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Distribution of *Bemisia tabaci* Genetic Groups in India

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ABSTRACT The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a phloem–feeding, economically important pest of crops worldwide. In addition to direct damage, it also vectors a number of plant viruses belonging to the family Geminiviridae. Its populations differ biologically with respect to insecticide resistance, virus transmission and host range. Therefore, understanding genetic variation among populations is important for management. We sequenced 850 bp of the mitochondrial *COI* (*mtCOI*) gene from *B. tabaci* populations surveyed across India. BLAST analysis of the *mtCOI* sequences generated in this study with sequences from the *mtCOI* dataset showed the presence of one invasive group, MEAM1, and eight other groups of *B. tabaci* in India. *mtCOI* sequence analyses showed the presence of Asia I, Asia I-India, Asia II-1, Asia II-5, Asia II-7, Asia II-8, and Asia II-11 genetic groups. We also found China-3 in a field in Birbhum district, West Bengal, India, suggesting a role of anthropogenic activities in the distribution of *B. tabaci*. Interestingly, more than one genetic group was found coexisting in the same field.

KEY WORDS *mtCOI*; *Bemisia tabaci*; genetic group

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a highly polyphagous pest affecting the world's agricultural economy. It reduces plant vigor by directly feeding on the phloem and also by transmitting Geminiviruses (Bedford 1994, Jones 2003, Briddon 2003, Naveed et al. 2007). It vectors >100 plant viruses and is the sole vector of begomoviruses (Czosnek et al. 2001).

The taxonomic status of the *B. tabaci* species complex is highly debatable. Studies on morphological, behavioral, and genetic variation have led to alternative designations, such as biotypes and genetic groups, for its different populations. However, Dinsdale et al. (2010) raised the status of genetic group differences in the *B. tabaci* complex to species level. Much of the available data from mating studies (Xu et al. 2010, Elbaz et al. 2010, Wang et al. 2010) support this proposition. However, due to the lack of such analyses from Indian samples, in this paper, we refrain from using the term "species'" and refer to them instead as genetic groups.

Different populations of *B. tabaci* are morphologically indistinguishable but display distinctive biological, physiological, and genetic variation, and thus are deemed a cryptic species complex (Boykin et al. 2007, Dinsdale et al. 2010, De Barro et al. 2011, Boykin et al. 2012, Tay et al. 2012). *B. tabaci* belonging to genetic group MED are highly resistant to many insecticides. Individuals of genetic group MEAM1 have very high fecundity (Pascual and Callejas 2004, Horowitz et al. 2005, Dalton 2006). Hence, *B. tabaci* belonging to these populations are highly invasive, i.e., they displace local populations and establish quickly in a new location. Such invasive populations have been recorded from many parts of the world. Thus, understanding the population structure of *B. tabaci* is required to control its spread and prevent the types of damage caused by its different populations.

Various molecular methods have been used to distinguish *B. tabaci* populations. However, the real insight into genetic variability within this insect was achieved using *mtCOI* and *ITS1* marker genes. Boykin et al. (2007) clustered the world *B. tabaci* populations into 12 major genetic groups by applying Bayesian phylogeny to *mtCOI* and *ITS1* gene sequences. Thereafter, Dinsdale et al. (2010) set quantifiable limits for grouping *B. tabaci* based on the mtCOI gene, which resolved the world population into 24 putative species based on 3.5% divergence. Using the same rule, Hu et al. (2011) added four new putative species to this cryptic species complex, and Reddy et al. (2012) added one more, making the total count of species 29.

B. tabaci was first recorded in India on cotton in 1905 (Misra and Lambda 1929). Previous studies of genetic structure of *B. tabaci* in India were limited to samples collected from smaller regions only (Brown et al. 1995, Perring 2001, Banks et al. 2001, Rekha et al. 2005, Lisha et al. 2003, Reddy et al. 2012, and Singh et al. 2012). In this study, we aim to present a

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more unified and thorough account of the current genetic structure of *B. tabaci* in India by including new sequences generated after an exhaustive survey. We have used the *mtCOI* marker gene for this purpose.

Materials and Methods

Sample Collection. In total, 101 *B. tabaci* samples collected across India between 2009 and 2011 from 45 districts (including 14 locations from our previous publication Singh et al. 2012) are included in the study (Table 1). At each location, insect samples were collected from three different fields, with each field being at least 1 km apart. Individuals were collected in separate microfuge tubes containing 100% ethanol and stored at −80°C till further use.

