Assessment of bacterial endosymbionts and the host, *Bemisia tabaci* (Hemiptera: Aleyrodoidea), using rRNA and mitochondrial cytochrome oxidase I gene sequences

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ABSTRACT
Endosymbionts are vital factor for arthropod ecology. The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodoidea) is a cryptic species complex composed of more than 34 putative species. Moreover to the primary endosymbiont *Portiera aleyrodidarum*, six secondary endosymbionts *Cardinium*, *Arsenophonus*, *Rickettsia*, *Wolbachia*, *Hamiltonella* and *Fritschea* are known in *B. tabaci*. Here, we tested four of the six secondary endosymbiont lineages (excluding *Fritschea* and *Hamiltonella*) from 180 whitefly individuals collected from six host plants belonging to families Solanaceae (Brinjal, Tomato and Potato) and Fabaceae (Soyabean, Mungbean and Subabool). Phylogenetic studies grounded on the mitochondrial cytochrome I gene revealed the presence of Asia 1, Asia II 1 and Asia II 7 genetic groups for *B. tabaci*. Specific primers targeting 16S rRNA and 23S rRNA gene were used for estimating the bacterial endosymbionts. As a primary endosymbiont *Portiera aleyrodidarum* was present in all the studied samples; whereas, an uneven distribution of secondary endosymbionts were recorded. Overall our finding exposes the variation and diversity of endosymbionts within the *B. tabaci* collected from different host plants and outlines the genetic groups of the insect pest. The study delivers a significant information concerning the circulation of secondary endosymbionts with host preferences of *B. tabaci* and provides suggestion for progressive studies on targeting the specific endosymbionts with respect to host for the control measures.

Introduction

The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodoidea) is a wide-reaching pest and has triggered substantial destruction to legumes, flowers, vegetables, grains and cotton production through direct nourishing, emitting honeydew, bringing host plant phytotoxic disorders, and communicating more than 120 plant viruses. [1,2,3] The taxonomic state of the *B. tabaci* species complex is very debatable. Plentiful revisions on morphological, behavioral and genetic variation have recommended diverse images, such as biotypes and genetic groups, for unlike populations of *B. tabaci*. Though, [4] drawn-out the rank of genetic group variances in the *B. tabaci* complex to species level. The inferences of mating revisions, [5,6,7] backed the proposal for presence of species level variances. *Bemisia tabaci* are morphologically indefinite but express characteristic biological, physiological and genetic variation, and consequently, are well-thought-out as cryptic species complex. [4,8,9,10,11] The genetic group Mediterranean (MED) are extremely unaffected to numerous insecticides, while Middle East Asia Minor 1 (MEAM1) have very extraordinary fecundity [12,13,14]. Hence, these genetic groups are vastly invasive, i.e. they move local populations and establish themselves quickly in a new spot. Such invasive populations have been known from several parts of the biosphere. Consequently, accepting the population structure of *B. tabaci* is critical to govern its blowout and to prevent the types of destruction triggered by the different species. Within 34 putative species1 of *B. tabaci* defined globally, nine have been recognized from India and the distribution outlines of *B. tabaci* specify that extreme range is established in Southern and Eastern India. Miscellany drops nearby the north and north-west, where both Asia II-1 or Asia I genetic groups dominate. Remarkably from Delhi, which harbour Asia II-7 and some areas of Gujarat harbour MEAM1 has been described. The spreading outline of *B. tabaci* may be influenced by numeral elements composed with host plants, geographical location and frequent anthropogenic-derived actions related with trade [15].
Endosymbionts are prevalent in environment, mainly predominant in arthropods and are of two types primary endosymbionts and secondary endosymbionts [16]. Primary endosymbionts are known to exist in all host individuals, vertically communicated and deliver vital nutrients to the hosts. Conversely, the Secondary endosymbionts are facultative and both vertically and horizontally communicated [17,18,19]. Secondary endosymbionts can have abundant effects on host phenotype and can improve host fitness through behavioral or physiological modifications [21,22,23]. The notorious and considered secondary endosymbiont is Wolbachia pipiensis, an Alphaproteobacteria predominant among various classes of arthropods and filarial nematodes [24]. In several insects, most bacteria identified are environmentally-inherited commensals or pathogens which reside the digestive tracts once they are used up by insects. Innumerable bacterial phyla are frequently present in insect guts, with γ-proteobacteria, α proteobacteria, β-proteobacteria, Bacteroidetes, Firmicutes and others [25].

