# **RESEARCH ARTICLE**





# Preference shifts in *Bemisia tabaci* (Aleyrodidae: Hemiptera) after the acquisition of Mungbean Yellow Mosaic Virus from *Vigna radiata*

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#### **Abstract**

The silver leaf whitefly, *Bemisia tabaci* (Aleyrodidae: Hemiptera), is a polyphagous pest and a major vector of plant viruses, causing significant economic losses globally. It exhibits a complex of cryptic species and is known to transmit various plant viruses affecting numerous crops globally. A study was conducted in Tamil Nadu, India, to identify the dominant cryptic species of *B. tabaci* on legume crops, leading to the establishment of laboratory colonies of the most common types. The research focused on the feeding preferences of these whiteflies on healthy green gram plants (*Vigna radiata* (L.) R. Wilczek) versus those infected with the Mungbean Yellow Mosaic Virus (MYMV). Biochemical changes in MYMV-infected green gram plants, compared to healthy ones, were analysed to understand their influence on whitefly feeding preference. The cryptic species Asia II 8 was found to be the most common in legume crops. The study assessed whether these whiteflies favoured healthy green gram plants or those infected with MYMV. Our findings revealed that when healthy whiteflies acquired the virus from the host, their preference shifted toward healthy plants. This shift is linked to the biochemical changes associated with the infection status of the host. Following viral infection, there was a decrease in total sugars and chlorophyll, while levels of protein, phenol, peroxidas and polyphenol peroxidase increased. These results suggest that the virus modifies the vectors' behaviour through a shared host, enhancing the pathogens' ability to disseminate within the ecosystem.

Keywords: Begomovirus; Bemisia tabaci; biochemical changes; cryptic species; green gram; Vigna radiata; vector-virus-host interactions

#### Introduction

Mungbean Yellow Mosaic Virus (MYMV; Geminiviridae; Begomovirus) is a devasting pathogen of legume crops worldwide, primarily transmitted by the whitefly vector *Bemisia tabaci* (Aleyrodidae: Hemiptera). The young leaves of virus-infected plants are covered in tiny, uneven yellow spots that eventually develop into mosaic patterns. Disease progression and symptom manifestation are influenced by seasonal factors such as temperature, humidity and rainfall patterns. Plants with severe infection displayed full yellowing of the leaves, stems and pods, as well as withering and drying of the leaves, which eventually impacted the production. The annual loss of productivity is projected to be between 80 and 100 percent, or USD 300 million (1, 2). Under field conditions, managing this disease is a difficult task and traditional breeding programs have their limits when it comes to producing resistant varieties (3).

*B. tabaci* is a polyphagous pest that causes direct damage to over 600 plant species and is responsible for the indirect spread of more than 120 plant viruses, notably those classified under the Begomovirus genus (family Geminiviridae). As a result, this leads to considerable yield losses in crops

worldwide (4). It ranks among the world's top 100 invasive organisms and exhibits species complexity (5-7). At least 40 cryptic species have been reported worldwide so far (8), seven of them (Asia I, Asia II 1, Asia II 2, Asia II 5, Asia II 7, Asia II 8 and MEAM 1) from India (9-13). During the early vegetative stage of the plant, female *B. tabaci* lays stalked eggs on the undersides of leaves. Over her lifespan, which ranges from 3 to 6 weeks, a single female can lay 100 to 300 eggs. It exhibits multivoltinism, producing 11 to 15 generations per year under favorable conditions.

*B.tabaci* spreads the virus in a circulative and persistent manner and the vector is capable of infecting another plant with a brief inoculation and access period, but it is advisable to adhere to a 24-hr interval for both acquisition and inoculation to ensure effective transmission of the virus (4). The primary transmission mode for plant viruses is through insect vectors, which frequently exhibit behavioural changes following the acquisition of the virus. These modifications facilitate the natural dissemination of the disease among plant populations. Various studies have documented this occurrence across different frameworks involving host and vector interactions

(14-17). Notable examples of well-established vector-virus-host relationships include aphid-luteovirus (15-18), thrips-tospovirus (19) and whitefly-begomovirus (20, 21); however, there is a relative scarcity of research focusing on legume crops in tropical environments. In light of this gap, a study was undertaken to identify the predominant cryptic species of *B. tabaci* associated with legume crops in Tamil Nadu, India. The most prevalent species were subsequently assessed on green gram to evaluate their feeding preferences in relation to the virus infection status and to examine the associated biochemical changes in green gram (*Vigna radiata*) plants.

