

Characterization and Confirmation of *Corchorus Golden Mosaic Virus* Associated with Jute in Bangladesh

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Abstract

Yellow mosaic disease is a major limiting factor for jute (*Corchorus capsularis* L.) cultivation in Bangladesh. We have cloned and sequenced three isolates of *Corchorus golden mosaic virus* (CoGMV) collected from different regions in Bangladesh. DNA A sequence of CoGMV-[BD:Mym:10] (BD1) shared highest identity (94.2%) with the Vietnam isolate of CoGMV, whereas DNA B shared a lower level of sequence identity (<73%) with the CoGMV isolates reported from Vietnam and India. Complete genome sequences of CoGMV-[BD: Ran: 10] [BD2] and CoGMV-[BD:Din:10] [BD3] showed at least 97% sequence identity with Indian isolates of CoGMV. The fact that the examined DNA A components of three isolates of CoGMV lack the AV2 open reading frame, indicated that BD1-3 share genetic features of New World begomoviruses. The pathogenicity of CoGMV-[BD:Din:10] [BD3] isolate was confirmed by agroinoculation and infectious clones of DNA A and DNA B induced characteristic yellow mosaic symptoms in jute plants. This is a first experimental demonstration of Koch's postulate for a begomovirus associated with jute yellow mosaic disease.

Keywords: Jute; CoGMV; Begomovirus; Agroinoculation

Introduction

Begomoviruses (family *Geminiviridae*) are plant viruses with small single-stranded circular DNA molecules encapsulated in twinned particles that are transmitted by the white fly *Bemisia tabaci* (Genn.) to dicotyledonous plants and have either monopartite (DNA A genome of about 2.7 kb, encoding six ORFs) or bipartite genomes (DNA A and DNA B genomes of 2.5-2.6 kb each, encoding eight ORFs) [1]. 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 infection, host range determination and symptom expression [2]. 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 [3].

Jute (*Corchorus capsularis* L. and *C. olitorius*) is an eco-friendly natural fiber-producing crop belonging to the family Tiliaceae, or more recently to Malvaceae. It is known for its versatility, fiber strength and biodegradability of lingo-cellulosic fibers and is almost exclusively cultivated in India and Bangladesh; nearly 98% of the world crop is grown in these two countries [4,5]. Jute plants are infected with many diseases [6]. Among them, jute yellow mosaic disease causes drastic reduction in the quality and yield of jute, and has been considered to be one of the most important limiting factors of jute cultivation. The disease was first reported by Finlow [7] in 1917, but the etiology was not known for many years, until Ha et al. [8,9] identified two begomoviruses, namely, *Corchorus yellow vein virus* (CoYVV) and *Corchorus golden mosaic virus* (CoGMV), from diseased plants in Vietnam. These bipartite viruses possess DNA A, which was found not to encode an AV2 open reading frame, and DNA B genomic components. Subsequent identification of CoGMV from India [10,11] has provided further evidence that CoGMV has a wide distribution in Asia. CoGMV infection reduces the levels of chlorophyll, total protein, catalase, peroxidase and esterase and also reduces plant height, so it lowers the quality and yield of jute fiber [12]. The incidence of the disease has been found to be around 50% on some of the leading *C. capsularis* cultivars in India. Ghosh et al. [11] also demonstrated that,

like other begomoviruses, CoGMV can be transmitted by whitefly and the efficiency of its transmission ranges from 20 to 60% for infestation with 3 and 10 viruliferous whiteflies. Considering the importance of the jute fiber and the economic impact of the viral disease, we designed our research work on molecular characterization and confirmation of the causal virus of jute yellow mosaic in Bangladesh.

Materials and Methods

Virus source

Naturally infected plants of *C. capsularis* cultivar CLV-1 showing symptoms of yellow mosaic disease and healthy plants were collected from Mymensingh, Rangpur and Dinajpur districts of Bangladesh (Figure 1A). The plants exhibiting symptoms of typical mosaic disease and healthy plants were then subjected to collect sample leaves. Sample leaves were dried using silica gel and assayed for virus infection and seed transmission at the plant virology laboratory of the Department of Agrobiolgy, Graduate School of Science and Technology, Niigata University, Japan.

