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Research Article

Molecular Analysis of Cytochrome Oxidase I in *Bemisia tabaci* (Gennadius) Populations Collected from Four Districts in the Special Region of Yogyakarta

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ABSTRACT

Bemisia tabaci is an agricultural pest that interferes plant growth, as well as being an insect vector of various types of viruses, one of which is the geminivirus group. *B. tabaci* is called *Cryptic Species Complex* due to their similar morphology but has different genetic profiles. Climate change and increased global trading of agricultural products could increase *B. tabaci* population and lead to the emergence of genetic disparity. This study aims to obtain the latest information on the population homogeneity of *B. tabaci* in four districts of the Yogyakarta and potential differences on their nucleotide arrangements. Molecular identification was performed using PCR and primers C1-J-2198/L2-N-3914 on *B. tabaci*. *B. tabaci* COI gene sequences were then compared using a phylogenetic analysis and similarities of nucleotide bases were determined. Results showed that the populations of *B. tabaci* in Yogyakarta were still homogeneous showed by biotypes similarity, namely the non-B biotype. *B. tabaci* from Yogyakarta have nucleotide base similarity of 100% with *B. tabaci* from Singapore (AY686095) and Thailand (AY686092) and 99.56% with species from Bangladesh (AJ748388). Results also showed no differences in the composition of both nucleotide bases and amino acids from the four districts of *B. tabaci* sampling location. The homogeneous population of *B. tabaci* and the high incidence of yellowing disease caused by Begomovirus in chili pepper plants in the Special Region of Yogyakarta prove that it is necessary to review the current methods of controlling *B. tabaci* pests.

Keywords: *Bemisia tabaci*; biotype; molecular identification

INTRODUCTION

Whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an insect pest of various plant species across the world and have shown high genetic variation. *B. tabaci* caused damage on more than 600 plant species by sucking plant fluid and transmitting viruses (de Barro *et al.*, 2011). *B. tabaci* is categorized as a Cryptic Species Complex. Cryptic Species Complex indicate species with similar morphological characteristics, but possess distinctly different genetic characters. In Indonesia, *B. tabaci* was first found in 1938 and recognized symptoms due to its attack were called *kerupuk* disease in Sumatera and Java tobacco plantations. This disease was believed to be transmitted from weeds, such as *Ageratum* sp., *Synedrella* sp., *Eupatorium odoratum*. However, damage caused by *B. tabaci* were considered insignificant at that time (Kalshoven, 1981).

Begomovirus is a virus pathogen on plants, known to be transmitted by *B. tabaci,* and belong to the Geminivirus family. In Indonesia, several diseases caused by *Begomovirus* have been often reported in horticultural fields. Examples of these diseases include *Pepper yellow leaf curl virus* (PYLCV) that has become an epidemic in chili pepper production centers in Indonesia, especially in Java from 2000 to 2003 with incidences reaching 100% (Sulandari, 2006), and *Mungbean yellow mosaic India virus* (MYMIV) that infect long beans with reported incidences of 80–100% in Java (Nurulita *et al.,* 2015). Pepper yellowing disease intensity in Yogyakarta have reached 100% and cause detrimental yield loss of 20–100%

(Ganefianti *et al.,* 2017). *Begomovirus* incidences were first most found in lowlands, but currently have been reported in highlands as well (Kusumaningrum *et al.,* 2015). *Begomovirus* can inhibit photosynthesis, plant growth, fruit formation, and decrease fruit quality.

Bemisia tabaci are known for its genetic diversity and are categorized into biotype complex. Biotypes of *B. tabaci* received attention as invasive variants of *B. tabaci* with distinct behavior from native variants in southern United States (de Barro *et al.,* 2011). *B. tabaci* diversity has been extensively studied including various biological or molecular characteristics, such as host preferences, fecundity, ability to transmit *Begomovirus*, dispersal ability, resistance against insecticide and DNA sequences of mitochondrial cytochrome oxidase I (mtCOI) (Perring, 2001; Simón *et al*., 2003; Tay *et al*., 2012; Firdaus *et al*., 2013). Currently there are 24 biotypes named A to T that have been reported (Perring, 2001; Simón *et al*., 2003) based on their ability to transmit *Begomovirus*, induce silver leaves on squash or yellow veins on honeysuckle and nightshade, host range, and other characteristics (de Barro *et al*., 2011). Biotype is a common term used by scientist to differentiate *B. tabaci* (Tay *et al*., 2012).

