Biological Forum – An International Journal **15(8): 148-158(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Elucidating Whitefly *Bemisia tabaci* Asia II 1 Transmission of MYMIV and the Possible Role of ORF AC4 in YMD of Soyabean

M.N. Rudra Gouda[1], Vineeth Vijayan[2*], Emmadi Venu[2], Bichhinna Maitri Rout[3] and Lokesha G.[4]

[1]Ph.D. Scholar, Division of Entomology, IARI, New Delhi, India.
[2]Ph.D. Scholar, Division of Plant Pathology, IARI, New Delhi, India.
[3]Ph.D. Scholar, Division of Vegetable Science, IARI, New Delhi, India.
[4]M.Sc. Scholar, Division of Plant Pathology, IARI, New Delhi, India.

(Corresponding author: Vineeth Vijayan)*
(Received: 01 June 2023; Revised: 28 June 2023; Accepted: 19 July 2023; Published: 15 August 2023)
(Published by Research Trend)

ABSTRACT: Soybean, an important legume crop, suffers from Mungbean Yellow Mosaic India Virus (MYMIV) stress. Whiteflies, *Bemisia tabaci* Genn., transmit MYMIV DNA A. This virus severely reduces crop productivity. In the absence of epidemiological information, no definite management strategy has been developed so far. Interaction between viruliferous whitefly and soybean at initial stages is crucial in epidemic development, and thus the interaction appears to be critical for developing management strategies. For this purpose, knowledge of the MYMIV virus-whitefly interaction is required. But the studies on the transmission of MYMIV by whiteflies in soybeans are limited. Thus, considering the importance of YMD in soybean, the present investigation was carried out to determine the MYMIV-whitefly relationship in soybean. MYMIV DNA A's complementary strand, ORF AC4, encodes a symptom-determinate protein. After artificially inoculating susceptible soybean cultivar JS 335 with viruliferous whiteflies (*Bemisia tabaci* Asia II 1) in a glasshouse, whitefly population and plant tissue maturity were linked to yellow mosaic disease (YMD). Thus, an average of five whiteflies and less than 15-day-old leaves were needed to produce a greater than 60% disease incidence. DNA A from MYMIV isolate VR2 New Delhi (accession: OQ473638) was grouped with strain Mu2. In *in silico* research, ORF AC4 found the catalytic domain in lysyl oxidases, a common and unique class of quinoenzymes that catalyse the oxidative deamination of primary amines to their aldehydes while reducing molecular oxygen to hydrogen peroxide. Thus, MYMIV ORF AC4 products cause chlorophyll yellowing through oxidation processes. The present study thus revealed that due to the viable potential primary inoculum delivered by whiteflies, it is essential for MYMIV to cause YMD in healthy soybeans and also that the genomic region of MYMIV encoded with the AC4 protein can oxidise functional groups of amino acids in proteins, which promotes the symptom development in soybeans in the form of a yellow mosaic.

Keywords: *Bemisia tabaci* Asia II 1, MYMIV, DNA A, and AC4.

INTRODUCTION

Grain legumes and pulses in India, Thailand, Bangladesh, Sri Lanka, and Pakistan are typically afflicted with yellow mosaic disease (YMD), which is caused by Mungbean yellow mosaic virus (MYMV), Mungbean yellow mosaic India virus (MYMIV), Dolichos yellow mosaic virus, and Horsegram yellow mosaic virus. These viruses are closely related and possess host ranges that are distinct but overlap, which are together known as yellow mosaic viruses (YMVs) (Qazi *et al.,* 2007; Malathi and John 2009). The term "Legumoviruses" has been applied to bipartite begomoviruses that infect legumes (Briddon *et al.,* 2010). Mungbean yellow mosaic virus (MYMV) and Mungbean yellow mosaic India virus (MYMIV) are prevalent across the Indian subcontinent, affecting the majority of legume crops, such as blackgram (Vigna mungo), cowpea (Vigna unguiculata), dolichos (Lablab purpureus), horsegram (Macrotyloma uniflorum), lima bean (Phaseolus lunatus), and mungbean (Qazi *et al.,* 2007; Varma *et al.,* 1992). The estimated annual loss attributable to YMD in leguminous crops is $300 million (Varma *et al.,* 1992). Generally, the Gemini viruses associate with a specific host for their multiplication and, hence, infection. However, the host specificity of this virus is currently described by many workers as developing into host non-specificity or a broader range of host specificity, demonstrating the expanding viral genome base over time. Such a host range expansion indicates evolution in the viral genome to diversify and thus establish infection in other crops that were earlier considered begomoviral non-host species (Pant *et al.,* 2022).

Soybean is an economically significant legume commodity in which YMD induces a 15–75% yield reduction (Sharma *et al.,* 2014). Usharani *et al.* (2004) designated the virus isolated from YMD-affected

soybean plants in northern and central India as a soybean isolate of MYMIV (MYMIV-[Sb]) based on the 89% similarity between its nucleotide sequence and that of Mungbean Yellow Mosaic India Virus (MYMIV). This virus is transmitted by the white fly, *Bemisia tabaci* Genn (Lazarowitz and Shepherd 1992). Both genomes encode essential components for replication, motility, and symptom development and range in size from 2.5 to 2.7 kb (Gutierrez 1999; Lazarowitz and Shepherd 1992).

The virus gets into the phloem cells of the host through the whitefly's proboscis. About two days before symptoms appear, viral aggregates form in the nucleus of the host cell (Thongmeearkom *et al.*, 1981). The first signs are scattered yellow spots on young leaves. Over time, these spots turn into a yellow mosaic pattern, and the leaves turn completely yellow, dry out, and die. Since the 1970s, MYMIV has been a big problem for growing soybeans in India, and its alarming spread across the country has been documented (Varma and Malathi 2003).

The first people to find MYMV particles in the leaf cells of mungbean were Thongmeearkom *et al.* (1981); Honda *et al.* (1983). The genomes of the Thailand isolates of MYMV (Morinaga *et al.*, 1993) and the isolate from North India (Mandal *et al.*, 1997) were discovered to share 89% similarity (Fauquet *et al.*, 2008), and were consequently regarded as separate species and afterwards designated as MYMIV.

Patterns of distribution and spread of Mungbean yellow mosaic India virus (MYMIV) begomoviruses (genus Begomovirus; family Geminiviridae) transmitted by the whitefly vector *Bemisia tabaci* (Genn.) As whitefly-transmitted geminiviruses have been recognised as emerging illnesses in worldwide agroecosystems, there has been an upsurge in the demand for large-scale epidemiological data. A recent study suggests that biological differences across populations of vectors may influence disease transmission. Crops typically infected by whitefly-transmitted viruses in the Eastern Hemisphere include cassava, brassicas, tobacco, tomato, and legumes (Vigna and Phaseolus species) (Muniyappa 1980; Mound, 1983); in the Western Hemisphere, hosts have included bean (Phaseolus vulgaris), cotton, soybean, tobacco, and occasionally tomato (Bird and Maramorosch 1978; Brown, 1994).