In whitefly Portiera aleyrodidarum is the only described primary endosymbiont, whereas a number of secondary endosymbionts, such as Cardinium, Rickettsia, Arsenophonus, Wolbachia, Hamiltonella and Fritschea are identified. Lately, one additional bacterium Candidatus Hemipterophilus asiaticus in the native B. tabaci cryptic species China 1 has been anticipated. Phylogenetic examination stranded on the 16S rRNA and gltA genes offered that the bacterium goes to the Alphaproteobacteria subdivision of the Proteobacteria and has a close connection with human pathogens of the genus Orientia and named as Orientia-like organism (OLO) [26]. Endosymbionts are obligatory for the survival, blowout and expansion of the B. tabaci [27]. Bacterial variety in B. tabaci considered by countless investigators from diverse part of the world [26,28,29,30] but from India slight figures are available [31,32,33,34] on the dispersal occurrence of secondary endosymbionts of B. tabaci. There are lot of studies available, which reveals the distribution of secondary endosymbionts with respect to their genetic groups and location but there is a lack of evidence on distribution frequency of endosymbionts with respect to the host preferences. Hence, the present study was aimed to define the comparative incidence of known endosymbionts of B. tabaci population from host plants belonging to family Solanaceae (brinjal, tomato and potato) and Fabaceae (soyabean, mungbean and subabool) collected from the fields of Indian Agricultural Research Institute, New Delhi, India. Additionally to finding the circulation frequency of endosymbionts with respect to host, the genetic groups of the collected B. tabaci were also determined.

Table 1. Primers used in PCR detection of endosymbionts and genetic groups.

| Targeted gene | Primer’s Sequence (5’→3’') | Annealing temp. (DC)/ Product size (bp) | Reference |
|---------------|----------------------------|----------------------------------------|-----------|
| Portiera      | F-GCCGGCCGCCGCGCCGCCGCCGCCTGGCCGCCGCCGCCGCCG | 60/550 | 45 |
| 16S rRNA      | R- CGTCAATACTTTAATTGTTT            | 58/400 | 46 |
| Cardinium     | F- GCGGTTAAATAGCGGTG              | 60/900 | 47 |
| 16S rRNA      | R- ACCTTTCTAATCTGGACCT            | 55/700 | 48 |
| Rickettsia    | F- GTCTGAAAGATGACGCTAC             | 60/600 | 27 |
| 16S rRNA      | R- GAAGGAAGACACATCTGC             | 60/500 | 35 |
| Wolbachia     | F- CCGGGAAAAATTTAATGGCT           | 60/600 | 39 |
| 16S rRNA      | R- ACCGTAAATCCAGAAGTAAA           | 55/700 | 30 |
| Arsenophonus  | F- CTGTGTGATCTCATATGC             | 55/700 | 31 |
| 23S rRNA      | R- GTTCCTCGCTGGTGTTCACCAAC        | 55/700 | 32 |
| B. tabaci     | F- TGATTITTTGTGCTCCAGAAT          | 52/800 | 33 |
| MCOI          | R- TCAATGCATATCTGCAATT            | 52/800 | 34 |

Results

Occurrence of endosymbionts

Endosymbionts were recognized by using genus specific primers for the amplification of 16S rRNA gene of Portiera, Rickettsia, Cardinium, Wolbachia, and the 23S rRNA gene of Arsenophonus (Table 1). The occurrence of endosymbiotic bacteria, viz., Portiera, Cardinium, Arsenophonus, Rickettsia and Wolbachia in B. tabaci were perceived with diagnostic PCR in the samples collected from six host plants belonging to family Solanaceae (brinjal, tomato and potato) and Fabaceae (soyabean, mungbean and subabool). The incidence of primary endosymbiont, Portiera was 100% in all the samples, which stipulates the high quality of DNA extractions. [Fig. 1] explain the dispersal frequency of secondary endosymbionts in individual insects from each host. Individuals from certain hosts were found infected with all the described secondary endosymbionts unevenly.

