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Whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) Cryptic Species is Closely Associated with Mungbean Yellow Mosaic Virus (Begomovirus: Geminiviridae) Hotspot Regions and Biochemical Changes in Host Plants

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Abstract:
The Greengram (Vigna radiata (L) Wilczek) or Mungbean is an important pulse crop cultivated in India. Mungbean Yellow Mosaic Virus (Begomovirus; Geminiviridae) is one of the majors constrains for the productivity and it is exclusively transmitted by the vector whitefly Bemisia tabaci (Gennadius), (Hemiptera: Aleyrodidae). The study reveals that the genetic diversity of B.tabaci is influenced by the begomovirus infection. The sequence analysis of Mitochondrial Cytochrome Oxidase (mtCOI) subunit I PCR products from 21 individual samples shown that exist of B. tabaci cryptic species Asia II 8 and Asia I. Where the Asia II 8 was closely associated with MYMV hotspot regions and Asia I was closely associated with vegetable crops grown in Tamil Nadu. Further laboratory experiment confirmed that the preference of B. tabaci (Asia II 8) was influenced by the MYMV infection status and the non-viruliferous whiteflies settled more (55%) on infected plants and the viruliferous whiteflies settled more (62.7%) on non-infected plants. Subsequent biochemical analysis revealed that the preferences are associated with biochemical changes after the virus infection in the plants. Found that the protein, phenol, peroxidase and polyphenol peroxidase contents were increased and the chlorophyll and total sugars were decreased after MYMV infection in greengram. MYMV specific primer was used to detect the virus in greengram and whiteflies.

INTRODUCTION

Mungbean yellow mosaic virus (MYMV) is a serious production constraint in all legume crops growing regions of Indian subcontinent and it is exclusively transmitted by the vector whitefly Bemisia tabaci (Gennadius), (Hemiptera: Aleyrodidae). The affected plants are with small yellow irregular specks, mosaic, leaf mottling, yellowing and drying and causes about 80-100% yield loss (Nene 1973) to the tune of $ 300 million every year (Varma et al. 1992; Varma and Malathi 2003). Two strains of MYMV had been reported in India (Tsai, 2013) one is Mungbean Yellow Mosaic India Virus (MYMIV) which is more predominant in northern, central and eastern regions (Usharani et al. 2004) and another one is MYMV in southern region particularly in Tamil Nadu (Karthikeyan et al. 2004; Girish and Usha 2005; Haq et al. 2011). Both the strains are transmitted by the vector B. tabaci in a persistent and circulative manner (Markham et al. 1994).

B.tabaci is a polyphagous, distributed worldwide and has species complexity; about 40 cryptic species had been reported so far (Hu et al., 2017). First B. tabaci was recorded in India during 1905 from cotton host (Rao et al., 1989; Misra and Lambda, 1929). Subsequent to this, the invasive biotype-B was discovered in South India (Banks et al., 2001; Rekha et al., 2005) Shankarappa et

al., 2007) and it changed the disease empidemiology in tomato and population dynamics of indigenous B. tabaci (Banks et al., 2001). Similarly, Palaniswami et al., (1995) observed exist of B. tabaci biotype in cassava and differed in transmission efficiency and feeding preference (Lisha et al., 2003; Palaniswami and Henneberry, 2011). The presence of host specific cluster in B. tabaci was reported and they classified into Group I, Group II and Group III (Sharma et al., 2008). Based on the mtCOI sequence analysis found 5 cryptic species in India (Chowda - Reddy et al., 2012; Ellango et al., 2015; Prasanna et al., 2015; Ram Kumar et al., 2017). However, B. tabaci cryptic species Asia II 1 was predominant in North India and Asia II 8 was predominant from south India (Nair et al., 2017).

The plant viruses are mostly transmitted by insect vectors for which the virus alter the behaviour of the vector in a manner to spread the disease from plant to plant by evolving special mechanism (Fereres and Moreno 2009; Ng & Falk 2006; Hogenhout, et al. 2008). Some of the recent studies demonstrated that the virus could manipulate the vector behaviour in ways favourable to the spread of viruses (Ingwell et al. 2012; Rajabaskar et al. 2013a, 2013b, 2013c; Roosien et al., 2013). With this view a study was carried out to identify cryptic species of B.tabaci in MYMV hotspot regions (Tamil Nadu, India) in greengram and to understand the preference of that cryptic species with infection status of MYMV and the associated biochemical changes.

