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Host induced genetic variation in legume pod borer, *Maruca vitrata*

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Abstract

Aim : The legume pod borer, *Maruca vitrata* Fab. is one of the major devastating threats to legume production in all pulse producing countries across the globe. However, it is necessary to acknowledge the role of host plants on *M. vitrata*.

Methodology : In the present study, the influence of major pulse hosts such as pigeonpea, greengram, blackgram, cowpea and lablab on *M. vitrata* was studied using RAPD, SSR, COI and *Wolbachia* specific primers.

Results : The results clearly revealed that RAPD primers produced 422 (100.0 % polymorphism) polymorphic alleles, whereas SSR primers produced 95 polymorphic alleles with the mean number of alleles detected / primer ranging from 9 to 16 and this showed the existence of wide genetic diversity. Dendrograms constructed using similarity index values for RAPD and SSR primers grouped the larval samples from above pulses into 8 and 6 clusters respectively. Larvae of *M. vitrata* also yielded specific fragments with mitochondrial COI primer (~800 bp). Additionally, presence of *Wolbachia* was also confirmed with specific primers.

Interpretation : The present study clearly indicated the existence of close proximity in the larval population of cowpea and lablab than other hosts.

Introduction

The legume pod borer, *Maruca vitrata* Fab. (Lepidoptera: Crambidae), is one of the major devastating threat to the legume production in both tropical and subtropical regions. It is a serious menace on greengram, blackgram, pigeonpea and common beans in sub-Saharan Africa (Sharma, 1998), Southeast Asia (Chinh *et al.*, 2000; Ulrichs, 2001) and India (Chandrayudu, 2008) and soybean, lima bean. The larval stages of *M. vitrata* feed on the tender parts of the plant stems, peduncles, flower buds, flowers and pods (Singh and Jackai, 1988). It feed on plant species belonging to 20 genera and 6 families, the majority of which belong to papilionaceae. At present, farmers mainly rely on synthetic chemical pesticides to overcome the problem of *M. vitrata*. But the level of control varies across India mainly due to chance of development of insecticide resistance, higher cost of chemical sprays in developing nations (Oparaeke, 2006; Kym, 2006) and hidden nature of larval feeding. Generally, insect resistance management (IRM) practices are crucial for effective and sustainable control of insect pests. Lack of sufficient population genetic data on DNA sequences, molecular markers, and population structure hinders the development of IRM protocols for *M. vitrata*. Variation in genetics and phenotypic characters are governed by natural selection through interaction of genetic forces and ever changing environments through space and time. Host plants also impose considerable role in this process. Many of the species exist within the genus *Maruca* are morphologically hard to distinguish because of their tough species complex (Brownlie and Johnson, 2009). Many scientific studies revealed the existence of different species complex in the genus *Maruca* due to active and passive dispersal means (Ferrari *et al.*, 2004; Guay *et al.*, 2000) which might lead to population variation and wide gene flow. Additionally, if host plants interfere with this process, there would be greater chance of the existence of variations in the species. This may help in understanding their behavior on damage, developmental process and scientific management. Hence, the present study was conducted to have an idea about the influence of host plants on the genetic variation in larvae of *M. vitrata*.

Materials and Methods

Isolation and checking of genomic DNA of *M. vitrata* : The present study on the influence of host plants on *M. vitrata* was conducted during 2012-13 at Molecular Ecology laboratory, Department of Plant Biotechnology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu. Larvae of *M. vitrata* of five pulse host plants such as pigeonpea, greengram, blackgram, cowpea and lablab were collected from the pulses fields of TNAU, Coimbatore, Tamil Nadu and DNA was extracted from individual larva using modified CTAB method (Doyle and Doyle, 1987) with the following chemical reagents Cetyl Trimethyl Ammonium Bromide (CTAB) extraction buffer (CTAB - 2% w/v), Tris HCl (pH 8.0) @ 100 mM, Sodium chloride @ 1.4 M, EDTA @ 20 mM, Tris EDTA (TE) Buffer (Tris HCl (pH 8.0) @ 10 mM and EDTA (pH 8.0) @ 1 mM), Ice cold Iso-propanol, chloroform and

Isoamyl alcohol (24:1) (v/v), Ethanol (70%), TE buffer, Proteinase K @ 25mgml⁻¹ and RNase A-10 mgml⁻¹. DNA was quantified on a Nanodrop Spectrophotometer (ND – 1000) and measured absorbance at 260 to 280 nm using 2µl of genomic DNA.. Based on the quantity of DNA, dilutions were made in TE buffer to a final concentration of 50 ngµl⁻¹ and stored in -20°C for further use. Agarose gels (0.8%) were used to analyze the quality of DNA samples and examined and documented using gel documentation (Bio-Rad, USA).

