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Records on Genetic Group of *Bemisia tabaci* (Gennadius) and Associated Endosymbionts Feeding on Potato Plants

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Abstract Potato (*Solanum tuberosum* L.) is one of the most significant food crops after wheat, maize and rice, contributing to feed and nutritional security in the world. A Study was conducted on distribution frequency of endosymbionts and genetic group of Bemisia tabasi on potato plants, collected from Central Potato Research Institute, Modipuram, India. The *B. tabaci* samples were investigated and settle along with Asia I population. The primary endosymbiont, Portiera aleyrodidarum was present in all the scanned samples and a variation was noted in the scattering frequency of secondary endosymbionts. The figures of irregular distribution of secondary endosymbionts and the genetic group of *B. tabaci* provides the basic information of this notorious pest and used for the study on the control measures of this insect pest over potato production.

Keywords *Bemisia tabaci*, Genetic group, Solanaceae, Endosymbionts.

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Introduction

Potato belongs to family Solanaceae, known as the world's fourth most significant food crop after rice, wheat and maize. India harvests 7.72% of the world's potatoes from 7.57% of the entire global potato-growing area, with through put levels higher than the world's average [1]. Potato crops are vegetatively propagated from tubers, that facilitate to carry pathogens and pests, due to which several pest complications have trailed potatoes to areas where they are grown [2]. The pests involved in damaging the potato plants by feeding on their leaves, reducing photosynthetic area and efficiency by attacking stems, weakening plants and inhibiting nutrient transport and by attacking potato tubers destined for consumption or use as seed [3].

Bemisia tabaci (Gennadius) is a wide-reaching economically vital insect pest involved in triggering significant crop damage [4]. It is a polyphagous insect that feeds on around 900 plants from vegetable, ornamental, agriculture and horticultural crops [4]. *Bemisia tabaci* is dispersed throughout the Northern and Western regions of the Indian subcontinent and has freshly arisen as a very serious pest in potato seed production, predominantly in the autumn crop in the Indo Gangetic plains [5]. Invasion is substantial on early potato crops planted in September and the extreme population on potato befalls in November, followed by a sharp decline by December [6].

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

Targeted gene	Primer's Sequence (5′→ 3′)	Annealing temp. (℃)/ Product size (bp)	Reference
Portiera 16S rRNA	F-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG R-CCGTCAATTCMTTTGAGTTT	60/550	29
Cardinium 16S rRNA	F-GCGGTGTAAAATGAGCGTG R-ACCTMTTCTTAACTCAAGCCT	58/400	10
Rickettsia 16S rRNA	F-GCTCAGAACGAACGCTATC R-GAAGGAAAGCATCTCTGC	60/900	11
Hamiltonella 16S rRNA	F-TGAGTAAAGTCTGGAATCTGG R-AGTTCAAGACCGCAACCTC	60/700	8
Wolbachia 16SrRNA	F-CGGGGGAAAAATTTATTGCT R-AGCTGTAATACAGAAAGTAAA	55/700	30
Fritschea 23S rRNA	F-TGGTCCAATAAGTGATGAAGAAAC R-GCTCGCGTACCACTTTAAATGGCG	60/600	31
Arsenophonus 23S rRNA	F-CGTTTGATGAATTCATAGTCAAA R-GGTCCTCCAGTTAGTGTTACCCAAC	60/600	9
B. tabaci MtCOI	F-TTGATTTTTTGGTCATCCAGAAGT R-TCCAATGCACTAATCTGCCATATTA	52/800	32

This pest is a phloem feeder, that is highly rich carbohydrate content and lacks essential amino acids needed for the growth of pest. Microbial community residing in the pest compensate the inadequacy of the deficient amino acids and nutritional content. *Portiera aleyrodidarum* is described as the only primary endosymbiont of whitefty [7], whereas the secondary endosymbionts have a figure of bacteria like *Wolbachia* (Rickettsiales) [8], *Arsenophonus* (Enterobacteriales) [9], *Cardinium* (Bacteroidetes) [10], *Rickettsia* (Rickettsiales) [11], Hamiltonella (Enterobacteriales) [8] and *Fritschea* (Chlamydiales) [12]. Secondary endosymbionts have been described to have numerous impression on the insects, such as heat tolerance [13], resistivity to parasitoids [14], ability of virus transmission [15] and susceptible to insecticides [16, 17]. Infection of *rickettsia* is stated to have upsurge fitness substantially and female bias in the host population [18].