DNA Isolation. Three *B. tabaci* individuals were analyzed from each field. DNA isolation was carried out as described by Singh et al. (2012). Briefly, each *B. tabaci* sample was washed with sterile water, and then homogenized in 14 µl of lysis buffer (100 mM Tris-Cl pH 8.0, 100 mM EDTA pH 8.0, 100 mM NaCl, and 1% SDS) and 1% proteinase K. The homogenate was incubated at 65°C for 30 min. Twenty-seven microliters of a pre-chilled mixture of 5 M potassium acetate and 6 M lithium chloride was added, followed by incubation on ice for another 15 min before centrifuging at 10,000 rpm at 4°C for 15 min. The supernatant was transferred to a fresh microfuge tube to which was added 0.6 volumes of isopropanol. This was again centrifuged at 10,000 rpm at 4°C for 15 min. The pellet thus obtained was washed with 70% ethanol. The air-dried pellet was dissolved in elution buffer (10 mM Tris-Cl, pH 8.0) followed by treatment with RNase A.

Genetic Identification Using the mtCOI Marker. The *mtCOI* gene sequence of three individuals from each field was analyzed for genetic identification. Approximately 850 bp of the *mtCOI* gene fragment was amplified using forward primer C1-J-2195 (5′-TTGATTTTTTGGTCATCCAGAAGT-3′) and reverse primer L2-N-3014 (5′-TCCAATGCACTAATCTGCCATATTA-3′; Simon et al. 1994). The 25-µl PCR mix contained 1× taq buffer, dNTP (2.5 mM), primers (7.5 pico moles each), Taq polymerase (1 Unit), 20 ng of insect DNA, and water to make up volume. PCR was performed with an initial denaturation at 94°C for 30 s followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 40 sec, and a final extension at 72°C for 5 min. The PCR product was cloned into pGEM-T Easy sequencing vector (Promega, Madison, Wisconsin, USA). Plasmids from three positive transformants were sequenced at Macrogen Inc., Korea.

Sequence Analysis. Analyses were carried out on 657 bp of *mtCOI* gene sequences from 164 taxa (Lee et al. 2013, Boykin and De Barro 2014). These were added to 850 bp of sequence of the *mtCOI* gene from samples we collected in this study to identify the different *B. tabaci* populations in India. The *mtCOI* DNA sequences generated in this study were submitted to the NCBI database. All sequences were translated into putative amino acid sequence before the sequence analysis to check for any stop codons. All sequences were subjected to a BLAST search of the NCBI GenBank database (Altschul et al. 1990).

Results

Genetic Identification Based on Mitochondrial Marker. Information from our survey, as well as analysis of sequences from GenBank, shows that only one type of invasive genetic group, MEAM1, is present in India. In our survey, MEAM1 was found only in two fields of Bangalore and one field in the Kolar region of Karnataka, areas where these have been previously reported (Banks et al. 2001 and Rekha et al. 2005). Presence of MEAM1 has also been reported from some regions of Gujarat (Reddy et al. 2012; Fig. 1 and Table 2).

Apart from this invasive group, *B. tabaci* populations belonging to eight different groups were also found, either from our survey or from GenBank records, to be present in India. Of these, five were previously recorded (Dinsdale et al. 2010, Boykin et al. 2012). Asia I-India was reported by Reddy et al. (2012) from Coimbatore.

Individuals from Dharwad and Gadag belong to Asia II-11. This group has been reported only from this region of India. Also notable was the presence of individuals belonging to China-3 recorded for the first time in India from a field in the Birbhum region of West Bengal.

Distribution of *B. tabaci* Across India. Distribution of *B. tabaci* genetic groups across India is shown in Figure 1 and Table 2. Asia I is the most widely distributed genetic group, recorded from 28 locations, followed by Asia II-1 which was found in 20 locations. Asia II-8, Asia II-7, and Asia II-5 are localized in six, two, and five locations, respectively. China-3 and Asia I-India were each recorded from only one location. The newly identified genetic group Asia II-11 was found in two locations. Interestingly, more than one genetic group of *B. tabaci* was found inhabiting neighboring fields in many locations (Table 2). In some locations, such as Muzaffarpur and Salem, we found *B. tabaci* belonging to two different genetic groups coexisting in the same field.

Discussion

This study presents the diversity and distribution of *B. tabaci* across India. Of the 29 putative species of *B. tabaci* reported globally, 9 have been recorded from India. All these samplings were conducted at different times due to different cropping seasons in India. To evaluate whether the different genetic groups we identified are a result of sampling time variation, we collected samples throughout the year from a single field in Delhi (which has many seasonal variations) and found the same genetic groups present throughout the year (S.T.S., unpublished data).

The distribution patterns of *B. tabaci* suggest that most diversity is present in southern and in eastern India. Diversity declines toward the north and northwest, where either Asia II-1 or Asia I predominate.

