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THE THIRD GENOTYPIC CLUSTERS OF *Bemisia tabaci* (GENNADIUS) (HEMIPTERA: ALEYRODIDAE) FOUND IN BANGLADESH

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Abstract

The sweetpotato whitefly, *Bemisia tabaci* is a species complex that possessed several biotypes including different genotypic clusters within species, which may differ from each other genetically and physiologically but morphologically alike. This study was performed by molecular analysis for easy identification of whitefly and describes its biotype throughout Bangladesh. Whiteflies have been identified from different places of Bangladesh based on mitochondrial cytochrome oxidase subunit I (mtCOI) gene and 16S ribosomal RNA gene sequences analysis. The mtCOI sequences of BW3 (collected from eastern part of Bangladesh) whitefly were diverged by 14.5% and 15.1% compared with B and Q biotypes from Korea and it also diverged by 15.4% and 13.7% from each other compared to BW1 (collected from southern part of Bangladesh) and BW2 (collected from northern part of Bangladesh), respectively within the country. The 16S rRNA sequences of BW3 whitefly were more deviated by 41.5%, 10.7%, 42.7% and 12.6% compared with the country populations from BW1, BW2, B and Q biotypes, respectively. Moreover, it showed high divergences from indigenous whiteflies of southern and northern part of Bangladesh which clustered in a different clade on both mtCOI and 16S rRNA phylogeny. Therefore, till date three genotypic cluster of indigenous whitefly BW1, BW2 and BW3 are identified from Bangladesh.

Keywords: *Bemisia tabaci*, BW3, mtCOI gene, 16S rRNA gene, indigenous whitefly.

Introduction

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a very complex species consists of at least 24 biotypes in tropical and sub-tropical region around the World (Ahmed *et al*., 2009). This devastating global insect pest caused damage directly by sucking the plant sap from phloem, indirectly by excreting honeydews that produce sooty mould, and by spreading 111 plant virus diseases (Martin *et al*., 2000; Jones, 2003; Mughra *et al*., 2008). *Bemisia tabaci* is a genetically different groups of insect that morphologically indistinguishable (Frohlich *et al*., 1999; De Barro *et al*., 2000; Boykin *et al*., 2007). Significant

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attempt has been committed to the analysis of the biological and genetic variation of *B. tabaci* and has been recognized different biotypes into the species (Perring, 2001). Among them, two predominantly aggressive biotypes, known as B and Q, are distributed everywhere around the World (De Barro, 2005; De Barro *et al*., 2005; Chu *et al*., 2006; Martinez-Carrillo & Brown, 2007) whereas, in Bangladesh yet B and/ or Q biotype are absent but indigenous biotype BW1 and BW2 recorded recently (Maruthi *et al*., 2007; Jahan *et al*., 2011; Jahan, 2012). The *B. tabaci* is not genetically consistent. Based on mitochondrial DNA markers, the *B. tabaci* complex can be placed into five major groups according to their geographical origin: (1) New World (US, Mexico, Puerto Rico), (2) Southeast Asia (Thailand, Malaysia), (3) Mediterranean basin (Southwest Europe, North Africa, Middle East), (4) Indian subcontinent (Bangladesh, India, Myanmar, Nepal and Pakistan), (5) Equatorial Africa (Cameroon, Mozambique, Uganda, and Zambia); an additional more distantly related group contained the B relatives from many world sites and the non-B relatives from the Middle East (Frohlich *et al*., 1999). At least three distinct genotypes, apparently indigenous to India, which are also present in China, Malaysia, Nepal, Pakistan and Thailand. These coexist with the B biotype, which was first reported in India in 1999, and has since spread rapidly to other states in south India (Rekha *et al*., 2005). This may support the conclusion of De Barro *et al*. (2005) that using mtCOI and internal transcribed spacer region 1 (ITS1) markers, some individuals from Asia did not fit into either of the two clusters (called Asia1 and Asia2), and they remained classified as 'unresolved' in the Asia group. In a similar study, two indigenous *B. tabaci* genotypes have been reported from neighboring Pakistan, and one from the Punjab area was similar to the Indian populations (Brown, 2001; Simon *et al*., 2003), suggesting their wider occurrence. The mtCOI DNA diversity of Asian *B. tabaci* therefore is comparable to that from African populations, where at least five distinct clades of *B. tabaci* have been recorded on cassava (Legg *et al*., 2002; Berry *et al*., 2004).

In this study, the genetic differences between the *B. tabaci* B and Q biotype populations from Korea and indigenous whitefly from Bangladesh which was collected from various places on different host-plants as bean, eggplant, and okra were investigated for identification.

Materials and Method

Whiteflies collection

Samples of adult *B. tabaci* were collected from bean, eggplant and okra of different places of eastern part of Bangladesh such as, Chittagong and Cox's Bazar in 2012 (Table 1) and were immediately preserved in 99% ethanol (alcohol) and stored at -20^{0}C. Adults of *B. tabaci* B and Q biotypes were collected from whitefly rearing house in Insect Molecular Physiology Laboratory

at Kyungpook National University in South Korea on cucumber and tomato plants in 2011 for morphological and genetic sequences comparison of Bangladesh whiteflies.