DNA extraction

Nucleic acids were extracted from symptomatic leaves (20 mg) by a modified Cetyl Trimethylammonium Bromide (CTAB) method [13]. The total DNA was suspended in 15 μ l of sterile distilled water and used in PCR procedures.

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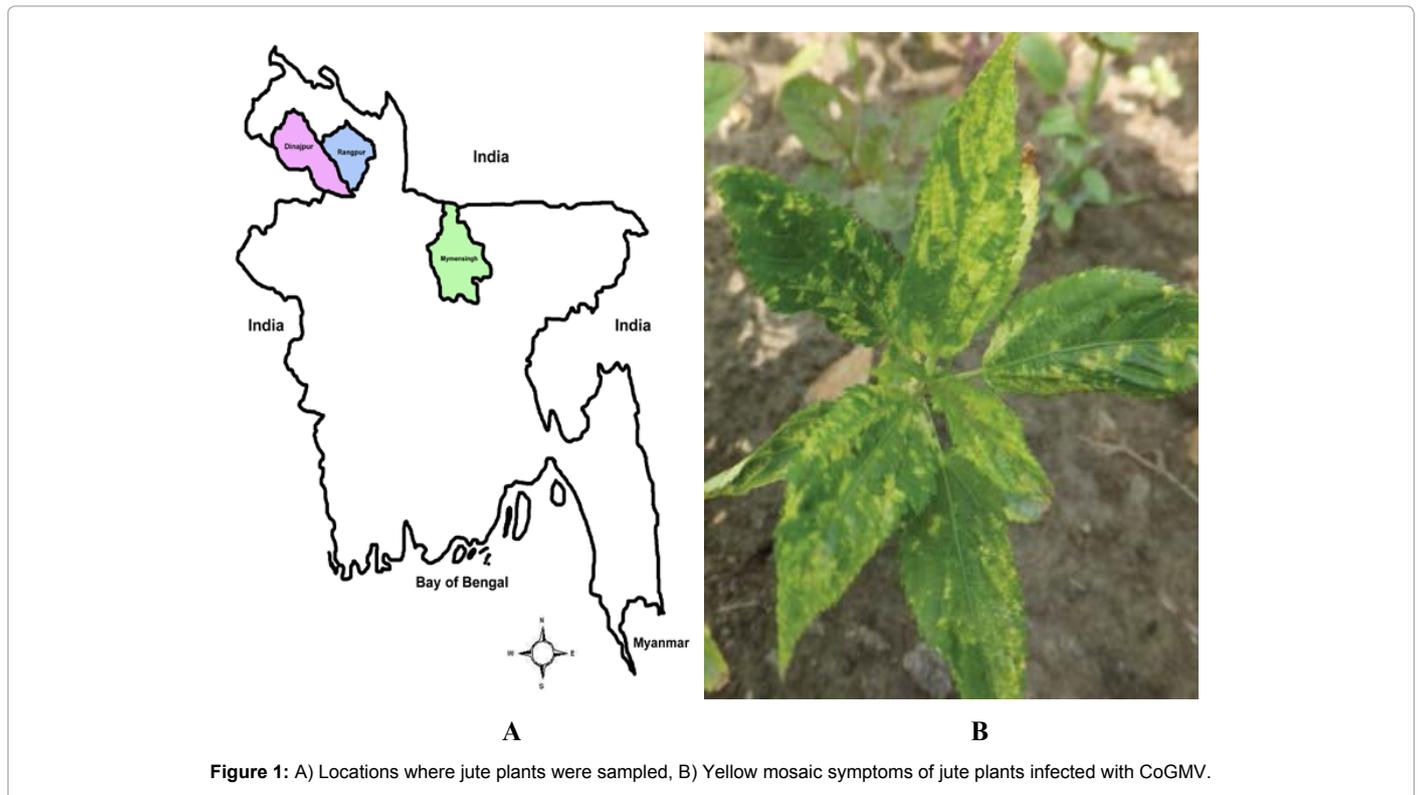


Figure 1: A) Locations where jute plants were sampled, B) Yellow mosaic symptoms of jute plants infected with CoGMV.

