Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(1): 487-491

Received: 26-11-2017
Accepted: 27-12-2017

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Characterization of *Mung bean yellow mosaic virus* transmission by Asia I and Asia II-1 genetic group of *Bemisia tabaci* Gennadius

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Abstractf
Whitefly, *Bemisia tabaci* (Genn.) believed to be originated from India has drawn global attention in recent past with its wide distribution across the world. It causes severe damage to crops directly by sucking the sap from plants and indirectly as vector of several viral diseases. The largest diversity of *B. tabaci* genetic groups is present in Asia with the distribution of about 16 out of 34 genetic groups reported so far in Asian countries whereas in India as many as nine genetic groups are reported, among which Asia I and Asia II-1 are two predominant genetic groups of *B. tabaci* distributed widely in different agro-climatic zones of India. The present investigation focuses on the characterizing the virus vector relationship between most prevalent begomoviruses *viz*., *Mung bean yellow mosaic virus* (MYMV) and two predominant vector genotypes, Asia I and Asia II-1. From the result it was observed that Asia I has shown significantly higher transmission efficiency *i.e.* 36.6% and 66.6% as compared to Asia II-1 23% and 56% when one and five whiteflies per plant per inoculation were used respectively. They have also shown significantly higher virus transmission efficiency at different acquisition and inoculation access periods as well. This study was conducted in the year of 2013-2015 in Insect proof climatic control chamber, Division of Entomology IARI, New Delhi – India.

Keywords: *Bemisia tabaci*, begomoviruses, *Mung bean yellow mosaic virus* (MYMV)

Introduction
B. tabaci is a polyphagus pest being possibly of Indian origin infecting broad host range over 700 plant species including horticultural and agriculturally important crop [12]. It is well known that there is a genetic structure which breaks up *B. tabaci* into a series of well-defined subgroups [9]. As per the present understanding *B. tabaci* is regarded as a species complex comprised of at least 34 morphologically indistinguishable genetic groups, [10, 9, 1]. These *B.tabaci* genetic groups vary with respect of biological characteristics such as host plant range, ability to resist insecticides, dispersing capability, virus transmission ability and esterase profile patterns [4, 23, 29, 30, 3].

Bemisia tabaci can transmit 114 virus species [29] belong to multiple genera, *Begomovirus* account 90% and 4% belong to *Carlavirus*, *Crinivirus Closterovirus* and *Ipomovirus* [18]. *Begomovirus* genus is largest genus of family geminiviridae among 9 genera consisting of >320 recognised species and > 500 isolate majorly infecting economic important dicot plants [11, 32]. Begomoviruses are monopartite or bipartite, whitefly-transmitted geminiviruses that are found in the Old (both genome types) and New Worlds (mostly bipartite genomes, with one recently described monopartite genome virus [3]. Among all *B. tabaci* transmitted virus species of begomovirus some are predominantly transmitted and some are poorly transmitted. This vector specificity with begomovirus species is associated with specific amino acid sequences in the viral coat protein [2].

Mungbean is a major pulse crop grown in India. It is prone to a number of viral diseases transmitted by *B. tabaci*. *Mungbean yellow mosaic virus* is a serious virus disease affecting major leguminous crops *viz*., black gram, mungbean, soybean etc. with an estimated yield loss of $300 million [31]. MYMV was first observed in New Delhi during 1950s [20]. This disease is characterized by yellow mosaic symptoms and it is easily vectored by *B. tabaci* [21]. Many of the yellow mosaic viruses reported from India are not sap-transmissible. The aim of study this study is to see the transmission efficiency of two different genotype of *B. tabaci* which are demographically isolated, which can provide basis for further research in biotype specific virus interaction.