The YMD was first reported in India in 1960 (Nariani, 1960; Nene, 1972), and it is a major limitation to soybean cultivation in many states like Karnataka, Uttarakhand, Punjab, Madhya Pradesh, Delhi, Haryana, Uttar Pradesh, and Rajasthan. In the absence of epidemiological and viral genome information about YMD in soybean, so far no definite management strategy has been developed. Interaction between viruliferous whitefly and soybean at initial stages and knowledge about the viral genome are crucial for the development of an epidemic, and thus the information about MYMIV transmission by the whitefly and the viral genome appears to be critical for developing management strategies. For this purpose, knowledge of the transmission of MYMIV by whiteflies and viral genome information about disease development is required. But the studies on transmission of MYMIV by whiteflies and MYMIV genome information in soybeans are limited. Thus, considering the importance of YMD in soybean, the present investigation was carried out to know the transmission characteristics of MYMIV by the whitefly *Bemisia tabaci* Asia II 1 and to elucidate the possible role of ORF AC4 in YMD development in soybean.

Therefore, we have undertaken the following study in order to know the efficiency, i.e., the number of vectors needed to transmit MYMIV, and also to find possible virus genome fragments actually governing the disease incidence in susceptible soyabean cultivars.

MATERIAL AND METHODS

A virus-free clonal population of whiteflies was maintained by collecting field populations and rearing them for two generations on a soyabean plant that is free from viruses. Eight soyabean plants in 8 cm × 8 cm × 8 cm plastic pots were maintained in a 40 cm × 25 cm × 60 cm acrylic cage. The plant-containing tray is periodically watered in order to maintain plant life. Release a single *B. tabaci* Asia II 1 into the rearing cage and maintain it at 28±2ºC, 30–50% relative humidity, 8 hours of darkness, and 22,200 lux illumination in the whitefly chamber. And these cages should remain undisturbed for a few days or until a substantial number of adults from the next generation can be observed. An experiment was carried out in 2021–2022.

Delivery of MYMIV by whitefly. Whiteflies were transformed from aviruliferous to viruliferous by feeding on a host plant infected with begomoviruses (MYMIV), such as soybean. In the first step, viruses are transferred from the field to controlled conditions using a stock of whiteflies, which have the ability to bring begomoviruses into susceptible cultivars under controlled conditions. As a result, the host carrying viruses served as the source of further infective studies in this experiment. The twig of a cultivar with MYMIV symptoms was used to acquire the virus by whiteflies reared in a cage system, and they were allowed to feed on it for an acquisition access period (AAP) of about 24 hours in a room with diffuse light conditions.

Assessing disease severity in controlled conditions. A separate set of experiments was carried out on tissue-specific disease severity analysis, which was collected using the rating scale 28 days after inoculation (dpi) (Akhtar *et al.*, 2011) (Table 1). Although the number of whiteflies had an effect on disease and the cultivar's susceptibility to disease from whiteflies carrying virus particles, the two factors that were correlated in terms of disease severity were maturity and viral load in tissues.

Table 1: Scale for categorising soybean genotypes' responses to artificial inoculation for the yellow mosaic disease under controlled conditions.

Symptoms	Severity grade	% Disease severity[a,b]	Disease reaction
Complete absence of symptoms	0	0	Immune (I)
Few small yellow specks or spots on few leaves seen after careful observations	1	0.01-10	Highly resistant (HR)
Bright yellow specks or spots common on leaves, easily observed and some coalesced	2	10.01-25	Resistant (R)
Mostly coalesced bright yellow specks or spots common on leaves, but no or minor reduction in yield	3	25.01-40	Tolerant (T)
Plants showing coalesced bright yellow specks or spots on all leaves, with no or minor stunting and set fewer normal pods	4	40.01-60	Susceptible (S)
Yellowing or chlorosis of all leaves on whole plant followed by necrosis, shortening of internodes, severe stunting of plants with no yield or few flowers and deformed pods produced with small, immature and shrivelled seeds	5	>60.01	Highly susceptible (HS)

Initial source material for pure viral DNA. The initial source material was soybean plants (JS335) grown in a glass greenhouse under controlled conditions with a prominent yellow mosaic symptom in order to obtain a DNA sample that also contained the genomes of MYMIV, which was used to establish the disease from the field to the controlled environment of the glasshouse. Initially, young leaves have mild, scattered yellow spots, and the next trifoliate leaves emerging from the growing apex have irregular yellow and green patches that alternate with each other. Spots gradually grow in size, and some leaves eventually turn completely yellow. Infected leaves exhibit necrotic symptoms as well. The third or fourth leaf from the meristematic region of the plant was chosen for isolating the DNA sample that was used in this study.

DNA extraction. Total DNA was extracted from soyabean plants showing yellow mosaic and deformation symptoms by using the cetyl trimethylammonium bromide (CTAB) method originally described by Lodhi *et al.,* (1994) and later modified (Maruthi *et al.,* 2002). All DNA extracts were diluted 1:100-fold in sterile distilled water (SDW) before being used in RCA amplifications. In a NanoDropTM 1000 Spectrophotometer (Thermo Scientific, USA), isolated DNA was quantified and diluted to a concentration of 0.1 g/l. Loading 1 l of sample DNA and 1 l of control genomic DNA 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 MYMIV genomes

One set of primers (Afl1: 5'GGATCCATTGTTGAACGACTTTCC3'; Afl2: 5' GGATCCCACATTGTTAGTGGGTTC3') having overlapping sequences 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 clone pTOPO-A. The recombinant E. coli strain DH5 was 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.

ORF and Gene structure prediction of DNA-A in MYMIV. The operational reading frame (ORF) of a gene was found using the NCBI ORF finder tool. Then we predicted the possible amino acids coded by the obtained ORFs that are produced during the translation process using the NCBI Blastx tool. Gene structure, exons, and introns were obtained by comparing open reading frames (ORFs) and genomic sequences. Structures were displayed using GSDS 2.0 (http://gsds.cbi.pku.edu.cn) (Guo *et al.,* 2007). With this structure, DNA-A of MYMIV was drawn.

Phylogeny analysis. 92 MYMIV DNA-A sequences were used to construct the phylogenetic tree. Sequences were aligned using the programme Clustal W with default gap penalty parameters of gap opening 10 and extension 0.2. A neighbour-joining tree was constructed using the programme MEGA 6.0 with a p-distance model and a pairwise deletion of gaps (Tamura *et al.,* 2013). The bootstrap support of tree branches was assessed by re-sampling amino acid positions 1000 times.

Motif Analysis. Based on the obtained blast results, the protein that has a key role in disease incidence (symptom determinant protein) was used for motif discovery and pattern analysis. The MEME software (version 4.12.0) on the line server (http://meme-suite.org/index.html) was used to discover and analyse the motifs in this analysis. The parameters used were as follows: minimum width = 6, maximum width = 10, and the maximum number of motifs to find = 6.

Protein structure prediction of the MYMIV symptom determinant protein. Phyre2 (Protein Homology/Analogy Recognition Engine V 2.0) (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id

=index) was used to predict the structure of the MYMIV symptom determinant protein. Along with that, the possible functional role of the protein was also known.

