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Begomovirus characterization from both soybean and insect vectors and their correlation by phylogenetic analysis in yellow mosaic disease development

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Abstract
The leaf sample from soybean plants showing the yellow vein mosaic disease symptoms was collected in New Delhi, India. The genome of the virus was amplified, cloned and sequenced. Sequence analysis revealed that the viral genome is 2747 nucleotides in length and genome is similar to that of monopartite begomoviruses originating from the Old World, with seven conserved ORFs. Further nucleotide (nts) sequence comparisons showed that the genome has the highest sequence identities of 99% the same as the sequence that had already been reported. These results suggested that the isolates are a strain of MYMIV also strongly associated with the whitefly population emerging during *Kharif* season in the year 2018. The isolates have obtained from sources like plant and whitefly having closely related with each other in isolated during initial period of *Kharif* from whiteflies and final period being considered for isolating from soybean tissue. In the study we have proved that insect vector bringing primary inoculum.

Keywords: Both soybean, vectors, phylogenetic analysis, yellow mosaic

Introduction
Soybean *Glycine max* (L.) Merr. (2n = 40) is a rare grain legume that serves as both a pulse and an oilseed, yielding both high-quality protein (38–44%) and oil (18-22%). In addition to being consumed as soya chunks and oils, soybeans are a popular source of traditional food among the ethnic communities of North East India, despite not being farmed commercially in this region. The majority of plant viruses require a biological vector to transmit from plant to plant in the wild. The majority of the 23 biotic vectors for plant viruses are hemipteroid insects feeding on phloem, such as whiteflies. Among various biotic stresses, Yellow Mosaic Virus Disease (YMD) is one of the most prevalent viral diseases, especially in the northern, northeastern, and central regions of India, where it causes yield losses of up to 80 percent. Yellow Mosaic Virus Disease (YMD) is a viral disease that is transmitted by the *Bassimia tabacci*. The begomovirus that causes YMD has two species: *Mungbean Yellow Mosaic India Virus and India Virus* (MYMIV) and *Mungbean Yellow Mosaic Virus* (MYMV) (Fauquet *et al*., 2003) [2]. At least four different species of whitefly (*Bemisia tabaci* Genn.)-transmitted begomoviruses (WTV, family Geminiviridae) cause YMD. These viruses are called legume yellow mosaic viruses (LYMVs; Qazi *et al*., 2007) [3] or "Legumovirus" (Ilyas *et al*., 2009) [4] and have a very limited natural host range within legumes. It has been said that synthetic and natural insecticides can be used to control vectors as a way to manage YMD, but this is neither a long-term solution nor a good way to make money. Also, pesticides can be dangerous if they are used without thinking. Vectors can become resistant to them and they can pollute the environment. So, the only way to stop YMD from happening in places where it is a big problem for growing grain legumes is to use resistant varieties. This is because resistant varieties are practical, effective, cheap, good for the environment, and long-lasting. Even though conventional breeding has led to many types of black gramme and mungbean that are resistant to LYMVs (Gupta *et al*., 2005) [1].

Material and Methods
During the 2018 kharif season, the experiment was conducted at the Research Farm in New Delhi using an augmented design. In each replication, the genotypes of susceptible cultivar (JS335) were grown in 2m-long rows with a row-to-row and plant-to-plant spacing of 40 cm and 10 cm, respectively. Within a row, seeds were planted 10 cm apart by hand. The crop was cultivated using a standard set of procedures.

Ten competitive plants were chosen at random from each treatment in each replication. That experimental set up was needed for developing sick plot of soybean for development of YMD.

Viral genome infectious matter

Starting material source for pure viral DNA Initial source material consisted of soybean plants (JS335) with a prominent yellow mosaic symptom that were grown in sick plots under controlled conditions in order to obtain a DNA sample that also contained the genomes of begomoviruses, which was used to establish the disease from the field to the controlled environment of the field. Originally, immature leaves feature scattered yellow spots, and the subsequent trifoliate leaves that emerge from the rising apex have alternating yellow and green spots. Spots expand over time, and finally some leaves turn entirely yellow. Moreover, infected leaves develop necrotic symptoms. The DNA sample used in this investigation was extracted from the third or fourth leaf from the plant's meristematic area.