In the studied individuals, the incidence of Portiera was 100% though a wide-ranging distribution frequency of secondary endosymbionts was noticed. Incidence of Cardinium in family Solanaceae was 20% in brinjal, 100% in tomato and 93.33% in potato; whereas, in family
Fabaceae it was 76.67% in soyabean, 96.67% in mungbean and 13.34% in subabool. Incidence of *Rickettsia* in family Solanaceae was 90% in brinjal, 43.33% in tomato and 50% in potato; whereas, in family Fabaceae it was 23.33% in soyabean, 30% in mungbean and 36.66% in subabool. Incidence of *Arsenophonus* in family Solanaceae was 16.66% in brinjal, 56.66% in tomato and 66.67% in potato; whereas, in family Fabaceae it was 60% in soyabean, 63.33% in mungbean and 90% in subabool. Incidence of *Wolbachia* in family Solanaceae was 33.33% in brinjal, absent in tomato and 10% in potato; whereas, in family Fabaceae it was 3.33% in soyabean, 3.33% in mungbean and 16.66% in subabool.

Single factor ANOVA was used to find the disparity of the endosymbiont infection in selected host populations. Among the family Solanaceae and Fabaceae it was noticed that there is no any significant variation found in the distribution frequency of *Arsenophonus* and *Wolbachia*; Whereas, a significant variation was observed in the distribution frequency of *Cardinium* and *Rickettsia* with the p-values as 0.0003 and 0.001 respectively.
Phylogenetic analysis

The mtCOI sequences of *B. tabaci* were studied for the determination of genetic group. Family Solanaceae and Fabaceae were showed the presence of diverse range of genetic groups, as the samples from the families aligned to Asia 1, Asia II 1 and Asia II 7 genetic groups (Fig. 2). Samples from tomato, potato, soyabeen and mungbean were aligned to Asia II-1 genetic group (Fig. 3), samples collected from subabool was aligned to Asia II-7 genetic group (Fig. 4) and the samples from brinjal were settled down with the Asia 1 genetic group (Fig. 5).

Discussion

The *B. tabaci* individuals collected from solanaceous and fabaceous hosts were settled with Asia 1, Asia II 1 and Asia II 7 genetic group after phylogenetic analysis. From the solanaceous hosts brinjal individuals were aligned to Asia 1 genetic group; tomato and potato individuals were aligned to Asia II 1 genetic group. While from fabaceous hosts soyabeen and mungbean individuals were aligned to Asia II 1 genetic group; subabool individuals were aligned to Asia II 7 genetic group. The results are in agreement with the conclusions of Ellango et al. (2015) [15], which states that the Asia II 1 and Asia II 7 are the notable genetic group in Delhi. His interpretation also established that out of 34 putative species only nine species have been recognized from India. They also suggested that maximum variety is present in southern and eastern India.

An investigation of individuals from solanaceous and fabaceous hosts revealed a divergence in the distribution frequency of secondary endosymbionts with host preferences and genetic groups. This study recommends the relation among the symbiotic bacterial populations and the genetic groups of *B. tabaci* as prior demarcated by the researchers [28,29,39]. The consequence found between whitefly

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Figure 2. Phylogram of the evaluated *Bemisia tabaci* samples with well-assigned homologous sequences of the *B. tabaci* genetic groups from the consensus sequence database by means of maximum likelihood (ML) tree method and the kimura 2-parameter distances mitochondrial COI sequences.
Figure 3. Subtree of evaluated *Bemisia tabaci* samples aligning with Asia II 1 genetic group with well-assigned homologous sequences from the consensus sequence database by means of maximum likelihood (ML) tree method and the kimura 2-parameter distances mitochondrial COI sequences.

Figure 4. Subtree of evaluated *Bemisia tabaci* samples aligning with Asia II 7 genetic group with well-assigned homologous sequences from the consensus sequence database by means of maximum likelihood (ML) tree method and the kimura 2-parameter distances mitochondrial COI sequences.
biotypes and secondary symbionts proposes a believable result of these bacteria to host features such as insecticide resistance, host range, virus transmission and speciation [28].

Insects are identified for having endosymbiotic microorganisms producing a diversity of symbiotic relations stretching from mutualism to parasitism [40,41]. The mutualistic link of symbiotic bacteria express a noteworthy appeal by providing crucial nutrients which are absent in arthropods because of nourishing on imbalanced foods such as plant sap [42]. Besides providing nutrients, these bacterial endosymbionts are moreover documented to have a range of effect on their hosts, viz., increasing the range of temperature tolerance [43], enhanced resistance to parasites [21] and possibly have a role in the sexual assortment of its insect hosts [44].