Current study, will explore the genetic group and the distribution frequency of endosymbionts residing in *B. tabaci* samples feeding on the potato plants from Modipuram, India. Study will provide elementary evidence on the endosymbionts and genetic group in this region on potato and helps as a supportive figure for the control measure of this pest over potato crops.

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

Endosymbionts	Cycling conditions				
	Pre-Denaturation	Denaturation	Annealing	Extension	Cycles
Portiera	94°C (4 Min)	94°C (30 s)	56°C (2 Min)	72°C (2 Min)	35
Hamiltonella	94°C (4 Min)	94°C (30 s)	52°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 mtCOI	94°C (1 Min)	94°C (1 Min)	55°C (1 Min)	72°C (1 Min)	35

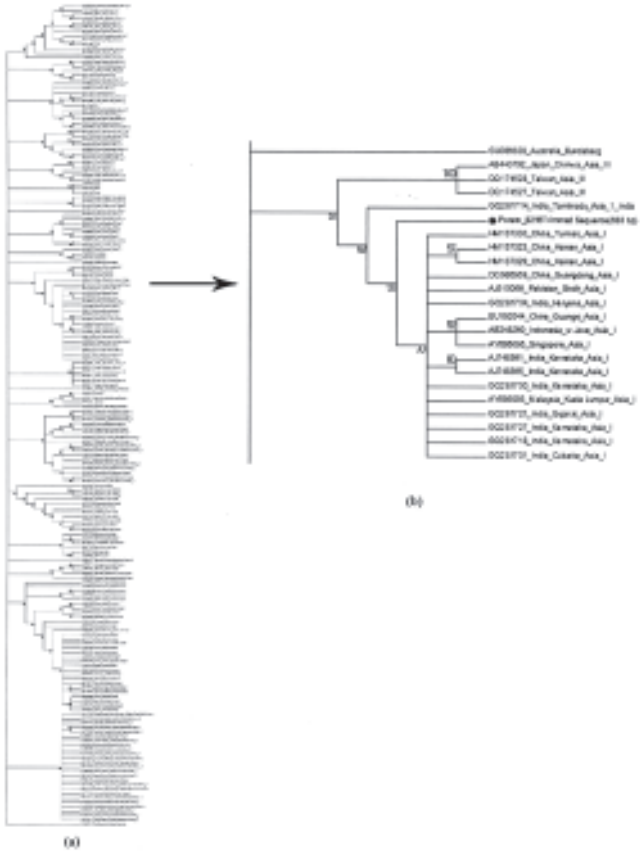

Fig. 1. (a) Representing the phylogenetic status of *B. tabaci* collected from potato plants ; (b) Mahnified tree.

Materials and Methods

Sampling and DNA extraction

Bemisia tabaci samples used in the current investigation were collected from arenas of Central Potato Research Institute, Modipuram, U. P, India. Total of 60 individuals were handled as samples. DNA was extracted through DNA Sure Tissue Mini Kit (Nucleopore, Genetix) as per manufacturer's protocol. DNA of each replicate was kept at-20°C for further handling.

Identification of *B. tabaci* genetic group

Molecular interpretation of *B. tabaci* for accreditation of the genetic group was prompted based on mitochondrial cytochrome oxidase 1 sequences after PCR reaction with universal primers (Table 1). The PCR program for the reaction is specified in Table 2. The products were scrutinized 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 used for sequencing. Records for sequences were explored using the BLAST algorithm in NCBI Gene Bank and were aligned using Bio Edit version 7.2.5. Distance was calculated using the Kimura 2-parameter model of MEGA 6.