DNA extraction

Total DNA was taken from the above soybean plants that were causing yellow mosaic and deformation symptoms in soybean using a method first described by basic research and later changed. Before being used in full genomic amplification of plant virus, all DNA extracts were diluted 100 times in sterile distilled water (SDW). Using a Nano Drop TM 1000 Spectrophotometer (Thermo Scientific, USA), isolated DNA was measured and diluted to a concentration of 0.1 g/l. Loading 1 l of sample DNA and 1 l of control genomic DNA (0.1 g/l; provided with Genome Walking kit, Clontech) on a 0.6% agarose/EtBr gel with a 1 kb Plus DNA ladder confirmed the size and purity.

PCR amplification and cloning of full length Begomovirus genomes

One set of primers (Afl1: 5'GGATCCATTGTTGAACGACTTTCC3'; Afl2: 5' GGATCCCACATTGTTAGTGGGTTC3') having overlapping sequence with Bam HI sites at their 5 ends were designed from published sequences (GenBank: DQ389153) for a PCR reaction to amplify full-length MYMIV DNA-A. Pfu polymerase (Stratagene) amplification reactions were carried out according to the manufacturer's instructions. Using the Zero Blunt-TOPO PCR Cloning Kit, amplified products were cloned into the pCR4Blunt-TOPO plasmid vector (Invitrogen). *E. coli* DH5 was given the resulting clones pTOPO-A. The recombinant E. coli strain DH5was selected on ampicillin (50 µg/ml), X-gal (40 µg/ml) and IPTG (40 µg/ml) medium. Plasmid DNA was purified using the Qiaprep kit via alkaline lysis (Qiagen). DNA- A was cloned using restriction digestion, PCR, and sequencing.

Detailed study through Phylogeny analysis

Limited number of MYMIV DNA-A sequences was used to construct phylogenetic tree. Sequences were aligned using the program Clustal W with default gap penalty parameters of gap opening 10 and extension 0.2. A neighbor-joining tree was constructed using the program MEGA 6.0 with a p-distance model and a pairwise deletion of gaps (Tamura *et al*., 2013) [5]. The bootstrap support of tree branches was assessed by re-sampling amino acid positions 1000 times.

Result

Total DNA was taken from soybean (G. max) leaves that showed signs of yellow mosaic. To use Pfu polymerase to make more copies of the genome, a pair of oligonucleotide primers made from the published DNA-A sequence of MYMIV (GenBank accession number: DQ389153) were used. The cloned PCR product was sequenced and matched up with the sequence that had already been published. The DNA-A product (GenBank accession number: OQ473638) was made from 2747 nucleotides that were 99% the same as the sequence that had already been reported.

On the viral strand, the coat protein gene (ORF AV1) and the precoat protein gene are coded by DNA A. (ORF AV2). On the opposite strand, DNA A codes for replication-associated proteins (ORF AC1 and AC3), transcription activator protein (ORF AC2), and symptom-determinant protein (ORF AC4). With the help of the some limited MYMIV DNA-A isolates, a phylogenetic tree of the MYMIV DNA-A genome was made. Even though the tree showed several subgroups, the roots of most of the major subgroups had weak bootstrap support, so we couldn't use this phylogenetic analysis to come up with a naming scheme for MYMIV DNA-A genome groups. In the neighbor-joining tree, our isolate VR2 New Delhi and isolate Mu2 New Delhi were grouped together. This showed that our isolate was more like the one that was already known to be Mu2 New Delhi isolate (Figure: 1)