MATERIALS AND METHODS

Sample Collection

B.tabaci adults were collected from MYMV hotspot regions of Tamil Nadu viz., Vamban, (Puthukottai), Aaduthurai (Thanjavur), Navalurkuttapadu (Trichy), Panpolil (Thirunelvli) and Coimbatore. The whiteflies were collected at the early morning hours by using aspirator then transferred to 1.5 ml eppendorf tubes containing 95 per cent ethanol with the help of camel hair brush (000 size) and stored in -20 °C until for further use (Ellango et al.,2015).

Vector Rearing

B.tabaci was reared as described by Butter and Rataul, (1977).The pure culture of whiteflies, B. tabaci (Asia II 8) was originally collected from the greengram (variety CO 8) field (Department of Pulses, Coimbatore) with help of an aspirator (50 ml) and maintained in a bugdorm (MegaView, Taiwan) under the screenhouse conditions. The brinjal (Solanum melongena L) plants (variety CO 6) were used for rearing whiteflies and fresh brinjal plants were introduced to the cage at 10 days interval and allowed to move the whiteflies to new one and the old plants were removed periodically. The adult non viruliferous whiteflies were collected 5 months after release and used for all the experimental studies. The whiteflies adult samples were taken from the bugdorm randomly and the DNA was analysed using mtCOI primer, sequenced and the cryptic species was identified by exploring NCBI database.

DNA Isolation

Five B.tabaci were analysed from each field. DNA was extracted from single whitefly by using lysis buffer **(**Zeidan and Czosnek, (1991); Singh et al. 2012). The DNA was extracted from single adult whitefly by using lysis buffer (5 µl Tris 1M (pH 8) @ 100 µl , 1 µl EDTA 0.5 M (pH 8) @ 20 µl, 5 µl Igepal (Triton X 100) @ 100 µl , 50 µl proteinase-K (20 mg/ml) @ 1000 µl, 939 µl Distilled water @ 18780 µl). Briefly a petri dish (90mm dia) was first wrapped up with aluminum foil (Mirage foil-10.5µ thickness) then with parafilm (American National Can^{TM}). 5 µl of lysis buffer was spotted on the centre of the parafilm. A single adult whitefly was placed on the buffer spot using camel hair brush (size 000) and crushed with edge of a sterile PCR tube (1.5ml). After crushing, the entire

content (including the washing from edge of the PCR tube) was transferred to sterile PCR tube (1.5.ml) and kept in an ice box for 5 minutes. Subsequently it was incubated in a water bath (Bio Equipment,) at 65°C for 15 minutes followed by 95°C for 10 minutes then kept in a refrigerator () for 3 min. Finally, the sample was vortexed for five seconds and proceeded for PCR analysis.

Sequencing of PCR Product:

The amplified PCR product (20 µl) was sent to the sequencing facility, J.K Scientific Company, Coimbatore,TN,India for single pass DNA sequencing with mtCOI forward and reverse primers (5 µl for each sample). The sequenced data were assembled and analysed as described by Hall, 1999 using a software programme (Bioedit v 7.0.5). Multiple alignments and predicted amino acid sequence alignment were done in CLUSTAL X programme. After that trimmed sequence data was analysed in the NCBI (). The neucleotite sequence similarity was checked with the data base available in the GenBank (NCBI). Then the dendrogram was constructed using the neighbour-joining method with bootstrapping (500 replicates) in MEGA software version 4.0 (Tamura et al., 2007).

Virus Maintenance

The non-viruliferous B.tabaci adults were collected from the brinjal plants reared under the bugdorm and kept 30 min in a petridish for starvation. Ten whiteflies' adults were shifted to clip cage (1.5 cm x 1.3 cm) and fixed on MYMV infected greengram plants (symptomatic) for virus acquisition. After 24 h of Acquisition Access Period (AAP), shifted to 10 days old greengram seedlings and kept 24 h for inoculation. From these symptomatic leaves were used for bioassay experiments. The presence of MYMV in the whiteflies and greengram plants was checked periodically using PCR analysis with MYMV specific primers (RHA-F 5' TCAAGCTCCCGGTGCATGTTGCA 3'and AC-abut 5' GTAAAGCTTTACGCATAATG 3').