#### **Materials and Methods**

#### **Diversity analysis**

#### Sample collection

Adult *B. tabaci* specimens were collected from legume crops (green gram, black gram, red gram, horse gram, chickpea, groundnut) and vegetables (brinjal, bhendi, cauliflower, capsicum) at five sites in Tamil Nadu, India: Vamban (10.3704° N, 78.9272° E), Aduthurai (11.0140° N, 79.4751° E), Trichy (10.7634° N, 78.5945° E), Coimbatore (11.0122° N, 76.9354° E) and Panpoli (9.0225° N, 77.2506° E). The collection was carried out with a handheld aspirator in the early morning hr, when the feeding activity of *B. tabaci* is more due to favourable environmental conditions such as temperature, wind speed and humidity. The samples were subsequently transferred into 1.5 mL Eppendorf tubes containing 95 % ethanol and stored at -20 °C until they were required for DNA extraction (11).

#### **DNA** Isolation

Total DNA was extracted from adult B. tabaci using a lysis buffer containing Tris (1M, pH 8), EDTA (0.5M, pH 8), Triton X-100, proteinase-K (20 mg/mL) and distilled water in standardized proportions. The procedure commenced with the wrapping of a 90 mm diameter petri dish in aluminium foil (Mirage foil, 10.5 µm thickness), followed by a layer of parafilm. A 5 µL drop of the lysis buffer was applied to the centre of the parafilm, where a single adult whitefly was placed using a size 000 camel hair brush and subsequently crushed with the edge of a sterile 1.5 mL PCR tube (23). Upon completion of the crushing, the entire contents, including the wash from the PCR tube's edges, were transferred to a sterile PCR tube (1.5 mL) and placed in an ice box for five minutes. The sample was then incubated in a water bath at 65°C for 15 min, followed by a10min incubation at 95°C and subsequently stored in a refrigerator for three minutes. The sample was vortexed for five sec before proceeding with PCR analysis. The mtCOI is a widely used DNA marker for species identification in insects. Therefore, the mtCOI forward primer 5'TTGATTTTTTGGTCATCCAGAA3' and reverse primer 5' TCCAATGCACTAATCTGCCAT 3' (Sigma, St. Louis, MO, USA) were used for the PCR amplification process (24).

### Sequencing and diversity analysis

The amplified PCR product, totalling 20  $\mu$ L, was sequenced utilizing mtCOI forward and reverse primers. The resulting sequence alignment data were compiled and analyzed through the Bioedit software program (version 7.0.5). Multiple sequence alignments and predicted amino acid alignments

were performed using the CLUSTAL X program to assess genetic relationships. Nucleotide sequence similarity was evaluated against the GenBank database (NCBI) using BLAST. A dendrogram was constructed using the neighbour-joining method with 500 bootstrap replicates, performed in MEGA software (version 4.0) (25).

#### **Preference test**

#### **Insect rearing**

B. tabaci was reared following the method described by Butter and Rataul (26), with modifications as necessary for this study. The most abundant cryptic species, B. tabaci (Asia II8), identified through phylogenetic analysis, was used to establish colonies for preference studies between infected and healthy plants. A colony of B. tabaci (Asia II 8) was initially sourced from a green gram field (variety CO 8) located at the Experimental Farm of the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, India. This colony was subsequently maintained within a Bugdorm (Mega View, Taiwan) in a plant growth chamber (Percival, USA). The environmental conditions for the plants were regulated to a daytime temperature of 25 ± 0.5°C and a nighttime temperature of 22 ± 0.5°C, with a relative humidity of 70%. The photoperiod was set to 12 hours of light followed by 12 hours of darkness. Brinjal (Solanum melongena L.; variety CO 6) plants served as hosts for whitefly rearing. Fresh plants were introduced every 10 days and older plants were removed after the whiteflies migrated to the new plants. A total of twenty adult whiteflies were randomly collected from the plants housed within the Bugdorm using a handheld aspirator, with each collection occurring in a separate test tube for the choice test. Random sampling was employed to eliminate any potential bias associated with selecting leaf feeding sites (top, middle, bottom).

