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Molecular evidence for existence of a New World begomovirus associated with yellow mosaic disease of *Corchorus capsularis* in India

R. Ghosh[A], *S. Paul*[A], *S. Das*[A], *P. Palit*[A], *S. Acharyya*[A], *A. Das*[A], *J. I. Mir*[A], *S. K. Ghosh*[A] *and A. Roy*[A,B]

[A]Plant Virus Laboratory & Biotechnology Unit, Division of Crop Protection, Central Research Institute for Jute and Allied Fibres, Kolkata 700120, India.

[B]Corresponding author. Email: anirbanroy75@yahoo.com

Abstract. Yellow mosaic disease of jute (*Corchorus capsularis*) was found to be associated with a whitefly-vectored begomovirus. Sequencing of part of the begomovirus DNA-A indicated the presence of a New World begomovirus which has been detected for the first time in India.

Jute (*Corchorus capsularis* L. and *Corchorus olitorius* L.), belonging to the family Tiliaceae, is one of the most important fibre crops of India. It has an average production of over 1 620 000 tonnes of jute goods per annum with an average export of 200 000 tonnes/year earning of Rs. 7500 million/year. (http://pib.nic.in/release/release.asp?relid=9038). Almost 80% of India's raw jute is produced in West Bengal. Apart from making hessians, sacking, gunny bags, carpets, mat and rope, jute is also used for making many and diverse products for domestic consumption. Jute plants in India are infected by many diseases (Ghosh and Som 1998). A whitefly-vectored yellow mosaic disease is known to have infected *C. capsularis* since 1975 (Ahmed 1978). The disease is characterised by symptoms such as small yellow flakes on the lamina during the initial infection stage which gradually increase in size to form green and chlorotic intermingled patches producing a yellow mosaic appearance (Fig. 1). Over the last 4 years we have conducted a survey on the disease within different jute growing regions of India. The results of this survey indicate that the incidence of the disease has increased from nearly 20% in 2004 to above 40% in 2007 (Table 1). The incidence of the disease has been found to be around 50% on some of the leading *C. capsularis* cultivars such as JRC 7447 and JRC 212. It was also observed from the survey that infection reduces plant height to the extent of 20% and thus adversely affects the yield of the fibre.

The diseased jute plants showing typical yellow mosaic symptoms, collected from the experimental fields of Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata, India were used as initial source of virus inoculum. For virus transmission studies, a virus free stock of whitefly (*Bemisia tabaci* Genn.) was reared on healthy tobacco plants (*Nicotiana tabacum*) in insect-proof wooden cages. The adult whiteflies that emerged from nymphs grown on the *N. tabacum* were used for transmission. Healthy jute plants (*C. capsularis* cv. JRC 7447 and JRC 212) were raised under glasshouse conditions with five seedlings per pot. Sets of 10 healthy whiteflies were collected with an aspirator into polyvinylchloride (PVC) bottles containing a leaf of a jute plant showing typical yellow mosaic symptom and allowed to feed for 24 h. After virus acquisition, each set of viruliferous whiteflies was released onto 30 healthy jute seedlings (five seedlings per pot) and was contained by a small plastic cylindrical cage (7.5×2.5 cm). After a 12-h transmission period, the plants were sprayed with 0.2% dimethoate (Rogor) and kept in an insect proof rectangular cage until symptom development. The experiment was repeated three times with 30 plants in each case. The same numbers of healthy plants were also inoculated with non-viruliferous whitefly as controls in each replication and no symptom expression was observed in these plants. Typical yellow mosaic symptoms of the disease were observed on glasshouse-grown healthy plants of cv. JRC 7447 and JRC 212 after 10 days of viruliferous whitefly transmission. It has been observed that when 10 whiteflies were used for transmission the efficiency was 60%, but the transmission efficiency was as low as 20% when only three viruliferous whiteflies were used.

Fig. 1. Yellow mosaic disease of *Corchorus capsularis* plants grown under field conditions. (Inset) a close view of the symptoms on leaves.

