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Genetic characterisation and associated endosymbionts of insect pest *Bemisia tabaci* nourishing on mungbean

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Abstract
Current study outlines the incidence of bacterial endosymbionts and the genetic group of *Bemisia tabaci* nourishing on mungbean plantation in New Delhi, India. Genetic group analysis based on mitochondrial cytochrome oxidase 1 (mtCO1) gene presented the presence of Asia II 1 genetic group on mungbean plantation. Individuals were examined for symbiotic bacterial infection using specific primers amplifying the 16S rRNA gene for *Rickettsia*, *Cardinium* and *Wolbachia*, and the 23S rRNA gene for *Arsenophonus*. The primary endosymbiont, *Portiera aleyrodidarum* was present in all the examined samples and a disproportion was detected in the incidence frequency of secondary endosymbionts. The figures of unbalanced incidence of secondary endosymbionts and genetic group of *B. tabaci* conveys the elementary data of this notorious pest for progressive studies on the control measures of this insect pest over mungbean plantations.

Keywords: Whitefly, endosymbionts, genetic group, pest, crops

Introduction
India has wide-ranging spectrum of core crops and vegetables, which struggles from numerous insect pests. Amongst these insect pests, whitefly, *Bemisia tabaci* (Gennadius) defined as a pest of tobacco in 1889 [1] is a leading threat worldwide nourishing on vegetables, fruit crops [2] and pulses from 86 botanical families [3]. Adults and nymphs are damaging stages and habitually found hidden underneath of the leaves. Other than sap sucking [4] and excreting honey dew [5], whitefly have a role in transmission of more than 115 types of virus [6] to the commercial crops amongst which 90% belong to *Begomovirus* genus [7]. The taxonomic position of the *B. tabaci* species complex is very debatable. Studies on morphological, behavioural, and genetic variation have directed to diverse designations, such as biotypes and genetic groups, for its different populations. However, Dinsdale *et al*., [8] extended the status of genetic group variances in the *B. tabaci* complex to species level. Plentiful of the accessible data from mating studies [9-11] support this suggestion. However, due to the shortage of such studies from Indian samples, this article refrains from using the term "species" and refers to them as genetic groups. Several populations of *B. tabaci* are morphologically indistinguishable but express typical biological, physiological, and genetic variation, and thus are considered as cryptic species complex [8, 12-15]. The genetic group Mediterranean (MED) are highly resistant to many insecticides whereas Middle East-Asia Minor 1 (MEAM1) has a very high fecundity [16-18]. Hence, these genetic groups are extremely invasive, i.e., they shift to local populations and establish rapidly in a new site. Such aggressive populations have been recognized from many parts of the world. Therefore, understanding the population structure of *B. tabaci* is essential to control its blowout and avert the types of damage caused by its different populations. Out of 34 putative species [15] of *B. tabaci* described worldwide, nine have been documented from India and the dispersal patterns of *B. tabaci* indicate that maximum diversity is found in southern and in eastern India.
Microbial community have and essential role in the pest for compensation of the insufficient and scarce amino acids and nutritional content [19]. The only well-known primary endosymbiont of whitefly is *Portiera aleyrodidarum* [20], while the secondary endosymbionts have several bacteria like *Wolbachia*, *Arsenophonus*, *Cardinium*, *Rickettsia*, *Hamiltonella* and *Fritschea*.

Secondary endosymbionts have been considered to have abundant role on the insects, such as heat tolerance, resistivity to parasitoids, skills of virus transmission, and vulnerable to insecticides. Invasion of *Rickettsia* is specified to have improvement in fitness substantially and female biasness in the host population [21]. The symbionts act as both mutualist and reproductive manipulator for the host insect, with logical positive impact on host population increases as well as the spread of symbiont in the fields.

Present study, lookout the genetic characterization and the incidence frequency of secondary endosymbionts be inherent in *B. tabaci* samples feeding on mung bean plantation in New Delhi, India. The study will bring basic evidences on the dispersal frequency of endosymbionts and genetic group on mung bean plantation and aids as a supportive data for the control measure of this pest over mung bean crops.

Material and Methods

Sampling and DNA Extraction

Samples of *B. tabaci* used in the existing investigation were collected from arenas of Indian Agricultural Research Institute, New Delhi, India. A total of 30 individuals were handled as samples. Separately flies were cleaned twofold with sterile distilled water and total genomic DNA was take out through DNASure Tissue Mini Kit (Nucleo- pore, Genetix) as per manufacturer's protocol. The extracted genomic DNA of each replicate was kept at -20^{0}C.