#### Virus maintenance

Non-viruliferous *B. tabaci* adults were extracted from the colony and subjected to a starvation period of 30 min in a petri dish. Ten whiteflies were placed in a clip cage (1.5 cm x 1.3 cm) and attached to a symbiotic green gram plant infected with MYMV. This setup facilitated virus acquisition during the exposure period. After a 24-hr Acquisition Access Period (AAP), the whiteflies were moved to healthy green gram seedlings that were 10 days old and kept for an additional 24 hr to promote inoculation under controlled conditions. Twenty-five days after the inoculation process, symptomatic leaves were collected for bioassay experiments.

The detection of MYMV in both whiteflies and green gram plants was conducted at 15-day intervals through PCR analysis utilizing primers that are specific to MYMV. For the identification of begomovirus, Roja's primers (PALIr772 5'GGNAARATHTGGATGGA 3' and PALIC1960 5'ACNGGNAARACNATGTGGGC 3') were employed. Additionally, specific primers designed for the coat protein gene (DNA A- F- 5'-ATGGGKTCCGTTGTATGCTTG-3' and R-5'-GGCGTCATTAGCATAGGCAAT-3') and the movement protein gene (DNA B- F- 5'-ATGGAGAATTATTCAGGCGCA-3' and R- 5'-GGCGTCATTAGCATAGGCAAT-3') were utilized for the detection of MYMV (Sigma, St. Louis, MO, USA). The coat protein of begomoviruses is crucial for guiding the viral genome into the nucleus and facilitating its subsequent export from the nucleus in host plant cells, that is essential for viral replication.

Additionally, the movement proteins encoded by plant viruses are fundamental for the distribution of viral genomes across the infected plant.

#### Choice test for whitefly preference

The settling preference of whiteflies was examined in relation to the MYMV infection status of green gram. Approximately 500  $B.\ tabaci$  adults were collected from a brinjal plant maintained in the plant growth chamber. After one hour of starvation, 20 whiteflies were introduced into the bioassay arena, which contained either MYMV-infected or healthy green gram leaves in a no-choice setup. The bioassay arena consisted of a plastic Petri dish (125 mm diameter x 1.2 cm height) and covered with a lid. The top of the petri dish lid was cut and replaced with a nylon mesh (100 mm diameter; mesh size, 63  $\mu$ m). A leaf bouquet was prepared from a healthy or virus-infected plant. The third leaf from the top was detached from the plant and placed abaxial side up on the petri dish. The cut end of the petiole was wrapped with moist cotton to prevent wilting (27).

# **Biochemical analysis from test plants**

Chlorophyll content in green gram leaves, both infected with MYMV and healthy, was performed following the Bruinsona method (28). The estimation of total sugars was carried out using the anthrone method, as detailed by Hedge and Hofreiter (29). Phenolic compounds were quantified using the Folin-Ciocalteu reagent, following the methodology of Malick and Singh (30). Total protein levels were assessed using a modified Lowry method as described by Hartree (31). The results from the preference assessments and the related biochemical changes in the infected and healthy plants were

subjected to t-test analysis using SPSS Statistics version 17.0.

#### **Results**

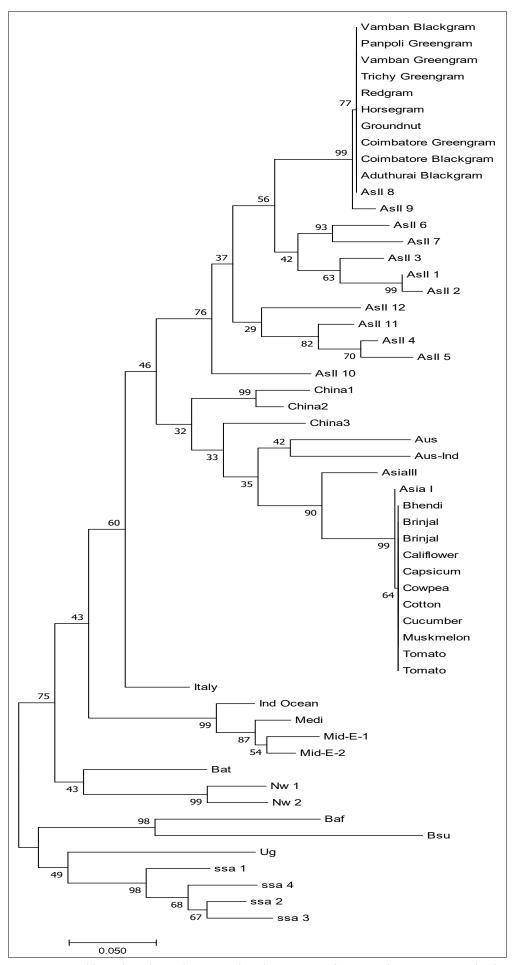
# **Ecological diversity associated with** *Bemisia tabaci*