DNA extraction

Total genomic DNA was extracted from individual *B. tabaci* according to protocol supplied by Invitrogen Purelink Genomic DNA mini kit. After removing from ethanol the sample had been washed with double-distilled water to remove alcohol, individual whiteflies were homogenized in 180 µl genomic digestion buffer using a 1.5 ml microcentrifuge tube and micropestle (homogenizer). Then added 200 µl genomic lysis/ binding buffer (1% SDS, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 25 mM NaCl, Proteinase K 200 mg/ml) and after that immediately added 200 µl absolute ethanol. Subsequently added wash buffer into the genomic column and finally added 20 µl genomic elusion buffer (Invitrogen Purelink, Carisbad, CA, USA). After 1 min incubation at room temperature, samples were centrifuged at about 12000 rpm for 1 min, and the supernatants/pellets were directly used for PCR detection of the secondary endosymbionts or were stored at -20°C for later use. These procedures were followed by Dellaporta *et al*., 1983 and Jahan *et al*., 2011.

Table 1. List of collection with GenBank accession no. for the sequences of collected *B. tabaci* in Bangladesh.

Name of Specimen	Host plant	Location	Year	Gene	GenBank Accession no.
B. tabaci	Bean	Chittagong	2012	mtCOI	JX417071
B. tabaci	Bean	Chittagong	2012	16S rRNA	JX417075
B. tabaci	Bean	Patuakhali	2011	mtCOI	JN018067
B. tabaci	Bean	Patuakhali	2011	16S rRNA	JQ305697
B. tabaci	Eggplant	Chittagong	2012	mtCOI	JX417072
B. tabaci	Eggplant	Chittagong	2012	16S rRNA	JX417076
B. tabaci	Eggplant	Kurigram	2011	mtCOI	JQ305088
B. tabaci	Eggplant	Kurigram	2011	16S rRNA	JQ305699
B. tabaci	Eggplant	Cox's Bazar	2012	mtCOI	JX417073
B. tabaci	Eggplant	Cox's Bazar	2012	16S rRNA	JX417077
B. tabaci	Okra	Cox's Bazar	2012	mtCOI	JX417074
B. tabaci	Okra	Cox's Bazar	2012	16S rRNA	JX417078

Primer design and PCR amplification

Bemisia tabaci was determined using the genomic DNA which was collected from Bangladesh with the primers listed in Table 2, using Polymerase Chain

Reaction (PCR). All PCR reaction mixture performed in 20 µl volume that included 1 µl of each primer (Forward and Reverse), 1 µl of DNA template and 17 µl smart buffer which were supplied by the manufacturer (Smart taq pre-mix). All PCR reactions were carried out on the PTC-200 DNA engine thermal cycler (MJ Research PTC-200 DNA Engine Thermal Cycler PCR).

Table 2. Nucleotide sequences of primers listed for *B. tabaci* identification.

Gene	Primer Name	Primer Direction	Primer Sequence (5' to 3')	Size (bp)	Reference	Tm. (°C)
mtCOI	C1-J-2195 L2-N-3014	Forward Reverse	TTGATTTTTTGGTCATCCAGAAGT TCCAATGCACTAATCTGCCATATTA	860	Simon *et al*. 1994	52°C
16S rRNA	LR-J-12887 LR-N-13398	Forward Reverse	CCGGTTTGAACTCAGATCATGT CGCCTGTTTAACAAAAACAT	520	Simon *et al*. 1994	55°C

PCR condition

The mixtures with the cytochrome oxidase sub-unit1 (CO1) primer C1-J-2195(F) and L2-N-3014(R) were amplified in a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) with a 1 minute initial denaturation at 95°C, 35 cycles (1 min at 94°C, 30 sec at 52°C, 2 min at 72°C), and finally by a 5 min extension at 72°C. For 16S rRNA primers LR-J-12887 (F) and LR-N-13398 (R) were also amplified with a 2 min initial denaturation at 94°C, 35 cycles (30 sec at 94°C, 1 min at 55°C, 1 min at 72°C), and finally by a 10 min extension at 72°C.

Gel-electrophoresis

Electrophoresis of amplified PCR products (5 µl) were done using 1.0% agarose gels with 1% TAE buffer at 100 V for 30 minutes with 100 bp ladder as DNA marker and the gels were then stained by 10 µl Ethidium Bromide for 20 minutes. When fragments with the expected size were visible on the gels, then the rest of 15 µl of PCR products were used for sequencing.

Sequence analysis

DNA sequences were aligned using CLUSTAL W (Thompson *et al*., 1994). The aligned sequences were checked and compared the sequences similarity with the online published sequences using BLAST in the National Centre for Biotechnology Information (NCBI). Sequences were aligned and arranged using

the Clustal W2 multiple alignments in BioEdit (version 7.0). The sequences divergences calculated by Molecular Evolutionary Genetics Aanlysis (MEGA) among intraspecific, interspecific species, based on Kimura-2-parameter (K2P) distances (Tamura *et al*., 2007). Phylogenetic relationships were inferred by MEGA Software Version 4.0 (Tamura *et al*., 2007) using neibour-joining method (NJ). Bootstrap values were obtained from 1000 replicates. The sequences are deposited in the GenBank database.