RESULTS AND DISCUSSION

Begomoviruses are a collection of plant viruses that have become a significant threat to numerous vegetable, root, and fibre crops in tropical, subtropical, and temperate regions of the globe (Navas-Castillo *et al.,* 2011). The resurgence of scientific interest in the study of begomovirus biology, especially transmission by whitefly vectors, is due to the virus's increasing significance in modern agriculture. Although there are currently just a handful of extensive investigations on the transmission of begomoviruses by whiteflies, patterns are beginning to emerge, including differential transmission based on the *B. tabaci* species. Begomoviruses are typical persistent viruses transmitted by the *B. tabaci* complex of whiteflies; nevertheless, it has been contested whether their transmission is circulative, nonpropagative (i.e., the virus does not multiply in the insect vector), or propagative (i.e., the virus replicates in the insect vector). There is mounting evidence that the begomovirus tomato yellow leaf curl virus (TYLCV) can replicate in *B. tabaci* (Pakkianathan *et al.,* 2015; Wang *et al.,* 2016). In one of the initial attempts to analyse the differential transmission of begomoviruses, Bedford *et al.,* (1994) found that whiteflies of what we now consider species of the *B. tabaci* complex—at the time, biotypes—exhibited differential transmission capacity for a variety of begomoviruses. Subsequently, Sánchez-Campos *et al.,* (1999) found that TYLCV and tomato yellow leaf curl Sardinia virus (TYLCSV) were transmitted with differing efficiency by whiteflies of the Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species, formerly biotypes B and Q, respectively. The relative prevalence of both begomoviruses in tomato test plants confirmed that MED is a more efficient transmitter than MEAM1 (Ning *et al.,* 2015). Many experiments have been undertaken since then, and the results confirm the theory that numerous begomoviruses are transmitted differently by distinct species of the *B. tabaci* complex. Various species of whiteflies have been found to transmit TYLCV, Chino del Tomate virus, tomato yellow leaf curl China virus (TYLCCNV), tomato leaf curl Bangalore virus (ToLCBaV), papaya leaf curl China virus (PaLCuCNV), tomato leaf curl Taiwan virus (ToLCTWV), tomato yellow leaf curl Thailand virus (TYLCTHV), cotton leaf curl Multan virus (CLCuMuV), and euphorbia yellow mosaic virus (Chen *et al.,* 2016; Chowda-Reddy *et al.,* 2012; De Marchi *et al.,* 2017; Guo *et al.,* 2015; Idris *et al.,* 2001; Li *et al.,* 2010; Jiu *et al.,* 2006; Pan *et al.,* 2018a,b; Venkataravanappa *et al.,* 2017; Wei *et al.,* 2014; Weng *et al.,* 2015).

In the case of MYMIV, under controlled conditions of viral transmission, yellow mosaic symptoms were arbitrarily associated with the whitefly population implicated in the transmission of viral particles in soybean. Whitefly *Bemisia tabaci* Asia II 1 has been prevalent in the Delhi region and is involved in the transmission of MYMIV, one of the important groups of viruses causing yellow mosaic in soybeans. The ability of whiteflies to transmit viruses may vary due to vector parameters such as biotype, feeding, and movement pattern. Moreover, this study has revealed that the population of whiteflies has a direct impact on disease severity in plant tissue. The minimum number of whiteflies needed for infectivity investigations should be five in order to provide more accurate pathogen-vector-based research under controlled conditions. The number of whiteflies utilised ranged from 20 to 5, which contributed to disease severity exceeding 61% (Table 2). The primary inoculum load of MYMIV delivered by the whitefly population was clearly linked to disease severity developing in plant tissues within 28 days. When the number of whiteflies used to inoculate MYMIV was massive, the time duration for disease development in susceptible varieties (JS335) was inversely proportional. In our experiment, we encountered whitefly populations in gradinet sets of 20, 15, 10, and 5 individuals involved in virus genome delivery into plant systems, resulting in symptom development in 11 to 14 days (Fig. 1, Table 2). The results were in conformity with the findings of Swathi *et al.* (2023), who reported that a single viruliferous whitefly per plant can transmit virus to inoculated plants in 16 days, and the percent transmission increased with an increase in the number of viruliferous whiteflies per plant. However, the minimum number of whiteflies required for 69% disease incidence was 10. The incubation period decreases with an increase in the number of whiteflies released per plant. Primary inoculum for viral infection in plant systems has been quantitatively related to disease intensity parameters and inversely related to time duration, with the initial period in the susceptibility cultivar (JS335) of soybean.

Whitefly feeding habits changed along with plant maturity, and experimental data suggested that plant maturity might have a negative impact on the delivery of viral particles into plant tissues due to challenges in cell wall components and chemical barriers like alkaloids and phenol levels in plant tissues. The level of disease incidence was shown to be 86 percent in the case of young leaves that developed within 5 days after sowing (DAS), while it was 41 percent in the case of leaves that developed within 25 days after sowing (DAS) (Table 3, Fig. 2). This indicated that the development of yellow mosaic symptoms is inversely correlated with the maturation of plant tissue and the delivery efficiency of the whitefly population.

We discovered that a minimum of five whiteflies were required to transmit and induce symptoms within 11–14 days. When whiteflies number below 5, it will take at least 16 to 18 days with a disease incidence much lower than 50 percent, so by this time the plant will escape a severe infection by growing and maturing fast. Nonetheless, we discovered that leaf age is inversely related to disease incidence, i.e., the younger the leaf, the higher the disease incidence. So for greater than 60

percent of disease incidence, an average of five whiteflies along with a minimum of less than 15-day-old leaves is needed.

Phylogeny analysis. From soybean (G. max) leaves exhibiting yellow mosaic symptoms, total DNA was isolated. To amplify the genome with Pfu polymerase, a pair of oligonucleotide primers created from the sequences of DNA-A of MYMIV that have been published (GenBank accession number: DQ389153) were used. Sequencing and alignment with the published sequence were done on the cloned PCR product (Fig. 3).

The DNA-A product (GenBank accession number: OQ473638) was made with 2747 nucleotides that were 99% the same as the sequence that had already been reported. On the viral strand, DNA A codes for the coat protein gene (ORF AV1) and the precoat protein (ORF AV2). On the complementary strand, DNA A codes for replication-associated proteins (ORF AC1 and AC3), transcription activator proteins (ORF AC2), and symptom-determinant proteins (ORF AC4). A phylogenetic tree of the MYMIV DNA-A genome was constructed using the 92 isolates of MYMIV DNA-A. Although several sub-groups were identified on the tree, the poor bootstrap support for the roots of most major sub-groups did not allow us to propose an MYMIV DNA-A genome group nomenclature based on this phylogenetic analysis. In the neighbour-joining tree, our isolate VR2 New Delhi was clustered together with isolate Mu2 New Delhi. This indicated that our isolate clearly shares more similarity to an isolate of a previously identified Mu2 New Delhi isolate (Fig. 4).

