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Characterization of West Bengal isolate of Rice Tungro bacilliform virus (RTBV)

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ABSTRACT

Tungro is a devastating disease of rice caused by rice tungro bacilliform virus (RTBV). It is transmitted by *Nephotettix virescens* commonly known as green leaf hopper (GLF). Present investigation was undertaken to study the plasmid blue script having movement protein and coat protein clones (PCR amplified products). These were isolated and digested with different restriction enzymes and were separated by 1% agarose gel electrophoresis. Gel was blotted on nitrocellulose membrane and Southern blots were hybridized with different radio labeled probes of different fragments of RTBV genome. Autoradiography was also carried out. Based on present hybridization studies it is concluded that both the putative clones are identical and they carry some part of coat protein and some part of movement protein encoding gene.

Keywords : Rice, RTBV, Tungro, Molecular characterization, Autoradigraphy

Rice (*Oryza sativa* L.) is the world's single most important food crop and a primary food for more than a third of the world's population. Rice tungro, the most important virus disease of rice in South and Southeast Asia. Rice tungro disease is caused by an infection of two different viruses, rice tungro spherical virus (a (+) sense RNA virus) and rice tungro bacilliform virus with a genome of circular double-standard DNA Rongda Qu *et al.* 2004) It is a plant pararetrovirus of the family Caulimoviridae. Tungro means 'degenerated growth' and was first observed in Philippines and first described by Saito *et al.* (1975) In Indonesia. In South and Southeast Asia, rice tungro bacilliform virus, which is transmitted by green leafhoppers, causes one of the most serious diseases of rice with the assistance of rice tungro spherical virus (Nagano *et al.*2002)

Based on symptoms expressed in rice plants, different isolates were obtained from different parts of India which were characterized on the basis of symptoms severity and insect transmission (Shastry *et al.* 1971, Anjanlyulu & John 1972, Mishra *et al*. 1976). The genome of RTBV obtained from different geographical locations shows considerable differences at the nucleic acid level. Thus, for developing transgenic for a particular geographic area, the knowledge of the nucleic acid sequence of the regional isolate becomes necessary. In the present study the coat protein and movement protein genes of West Bengal isolate were characterized which will help in designing strategies for developing genetic engineering plants (transgenics) against rice tungro disease.

METHODS & MATERIALS

The PCR products of West Bengal isolate amplified using primer 13, 14 (movement protein part) and amplified using primer 15, 16 (coat protein part) were taken as source material (Tables 1 & 2).

Table 1— Details of the primers used in the study.

S. No.	Primer	Position in the RTBV DNA Sequence	sequence
1	13	5'2081-2063 3'	5' GGATCCTCATC TTCTATGACCGC C 3'
2	14	5'1018-1001 3'	5' GGTCGAGTCT TAGACCGTTT AG 3'
3	15	5'2358-2375 3'	5' GGTACCGAA ACTTAGAAGGA GCCA 3'
4	16	5'3370-3390 3'	5' GGCTGCAGCT CTAGCTTGATTG ACAT 3'

Table 2— Expected size of PCR products using given pairs of primers and the genes/ DNA fragment flanked by the primers.

S.No.	Primer	Size	Primer/ DNA fragment
1	13-14	1008 bp	Putative movement protein
2	15-16	1032 bp	Putative coat protein

These PCR products were cloned in plasmid bluescript and transformed in DH5a strain of *E.coli*. Plasmid bluescript having coat protein and movement protein gene (PCR amplified product) were isolated using alkaline lysis method of Birmboim & Dolly (1997) as described by Sambrook *et al.* (1989). Agarose gel (1-1.2% w/v) was prepared and plasmid DNA samples were electrophoresed at a constant voltage of 80-90. The plasmid DNA was then digested with different restriction enzymes (EcoRI, Hind III, SalI, BamHI, XbaI, KpnI and PstI). The restriction mixture was then electrophoresed on 1% agarose gel. Gels were then stained with ethidium bromide and computer generated image was taken on geldoc system. Each gel containing restriction digests were then blotted on hybond membrane following the method described by Southern (1975) which include four steps – Depurination, Denaturation, Neutralization and capillary blotting. Southern blots wee hybridized with different radiolabelled probes. These probes were different fragments of RTBV genome.