2. Materials and Methods

2.1 Collection and maintenance of avirulifrous *B. tabaci* genetic group and *Mungbean yellow mosaic virus* source plant for study

The whitefly, *Bemisia tabaci* populations evaluated in the study were originally collected from tomato fields of Guntur (Andhra Pradesh, India : 16.3008° N, 80.4428° E) and from New Delhi, India (28° 38' 4.790" N, 77° 10' 1.590" E) were reared on healthy tomato plant. Whitefly cultures were maintained at favourable temperature 25±2 ºC with 60±5% Relative Humidity in Insect Proof Climate Control Chamber (IPCCC) at Division of Entomology Indian agricultural research institute (IARI), New Delhi, India. Species authentication of *B. tabaci* species complex was done by using distinct taxonomic characters [16] and genetic group status of *B. tabaci* populations was ascertained by partial sequencing of *mitochondrial Cytochrome Oxidase 1 (mtCO1) gene* [10]. From sequencing *mtCO1gene* it was found that populations collected from Guntur and New Delhi were identified as Asia I and Asia II-1 respectively. Aviruliferous whitefly cultures were maintained in quarantined chambers in the IPCCC throughout the study period and the virus free status was confirmed by PCR amplification of universal marker for begomovirus in randomly selected *B. tabaci* adults [5]. Adults of both the population irrespective of sex were used for MYMV transmission studies. Source mungbean plant of begomovirus (genus: Begomovirus, family: Geminiviridae) was collected from the fields of Indian Agriculture Research Institute (IARI), New Delhi and maintained in IPCCC.

2.2 Developing the isofemale lines of Asia I and Asia II-1 population

The aviruliferous culture of both the genotype was developed from a single isofemale line by using clip cages. One pair of male and female of whitefly was transferred into a clip cage soon after emergence and allowed to proliferate for further generation and population generated from that particular cage was transferred for further multiplication on healthy tomato plant. Aviruliferous condition was maintained throughout the experiment by keeping the culture in double wire mesh case. The cage was again placed in isolated glass chamber to prevent cross contamination.

2.3 Determination of the rate of MYMV transmission by different numbers of whitefly

For transmission studies, 200 aviruliferous whitefly adults were transferred to virus source plant and allowed to feed up to 24 hour acquisition access period (AAP) and released on 12 day old plants for 24hr inoculation access period (IAP). After completion 24 hour of IAP the insects were killed by foliar spraying of imidachlorprid 17.5% SL @200ml/l. Subsequent spraying was done after one week to kill the nymph, prepupa and pupa, if any, perpetuated from fly to ensure virus transmission by released number of whiteflies only. Rate of transmission by different number of fly was recorded based on symptomatic progression. Test plant was covered with acrylic chimneys to avoid cross contamination if any and placed in screen house for 30 days to observe the development of viral disease symptoms. Same experiment performed for both Asia I and Asia II-1 genotype.

2.4 Determination of acquisition access periods (AAP) and inoculation access periods (IAP)

To determine the AAP aviruliferous population of Asia I and Asia II-1were selected and exposed to different AAP i.e. 10, 15, 30, 60, 90, 120, 240, 360 min by maintaining a constant IAP of 24h. The same way IAP was also determined by keeping the different IAP i.e. i.e. 10, 15, 30, 60, 90, 120, 180 and 240 min and maintaining a constant AAP of 24h. After feeding at different AAP and IAP flies were killed by foliar spraying of imidacholprid 17.8 SL @200ml/l. After one weak repeated spraying is done to kill the nymph, prepupa and pupa, if any, perpetuated. Plants were observed on daily basis for symptom progression.