PCR amplification of DNA A and DNA B components

Extracted DNAs from four samples were used in Polymerase Chain Reaction (PCR) to amplify the complete viral DNA A and DNA B genomes of CoGMV using the 3 abutting primer pairs, CoGMVAF/CoGMVAR, CoGMVB1F/CoGMVB1R and CoGMVBF/CoGMVBR, were designed according to the determined sequences (sizes 2320, 2190, 260 and 138 bp for Co-V1/R1, Co-V1/R2, Co-V2/R1 and Co-V2/R2 primer pairs, respectively). The primers used in this study are listed in Supplementary file 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 50-58°C and chain extension at 72°C (25 cycles). 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 were cloned into T-vector pMD20 and used to transform *Escherichia coli* (JM109) following the manufacturer's instructions (Takara Bio Inc., Japan). Selected clones were completely sequenced 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. Multiple sequence alignments of viral genomic sequences and pairwise comparisons were carried out with the help of ClustalW software [14]. Phylogenetic trees were generated with Molecular Evolutionary Genetics Analysis (MEGA) software, version 6 [15] using the neighbor-joining method.

Construction of agroinfectious clones

For construction of CoGMV-[BD:Din:10] [BD3] DNA A

infectious clone, the *EcoRV*-to-*KpnI* (about 1.7 kb) fragment of DNA A clone (1.0 mer) was separated and removed by gel electrophoresis and the remaining (0.4 mer) of the clone was purified, treated with T4 polymerase and self-ligated which gives pMD20A0.4mer. The viral fragment was confirmed, and a full length DNA A component released as *NheI* fragment was further ligated with the *NheI* linearized pMD20A0.4mer to generate pMD20A1.4mer. The orientation of the constructs was confirmed by restriction digestion with *AflII*, which was expected to release 2.7 kb fragment. The 3.7 kb band (1.4 mer), released by digestion with *HindIII* and *EcoRI* from the recombinant pMD20 clone of DNA A was subsequently cloned at the same restriction sites of a binary vector pRI201-AN-GUS and the final clone was named pRI1.4A.

For construction of CoGMV-[BD: Din: 10] [BD3] DNA B infectious clone, a similar strategy was adopted. The *BglII*-to-*KpnI* (0.5 kb) fragment of DNA B clone (1.0 mer) was separated and removed by gel electrophoresis and the remaining (0.8 mer) of the clone was purified, and treated with T4 polymerase and self-ligated to give pMD20B0.8mer. The viral fragment was confirmed and a full length DNA B component released as a *FbaI* fragment was further ligated with the *FbaI* linearized pMD20B0.8mer to generate pMD20B1.8mer. The orientation was confirmed by restriction digestion with *PvuII*, which was expected to release 2.7 kb fragment of DNA B. The 4.9 kb band (1.8 mer) released by digestion with *HindIII* and *EcoRI* from the recombinant pMD20 clone of DNA B was subsequently cloned at the same restriction sites of a binary vector pRI201-AN-GUS and the final clone was named pRI1.8B.

Agroinoculation

Jute (*Corchorus capsularis*) seedlings of the cultivar CVL-1 were grown in vermiculite inside a temperature controlled growth chamber maintained at 22±2°C and a 16/8 h light/dark cycle. Third and fourth