Several studies have been done to identify genetic characteristics of *B. tabaci* in Indonesia. Hidayat *et al.* (2008) detected B (MEAM1) dan non-B *B. tabaci* biotypes by using PCR. Another study analyzed COI sequence and found different native genotypes in Indonesia, including Asia I, Asia II 5, Asia II 6, Asia II 7, Asia II 12, and Australia/Indonesia. Asia I is the dominant *B. tabaci* genotype found across Indonesia (Srinivasan *et al*., 2013; Rahayuwati *et al*., 2016), while Asia II 5 was detected in Bandung and Bogor, Asia II 7 in Bogor, West Java (Lestari *et al*., 2021), Asia II 6 in Cirebon, West Java (Shadmany *et al*., 2019), Asia II 7 in west areas of Kalimantan, and Asia II 12 in the western areas of Java (Firdaus *et al*., 2013). Australia/ Indonesia genetic group have been identified in Indonesia (Dinsdale *et al*., 2010).

Climate change and increase of agricultural product global trade are factors that increase *B. tabaci* population and the possibility for different or new biotypes occurrence. Incidence of viral diseases transmitted by *B. tabaci* are high in Yogyakarta. *B. tabaci* is a Cryptic Species Complex and therefore molecular identification are needed to understand *B. tabaci* population homogeneity across Yogyakarta based on its mitochondrial COI sequence.

MATERIAL AND METHODS

Insect Collection

Insect were field collected from four districts across Yogyakarta, namely Sleman, Gunungkidul, Bantul, and Kulon Progo (Figure 1). Twenty insects were collected on host plants in each location. *B. tabaci* of each location were placed in one Eppendorf tube with 90% ethanol and stored in a -4ºC cooler. Collected *B. tabaci* were used to identify genetic diversity.

Bemisia tabaci DNA Extraction

DNA was extracted from three individuals of each location using a Genomic DNA Mini Kit (Tissue) Geneaid. Individual *B. tabaci* samples were extracted by homogenizing insect in 200 µL GT buffer and 20 µL *proteinase* K. Extracted DNA samples were purified using a GD spin column as instructed by kit's manual. Total DNA samples were stored in -20 °C or directly used for PCR testing.

Amplification of mtCOI Fragments using PCR

The COI gene fragments were first amplified using LCO-1490/HCO-2198 universal primer (Folmer *et al.,* 1994); DNA where then amplified using a C1-J-2195/L2-N-3014 specific primer (Frohlich *et al.,* 1999). This primer enhances regions between 725 and 1560 of mtCOI genes. PCR was done using a 10 µL total volume consisting of 5 µL Mytaq Red Mix, 2 µL Nuclease-Free Water (NFW), 1 µL of each primer (10pmol/µL), and 1 µL DNA template. Amplification was done using PCR T100 Thermal Cycler (Bio–Rad USA) and reaction program of pre-denaturation 95°C for 3 minutes, followed by 35 denaturation cycle of 95°C for 15 seconds, annealing 50°C (LCO-1490/HCO-2198) and 53°C (C1-J-2195/L2-N-3014) for 30 second, extension 72°C for 1 minute, and final elongation of 72°C for 5 minutes. Amplification of DNA mtCOI fragments were detected using electrophoresis on 2% gel agarose with florosafe DNA stains, and 1 kb DNA ladder that was later used of nucleotide sequencing.