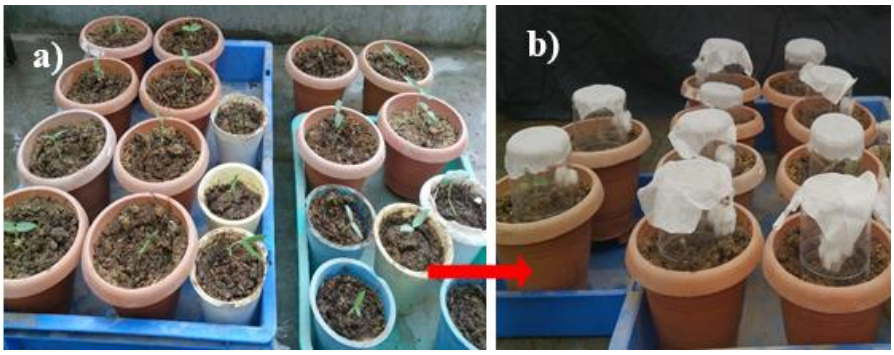

Fig 1: a) Healthy mungbean seedling b) MYMV inoculation to healthy seedling

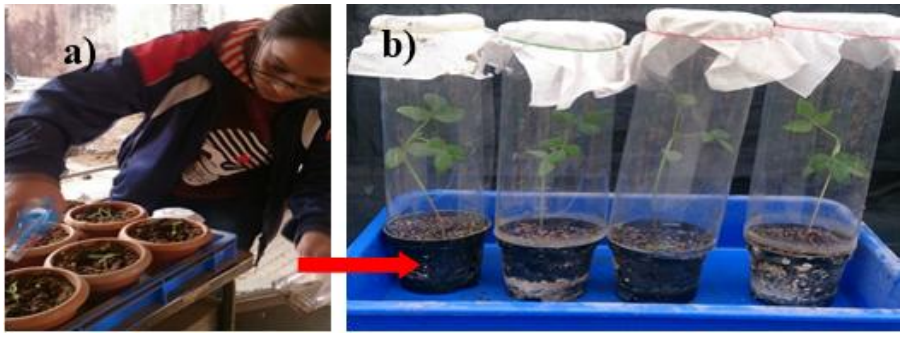

Fig 2: a) Spraying to kill whitefly after defined IAP b) Plant protected in acrylic chimney which was again placed in IPCCC to prevent cross contamination.

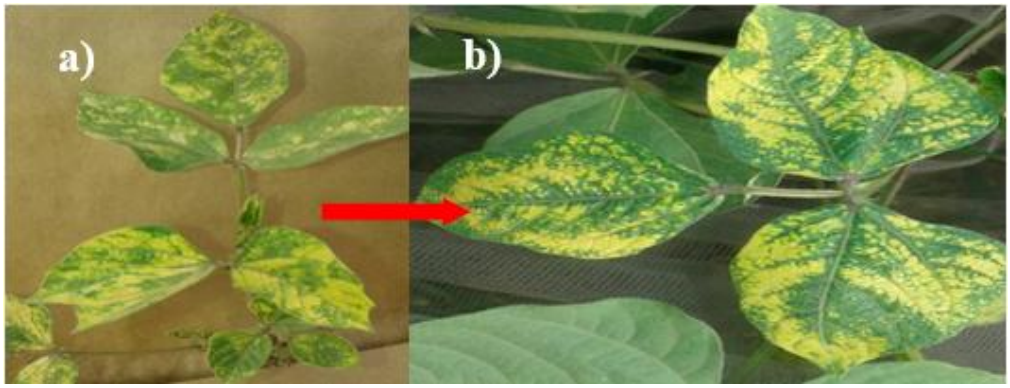

Fig 3: a) and b) Typical mung bean yellow mosaic symptoms at early and late stage of infection respectively

2.5 Data Analysis
Virus transmission parameter in both the genotype were analyzed by Students *t*-test and tukey test.

3. Results
3.1 Determination of the rate of virus transmission by different numbers of whitefly
Asia I has shown significantly higher transmission efficiency 36.6% and 66.6% as compared to Asia II-1 23% and 56% when one and five whiteflies per plant per inoculum were used respectively. To achieve 100 percent transmission efficiency minimum of fifteen whiteflies were required for both the genetic groups (fig-4)

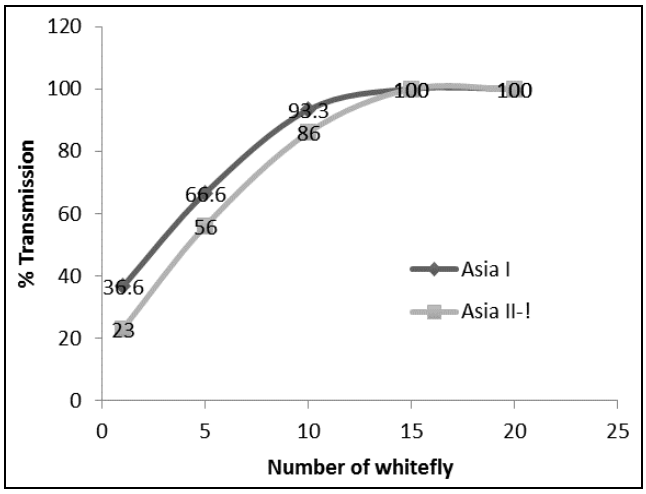

Fig 4: Rate of MYMV transmission by different numbers of whitefly genetic groups.