Virus -Vector- Host Interactions

Settling Preference:

The settling preference of B. tabaci, Asia II 8 was examined in relation to MYMV infection status in greengram. About 500 B.tabaci adults were collected from the brinjal plant which was maintained under the screenhouse. After 1 h of starvation each 20 whiteflies were shifted into the bottom of petridish (125mm dia x --h) containing MYMV infected and non-infected leaves separately. The covering lid top portion was removed and fixed with a nylon mesh (diameter, 34 mm; mesh size, 63 µm). The infected non infected greengram leaves were collected from the plants maintained at the screenhouse and a leaf bouquet was prepared. The detached trifoliate leaf's petiole was covered with moist cotton in order to avoid wilting of leaf. Then the bouquet leaf (facing the abaxial portion upper side) was kept into the petridish over a moist filter paper (No.46). The 20 whiteflies were placed into petridish, with the help of camel hair brush after 5 min refrigeration. Then the areana was covered with lid and kept inside a Enviromental plant growth chamber (Percival, USA). The experiment was completely randomized with four tratments consist of viruliferous and non-viruliferou B.tabaci and MYMV infected and healthy greengram leaves. Each treatment was replicated for 15 times. Number of whiteflies settled on the infected and non-infected leaves were observed 1, 3, 6, 12 and 24 h after release. The mobile torch light was used for counting under dark condition. After the bioassay the samples were kept in a zip lock bag individually, properly labeled and stored under -80 for further biochemical and PCR analysis.

Biochemical Analysis

The chlorophyll content present in the MYMV infected and healthy greengram leaves were analysed as described by Bruinsona, (1963), the total carbohydrate was estimated using anthrone method (Hedge and Hofreiter, 1962). Phenols were estimated using folin reagent as described by Malick and Singh, 1980, Protein was estimated using modified Lowry method (Hartree, 1972).

Statistical Analysis

The data observed from preference and the associated biochemical changes in the infected and non-infected plants were analyzed with student's t-test by using SPSS Statistics ver.17.0.

RESULTS

B.tabaci Cryptic Species Identification in MYMV Hotspot Regions

B.tabaci adult samples were collected from 5 major MYMV hotspot regions of Tamil Nadu. A summary of geographical locations, host plants, date of collections, species identified and the gene bank accession number is presented in Table 2. The sequence analysis of Mitochondrial Cytochrome Oxidase (mtCOI) subunit I PCR products from 21 individual sample shown that presence of B. tabaci cryptic species Asia II 8 and Asia I where Asia II 8 is closely associated with MYMV hotspot regions and Asia I is closely associated with vegetable crops grown in Tamil Nadu (Table 1).

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

S.NO	Sample Name	Genetic Group	Host	Place	Coordinates	Collection Date	Genbank Accession No
1.	VBNBG	Asia II 8	Vigna mungo	Vamban	N 11° 30' , E 79° 26'	6.3.18	MH374156
2.	VBNGG	Asia II 8	Vigna radiata	Vamban	N 11° 30' , E 79° 26'	6.3.18	MH374157
3.	ADTBG	Asia II 8	Vigna mungo	Aduthurai	N 10.9985° , E 79.4801°	7.3.18	MH356716
4.	TRYGG	Asia II 8	Vigna radiata	Trichy	N 10°45' , E 78°36'	7.3.18	MH374155
5.	CBEBG	Asia II 8	Vigna mungo	Coimbatore	N 11° 07' ,E 76° 59'	28.12.17	MH374139
6.	CBEGG	Asia II 8	Vigna radiata	Coimbatore	N 11° 07' ,E 76° 59'	28.12.17	MH374148
7.	CBERG	Asia II 8	Cajanus cajan	Coimbatore	N 11° 07' ,E 76° 59'	4.10.17	MH374152
8.	CBEHG	Asia II 8	Macrotyloma uniflorum	Coimbatore	N 11° 07' ,E 76° 59'	20.12.17	MH374150
9.	CBEGNUT	Asia II 8	Arachis hypogaea	Coimbatore	N 11° 07' ,E 76° 59'	22.12.17	MH374149
10.	CBEBRI	Asia 1	Solanum melongena	Coimbatore	N 11° 07' ,E 76° 59'	19.9.17	MH374141
11.	CBEBRI1	Asia 1	Solanum melongena	Coimbatore	N 11° 07' ,E 76° 59'	20.9.17	MH374142
12.	CBEBI	Asia 1	Abelmoschus esculentus	Coimbatore	N 11° 07' ,E 76° 59'	13.10.17	MH374140
13.	CBECALI	Asia 1	Brassica oleracea var. botrytis	Coimbatore	N 11° 07' ,E 76° 59'	31.10.17	MH374143
14.	CBECAP	Asia 1	Capsicum annuum	Coimbatore	N 11° 07' ,E 76° 59'	14.9.17	MH374144
15.	CBECWA	Asia 1	Vigna unguiculata	Coimbatore	N 11° 07' ,E 76° 59'	4.11.17	MH374145
16.	CBECT	Asia 1	Gossypium hirsutum	Coimbatore	N 11° 07' ,E 76° 59'	8.10.17	MH374146
17.	CBECU	Asia 1	Cucumis sativus	Coimbatore	N 11° 07' ,E 76° 59'	14.12.17	MH374147
18.	CBEMM	Asia 1	Cucumis melo	Coimbatore	N 11° 07' ,E 76° 59'	16.12.17	MH374151
19.	CBETOM	Asia 1	Solanum lycopersicum	Coimbatore	N 11° 07' ,E 76° 59'	19.9.17	MH374153