Probe 1 (KB8) :- pTBKB8 (cloned RTBV DNA from West Bengal isolate)

Probe 2 (KB8-I) :- pTBKB8 digested with NcoI and ClaI

Probe 3(KB8-II) :- pTBKB8 digested with Bam HI and PstI

Probe 4 (KB8-III) :- pTBKB8 digested with PstI and XbaI

Autoradiography was carried out in which hybond membrane were exposed to Kodak X-omat diagnostic film in cassettes with screen (Amersham International Inc. U.K.)./ This film was developed in dark room. Finally the film was washed in running water, dried and preserved for further studies.

RESULTS & DISCUSSION

The infection by RTBV causes stunted growth of plants, discoloration of leaves and reduction in tiller number and sterile or partly filled grains. In an earlier work movement protein part and coat protein part from West Bengal isolate of RTBV were amplified and these PCR products were cloned in plasmid bluescript and transformed in DH5a strain of *E .coli*.

Plasmid blue script containing putative movement protein and coat protein clones was isolated and isolated plasmid (linear, coiled, and supercoiled) was observed on agarose gel which carries the movement protein and coat protein clones.

Plasmid bluescript containing movement protein clones and coat protein clones on digesting with Bam HI and Sal I and Kpn I and Pst respectively produced two bands. It indicated that upper 30 Kb band is plasmid blue script and lower one of 1.7 Kb is the insert (Fig. 1).

On digestion of plasmid bluescript containing putative movement protein gene two band with Sal I + Bam HI two bands with Eco RI, one band with Hind III and two bands with Xba I were produced (Fig. 2). On digestion of plasmid bluescript containing putative coat protein gene two bands with Kpn I + Pst I, two bands with two bands with Eco RI, one band with Hind III and two bands with Xba I were produced (Fig. 3).

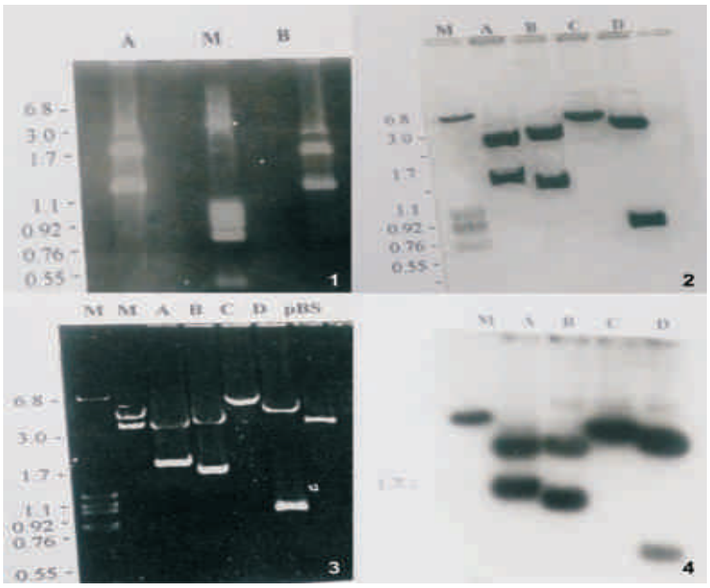

Fig.1— Plasmid bluescript containing PCR amplified products from W.B. isolate using primer 13, 14 (movement protein) and primer 15,16 (coat protein) digested with Bam HI and Sal I (lane A)and Kpn I and Pst I (Lane B). **2—** Plasmid blue script containing PCR amplified products from W.B. isolate using primer 13,14 (movement protein) digested with Sal I + Bam HI (Lane A), Eco RI (Lane B), Hind III (Lane C) and Xba I (Lane D). **3—** Plasmid blue script containing PCR amplified products from W.B. isolate using primer 15,16 (coat protein) digested with Kpn I + Pst I (Lane A), Eco RI (Lane B), Hind III (Lane C) and Xba I (Lane D). **4—** Autoradiogram of hybridization of Fig 2 bands with probe I (KB8).