Polymerase chain reaction (PCR) analysis : DNA samples from *Maruca* larvae collected from different hosts were amplified using a set of 24 Random Amplified Polymorphic DNA (RAPD) primers (Williams *et al.*, 1994) (Table 1) and COI primer, *Wolbachia* primers (Dyson *et al.*, 2002 and Prakash and Puttaraju, 2007) and 6 SSR primers (Agunbiade *et al.*, 2012) (Table 2). PCR were performed in 25 µl reaction volumes that contained 2 µl of template DNA, 10x PCR buffer (2.5 µl of 1.2 µM), MgCl₂ (0.5 µl of 1.2 µM), dNTPs (1 µl mixture of dATP, dCTP, dGTP and dTTP @ 200µM of each dNTP), forward and reverse primers (each 1 µl of 10µM), Taq DNA polymerase (0.5 µl of 1.5 unit), and sterile distilled water (16.5 µl). Thermal cycling was carried out on an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany). The PCR cycle programmes for different primers were performed as follows.

In case of RAPD primers, PCR cycle programme was performed at 94°C for initial denaturation for 5 min followed by 40 cycles @ 94°C for 1 min, annealing @ 40°C for 1 min, extension @ 72°C for 2 min with a final extension of 72°C for 7 min. Whereas, PCR cycle programme for COI primer was done at 94°C for 2 min (initial denaturation), 94°C for 1 min, 52 to 57°C (Table 3) for 1 min (annealing) and 72°C for 1 min (extension) and finally 72 for 2 min (final extension) with the repetition of 40 cycles. PCR programme for *Wolbachia* primers includes 94°C for denaturation step for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, followed by final extension cycle of 72°C for 7 min. SSR primers were amplified at a temperature programme of 94°C for 2 min (initial denaturation), 94°C for 1 min, 52 to 57°C (Table 2) for 1 min (annealing) and 72°C for 1 min (extension) and finally 72 for 2 min (final extension) with the repetition of 40 cycles

Agarose gel electrophoresis for RAPD primers, COI primer and *Wolbachia* specific primers : A 2 µl aliquot of PCR amplified products with RAPD primers, COI primer and *Wolbachia* specific primers were separated on a 2% agarose gel that contained 0.5 µgml⁻¹ ethidium bromide at 90 V for 45 min and viewed under UV light and data acquired on a image documentation system (Bio-Rad USA). The size of individual DNA fragments was compared with a co-migrating 100 bp DNA ladder (MBI Fermentas).

Polyacrylamide gel electrophoresis for SSR primers : The amplified PCR products of SSR primers of *M. vitrata* larval samples were run on 6% Polyacrylamide gel, using a gel apparatus. It was prepared from the stock solution (acrylamide -

Table 1: List of RAPD primers used for characterization of *M. vitrata*

S. No.	Primer	Sequence (5'- 3')
1.	OPA 01	CAGGCCCTTC
2.	OPA 07	GAAACGGGTG
3.	OPA 08	GTGACGTAGG
4.	OPA 13	CAGCACCCAC
5.	OPA 15	TTCCGAACCC
6.	OPA 20	GTTGCGATCC
7.	OPAF 12	GACGCAGCTT
8.	OPAF 13	CCGAGGTGAC
9.	OPAF 14	GGTGCGCACT
10.	OPAG 10	ACTGCCCGAC
11.	OPBB 03	TGGCGCACAC
12.	OPBB 13	CCTACCGTGG
13.	OPBB 15	AAGTGCCCTG
14.	OPC 02	GTGAGGCGTC
15.	OPC 04	CCGCATCTAC
16.	OPC 08	TGGACCGGTG
17.	OPC 15	GACGGATCAG
18.	OPE 04	GTGACATGCC
19.	OPE 08	TCACCACGGT
20.	OPE 15	ACGCACAACC
21.	P 210	AAATGCGGCA
22.	P 225	AGTGGTCGCG
23.	P 443	GCCGTGATAG
24.	S 34	GATAGCCGAC