Results

Identification of *B. tabaci* in Bangladesh

The sweetpotato whitefly, *B. tabaci* was identified from the collected specimen in Bangladesh using PCR, and compared the similarity of mtCOI and 16S rRNA gene sequences with related information published in NCBI database. The DNA fragment at 860 bp to C1-J-2195(F) and L2-N-3014(R) and at 520 bp to LR-J-12887 (F) and LR-N-13398 (R) primer sets were shown in PCR amplification (Figure 1). The partial mtCOI and 16S rRNA gene sequences have been provided here that was submitted for registration to the NCBI GenBank database (Table1). Moreover, we provide here the comparison of similarity using both mtCOI and 16S rRNA nucleotides in total length of sequences (Table 3). We found that all sequences showed maximum similarity with *B. tabaci* around the World.

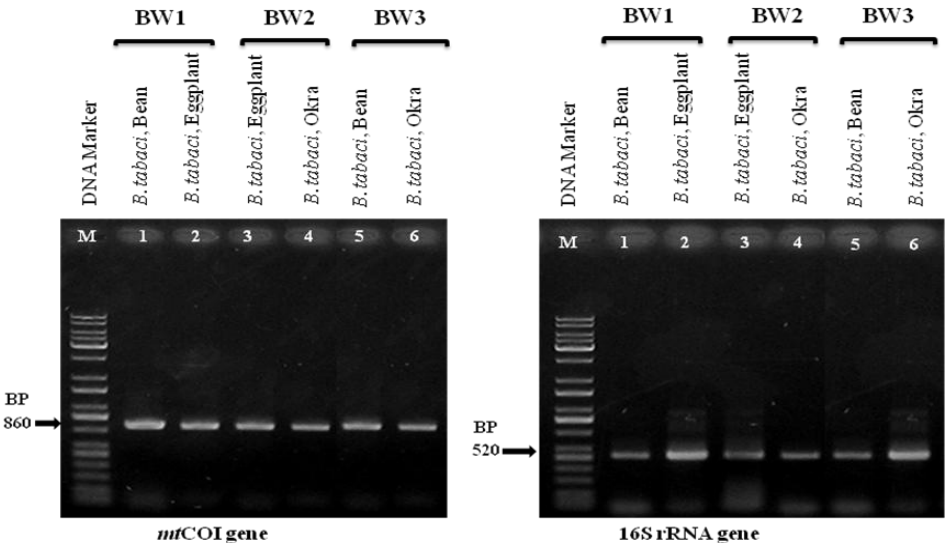

Figure1. PCR Amplification of mtCOI gene region of *B. tabaci* with C1-J-2195 and L2-N-3014 primers and 16S rRNA gene with LR-J-12887 and LR-N-13398 primer set to distinguish *B. tabaci*. M: molecular weight standard-DNA marker (100 bp ladder).

Biotype detection from Bangladesh whiteflies

Biotype of *B. tabaci* collected from Bangladesh has been examined using PCR amplification with 16S rRNA gene sequence digested with restricted enzyme Hinf1 (Figure 1). The result revealed that collected whiteflies from eastern part of Bangladesh are indigenous (native) BW3 biotype (Figure 4 & 5). More interesting things that collected samples from eastern part were shown very dissimilarity from B biotype, Q biotype and previously collected BW1 BW2 whitefly but they all are truly indigenous whitefly (Figure 4 & 5). We revealed another new genotypic cluster of *B. tabaci* (BW3) in Bangladesh. This new genotype of whiteflies showed score of similarity 85.51%, 84.87%, 84.61% and 86.32% with B and Q biotype (Korea), BW1 (southern part of Bangladesh) and BW2 (northern part of Bangladesh), respectively for mtCOI gene (Table 3). The 16S rRNA sequences of BW3 (newly collected from eastern part of Bangladesh) whitefly were shown similarity by 57.32%, 87.38%, 58.54% and 89.29% compared with B biotype, Q biotype, BW1 and BW2, respectively (Table 3).

Table 3. Similarity of both mtCOI and 16S rRNA nucleotide sequences of *B. tabaci* from each other with another biotype.

Sequence of *B. tabaci* (A)	Sequence of *B. tabaci* (B)	Score of Similarity of mtCOI (%)	Score of Similarity of 16S rRNA (%)
B-biotype, Korea	Q-biotype, Korea	94.73	50.0
B-biotype, Korea	BW1, Bangladesh(South)	84.06	91.46
B-biotype, Korea	BW2, Bangladesh(North)	84.85	53.66
B-biotype, Korea	BW3, Bangladesh(East)	85.51	57.32
Q-biotype, Korea	BW1, Bangladesh(South)	83.55	54.15
Q-biotype, Korea	BW2, Bangladesh(North)	84.34	90.24
Q-biotype, Korea	BW3, Bangladesh(East)	84.87	87.38
BW1, Bangladesh(South)	BW2, Bangladesh(North)	90.26	54.88
BW1, Bangladesh(South)	BW3, Bangladesh(East)	84.61	58.54
BW2, Bangladesh(North)	BW3, Bangladesh(East)	86.32	89.29