Samples of adult *B. tabaci* were collected from five key MYMV hotspot regions in Tamil Nadu, India, as outlined in Table 1 to analyze their ecological diversity. This table summarizes the geographical locations, host plants, species identified and their respective gene bank accession numbers, highlighting the diversity of *B. tabaci* across regions. Sequence analysis of the Mitochondrial Cytochrome Oxidase (mtCOI) subunit I PCR products from 20 locations confirmed the presence of *B. tabaci* cryptic species Asia II 8 and Asia I. Asia II 8 was the only cryptic species found in all MYMV hotspot regions. In contrast, Asia I was detected exclusively in vegetable crops, as indicated in Table 1, suggesting a host-specific distribution pattern.

The sequences, with a maximum alignment length of approximately 605 base pairs, were compared following the methodology described by Dinsdale et al. (32), ensuring accurate cryptic species identification. The phylogenetic analysis revealed that the *B. tabaci* sequences were organized into two distinct clades (Fig. 1), reflecting the genetic divergence between Asia II 8 and Asia I. Specifically, the whitefly populations sourced from the MYMV hotspot in pulse crops, namely VBNBG, VBNGG, ADTBG, TRYGG, CBEBG, CBEGG, CBERG, CBEHG, CBEGNUT and PAPGG (Table 1), showed a 99% identity with the Asia II 8 genotype. In contrast, the populations collected from vegetable crops, including CBEBRI, CBEBRI1,

Table 1. Details of survey, sample collection, host, locations, and Bemisia tabaci genetic group in MYMV hotspot regions of Tamil Nadu, India.

S.NO	SAMPLE NAME	<i>B. tabaci</i> strains	ноѕт	PLACE	COORDINATES	GENBANK ACCESSION NO.
1.	VBNBG	Asia II 8	Vigna mungo	Vamban, Tamil Nadu	N 11° 30', E 79° 26'	MH374156
2.	VBNGG	Asia II 8	Vigna radiata	Vamban, Tamil Nadu Aduthurai,	N 11° 30', E 79° 26'	MH374157
3.	ADTBG	Asia II 8	Vigna mungo	Tamil Nadu	N 10.9985°, E 79.4801°	MH356716
4.	TRYGG	Asia II 8	Vigna radiata	Trichy, Tamil Nadu	N 10°45′, E 78°36′	MH374155
5.	CBEBG	Asia II 8	Vigna mungo	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374139
6.	CBEGG	Asia II 8	Vigna radiata	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374148
7.	CBERG	Asia II 8	Cajanus cajan	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374152
8.	CBEHG	Asia II 8	Macrotyloma uniflorum	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374150
9.	CBEGNUT	Asia II 8	Arachis hypogaea	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374149
10.	CBEBRI	Asia 1	Solanum melongena	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374141
11.	CBEBRI1	Asia 1	Solanum melongena	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374142
12.	CBEBI	Asia 1	Abelmoschus esculentus	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374140
13.	CBECALI	Asia 1	Brassica oleracea var. botrytis	Coimbatore, Tamil Nadu	N 11° 07 ', E 76° 59'	MH374143
14.	CBECAP	Asia 1	Capsicum annuum	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374144
15.	CBECWA	Asia 1	Vigna unguiculata	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374145
16.	CBECT	Asia 1	Gossypium hirsutum	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374146
17.	CBECU	Asia 1	Cucumis sativus	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374147
18.	СВЕММ	Asia 1	Cucumis melo	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374151
19.	CBETOM	Asia 1	Solanum lycopersicum	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374153
20.	CBETOM1	Asia 1	Solanum lycopersicum	Coimbatore, Tamil Nadu	N 11° 07', E 76° 59'	MH374154



**Fig. 1.** Phylogenetic tree generated from aligned partial mtCOI nucleotide sequences of *Bemisiatabaci* genotypes with other selected whitefly genotypes. The tree was generated by the neighbour joining method by aligning the sequences in MEGA 7 Clustal X. Vertical branches are arbitrary; horizontal branches are proportional to calculated mutation distances; values at nodes indicate percentage bootstraps values (1000 replicates).