In Silico analysis. The gene bank accession number for our DNA-A chromosome of MYMIV is OQ473638. And from this obtained sequence, we found primarily seven ORFs that code for a possible prominent protein, along with their domain and gene annotation function (Table 4). Based on that, we found precoat protein, capsid protein, AC5 protein, replication associated protein, transcriptional activator protein, replication enhancer protein, and symptom determinant protein. Of the proteins obtained, we mainly targeted a symptom-determinant protein and drew the gene structure by highlighting only this target protein (Fig. 5). In this background, it has been earlier established that certain domains of viral proteins are essential for pathogenicity (Kang *et al.,* 2016; Matic *et al.,* 2016). Similar necrosis-like symptoms were also observed in N. benthamiana overexpressing the TGB3 and polymerase domains of the RdRp protein of the pepino mosaic virus (Samperi *et al.,* 2016). The results were in conformity with the findings of Roshan *et al.* (2018) that amino acids in the N-terminal region of AV2 of the tomato leaf curl palampur virus are important for the development of necrotic symptoms, signifying their role in symptom severity.

Motif analysis. In DNA-A of MYMIV, we found six motif patterns, namely QLEEVN, LDQSWR, NCRKSKG, PDADRH, DELIME, and PSKRMF (Fig. 6). The motif pattern for DNA-A is DELIME, NCRKSKG, NCRKSKG, PDADRH, PSKRMF, QLEEVN, LDQSWR, DELIME, PSKRMF, QLEEVN, PDADRH, and LDQSWR with a P-value of 7.23e-26 (Fig. 7).

Protein structure and possible role of symptom determinant protein (ORF AV4). ORF AC4 of MYMIV-encoded peptide structures, yellow being β-sheet and blue being α-helix and a green dot in the chain, are indicated in the secondary structure of the protein that was used in bond formation for folding the peptide chain (Fig. 8). The x-ray diffraction structure of AC4-encoded protein (symptom-determining protein), which encodes a protein vesting with different domains containing lysyl oxidase as a macromolecule, beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose as oligosaccharides and ligands like imidazole (IMD), 2-acetamido-2-deoxy-beta-D-glucopyranose (NAG), and ligands like Ca^{2+}, Mg^{2+}, Cu^{2+}, and Cl^{-1} ions (Fig. 9 and Table 5). The N2 domain is the first or second structural domain in copper amine oxidases. The catalytic domain can be found in Copper amine oxidases are a common and novel class of quinoenzymes that catalyse the oxidative deamination of primary amines to the corresponding aldehydes while simultaneously reducing molecular oxygen to hydrogen peroxide. The enzymes are dimers of identical 70–90 kDa subunits, each of which contains a single copper ion and a covalently bound cofactor formed by post-translational modification of a tyrosine side chain to 2,4,5-trihydroxyphenylalanine quinone (TPQ). N-acetyl-d-glucosaminidase is a mesophilic hydrolase that hydrolyzes N-acetyl-glucosides exclusively, and its primary function is to break down oligosaccharides in the presence of water. One of the enzyme's primary functions is to target and hydrolyze oligosaccharides. Imidazole is amphoteric, which means it can act as both an acid and a base. Imidazole has a pKa of 14.5, making it slightly less acidic than carboxylic acids, phenols, and imides but slightly more acidic than alcohols. The proton that is bound to nitrogen is an acidic proton. Deprotonation produces the symmetrical imidazolide anion. The conjugate acid's pKa as a base is approximately 7, making imidazole approximately sixty times more basic than pyridine. The nitrogen with the lone pair is the basic site (and not bound to hydrogen). Protonation produces the symmetrical imidazolium cation. Imidazole-based histidine compounds are essential for intracellular buffering. Copper amine oxidases do this by catalysing the conversion of primary amines into aldehydes, which releases ammonia and hydrogen peroxide as well as an essential copper ion per subunit and topaquinone as a cofactor. High concentrations of NH^{4+} reduce the accumulation of Ca^{2+} and Mg^{2+} in plant cells, whereas the exclusive accumulation of ammonia is detrimental to many plants and may cause ion imbalances. Lower plant yields, changes in the concentrations of several metabolites, leaf chlorosis, and decreased net photosynthesis (Britto and Kronzucker 2002). Chlorosis, which causes tissue to change colour from green to yellow and attract whiteflies to plants, may be induced by the copper amine oxidase activity of AV4 gene products during infection.

Table 2: Disease severity studies of YMD corresponding with whitefly population and disease severity development in the selected plants.

Number of whiteflies used for transmission MYMIV	Soybean seedlings were used for inoculation	Soybean seedlings occurred yellow mosaic symptoms by infection	Disease incidence percentage (average)	Symptoms after DAI (average)
20	10	9	85	11
15	10	9	73	12
10	10	8	69	13
5	10	9	61	14
3	10	8	43	16
2	10	5	32	18
1	10	5	25	18

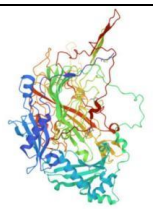
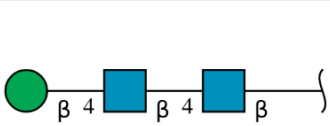
Table 3: Disease severity level and plant tissue maturity in disease development with a fixed population of whiteflies.

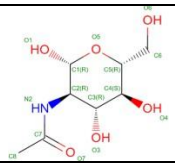
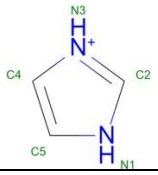
Plant part inoculated based on maturity	Number of plants used for the experiment	Disease incidence percentage (average)	Major plant responses be gomo viruses
Highly mature (25days old leaves)	5	41	Yellowing with limited spread In inoculated leaves
Medium mature (15 Days old leaves)	5	62	Yellowing with less deformation
Young portion (5 days old leaves)	5	86	Yellowing and Yellow mosaic

Table 4: Showing ORFs of DNA-A of MYMIV with domain on its function.

ORF	(nt/aa)	Start	Stop	Best Blast × match	Pfam	GO function	Gene
1	341/113	156	497	Precoat protein	Gemini_V2 domain	Hostcellcytoplasm (GO:0030430)	AV2
2	773/257	316	1089	capsid protein	Gemini_coat domain	Structural molecule activity(GO:0005198)	AV1
3	251/83	733	984	AC5protein	Gemini_AC4_5	ND	AC5
4	404/134	1086	1490	Replication enhancer protein	Gemini_AL3doma in	Viral process(GO:0016032)	AC3
5	452/150	1228	1680	Transcription active at or protein	Gemini_AL2doma in	Structural molecule activity(GO:0005198)	AC2
6	1088/362	1538	2626	Replication initiation protein/replication associated protein	Gemini_AL1_Mdo main	Endodeoxyri bonuclease activity, producing 5'-phosphomonoesters (GO:0016888)	AC1
7	299/99	2176	2475	Symptom determinant protein	Gemini_C4domain	ND	AC4

Table 5: Detailed analysis of proteins encoded by ORF AC4 in possible function of domain in their structure system.

