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First report of mesta yellow vein mosaic virus infecting okra in West Bengal

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ABSTRACT

Bhendi Yellow Vein Mosaic Virus (BYVMV) is predominantly occurring in okra across India wherever okra is grown but in West Bengal many fields are showing this typical yellow vein mosaic symptom in combination with severe leaf yellowing, enation and curling of leaves which is creating a confusion with the infection of Mesta yellow vein mosaic virus (MYVMV) and Okra enation leaf curl viruses (OELCV). Virus isolate Krish1 was obtained from Okra showing typical yellowing symptoms in Nadia, West Bengal. The complete nucleotide sequence of DNA-A was determined, it contains 2743 bp nucleotides (MN005041), with two ORFs in virion-sense DNA and five ORFs in complementary- sense DNA. Total DNA-A and deduced amino acid sequences of individual ORFs showed that 99.59% identity with Mesta Yellow Vein Mosaic Virus, Jalgaon, Maharashtra isolate.These are actually emerging in association with yellow vein mosaic symptoms in severe form that may evolve laterly by recombination with BYVMV and OELCV which is frequently occurring in this state. Here we also detected the presence of Beta-satellite of BYVMV in okra with MYVMV in the field of Nadia district which is highly significance in suppression of host resistance. To the best of our knowledge, this is first report of Mesta yellow vein mosaic virus (MYVMV) in okra from West Bengal.

Keywords: *Begomo virus, beta-satellite, detection, characterization, okra,*

Okra [*Abelmoschus esculentus* (family:Malvaceae)] commonly called lady's finger or bhendi is a very popular vegetable crop grown in West Bengal, India. In 2017, a vein yellowing mosaic disease of okra was observed in farmers' field of Nadia district of West Bengal, India (Fig.1). Symptoms were similar to those of okra yellow vein mosaic disease, a suspected viral disease of okra transmitted by vector white fly (*Bemiciatabaci*) (Rana*et al*., 2006). A 100% crop loss, severe mottling of leaves and yellowing of fruits were observed. Plants were severely stunted and fruits were unmarketable. In 2018, similar symptoms were observed in other fields of the same district in West Bengal. Samples were collected from the diseased okra fields showing visible symptoms of yellow vein mosaic virus disease. Genomic DNA was extracted and used for detection of the ssDNA associated with the disease specific primers SPG1 and SPG2 (Li *et al*., 2004) and also the associated beta satellite. PCR amplified bands of expected size 850 bp (Fig.2) was obtained from the concerned virus infected sample but not from healthy okra plant. DNA Amplification Kit, illustra™ TempliPhi (GE Healthcare, UK) was used for isothermal rolling circle amplification (RCA) to efficiently prepare DNA subjected to perform RFLP with *Kpn*I (Fig.2).The amplified products was cloned followed by sequencing by Chromous Biotech Pvt. Ltd., Bangalore. Annotation of nucleic acid (NA) sequence was done and submitted to NCBI. Accession number MN005041 has been assigned.Affinities of DNA-A of Krish1 isolate (MN005041) from okra to other begomo viruses were performed by comparing the sequence with other Begomovirus sequences of Okra in the Gen Bank data base.Whole genome sequence of Krish1 isolate MN005041 (nt 2743 bp) showed 99.59% identity with Mesta Yellow Vein Mosaic Virus, Jalgaon, Maharashtra isolate. Based on comparison with the previous reports available on NCBI, the virus is identified as MYVMV. The Krish1 isolate (MN005041) was also compared with the published sequences of Okra Yellow Vein Mosaic Virus (OYVMV), Okra Enation Leaf Curl Virus (OELCV), Mesta Yellow Vein Mosaic Virus (MYVMV). In all cases Tomato Leaf Curl Virus was considered as outgroup. Fig.3A shows <90% similarity of Krish1 isolate with all BYVMV sequences except that from Haryana showing 93% similarity. Fig.3B shows >91% similarity of MN005041 with all OELCV sequences. Although the symptoms were that of yellow vein mosaic virus, the genome showed greater similarity with the published database of OELCV than BYVMV. Fig.3C shows 100% similarity of MN005041 with MYVMV Jalgao and <90% similarity with other MYVMV sequences. Association of Beta-Satellite [MK844301.1 (NadiaBeta1 isolate)(1.35kb)] is confirmed with the infection of MYVMV which showed 94-98% similarity several BYVMV Beta isolates of India (Fig.3D). The

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Fig.1: Yellow vein mosaic disease effected field in Krishnanagar, Nadia, West Bengal
1a. Okra field is showing 100% infection with severe yellowing of plants;
1b. Yellowing of veins with complete bleaching of interveinal tissues of leaves

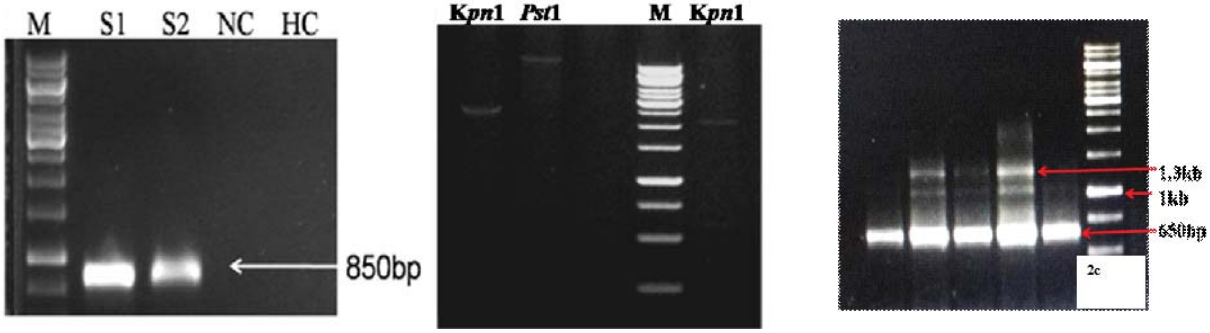

Fig.2: Agarose gel electrophoresis of PCR and RCA products
2a.shows ~850bp bands with degenerated primers, SPG1/SPG2;
2b.RFLP of RCA product with restriction enzymes amplified ~2.7kb band;
2c. Amplification of Beta Satellite with primers âC1/âC2 showing 1.3kb band and â helper band at 650bp M: Marker

