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KASAWVNRPMY 42
BD 2-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
BD 3-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGH---KATAWVNRPMY 42
BD 4-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
BD 5-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
BD 6-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
BD 7-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
BD 8-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
BD 9-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
BD 10-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
BD 11-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
IND1-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
IND2-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KASAWVNRPMY 42
IND3-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KASAWVNRPMY 42
IND4-AV1	1:-----MM KREAPWRTNAGT SKVRRSLNFSPRSG-----LGP---KATAWVNRPMY 42
VIET-AV1	1:-----MM KREAPWRTNAGT SKVRRALNFSPRSG-----LGP---KASAWVNRPMY 42

Figure 3. N-terminal nuclear localizing signal (NLS) of deduced coat protein amino acid sequences of CoGMV of Bangladesh, India and Vietnam. Shaded NLS (Harrison *et al.*, 2002) of deduced coat protein amino acid sequences unique to the New World viruses and basic domains of the NLS (Ha *et al.*, 2006; Ha *et al.*, 2008) are shown in bold.



Figure 4. Putative stem-loop domains of the intergenic regions of CoGMV, A. Vietnam and B. Indian isolates (Red circle showed the difference between Vietnam and Indian isolates).

Table 1. Nucleotide sequence identity of CoGMV isolates with other Indian and Vietnamese CoGMV isolates for DNA A and DNA B at the nucleotide level (lower left: DNA A / upper right: DNA B).

A\B	BD1	BD2	BD3	BD4	BD5	BD6	BD7	BD8	BD9	BD10	BD11	IND1	IND2	IND3	IND4	VIE T
BD1		98.6	97.3	97.2	97.2	98.5	97.1	97.1	96.4	98.0	97.5	96.2	96.8	96.8	96.2	92.2
BD2	98.4		97.5	97.3	97.1	98.9	97.2	97.1	96.8	98.6	97.5	96.1	97.0	97.0	96.1	92.3
BD3	98.4	99.3		97.3	97.1	97.4	97.3	97.0	96.4	97.2	96.9	95.7	96.7	96.7	95.7	91.6
BD4	97.8	98.5	98.6		99.7	97.4	97.1	97.9	96.2	97.0	97.4	96.4	97.6	97.6	96.4	92.1
BD5	97.8	98.5	98.6	99.9		94.3	97.0	98.0	96.2	96.8	97.5	96.6	97.7	97.7	96.6	92.1
BD6	98.1	98.6	98.7	99.2	99.2		97.1	97.3	96.7	98.3	97.6	96.1	96.8	96.8	96.1	91.3
BD7	98.2	99.0	98.8	98.9	98.9	99.1		97.2	97.6	97.4	96.4	95.4	96.4	96.4	95.4	91.5
BD8	98.0	98.6	98.6	99.2	99.2	99.4	99.2		96.3	96.8	97.5	96.1	96.9	96.9	96.1	91.9
BD9	98.1	98.9	98.8	98.9	98.9	99.0	99.7	99.1		96.3	95.7	94.3	95.5	95.5	94.3	91.3
BD10	97.3	97.9	98.0	98.7	98.7	98.8	98.3	98.8	98.2		97.1	95.6	96.7	96.7	95.6	91.9
BD11	98.4	99.2	99.1	98.3	98.3	98.5	98.8	98.6	98.7	97.9		96.2	97.0	97.0	96.2	92.1
IND1	98.7	98.9	98.8	98.3	98.3	98.6	98.7	98.5	97.9	97.9	98.8		97.5	97.5	100	91.1
IND2	99.1	98.4	98.3	97.8	97.8	98.1	98.2	97.9	97.3	97.3	98.3	98.8		100	97.5	92.1
IND3	99.1	98.4	98.3	97.8	97.8	98.1	98.2	97.9	97.3	97.3	98.3	98.8	100		97.5	92.1
IND4	98.7	98.9	98.8	98.3	98.3	98.6	98.7	98.5	97.9	97.9	98.8	100	98.8	98.8		91.1
VIET	94.3	94.1	94.0	94.0	94.0	94.1	94.1	93.9	94.1	94.1	94.0	94.3	94.4	94.4	94.3	

(BD3), 2667 nt (BD4), 2668 nt (BD5), 2661 nt (BD6), 2638 nt (BD9), 2662 nt (BD10) and 2664 nt (BD11) in length and encoded two ORFs, BV1 in virion sense and BC1 in complementary sense orientation. The BV1 and BC1 of all three isolates encoded proteins with 255 aa and 292 aa, respectively. The complete DNA B sequence of all BD isolates shared sequence identity at >94.3% with CoGMV Indian isolates (Table 1). The percent nucleotide identities of the complete DNA B and the two ORFs with the respective sequences of different begomoviruses used for analysis are listed in supplementary file table 2-8.