ID	Chains	Name/Formula/In ChI Key	2D Diagram
Macromolecule			
Lysyloxidase	A		
Oligo molecules			
beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose	B		
Small molecules			
NAG	Q[authA], R[authA], S[authA],T[authA]	**2-acetamido-2-deoxy-beta-D-glucopyranose**C8H15N O6OVRNDRQMDRJTHS-	

		FMDGEEDCSA-N	
IMD	P[authA]	**IMIDAZOLE** C3H5N2RAXXELZNTBOGNW-UHFFFAOYSA-O	
CU	C[authA]	**COPPER(II)ION** CuJPVYNHNXODAKFH-UHFFFAOYSA-N	
CA	K[authA], L[authA], M[authA], N[authA],O[authA]	**CHLORIDEION** ClVEXZGXHMUGYJMC-UHFFFAOYSA-M	
MG	F[authA], G[authA], H[authA], I[authA],J[authA]	**MAGNESIUM ION** MgJLVVSXFLKOJNIY-UHFFFAOYSA-N	
CL	K[authA], L[authA], M[authA], N[authA],O[authA]	**CHLORIDEION** Cl VEXZGXHMUGYJMC-UHFFFAOYSA-M	

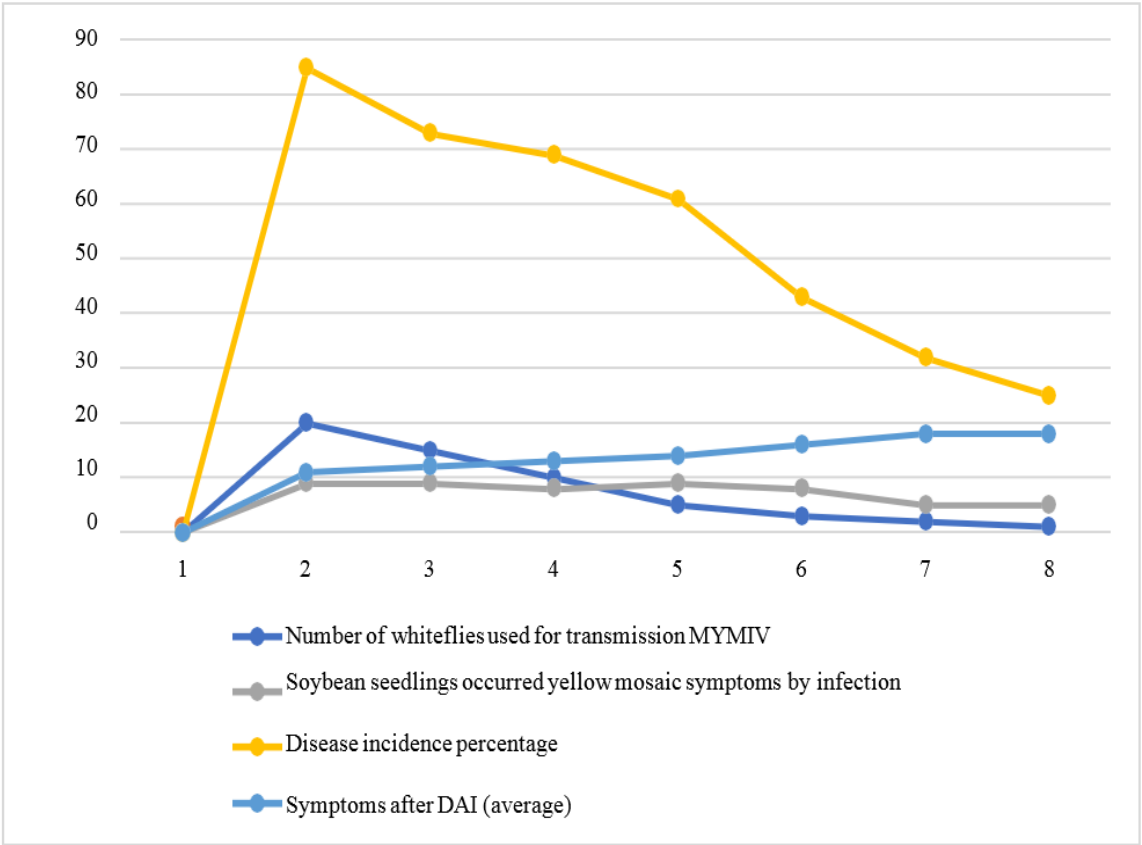


Fig. 1. The primary inoculum of begomoviruses delivered by white flies in a fixed plant population of soybean and its disease severity level.

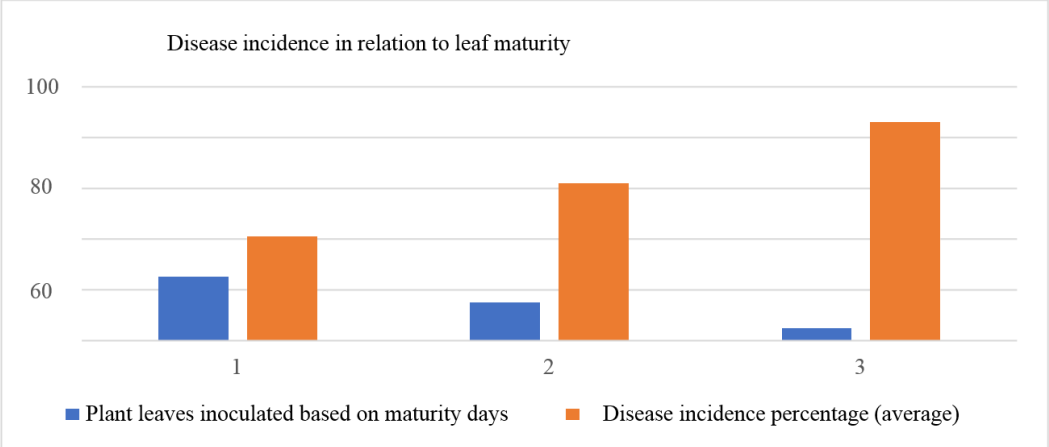


Fig. 2. Showing disease incidence percentage in relation to age of plant leaves.

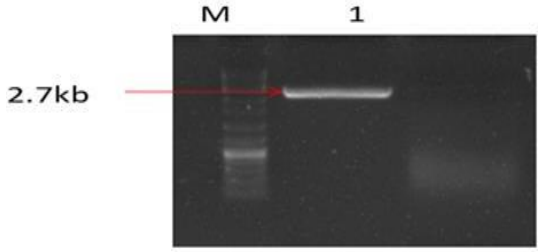


Fig. 3. Agarose gel electrophoresis for colony PCR, Lane M-1 kb DNA ladder, and DNA-A MYMIV of soybean showed an amplicon size of about 2.7 kbps.