20.	CBETOM1	Asia 1	Solanum lycopersicum	Coimbatore	N 11° 07' ,E 76° 59'	20.9.17	MH374154
21.	PAPGG	Asia II 8	Vigna mungo	Panpoli	N 8°67', E 77° 70'	6.3.18	MH476485

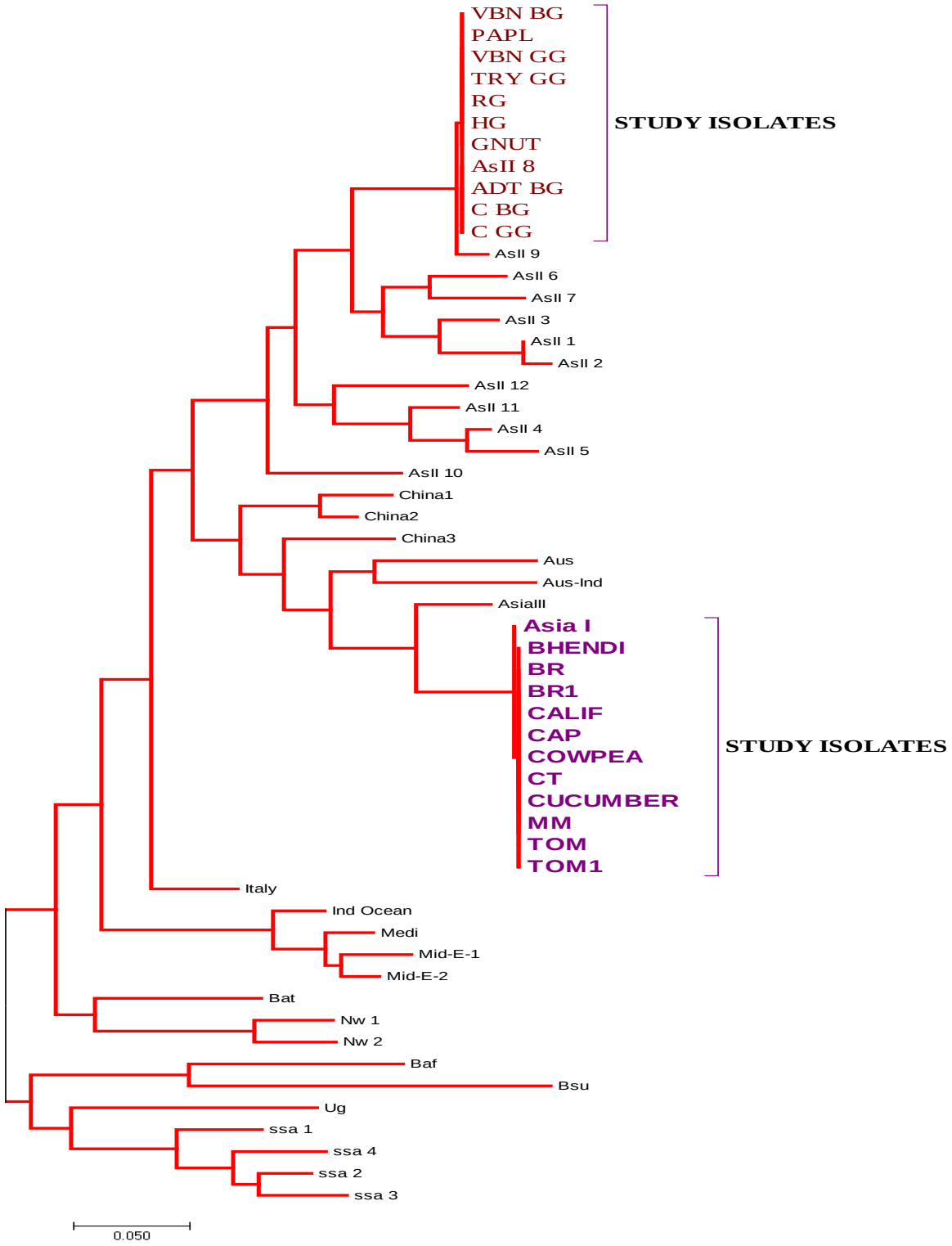

Fig.1.Phylogenetic dendrogram based on mtCOI partial sequences of Bemisia tabaci genotypes