Sequence analysis

The profile of PCR amplification of 16S rRNA gene with primer, LR-J-12887 (5'- CCGGTTTGAACTCAGATCATGT-3'), LR-N-13398 (5'-CGCCTGTTTAACAAAAACAT-3') showed consistent and single fragment of DNA in the length of 520 bp. Similarly, the primer set of C1-J-2195(F) and L2-

N-3014(R) demonstrated consistent and single DNA fragment of 860 bp to distinguish the whiteflies including all biotypes (Figure 1). The sequences for all samples were obtained by purification of PCR product using ProMega Gel purified kit and can compare easily among the all sequences by clustalW2 alignment (Figure 2 & 3). Here, it was found dissimilarity score of BW3 are 15.39% and 13.68% for mtCOI gene and 41.46% and 10.71% for 16S rRNA gene compared with BW1 and BW2, respectively (Table 3). Moreover, the sequences of mtCOI and 16S rRNA for BW3 were analyzed based on pairwise distance (character difference) and nucleotide different. It revealed that BW3 was highly divergent from B & Q biotype (Korea), BW1 and BW2, which expressed 110 and 198 nucleotides were different from B biotype, 115 and 51 nucleotides were different from Q biotype, 117 and 204 nucleotides were different from BW1, 103 and 43 nucleotides were different from BW2 in mtCOI and 16S rRNA gene sequences, respectively (Table 4 & 5). These sequences of BW3 had shown the proportions of A+T and G+C in residue compositions of 69.0% and 31.0% for mtCOI as well as 73.7% and 26.3% for 16S rRNA, respectively. The average proportion of T: C: A: G was 42.9: 13.0: 26.1: 18.0 for mtCOI and 30.4: 12.7: 43.3: 13.6 for 16S rRNA, respectively with a narrow standard error around means, but base composition varied substantially in different portions within the sequences of Bangladesh indigenous whiteflies. Among these 760 bp nucleotide, 568 characters were conserved and 192 characters were variable (Table 6 & 7). The sequence divergence in pairwise comparisons revealed that BW3 was very diverse group of whitefly in phylogenetic tree where number of nucleotide changed 33-210 from each other, and lowest distance value was 0.053 and highest was 0.532 among all tested population (Table 4 & 5).

Table 4. Pairwise distance among 5 different biotypes of *B. tabaci* in Bangladesh based on sequences of the fragment of mtCOI gene.

	1	2	3	4	5
[1]		0.099	0.154	0.159	0.165
[2]	75		0.136	0.152	0.157
[3]	117	103		0.145	0.152
[4]	121	115	110		0.053
[5]	125	119	115	40	

Table 5. Pairwise distance among 5 different biotypes of *B. tabaci* in Bangladesh based on sequences of the fragment of 16S rRNA gene.

	1	2	3	4	5
[1]		0.532	0.516	0.084	0.527
[2]	210		0.109	0.514	0.101
[3]	204	43		0.501	0.129
[4]	33	203	198		0.511
[5]	208	40	51	202	

(*Bemisia tabaci* from Southern part of Bangladesh (1), Northern part of Bangladesh (2), Eastern part of Bangladesh (3), B biotype from Korea (4); and Q biotype from Korea (5)). Distance in-between mtCOI gene for 5 different biotypes of *B. tabaci* in Bangladesh (below diagonal: total nucleotide differences, above diagonal: mean character differences) using Kimura 2-parameter (*Bemisia tabaci* from Southern part of Bangladesh (1), Northern part of Bangladesh (2), Eastern part of Bangladesh (3), B biotype from Korea (4); and Q biotype from Korea (5)). Distance in-between 16S rRNA gene for 5 different biotypes of *B. tabaci* in Bangladesh (below diagonal: total nucleotide differences, above diagonal: mean character differences) using Kimura 2-parameter