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Table 1. Survey on jute mosaic disease

CRIJAF, Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata, India

Location	Total no. of plants surveyed	Disease incidence[A]			Disease severity[B,C]			Plant height (cm)[C]			
		2004–05	2005–06	2006–07	2004–05	2005–06	2006–07	Non-symptomatic	Symptomatic	Reduction (%)	Varity cultivated
CRIJAF Main farm											
Plot I	525	19.50	29.95	33.57	66.45 ± 3.30	62.70 ± 1.02	71.57 ± 1.63	245.34 ± 2.33	191.66 ± 1.57	21.88	JRC 321
Plot II	400	20.42	18.74	50.18	74.89 ± 2.97	70.12 ± 2.85	62.80 ± 2.76	213.98 ± 2.99	182.95 ± 1.95	14.50	JRC 212
Plot III	567	19.89	37.33	36.1	59.29 ± 1.24	65.87 ± 1.09	65.56 ± 1.27	263.35 ± 1.33	201.91 ± 2.34	23.33	JRC 4444
CRIJAF North farm											
Plot I	312	22.67	27.43	51.03	71.22 ± 2.23	70.87 ± 1.03	70.70 ± 2.3	245.21 ± 1.87	195.11 ± 2.33	20.43	JRC 7447
Plot II	298	18.99	30.00	37.15	69.88 ± 1.97	67.57 ± 0.97	70.10 ± 2.9	276.78 ± 1.98	211.95 ± 1.03	23.42	UPC 94
Plot III	397	21.40	28.90	40.78	79.75 ± 3.46	74.6 ± 3.93	76.18 ± 3.59	257.70 ± 0.98	214.79 ± 0.79	16.65	CEX 3

[A]Disease incidence: % of surveyed plants that were symptomatic.

[B]Disease severity: % of symptomatic leaves on a plant.

[C]Mean average ± s.e. from 40 plants in each case.

Back-inoculation to a new set of healthy plants produced similar yellow mosaic symptoms and confirmed the whitefly transmissibility of the disease. The whitefly transmissibility and the type of symptomatology highlighted the probable association of a whitefly-vectored begomovirus with the disease.

To confirm the association of a begomovirus with the yellow mosaic disease of jute, total nucleic acid was isolated and purified following the method of Doyle and Doyle (1987) from the leaves that showed distinctive yellow mosaic symptoms under glasshouse conditions (nine samples) and field conditions (three samples). To remove the mucilaginous substances, the isolated total nucleic acid was further purified through a DNeasy Mini Spin Column (Qiagen). Begomovirus-specific primers (Rojas *et al*. 1993) were used to amplify the corresponding genomic fragments of the virus. The primers PAL1v1978 and PAR1c496 amplified the expected 1.2-kb segment of DNA-A from all the 12 samples tested (Fig. 2). Gel-eluted amplicons (eight amplicons from glasshouse samples and two amplicons from field samples) were cloned into the pJET1 positive selection vector using the GeneJET™ PCR Cloning Kit (Fermentas) following the manufacturer's protocol and competent *Escherichia coli* cells (strain DH5-α) were transformed following standard molecular biology procedures. Sequencing of a representative clone from each of the 10 amplicons revealed that all the inserted fragments were 1263 nucleotides in length and identical in sequence except for two clones in which only two nucleotides were found to vary. These sequence variations may be due PCR or sequencing error. One representative sequence was deposited in the GenBank database under the accession number EU047706.

Sequence analysis of accession number EU047706 revealed that it has a 91.2% nucleotide identity with *Corchorus golden mosaic virus* (DQ641688), a New World begomovirus that was recently reported from Vietnam (Ha *et al*. 2008) (Table 2). Interestingly, the nonanucleotide sequence at the origin of replication was CATTATTAC in the jute isolate instead of the conventional TAATATTAC which has previously been determined as the most conserved feature within the Geminiviridae family (Roberts and Stanley 1994). This unique change was consistently observed in all of the 10 clones, obtained both from the glasshouse samples (eight clones, Lanes 1–8 of Fig. 2) and field samples (two clones, Lanes

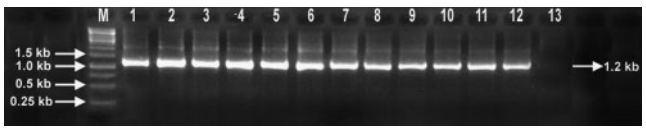

Fig. 2. 1.2 kb amplicon of DNA A component of the begomovirus associated with the yellow mosaic disease of *C. capsularis*. Lanes: 1–9: glasshouse grown plants; lanes 10–12: field samples; and lane 13: healthy control; M: DNA 1 kb DNA ladder (Fermentas).

