Original Research Article

Characterization of begomovirus isolates causing YMD in soybean and analyzing the relation with early reported strains of begomovirus from New Delhi region

ABSTRACT

This work was focused on the exact characterization of begomovirus correlated with yellow mosaic disease (YMD) in soybean during the monsoon season. This information helped to understand the time-bound status of the viral group. Augmented design and was carried out at the research field. 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 samples that also contained the genomes of begomovirus. The experiment used an augmented design and was carried out at the research field in New Delhi during the 2018 kharif season. In each replication, the susceptible cultivar (JS335) genotypes were planted for the establishment of a sick soybean plot for YMD development purposes. A limited number of MYMIV DNA-A sequences was used to create the phylogenetic tree. Cluster W was used to align the sequences, with the default gap penalty values of gap opening 10 and extension 0.2. A neighbor-joining tree was built using MEGA 11.0, a p-distance model, and pairwise gap deletion. The viral genome was amplified and sequenced. The sequence analysis revealed that the viral genome is 2747 nucleotides long, similar to monopartite begomoviruses, and has seven conserved ORFs. Further nucleotide (nts) sequence comparisons revealed that the genome had the maximum sequence identity of 99%, which is identical to the previously reported sequence. The DNA-A of MYMIV (GenBank accession number: OQ473638) was made from 2747 nucleotides that were 99% the same as the sequence that had already been reported. Previously, that Mungbean yellow mosaic virus (MYMV) and Mungbean yellow mosaic India virus (MYMIV) are the primary begomoviruses causing yellow mosaic disease (YMD) in soybeans in India. This study also demonstrated that our isolate of MYMIV was more similar to the one that was already recognized to be Mu2 New Delhi isolate. This information would be useful in more detailed screening processes under controlled condition and evaluation of soybean genotypes as part of variety development for sustainable cultivation of soybean.

1.INTRODUCTION

The soybean plant is of great economic importance because it is used to produce soybean oil, animal feed, etc. Soybeans are grown in different countries such as USA, Brazil, Argentina, China, and India. Thirty-five diseases have been described in soybean plant, of which fourteen have been reported causing significant yield and productivity losses in India (Wrather et al., 2010). India is the fifth largest soybean grower, with a production of 10.45 million metric tons on 12.7 million hectares (FAOSTAT, 2022). Yellow mosaic disease is a major soybean disease that affects Uttarakhand, Punjab, Haryana, Madhya Pradesh, Uttar Pradesh, Rajasthan, Delhi, and Karnataka (Gaikwad et al., 2021; Amrate et al., 2023). Yellow mosaic disease (YMD) is one of the most damaging diseases affecting soybean output over time. 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 (Maranna et al., 2023). The disease is mostly caused by the mungbean yellow mosaic India virus (MYMIV), the mungbean yellow mosaic virus (MYMV), the horsegram yellow mosaic virus (HgYMV), and the dolichos yellow mosaic virus. These viruses, generally known as legume yellow mosaic viruses (LYMVs), generate distinctive yellow mosaic patterns on leaf surfaces (Qazi et al., 2007). The most definitive symptom of soybean YMD is the presence of contrast yellow green spots (mottles) on the leaves, and under severe conditions, diseased leaves turn yellow, leaving the veins green. Yellow dots or mottles of varying sizes emerged on juvenile leaves. As the infection worsened, the affected leaves went virtually yellow, while the main veins remained green. Yellow green patches (mottles) on the leaves were the most noticeable symptoms on all infected lines. Rusty necrotic patches were also observed on heavily diseased leaves (Amrate et al., 2020).

YMD was limited to India's northern plains until the early 1970s, when it began to spread to central India gradually. Nonetheless, no prevalent varieties growing in central India are resistant to YMD, which is concerning for soybean growers and companies throughout the region (Amrate et al., 2023). The disease was successfully spread by the whitefly (*Bemisia tabaci* Genn.). The whitefly is a damaging insect pest that sucks phloem sap from the lower surface of leaves and serves as a vector for the spread of mungbean yellow mosaic virus disease in soybeans (Ansari et al., 2017). It has been suggested that synthetic and natural pesticides can be employed to control vectors in order to manage YMD, but this is neither a long-term solution nor a profitable business opportunity. Pesticides can also be hazardous if used improperly. Vectors can become resistant to them, causing pollution in the environment. So, the only approach to prevent YMD in areas where it is a major issue for cultivating grain legumes is to adopt resistant types. This is because resistant variants are practical, effective, inexpensive, environmentally friendly, and long-lasting. Although traditional breeding has resulted in various kinds of blackgram and mungbean that are resistant to LYMVs (Mishra et al., 2020; Bag et al.,2014). This work was focused on the exact characterization of begomovirus correlated with YMD in soybean during the monsoon season of 2018-19. This information helped to understand the time-bound status of the viral group.