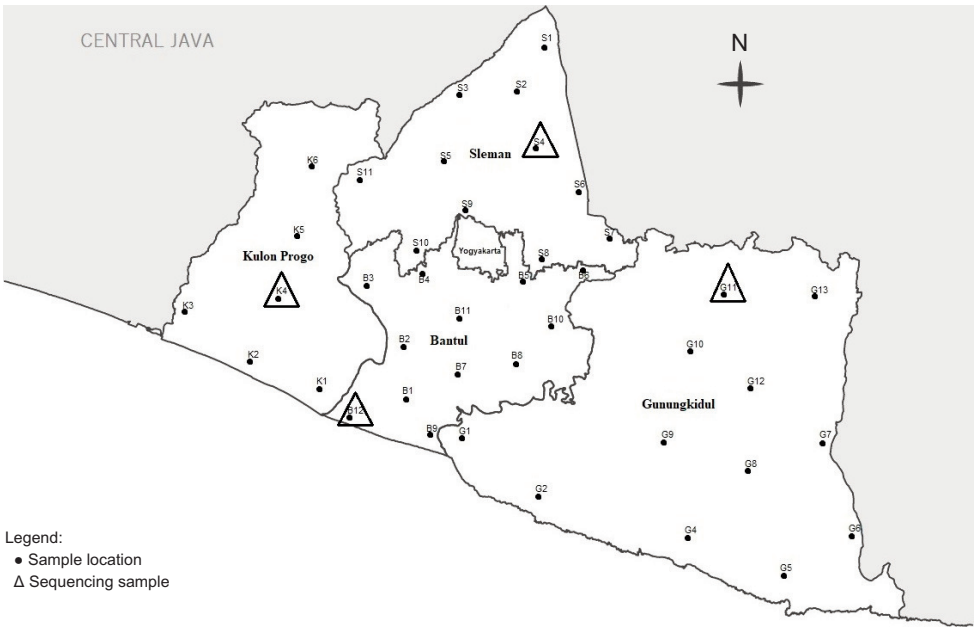


Figure 1. *Bemisia tabaci* sampling location across four districts of Yogyakarta

Table 1. Location of samples used for sequencing

No.	District	Elevation (mdpl)	Location coordinates	Plant host	Sample Code
1	Bantul	5	7°59'26.1"S 110°14'15.4"E	Eggplant	B12
2	Kulon Progo	13	7°52'53.6"S 110°08'29.7"E	Eggplant	K4
3	Sleman	261	7°42'38.3"S 110°24'54.6"E	Eggplant, Chili pepper, Cucumber	S4
4	Gunungkidul	190	7°51'15.5"S 110°38'55.1"E	Eggplant, Chili pepper	G11

Nucleotide Sequencing

Purified DNA fragments and mtCOI sequencing was done at 1st Base Company, Malaysia (Table 1). Primers used were C1- J-2195 for primer forward and L2-N-3014 for primer reverse (Frohlich *et al.,* 1999). Results of mtCOI sequences from for districts in Yogyakarta were submitted to GenBank NCBI (http://www.ncbi.nlm.nih.gov).

Phylogenetic Analysis

Phylogenetic trees were done by comparing fragment samples of *B. tabaci* from Yogyakarta to 53 COI fragments found on GenBank NCBI (National Center of Biotechnology Information) and access through http://blast.ncbi.nlm.nih.gov/Blast.cgi. The method used to construct phylogenetic tree was Maximum Likelihood with TN93 (Tamura-Nei) + G + I substitution method and 1000 bootstrap performed on MEGA 6 (Tamura *et al*., 2013). The best method was selected based on lowest Bayesian Information Criterion (BIC) value (Nei & Kumar, 2000).

RESULTS AND DISCUSSION

Phylogenetic Analysis

Bemisia tabaci from four districst across Yogyakarta were analyzed to obtain their mtCOI sequence. Relative position of mtCOI to *B. tabaci* mitochondrial genome was between 725−860. Phylogenetic analysis of *B. tabaci* were divided into 4 clades. The first clade consisted of invasive *B. tabaci* biotype B and Q, but this clade also contained biotype Ms that are distributed at eastern areas of Africa. The second clade consisted of *B. tabaci* non-B biotype and all four samples of this study were included in this clade. The third and fourth clade each consisted of one biotype that were non-B and A biotype, respectively. *Bemisia tabaci* from Sleman, Bantul, Gunungkidul, and Kulon Progo grouped well with *B. tabaci* from various area in Indonesia and Singapore, Thailand, Bangladesh, India, and Malaysia (Figure 2). Cladogram showed that *B. tabaci* from Yogyakarta and Indonesia had similar ancestry with ones from South East