Begomovirus	CoGMV-[BD:Mym:10] (AB849288-9) (BD1)						CoGMV-[BD:Ran:10] (AB849290-1) (BD2)					
	DNA A	AV1	AC1	DNA B	BV1	BC1	DNA A	AV1	AC1	DNA B	BV1	BC1
CoGMV-[BD:Din:10] (AB849292-3) (BD3)	92.1	95.1	91.8	72.1	78.0	91.1	98.8	100.0	99.4	97.0	99.2	100.0
CoGMV-[IN:Bah:08]	92.7	95.1	92.1	72.6	78.8	90.7	98.2	99.2	98.3	97.1	99.6	99.6
CoGMV-[IN:Bah2:08]	92.7	95.1	92.1	72.6	78.8	90.7	98.2	99.2	98.3	97.1	99.6	99.6
CoGMV-[VN:Han:05]	94.2	96.3	94.4	72.1	79.2	91.4	93.9	98.8	93.8	92.2	99.2	98.9
CoGMV-[IN:Bar:08]	92.6	95.1	91.6	72.2	76.5	90.4	98.8	100.0	98.0	95.9	95.3	98.9
CoGMV-[IN:Bar2:09]	92.6	95.1	91.6	72.2	76.5	90.4	98.8	100.0	98.0	95.9	95.3	98.9
CoYVV-[VN:Ho:00]	76.6	84.9	80.4	64.6	63.1	89.7	75.1	83.6	79.2	63.9	60.8	86.3
MacMPRV-[PR:Bea:98]	68.1	85.8	64.9	59.6	50.6	75.3	67.5	86.7	64.3	57.9	50.2	72.9
DiYMoV-[US:Flo:98]	65.4	86.3	56.8	58.6	47.7	74.3	64.9	85.6	56.5	57.8	49.2	70.5
CoYSV-[MX:Yuc:05]	65.9	84.5	57.6	58.1	50.0	72.3	65.1	85.9	57.9	58.2	50.4	71.2
ToMoTaV-[CU]	65.0	86.3	55.5	58.4	48.6	71.9	64.7	86.4	55.5	57.9	50.6	70.5
BGMV-[BR:Cam1:78]	64.0	84.4	56.6	58.7	50.2	73.3	64.3	84.7	56.6	56.9	49.0	72.2
CLCrV-AZ-[MX:Son:91]	62.6	83.5	50.7	58.3	50.8	73.4	62.8	84.1	49.6	58.1	51.2	73.1
BDMV-[CO:87]	64.2	84.3	54.3	57.6	50.6	73.9	63.5	84.5	54.9	57.3	53.7	71.9
CabLCV-[US:Flo:96]	63.8	84.5	51.1	58.6	48.8	72.1	64.7	85.5	51.1	58.2	50.2	70.1
SiGMCRV-[CR]	64.9	84.3	55.2	57.7	50.9	72.6	63.8	85.6	55.8	57.3	51.6	71.2
BCaMV-[MX:Son:86]	63.6	84.3	48.1	58.3	50.2	71.2	63.7	85.4	46.9	57.5	50.4	69.5
PYMV-Po-[VE:91]	65.1	85.9	56.7	55.3	47.5	71.9	64.8	86.5	57.1	57.6	50.4	71.6
SMLCuV-[US:IV:79]	64.1	86.0	49.8	52.0	45.7	70.2	63.1	86.7	48.4	52.9	46.5	68.8

Table 1: Nucleotide sequence identity of CoGMV isolates with other begomoviruses and comparison of ORF-wise amino acid sequence identity for DNA A and DNA B at the nucleotide level. Highest values are indicated in bold.

Infectious constructs	Symptomatic plants/Inoculated plants			Types of symptoms
	1 st experiment	2 nd experiment	3 rd experiment	
pRI1.4A	0/5	0/5	0/5	No symptoms
pRI1.8B	0/5	0/5	0/5	No symptoms
pRI1.4A + pRI1.8B	6/14	12/21	12/22	Mosaic and leaf curling
Control	0/5	0/5	0/5	No symptoms

Table 2: Infectivity and symptom induced by CoGMV-[BD:Din:10] (BD3) and the number of symptomatic plants as confirmed by PCR.

leaf stage jute seedlings were used for agroinoculation. CoGMV DNA A was agroinoculated either alone or with cognate DNA B. *Agrobacterium tumefaciens* strain C58C1 cells containing the infectious constructs pRI1.4A and pRI1.8B were grown for 48 hours on Luria Bertani medium (pH 6.8) containing kanamycin (50 µg/ml) and rifampicin (100 µg/ml). *Agrobacterium* cells were harvested and resuspended in MES buffer (10 mM 2-[N-morpholino] ethanesulfonic acid (MES), 10 mM Magnesium chloride (MgCl₂) and 100 µM acetosyringone). DNA A and DNA B were mixed at equal concentrations (1.0 OD) and introduced by cutting of the leaf petiole with sharp blade and agroinoculated plants were grown in an insect free growth chamber at 22 ± 2°C with 12/12 hour light/dark cycle and observations were recorded periodically.