Screening of endosymbionts

All the samples were scanned independently for the occurrence of endosymbiotic bacteria using specific

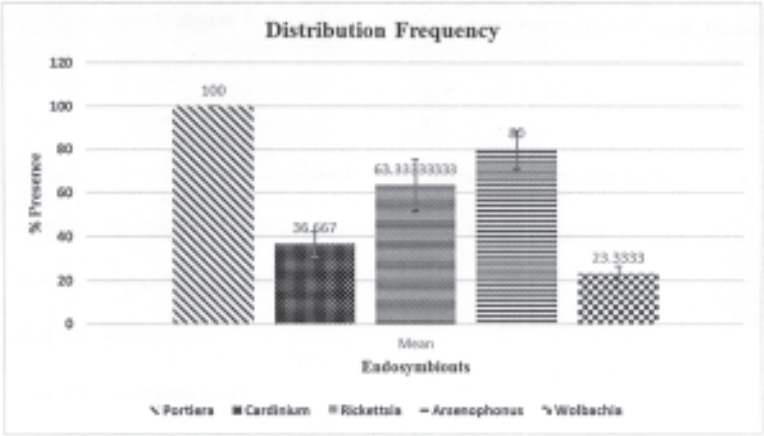

Fig. 2. Circulation frequency of endosymbionts of *B. tabaci* on potato plants.

primers amplifying the 16 S r RNA and the 23 S r RNA gene (Table 1). The existence Portiera was checked to favor the quality of DNA extraction. PCR reaction mixture's finishing volume of 25µl, includes of 12.5µl. Thermo Scientific maxima hot start PCR master mix, 8.5µl molecular grade water, 1µl of each forward and reverse primers and 2 µl genomic DNA. The PCR program for the endosymbionts is listed in Table 2. With the expected band size on the gels, the products were used for sequencing (Table 1). The obtained sequences were linked to the available sequences in the data bank using BLAST algorithm in NCBI.

Results and Discussion

The population of *B. tabaci* collected from potato plants from Modipuram was analyzed with the reference sequences from NCBI and the phylogenetic analysis confirms, the specimens from potato belongs to Asia I genetic group (Fig. 1). The study ropes with the previous finding that the diversity of genetic group in North and North-West India is constrained to Asia II 1, and Asia I with exceptional incidence of Asia II 7 in Delhi and the occurrence of MEAMI in some pockets of Gujrat [19—21].

The consequences of the present study update the circulation incidence of seven known endosymbionts in the studied flies from potato and exposed a miscellaneous spreading array. All the individuals were found positive with the infestation of *Portiera* (Primary endosymbiont) that also measured as the positive control for the class extraction of DNA. Figure 2 is the graphical demonstration of the spreading frequency of secondary endosymbionts in the studied host of *B. tabaci*. Excluding *Fritschea* and *Hamiltonella*, individuals were found infested with rest known secondary endosymbionts disproportionately. The individuals from potato were found infected with *Rickettsia* (63.333%), *Cardinium* (36.667%), *Arsenophonus* (80%) and *Wolbachia* (23.33%).

For the endurance, flow and progression of *B. tabaci*, the bacterial endosymbionts showed a substantial role [9]. The investigation intended on the linked endosymbionts of *B. tabaci* has been done by numerous of investigators around the globe [22—25] but a very limited effort from India has been described [20, 21, 26, 27]. Consequently, this study was supported out to stretch some extension in the evidence of accompanying endosymbionts of *B. tabaci* feeding on potato plants from Modipuram, India.

This study discloses the percentage circulation frequency of secondary endosymbionts in the flies

feeding on points plants. Fallouts exposed the 100% presence of primary endosymbionts, *Portiera* and a contrast was noticed in the percentage circulation of secondary endosymbionts on the studied host plant. Output of the study have a resemblance with the work earlier done on the host belongs to same family *Solanaceae* and [20, 21, 26]. Accordingly, this results authorizes the connotation between the endosymbiotic bacterial groups and the genetic groups of *B. tabaci* and have an agreement with earlier works [22, 23, 28].

The present study was highlighted in the track of recording of the endosymbiont range associated with *B. tabaci* on potato plants. The outcomes agree that there is a lacuna present in the data of spreading of secondary endosymbionts with respect to the host plants and genetic groups ; and proposes an obligation for progressive revisions on circulation of secondary endosymbionts and its term with several genetic groups of *B. tabaci*. A forward-thinking and relative inspection is required to disclose the facts regarding the role of these endosymbionts and the origin of uneven circulation frequency of these secondary endosymbionts for working on the control measure of this devastating insect pest.

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