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Endosymbionts and Genetic Characterisation of whitefly on Soyabean

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ABSTRACT
Soybean (Glycine max L.) is an adaptable and captivating crop with countless potentials of not only enlightening agriculture but also assisting industries. It is an amusing source of lysine (6.4%) in count to further crucial amino acids, vitamins. Alike other economically significant crops soybean is vulnerable to diverse diseases caused by viruses. Whitefly Bemisia tabaci is one of the most important pest that is involved in the transmission of several plant viruses and described for damaging the numerous crops. Investigation was continued to record the distribution frequency of associated bacterial endosymbionts and the genetic group of Bemisia tabaci feeding on Soyabean, collected from Indian Agricultural Research Institute, New Delhi, India. Samples of Bemisia tabaci were scrutinized grounded on mtCOI sequences and settle down along with Asia II 1 genetic group. Distribution frequency of seven known endosymbionts were documented. The primary endosymbiont, Portiera aleyrodidarum was noted in all the scanned samples and a disproportion was noted in the distribution of secondary endosymbionts. The figures of irregular dispersal of secondary endosymbionts and the genetic group of B. tabaci delivers the elementary data of this notorious pest for advance studies on the control measures of this insect pest over Soyabean.
Keywords: *Whitefly, genetic group, crops, endosymbionts, distribution frequency.*

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INTRODUCTION

Soybean (*Glycine max* L.) also called as "Golden Bean" is a native of North China, Asia belongs to family Fabaceae. It is a rich source of lysine (6.4%) in addition to other essential amino acids, vitamins. India has widespread spectrum of core crops and vegetables including Soyabean that struggles from numerous insect pests. Amongst these insect pests, whitefly, *Bemisia tabaci* (Gennadius) demarcated as pest of tobacco in 1889 [1] is a foremost hazard worldwide nourishing on vegetables, fruit crops and pulses from 86 botanical families. Adults and nymphs are destructive stages and usually found resting underside of the leaves. Other than sap sucking and excreting honey dew, whitefly have a role in transmission of more than 115 types of virus to the commercial crops amongst which 90% belong to *Begomovirus* genus [2].

Microbial community have and essential role in the pest for compensation of the insufficient and scarce amino acids and nutritional content [3]. The only well-known primary endosymbiont of whitefly is *Portiera aleyrodidarum*, 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, skill 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 [4]. 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 fields.

Present study, allocate the genetic group and the distribution frequency of endosymbionts residing in *B. tabaci* samples feeding on Soyabean plantation in New Delhi, India. Study will convey basic notions on the distribution frequency of endosymbionts and genetic group in this region on Soyabean plantation and aids as a supportive data for the control measure of this pest over Soyabean 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. 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

Molecular clarification of *B. tabaci* for consent of the genetic group was driven based on mitochondrial cytochrome oxidase I (mtCOI) sequences after PCR reaction with universal primers (Table 1). The products were inspected in 1.0% agarose gel containing ethidium bromide under UV illumination after a passage of 45 minute at 80 V. Through the predicted band size (Table 1) on the gels, the products (20 µl) were sent for outsource sequencing. Sequences were explored using the BLAST algorithm in NCBI Gene Bank, and were aligned using BioEdit version 7.2.5. Distance was calculated using the Kimura 2-parameter model of MEGA 6.

Screening of Endosymbionts

All the samples were checked separately for the occurrence of endosymbiotic bacteria by means of specific primers amplifying the 16S rRNA gene for *Portiera*, *Cardinium*, *Rickettsia*, *Wolbachia* and *Hamiltonella*, and the 23S rRNA gene for *Arsenophonus* and *Fritschea* (Table 1). PCR reaction mixture's final volume of 25µl, embraces of 12.5 µl Thermo Scientific maxima hot start PCR master mix, 8.5 µl molecular grade water, 1 µl of each forward and reverse primers and 2 µl genomic DNA. The products were visualized in 1.0% agarose gel containing ethidium bromide under UV illumination after a migration of 45 minute at 80 V. With the anticipated band size on the gels, the products were used for outsource sequencing (Table 1). The gained sequences were allied to the available sequences in the databank using BLAST algorithm in NCBI.

RESULTS

The population of *B. tabaci* collected from Soyabean plantation were compared with the reference sequences from NCBI and the phylogenetic analysis settles them to Asia II 1 genetic group (Fig. 1).