Table 1. Details of field survey for collection of *B. tabaci* samples across India

Sr. no.	State	District	Field	Host plant	*Bemisia tabaci* genetic group	GenBank accession number
1	Jammu & Kashmir	Jammu	I and II	Okra and cucurbit	Asia II 1	JQ995278
3	Himachal Pradesh	Hamirpur	I	Brinjal	Asia II 1	JQ995277
4	Punjab	Ludhiana	I	Cotton	Asia I	JX993213
5			II	Cotton	Asia II 1	JX993212
6		Faridkot	I and II	Cotton	Asia II 1	JX993200
8		Barnala	I, II, and III	Cotton	Asia II 1	JX993186
14		Abohar	I, II, and III	Cotton	Asia II 1	JX993179
17	Rajasthan	Sriganganagar	I, II, and III	Cotton	Asia II 1	JX993219
20	Haryana	Sirsa	I, II, and III	Cotton	Asia II 1	JX993220
23	New Delhi	Delhi	I	*Leucaena leucocephala*	Asia II 7	JX993196
24	Utter Pradesh	Lucknow	I	Brinjal	Asia II 1	JX993210
J			II and III	Brinjal	Asia I	JX993211
27		Kanpur	I	Brinjal	Asia I	JX993204
28			II	Brinjal	Asia II 1	JX993205
29		Varanasi	I, II, and III	Brinjal	Asia II 1	JX993225
32	Bihar	Patna	I and II	Brinjal	Asia I	JX993218
34		Muzzaffarpur	I	Brinjal	Asia I / Asia II 1	JX993215
35	Gujarat	Anand	I, II, and III	Cotton	Asia II 1	JX993178
38		Surat	I and II	Cotton	Asia II 1	JX993223
40	West Bengal	Birbhum	I and III	Brinjal	Asia I	JX993190
41			II	Parwal/pointed gourd	China 3	JX993191
43		Bardhaman	I	Brinjal	Asia I	JX993187
44		Nadiya	I, II,III, and IV	Brinjal	Asia I	JX993216
48		Kolkata	I, II	Brinjal and beans	Asia II 5	JX993207
50	Madhya Pradesh	Indore	I, II, and III	Cotton	Asia I	JX993209
53	Maharashtra	Nagpur	I, II, and III	Cotton	Asia II 1	JX993217
56		Amravati	I, II, and III	Cotton	Asia I	JX993182
59		Akola	I, II, and III	Cotton	Asia I	JX993180
62	Andhra Pradesh	Guntur	I, II, and III	Brinjal and cotton	Asia I	JX993202
65		Prakasam	I	Cotton	Asia I	JX993184
66			II and III	Groundnut and mung	Asia II 1	JX993185
68	Karnataka	Dharwad	I	Cotton	Asia II 11	JX993197
69			II	Cotton	Asia II 8	JX993198
70			III	Cotton	Asia II 11	JX993199
71		Gadag	I	Cotton	Asia II 11	JX993201
72		Uttar Kannada	I	Brinjal	Asia I	JX993224
73		Havery	I	Chilly	Asia I	JX993203
74		Belgaum	I	Brinjal	Asia I	JX993188
75		Bagalkot	I	Cotton	Asia I	JX993189
76		Bangalore	I and II	Cauliflower	MEAM1-B	JX993192
78			III	Brinjal	Asia I	JX993193
79		Chikkabellapura	I	Brinjal	Asia II 1	JN410778
80			II	Tomato	Asia II 8	JN410779
81		Kolar	I	Brinjal	Asia I	JX993206
82			II	Cabbage	MEAM1-B	JX993208
83			III	Cotton	Asia I	JN410801
84	Tamil Nadu	Coimbatore	I and III	Brinjal	Asia I	JX993194
85			II	Sunflower	Asia II 5	JX993195
87		Salem	I	Cotton	Asia II 8 / Asia I	JX993221, JX993222
88			II	Brinjal	Asia I	JN410800
89		Erode	I	Brinjal	Asia II 8	JN410785
90		Dindigul	I and II	Cotton	Asia I	JN410786
92		Vridhunagar	I	Cotton	Asia I	JN410788
93		Madurai	I and II	Cotton	Asia I	JN410789
95	Kerala	Thiruvananthapuram	I	Cassava	Asia II 5	JQ995242
96			II	Tobacoo	Asia II 7	JN410713
97			III	Yellow bauhinia	Asia II 8	JQ995251
98		Alappuzha	I	Cassava	Asia II 5	JQ995243
99		Kottayam	I	Cassava	Asia II 5	JQ995244
100			II	Mulberry	Asia II 1	JQ995250
101			III	Yellow bauhinia	Asia II 8	JN410715

Exceptions are Delhi, which has Asia II-7, and some pockets of Gujarat which harbor MEAM1. The distribution pattern of *B. tabaci* may be influenced by a number of factors including host plant, geographical location, and various anthropogenic-derived movements associated with trade. For instance, the presence of Asia II-7 in Delhi, while neighboring regions harbor Asia II-1 and Asia-I, begs for possible explanations.