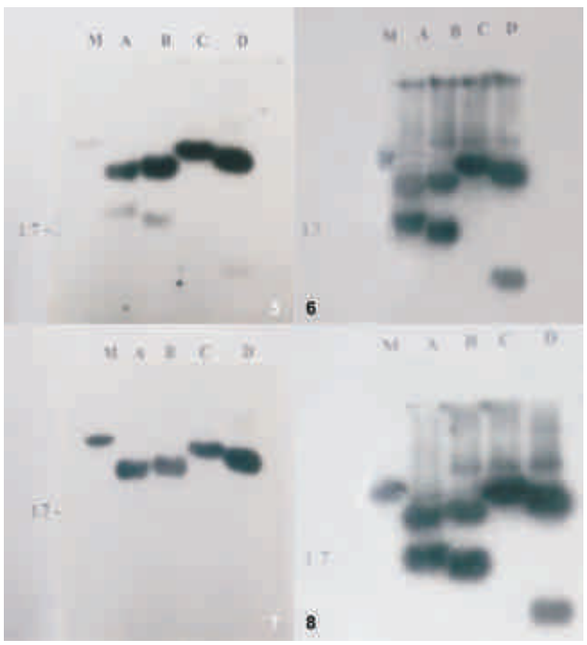

Fig. 5— Autoradiogram of hybridization of Fig. 2 bands with probe II (KB8-I). **Fig. 6—** Autoradiogram of hybridization of Fig. 2 bands with probe III (KB8-II). **Fig.7—** Autoradiogram of hybridization of bands shown in Fig. 2 with probe IV (KB8-III). **Fig.8—** Autoradiogram of hybridization of bands shown in Fig.3 with probe I (KB8).

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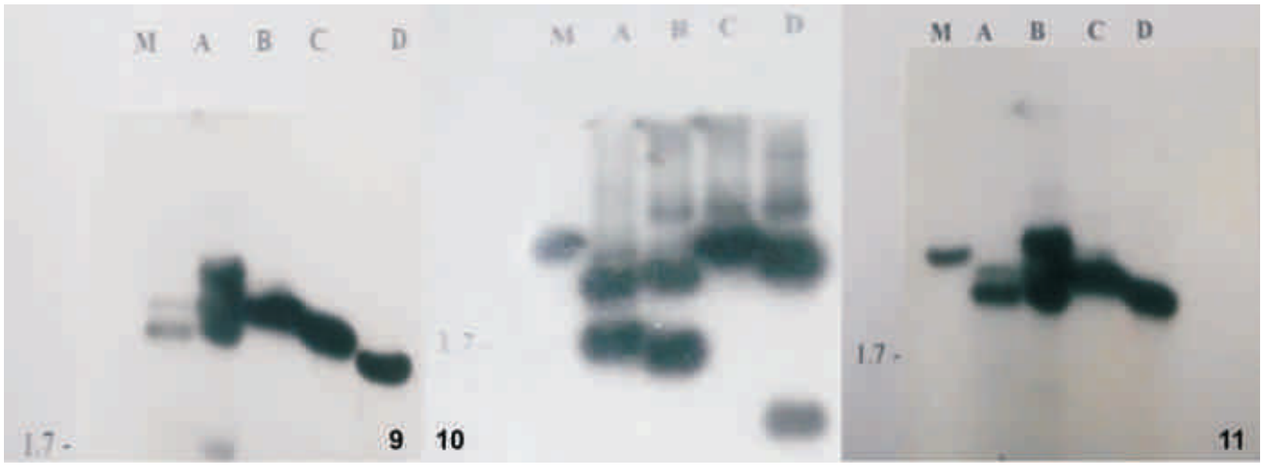

Fig.9 — Autoradiogram of hybridization of bands shown in Fig. 3 with probe II (KB8-I). **Fig.10 —** Autoradiogram of hybridization of Fig 3 bands with probe III (KB8-II). **Fig.11 —**Autoradiogram of hybridization of Fig 3 bands with probe IV (KB8-III).