It should be noted that the conserved nonanucleotides in the putative stem-loop domains of the intergenic regions of Vietnam and Indian CoGMV isolates were TATTATTAC and CATTATTAC, respectively, in place of the most conserved TAATATTAC feature within the *Geminiviridae* family (Ghosh *et al.*, 2008; Roberts and Stanley, 1994) (Fig. 4). CoGMV of all BD isolates had the nonanucleotide sequence CATTATTAC like as Indian isolates.

Phylogenetic analysis: The complete nucleotide

sequences of CoGMV (BD1-11) were aligned with the corresponding sequences of other CoGMV isolates reported from India and Vietnam (Supplementary file table 9). The neighbor-joining phylogenetic analysis using complete DNA A and DNA B sequences of Bangladeshi CoGMV isolates with the other CoGMV reported from India and Vietnam (100% bootstrap support) (Fig. 5). All the CoGMV DNA A and DNA B sequences taken for comparison formed two major clusters. The Bangladeshi isolates and Indian isolates of CoGMV formed the first cluster, while the second cluster composed of CoGMV obtained from Vietnam.

Genetic variability of CoGMV populations from Bangladesh, India and Vietnam: Genetic variability analysis revealed that the DNA A of CoGMV isolates originating from Bangladesh have higher genetic variability when compared to the Indian isolates. The DNA B of CoGMV Bangladeshi isolates showed higher genetic variability than the Indian isolates. Each isolate perform as one haplotype, which indicates the higher genetic variation of the viral population within each designated group (Table 2).