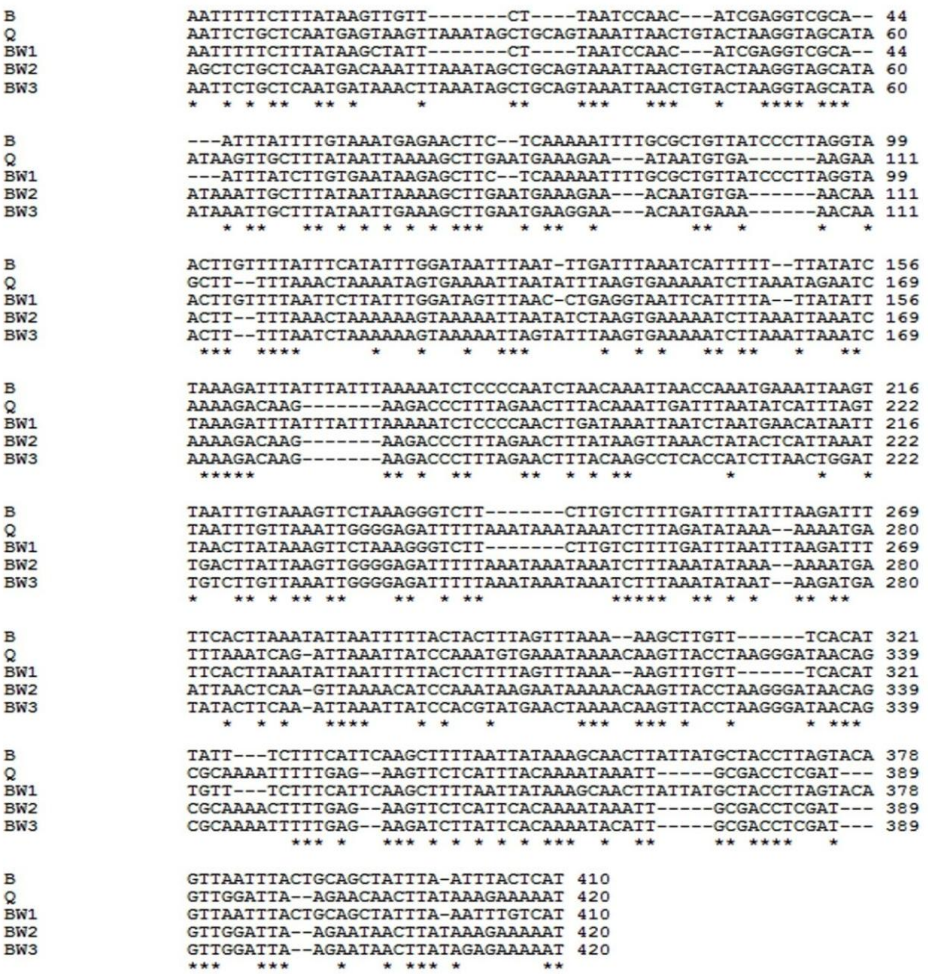

Fig. 2. Sequence alignment of Bangladesh whitefly (BW1, BW2 and BW3) and compared with B and Q biotype of Korea using 16S rRNA gene nucleotide sequences by ClustalW2 program.