3.2 Estimation of MYMV percent transmission at different acquisition access period (AAP) by Asia I and Asia II-1 genetic group
Experiments for determination of AAP revealed that both the *B. tabaci* genetic groups required a minimum AAP of 30 minutes to become viruliferous and at this AAP Asia I was found to have significantly higher transmission efficiency *i.e* 15 percent in comparison to Asia II-1 where transmission efficiency was found 11 percent. At 60 min AAP, transmission efficiency was found 35 percent and 30 percent respectively for Asia I and Asia II-1. There was no difference in transmission efficiency was observed between the genotypes when AAP was increased above the 90 min whereas at 240 minute and above the transmission efficiency was obtained 100 percent. Graphical representations of percent transmission given in Fig-5

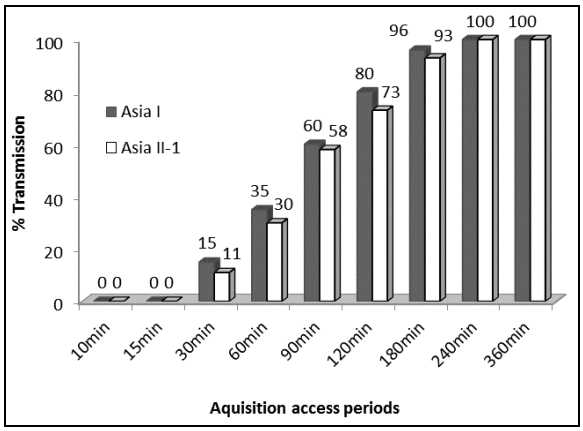

Fig 5: percent transmission of MYMV transmission at different AAP by whitefly genotype Asia I and Asia II-1.

3.3 Estimation of MYMV percent transmission at different inoculation access period (IAP) by Asia I and Asia II-1 genetic group
Experiments for determining IAP revealed that both the *B. tabaci* genetic groups required a minimum IAP of 15 minutes and significant different in transmission efficiency was observed at 15, 30, 60 and 90 minutes where Asia I has shown 16%, 38%, 57% and 70% respectively whereas at same IAP Asia II-1 has shown significantly lower transmission efficiency i.e. 13%, 29%, 33.3% and 66%. Beyond 90 minutes there is no significantly difference was obtained in both the genotype, whereas 100% transmission efficiency was achieved at 180 min IAP. Graphical representations of percent transmission given in Fig-6

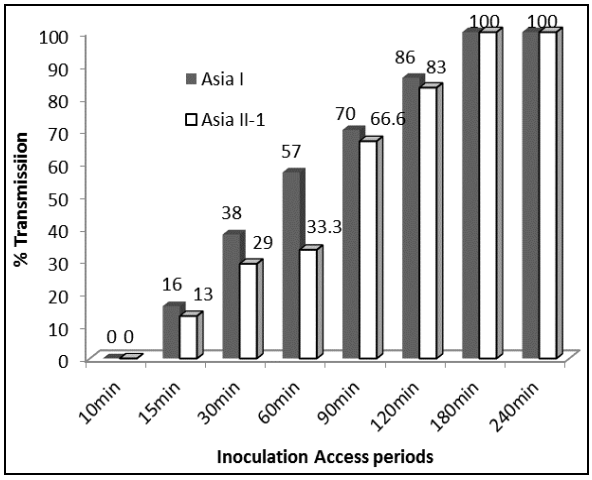

Fig 6: percent transmission of MYMV transmission at different IAP by whitefly genotype Asia I and Asia II-1