On digestion with EcoR I two bands of sizes 3.1 Kb and 1.45 Kb were produced indicating that insert has one EcoR I site. On digesting with Hind III only one band of 4.45 was produced indicating absence of Hind III site is insert. On the other hand, on digestion with XbaI, two bands of 3.3 Kb and 0.66 Kb were produced indicating that insert has one Xba I site. Same pattern was observed in case of coat protein clone.

In southern blotting fragments were transferred from gel to Hybond membrane through capillary blotting. The blots of Fig 2 having movement protein gene, were hybridized with different probes (KB8, KB8-1, KB8-II and KB8- III). Results of autoradiography showed that hybridization of the bands with KB8 probe indicates that 1.7 Kb insert is of viral origin (Fig. 4).

The blots when hybridized with different fragments of KB8 insert mainly KB8-I (Fig. 5) and KB8-II (Fig. 6) it was found that the insert may contain some part of coat protein and some part of movement protein as these blots were not hybridized with another probe KB-III (Fig. 7).

The blots of Fig. 3 having coat protein gene, when hybridized with different probes same pattern was observed (Figs. 8, 9, 10, 11).

Galves (1968) purified the virus and reported that the particles are polyhedral with a diameter of 30-33 nanometers. The virus withstands temperatures below 63° C for 10 minutes. The work done on the molecular structure of RTBV has been reviewed by Hull (1996). It is a plant pararetrovirus with bacilliform particles, the structure of which is based on T = 3 icosahedral symmetry cut across the threefold axis. The particles encapsidate a circular double-stranded DNA of 8 kbp which encodes four proteins. The current information on the properties, functions, and expression of these proteins is discussed, as is the evidence for replication by reverse transcription. Two major strains of RTBV have been recognized, one from the Indian subcontinent and the other from Southeast Asia. RTSV particles contain a single-stranded RNA genome of 12 kb that encodes a large polyprotein and possibly one or two smaller proteins. The properties and processing of the polyprotein are described and the resemblance to picornaviruses noted. Fan *et al.* (1996) have isolated genomes of isolates of rice tungro bacilliform virus from Bangladesh, India, Indonesia, Malaysia and Thailand. These were cloned and compared with that of the isolate from the Phillipines. Restriction endonuclease map revealed differences between the isolates and cross-hybridization showed that they fell into two groups, those from Indian sub-continent and those from south-east Asian countries. These genome of isolates from the Indian sub-continent contained a deletion of 64 hp when compared with those from south-east Asia.

Malathi *et al.* (2015) studied the sequence divergence and evolution of RTBV isolates present in India and other countries. Phylogenetic analysis based on coat protein (CP) sequences of RTBV generated in this study showed distinct divergence of Indian and non-

Indian RTBV isolates into two clusters. Further, Indian RTBV isolates formed two groups- one consisted isolates from Andhra Pradesh and Kanyakumari, and other included isolates from Hyderabad, Punjab, and West Bengal. The results obtained from phylogenetic analysis were further supported with the single nucleotide polymorphisms (SNPs), insertion and deletions (INDELs) and evolutionary distance analysis. Signature sequences and amino acid motifs were identified which showed distinct difference between Indian and non Indian isolates. This study will help in understanding the geographical evolution and adaptation of RTBV in different rice ecosystems.

On the basis of present study it is concluded that both 13,14 and 15,16 amplified products do not contain purely movement protein and coat protein part and this 1.7 Kb insert codes for a part of movement protein and coat protein. It can also be concluded that both the putative clones are identical clones.

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