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Evaluation Of Endosymbionts In Different Developmental Stages Of Three Population Of Bemisia Tabaci

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Abstract: ***Bemisia tabaci is a most devastating and economically important insect pest; presently considered as a species complex comprising of about 36 morphologically indistinguishable genetic groups. At least 7 species of endosymbiont with different combination have been found infecting all members of the B. tabaci complex. The origin and co-evolution between specific endosymbionts and their whitefly hosts is unknown. The present study aimed for validating the vertical transmission ability of endosymbionts in most dominant Asia II 1 genetic group of B. tabaci in Indian subcontinents. Here we investigate the spatial distribution of Portiera, Cardinium, Arsenophonus, Rickettsia, Wolbachia and Hamiltonella in all life stages of three isofemale strains of Asia II 1 genetic group B. tabaci from Punjab, Rajasthan and Delhi. The result explores that there is no significant variation was found in endosymbionts combination in scanned life stages of Asia II 1 genetic group of B. tabaci.***

I. INTRODUCTION

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodoidea) is the most economically important whitefly species worldwide (De Barro et al., 2011) and reported for a considerable damage to vegetables, flowers, grain legume and cotton production. It has piercing/sucking mouthparts which facilitate feeding on phloem sap that results in two types of damage: direct damage i.e. the result of the extraction of large amounts of sap from host plants and indirect damage by excreting honeydew, inducing host plant phytotoxic disorders, and transmitting more than 120 plant viruses (Boykin et al., 2013). The insect pest is highly polyphagus and feeds on the phloem sap of a variety of crops like cotton, tomato, brinjal, tobacco, etc. As we know that phloem sap is highly rich in carbohydrate and lacks essential amino acids, required for the development of insect. This inadequacy is compensated by the microbial community residing in the insect (Douglas, 1998). These microbial communities not only compensate the inadequacy of nutrient but also have a variety of effect on their host; like increased resistance to parasite (Oliver et al., 2003) increasing the range of temperature tolerance (Montllor et al., 2002) and might also be contributing to the sexual selection of the insect hosts.

It is reported that microbial endosymbionts are widespread in nature and particularly prevalent in arthropods (Bauman, 2005). These endosymbionts have been divided into obligate or primary endosymbionts (P-endosymbionts) and facultative or secondary endosymbionts (S-endosymbionts). Primary endosymbionts are known to be

present in all host individuals and vertically transmitted in the life cycle and provides the essential nutrients to the host insect. Like many sap-feeding insects, B. tabaci also harbors P-endosymbionts, Candidatus *Portiera aleyrodidarum* (Oceanospirillales), which is located in whitefly's bacteriocytes (Baumann, 2005; Thao & Baumann, 2004), and many S-endosymbionts. Out of many S-endosymbionts only six have been identified till date, that includes *Hamiltonella* (Enterobacteriaceae), *Arsenophonus* (Enterobacteriaceae), *Wolbachia* (Rickettsiales), *Rickettsia* (Rickettsiales), *Cardinium* (Bacteroidetes) and *Fritschea* (Chlamydiales) (Everett et al., 2005; Gottlieb et al., 2006; Nirgianaki et al., 2003; Thao et al.,2003; Thao& Baumann, 2004; Weeks et al., 2003; Zchori-Fein & Brown, 2002).

The current study will reveal the co-infection and vertical transmission pattern of secondary endosymbionts in Asia II 1 genetic group of *B. tabaci*. This will be a necessary footstep for understanding the biology and evolution of this pest on the basis of microbial influential partners.

II. MATERIAL AND METHODS

A. SAMPLE COLLECTION

B. tabaci samples used in the present study were collected from farmer's fields of Ludhiana (Punjab), Sriganganagar (Rajasthan) and Indian Agricultural Research Institute (New Delhi). All the samples were allowed to grow for the formation of colony on cotton seedlings in the different chambers of IPCCC (Insect proof climate control chambers), Division of Entomology, I.A.R.I, New Delhi. For establishing the isofemale population of the mentioned three locations, 30 females were allowed to lay eggs in individual leaf cages. The adults which emerges from the eggs laid by the females were pooled to establish the desired populations for the current study. The samples for this study were drawn from the maintained isofemale population of all the three location in the IPCCC. Samples of eggs, first to fourth larval instars and adults were drawn from the lower surface of cotton leaves and examined under Leica EZ4 stereo zoom microscope for the confirmation. The sampling was done by collecting individual sample of all the life stages in the replicates of 10.