CBEBI, CBECALI, CBECAP, CBECWA, CBECT, CBECU, CBEMM, CBETOM and CBETOM1, exhibited a 99% identity with the Asia I genotype (Table 1).

#### Preference of B. tabaci in relation to MYMV infection

The preference bioassay revealed that non-viruliferous whiteflies settled more frequently on infected plants than on healthy ones at 12 and 24 hr following their release, suggesting a potential attraction to virus-induced plant changes. Conversely, viruliferous whiteflies showed a preference for healthy plants during these time intervals (Fig. 2). Non-viruliferous whiteflies showed a statistically significant preference for MYMV-infected plants at both 12 hr (t = 1.571, d.f. = 28, P = 0.027) and 24 hr (t = 3.374, d.f. = 28, P = 0.002). In contrast, viruliferous whiteflies displayed a significant preference for healthy plants at the 24-hr interval (t = 1.071, d.f. = 28, P = 0.029) (Fig.3).

# Biochemical changes in MYMV-infected and healthy green gram plant

Biochemical analysis revealed significant changes in green gram plants infected with MYMV. Protein (t = 2.145, df = 8, P = 0.064), phenol (t = 3.396, df = 8, P = 0.009), peroxidase (t = 0.808, df = 8, P = 0.443) and polyphenol peroxidase (t = 2.587, df = 8, P = 0.032) levels increased following infection. Conversely, chlorophyll (t = 10.038, df = 9.69, P = 0.001) and total sugar levels (t = 2.996, df = 8, P = 0.017) significantly decreased (Fig. 4-11).

# **Discussion**

The adaptive significance of *B. tabaci* cryptic species composition and their impact on virus transmission are pivotal for creating sustainable and effective vector-virus management practices in legume crops. Molecular research has demonstrated the existence of approximately 40 cryptic species complexes of *B. tabaci* that are morphologically indistinguishable and reproductively isolated on a global scale (5). In India, seven cryptic species were identified, namely Asia I, Asia II 1, Asia II 2, Asia II 5, Asia II 7, Asia II 8 and MEAM 1, based

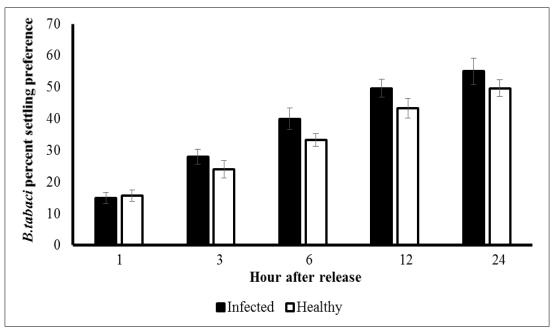
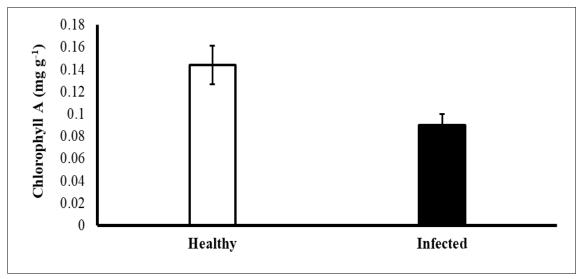


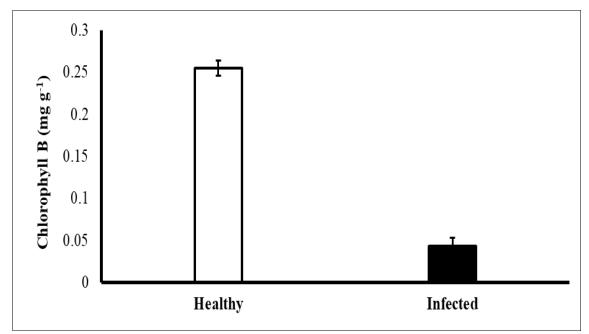
Fig. 2. Mean percent (± SEM) settling preference of non-viruliferous *B. tabaci* towards healthy and MYMV infected plants 1, 3, 6, 12 and 24 h after release.