29.0 g, acrylamide - 1.0 g and distilled water) by adding 20 ml of Polyacrylamide stock solution, 10.0 ml of 10 x TBE buffer, 900 µl of ammonium persulphate (APS) and 100 µl of tetra methyl ethylene diamine (TEMED). The gel was immediately poured into the glass plates immediately after adding TEMED (to avoid solidification) without any air bubbles. The gel was pre-run for about 20 min before loading the sample and maintained at 70 W for 2 hrs. After electrophoresis, the gel was separated from the plates and treated for 15 min in fixation solution (double distilled water and 0.5% v/v acetic acid) with gentle shaking, the gel was washed 2-times with distilled water for 2 min. The gel was stained in staining solution (0.1% w/v silver nitrate), the gel was washed two times with distilled water for 2 min, and then the gel was transferred to developing solution (1.5% w/v sodium hydroxide, 0.3% formaldehyde) to develop the silver-staining DNA bands. The reactions were stopped by using stopper solution (double distilled water and 0.5% v/v acetic acid). The bands were then visualized by silver staining (Bassam *et al.*, 1991; Chalhoub *et al.*, 1997) dried overnight and photographed. The size of individual DNA fragments was compared with a co-migrating 100 bp DNA ladder (MBI Fermentas).

Statistical analysis : Clearly resolved, unambiguous polymorphic bands from RAPD and SSR primers amplifications were scored with the help of Alpha Ease® FC Software (Version 6.0) for their presence or absence. The scores were obtained in

the form of a matrix with '1' and '0', which indicate the presence and absence of bands in each location, respectively and 0 and 1 matrices were developed. The sizes of the alleles are approximated by comparing with the band migration distance of the standard 100bp ladder. The per cent polymorphism was calculated as follows :

$$\text{Percent polymorphism} = \frac{\text{Total number of polymorphic alleles}}{\text{Total number of alleles}} \times 100$$

The polymorphism information content (PIC) values were calculated for each RAPD and SSR primers using the following algorithm :

$$\text{PIC} = 1 - \sum p_i^2 \quad i = 1$$

where p_i is the frequency of i^{th} allele (Hildebrand *et al.* 1992). The binary data scored was used to construct a dendrogram. Similarity matrix was generated using the SIMQUAL programme of NTSYS-pc software, version 2.02 (Rohlf, 1998). The similarity coefficients were used for cluster analysis and dendrograms were constructed by the Unweighted Pair-Group method using Arithmetic Average (UPGMA) (Sneath and Sokal, 1973).

Results and Discussion

Analysis genetic diversity in *M. vitrata* using RAPD and SSR primers : The RAPD analysis of genetic variation revealed that among the 24 RAPD primers (in series OPA, OPAF, OPAG, OPBB, OPC, OPE and few specific primers) screened, 20 primers has produced clear and unambiguous scorable alleles and were detected as polymorphic (Fig 1). In total, 422 polymorphic alleles were detected and all were found to be polymorphic and hence, the polymorphism was 100.0 % with a mean of 21 alleles per primer (Table 3). A maximum of 36 polymorphic alleles per primer were detected from OPC 04 and OPC 08 followed by 32 alleles in OPA 32 and the least (9 alleles) was recorded in the primer P 210. The amplicons size varied from 0.21 to 3.37 kb. The Polymorphism Information Content (PIC) value ranged from 0.031 to 0.210.

The binary data from the polymorphic primers were used for computing similarity indices. The similarity coefficients based on 20 primers revealed that among the five pulses, the highest similarity index (0.606) was observed between *Maruca* larvae collected from cowpea and lablab and the lowest similarity index of 0.523 between pigeonpea and blackgram. The mean similarity index was 0.567.

Cluster analysis was performed on the similarity matrix calculated using 20 RAPD primers using Jaccard's coefficient (Fig. 2). The dendrogram differentiated the larvae collected from five pulse hosts in to two principal clusters A and B, with a similarity of 53.4 %. Cluster A had larvae from pigeonpea. Cluster B was further divided into two sub clusters C and D (blackgram populations) and a similarity of 57.8% obtained between them. Sub cluster C further divided in to sub clusters E and F and sub cluster E comprised of larval populations from greengram had 58.9 % similarity with cluster F, which ultimately differentiated into two clusters (sub G and H).