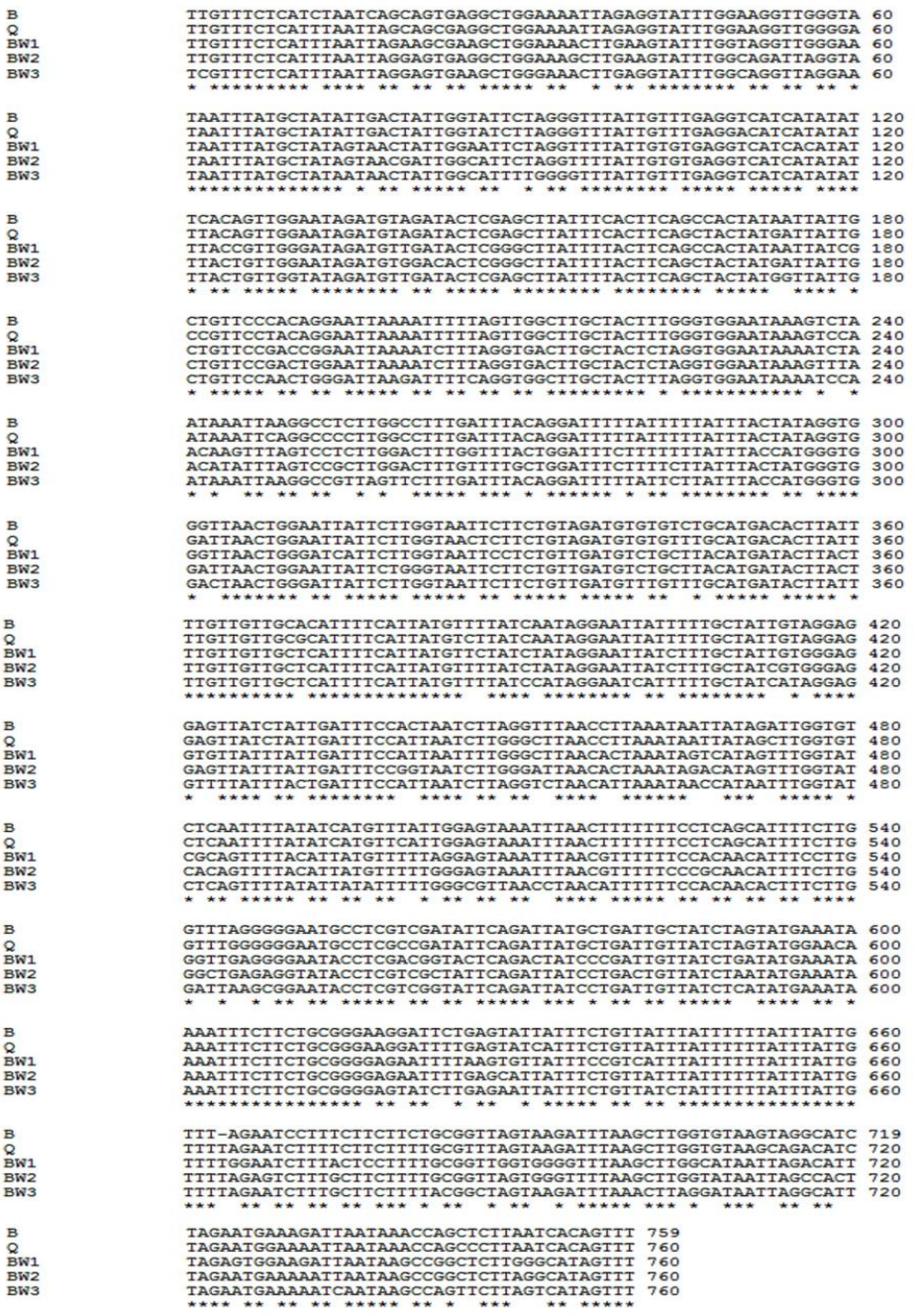

Fig. 3. Sequence alignment of Bangladesh whitefly (BW1, BW2 and BW3) and compared with B and Q biotype of Korea using mtCOI region nucleotide sequences by ClustalW2 program

Phylogenetic analysis

The Neibour-joining phylogenetic tree reconstruction based on 21 mitochondrial cytochrome oxidase subunit-I (mtCOI) sequences (B biotype from Israel, Viet Nam, China and Korea; Q biotype from Costa Rica, China, Egypt and Korea; BW1 from southern part of Bangladesh, BW2 from northern part of Bangladesh and newly collected Indigenous *B. tabaci* from eastern part of Bangladesh were compared) is shown in Figure 6. It revealed that mtCOI sequence of *B. tabaci* of eastern part of Bangladesh from Chittagong and Cox's Bazar which collected from bean, eggplant and okra were clustered together individually with high distance from each other (Figure 4). It was clear that third genotypic cluster was present in Bangladesh indigenous whiteflies which was BW3. Here mtCOI sequence of *Aleurodicus dispersus* was used as out group of phylogeny.

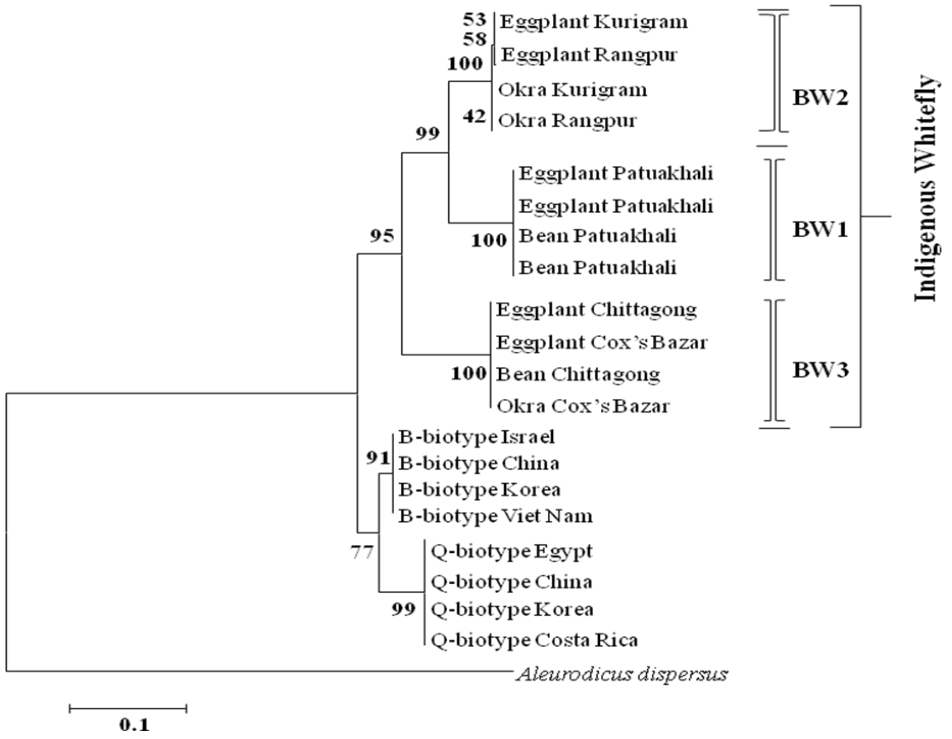

Fig. 4. Phylogenetic relationships of *B. tabaci* populations based on a fragment (~860 bp) of the mitochondrial COI sequences. Neibour-joining phylogenetic tree reconstructed using the whitefly mitochondrial cytochrome oxidase subunit-I (mtCOI) sequences as a molecular marker according to the Bayesian method. The numbers placed at each node indicate the bootstrap support for values > 50. The horizontal branch length is drawn to scale, and the bar indicates the distance of 0.1 nt replacements per site.

Table 6. Percentage of nucleotide frequencies in variable DNA sites of 5 different biotypes of *B. tabaci* in Bangladesh based on sequences of the fragment of mtCOI gene.