Fig. 3. Mean percent (± SEM) settling preference of viruliferous *B. tabaci* towards healthy and MYMV infected plants 1, 3, 6, 12, and 24 h after release.



**Fig.4.** Mean ( $\pm$  SEM) chlorophyll A (mg g<sup>-1</sup>) in healthy and MYMV infected leaves



**Fig.5.** Mean ( $\pm$  SEM) chlorophyll B (mg g<sup>-1</sup>) in healthy and MYMV infected leaves

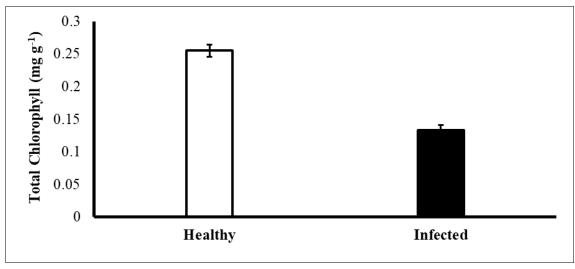


Fig.6. Mean (<u>+</u> SEM) total chlorophyll (mg g<sup>-1</sup>) in healthy and MYMV infected leaves

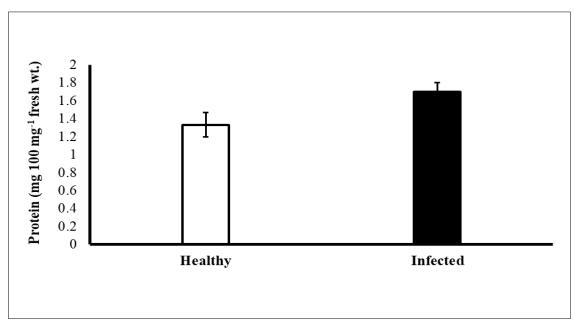
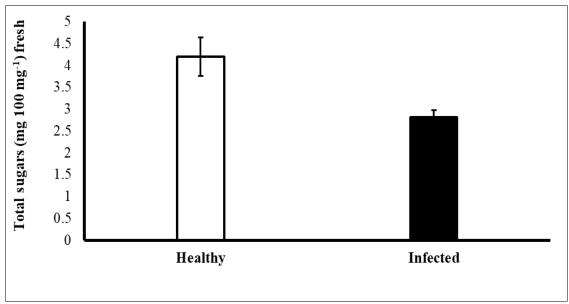


Fig.7. Mean (± SEM) total protein (mg 100 mg<sup>-1</sup> fresh wt.) in healthy and MYMV infected leaves samples



 $\textbf{Fig.8.} \ \text{Mean} \ (\underline{\textbf{+}} \ \text{SEM}) \ \text{total sugars} \ (\text{mg } 100 \ \text{mg}^{\text{-}1} \ \text{fresh wt.}) \ \text{in healthy and MYMV infected leaves}$ 

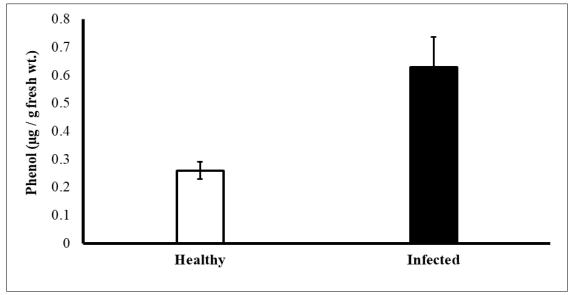


Fig. 9. Mean ( $\pm$  SEM) Phenol content ( $\mu g$  / g fresh wt.) healthy and MYMV-infected leaves