Table 2 : List of SSR, COI and *Wolbachia* primers used for *M. vitrata*

S. No.	Primer	Sequence (5'-3')	Annealing temperature
A. SSR Primer			
1.	C32008 ^E	F-(MAX)AAAAAGCGCTTATATGTTTGTATAGT R-GAAATTTTAAACGGAGATACAATCA	57 °C
2.	7_02K06 ^A	F-(FAM)ATTTGTCAGAATGGTATCTTACGT R-CCTCTGGGTCATAATTATATTGTTCA	52 °C
3.	C3393 ^{E1}	F-(ROX)AGACCCCCAAAGTGGAGAA R-ACGTTACGAACCTCCTGTT	55 °C
4.	C0444 ^{E1}	F-(FAM)AAAGGAACTACGCCGTCAGG R-GTTGAGCGATCTTGGCACAG	57 °C
5.	C0241 ^E	F-(TAM)GACGAAACAAGGCCTACCAG R-GGTACTTCYGACGTTGTTG	57 °C
6.	C0325 ^E	F-(ROX)CGAAAAGAAACACCGCTCTG R-CAGTCTGTTTCAGWCTCTTCAGTGG	57 °C
B. COI Primer			
1.	C1-J-2183	TT-GATTTTTTGGTCATCCAGAAGT	72 °C
2.	L2-N-3014	TCCAATGCACTAATCTGCCATATT	
C. <i>Wolbachia</i> Primer			
1.	wsp81f	TGGTCCAATAAGTGATGAAGAAAC	72 °C
2.	wsp691r	AAAAATTAAACGCTACTCCA	
3.	ftsZAdf	CTCAAGCACTAGAAAAGTCG	

Table 3 : RAPD primer profile of *M. vitrata* larvae collected from different pulse hosts and percent polymorphism observed

S. No.	Primer	Number of alleles	Number of polymorphic alleles	Number of polymorphic alleles	Polymorphism (%)	PIC value
1.	OPA 07	23	23	0	100.0	0.070
2.	OPA 08	32	32	0	100.0	0.063
3.	OPA 13	28	28	0	100.0	0.064
4.	OPA 20	13	13	0	100.0	0.077
5.	OPAF 13	24	24	0	100.0	0.063
6.	OPAF 14	28	28	0	100.0	0.051
7.	OPAG10	23	23	0	100.0	0.081
8.	OPBB 03	11	11	0	100.0	0.124
9.	OPBB 15	19	19	0	100.0	0.069
10.	OPC 02	9	9	0	100.0	0.210
11.	OPC 04	36	36	0	100.0	0.039
12.	OPC 08	36	36	0	100.0	0.031
13.	OPC 15	23	23	0	100.0	0.047
14.	OPE 04	25	25	0	100.0	0.043
15.	OPE 08	13	13	0	100.0	0.124
16.	OPE 15	20	20	0	100.0	0.070
17.	P 210	9	9	0	100.0	0.111
18.	P 225	21	21	0	100.0	0.061
19.	P 443	12	12	0	100.0	0.097
20.	S 34	17	17	0	100.0	0.073
Total		422	422	0	-	-
Mean		21	21	0	100.0	-

Table 4 : Polymorphism information content for SSR primers amplified with larvae of *M. vitrata* collected from different pulses

S. No.	Primer	Number of alleles	Number of polymorphic alleles	Number of polymorphic alleles	Polymorphism (%)	PIC value
1.	C32008 ^E	21	16	5	76.2	0.061
2.	7_02K06 ^A	13	13	0	100.0	0.101
3.	C3393 ^{E,1}	16	16	0	100.0	0.102
4.	C0444 ^{E,1}	9	9	0	100.0	0.358
5.	C0241 ^E	21	21	0	100.0	0.079
6.	C0325 ^E	15	10	5	66.7	0.084
Total		95	85	10	-	-
Mean		16	14	5	90.5	-

Lane L - 1 kb ladder, Lane 1 - Larva of pigeonpea, Lane 2 - Larva of greengram,
Lane 3 - Larva of blackgram, Lane 4 - Larva of cowpea, Lane 5 - Larva of lablab

Fig.1 : RAPD primer profiles of *M. vitrata* larvae from different pulses

Larvae collected from cowpea (sub cluster G) and lablab (sub cluster H) had maximum similarity (61.2 %).