The percentage distribution of Cardinium was higher in individuals belonging to Asia II 1 genetic group. As the results describes the percentage presence of Cardinium in tomato, potato, soyabean and mungbean individuals were 100%, 93.33%, 76.67% and 96.67% respectively. The lowest presence of Cardinium was noted in Asia II 7 genetic group (subabool, 13.34%), followed by Asia 1 genetic group (brinjal, 20%). For Rickettsia percentage presence was higher in Asia 1 genetic group (brinjal, 90%), whereas an average percentage distribution was recorded in Asia II 1 and Asia II 7 genetic groups (Fig. 1). In case of Wolbachia, again the presence was higher in Asia 1 genetic group (brinjal, 33.33%), whereas in Asia II 1 genetic group it was found in least proportion as the potato harbors 10%, soyabean and mungbean harbors 3.33% and interestingly it was totally absent in tomato individuals. The percentage distribution of Wolbachia was 16.66% in Asia II 7 genetic group (subabool). The distribution frequency of Arsenophonus was recorded higher in subabool individuals as 90% belonging to Asia II 7 genetic group. Whereas, the percentage presence of Arsenophonus was least recorded in the brinjal individuals as 16.66% belonging to Asia 1 genetic group. These outcomes are in contract with the earlier reviews by Chiel et al. (2007), Gueguen et al. (2010)

**Table 2.** PCR programs used to detect the prevalence of primary and secondary endosymbionts in *Bemisia tabaci*.

| Endosymbionts | Pre- denaturation | Denaturation | Annealing | Extension | Cycles |
|---------------|------------------|--------------|-----------|-----------|--------|
| Portiera      | 94°C (4 Min)     | 94°C (30 s)  | 56°C (2 Min) | 72°C (2 Min) | 35     |
| Wolbachia     | 94°C (4 Min)     | 94°C (30 s)  | 55°C (2 Min) | 72°C (2 Min) | 35     |
| Arsenophonus  | 94°C (4 Min)     | 94°C (30 s)  | 56°C (2 Min) | 72°C (2 Min) | 35     |
| Cardinium     | 94°C (4 Min)     | 94°C (30 s)  | 52°C (2 Min) | 72°C (2 Min) | 35     |
| Rickettsia    | 94°C (4 Min)     | 94°C (30 s)  | 58°C (2 Min) | 72°C (2 Min) | 35     |
| *B. tabaci*   | 94°C (1 Min)     | 94°C (1 Min) | 55°C (1 Min) | 72°C (1 Min) | 35     |
Table 3. Showing GenBank Accession numbers, along with locality data of mtCOI.