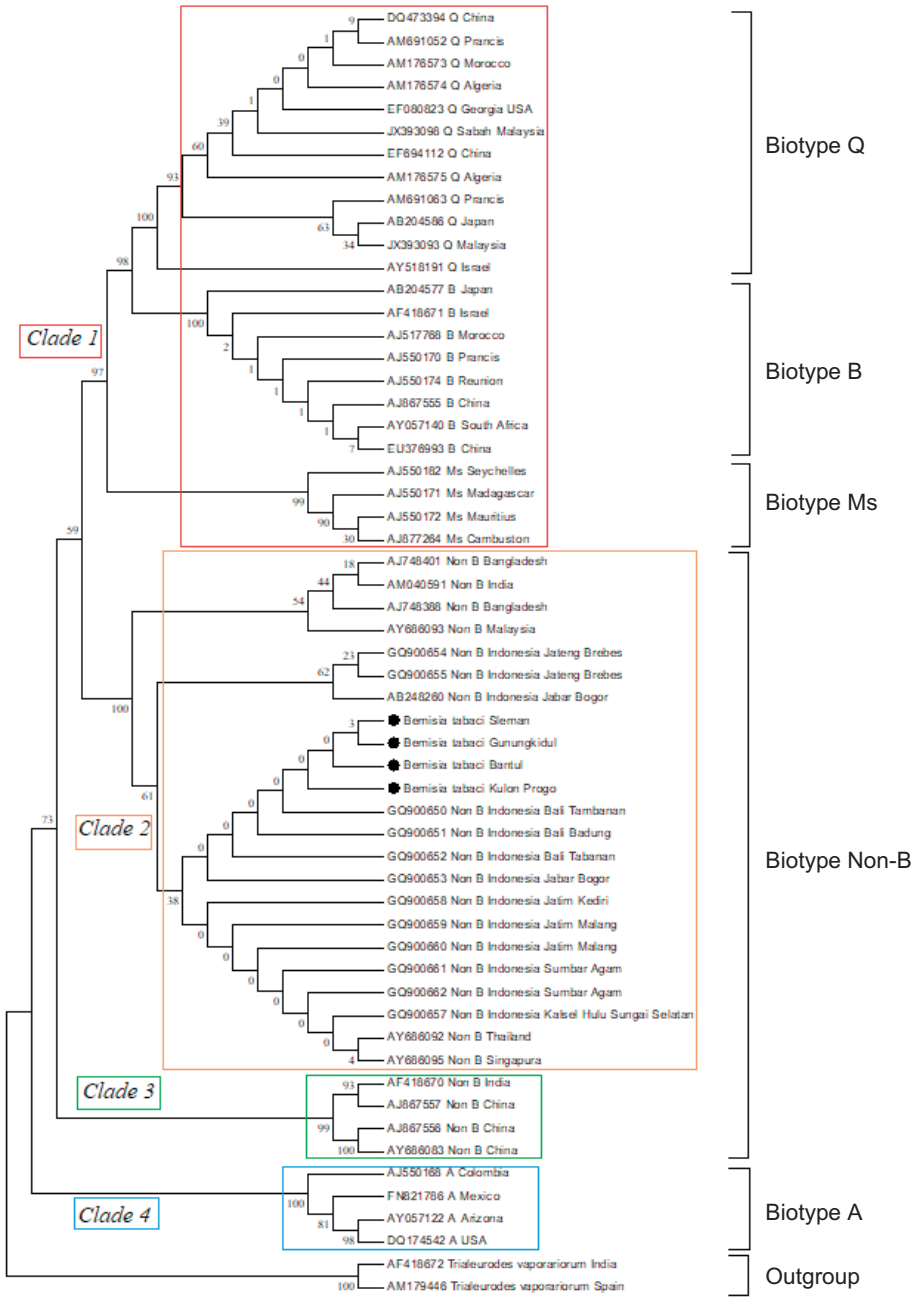


Figure 2. Phylogenetic tree of *Bemisia tabaci* mtCOI fragments from Yogyakarta compared to ones deposited on NCBI. *Trialeurodes vaporariorum* accession AF418672 and AM179446 were used as outgroups; phylogenetic tree was constructed based on a maximum likelihood model TN93 (Tamura-Nei) + G + I (1000 bootstrap) as the best model. *Bemisia tabaci* from this study were labelled using a square icon

Asia (Singapore, Thailand, and Malaysia), and some countries from South Asia (Bangladesh and India). This was supported by the bootstrap values of 100%. Nucleotide similarity percentage from the clade containing *B. tabaci* samples of this study were 100%. Phylogenetic tree showed that *B. tabaci* in Indonesia were still homogenous.