Phylogenetic tree generated from aligned partial mtCOI nucleotide sequences of Bemisia tabaci genotypes with other selected whitefly genotypes. Tree was generated by neighbor joining

method by aligning the sequences in MEGA 7 ClustalW. Vertical branches are arbitrary; horizontal branches are proportional to calculated mutation distances; values at nodes indicate percentage boot straps values (1000 replicates).

A phylogenitic relationship of 21 B. tabaci samples from 5 major locations was presented in the Table 2. The Bayesian phylogenitic analysis revealed that the B.tabaci sequences were grouped into two different clades (Fig 1). The sequences were characterized and assigned to two groups as described by Dinsdale et al., (2010). When the sequences of about 605bp (the maximum sequence which got aligned) were compared, the whitefly populations VBNBG, VBNGG, ADTBG, TRYGG, CBEBG, CBEGG, CBERG, CBEHG, CBEGNUT and PAPGG showed 99 % identity with Asia II 8 genotype and CBEBRI, CBEBRI1, CBEBI, CBECALI, CBECAP, CBECWA, CBECT, CBECU, CBEMM, CBETOM and CBETOM1 showed 99 % identity with Asia I genotype (Table 1).

Table.2. Preference of non-viruliferous B. tabaci (Asia II 8) to MYMV infected and healthy Greengram hosts

Replication	% Settling preference at different time intervals									
	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy
	1 h	1 h	3 h	3 h	6 h	6 h	12 h	12 h	24 h	24 h
R1	15	10	50	50	65	40	75	50	85	70
R2	10	25	25	30	50	45	40	40	55	65
R3	10	15	35	30	30	40	50	50	70	45
R4	5	5	30	20	20	35	40	40	55	55
R5	10	10	30	15	50	40	55	45	75	55
R6	5	5	25	20	60	30	50	50	70	50
R7	20	20	20	30	35	35	40	25	45	45
R8	15	10	25	40	45	30	55	15	50	35
R9	25	20	40	20	50	30	55	60	40	35
R10	10	10	15	15	35	20	55	45	45	60
R11	15	15	20	20	40	40	50	50	70	50
R12	20	20	20	10	35	40	60	55	55	45
R13	20	25	25	20	25	30	30	30	25	40
R14	30	25	30	25	25	20	40	40	50	50
R15	15	20	30	15	35	25	50	55	35	45
Mean	15.00	15.67	28.00	24.00	40.00	33.33	49.67	43.33	55.00	49.67
Standard Error	1.83	1.82	2.28	2.73	3.38	1.99	2.78	3.15	4.23	2.60
P value	0.789		0.270		0.137		0.027*		0.002**	

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

MYMV-Bemisia tabaci (Asia II 8)-Mungbean Interactions

Preference of B.tabaci (Asia II8) Pertaining to MYMV Infection Status:

The per cent preferences of non-viruliferous and viruliferous B.tabaci (Asia II8) to MYMV infected and healthy greengram plants were given in Table 4 & 5. The result indicated that the per cent settling of non-viruliferous whiteflies was 55 % on infected plants and 49.67 % on healthy plants 24 h after release (Table 4). The per cent settling of viruliferous whiteflies was 49.67 % on infected plants and 62.67% on healthy plants 24 h after release (Table 5). The preference was statistically

significant at 12 h (t = 1.571, d.f. = 28, P=0.027) and 24 h (t = 3.374, d.f. = 28, P=0.002) after release for non-viruliferous whiteflies and the preference was significant at 24 h (t = 1.071, d.f. = 28, P=0.029) after release for viruliferous whiteflies (Table 2 & 3).