| S.No. | Species       | Genetic group | Location     | GenBank Sequence ID |
|-------|---------------|---------------|--------------|---------------------|
| 1.    | *Bemisia tabaci* | Asia 1        | India: Tamilnadu | GQ281714          |
| 2.    | *Bemisia tabaci* | Asia 1        | China: Guangxi | EU192044           |
| 3.    | *Bemisia tabaci* | Asia 1        | Malaysia: Kuala Lumpur | AY686093       |
| 4.    | *Bemisia tabaci* | Asia 1        | India: Gujarat | GQ281721           |
| 5.    | *Bemisia tabaci* | Asia II       | China: Jiangsu | AY686088           |
| 6.    | *Bemisia tabaci* | Asia II       | China: Guangxi | EU192045           |
| 7.    | *Bemisia tabaci* | Asia II       | China: Zhejiang | DQ309074          |
| 8.    | *Bemisia tabaci* | Asia II       | China: Guangdong | AY686083       |
| 9.    | *Bemisia tabaci* | Asia II       | India: Karnataka | AJ748376         |
| 10.   | *Bemisia tabaci* | Asia II       | India: Karnataka | AF418666         |
| 11.   | *Bemisia tabaci* | Asia II       | Taiwan        | DQ174520           |
| 12.   | *Bemisia tabaci* | Asia II       | China: Guangxi | HM137338           |
| 13.   | *Bemisia tabaci* | Asia II       | China: Yunnan | HQ196813           |
| 14.   | *Bemisia tabaci* | Asia II       | India: Karnataka | AJ748358        |
| 15.   | *Bemisia tabaci* | Asia II       | India: Karnataka | AJ748374        |
| 16.   | *Bemisia tabaci* | Asia II       | India: Gujarat | GQ281733           |
| 17.   | *Bemisia tabaci* | Asia II       | China: Hunan  | HM137313           |
| 18.   | *Bemisia tabaci* | Asia II       | China: Hunan  | HM137345           |
| 19.   | *Bemisia tabaci* | Asia II       | China: Guangdong | HM137356       |
| 20.   | *Bemisia tabaci* | Asia II       | China: Guangdong | HM137339       |
| 21.   | *Bemisia tabaci* | Asia II       | China: Guangdong | HM137326       |
| 22.   | *Bemisia tabaci* | Asia II       | Pakistan: Faisalabad | GUS58369      |
| 23.   | *Bemisia tabaci* | Asia II       | Syria: Hims  | FJ802389           |
| 24.   | *Bemisia tabaci* | Asia II       | Taiwan: Chiayi | DQ174519           |
| 25.   | *Bemisia tabaci* | Asia II       | Pakistan: Punjab | GUS58372       |
| 26.   | *Bemisia tabaci* | Asia II       | China: Guangdong | AY686064       |
| 27.   | *Bemisia tabaci* | Asia II       | Taiwan        | AY686075           |
| 28.   | *Bemisia tabaci* | Asia II       | India: Tamil Nadu | DQ116660     |
| 29.   | *Bemisia tabaci* | Asia II       | Taiwan        | DQ174521           |
| 30.   | *Bemisia tabaci* | Asia II       | China: Guangdong | DQ174523       |
| 31.   | *Bemisia tabaci* | Asia II       | China: Fujian | GQ139492           |
| 32.   | *Bemisia tabaci* | Asia II       | India: Andhra Pradesh | DQ116650     |
| 33.   | *Bemisia tabaci* | Asia II       | India: Tamil Nadu | DQ116661     |
| 34.   | *Bemisia tabaci* | Asia III      | India: Tamil Nadu | AM408899     |
| 35.   | *Bemisia tabaci* | Asia III      | Japan: Okinawa | AB440792           |
| 36.   | *Bemisia tabaci* | Asia III      | Taiwan        | DQ174527           |
| 37.   | *Bemisia tabaci* | Asia III      | Taiwan        | DQ174528           |
| 38.   | *Bemisia tabaci* | Asia III      | Australia     | GU086328           |
| 39.   | *Bemisia tabaci* | Asia III      | Indonesia     | GU086325           |
| 40.   | *Bemisia tabaci* | Asia III      | China          | GUS57045           |
| 41.   | *Bemisia tabaci* | Asia III      | China: Sichuan | HM137315           |
| 42.   | *Bemisia tabaci* | Asia II       | China: Guangdong | AY686072       |
| 43.   | *Bemisia tabaci* | Asia II       | China: Yunnan | HQ196820           |
| 44.   | *Bemisia tabaci* | Asia II       | China: Yunnan | EU192050           |
| 45.   | *Bemisia tabaci* | Indian Ocean  | Madagascar    | AJ550171           |
| 46.   | *Bemisia tabaci* | Indian Ocean  | Reunion       | AJ550179           |
| 47.   | *Bemisia tabaci* | Indian Ocean  | Uganda: Busukuma | AY903537     |
| 48.   | *Bemisia tabaci* | Indian Ocean  | Uganda: Namulong | AY903523     |
| 49.   | *Bemisia tabaci* | Indian Ocean  | Italy          | AY827596           |
| 50.   | *Bemisia tabaci* | Italy         | Italy          | AY827599           |
| 51.   | *Bemisia tabaci* | Italy         | Italy          | AY827602           |
| 52.   | *Bemisia tabaci* | Japan 2       | Japan: Hiroshima | AB240967     |
| 53.   | *Bemisia tabaci* | Japan 2       | Japan: Kumamoto | AB308115      |
| 54.   | *Bemisia tabaci* | Japan 2       | Japan: Okta   | AB308117           |
| 55.   | *Bemisia tabaci* | Japan 2       | Burkina Faso  | FJ766384           |
| 56.   | *Bemisia tabaci* | Japan 2       | Burkina Faso  | FJ766408           |
| 57.   | *Bemisia tabaci* | Mediterranean | Cameroon       | EU760741           |
| 58.   | *Bemisia tabaci* | Mediterranean | China: Yunnan | EU192049           |
| 59.   | *Bemisia tabaci* | Mediterranean | Croatia        | GU086336           |
| 60.   | *Bemisia tabaci* | Mediterranean | Czech Republic | GU086330           |
| 61.   | *Bemisia tabaci* | Mediterranean | Turkey         | AY827619           |
| 62.   | *Bemisia tabaci* | Mediterranean | Uganda         | AY903579           |
| 63.   | *Bemisia tabaci* | Mediterranean | Zimbabwe       | AF344285           |
| 64.   | *Bemisia tabaci* | Middle East Asia Minor 1 | Argentina: Buenos Aires | AF340215        |
| 65.   | *Bemisia tabaci* | Middle East Asia Minor 1 | China: Fujian | AY686062           |
| 66.   | *Bemisia tabaci* | Middle East Asia Minor 1 | Dominican Republic | GU086349       |