The upshots of the study apprise the distribution frequency of 7 known endosymbionts in the Soyabean 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. The distribution frequency of secondary endosymbionts in the studied *B. tabaci* from Soyabean plantation is obtainable in figure 2. Apart from *Fritschea* and *Hamiltonella*, individuals were found infested with rest known secondary endosymbionts irregularly. The populations from Soyabean were found infested with *Cardinium* (76.66%), *Arsenophonus* (60%), *Rickettsia* (23.33%) and *Wolbachia* (3.33%).

Table I. 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	[13]
Cardinium 16S rRNA	F- GCGGTGTAAAATGAGCGTG R- ACCTMTTCTTAACTCAAGCCT	58/ 400	[14]
Rickettsia 16S rRNA	F- GCTCAGAACGAACGCTATC R- GAAGGAAAGCATCTCTGC	60/ 900	[15]
Hamiltonella 16S rRNA	F- TGAGTAAAGTCTGGAATCTGG R- AGTTCAAGACCGCAACCTC	60/700	[16]
Wolbachia 16S rRNA	F- CGGGGGAAAAATTTATTGCT R- AGCTGTAATACAGAAAGTAAA	55/ 700	[17]
Fritschea 23S rRNA	F- TGGTCCAATAAGTGATGAAGAAAC R- GCTCGCGTACCACTTTAAATGGCG	60/ 600	[18]
Arsenophonus 23S rRNA	F- CGTTTGATGAATTCATAGTCAAA R- GGTCCTCCAGTTAGTGTTACCCAAC	60/ 600	[8]
B. tabaci MtCOI	F- TTGATTTTTTGGTCATCCAGAAGT R- TCCAATGCACTAATCTGCCATATTA	52/ 800	[19]