Dinsdale *et al.* (2010) stated that the Asia I group included *B. tabaci* from Indonesia, Malaysia, Singapore, Thailand, Pakistan, India, and China. Dinsdale *et al.* (2010) used an Indonesia sample with accession number AB28260 and this same sample was used in this study to construct our phylogenetic tree. The AB28260 COI fragment was 820 bp (Hidayat *et al.*, 2008) and from species collected on soybeans from Dramaga Bogor, West Java, and grouped into the same clade with the four samples used in this study and other *B. tabaci* samples from Indonesia from Genbank.

This implies that samples from Yogyakarta used in this study were non-B biotype from the Asia I phylogenetic group. Although only one genotype was discovered, yellow disease incidence on chili pepper was high and has caused significant yield loss in Yogyakarta. Divergence of *B. tabaci* genetic structure that was first suspected to cause this high incidence in Yogyakarta was not proven based on the cladogram results of this study.

Analysis of Molecular Structure

Alignments can be used to determine whole or partial homology and genetic relationship between species. Alignment results (Figure 3) were obtaining by analyzing *B. tabaci* DNA sequence from samples of this study and several *B. tabaci* from Genbank. Results showed that *B. tabaci* from Indonesia, especially Yogyakarta, had nucleotide similarity of 100% (homogenous) and close genetic relationship with *B. tabaci* from Bangladesh (AJ748401) that showed 99.56% similarity (Table 2). Meanwhile, other sequences of non-B biotypes from China (AJ 867556), Q Malaysia (JX393093), and A Colombia (AJ550168) had lower similarity of 79.35%, 83.55%, and 82.19%, respectively.

Amino acid sequence reading (Figure 4) showed no difference between amino acids from *B. tabaci* non-B biotype from Indonesia or *B. tabaci* from Bangladesh (AJ748388). *B. tabaci* non-B biotype from China (AJ867556), A from Colombia (AJ5501 68) had amino acid similarity of 95.45% and 97.78%. *B. tabaci* Q biotype from Malaysia (JX393 093) had different amino acid from Indonesian non-B.

Nucleotide sequence alignment (Figure 3) and amino acid alignment (Figure 4) showed no difference between for *B. tabaci* non-B biotype of this study. Figure 5 showed nucleotide composition between *B. tabaci* sequences from this study with one from Bangladesh (AJ748388). Results showed no signi-

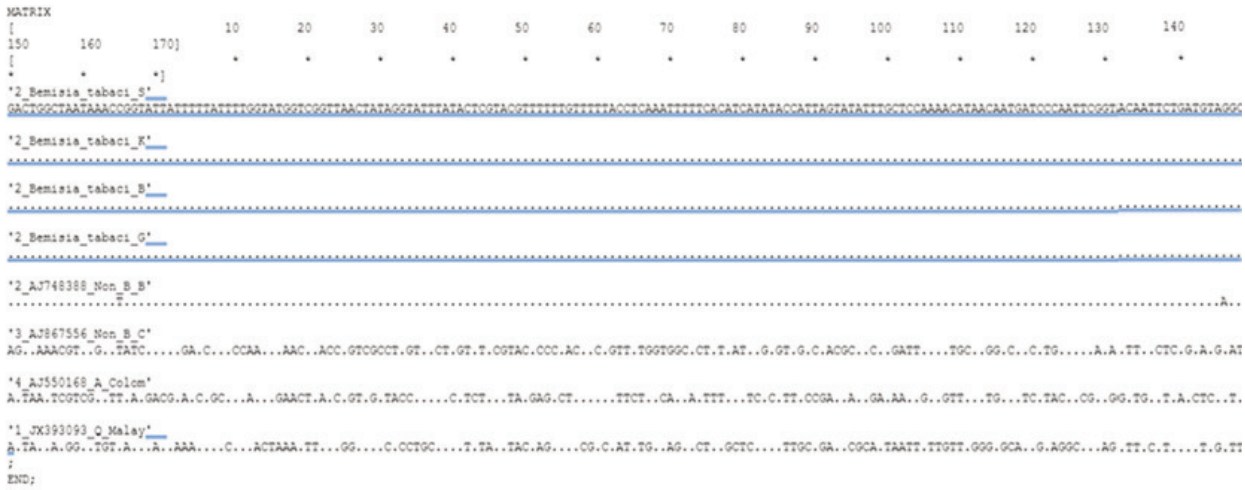


Figure 3. Nucleotide arrangement of *Bemisia tabaci* polymorphic site

Table 2. Nucleotide similarity percentage of *Bemisia tabaci* samples compared to several isolates from different clades