Identification of *B. tabaci* Genetic Group

The characterisation of the genetic group was based on mitochondrial cytochrome oxidase 1 (mtCO1) after a PCR reaction with universal primers [22] (Table 1). The final volume of the PCR mixture was 25 µl consisting 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 amplification of the samples. A PCR program used for the amplification of mtCO1 region is shown in Table 2. The amplified products were determined in 1% agarose gel, stained by ethidium bromide and visualized in a gel documentation system (DNr, Bio-Imaging systems, MiniLumi). By the expected band (Table 1) size of the gels, the products were used for sequencing.

Genetic group determination was obtained through sequence evaluations using the web-based Basic Local Alignment Search Tool algorithm of NCBI. The genetic group identity was additionally confirmed by the phylogenetic and molecular evolutionary analysis with well-assigned homologous sequences of the *B. tabaci* genetic groups from the consensus sequence database using MEGA version 6 [23]. Sequence divergences between *B. tabaci* samples were calculated using the Kimura 2-Parameter distance model [24] and graphically displayed in a maximum likelihood (ML) tree by the program MEGA 6 [23]. Tree robustness was assessed by bootstrapping with 1,000 replicates with the *Bemisia afer*, *Bemisia atriplex*, *Bemisia berbericola*, *Bemisia subdecipiens*, *Bemisia tuberculate* and *Trialeurodes vaporariorum as* outgroups.

Screening of Endosymbionts

Genus specific primers amplifying the 16S rRNA gene for *Portiera*, *Rickettsia*, *Cardinium* and *Wolbachia*, and the 23S rRNA gene for *Arsenophonus* (Table 1) were used for the detection of endosymbionts infection frequency in the individuals. The PCR programs used for the amplification of bacterial endosymbionts are shown in Table 2. The products were visualized in 1.0% agarose gel comprising ethidium bromide and visualized in a gel documentation system. With the expected band size (Table 1) on the gel, products were used for sequencing. The obtained sequences were compared with the sequences on Gene Bank using the BLAST algorithm in NCBI.

Table 1: Oligonucleotide primers used in PCR detection of endosymbionts and genetic group**.**

Targeted gene	Primer's Sequence (5'-› 3')	Annealing temp. (0C)/ Product size (bp)	Reference
Portiera 16S rRNA	F-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG R- CCGTCAATTCMTTTGAGTTT	60/ 550	[35]
Cardinium 16S rRNA	F- GCGGTGTAAAATGAGCGTG R- ACCTMTTCTTAACTCAAGCCT	58/ 400	[36]
Rickettsia 16S rRNA	F- GCTCAGAACGAACGCTATC R- GAAGGAAAGCATCTCTGC	60/ 900	[37]
Wolbachia 16S rRNA	F- CGGGGGAAAAATTTATTGCT R- AGCTGTAATACAGAAAGTAAA	55/ 700	[38]
Arsenophonus 23S rRNA	F- CGTTTGATGAATTCATAGTCAAA R- GGTCCTCCAGTTAGTGTTACCCAAC	60/ 600	[28]
B. tabaci MtCOI	F- TTGATTTTTTGGTCATCCAGAAGT R- TCCAATGCACTAATCTGCCATATTA	52/ 800	[22]

Table 2: PCR programs for the detection of prevalence of Primary and Secondary endosymbionts in *B. tabaci*.

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	94 ^{0}C (1 Min)	94 ^{0}C (1 Min)	55 ^{0}C (1 Min)	72 ^{0}C (1 Min)	35