Table.3. Preference of viruliferous B. tabaci (Asia II 8) to MYMV infected and healthy Greengram hosts

Replication	% Settling preference at different time intervals									
	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy
	1 h	1 h	3 h	3 h	6 h	6 h	12 h	12 h	24 h	24 h
R1	10	15	50	50	40	65	50	75	70	65
R2	25	10	30	25	45	50	40	40	65	55
R3	15	10	30	35	40	30	50	50	45	70
R4	5	5	20	30	35	20	40	40	55	55
R5	10	10	15	30	40	50	45	55	55	75
R6	5	5	20	25	30	60	50	50	50	70
R7	20	20	30	20	35	35	25	40	45	45
R8	10	15	40	25	30	45	15	55	35	50
R9	20	25	20	40	30	50	60	60	35	65
R10	10	10	15	15	20	35	45	55	60	45
R11	15	15	20	20	40	40	50	50	50	70
R12	20	20	10	20	40	35	55	60	45	75
R13	25	20	20	25	30	25	30	30	40	60
R14	25	30	25	30	20	25	40	40	50	80
R15	20	15	15	30	25	35	55	50	45	60
Mean	15.67	15.00	24.00	28.00	33.33	40.00	43.33	50.00	49.67	62.67
Standard Error	1.82	1.83	2.73	2.28	1.99	3.38	3.15	2.85	2.60	2.84
P value	0.798		0.266		0.744		0.633		0.049*	

*. Correlation is significant at the 0.05 level (2-tailed).

Biochemical Analysis of MYMV Infected and Healthy Mungbean Plants

Chlorophyll:

The result showed that the chlorophyll-A content was ranged from 0.103 to 0.207 mg g^{-1} in healthy plants and 0.06 to 0.11 mg g^{-1} in MYMV infected greengram plants (t= 2.7, df= 8, p= 0.03), the chlorophyll-B content was ranged from 0.224 to 0.280 mg g^{-1} in healthy plants and it was 0.013 to 0.073 mg g^{-1} in infected plants (t= 15.528, df= 8, p= 0.001) and the total chlorophyll content in healthy leaves was ranged from 0.224 to 0.280 mg g^{-1} and it was 0.105 to 0.149 mg g^{-1} in infected leaves (t= 10.038, df= 9.69, P=0.001). The chlorophyll-A (0.14), chlorophyll-B (0.009) and total chlorophyll (0.009) content were higher in healthy plants compared to MYMV infected plants where the chlorophyll-A (0.01), chlorophyll-B (0.01) and total chlorophyll (0.01) and these were statistically significant (Table 4)

Table4.4 Chlorophyll content in healthy and MYMV infected Greengram leaves

Replication	Chlorophyll- a content (mg g^{-1})		Chlorophyll -b content (mg g^{-1})		Total chlorophyll content (mg g^{-1})	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
1	0.129	0.062	0.224	0.043	0.224	0.105
2	0.149	0.105	0.280	0.038	0.280	0.143
3	0.103	0.097	0.262	0.052	0.262	0.149
4	0.207	0.114	0.251	0.013	0.251	0.127
5	0.131	0.070	0.258	0.073	0.258	0.143
Mean	0.144	0.090	0.255	0.044	0.255	0.133
S.E	0.017	0.010	0.009	0.010	0.009	0.008
P value	0.027*		0.001**		0.001**	

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Protein, Total Sugars and Phenols:

The protein content present in the MYMV infected and healthy greengram leaves are presented in the Table 5. It was varied from 0.98 to 1.76 mg/100 mg fresh wt. and in infected plant total protein was 1.16 to 1.92 mg/100 mg fresh wt. The protein content was more in MYMV infected leaves (1.7 mg/100 mg fresh wt) and compared to healthy leaves (1.7 mg/100 mg fresh wt) which is statistically significant (t= 2.145, df= 8, P= 0.064) (Table 7).

Table.5. Total protein, total sugars and phenol content in healthy and MYMV infected Greengram leaves

Replication	Protein (mg 100 mg^{-1})		Total sugars (mg 100 mg^{-1})		Phenol content (µg / g)	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
1	1.35	1.87	4.64	2.34	0.32	0.79
2	0.98	1.34	2.76	3.12	0.18	0.65
3	1.12	1.72	5.43	2.74	0.34	0.88
4	1.76	1.64	4.26	2.63	0.25	0.26
5	1.45	1.92	3.87	3.21	0.21	0.57
Mean	1.332	1.698	4.192	2.808	0.260	0.630
S.E	0.135	0.103	0.441	0.160	0.031	0.107
P value	0.064		0.017*		0.009**	

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

The total sugars were higher in healthy leaves (4.19 mg/100 mg) which is statistically significant (t= 2.996, df= 8, P= 0.017) compared to MYMV infected leaves (2.81 mg/100 mg (Table 7).