B. tabaci is not known to fly long distances; hence, it was expected that nearby fields of a particular location would contain the same genetic group. In contrast, more than one genetic group of *B. tabaci* was

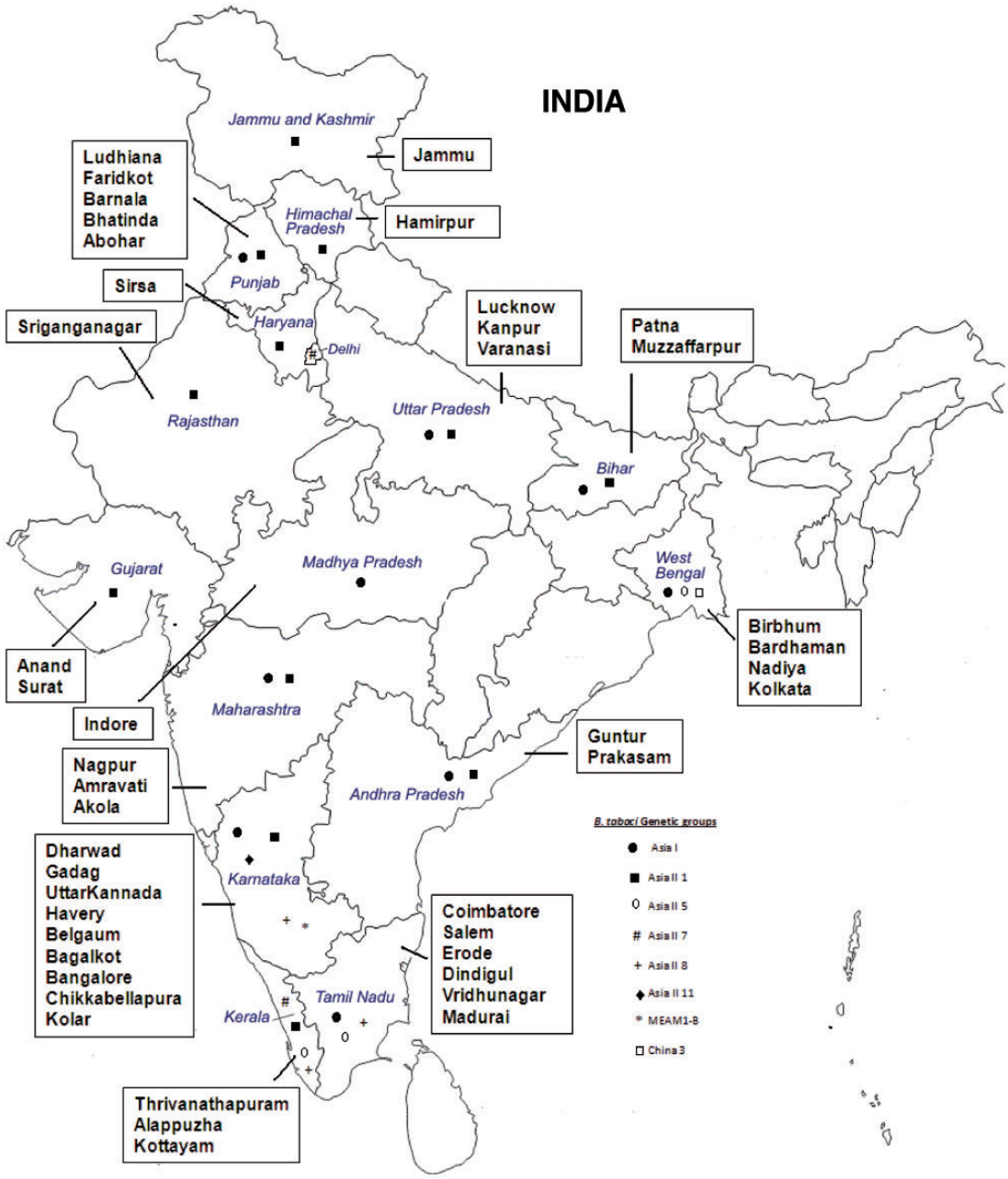

Fig. 1. Collection locations across India surveyed for *B. tabaci*.

