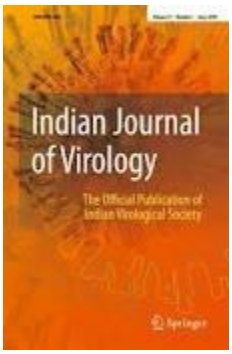


# Detection of Corchorusgolden mosaic virus Associated with Yellow Mosaic Disease of Jute (Corchorus capsularis)

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**Detection of *Corchorus golden mosaic virus* Associated with Yellow Mosaic Disease of jute (*Corchorus capsularis*)**

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## Abstract

Yellow mosaic disease, caused by a whitefly transmitted New World begomovirus, named *Corchorus Golden Mosaic Virus* (CoGMV), is emerging as a serious biotic constraint for jute fibre production in Asia. For rapid and sensitive diagnosis of the

begomovirus associated with this disease, a non-radiolabelled diagnostic probe, developed against the DNA A component of the east Indian isolate of CoGMV, detected the presence of the virus in infected plants and viruliferous whiteflies following Southern hybridization and nucleic acid spot hybridization tests. Presence of the virus was also confirmed when polymerase chain reaction amplification was performed using virus-specific primers on DNA templates isolated from infected plants and viruliferous whiteflies.

*Geminiviridae* is a family of plant viruses whose members cause serious damage to several crops worldwide, especially in tropical and subtropical regions [3,27,29,33]. Viruses of *Geminiviridae* family are grouped into four genera (*Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus*), which differ in genome organization, host range and vectors [10]. Begomoviruses are transmitted by whitefly (*Bemisia tabaci*) and infect dicotyledonous plants. Genome of begomoviruses are either bipartite or monopartite [38]. In bipartite begomoviruses, DNA A encodes proteins needed for replication and encapsidation of the genome [18,24,39]. DNA A typically has six open reading frames (ORFs): AV1/V1 (coat protein, CP) and AV2/V2 (AV2/V2 protein) on the virion-sense strand, and AC1/C1 (replication initiation protein, Rep), AC2/C2 (transcriptional activator protein, TrAP), AC3/C3 (replication enhancer protein, REn) and AC4/C4 (AC4/C4 protein) on the complementary-sense strand. DNA-B has two ORFs encoding proteins necessary for virus movement: BV1 (nuclear shuttle protein, NSP) on the virus-sense strand and BC1 (movement protein, MP) on the complementary-sense strand [33,36].

Based on phylogenetic studies and genome arrangement, bipartite begomoviruses have been divided broadly into two groups: the Old World (OW) viruses (eastern hemisphere, Europe, Africa, Asia) and the New World (NW) viruses (western hemisphere, the Americas) [30,31,34]. Begomovirus genomes have a number of characteristics that distinguish OW and NW viruses. DNA-A of bipartite begomoviruses from the NW lacks an AV2 ORF [34,38]. NW begomoviruses also have an N-terminal PWRsMaGT motif in the CP that is absent from OW viruses [18]. Until recently, it was thought that NW viruses arose more recently than OW viruses, evolving after continental separation of the Americas from Gondwana [34].

Rapid and accurate detection of the causal virus is an important prerequisite to monitoring plant virus epidemics [23]. Techniques based on polymerase chain reaction (PCR) and nucleic acid hybridization were used for the detection of these viruses [37]. PCR allows the detection of very small amounts of plant viruses in their hosts and vectors [19,21,22]. As PCR amplifies the viral nucleic acid, this approach is extremely useful in bypassing problems associated with serology.