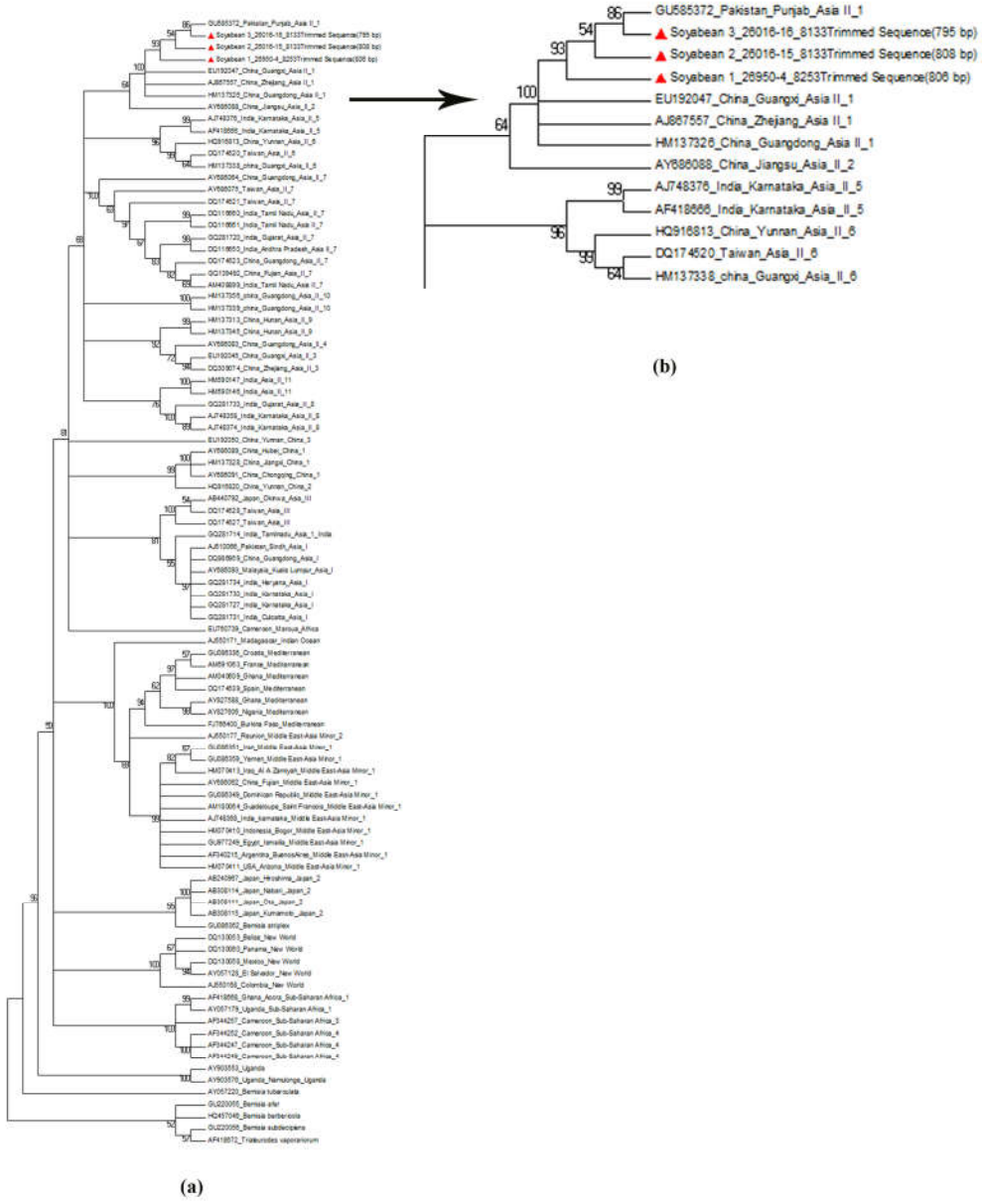

Figure 1. (a) Representing the phylogenetic status of *B. tabaci* collected from mung bean plantation; **(b)** Magnified tree.

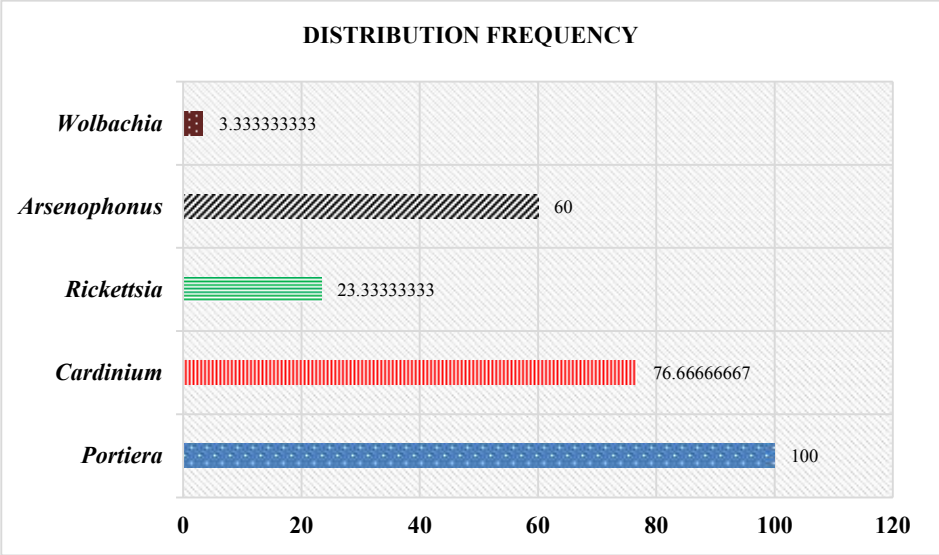

Figure 2. Dispersal frequency of endosymbionts of *B. tabaci* on mung bean plantation.

DISCUSSION

In the current study, samples were collected from grounds of 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 Soyabean belongs to family Fabaceae. The inspection enlightenment reveals that the specimens from Soyabean belongs to most prevalent genetic group in the region i.e. Asia II 1 genetic group. The study showed a relevance 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 exceptional presence of Asia II 7 in Delhi and the occurrence of MEAM1 in some pockets of Gujrat [5], [6], [7].

For the survival and evolution of *B. tabaci*, the bacterial endosymbionts demonstrate a significant role ([8]. The study intended on the accompanying endosymbionts of *B. tabaci* has been done by many of researchers around the globe [9], [10] but a very inadequate work from India has been reported [5], [6], [7], [11], [12]. Consequently, this study was carried out to give some extension in the indication of associated endosymbionts of *B. tabaci* feeding on Soyabean plantation in New Delhi, India.

The study discloses the percentage dispersal frequency of secondary endosymbionts in the flies feeding on Soyabean. Results disclosed the 100% presence of primary endosymbionts, *Portiera* and a variation was spotted in the percentage distribution of secondary endosymbionts. The flies feeding on Soyabean belongs to Asia II 1 genetic group and it harbors *Arsenophonus* (60%), *Rickettsia* (23.33%), *Wolbachia* (3.33%) and *Cardinium* (76.66%), chains the finding previous reported on the host belongs to family Solanaceae and cotton [6], [7], [11].

The current study was highlighted in the direction of recording of the endosymbiont range associated with *B. tabaci* on Soyabean plantation in New Delhi, India. The consequences specify, 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. For working on the control measure of this devastating insect pest A substantial and comparative investigation is required to disclose the facts regarding the role of these endosymbionts and the origin of uneven circulation.

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