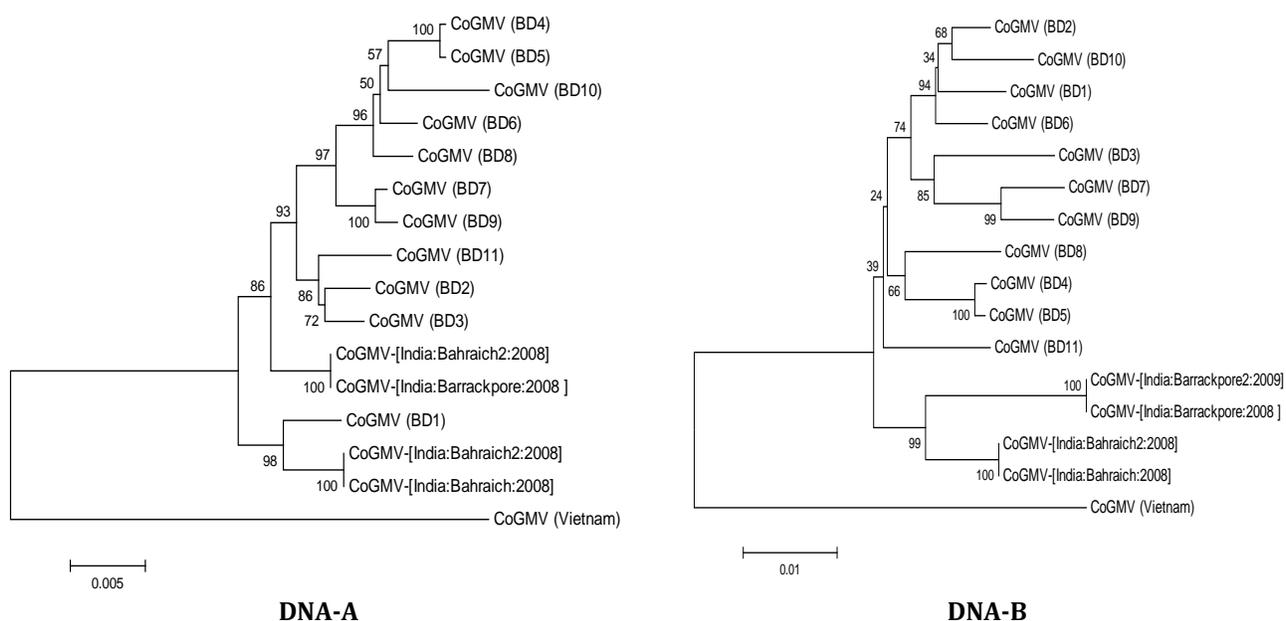


Figure 5. Phylogenetic analysis of CoGMV isolates based on the alignment of complete nucleotide sequences of DNA A (A) and DNA B(B). Phylogenetic analyses were performed with MEGA (Molecular Evolutionary Genetics Analysis), version 6 (Tamura *et al.*, 2013). Dendrograms were constructed by the neighbor-joining method. Bootstrap values (1,000 replicates) are given at the branch nodes. The scale at the base of the diagrams is the pairwise distance expressed as percentage dissimilarity.

Table 2. Genetic variability determinants of CoGMV isolates from Bangladesh, India and Vietnam based on their geographical origin.

Population	No. of sites	No. of sequences	S ^a	Eta ^b	K ^c	Π ^d	H ^e	Hd ^f
For DNA A								
CoGMV (BD)	2688	11	117	121	33.018	0.01231	11	1.000
CoGMV (IND)	2686	4	32	32	21.333	0.00794	2	0.667
CoGMV (BD, IND and VIET)	2688	16	234	247	49.658	0.01856	14	0.983
For DNA B								
CoGMV (BD)	2671	11	203	218	61.364	0.02332	11	1.000
CoGMV (IND)	2666	4	65	65	43.333	0.01625	2	0.667
CoGMV (BD, IND and VIET)	2672	16	360	394	84.308	0.03226	14	0.983

DISCUSSION

Yellow mosaic disease has immense economic importance, including through its association with jute plants in Bangladesh. Although two begomoviruses, CoYVV and CoGMV, were subsequently identified in Vietnam, the infectivity using cloned DNA has been demonstrated only for CoYVV by microprojectile bombardment on tobacco culture cells (Ha *et al.*, 2008; Ha *et al.*, 2006). The mechanical transmission of the yellow mosaic disease has not been demonstrated, presumably due to the presence of a large amount of mucilage and phenolics in jute plant. Begomoviruses can

be transmitted by the whitefly (*B. tabaci*) and sometimes by mechanical inoculation (King *et al.*, 2012). Transmission is an effective survival strategy and is important for virus perpetuation and dissemination; however, it also provides an inoculum source that may have a considerable impact on crop production (Yang *et al.*, 1997). Annual jute crop is the only known natural host of CoGMV; it is harvested after a 4-month growing season.

The genetic variability analysis of CoGMV from Bangladesh showed the highest haplotype diversity (Hd =1.00), so each Bangladeshi isolate occurs as a distinct

variant (Table. 2). High mutation rate ($\text{Eta}=121$) and high nucleotide diversity ($p=0.1231$) indicate a higher genetic diversity in Bangladeshi CoGMV variants than CoGMV variants from India.

In phylogenetic analyses, DNA A sequences of CoGMV BD1-11 isolates were clustered into a group of other CoGMV isolates reported from India. Based on DNA A sequence similarity, BD isolates were closely related to Indian isolates (97.3-99.1%). The level of nucleotide identity (97.3-99.1%) found between DNA A sequences of Bangladeshi and other Indian CoGMV isolates was higher than the strain demarcation level of 93% (Fauquet *et al.*, 2008). However, the DNA A of Vietnamese isolate exhibited intermediate levels of nucleotide identity of (93.9-94.3%) with Bangladeshi isolates, indicating that Bangladeshi, Vietnamese and the other CoGMV isolates should be regarded as variants. It is also noteworthy that DNA B of Bangladeshi isolates had 91.3-97.7% nucleotide identity with other CoGMV isolates. Possible recombination events could not be detected in DNA A and DNA B of Bangladeshi isolates with other CoGMV and CoYVV isolates, using Recombination Detection Program (RDP) ver. 4 (Martin *et al.*, 2010) (results not shown). DNA B component, by virtue of encoding no overlapping genes, has a greater capacity for variation (Harrison, 1985). The geographic location of Bangladesh is a south Asian small country. Three sides of Bangladesh are covered with India, thereby providing opportunities for easy transmission of the virus from India to Bangladesh.

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