(Continued on next page)
Table 3. (Continued)

| S.No. | Species                      | Genetic group               | Location                      | GenBank Sequence ID         |
|-------|------------------------------|-----------------------------|-------------------------------|----------------------------|
| 71.   | *Bemisia tabaci*             | Middle East Asia Minor 1    | Egypt: Ismailia              | GU977249                   |
| 72.   | *Bemisia tabaci*             | Middle East Asia Minor 1    | Guadeloupe: Saint Francois    | AM180064                    |
| 73.   | *Bemisia tabaci*             | Middle East Asia Minor 1    | India: Karnataka              | AJ748368                    |
| 74.   | *Bemisia tabaci*             | Middle East Asia Minor 1    | Pakistan: Sindh               | AJ510081                    |
| 75.   | *Bemisia tabaci*             | Middle East Asia Minor 1    | Pakistan: Sindh               | GU977268                    |
| 76.   | *Bemisia tabaci*             | Middle East Asia Minor 1    | Reunion: Petiteille           | AJ877260                    |
| 77.   | *Bemisia tabaci*             | Middle East Asia Minor 1    | Saudi Arabia                  | GU086357                    |
| 78.   | *Bemisia tabaci*             | Middle East Asia Minor 2    | Reunion                       | AJ550177                    |
| 79.   | *Bemisia tabaci*             | New World                   | Belize                        | DQ130053                    |
| 80.   | *Bemisia tabaci*             | New World                   | Mexico                        | DQ130058                    |
| 81.   | *Bemisia tabaci*             | New World                   | Panama                        | DQ130060                    |
| 82.   | *Bemisia tabaci*             | New World 2                 | Argentina                     | JF901837                    |
| 83.   | *Bemisia tabaci*             | New World 2                 | Argentina                     | JF901844                    |
| 84.   | *Bemisia afer*               |                             | Outgroup                      | GJ220055                    |
| 85.   | *Bemisia afer*               |                             | Outgroup                      | GU086362                    |
| 86.   | *Bemisia atriplex*           |                             | Outgroup                      | HQ457046                    |
| 87.   | *Bemisia berbericola*        |                             | Outgroup                      | GU220056                    |
| 88.   | *Bemisia subdecipiens*       |                             | Outgroup                      | AF418672                    |
| 89.   | *Bemisia tuberculata*        |                             | Outgroup                      | KY775540                    |
| 90.   | *Trialeurodes vaporariorum*  |                             | Outgroup                      | KY775541                    |
| 91.   | *Bemisia tabaci*             | Sub-Saharan Africa 1        | Uganda                        | KY775542                    |
| 92.   | *Bemisia tabaci*             | Sub-Saharan Africa 1        | Uganda: Mbarara               | KY775543                    |
| 93.   | *Bemisia tabaci*             | Sub-Saharan Africa 1        | Uganda: Namulonge             | KY775544                    |
| 94.   | *Bemisia tabaci*             | Sub-Saharan Africa 1        | Uganda                        | KY775545                    |
| 95.   | *Bemisia tabaci*             | Sub-Saharan Africa 2        | Mali                          | KY775546                    |
| 96.   | *Bemisia tabaci*             | Sub-Saharan Africa 2        | Nigeria                       | KY775547                    |
| 97.   | *Bemisia tabaci*             | Sub-Saharan Africa 2        | Spain                         | KY775548                    |
| 98.   | *Bemisia tabaci*             | Sub-Saharan Africa 3        | Cameroon                      | KY775549                    |
| 99.   | *Bemisia tabaci*             | Sub-Saharan Africa 4        | Cameroon                      | KY775550                    |
| 100.  | *Bemisia tabaci*             | Sub-Saharan Africa 4        | Cameroon                      | KY775551                    |
| 101.  | *Bemisia tabaci*             | Sub-Saharan Africa 4        | Cameroon                      | KY775552                    |
| 102.  | *Bemisia tabaci*             | Sub-Saharan Africa 4        | Cameroon                      | KY775553                    |
| 103.  | *Bemisia tabaci*             | Sub-Saharan Africa 4        | Cameroon                      | KY775554                    |
| 104.  | *Bemisia tabaci*             | Sub-Saharan Africa 1        | Uganda: Mbarara               | KY775555                    |
| 105.  | *Bemisia tabaci*             | Sub-Saharan Africa 1        | Uganda: Namulonge             | KY775556                    |
| 106.  | *Bemisia tabaci*             | Sub-Saharan Africa 1        | Uganda                        | KY775557                    |
| 107.  | *Bemisia tabaci*             | Asia II 11                  | India                         | KY775558                    |
| 108.  | *Bemisia tabaci*             | Asia II 11                  | India                         | KY775559                    |
| 109.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775560                    |
| 110.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775561                    |
| 111.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775562                    |
| 112.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775563                    |
| 113.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775564                    |
| 114.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775565                    |
| 115.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775566                    |
| 116.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775567                    |
| 117.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775568                    |
| 118.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775569                    |
| 119.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775570                    |
| 120.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775571                    |
| 121.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775572                    |
| 122.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775573                    |
| 123.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775574                    |
| 124.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775575                    |
| 125.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775576                    |
| 126.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775577                    |
| 127.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775578                    |
| 128.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775579                    |
| 129.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775580                    |
| 130.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775581                    |
| 131.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775582                    |
| 132.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775583                    |
| 133.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775584                    |
| 134.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775585                    |
| 135.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775586                    |
| 136.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775587                    |
| 137.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775588                    |
| 138.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775589                    |
| 139.  | *Bemisia tabaci*             | Asia II                     | India                         | KY775590                    |