The phenol content was higher in infected leaves (0.63 µg / g) and it is statistically significant (t= 3.396, df= 8, P= 0.009) whereas the phenol content was lower in healthy leaves (0.26 µg / g) (Table 7).

Peroxidase and Polyphenol Oxidase:

The peroxidase and polyphenol oxidase activities in the MYMV infected and healthy leaves are presented in the table 6. The peroxidase content was higher in infected leaves (42.4 units / min) and it is statistically significant (t= 0.808, df= 8, P= 0.443) whereas the phenol content was lower in healthy leaves (32.4 units / min) (Table 8). The polyphenol peroxidase content was higher in infected leaves (6.4 units / min) and it is statistically significant (t= 2.587, df= 8, P= 0.032) whereas the phenol content was lower in healthy leaves (3.6 units / min).

Table.6. Peroxidase and Polyphenol oxidase content in healthy and MYMV infected greengram leaves

Replications	Peroxidase (units/minute)		Polyphenol oxidase (units/minute)	
	Healthy	Infected	Healthy	Infected
1	10.26	13.27	4.37	5.34
2	36.71	48.62	1.28	6.12
3	54.28	62.58	5.21	4.23
4	33.12	52.85	2.63	7.82
5	27.16	34.61	4.26	8.24
Mean	32.306	42.386	3.550	6.350
S. E	7.125	8.557	0.705	0.752
P value	0.0443*		0.032*	

*. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Genetic Diversity of Whiteflies

Cryptic species composition and their diversity within the B. tabaci complex are the critical factors for developing sustainable and effective approaches for B. tabaci and MYMV management in pulses. The genetic diversity of whitefly was presented in (Fig 1) and Asia II 8 was found to be predominantly associated in the MYMV hotpot areas of Tamil Nadu and Asia I was closely associated with the vegetable ecosystem. These close association might be influenced by the virus for its own spread and survival () and this could be evidenced from the previous works where the introduction of new bio type had displaced the indigenous biotype in tomato-ToYLCV pathosystem in India and that affected the disease epidemiology (Ramappa et al., 1998; Colvin et al., 2006; Banks et al.,2001)

The genetic diversity of B.tabaci and its complexity had been reported earlier from India on different hosts (Ramappa et al., 1998; Rekha et al., 2005; Chowda-Reddy et al., 2012, Prasanna et al. 2015; Ellango et al., 2015; Nair et al., 2017). They followed mitochondrial COI method (Frohlich et al., 1999 and Brown, 2000) which is widely accepted and differentiation was on the basis of nucleotide sequence of mitochondrial cytochrome oxidase gene subunit I (mtCOI). They documented exists of 7 cryptic species in India viz., Asia I, Asia II 1, Asia II 2, Asia II 5, Asia II 7, Asia II 8 and MEAM 1.

In the present study showed that Asia II 8 was predominant in MYMV hotspot regions and Asia I was more predominant in vegetables crops with a possibility of associating other begomovirus which needs to be explored further. Similar findings were reported earlier (Ellango et al., 2015; Prasanna et al., 2015; Nair et al., 2017 and) where they reported that Asia II 8 population was documented in greengram and blackgram from south India and Asia I population was reported in eggplant (Prasanna et al., 2015) and cotton (Ellango et al., 2015) in Tamil Nadu, Karnataka and

Maharashtra. Considering the record of whitefly population in different crops in India, limited information is available on the cryptic species involved in MYMV transmission in pulses.

MYMV-*Bemisia tabaci* (Asia II 8)-Mungbean Interactions

Preference of B. tabaci (Asia II 8) Pertaining to MYMV Infection Status:

The result shown that the preference of non-viruliferous B.tabaci (Asia II8) adults was greater towards MYMV infected plants compared to non-infected plants. This might be due to the influence of virus on vector behaviour in order to spread the disease in the field condition. Similar results were reported especially persistently transmitted pathosystem viz., aphid-leuteovirus pathosystem (Eigenbrode et al.,2002;2017;Jiménez-Martínez et al., 2004; Srinivarsan et al., 2008; Werner et al.,2009; Rajabaskar et al.,2013a,2013b, 2013c; 2014; Ingwell et al., 2013), whitefly-begomovirus pathosystem (Liu et al.,2010; Moreno-Delafuente et al. 2013; Fang et al.,2013; He et al.,2015; Fereres et al.,2016; Legarrea et al., 2016; Shi et al.,2018) and thrips-tospovirus pathosystem (Maris et al., 2004; Ogada et al.,2012; Shrestha et al., 2012; Shalileh et al., 2016; Daimei et al., 2017; Wilson et al., 2017).