B. tabaci biotypes	Nucleotide composition (%)				Conserved sites (%) (568/760)				Variable sites (%) (192/760)				Parsim-info sites (%) (92/760)				Total
	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G	
BW1	41.3	13.9	24.1	20.7	43.3	12.3	25.2	19.2	35.4	18.8	20.8	25.0	25.0	20.7	26.1	28.3	760
BW2	42.4	13.0	24.2	20.4	43.3	12.3	25.2	19.2	39.6	15.1	21.4	24.0	25.0	20.7	22.8	31.5	760
BW3	42.9	13.0	26.1	18.0	43.3	12.3	25.2	19.2	41.7	15.1	28.6	14.6	33.7	10.9	34.8	20.7	760
Bt-B	42.4	12.5	26.1	19.0	43.2	12.3	25.2	19.2	40.1	13.0	28.6	18.2	30.4	12.0	32.6	25.0	759
Bt-Q	42.1	13.0	25.5	19.3	43.3	12.3	25.2	19.2	38.5	15.1	26.6	19.8	27.2	15.2	30.4	27.2	760
Avg.	42.2	13.1	25.2	19.5	43.3	12.3	25.2	19.2	39.1	15.4	25.2	20.3	28.3	15.9	29.3	26.5	759.8

Table 7. Percentage of nucleotide frequencies in variable DNA sites of 5 different biotypes of *B. tabaci* in Bangladesh based on sequences of the fragment of 16S rRNA gene.

B. tabaci biotypes	Nucleotide composition (%)				Conserved sites (%) (185/427)				Variable sites (%) (242/427)				Singleton sites (%) (55/427)				Total
	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G	
BW1	44.9	14.1	30.6	10.4	44.2	5.5	44.2	6.1	45.4	20.0	21.3	13.3	37.7	20.8	28.3	13.2	427
BW2	28.9	12.0	46.9	12.2	41.8	6.8	44.6	6.8	19.5	15.8	48.5	16.2	25.5	14.5	49.1	10.9	427
BW3	30.4	12.7	43.3	13.6	41.8	6.8	44.6	6.8	22.0	17.0	42.3	18.7	34.5	18.2	30.9	16.4	427
Bt-B	45.4	13.6	31.4	9.6	44.2	5.5	44.2	6.1	46.3	19.2	22.5	12.1	49.1	9.4	34.0	7.5	427
Bt-Q	31.7	9.6	44.4	14.4	42.0	6.8	44.3	6.8	24.1	11.6	44.4	19.9	34.5	5.5	38.2	21.8	427
Avg.	36.2	12.4	39.4	12.1	42.8	6.3	44.4	6.5	31.4	16.7	35.8	16.0	36.2	13.7	36.2	14.0	427.0

Bemisia tabaci from Southern part of Bangladesh (BW1), Northern part of Bangladesh (BW2), Eastern part of Bangladesh (BW3), B biotype from Korea (Bt-B); and Q biotype from Korea (Bt-Q).

The same analysis constructed by Neibour-joining phylogenetic tree based on twenty three 16S ribosomal RNA (16S rRNA) sequences (B biotype from Israel, China and Korea; Q biotype from Spain, China and Korea; Indigenous *B. tabaci* BW1 and BW2 from southern part and northern part of Bangladesh, China, India, Pakistan, Sri Lanka and Japan were compared with newly collected whitefly from eastern part of Bangladesh) is shown in Figure 5. It revealed that ribosomal RNA (16S rRNA) sequences of *B. tabaci* of eastern part of Bangladesh which was collected from bean, eggplant and okra were clustered together individually with high distance from each other (Figure 5). It is undoubtedly revealed that third genotypic cluster of *B. tabaci* in Bangladesh which is BW3.