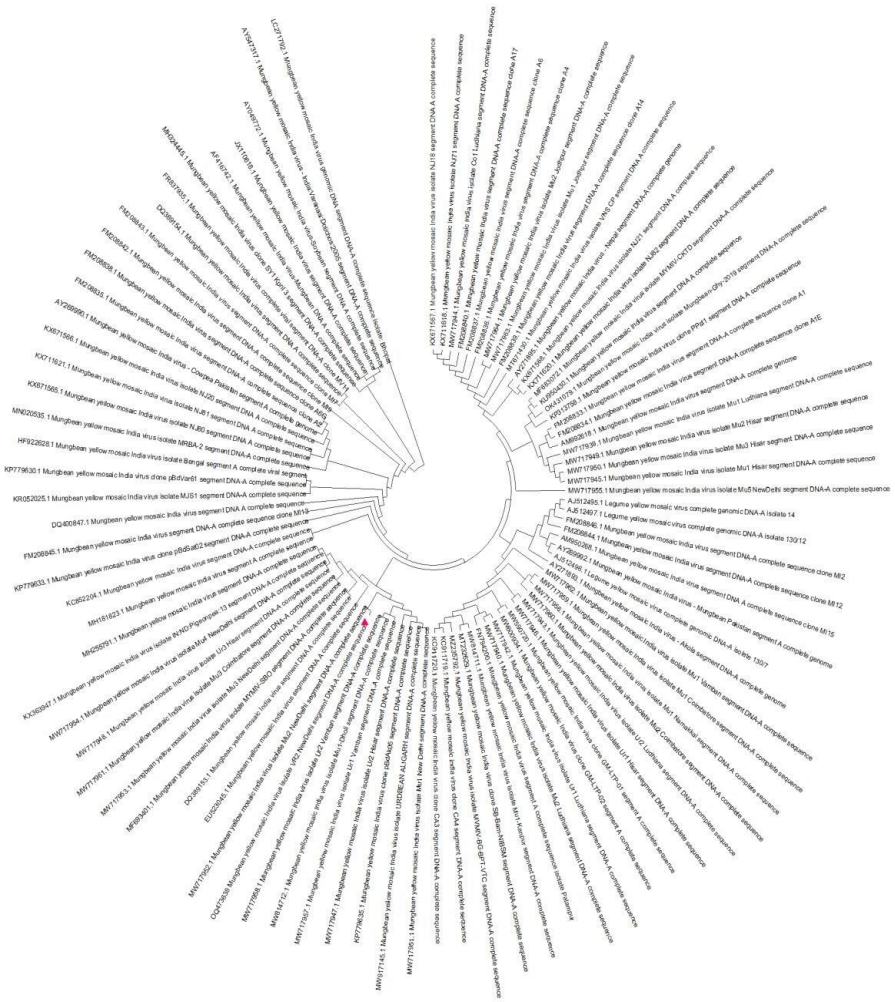


Fig. 4. Phylogenetic analysis of the amino acid sequences of the MYMIV DNA-A genome (our accession is indicated by the triangle) in the context of various MYMIV DNA-A. The DNA-A of MYMIV was used to create a neighbor-joining tree, which was based on the nucleotide sequences of 92 different isolates. Boots trap values were calculated with 1000 replications, and those are marked on the nodes.

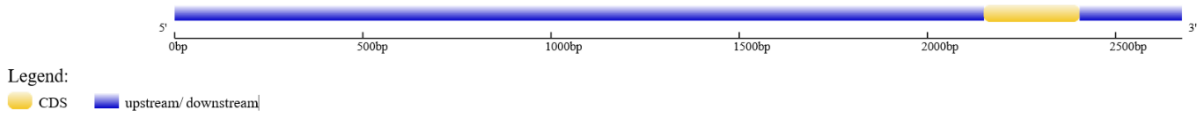


Fig. 5. Showing structure DNA-A of MYMIV with CDS region of symptom determinant protein.

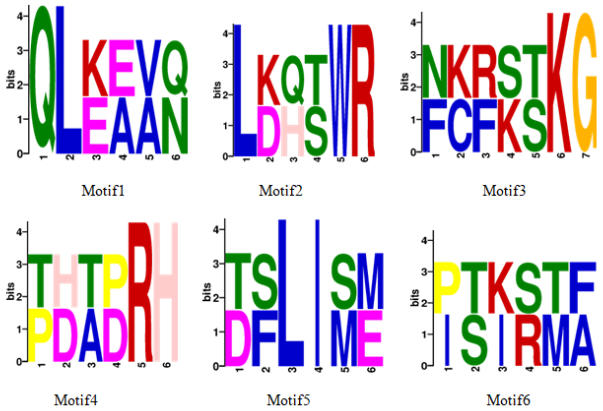


Fig. 6. Showing motifs of the symptom determinant protein of DNA-A of MYMIV.

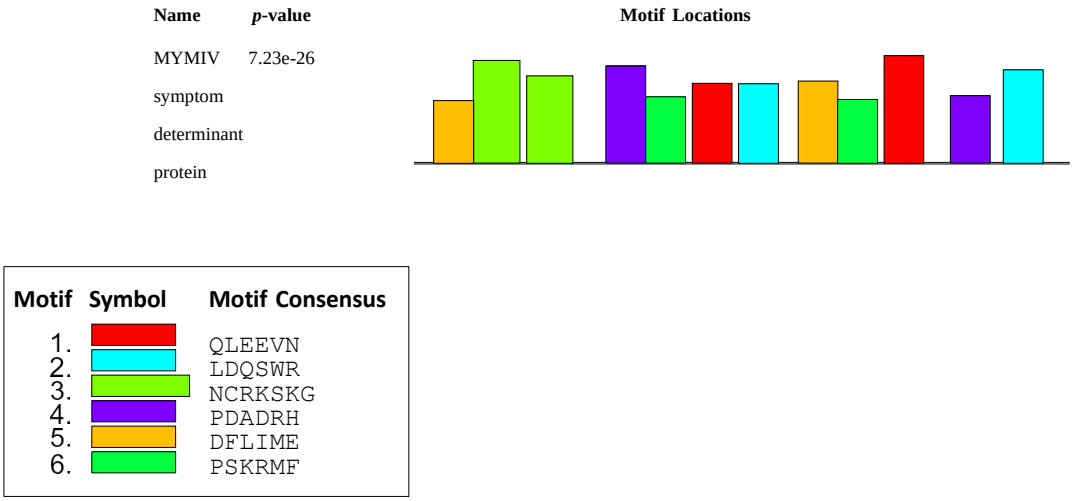


Fig. 7. Showing motif pattern in symptom determinant protein of DNA-A of MYMIV.