*Sequences obtained from the current study.*
and Gnankine et al. (2013) [28,29,39] which enumerated that inside Q genetic group, most Q 1 individual’s harbour Hamiltonella and rarely low frequencies of Wolbachia and Cardinium, while Q 3 individuals harbour commonly Arsenophonus with a high level of co-infection with Rickettsia. In added words, there is an inequality in the distribution of secondary endosymbionts inside varied genetic groups of B. tabaci.

The study is trying to document the distribution incidence the secondary endosymbionts related with host’s preference of B. tabaci in New Delhi, India. The results point out an inevitability for groundbreaking revisions on the host wise occurrence of secondary endosymbionts and its link with different genetic groups of B. tabaci and their impact in the polyphagous nature of this insect pest.

Material and methods

Sample collection and DNA extraction

Bemisia tabaci samples were collected from fields of the Indian Agricultural Research Institute, New Delhi, during 2016 and 2017, and preserved particularly in Eppendorf tubes with absolute alcohol at -20°C until further handling. Sample size of 180 individuals from six hosts of two families were used for the study. Whole genomic DNA of each adult single fly was retrieved by means of DNASure Tissue Mini Kit (Nucleo- pore, Genetix) as per manufacturer’s protocol and kept at -20°C. The extracted DNA was used in the succeeding experiments.

Identification of genetic group

The identification of the genetic group of B. tabaci was concluded aground on mitochondrial cytochrome oxidase I (mtCOI), subsequently a PCR reaction with universal primers [35] (Table 1). The finishing size of the PCR mixture was 25 μl comprising of 12.5 μl of Thermo Scientific maxima hot start PCR master mix, 8.5 μl of molecular grade water, 1 μl each forward primer CI-J-2195 and reverse primer TL2-N-3014 and 2 μl of genomic DNA. Ventri® 96- well thermal cycler (Applied Biosystems® Life Technologies) was used for the extension of the samples. PCR program used for the amplification of mtCOI region is presented in Table 2. The amplified products were determined in 1% agarose gel, stained by ethidium bromide and envisaged in a gel documentation system (DNr, Bio-Imaging systems, MiniLumi). By the estimated band (Table 1) size of the gels, the products were used for sequencing.