Among the six SSR primer pairs tested with larvae collected from different pulse hosts, four primer pairs (7_02K06^A, C3393^{E,1}, C0444^{E,1} and C0241^E) showed amplification of alleles all the loci (Fig 3) indicted variation and were found to be completely polymorphic (Table 4). A total of 95 alleles were detected across six loci with the number of alleles per locus per host ranging from nine in primer C0444^{E,1} to 21 in C32008^E and C0241^E with an average of 16. In total 85 alleles were polymorphic. The size of the amplicons ranged from 88 to 924 bp. The PIC value was highest for the SSR primer C0444^{E,1} (0.358) and lowest for the primer C32008^E (0.061).

Similarity index values obtained for each pair wise comparison among the larvae collected from different pulse hosts based on six SSR primers ranged from 0.470 to 0.651. Of the 10 pair wise combinations, *Maruca* larvae from pigeonpea and lablab and blackgram and pigeonpea showed the highest similarity index of 0.470, while greengram and blackgram showed the lowest similarity index (0.651) with the mean of 0.549.

Dendrogram based on six SSR primers differentiated the *M. vitrata* larvae into two major clusters (A and B) with a similarity of 29.8 % (Fig. 4). Cluster A and B subdivided in to sub clusters such as C and D (greengram larval populations) and sub cluster B further differentiated into sub clusters E (cowpea larval populations) and F (lablab larval populations), respectively. A similarity of 82.5 % was obtained between sub clusters C and D and also between sub clusters E and F. Sub cluster C finally differentiated into clusters G and H comprised of larval populations of pigeonpea and blackgram respectively and had highest similarity of 100 %.

Over all, the dendrogram of larvae collected from five pulses using RAPD and SSR primers yielded eight clusters.

Based on the cluster analysis, it was observed that the greengram population of *M. vitrata* was slightly different from other hosts. However, in both RAPD and SSR primer analysis individuals from cowpea and lablab also pigeonpea, balckgram and greengram clustered together among themselves, but spread across the hosts indicating their varied degrees of similarity to them. It might be due to their continuous feeding on these crops generations after generations and breeding largely among themselves and also cross contamination of breeding populations when the host crops were cultivated in nearby areas. This indicated that individuals occurring on these crops were genetically similar compared to those occurring on other crops and may lead to specific genetic speciology. The present results of cluster analysis also clearly showed that there is comparatively a close association between larvae collected from cowpea and lablab followed by pigeonpea and blackgram.

Among five pulses, the highest similarity index (0.606) existed between *Maruca* larvae from cowpea and lablab and this showed close relatedness between larval populations than other hosts studied. Fakrudin *et al.* (2004a) recorded similarity coefficient of 0.75 to 0.82 in RAPD-PCR across *Helicoverpa armigera* Hubner population in South Indian cotton ecosystems. Similar levels of genetic variability have been reported within the individuals of *H. armigera* in China (Lei *et al.*, 1995) and Israel (Zhou *et al.*, 2000) and *Spodoptera litura* Fab. in China (Janarthanan *et al.*, 2002).

In the present investigation, the average number of polymorphic fragments was 21 in larval populations from different hosts and polymorphism level of nearly 100 %. This showed existence of many variations in the population of *M. vitrata* collected from different hosts. But, the phylogenetic analysis done by Periasamy *et al.* (2015) showed no difference among the *M. vitrata* population from different host plants collected from Asia and Sub Saharan Africa. Hence, it may be believed that the existence of host induced genetic variation in the larvae of *M.*

Fig. 2 : Dendrogram of larvae of *M. vitrata* collected from different pulses based on 20 RAPD primers constructed using UPGMA

Lane L– 100 bp ladder, Lane 1 - Larva of pigeonpea, Lane 2 - Larva of greengram
Lane 3 - Larva of blackgram, Lane 4 - Larva of cowpea, Lane 5 - Larva of lablab

Fig. 3 : SSR primer profiles of *M. vitrata* larvae of different pulses

Fig. 4 : Dendrogram of larvae of *M. vitrata* collected from different pulses based on six SSR primers constructed using UPGMA

Fig. 5 : COI primer profile *M. vitrata* larvae of different pulses

vitrata might be due to high diversification of host plants in the study area. Like above studies, any specific primer analysis is helpful on evaluation of influence of host plants on occurrence and diversity of insect pests at genome level.