Jute (*Corchorus capsularis* and *C. olitorius*) is one of the most important commercial crops in India. The raw fibre obtained from the bark tissue is being utilized in packaging industries for more than hundred years. Besides, jute fibre also has the potential utilization in various value added products in textile industries. Jute plants in India are infected by many diseases [15]. A yellow mosaic disease is known to have infected *C.*

*capsularis* since 1917 [11]. Effect of jute mosaic disease on jute production was investigated and it has been considered to be one of the most important limiting factors of jute cultivation [8]. During the initial infection stage the disease is characterized by symptoms on the leaf lamina such as small yellow flakes which gradually increase in size to form green and chlorotic intermingled patches producing a yellow mosaic appearance. The symptom bearing true leaves crinkled, leathery and sometimes, at the top of the plant, some-what needle like. The floral organs are more or less deformed. Internodes and branches become proliferated. Leaf mosaic infected plants have significantly lower percentage of cellulose, lignin, and pectin, thus the fibre strength become weak [4]. Whitefly (*Bemcia tabaci*) transmission of the disease has been reported by several workers [1,2,12,16,40]. Management of jute leaf mosaic through vector control and cultural practices were carried out in Bangladesh [20]. Due to unavailability of proper diagnostic tool there was is a lot of confusion about the exact identity of the causal agent of yellow mosaic of jute. Many workers believed that the causal agent is a virus [16,26]. Side by side, there were claims that the causal agent could be mycoplasma or rickettsea [5,6,32]. Recently, association of a New World bipartite begomovirus named *Corchorus golden mosaic virus* (CoGMV) with the disease was established from Vietnam [17]. In the same year the association of CoGMV with the yellow mosaic disease of jute from India was reported [12]. Both the DNA A and DNA B components of east and north Indian isolates of CoGMV were amplified using a rolling circle amplification strategy, cloned and sequenced [14]. Sequences were deposited in nucleotide sequence database under the Accession No. EU636712, FJ790326 (DNA A from east Indian isolate); FJ463902, GQ183863 (DNA A from north Indian isolate); FJ455448, FJ790327 (DNA B from east Indian isolate); EU636712, FJ790326 (DNA B from north Indian isolate). We report here the molecular diagnosis of the CoGMV infecting jute crop in India.

Jute plants showing typical yellow mosaic symptoms, were collected from different farmers fields of West Bengal (east Indian isolate) and Uttar Pradesh (north Indian isolate) and were re-established in healthy *C. capsularis* plants under glasshouse condition through whitefly transmission. Non-viruliferous whiteflies reared on healthy tobacco plants were allowed to feed on infected jute plants with acquisition access period of 24 h. The viruliferous whiteflies were then collected with the help of an aspirator and approximately 10 insects/ plant were released to healthy jute plants, grown under controlled conditions, for an inoculation access period of 24 h. The whiteflies were then collected and killed by spraying with the insecticides. The inoculated plants were maintained in an insect-proof glasshouse and observed for symptom development up to 4 weeks.

In a separate experiment, viruliferous whiteflies were collected after acquisition access period of 24 h to test the presence of virus in them. The whiteflies were then stored in sterilized small glass tube at $-20^0$C for further processing. Likewise, healthy whiteflies served as controls for succeeding experiments were also collected.

Total DNA was isolated from 100 mg leaf tissues of experimentally inoculated jute plants maintained under glasshouse and field samples as per modified CTAB method [13]. Total DNA was also isolated from groups of 10 viruliferous whiteflies following CTAB

method [9]. DNA isolated from the healthy leaf tissues and non-viruliferous whiteflies using the above mentioned methods were used as negative controls. Recombinant plasmid containing the DNA A component of the east Indian isolate of CoGMV (reference clone, Accession No. EU636712) was used as positive control.

The genomic DNA, obtained from leaf tissues of infected and healthy jute plants was electrophorased in 0.8% agarose gel and then was transferred onto nitrocellulose membrane by capillary transfer method for Southern hybridization using the protocol of Sambrook and Russell, 2001 [35]. For the nucleic acid spot hybridization (NASH) test, the blot was prepared by placing 100 ng total DNA (5µl) directly on the membrane and crosslinked in UV crosslinker. Different dilutions of DNA (1 : 1, 1 : 10, 1 : 100 and 1 : 1000) from infected and healthy leaf tissues and whiteflies were used for NASH test.