2. MATERIAL AND METHODS

2.1 Field conditions: sick plot

The experiment used an augmented design and was carried out at the research field in New Delhi during the 2018 kharif season. In each replication, the susceptible cultivar (JS335) genotypes were planted in 2m-long rows with a 40 cm row-to-row and 10 cm plant-to-plant spacing. Seeds were hand-planted 10 cm apart in a row under area of 0.26 ha. The crop was grown according to a standard set of practices. Ten competitive plants were randomly selected from each treatment in each repetition with insecticide treated small population JS335 as control of treatment. That experimental setup was required for the establishment of a sick soybean plot for YMD development purposes.

2.2 Sample collection and isolation of viral DNA

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 (**Fig.1**).

Fig.1: 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

2.3 DNA extraction from plant samples

Total DNA was extracted from the soybean plants listed above that were creating yellow mosaic and deformation symptoms in soybean, using a method first reported by basic research and then modified (Youssef et al., 2015). Before being Whole Genomic Amplification (WGA) of plant viruses, all DNA extracts were 100 times diluted in sterile distilled water (SDW). Using a Nano Drop TM 1000 Spectrophotometer (Thermo Scientific, USA), extracted DNA was quantified and diluted to 0.1 g/l. Loading 1 µl of sample DNA and 1 µl of control genomic DNA (0.1 mg/l; included with Clontech's Genome Walking kit) onto a 0.6% agarose/EtBr gel with a 1 kb Plus DNA ladder confirmed the size and purity.

2.4 Detailed phylogeny analysis of MYMIV genome

A limited number of MYMIV DNA-A sequences was used to create the phylogenetic tree. Clustal W was used to align the sequences, with the default gap penalty values of gap opening 10 and extension 0.2. A phylogenetic tree was created using the neighbor-joining method and the p-distance model, and pairwise gap deletion (Tamura et al., 2013). The bootstrap support of tree branches was determined by resampling amino acid locations 1000 times. The selected mymiv sequences were used for preparing phylogenetic tree namely, MW717952.1 (isolate Mu2_NewDelhi), AJ416349.1 (isolate SoybeanTN), MW717962.1 (isolate Mu1_Vamban), OQ107481.1 (isolate Mu9_Bhopal), OQ107483.1 (isolate Mu2_Phanda), OQ107487.1 (isolate

Mu1_Hoshangabad), OQ107477.1 (isolate Mu4_Bhopal), OQ107492.1 (isolate Mu1_Harda) as per the genbank submission.

3. RESULT AND DISCUSSION

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 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% similar to that of sequence that had already been reported.

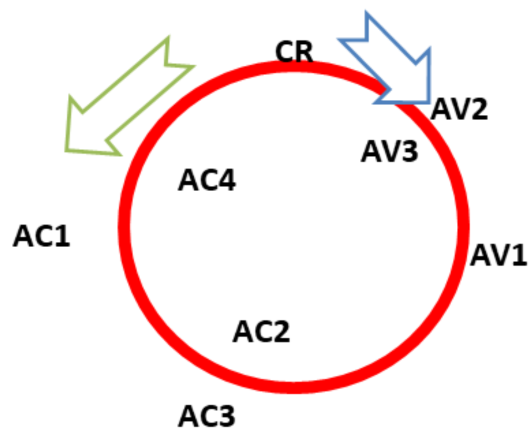

Fig 2: DNA A encodes replication-associated proteins (ORF AC1 and AC3), transcription activator protein (ORF AC2), and symptom-determinant protein (ORF AC4) and also the coat protein gene (ORF AV1) and the precoat protein gene (ORF AV2) on the viral strand of MYMIV DNA-A (Isolates VR2 New Delhi).