No.	Isolate	1	2	3	4	5	6	7	8
1	*Bemisia tabaci* Sleman	ID							
2	*Bemisia tabaci* Gunungkidul	100	ID						
3	*Bemisia tabaci* Bantul	100	100	ID					
4	*Bemisia tabaci* Kulon Progo	100	100	100	ID				
5	AJ748388 *Bemisia tabaci* Non B Bangladesh	99.694	99.694	99.694	99.694	ID			
6	AJ867556 *Bemisia tabaci* Non B China	80.313	80.313	80.313	80.313	80.309	ID		
7	AJ550168 *Bemisia tabaci* A Colombia	82.004	82.004	82.004	82.004	82	78.803	ID	
8	JX393093 *Bemisia tabaci* Q Malaysia	81.752	81.752	81.752	81.752	81.261	79.558	80.286	ID

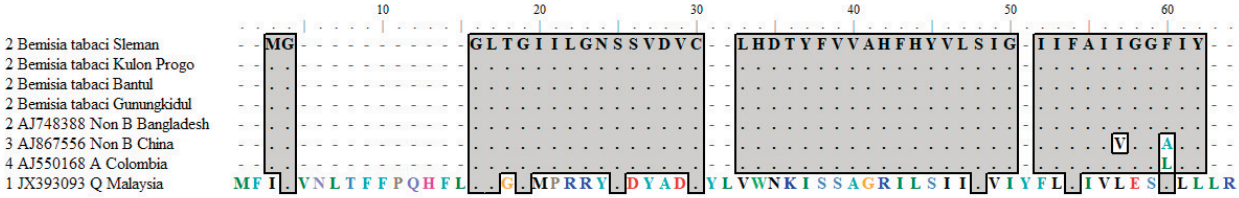
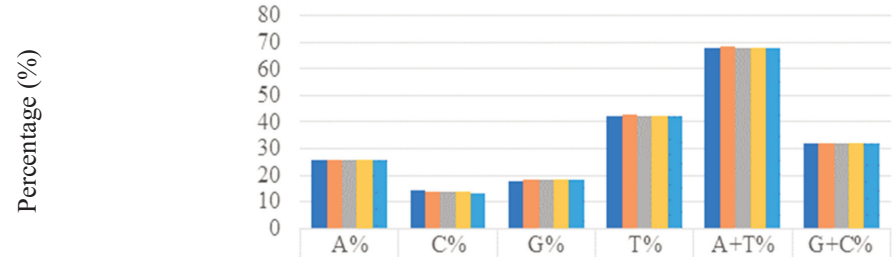


Figure 4. Alignment of *Bemisia tabaci* amino acid



	A%	C%	G%	T%	A+T%	G+C%
Bemisia tabaci Bantul	25.71	14.04	18.01	42.24	67.95	32.05
Bemisia tabaci Sleman	25.59	13.54	18.26	42.61	68.2	31.8
Bemisia tabaci Kulon Progo	25.66	13.64	18.4	42.3	67.96	32.04
Bemisia tabaci Gunungkidul	25.82	13.6	18.51	42.07	67.88	32.12
Bemisia tabaci Non-B Bangladesh	25.75	13.43	18.53	42.29	68.03	31.97

Figure 5. Nucleotide percentage composition of *Bemisia tabaci* from Yogyakarta

ficant differences between them. According to Simon *et al.* (1994), higher percentage of A+T nucleotide is a characteristic of insect mitochondrial DNA.

Previous study on *B. tabaci* genetic diversity in Yogyakarta also showed the discovery of only non-B biotype (Rahayuwati *et al.,* 2016). No differences between nucleotide and amino acid sequence composition were found. High yellow disease incidence on chili pepper crops in Yogyakarta and no biotype differences with previous studies indicate that current management strategies require improvement.

CONCLUSION

B. tabaci populations across Yogyakarta were still homogeneous based on DNA and amino acid sequences. Primer C1-J-2198 and L2-N-3014 were stable primers to identify *B. tabaci* biotypes. Only non-B biotype was discovered and was included in Asia I group. In this study no nucleotide arrangements differences were discovered between samples of four districts.

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