B. DNA EXTRACTION

Each replicates containing individual Samples of eggs, first to fourth larval instars and adults were washed twice with the sterile distilled water and the total genomic DNA was isolated from each replicate by using DNASureTissue Mini Kit (Nucleopore, Genetix brand) as per manufacturer's protocol. The isolated Genomic DNA of each replicate was Stored at -20 ^{0}C for further processing.

C. GENETIC IDENTIFICATION OF *B. TABACI* POPULATIONS

Molecular characterization of B. tabaci for identification of the genetic group was performed based on mitochondrial cytochrome oxidase 1 (mtCO1) after PCR reaction using universal primers (Khasdan et al., 2005) (Table 1). PCR was performed with a final volume of 25 µl consisting of Thermo Scientific maxima hot start PCR master mix (12.5 µl), molecular grade water (8.5 µl), forward and reverse primers (10 pmol each 1 µl) and 2 µl of genomic DNA. Samples were amplified using a Ventri® 96- well thermal cycler (Applied Biosystems® Life Technologies). The PCR program for amplification of mtCO1 is given in Table 2. The products (5 µl) were visualized in 1.0% agarose gel containing ethidium bromide under UV illumination after a migration of 45 minute at 80 V. With the expected band size (Table 1) on the gels, the product (20 µl) was used for sequencing.

Databases for sequences were searched using the BLAST algorithm (Altschul et al., 1997; Schäffer et al., 2001) in NCBI Gene Bank (NCBI), and were aligned using BioEdit version 7.2.5 and Clustal W (Thompson et al. 1994). Distance was calculated using the Kimura 2-parameter model of MEGA 6.

Targeted gene	Primer's Sequence (5'-› 3')	Annealing temp. (^{0}c)/ Product size (bp)	Reference
***Portiera* 16S rRNA**	F- CGCCCGCCGCGCCCCGCGCCCGTCCCGCCG CC CCCGCCCG R- CCGTCAATTCMTTTGAGTTT	60/ 550	Muyzer et al., 1996
***Cardinium* 16S rRNA**	F- GCGGTGTAAAATGAGCGTG R- ACCTMTTCTTAACTCAAGCCT	58/ 400	Weeks *et al*., 2003
***Rickettsia* 16S rRNA**	F- GCTCAGAACGAACGCTATC R- GAAGGAAAGCATCTCTGC	60/ 900	Gottlieb *et al*., 2006
***Wolbachia* 16S rRNA**	F- CGGGGGAAAAATTTATTGCT R- AGCTGTAATACAGAAAGTAAA	55/ 700	Heddi et al., 1999
***Hamiltonella* 16S rRNA**	F- TGAGTAAAGTCTGGAATCTGG R- AGTTCAAGACCGCAACCTC	60/ 700	Zchori-Fein & Brown, 2002
***Arsenophonus* 23S rRNA**	F- CGTTTGATGAATTCATAGTCAAA R- GGTCCTCCAGTTAGTGTTACCCAAC	60/ 600	Thao & Baumann, 2004
***B. tabaci* MtCOI**	F- TTGATTTTTTGGTCATCCAGAAGT R- TCCAATGCACTAATCTGCCATATTA	52/ 800	Khasdan *et al*., 2005

Table 1: PCR primers and conditions used in the study

Endosymbionts	Pre-denaturation	Denaturation	Cycling conditions		
			Annealing	Extension	Cycles
Portiera	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	56 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Hamiltonella	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	52 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Wolbachia	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	55 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Arsenophonus	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	56 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Cardinium	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	52 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Rickettsia	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	58 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
***B. tabaci* mtCOI**	94 ^{0}C (1 Min)	94 ^{0}C (1 Min)	55 ^{0}C (1 Min)	72 ^{0}C (1 Min)	35

Table 2: PCR programs used to detect the prevalence of Primary and Secondary endosymbionts in B. tabaci.