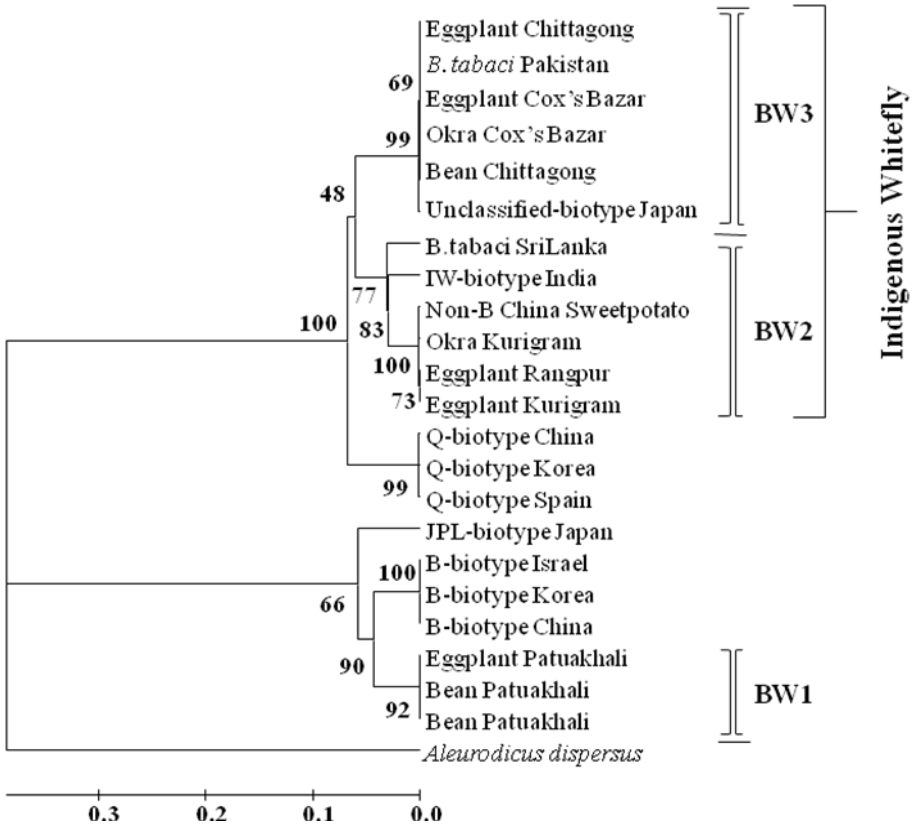

Fig. 5. Phylogenetic relationships of *B. tabaci* populations based on a fragment (~520 bp) of the mitochondrial 16S rRNA sequences. Neibour-joining phylogenetic tree reconstructed using the whitefly 16S ribosomal RNA (16S rRNA) sequences as a molecular marker according to the Bayesian method. The numbers placed at each node indicate the bootstrap support for values > 50. The horizontal branch length is drawn to scale, and the bar indicates 0.0-0.3 nt replacements per site.

Discussion

Bemisia tabaci populations revealed high genetic diversity in southern Asia. Phylogenetic analysis of mtCOI and 16S rRNA gene sequence data can be separated clearly the *B. tabaci* samples into north, south and east indigenous groups of Bangladeshi whitefly (Figure 4 & 5). Different cropping pattern and diverse climatic conditions in the northern, eastern and southern regions of Bangladesh may be responsible for the apparent diversity in *B. tabaci*, which otherwise grouped in geographic location. Tobacco, potato, and vegetables are grown almost as monocultures in the dry arid conditions in Kurigram, northern part of Bangladesh whereas the south is relatively cool and mixed crops of cereals, pulses, and vegetables predominate. However, divisions were not based on the host-plant from which the samples were collected, which was one of the criteria used to identify two *B. tabaci* biotypes (cassava and sweet potato) in south India (Lisha *et al*., 2003). The RAPD-PCR technique has been used before to distinguish indigenous populations of *B. tabaci* from those of the introduced B biotype (Gawel & Bartlett, 1993; Perring *et al*., 1993; De Barro & Driver, 1997; Guirao *et al*., 1997; Moya *et al*., 2001). Based on phylogenetic analyses of mtCOI and 16S rRNA gene sequences, the Asian *B. tabaci* formed three clusters each supported with a high bootstrap score, which indicate the existence of at least two genotypic clusters (BW1 and BW2) of *B. tabaci* indigenous to Bangladesh. This may support the conclusion of De Barro *et al*. (2005) that using mtCOI and internal transcribed spacer region 1 (ITS1) markers, some individuals from Asia did not fit into either of the two clusters (called Asia1 and Asia2), and they remained classified as 'unresolved' in the Asia group.

This research work has been successfully carried out for monitoring *B. tabaci* and detecting its genotypic clusters from Bangladesh. Jahan *et al*. (2011) previously mentioned that all Bangladeshi whiteflies were indigenous and there was apparently absent B and Q biotype of *B. tabaci*. The present findings also supported the previous report of Jahan *et al*., 2011. In the present study for detecting the third genotypic cluster of *B. tabaci*, it was found that whiteflies of eastern part of Bangladesh make a distinct clade in Phylogenetic tree based on mtCOI and 16S rRNA sequences separately (Figure 4 & 5). Although, Jahan (2012) reported two genotypic clusters present in Bangladesh. However, all sequences data currently found in GenBank were compared for preparing the phylogeny of whiteflies using mtCOI sequences of different biotypes including indigenous whiteflies from different countries.

Some Bangladeshi *B. tabaci* shared about 99% mtCOI sequence identity with populations from other Asian countries like India, Pakistan, Myanmar, Japan and Nepal. There may be several explanations for this, including the existence of a cline of distinct *B. tabaci* genotypes across Asian countries that allow gene flow between them. Huge geographical distances and natural barriers, such as the Himalayan mountain range, however, represent significant physical barriers for

natural movement and therefore probably restrict gene flow, for example, between the Bangladesh and Myanmar *B. tabaci* populations. The most likely reason for the similarity of these populations may be the movement of *B. tabaci* between the countries as a result of human activities. The most recent example of such introduction has been the arrival of the B biotype in India, Pakistan, and China (Banks *et al*., 2001; Simon *et al*., 2003; Zhang *et al*., 2005), and more recently the Q biotype in China (Zhang *et al*., 2005). The data, which presented here, highlight the real and increasing threat posed by the movement of *B. tabaci* and potentially new viruses to agriculture in Asia.

Phylogenetic analysis of mtCOI and 16S rRNA gene sequences with reference *B. tabaci* sequences from other countries divided them into two genotypic clusters. Each cluster supported with high bootstrap values (55–100%) and the individuals belonging to each cluster shared high nucleotide identities (up to 100%) (Figure 6 & 7). This result supported by Rekha *et al*. (2005) who reported that at least three distinct genotypes, of indigenous whitefly to India, which are also present in China, Malaysia, Nepal, Pakistan, Japan and Thailand.

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