Results

Symptoms and detection

Yellow flakes appeared on the lamina of young leaves of CoGMV infected plants, which gradually increased in size to form green and chlorotic intermingled patches (Figure 1B). The full-length DNA A and B clones of three CoGMV isolates, namely, BD1 (Mymensingh), BD2 (Rangpur) and BD3 (Dinajpur), were amplified and fully sequenced, using the primer pairs CoGMVAF and CoGMVAR and CoGMVBF and CoGMVBR. Our attempts to amplify CoYVV and sub-genomic components from the jute samples using primer pairs targeting the CoYVV, alphasatellite [16] or betasatellite [17] were unsuccessful.

Characterization of CoGMV DNA A and DNA B

The complete nucleotide sequences of CoGMV DNA A were

determined to be of 2676 nt [BD1, Accession no. AB849288] and 2687 nt [BD2, Accession no. AB849290 and BD3, Accession no. AB849292] in length. The complete BD1 DNA A sequence shared the highest nucleotide sequence identity at 94.2% with CoGMV DNA A from Vietnam (Table 1). The DNA A sequences of BD2 and BD3 shared the highest nucleotide sequence identities at 98.8 and 98.5%, respectively; with Indian isolate of CoGMV DNA A (Table 1). The DNA A component of CoGMV had a typical New World bipartite begomovirus genome organization; all three isolates lacked the AV2 ORF that is present in Old World begomoviruses.

The complete nucleotide sequences of CoGMV DNA B were determined to be of 2668 nt [BD1, Accession no. AB849289], 2658 nt [BD2, Accession no. AB849291] and 2667 nt [BD3, Accession no. AB849292] in length and to encode two ORFs, BV1 in virion sense and BC1 in complementary sense orientation. 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 Table 1. The DNA B of BD1 shared the highest sequence identity at 72.6% with two Indian isolates of CoGMV. The complete DNA B sequence of other isolates [BD2 and BD3] shared the highest sequence identity at 97.1% with CoGMV Indian isolate.

The complete nucleotide sequences of CoGMV [BD1-3] were aligned with the corresponding sequences of other begomoviruses showing similarities in BLAST search (Supplementary file Table 2). The neighbor-joining phylogenetic analysis using complete DNA A and DNA B sequences of three Bangladeshi CoGMV isolates with the other CoGMV reported from India and Vietnam formed a distinct cluster