D. SCREENING FOR THE PRESENCE OF ENDOSYMBIONTS

To study the bacterial endosymbiont diversity in different life stages of *B. tabaci* the16S and 23S subunit of the rRNA gene was amplified using the genus specific primers (Table-1). The 25µl reaction mix consisted of Thermo scientific maxima hot start PCR master mix (12.5 µl), molecular grade water (8.5 µl), forward and reverse primers (10pmol each 1 µl) and 2 µl of genomic DNA sample. Samples were amplified using a Ventri®96- well thermal cycler (Applied biosystems® by life technologies), and the PCR programs for the primary and secondary endosymbionts are shown in Table 2. A negative control containing no DNA template was also kept with each experiment. The PCR products (5 µl) were electrophoresed in 1.0% agarose gel and visualized using ethidium bromide staining. When bands with the expected size were visible on the gels, the rest 20µl of PCR products were used for

sequencing. The obtained sequences were compared to the available sequences in the database using the BLAST algorithm in NCBI.

III. RESULTS

The mtCO1 sequences of *B. tabaci* populations from Punjab, Rajasthan and Delhi were analyzed and revealed that the studied populations belong to the Asia II 1 genetic group.

The three populations of *B. tabaci*, showed a uniform distribution of endosymbionts in all the life stages. All the scanned life stages of *B. tabaci* from three different locations were uniformly infected with the reported primary (*Portiera aleyrodidarum*) and secondary endosymbionts (*Cardinium, Arsenophonus, Rickettsia, Wolbachia* and *Hamiltonella*). The results revealed that apart from *Hamiltonella* and *Wolbachia* all the endosymbionts were present in the scanned samples of three locations.

We found that all the endosymbionts that were present in the eggs were present up to adults in all the three populations of Punjab, Rajasthan and Delhi. The results of this study are confirming the concept of vertical transmission of endosymbionts in *Bemisia tabaci*.

IV. DISCUSSION

Arthropods have endosymbiotic bacteria for establishing a diversity of symbiotic associations ranging from mutualism to parasitism (Moran, 2007; Moya et al., 2008). These symbiotic bacteria play a significant role by providing crucial nutrients which are lacking in arthropods feeding on imbalanced foods like plant sap (Douglas, 1998). Besides providing nutrients, these endosymbionts have also been recorded to have a variety of effect on their hosts, viz., increased resistance to parasites (Oliver et al., 2003), increasing the range of temperature tolerance (Montllor et al., 2002) and also possibly have a role in the sexual selection of its insect hosts (Gherna et al., 1991).

Bacterial endosymbionts are essential for the survival, spread and evolution of *B. tabaci* (Thao & Baumann, 2004).

Bacterial diversity in *B. tabaci* has been studied by many hands from different part of the world (Chiel et al., 2007; Gueguen et al., 2010; Chu et al., 2011; Bing et al., 2013) but from India very few reports are available (Singh et al., 2012; Roopa et al., 2014). Thus, the present study was undertaken to study the bacterial endosymbiont diversity in different life stages of *B. tabaci* collected from three locations of India *viz.* Punjab, Rajasthan and Delhi.

During the present study, *B. tabaci* life stages from three different locations were collected and were identified as belonging to the Asia II 1 genetic group. A scan for identifying the symbionts diversity in all the life stages of *B. tabaci* from the studied locations showed a uniform distribution of reported endosymbionts. As the results describes that apart from *Hamiltonella* and *Wolbachia*, the samples of three locations from egg to adults were infected with all the endosymbionts. The uniform presence of endosymbionts in all the life stages from eggs to adult strengthens the concept of vertical transmission (Moran and Baumann, 2000). Due to the vertical transmission of endosymbionts from one generation to next generations all the endosymbionts that were present in the parent population were transferred to offspring's and the populations from Punjab, Rajasthan and Delhi becomes homogenous for the diversity of endosymbionts.

V. CONCLUSION

The results of the current study explored and strengthens the concept of vertical transmission and the formation of homogenous population. According to the concept of vertical transmission the endosymbionts are transferred from one generation to next generations. In the current study all the endosymbionts were uniformly distributed in all the life stages of *B. tabaci* collected from three different locations and supporting the concept of vertical transmission.

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