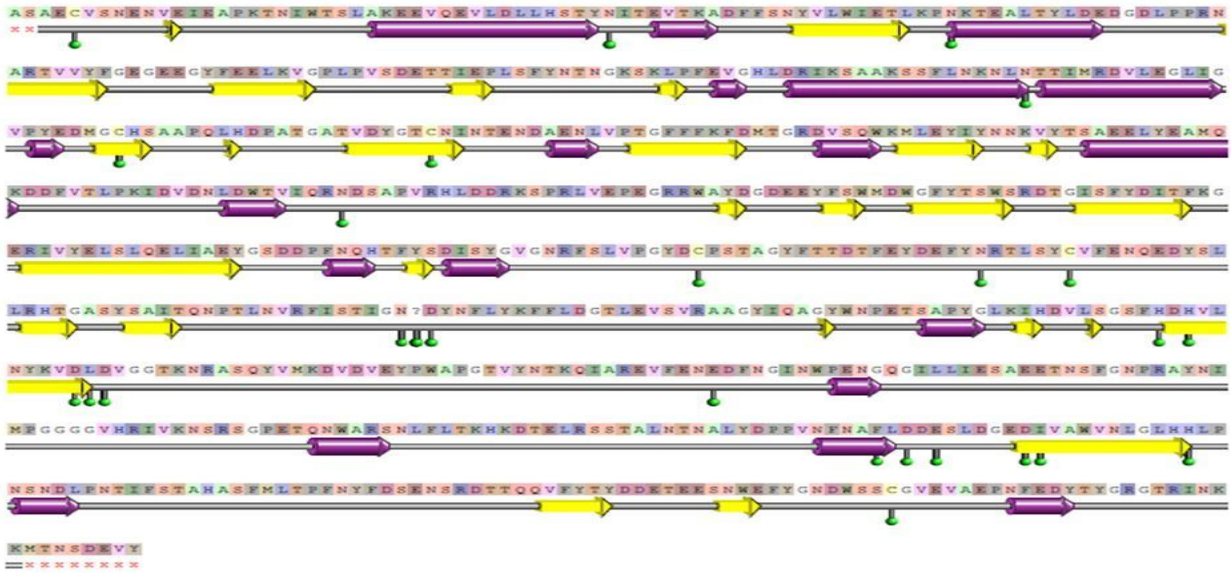


Fig. 8. Color schemes according to secondary structural conformations: Yellow: β-sheet, blue: α-helix and green dot in the chain indicate the secondary structure of proteins that are used in bond formation for folding peptide chains into the biochemically active domain of proteins for converting biomolecules into another product that is involved in disease development in plant tissues.

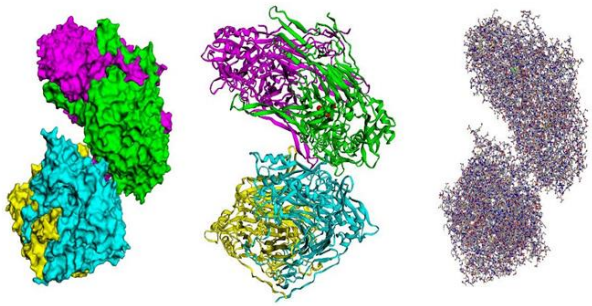


Fig. 9. 3D structure of a macromolecule (lysyl oxidase) of the symptom determinant protein MYMIV. a) Surface structure; b) four chains of protein, each colour showing a different chain; c) amino acid-based construction of molecules.

CONCLUSIONS

Thus, the present study revealed that due to viable potential primary inoculum delivered by whiteflies, it is essential for MYMIV to cause YMD in healthy soybean. A minimum of five whiteflies can be used for studying disease under controlled conditions. The genomic region of MYMIV encoded with the AC4 protein has the ability to oxidize functional groups of amino acids in proteins, which promoted symptom development in soybeans in the form of a yellow mosaic.

FUTURE SCOPE

Future research on MYMIV and whitefly transmission should focus on understanding different whitefly biotypes' transmission efficiency, unraveling the molecular interactions between the virus and whitefly vectors, assessing the impact of plant maturity on transmission, developing sustainable management strategies, and exploring the genetic diversity and evolution of MYMIV.

Conflict of interest. None.

REFERENCES

Akhtar, K. P., Sarwar, G., Abbas, G., Asghar, M. J., Sarwar, N., & Shah, T. M. (2011). Screening of mungbean germplasm against Mungbean yellow mosaic India virus and its vector *Bemisia tabaci*. *Crop Protection*, *30*(9), 1202-1209.

Bedford, I. D., Briddon, R. W., Brown, J. K., Rosell, R. C., Markham, P. G. (1994). Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Annals of Applied Biology*, *125*(2), 311-325.

Bird, J., & Maramorosch, K. (1978). Viruses and virus diseases associated with whiteflies. Advances in *Virus Research*, *22*, 55-110.

Briddon, R. W., Patil, B. L., Bagewadi, B., Nawaz-ul-Rehman, M. S., & Fauquet, C. M. (2010). Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses. *BMC Evolutionary Biology*, *10*(1), 97.

Britto, D. T., & Kronzucker, H. J. (2002). NH^{4+} toxicity in higher plants: A critical review. *Journal of Plant Physiology*, *159*(6), 567-584.

Brown, J. K. (1994). Current status of *Bemisia tabaci* as a plant pest and virus vector in agroecosystems worldwide. *FAO Plant Protection Bulletin*, *42*(1/2), 3-2.

Chen, T., Tang, Y. F., Zhao, R., He, Z. F., & Lü, L. H. (2016). Identification of the cryptic species of *Bemisia tabaci* transmitting Cotton leaf curl Multan virus. *Journal of Plant Protection*, *43*, 91-98. (in Chinese with English abstract)

Chowda-Reddy, R. V., Kirankumar, M. & Seal, S. E. (2012). *Bemisia tabaci* phylogenetic groups in India and the relative transmission efficacy of Tomato leaf curl Bangalore virus by an indigenous and an exotic population. *Journal of Integrative Agriculture*, *11*(2), 235-248.

De Marchi, B. R., Marubayashi, J. M., Favara, G. M., Yuki, V. A., Watanabe, L. F. M., Barbosa, L. F., ... & Krause-Sakate, R. (2017). Comparative transmission of five viruses by *Bemisia tabaci* NW2 and MEAM1. *Tropical Plant Pathology*, *42*, 495-499.

Fauquet, C. M., Briddon, R. W., Brown, J. K., Moriones, E., Stanley, J., Zerbini, M., & Zhou, X. (2008). Geminivirus strain demarcation and nomenclature. *Archives of virology*, *153*, 783-821.

Guo, T., Guo, Q., Cui, X. Y., Liu, Y. Q., Hu, J., & Liu, S. S. (2015). Comparison of transmission of Papaya leaf curl China virus among four cryptic species of the whitefly *Bemisia tabaci* complex. *Scientific Reports*, *5*, 15432.

Gutierrez, C. (1999). Geminivirus DNA replication. *Cellular and Molecular Life Sciences*, *56*(3-4), 313-329.

Honda, Iwaki, M., & Saito, Y. (1983). Mechanical transmission, purification and some properties of whitefly-borne mungbean yellow mosaic virus in Thailand. *Plant Disease*, *67*(7), 801-804.

Idris A. M., Smith, S. E., and Brown, J. K. (2001). Ingestion, transmission, and persistence of Chino del tomate virus (CdTV), a New World begomovirus, by Old and New World biotypes of the whitefly vector *Bemisia tabaci*. *Ann. Appl. Biol*., *139*, 145-154.

Jiu, M., hou, X. P., & Liu, S. S. (2006). Acquisition and transmission of two begomoviruses by the B and a non-B biotype of *Bemisia tabaci* from Zhejiang, China. *Journal of Phytopathology*, *154*(10), 587-591.

Kan, S. H., Qu, F. & Morris, T. J. A spectrum of HRT-dependent hypersensitive responses elicited by the 52 amino acid N-terminus of turnip crinkle virus capsid protein and its mutants. *Virus Res.* 200, 30–34 (2016).

Lazarowitz, S. G., & Shepherd, R. J. (1992). Geminiviruses: genome structure and gene function. *Critical Reviews in Plant Sciences*, *11*(4), 327-349.