The DIG-labelled probe was prepared against the reference clone using DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics, GMBH, Mannheim Germany) followed by hybridization as described in the manufacturer's protocol.

Polymerase chain reaction was performed for rapid detection of CoGMV in infected jute plants collected from eastern and northern India and also from viruliferous whiteflies. DNA A-specific abutting primer pair (JMFL114F:5'-CGTACGAATGCTGGGACCTCC-3'/ JMFL112R:5' CCATGGGGCCTCACGTTTCATC-3') designed from the nucleotide co-ordinate 230-270 of the reference clone was used for PCR amplification. PCR was done in 50 µl volumes, each containing 20 ng of template DNA, 100 ng of primers, 1X PCR buffer, 10mM dNTP, 1.5 mM $MgCl_2$ and 1 U *Taq* DNA polymerase (Fermentas, MD, USA). PCR products were visualized after electrophoresis in a 1.0% w/v agarose gel at 2 v/cm for 45 min under ultraviolet light.

Symptomatic jute samples obtained from glasshouse and different fields of eastern and northern India showed positive hybridization signal in Southern blot analysis (Fig. 1) and in the NASH test (Fig 2) using specific probes prepared against the reference clone. In jute plants, viral DNA A could be detected up to a dilution of 1 : 100 by NASH, although hybridization at this dilution was found weaker than that obtained with 1 : 1 and 1 : 10 dilutions (Fig. 2). The viruliferous whiteflies showing positive hybridization signal in NASH test indicated the presence of the virus within whiteflies, the vector of this disease (Fig. 2). The hybridization obtained with north Indian diseased samples was the same as that obtained with the east Indian diseased sample. No hybridization was detected with any of the healthy control plants as well as with non-viruliferous whiteflies. The specificity of the probe was tested in a separate experiment in which DNA from the leaf samples of mungbean, bhendi and Ageratum plants infected with *Mungbean yellow mosaic virus*, *Bhendi yellow vein mosaic virus* and *Ageratum yellow vein virus*, respectively were used. None of these samples gave any cross reaction with the *Corchorus golden mosaic virus* probe developed in this study (data not shown), possibly due to very low sequence similarity of CoGMV with any of these viruses used in hybridization test.

In PCR, the full length DNA A (approximately 2.7 kb) was amplified from all the infected jute plants and viruliferous whiteflies, using the specific primer sets. No size difference was observed between the amplicons (Fig. 3). The healthy samples did not gave any amplified product.

The study showed that CoGMV could be detected by Southern hybridization, NASH and PCR amplification in all the experimentally and naturally infected jute plants showing yellow mosaic symptom. The probe developed in the study could specifically detect the virus even upto 1:100 dilutions from infected plants and viruliferous whiteflies in NASH test. The nucleic acid based hybridization is a very common method for detection of begomoviruses [25]. The specific primers developed in the study detected the presence of the virus from two geographical regions. Use of such primers for specific detection of begomoviruses has also been reported by several workers [7,28]**.**

The yellow mosaic disease of jute was known since 1917, but the causal virus could not be identified due to paucity of the detection tools. This study generated virus specific probe and primers which could be used as reliable diagnostic tool to identify the CoGMV, an emerging begomovirus in Asian continent, not only in jute samples but in other hosts that might be infected by this newly spreading virus in future.

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**Figure legend**:

**Fig. 1** Detection of DNA A in jute plants by Southern hybridization with non-radiolabelled probe of full length DNA A of CoGMV. 1: glasshouse sample of diseased *Corchorus capsularis* (east Indian isolate), 2: field sample of diseased *C. capsularis* (east Indian isolate), 3: glasshouse sample of diseased *Corchorus capsularis* (north Indian isolate), 4: field sample of diseased *C. capsularis* (north Indian isolate), 5: healthy *C. capsularis* (negative control), 6: PCR product of reference clone from which probe was developed (positive control)

**Fig. 2** Detection of DNA A in jute plants and in viruliferous whiteflies by probe prepared from clone of full length DNA A of CoGMV by nucleic acid spot hybridization. 1: *Corchorus capsularis* (east Indian isolates), 2: *Corchorus capsularis* (north Indian isolates), 3: viruliferous whiteflies, 4: reference clone from which probe was developed (positive control), 5: healthy *Corchorus capsularis* (negative control), 6: healthy whiteflies (negative control).