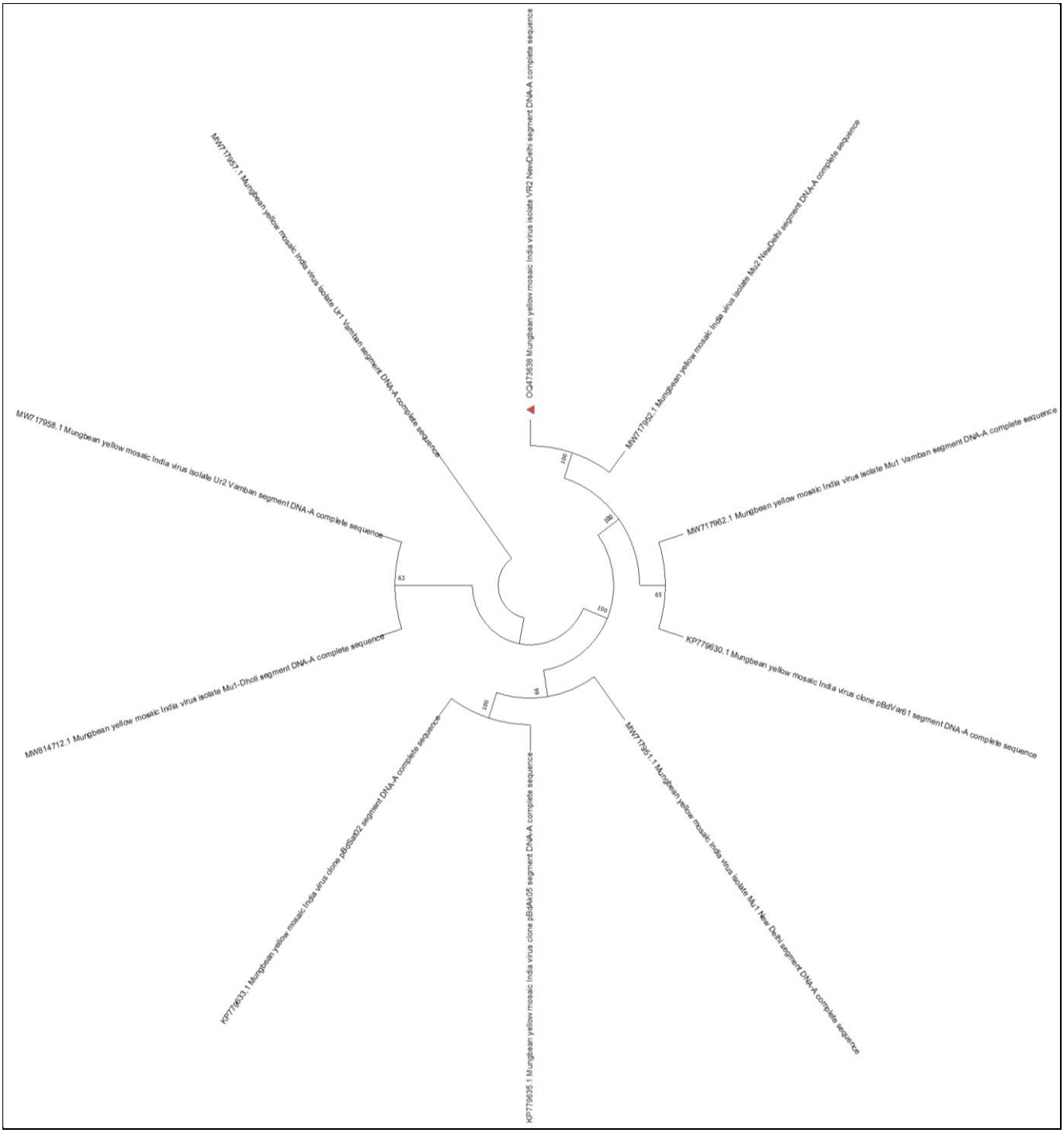

Fig 1: Phylogenetic analysis of the amino acid sequences of the MYMIV DNA-A genome (our accession is indicated by the triangle) in the context of various MYMIV DNA-A. The DNA-A of MYMIV was used to create a neighbor-joining tree, which was based on the nucleotide sequences of 10 different isolates. Bootstrap values were calculated with 1000 replications, and those are marked on the nodes.

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