Table 2. Percentage sequence identity of the present jute begomovirus isolate from India (accession number EU047706) with other begomoviruses reported from Old and New World

Group	Virus name	Acronym	Accession no.	% identity
New World	*Corchorus golden mosaic virus*	CoGMV	DQ641688	91
	Corchorus yellow vein virus	CoYVV	AY727903	57
	Sida mottle virus	SiMoV	AY090555	42
	Squash leaf curl virus	SLCV	M38183	40
	Tomato golden mottle virus	ToGMoV	AF132852	36
	Sida golden mosaic virus	SiGMV	AF049336	36
	Tomato mottle Taino virus	ToMoTV	AF012300	34
	Sida golden mosaic Costa Rica virus	SiGMCRV	X99550	34
	Cabbage leaf curl virus	CaLCuV	U65529	30
Old World	*Tomato leaf curl Laos virus*	ToLCLV	AF195782	45
	Tobacco leaf curl Yunnan virus	TbLCYNV	AJ566744	45
	Eupatorium yellow vein virus	EpYVV	AJ438936	45
	Tomato leaf curl virus	ToLCV	S53251	44
	Malvastrum leaf curl Guangdong virus	MLCV	AM503104	44
	Ageratum yellow vein virus	AYVV	X74516	44
	Tomato yellow leaf curl virus-Thailand	TYLCTHV	AY514630	43
	Indian cassava mosaic virus	ICMV	AJ314739	43
	Cotton leaf curl Rajasthan virus	CLCuRV	AF363011	43
	Tomato leaf curl Vietnam virus	ToLCVV	AF264063	42
	East African cassava mosaic virus	EACMV	AF126806	42
	Mungbean yellow mosaic virus	MYMV	D14703	41
	Mungbean yellow mosaic India virus	MYMIV	AF126406	41
	Squash leaf curl virus-China	SLCCNV	AF509743	39
	African cassava mosaic virus	ACMV	AF126802	35
	East African cassava mosaic virus	EACMV	AF126806	42
	Mungbean yellow mosaic virus	MYMV	D14703	41
	Mungbean yellow mosaic India virus	MYMIV	AF126406	41
	Squash leaf curl virus-China	SLCCNV	AF509743	39
	African cassava mosaic virus	ACMV	AF126802	35

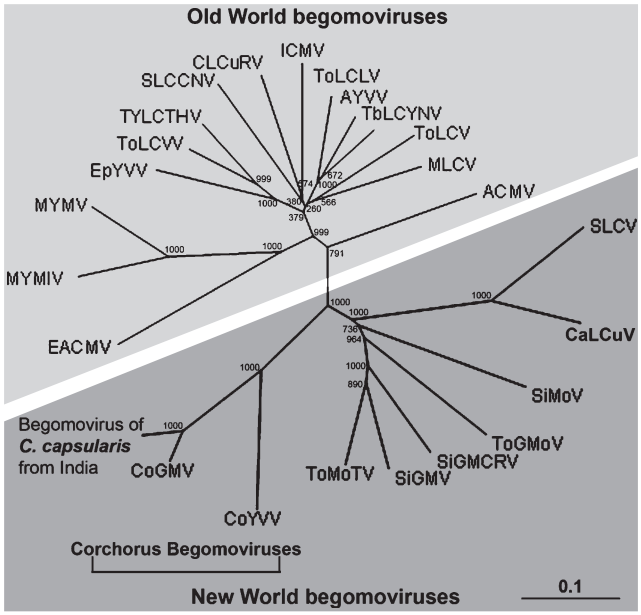

Fig. 3. Unrooted dendogram for phylogenetic analysis of the begomovirus associated with yellow mosaic disease of *Corchorus capsularis* in India. The begomoviruses isolated from *Corchorus* sp. form a separate subcluster within the New World begomovirus group that is separate from the Old World begomoviruses. The bootstrap value of each cluster is given at the nodes.

10–11 of Fig. 2). We observed that in *Corchorus golden mosaic virus* reported from Vietnam, the nonanucleotide sequence also has the modified nucleotides but in this case it is TATTATTAC and Ha and his co-workers did not comment on this observation.

To assess the relationships with other begomoviruses, the corresponding genomic regions of nine New World and 13 Old World begomoviruses were obtained from GenBank and a phylogenetic tree was constructed and bootstrapped after multiple alignment using the Neighbour Joining algorithm of Clustal X (version 1.8) software (Thompson *et al*. 1997) and viewed by Treeview software. Sequence identity matrix was generated for comparison using BioEdit Sequence Alignment Editor (version 5.0.9) (Hall 1999). Phylogenetic analysis revealed that this jute begomovirus isolate from India grouped with other begomoviruses reported to be associated with *Corchorus* sp. and clustered with the New World begomoviruses (Fig. 3). *Corchorus golden mosaic virus* was reported earlier from Vietnam (Ha *et al*. 2008), where this virus was shown to be a member of New World begomovirus group and this proposition has been approved by the International Committee on Taxonomy of Viruses. As the segment of sequence we report here shares the highest sequence identity with *Corchorus golden mosaic virus*, we propose that this Indian isolate of *Corchorus golden mosaic virus* is also a member of the New World begomoviruses. The present investigation thus constitutes the first report of the association of a begomovirus, related to New World begomoviruses, with yellow mosaic disease of *C. capsularis* in India.

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