Genetic variation in *M. vitrata* using COI primers : The results of COI Primers analysis on *Maruca* larvae from five different pulses clearly yielded specific fragments of ~800 bp length (Fig 5). Variation of DNA sequence in the mitochondrial cytochrome oxidase I (cox1) gene is important for species-level identification for many species (Scarborough *et al.*, 2005, Von-Burg, 2008, Vorburger *et al.*, 2010) and this might be one of the tools to check influence of host plants. Larvae of *M. vitrata* collected from five

different pulses yielded specific fragments of ~800 bp with mitochondrial COI primer. This showed significant genetic structuring and restricted gene flow among different populations. Taylor *et al.* (1996) characterized mitochondrial DNA variation in screwworm, *Cochliomyia hominivorax* (Coquerel) from Caribbean, North America and South America and found high variation of mtDNA, in 14 haplotypes of eighteen samples. Analysis of mtDNA variation in Spanish populations of *Ceratitis capitata* (L.) revealed a low to moderate levels of nucleotide diversity (Reyes and Ochando, 2004) due to recent colonization. Similarly, in white pine weevil, *Pissodes strobi* (Peck), Laffin *et al.* (2004) obtained 826bp fragment of the mitochondrial DNA (mtDNA) COI gene in 130 individuals from 11 locations across Canada. Shashank

Fig. 6 : *Wolbachia* specific primer profiles of *M. vitrata* larvae of different pulses

et al. (2015) reported the low level of genetic diversity in mitochondrial variation among the populations of *Leucinodes orbonalis* Guenee when charactering the larval populations from various parts of India using COI primer. Similarly, lower level genetic diversity was recorded among the populations of gypsy moth, *Limantria dispar* L. (Harrison *et al.*, 1983), monarch butterfly, *Danatus plexippus* L. (Brower and Boyce, 1991) and tobacco bud worm, *Heliothis virescens* Fab. (Roehrdanz *et al.*, 1994).

Detection of *Wolbachia* endosymbiont in *M. vitrata* : PCR amplified product of *wsp* gene specific primer (*wsp81f* and

wsp691r) indicated presence of few variation (Table 7) at nucleotide level among five pulses (Fig 6). However, the primer *ftsZ Adf* and *ftsZ Adr* produced alleles only in larvae collected from blackgram. The amplicons size of two primers was approximately 600 and 650 bp, respectively.

Inheritance of endosymbiotic bacteria is very common in insects. They are involved in many physiological processes ranging from provision of essential nutrients (Douglas, 1998), protection from natural enemies (Brownlie and Johnson, 2009) and altering the host's reproductive system (Engelsta"tter and

Hurst, 2009). Results of diversity analysis with *Wolbachia* specific primers showed that the presence of *Wolbachia* genes in *M. vitrata* larvae collected from all five pulses viz., pigeonpea, greengram, blackgram, cowpea and lablab studied. Infection of *Wolbachia* is already reported in many lepidopterans (Ankola et al., 2011). Fujii et al. (2001) demonstrated that the *Wolbachia* causing feminization in *O. scapularis*, induced male killing when transferred to the Mediterranean flour moth, *Ephestia kuehniella* Zeller. The present finding is in conformity with the findings of Ankola et al. (2011), who reported that *Wolbachia* might be responsible for sex ratio distortion in *Papilio polymnestor* Cramer and *Talicauda nyseus* Guerin, by causing male killing or feminization. Sumithra et al. (2012) reported the *Wolbachia* infection in insect populations of *Diaphania pyralis* Walker, *Bombyx mori* L., *Margarona pulverulentalis* Hampson, *Acanthacris* spp. and *Diacrisia obliqua* Walker using specific primers and Hiroki et al. (2004) also recorded similar results in *Eurema hecabe* Linnaeus. This may result in the production of more female individuals and sex ratio biased towards female population. Ramasubramanian and Sundara Babu (1989) and Sambathkumar and Durairaj (2012) recorded female biased sex ratio in the field populations of *M. vitrata* collected from different pulse hosts. It may result in production of more population under favourable environmental conditions. However, to ensure the ecological balance nature will maintain the ideal level of species population through various biotic and catastrophic factors.

Until now, there are no strong evidences from India on molecular identification or genetic relationship of *M. vitrata* from different host plants, and hence the present report is of first of its kind that discusses through areas of molecular characterization techniques with the target insect pest. This type of DNA based species characterization and species composition of *M. vitrata* can provide valuable information to carry out further research on association with host plants and host plant mediated interaction of *M. vitrata* with its natural enemies.

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