4. Discussion

Virus transmission ability of *B. tabaci* in *Pumpkin yellow vein mosaic virus* was studied where healthy pumpkin plant had inoculated with virus through *B. tabaci* and found that even a single viruliferous whitefly was able to cause 21.67 per cent infection, but 100 per cent infection was obtained when 15 whiteflies were used per plant with an acquisition and inoculation threshold periods 6 hr. and 3 hr., respectively [14] whereas in case of *jute leaf mosaic virus* transmission by whitefly *B. tabaci* 20 days old jute plants were inoculated. It was noticed that the minimum acquisition and inoculation feeding period required by whitefly for *Jute leaf mosaic virus* source was 30 and 15 min. which resulted in 12.5 and 10 per cent transmission, respectively. They also found that for 100 per cent transmission, there must be 48 h inoculation access on jute plants. A positive correlation between number of whiteflies and transmission efficiency was observed [8]. It was also reported that 15 whiteflies were required to cause 100 per cent transmission of yellow mosaic virus in lablab. Virus transmission efficiency of whitefly in different cultivar of Mungbean plant for *Mungbean mosaic virus* was also studied and they observed that the transmission capacity of whitefly differed with respect to different cultivars [13]. In another study it was found that five whiteflies caused 100 per cent infection in *squash leaf curl virus* infected squash plants, whereas later it was found that 15 to 20 whiteflies were required to cause effective transmission of yellow mosaic virus diseases of Urdbean and Soybean, respectively [7, 26, 24].

Significant differences in virus transmission efficiencies between B and Q biotypes and within biotype have been documented a decade ago [22, 27]. Populations of the B biotype have shown the great capacity to transmit begomoviruses as compare to 'A 'biotype [5].

The transmission efficiency obtained in this study is comparable with the findings of Senanayake [28] who reported that the transmission efficiency varied between 66%, and 83% whereas days to appear symptoms ranges from 13-18 when one and five whiteflies respectively were used for inoculation per plant respectively. [19] Muniyappa reported that there was 50% and 65% transmission efficiency found when one and five whiteflies are used in case of *Tomato leaf curl banglore virus* (ToLCV-Ban4). It was also found that there was increase in transmission efficiency from 20% to 30% when one and five whitefly was used [25]. 10 per cent transmission efficiency could be achieved by using one whitefly per plant per inoculation; 20, 50 and 90 per cent transmission could be achieved with 2, 5, 10, adults respectively; a minimum 30 min AAP was required to achieve 4 per cent transmission and with 24 h AAP the transmission could be increased up to 85 per cent [17]. From the previous results it was shown that transmission efficiency of indigenous biotypes was only 61 per cent compared to that of 86 per cent recorded in case of B-biotype. It was observed as the when time periods increased, transmission efficiency also increased [5].

We have observed existence of distinct differences in transmission of MYMV between Asia I and Asia II-1 genetic groups of *B. tabaci*. Asia I (*B. tabaci* Guntur population) was found to be more efficient than Asia II-1 (*B. tabci*, New Delhi population) in transmission of the MYMV. They have also shown great variation with respect to different acquisition access periods and inoculation access periods. These variations may probably be attributed to the demographic traits of the virus and the vectors of these locations. However, further studies are required to confirm the virus transmission parameters in these Asian genetic groups by including diverse geographic populations of *B. tabaci* belonging to these genetic groups and to identify the biological characteristics of these Asian genetic groups contributing to differences in transmission of MYMV and other Begomovirues.

5. Conclusion

Our studies have shown that there is an inverse relationship between incubation periods and number of whiteflies used per plant per inoculation. This study has also shown that there is a significant difference between the whitefly genetic groups Asia I and Asia II-1 observered with respect to transmission parameters like percent rate of transmission when different number of whitefly used per plant per inoculation, acquisition access periods (AAP) and inoculation access periods (IAP) while transmission of MYMV virus. So from this study it can be concluded that Asia I has significantly higher *mung bean yellow mosaic virus* transmission efficiency than Asia II-1.

Acknowledgment

This research was supported by Fellowship from PG School of, Indian Agricultural Research Institute, New Delhi. The support by Dr. N. C. Naveen in the conduct of the experiments is greatly acknowledged.

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