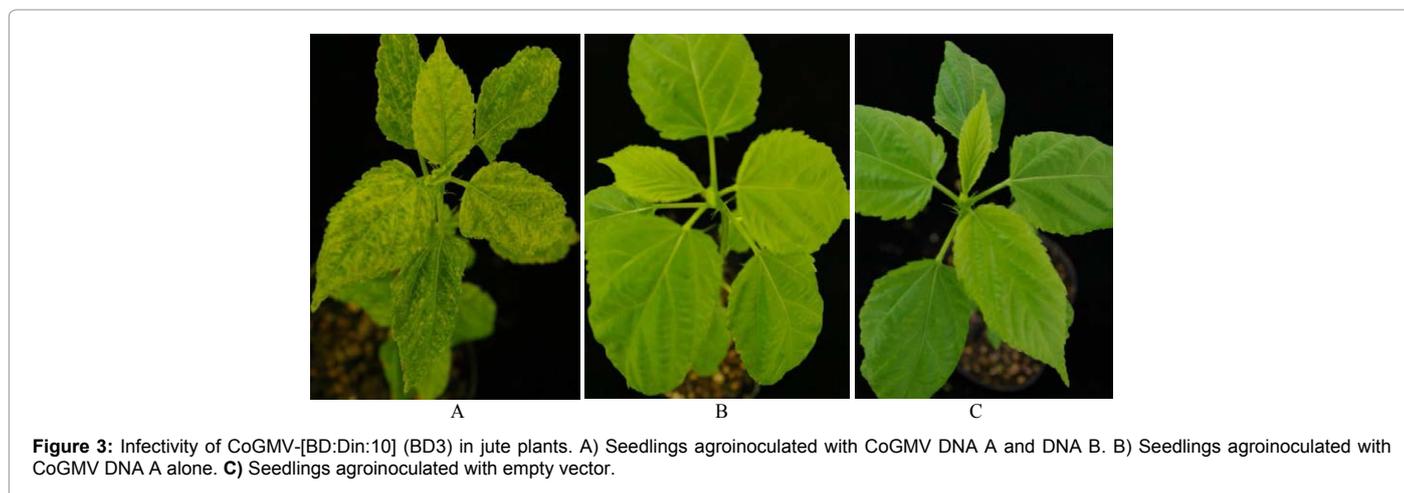
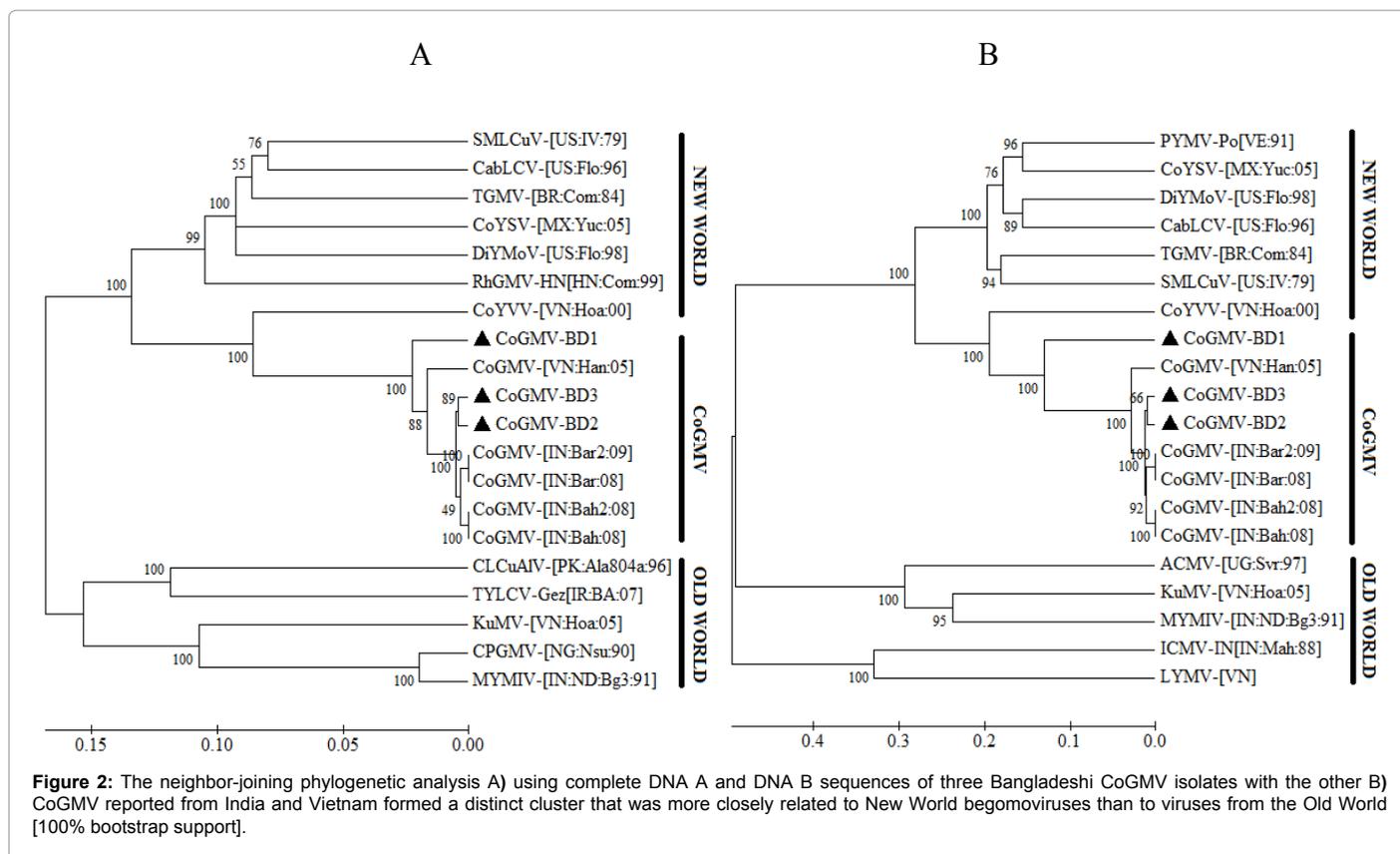
that was more closely related to New World begomoviruses than to viruses from the Old World (100% bootstrap support) (Figure 2).