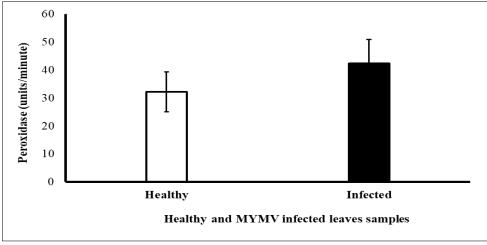


Fig. 10. Mean (± SEM) peroxidase (units/minute) healthy and MYMV-infected leaves

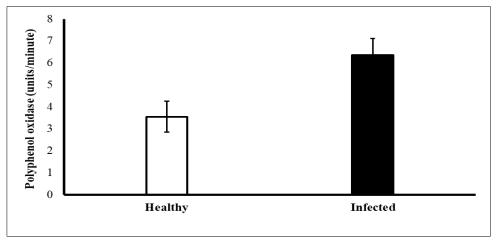


Fig. 11. Mean (± SEM) polyphenol oxidase (units/minute) healthy and MYMV-infected leaves

on their host plant associations and DNA barcoding methodologies (11,13).

Our research indicates that the cryptic species Asia II 8 is the most prevalent in legume crops. This strong association with the virus may be influenced by the virus reliance on shared hosts for its propagation and survival, as supported by previous studies on different pathosystems (15, 32). The infection of begomovirus in plant hosts was shown to attract whiteflies, enhancing their fitness in these shared hosts (33). Additionally, some research indicated that plant viral infections can have either negative or neutral impacts on vector performance (34, 35). Notably, positive fitness benefits were observed primarily in persistently transmitted viruses, such as the Tomato yellow leaf curl virus (TYLCV) in the *B. tabaci* and tomato Patho system; however, studies exploring these interactions in other pathosystems remain limited.

The dynamics of whitefly performance, driven by virus manipulation of host physiology and vector behaviour, are significantly influenced by host plant resistance, disease progression, inoculation timing, virus strains and the specific vector species or biotypes involved (17, 36). Notably, the introduction of biotype 'B' (MEAMI 1) has resulted in the replacement of the indigenous biotype in the tomato-ToYLCV pathosystem in India, thereby enhancing disease epidemiology and accelerating the population growth of the vector *B. tabaci* in virus-affected fields, leading to the predominance of biotype 'B' (22,23,24). Comparable findings were also reported in

Pakistan (37) and China (38). We have detected the *B. tabaci* Asia 1 cryptic species in various vegetable crops in these regions, but further exploration of the status of virus infection is warranted.

B. tabaci exhibits a polyphagous feeding behaviour, acting as a direct pest when it feeds and reproduces on the same plant species, including brinjal and cotton. It can also be categorized as both a direct and indirect pest, as it not only feeds and breeds but also transmits diseases on the same hosts, such as tomatoes, chilies and bhindi. Moreover, it serves as an indirect pest by facilitating the spread of diseases to a range of plants, including pulses like green gram, black gram, red gram, cowpea, lablab and horse gram, while also reproducing on various alternative hosts, including numerous weeds. The results reveal that MYMV-acquiring B. tabaci preferentially target plants that are not infected with the disease, while non-viruliferous B. tabaci tend to choose MYMVinfected plants (Fig. 2 & 3). This differential preference adds complexity to management efforts since MYMV is not a seedborne pathogen and is chiefly spread by B. tabaci, notably the cryptic species Asia II 8. This observation indicates that the change in feeding behaviour after virus acquisition significantly enhances the rate of virus transmission in field environments. The complex interactions among species are influenced by geographic factors, the availability of hosts, the dynamics of viruses and various environmental conditions (5,21). Comparable outcomes have been reported, particularly in Patho systems characterized by persistent transmission (15,16)

and are further supported by an ecological model related to aphid-luteovirus interactions (39).

The findings from our research demonstrate that infection with MYMV led to elevated concentrations of protein, phenol, peroxidase and polyphenol peroxidase. Conversely, levels of chlorophyll and total sugars experienced a decline following MYMV infection (Fig. 4-11), suggesting that viral infection may trigger the plant's defence mechanisms, which could subsequently influence the fitness and survival of the vectors. The presence of total phenols, ortho dihydroxy phenols, tannins and gossypol plays a crucial role in diminishing the reproductive success of adult whiteflies (40). The biochemical modifications resulting from these compounds are known to significantly deter the feeding preferences and reproductive viability of B. tabaci, suggesting that this insect may not be ideally adapted for reproduction on green gram hosts. This observation implies that viruses can manipulate vector behaviour to enhance the spread of pathogens (Fig. 9-11).