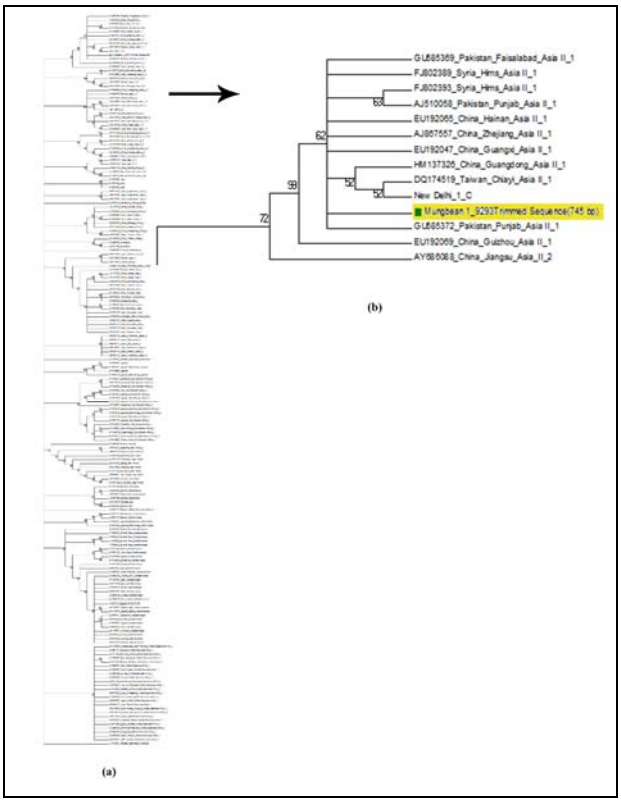

Fig 1: (a) Phylogram of the analyzed *Bemisia tabaci* samples with well-assigned homologous sequences of the *B. tabaci* genetic groups from the consensus sequence database by using maximum likelihood (ML) tree method and the kimura 2-parameter distances mitochondrial COI sequences. (b) Magnified tree.

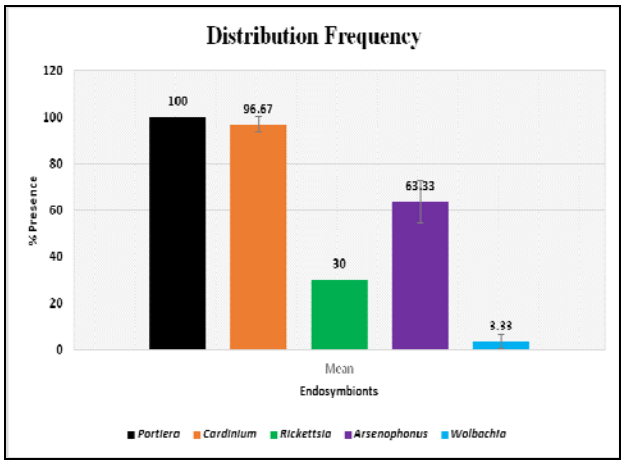

Fig 2: Dispersal frequency of endosymbionts of *B. tabaci* on mung bean plantation

Results

The sequence of *B. tabaci* collected from mungbean plantation was evaluated with the reference sequences from NCBI and the phylogenetic analysis settles, the specimens of mungbean to Asia II 1 genetic group (Fig. 1).

The fallouts of the current study describe the incidence frequency of seven known endosymbionts in the mung bean plantation and discovered a miscellaneous spreading array. All the individuals were positive with the invasion of *Portiera* (Primary endosymbiont) that correspondingly measured as the positive control for the class extraction of DNA. Figure 2 is the graphical display of the dispersal frequency of secondary endosymbionts in the examined *B. tabaci* from mung bean plantation. Apart from *Fritschea* and *Hamiltonella*, individuals were found infested with rest known secondary endosymbionts unevenly. The mung bean collected populations were found infested with *Arsenophonus* (63.33%), *Rickettsia* (30%), *Wolbachia* (3.33%) and *Cardinium* (96.66%).

Discussion

In the current study, samples were collected from the fields of the Indian Agricultural Research Institute, New Delhi. This is an elementary report on the genetic groups and their associated endosymbiotic microbiota examined on *B. tabaci* collected from one of the important crop plant mung bean belongs to family Fabaceae. The inspection enlightenment reveals that the specimens from mung bean belongs to most prevalent genetic group in the region i.e. Asia II 1 genetic group. The study chains with the previous conclusions that the range of genetic group in north and north-west India is restricted to Asia II 1, and Asia I with the exceptional presence of Asia II 7 in Delhi and the occurrence of MEAM1 in some pockets of Gujrat [25-27].

For the survival, passage and evolution of *B. tabaci*, the bacterial endosymbionts demonstrate a significant role [28]. The study intended on the accompanying endosymbionts of *B. tabaci* has been done by many of researchers around the globe [29-32] but a very inadequate work from India has been reported [25-27, 33, 34]. Consequently, this study was carried out to give some extension in the indication of associated endosymbionts of *B. tabaci* feeding on a mung bean plantation in New Delhi, India.

The current study was highlighted in the direction of recording of the endosymbiont range associated with *B. tabaci* on mung bean plantation in New Delhi, India. The consequences instruct, there is a lacuna present in the evidence of dispersal of secondary endosymbionts with respect to the host plants and genetic groups; and recommends an obligation for broadminded assessments on the host wise frequency circulation of secondary endosymbionts and its term with several genetic groups.

Disclosure

The authors have no conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject of this manuscript.

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