sometimes found in the same location. For instance, Asia I was recorded from a field in Ludhiana, whereas Asia II 1 was found in all neighboring locations. Interestingly, we also found *B. tabaci* of more than one genetic group within the same field. Both Asia II 1 and Asia I were identified from a field in Muzaffarpur, while a field in Salem harbored Asia I and Asia II 8. Thus, the presence of more than one genetic group in the same field and location suggests an impact of anthropogenic movements in India. Also interesting to note is the presence of China-3 in a field in Birbhum, whereas the other two fields sampled contained only Asia I. Hu et al. (2011) found that this group (indigenous to China) was present only on the China mainland and the island of Hainan. Its presence in India shows the impact of trade on movement of *B. tabaci* across Asia. We suggest that China-3 is slowly invading India.

Our survey as well as sequences from the dataset shows that the invasive MEAM1 genetic group is in pockets of Bangalore, Kolar, and some areas of Gujarat. The MEAM1 genetic group is the most invasive genetic group of *B. tabaci* and has high fecundity.

Table 2. Distribution of different *B. tabaci* genetic groups across India

Genetic group	Asia I	Asia I- India	Asia II 1	Asia II 5	Asia II 7	Asia II 8	Asia II 11	China 3	MEAM1
Locations	Ludhiana I	Coimbatore	Ludhiana II	Coimbatore II	Delhi I	Dharwad II	Dharwad I and III	Birbhum II	Bangalore I and II
	Amravati I, II, and III		Faridkot I and II	Kolkata I and II	Thiruvananthapuram II	Salem IB	Gadag I		Kolar II
	Akola I and II		Barnala I, II, and III	Alappuzha I	Ranibennaur	Chikkabellapura II			Dabhoi
	Lucknow II and III		Bhatinda I, II, and III	Thiruvananthapuram I	Bangalore	Erode I			Nagamangala
	Kanpur I		Sirsa I, II, and III	Kottayam I	Ahmedabad	Thiruvananthapuram III			Bangalore
	Indore I, II, and III		Sriganganagar I, II, and III	Pune	Pune	Kottayam III			
	Havery I		Abohar I, II, and III			Ranibennaur			
	Uttarkannada I		Nagpur I, II, and III						
	Prakasam I		Akola III						
	Guntur I, II, and III		Varanasi I, II, and III						
	Bangalore III		Lucknow I						
	Kolar I and III		Kanpur II						
	Coimbatore I and III		Surat I and II						
	Salem IA and II		Anand I, II, and III						
	Patna I, and II		Prakasam II and III						
	Muzaffarpur IA		Muzaffarpur IB						
	Birbhum I and III		Jammu I and II						
	Bardhman I		Hamirpur I						
	Nadiya I, II, III, and IV		Chikkabellapura I						
	Bagalkot I		Kottayam II						
	Belgaum I								
	Dindigul I								
	Vridhunagar I								
	Madurai I								
	Kristapur								
	Kalyani								
	Ahemadabad								
	Delhi								
	Ranibennaur								
	Hissar								

The locations highlighted in shading are from GenBank records.

Nevertheless, our survey revealed MEAM1 restricted to these pockets, and it was not detected in neighboring areas. Previously, the presence of the MEAM1 genetic group in India was reported only from Bangalore and Kolar regions (Banks et al. 2001, Rekha et al. 2005). Banks et al. (2001) and Rekha et al. (2005) reported MEAM1 *B. tabaci* from tomato hosts. However, in our survey, MEAM1 was recorded only from cruciferous vegetables such as cabbage and cauliflower. The reason for this host difference and its restriction to certain geographic pockets is beyond the scope of this paper but needs to be investigated because it might prove beneficial in controlling this invasive genetic group. It is possible that some of *B. tabaci*'s natural enemies are responsible for the confinement of MEAM1 to these regions of the country. For example, the greatest diversity of *B. tabaci* parasitoid species is found in India (Oliveira et al. 2001).

B. tabaci from Gadag and Dharwad belong to the Asia II 11 genetic group, which has not been reported from any other location globally. The sequences HM590146 and HM590147 attributed to Asia II 11 by Firdaus et al. (2012) are wrongly described as being from Gujarat and Andhra Pradesh, as these two sequences were a part of our survey of the Dharwad region, a district of Karnataka. This recent addition of a new genetic group to this cryptic species evidences the complexity of this species complex. It is possible that more new genetic groups are present whose revelation will require a more intensive sampling regimen.

This report on the patterns of spread and diversity of *B. tabaci* in India will provide useful insights into the discovery of new genetic groups, in addition to helping to better monitor and manage the spread of this insect in India.

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