Agroinoculation

In order to study the infectious nature of CoGMV-[BD:Din:10] [BD3], agroinfectious constructs of both genomes were introduced into the leaf petiole of jute using agroinoculation technique. Plain *Agrobacterium tumefaciens* cells were mock inoculated without any of the constructs as a control. Agroinoculation of constructs were carried out either individually or in combination. Initial small spots on leaf lamina was observed at 15 days post inoculation (dpi), which intensified further as typical yellow mosaic symptoms at 20 days post

inoculation (dpi) in jute plants when both the genomic components (DNA A and DNA B) were co-inoculated (Figure 3A). The DNA A genomic component failed to develop disease in jute when inoculated alone (Figure 3B). No noticeable phenotypic changes in jute plants were observed which were inoculated with plain *Agrobacterium tumefaciens* (control). The disease expressions of are summarized in Table 2.

Total DNA from systemic and symptomatic leaves of agro inoculated jute plants was extracted as described above and subjected to PCR using primer pair CoGMVAF/CoGMVAR. The expected 2.7 kb band was observed only in plants agro inoculated with both the genomic components. We could not observe any band when plants were inoculated with Co GMV DNA A alone.



Discussion

Yellow mosaic disease has immense economic importance, including through its association with jute plants in Bangladesh. In phylogenetic analyses, DNA A sequences of CoGMV BD1-3 isolates were clustered into a group comprising other isolates of CoGMV reported from India and Vietnam. On the basis of DNA A sequence similarity, BD1 was most closely related to Vietnam isolate (94.2%), whereas BD2 and BD3 were closely related to Indian isolates (98.2-98.8%). The level of nucleotide identity (92.1-92.8%) found between DNA A sequences of BD1 and other Indian and BD3 isolates of CoGMV was slightly lower than the strain demarcation level of 93% [18]. However, the DNA A of Vietnam isolate exhibited intermediate levels of nucleotide identity of 94.2% and 93.9% with BD1 and BD2, respectively, indicating that BD1, Vietnam and the other CoGMV isolates should be regarded as variants. It is also noteworthy that DNA B of BD1 had only 72.1-72.6% nucleotide identity with other CoGMV isolates. Possible recombination events could not be detected in DNA B of BD1 with other CoGMV and CoYVV isolates, using RDP ver. 3 [19] (results not shown). DNA B component, by virtue of encoding no overlapping genes, has a greater capacity for variation [20].

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. 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 [13]. Virus isolated from a plant may or may not be the cause of disease unless it satisfies the Koch's postulates. Therefore, to satisfy the Koch's postulates, the infectivity of CoGMV-[BD: Din: 10] [BD3] was established by inoculating the agro infectious clones in jute plant. The agro inoculation in jute plants resulted in the disease symptoms that are similar to those occurred in the virus infected jute plant in fields. Thus, we fulfilled the Koch's postulates and showed for the first time that CoGMV-[BD: Din: 10] [BD3] is responsible for the newly emerging yellow mosaic disease of jute in Bangladesh. Empty agrobacterium cells (control) or only DNA A of CoGMV-[BD: Din: 10] [BD3] inoculated plants did not show any symptom by 48 day dpi. This showed that only DNA A of CoGMV-[BD: Din: 10] [BD3] was neither able to sustain nor produce systemic infection in the host plants. This phenomenon is observed for most of the geminiviruses with the bipartite genomes [21].