**Fig. 3** Polymerase chain reaction (PCR) amplification of jute samples and whiteflies for full length DNA A (2.7 kb) molecules. DNA used in the PCR from the hosts: 1: *Corchorus capsularis* (east Indian isolate), 2: *Corchorus capsularis* (north Indian isolate), 3: viruliferous whiteflies, 4: reference clone from which primer was developed (positive control), 5: healthy *Corchorus capsularis* (negative control), 6: healthy whiteflies (negative control), M: 1 kb DNA ladder (Fermentas, MD, USA).

**Fig 1:**

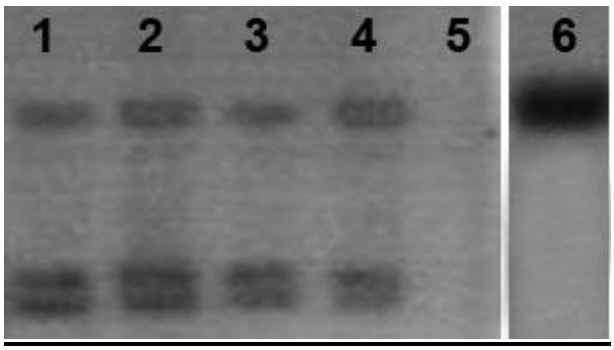


**Fig 2:**

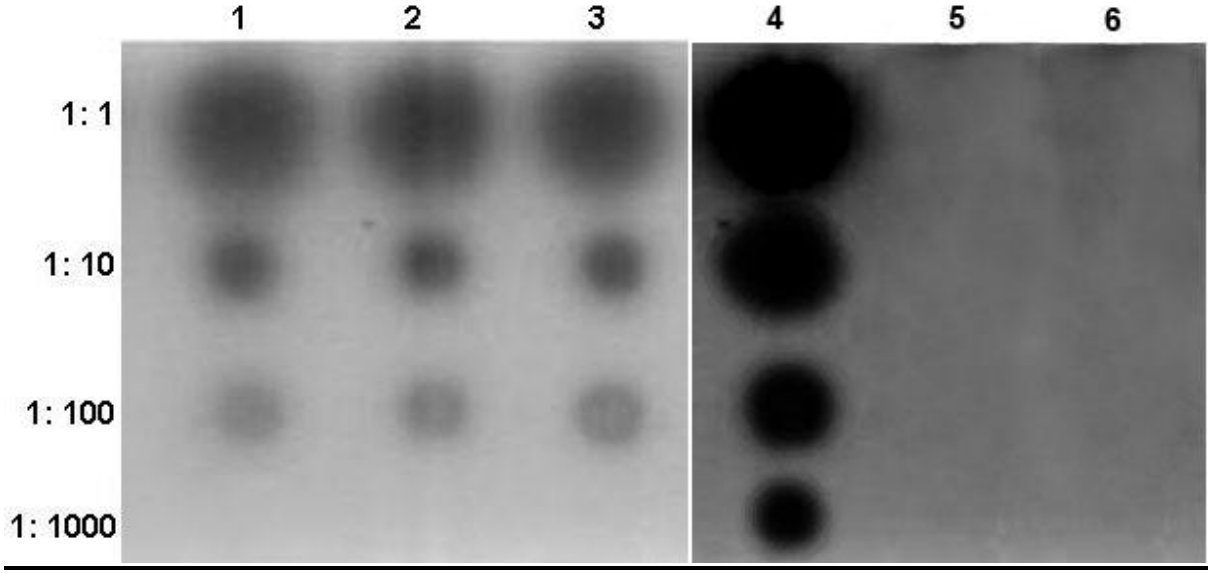


**Fig 3:**