Li, M., Hu, J., Xu, F. C., & Liu, S. S. (2010). Transmission of Tomato yellow leaf curl virus by two invasive biotypes and a Chinese indigenous biotype of the whitefly *Bemisia tabaci. International Journal of Pest Management*, *56*(3), 275-280.

Lodhi, M. A., Guang-Ning, Y., Norman, F. W., & Bruce, I. R. (1994). A Simple and Efficient Method for DNA Extraction from Grapevine Cultivars, Vitis species and Ampelopsis. *Plant Molecular Biology Reporter, 12*, 6-13.

Malathi, V. C., & John, P. (2009). Mungbean Yellow Mosaic Viruses. In Desk *Encyclopedia of Plant and Fungal Virology* (pp. 217-226).

Mandal, B., Varma, A., & Malathi, V. G. (1997). Systemic infection of Vigna mungo using the cloned DNAs of the blackgram isolate of mungbean yellow mosaic geminivirus through agroinoculation and transmission of the progeny virus by whiteflies. *Journal of Phytopathology, 145*(11-12), 505-510.

Maruthi, M. N., Colvin, J., Seal, S., Gibson, G., & Cooper, J. (2002). Co-adaptation between cassava mosaic geminiviruses and their local vector populations. *Virus Research, 86*(1-2), 71-85.

Matic, S., Pegoraro, M. & Noris, E. (2016). Te C2 protein of tomato yellow leaf curl Sardinia virus acts as a pathogenicity determinant and a 16-amino acid domain is responsible for inducing a hypersensitive response in plants. *Virus Res*., *215*(2), 12–19.

Morinaga, T., Ikegami, M., & Miura, K. (1993). The nucleotide sequence and genome structure of mungbean yellow mosaic geminivirus. *Microbiology and Immunology*, *37*(6), 471-476.

Mound, L. A. (1983). Biology and Identity of Whitefly Vectors of Plant Pathogens. pp. 305-313. En: Plumb, R. T., Thresh, J. M. (eds.). *Plantvirus Epidemiology*. Oxford, Blackwell.

Muniyappa, V. (1980). Whiteflies.

Nariani, T.K. (1960). Yellow mosaic of mungbean (Phaseolus aureus L.). *Indian Phytopathology*, *13*, 24-29.

Nene, Y.L. (1972). A survey of viral diseases of pulses in India. G. B. Pant University of Agriculture and Technology Research Bulletin. *4*, 191.

Navas-Castillo, J., Fiallo-Olivé, E., & Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual Review of Phytopathology, 49*, 219-248.

Ning, W., Shi, X., Liu, B., Pan, H., Wei, W., Zeng, Y., ... & Zhang, Y. (2015). Transmission of Tomato yellow leaf curl virus by *Bemisia tabaci* as affected by whitefly sex and biotype. *Scientific reports*, *5*(1), 10744.

Pakkianathan, B. C., Kontsedalov, S. & Lebedev, G. (2015). Replication of Tomato yellow leaf curl virus in its whitefly vector, *Bemisia tabaci. Journal of Virology, 89*(19), 9791-9803.

Pan, L. L., Chen, Q. F. & Guo, T. (2018). Differential efficiency of a Begomovirus to cross the midgut of different species of whiteflies results in variation of virus transmission by the vectors. *Science China Life Sciences*, *61*(10), 1254-1265.

Pant, P., Pant, P. & Patel, S. (2022). Expanding Host Range of Papaya Leaf Curl Virus (PLCV) into Agricultural Crops and Weeds Species. *Biological Forum-An international Journal*, *14*(3), 1186-1197.

Qazi, J., Ilyas, M., Mansoor, S., & Briddon, R. W. (2007). Legume yellow mosaic viruses: genetically isolated begomo viruses. *Molecular Plant Pathology, 8*(4), 343-348.

Roshan, P., Kulshreshtha, A., Kumar, S., Purohit, R., & Hallan, V. (2018). AV2 protein of tomato leaf curl Palampur virus promotes systemic necrosis in *Nicotiana benthamiana* and interacts with host Catalase2. *Scientific Reports, 8*(1), 1273.

Sánchez-Campos, S., Rodríguez-Negrete, E. A., & Cruzado, L. (2016). Tomato yellow leaf curl virus: no evidence for replication in the insect vector *Bemisia tabaci*. *Scientific Reports*, 6.

Sharma, A. N., Gupta, G. K., Verma, R. K. (2014). Integrated Pest Management for Soybean. Retrieved from http://www.ncipm.org.in.

Swathi, M., Gaur, N. and Singh, K. (2023). Virus vector relationship of yellow mosaic virus and whitefly, *Bemisia tabaci* (Gennadius) in soybean. *Legume Research-An International Journal*, *46*(7), pp.946-950.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*(12), 2725-2729.

Thongmeearkom, P., Honda, Y., Saito, Y., & Syamananda, R. (1981). Nuclear ultrastructural changes and aggregates of virus like particles in mungbean cells affected by mungbean yellow mosaic disease. *Phytopathology, 71*(1), 41-44.

Usharani, K. S., Surendranath, B., Haq, Q. M., & Malathi, V. G. (2004). Yellow mosaic virus infecting soybean in northern India is distinct from the species infecting soybean in southern and western India. *Current Science, 86*, 845-850.

Varma, A. (1992). MYMV transmission and its control in India. In Mungbean Yellow Mosaic Disease: Proceedings of an International Workshop, Bangkok (Vol. 1991, pp. 143-148). Asian Vegetable Research and Development Center.

Varma, A., & Malathi, V. G. (2003). Emerging geminivirus problems: A serious threat to crop production. *Annals of Applied Biology*, *142*(2), 145-164.

Venkataravanappa, V., Kodandaram, M. H., Lakshminarayana Reddy, C. N., Shankarappa, K. S., & Krishna Reddy, M. (2017). Comparative transmission of Bhendi yellow vein mosaic virus by two cryptic species of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *3 Biotech, 7*(5), 331.

Wang, L. L., Wang, X. R., Wei, X. M., Huang, H., Wu, J. X., Chen, X. X., ... & Wang, X. W. (2016). The autophagy pathway participates in resistance to tomato yellow leaf curl virus infection in whiteflies. *Autophagy*, *12*(9), 1560-1574.

Wei, J., Zhao, J. J., Zhang, T., Li, F. F., Ghanim, M., Zhou, X. P., ... & Wang, X. W. (2014). Specific cells in the primary salivary glands of the whitefly *Bemisia tabaci* control retention and transmission of begomoviruses. *Journal of Virology*, *88*(22), 13460-13468.

Weng, S. H., Tsai, W. S., Kenyon, L., & Tsai, C. W. (2015). Different transmission efficiencies may drive displacement of tomato begomoviruses in the fields in Taiwan. *Annals of Applied Biology, 166*(2), 321-330.

How to cite this article: M.N. Rudra Gouda, Vineeth Vijayan, Emmadi Venu, Bichhinna Maitri Rout and Lokesha G. (2023). Elucidating Whitefly *Bemisia tabaci* Asia II 1 Transmission of MYMIV and the Possible Role of ORF AC4 in YMD of Soyabean. *Biological Forum – An International Journal, 15*(8): 148-158.