dendrograms confirmed that MYVMV Krish1 isolate is closely related to MYVMV, Jalgaon, Maharashtra isolate and closely related to OELCV but very similarity with BYVMV.The Coat protein (CP) of MYVMV krish 1 isolate has very high amino acid sequence identity with OELCV and BYVMV (97%), suggesting that the CPs of these viruses have a common ancestor, and they may evolve by recombination with different begomo viruses.

Our study seconds the concept that various Gemini viruses are emerging as major threats to crops (Varma&Malathi, 2003), recombination and evolution of viruses urge the need for extensive studies of the newly associated virus with of yellow vein mosaic disease. Chatterjee *et al*., reported in 2005, that a begomo virus (transmitted by whitefly) in association with a â-DNA satellite produced yellow vein mosaic disease of mesta

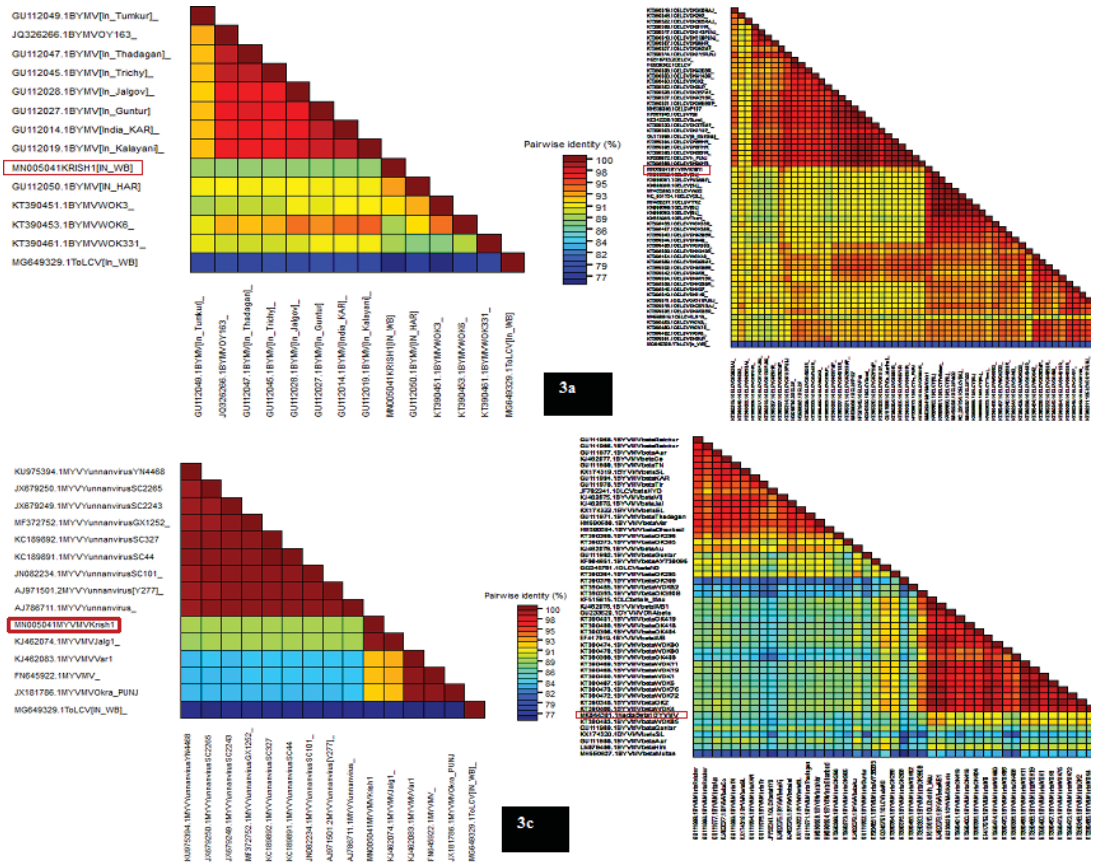

Fig.3: Similarity matrix.

3a showscomparative analysis of MN005041 (Krish1 isolate) with published data of Bhendi Yellow Vein Mosaic Virus,

3b shows comparative analysis of MN005041 (Krish1 isolate) with published data of Okra Enation Leaf Curl Virus,

3c shows comparative analysis of MN005041 (Krish1 isolate) with published data of Mesta Yellow Vein Mosaic Virus,3d shows comparative analysis of MK844301.1 (NadiaBeta1 isolate) with published data BYVMV Beta isolates.

(*Hibiscus cannabinus* and *Hibiscus sabdariffa*) in mesta growing regions of West Bengal, India. We report first time on the occurrence of Mesta yellow vein mosaic virus (MYVMV) in association of Betasatellite-BYVMV in okra from West Bengal.

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