It was observed that the chlorophyll content was less in the infected plant compared to healthy plants (Fig.4-6), which might be due to virus manipulation of the genomic expression of the plants and it affects the formation of plastids in young growing leaves and also the chlorophyll content in various host plants decreases following viral infection (40,41). The yellowing of leaves may also serve as a strategy employed by viruses to facilitate their transmission, as many non-viruliferous vectors are attracted to yellow colour (42).

The protein content in mungbean plants infected by the virus was found to be higher than that in healthy plants (Fig.7). This increase may be attributed to enhanced nitrogen absorption and respiration processes that facilitate amino acid production in the infected plants (43). The rise in amino acids or specific proteins could confer reproductive advantages following viral infection. For example, the MEAMI 1 or "B" biotype, when fed on plants infected with begomovirus, exhibited improved reproductive success due to elevated levels of vitellin and vitellogenesis (44). This phenomenon may explain the vector's need to pre-feed on less adapted hosts.

The biochemical analysis revealed that healthy leaves exhibited a higher total sugar content compared to those that were infected (Fig.8). This disparity may explain the initial attraction or preference of viruliferous whiteflies for non-infected plants during the early growth phase (seedling stage). However, following the manipulation of the host by the virus, the preference of whiteflies shifted, leading non-viruliferous whiteflies to favour infected plants for the further dissemination of the virus. During the course of viral infection, the excessive protein production required for the rapid synthesis of viral particles results in an accelerated breakdown of carbon compounds, which are redirected towards amino acid synthesis, ultimately leading to a reduction in carbohydrate levels in the plant leaves.

Interestingly, *B. tabaci* does not persist or reproduce on green gram hosts; however, the virus has specifically adapted the insect's behaviour to enhance its spread. Despite green gram being an unfavourable host for the whitefly in terms of feeding and reproduction, the virus manipulates the vector's

behaviour through this shared host. Consequently, this can lead to an accelerated disease transmission, even when vector management strategies are applied in the field.

The investigation of mitochondrial cytochrome oxidase (mtCOI) subunit I PCR products from 20 individual samples uncovered the existence of two cryptic species of Bemisia tabaci: Asia II8 and Asia I. Asia II8 was particularly associated with regions that identified as hotspots for the Mungbean yellow mosaic virus (MYMV), while Asia I was predominantly linked to vegetable crops grown regions. A study assessing the settling preferences of both non-viruliferous and viruliferous B. tabaci (Asia II8) on MYMV-infected versus healthy green gram plants revealed that non-viruliferous B. tabaci exhibited a greater preference for infected plants, in contrast to viruliferous B. tabaci, which favoured healthy plants. Furthermore, biochemical analyses of MYMV-infected and healthy green gram plants indicated alterations in nutritional status that could explain the differential preferences of B. tabaci in relation to MYMV infection. Our research aligns with previous studies indicating that whiteflies prefer begomovirusinfected plants, which positively influences their performance as vectors (20, 45). However, some investigations have reported negative or neutral impacts on vector performance (35). These performance dynamics are influenced by various host plant factors, including the timing of inoculation and the progression of the disease (36), plant varieties (27), the amount of volatile organic compounds released (16) and the level of host resistance (17, 46), as well as the specific vector species or biotypes involved (47). Collectively, these interactions among the virus, host and vector contribute to the acceleration of disease spread through shared manipulation of hosts and vectors (17).

#### Conclusion

This investigation is the first to illustrate a tripartite interaction among a vector, a virus and their mutual host, which is typically perceived as less favourable for the feeding and reproduction of its vector. In nature, such interactions can promote the rapid dissemination of diseases, posing significant challenges for vector and disease management in agricultural contexts. In this instance, the vector targets the host for temporary feeding rather than for establishing a lasting presence or reproduction on *V. radiata*, suggesting that the virus manipulates vector behaviour in order to shift from infected to non-infected plants for its survival. Furthermore, a more profound understanding of these vector-virus-host interactions and their coevolutionary implications will contribute to the formulation of improved strategies for vectorvirus management, essential for sustaining agricultural productivity and ensuring global food security.

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# **Authors' contributions**

DR designed the experiment, drafted and edited the manuscript, RR recorded the data, performed the statistical analysis, monitored the experiment and GK edited the manuscript and helped with molecular analysis.

## **Compliance with ethical standards**

**Conflict of interest:** Authors do not have any conflict of interest to declare.

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