Genetic group identification was done by sequence evaluations using the web-based Basic Local Alignment Search Tool algorithm of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The genetic group uniqueness was additionally recognized by the phylogenetic and molecular evolutionary analysis with well-assigned homologous sequences of the B. tabaci genetic groups from the consent sequence databank by MEGA version 6 [9,36]. The mtCOI sequences in FASTA format were bring together into the sequence alignment application of MEGA 6[36] and multiple sequence alignments were attained with the Clustal W[37] algorithm using default parameters. The sequences were submitted to NCBI for GenBank Accessions (Table 2). Sequence divergences among B. tabaci samples were assessed using the Kimura 2-Parameter distance model [38] and graphically revealed in a maximum likelihood (ML) tree by the program MEGA 6 [36]. Tree robustness was assessed by bootstrapping with 2,000 replicates with the Bemisia afer, Bemisia atriplex, Bemisia berbericola, Bemisia subdecipiens, Bemisia tuberculare and Trialeurodes vaporariorum as outgroups.

Screening of endosymbionts

Genus specific primers amplifying the 16S rRNA gene for Portiera, Rickettsia, Wolbachia and Cardinium and the 23S rRNA gene for Arsenophonus (Table 1) were used for the authorization of endosymbionts occurrence in the individuals from each host belonging to different families. The PCR programs for the extension of bacterial endosymbionts are shown in Table 3. The products were envisioned in 1.0% agarose gel comprising ethidium bromide. Through the expected band size (Table 1) on

| S.No. | Gene | Organism | GenBank Accession Number |
|-------|------|----------|--------------------------|
| 1.    | 16S rRNA | Portiera | KX161849 |
| 2.    | 16S rRNA | Portiera | KX161850 |
| 3.    | 16S rRNA | Portiera | KX161851 |
| 4.    | 16S rRNA | Portiera | KX161852 |
| 5.    | 16S rRNA | Portiera | KX161853 |
| 6.    | 16S rRNA | Cardinium | KX197216 |
| 7.    | 16S rRNA | Cardinium | KX197217 |
| 8.    | 16S rRNA | Cardinium | KX197218 |
| 9.    | 16S rRNA | Cardinium | KX197219 |
| 10.   | 16S rRNA | Cardinium | KX197220 |
| 11.   | 16S rRNA | Rickettsia | KX197221 |
| 12.   | 16S rRNA | Rickettsia | KX197222 |
| 13.   | 16S rRNA | Rickettsia | KX197223 |
| 14.   | 16S rRNA | Rickettsia | KX197224 |
| 15.   | 16S rRNA | Rickettsia | KX197225 |
| 16.   | 16S rRNA | Wolbachia | KX197226 |
| 17.   | 16S rRNA | Wolbachia | KX197227 |
| 18.   | 16S rRNA | Wolbachia | KX197228 |
| 19.   | 16S rRNA | Wolbachia | KX197229 |
| 20.   | 16S rRNA | Wolbachia | KX197230 |
| 21.   | 23S rRNA | Arsenophonus | KY630534 |
| 22.   | 23S rRNA | Arsenophonus | KY630535 |
| 23.   | 23S rRNA | Arsenophonus | KY630536 |
| 24.   | 23S rRNA | Arsenophonus | KY630537 |
the gel, products were used for sequencing. The acquired sequences were equated with the sequences on GenBank via BLAST algorithm in NCBI. For each endosymbiont, arbitrarily five samples were sequenced for reference band size and submitted to NCBI for GenBank Accessions (Table 4).

The divergences in comparative number of endosymbionts in B. tabaci were examined by means of one-way analysis of variance (ANOVA). Statistical assessments were completed with SPSS version 16.0.

Conclusion

The present study was concentrated on finding the endosymbiont array associated with B. tabaci on Solanaceae and Fabaceae host plants of New Delhi, India. The consequences originated an agreement that there is a breach in the facts of existence of secondary endosymbionts with respect to the host plants and genetic groups; and suggests an obligation for lenient enhancements on the host wise occurrence of secondary endosymbionts and its stretch with numerous genetic groups. A broad-minded and proportionate survey is obligatory to reveal the confirmations concerning the role of these endosymbionts and the source of uneven passage frequency of these secondary endosymbionts.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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