The volatiles and bio-chemicals components present in the plants are being altered by the virus after entering into the plants which would influence the host selection behaviour and fitness the vector (Bosque-Perez & Eigenbrode, 2011; Eigenbrode et al., 2017). It is important to consider that MYMV is a persistent circulative virus, this would have influenced the transmission, behavior, and preference B.tabaci, with a positive effects on viral dissemination, evidencing coevolution between virus and vector species (Eigenbrode et al., 2002; Bosque-Perez & Eigenbrode, 2011; Eigenbrode et al., 2017).

Biochemical Changes Pertaining to MYMV Infection Status

The biochemicals components viz., chlorophyll, phenols, protein, total sugars, peroxidase and polyphenoloxidase were analysed both in MYMV infected and non-infected plants and the results are given in table () and figures (). It was observed that the chlorophyll content was less in the infected plant compared to healthy plants which might be due to virus manipulation on the genomic expression of the plants and it affects the formation of plastids in young growing leaves and it is line with earlier report by Sinha and Srivastava, (2010). Similar reports were obtained by many workers where chlorophyll content was affected in many host plants due to virus infection (Pandey and Joshi 1989; Rathore and Agnihotri, 1995; Thind et al., 1996, Dantre et al., 1996; Sutha and Rajappan, 1998; Mali et al., 2000, Gill and Singh, 2000, Milavec et al., 2001; Funayama-Noguchi and Terashima 2006, Pineda et al., 2008, Arora et al. 2009 and Singh and Shukla, 2009). This is a kind of host manipulation by virus for spread where the loss of chlorophyll followed development of yellowing in host plants would also influence the host selection behaviour of the vector (Fig).

The phenol content had changed with virus infection where it was higher in infected plant compared to non-infected plants (Fig) and this might be due to the plant response to virus infection where the defense system of the plant would have been activated due to the virus infection which in turn affected the fitness and survival of the vector. It is very interesting to mention here the B.tabaci is not surviving or reproducing on greengram host but it behaviour has been specifically manipulated by the virus for spread. The presence of total phenols, ortho dihydroxy phenols, tannins and gossypol are having significant negative impact on reproductive success of whitefly adults (Rao and Panwar, 2001; Perveen et al., 2001; Raghuraman et al., 2004; Halder and Srinivasan, 2007).

The total protein content was increased in virus infected mungbean plants than healthy plants (Fig.). This might be due to increased nitrogen uptake and respiration for amino acid synthesis in infected plants (Szczepanski and Redolfi, 1985; Shivaprasad et al., 2005; Hofius et al., 2001). There is a possibility that the elevated aminoacids or specific proteins would have some positive relationship with the endosymbionts associated in the midgut of whitfly adult which favour the virus transmission.

The total sugars content was more in healthy leaves compared to infected leaves (Fig) and this might be reason for attraction or preference of viruliferous whitfly to non-infected plants at the early stage of plant growth (seedling stage) and after the host manipulation by the virus, the preference of whitefly shifted where the non-viruliferous whitefly prefer infected plant for further spread of the virus. During virus infection, abnormal amount of protein production needed for protein synthesis for rapid synthesis of virus particle resulted in faster breakdown of carbon compounds towards amino acid synthesis creates decreased production of carbohydrates level of plant leaves. The findings are in line with earlier works (Ashraf and Zafar, 2000; Goncalves et al., 2005; Handford and Carr, 2007; Adomako and Hutcheon, 2008; Goodman et al., 2008; Singh and Shukla, 2009).

The peroxidase and polyphenol oxidese are defense enzyme exist in the plant. The present study showed that these enzymes are increased in MYMV infected plants than healthy plants (Fig. & Fig.). Enhancement of peroxidase and polyphenol oxidase are responsible for many physiological processes like oxidation of secondary metabolites ie, phenol that are toxic to invading microorganisms and their increased activity might be due to the induced resistance to MYMV infection. Similar, reports were found on increased level of PPO and PO activities in infected leaves of resistant cultivar were higher than in susceptible (Tripathi et al. 1975; Stahman and Demorest, 1973; Kaur et al., 1991; Sohal and Bajaj, 1993; Jagdish Chandra and Tyagi, 1993) In contrast Sohal and Bajaj (1993) reported that the specific activities of PPO and PO decreased MYMV infected leaves compared to susceptible mungbean cultivars.

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