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References

1. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (2012) Family Geminiviridae. In *Virus taxonomy: Ninth Report of the International Committee on Taxonomy of viruses*, Elsevier, New York, pp. 351-373.
2. Lazarowitz SG (1992) Geminiviruses: Genome structure and gene function. *Critical Reviews in Plant Sciences* 11: 327-349.
3. Hanley-Bowdoin L, Settledge SB, Orozco BM, Nagar S, Robertson D (2000) Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. *Crit Rev Biochem Mol Biol* 35: 105-140.
4. Das M, Banerjee S, Dhariwal R, Vyas S, Mir RR, et al. (2012) Development of SSR markers and construction of a linkage map in jute. *J Genet* 91: 21-31.
5. Kundu BC (1951) Origin of jute. *Indian Journal of Genetics*. 2: 95-99.
6. Ghosh SK, Som D (1998) Diseases of jute and their control. In *Pathological problems of economic crop plants and their management*, pp. 329-344. Edited by Paul Khurana SM. Scientific Publishers, Jodhpur, India.
7. Finlow RS (1917) Historical notes on experiments with jute in Bengal. *Agricultural Journal of India* 12: 3-29.
8. Ha C, Coombs S, Revill P, Harding R, Vu M, et al. (2008) Molecular characterization of begomoviruses and DNA satellites from Vietnam: additional evidence that the New World geminiviruses were present in the Old World prior to continental separation. *J Gen Virol* 89: 312-326.
9. Ha C, Coombs S, Revill P, Harding R, Vu M, et al. (2006) *Corchorus* yellow vein virus, a New World geminivirus from the Old World. *J Gen Virol* 87: 997-1003.
10. Ghosh R, Palit P, Paul S, Ghosh SK, Roy A (2012) Detection of *Corchorus* golden mosaic virus Associated with Yellow Mosaic Disease of Jute (*Corchorus capsularis*). *Indian J Virol* 23: 70-74.
11. Ghosh R, Paul S, Das S, Palit P, Acharyya S, et al. (2008) Molecular evidence for existence of a New World begomovirus associated with yellow mosaic disease of *Corchorus capsularis* in India. *Australasian Plant Disease Notes* 3: 59-62.
12. Ghosh R, Palit P, Ghosh SK, Roy A (2011) A New World virus alters biochemical profiling of jute plants (*Corchorus capsularis*) upon infection. *International Journal of Science & Nature* 2: 883-885.
13. Hasan MM, Sano Y (2014) Genomic variability of *Corchorus* golden mosaic virus originating from Bangladesh. *International Journal of Phytopathology* 3: 81-88.
14. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.
15. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731-2739.
16. Bull SE, Briddon RW, Markham PG (2003) Universal primers for the PCR-mediated amplification of DNA 1: A satellite-like molecule associated with begomovirus-DNA β complexes. *Molecular Biotechnology* 23: 83-86.
17. Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG (2002) Universal primers for the PCR-mediated amplification of DNA beta: a molecule associated with some monopartite begomoviruses. *Mol Biotechnol* 20: 315-318.
18. Fauquet CM, Briddon RW, Brown JK, Moriones E, Stanley J, et al. (2008) Geminivirus strain demarcation and nomenclature. *Arch Virol* 153: 783-821.
19. Martin DP, Lemey P, Lott M, Moulton V, Posada D, et al. (2010) RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics* 26: 2462-2463.
20. Harrison B (1985) Advances in Geminivirus Research. *Annual Review of Phytopathology* 23: 55-82.
21. Padidam M, Beachy RN, Fauquet CM (1995) Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *J Gen Virol* 76: 25-35.