DNA A codes for both the coat protein gene (ORF AV1) and the precoat protein gene (ORF AV2) on the viral strand. On the other strand, DNA A encodes replication-associated proteins (ORF AC1 and AC3), transcription activator protein (ORF AC2), and symptom-determinant protein (ORF AC4) **(Fig. 2)**. The details of ORFs were given in GenBank submission (accession number: OQ473638). A phylogenetic tree of the MYMIV DNA-A genome was constructed using only a few MYMIV DNA-A isolates. DNA A, (ORF AV2) codes for the coat protein gene (ORF

AV1) and the pre-coat protein gene on the viral strand **(Fig. 2)**. DNA A opposing strand encodes replication-associated proteins (ORF AC1 and AC3), transcription activator protein (ORF AC2), and symptom-determinant protein (ORF AC4). A phylogenetic tree of the MYMIV DNA-A genome was created using a small number of MYMIV DNA-A isolates. Although the tree revealed multiple subgroups, the roots of the majority of the significant subgroups had inadequate bootstrap support, so we were unable to use this phylogenetic analysis to develop a naming scheme for MYMIV DNA-A genomic groups. In the neighbor-joining tree, our isolates VR2 New Delhi and Mu2 New Delhi were combined. This demonstrated that our isolate was more similar to the one that was already recognized to be Mu2 New Delhi isolate (**Fig.3**). Even though legume yellow mosaic viruses (LYMVs) cause significant yield loss in legume group, investigations on the evolutionary lineage of LYMVs are extremely rare. Previously, that Mungbean yellow mosaic virus (MYMV) and Mungbean yellow mosaic India virus (MYMIV) are the primary begomoviruses causing yellow mosaic disease (YMD) of soybeans in India. This study characterized the full genome sequence of begomovirus, which causes yellow mosaic disease of soybeans in the delhi region of India. MYMV is the most common isolate of yellow mosaic virus infecting mungbean in Western and Southern India, while MYMIV is more prevalent in India, Pakistan, Bangladesh, Nepal, and Vietnam (Malathi and John, 2009), Genome of DNA-A of MYMIV encoded in viral sense the coat protein gene (CP, ~29.7 kDa), pre-coat protein and movement protein (~12.8kDa) from AV2 and AV1 genes, respectively (Rouhibakhsh et al., 2011). The begomovirus causing yellow mosaic disease in blackgram in southern India were identified from blackgram that showed that nucleotide sequence of DNA A component occurred as the begomovirus isolate to be a variant of Mungbean yellow mosaic virus:–(MYMV-[IN:Vam:05]). Nevertheless, DNA B component of that virus group isolate has greater similarity (~ 92%) to Mungbean yellow mosaic India virus (Haq et al., 2011). Phylogenetic analyses were progressively being used in epidemiology, to know the pattern of virus infection in soybean plants, while the Recombination detection program has been also used to evaluate nucleotide sequence of viruses and to recognize the evidence of genetic recombination between the viruses infecting soybean (Gupta et al., 2022). Phylogenetic analyses based studies have been indicated that MYMV and MYMIV are most frequently identified virus group that were causing agent of YMD in soybean in different part of India. In our study, MYMIV was the major begomovirus causing YMD in experimental field of soybean.

Fig.3: Phylogenetic tree of the MYMIV DNA-A (Isolates VR2 New Delhi) constructed using the Maximum Likelihood method and Tamura-Nei model. The tree with the highest log likelihood (-4868.00) is shown. The percentage of trees in which the associated taxa clustered together is shown above the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Tamura-Nei model. This analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 2747 positions in the final dataset. Phylogenetic tree were conducted in MEGA11.

4. CONCLUSION

Previously, that Mungbean yellow mosaic virus (MYMV) and Mungbean yellow mosaic India virus (MYMIV) are the primary begomoviruses causing yellow mosaic disease (YMD) of soybeans in India. This study also confirmed that our isolate of MYMIV was more similar to the one that was already recognized to be Mu2 New Delhi isolate. In this study, Mungbean yellow mosaic India virus (MYMIV) has been considered as most dominate group of begomovirus than that of Mungbean yellow mosaic virus (MYMV). This information would be useful in more detailed screening processes under controlled condition and evaluation of soybean genotypes as part of variety development for sustainable cultivation of soybean.

Ethical Statement

Our manuscript entitled ‘Characterization of begomovirus isolates causing YMD in soybean and analyzing the relation with early reported strains of begomovirus from New Delhi region’ complies with the Ethical Rules applicable to the journal.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models and text-to-image generators have been used during the writing or editing of this manuscript. Although grammatical correction was done with AI tools.

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