Population Differentiation of Three Biotypes of *Bemisia tabaci* in China by DNA Polymorphism

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Abstract: The differentiation of three haplotypes of *Bemisia tabaci* populations in China was analyzed using RAPD-PCR in this study. Five *B. tabaci* populations were collected in the north, middle and south of China, respectively, including three B biotype populations, the indigenous haplotype populations (NaC haplotype) and a new haplotype population from ornamental plants (C_v haplotype). In addition, the greenhouse whitefly *Trialeurodes vaporariorum*, was used as a outgroup population in this analysis. The results of the cluster analysis using genetic distances indicated that: first, the population relationship between the NaC haplotype and the C_v haplotype was more close than that between B biotype and NaC haplotype, or that between B biotype and the C_v haplotype; second, the population relationships of the three haplotype of *B. tabaci* in China was intraspecific; and the C_v haplotype was potentially originated in China or the neighboring countries.

Key words: *Bemisia tabaci*; population differentiation; biotype; haplotype; DNA polymorphism
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中国 3种不同生物型烟粉虱的种群 DNA多态性研究

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摘要: 利用基因组 DNA的多态性研究了中国 3种不同生物型烟粉虱的种群分化. 烟粉虱种群分别采自北京、扬州和广州的不同寄主上, 烟粉虱生物型包括 B型、本地型(NaC)和一种新的生物型(C_V型). 对 3种生物型烟粉虱的 RAPD-PCR结果进行遗传距离聚类分析, 结果显示烟粉虱中国本地型和 C_V型的种群关系比较近缘, 二者与 B型种群的亲缘关系较远. 3个烟粉虱生物型的种群分化属于种内分化, 据此推测 C_V型烟粉虱可能起源于中国或其临近地区.

关键词: 烟粉虱; 种群分化; 生物型; 单模; DNA多态性

Bemisia tabaci (Gennadius) has been a worldwide serious pest of agriculture and horticulture over the last decade. It can seriously injure plants by sucking juices causing wilting, stunting, irregular ripening of fruits. In addition, the excretion of honeydew induces the growth of the sooty mold that can block sunlight from reaching the leaf surface, thus reducing photosynthesis and fruit marketability[1]. Adults can also transmit more than 70 viruses from infected to healthy plants, most of which are important mainly in commercial crops[2-3]. Up to now, at least 24 biotypes of *B. tabaci* have been reported, which differed in their biological, physiological

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and morphological characteristics and there are still many haplotypes of *B. tabaci* that have not been identified to biotypes[3-4]. Apparently, among them, the B biotype is the most destructive one[5-6]. *B. tabaci* was first record in China in 1949[7]. The outbreak of this pest in China in the middle of 1990's was thought to due to the invasion of a new biotype on ornamental plants[8]. This biotype was identified by RAPD-PCR and cytochrome oxidase I gene sequences (COI) to be B biotype[8-9]. *B. tabaci* B biotype has been reported in 25 provinces in China and the loss due to this pest on vegetables, fruits, and ornamentals is increasing[9-10]. In contrast to B biotype, the *B. tabaci* recorded earlier in China is though to be a native haplotype "NaC" (native of China). In addition to the B and the native biotype, another new haplotype (C_v haplotype) was found in Guangzhou on *Codiaeum variegatum*. This C_v haplotype differed biologically from the B biotype and NaC haplotype. All the studies focalized on *B. tabaci* were the B biotype identification till now[8-9, 11]. This paper is focusing on the description of the population relationships among three haplotypes of *B. tabaci* in China by their genomic DNA polymorphism.

1 Materials and methods

1.1 Insect collection

Bemisia tabaci populations were collected from different host plants in China (Tab. 1), and they were identified according to the basic characters of the fourth instar which is usually called "pupal cases"[12-13] with a stereo microscope (Leica MZ125). The haplotypes of these populations were then identified by a RAPD-PCR method[9]. The specimens used in this study were either fresh or $\varphi = 95\%$ ethanol soaked samples. In addition, *Trialeurodes vaporariorum* (T_v) was used as an outgroup.

Tab. 1 The host plant, location and collection date of the different whitefly populations

code	species	collection location	host plant	collection date	biotype/haplotype
NaC	*B. tabaci*	Yangzhou, Jiangsu	*Gossypium hirsutum*	Oct., 2002	native haplotype
B_1	*B. tabaci*	Yangzhou, Jiangsu	*Brassica oleraceal*	Oct., 2002	B biotype
B_2	*B. tabaci*	Haidian, Beijing	*Lycospersicum esculentum*	Aug., 2000	B biotype
B_3	*B. tabaci*	Guangzhou, Guangdong	*Brassica oleraceal*	Jun., 2003	B biotype
C_v	*B. tabaci*	Guangzhou, Guangdong	*Codiaeum variegatum*	Aug., 2002	C_v haplotype
T_v	*T. Vaporariorum*	Guangzhou, Guangdong	*Euphorbia pulcherrima*	Sept., 2001	

1.2 DNA extraction

DNA of the different whitefly populations was extracted from single specimen[6]. Samples were washed briefly in sterile distilled water and dipped in TE buffer for 4-6 h before homogenization. Whitefly individual was homogenized in a 200 μL micro centrifuge tube using a 100 μL head-melted pipette tip in 10 μL lysis buffer including 1% SDS, 10 mmol/L Tris-HCl, pH 8.0, 25 mmol/L NaCl, 25 mmol/L EDTA, 200 μg/mL proteinase K (Merck kGaA Co., Germany). After homogenization, another 20 μL lysis buffer was added. After that, the homogenized solution was incubated at 57 ℃ for 3 h, and then boiled at 95 ℃ for 5 min in order to inactivate the proteinase K, and then DNA samples were stored at −20 ℃ for use.

1.3 RAPD-PCR amplification

Four primers were used in the RAPD-PCR amplification, F_2(5′GAGGATCCCT 3′), F_{12}(5′ACGCTACCAG 3′), H_9(5′TGTAGCTGGG 3′) and H_{16}(5′TCTCAGCTGG3′) (synthesized in SBS Genetech Co., Ltd.), and 10 individuals of each whitefly population were test. Polymerase chain reactions were done in PTC-100 thermocycler (MJ Research Co. Ltd.). The amount of reagents were 25 μL containing double sterile distilled water, 1× PCR buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 15 pmol/L PCR primer, 2 unit *Taq* DNA polymerase (Sino-America Biotech, China) and DNA template 10-20 ng. Amplification was done using the following procedures: one cycle of 5 min at 94 ℃, 2 min at 40 ℃ and 3 min at 72 ℃, followed by 39 cycles of 1 min at 94 ℃, 1.5 min at 40 ℃ and 2 min at 72 ℃. PCR products were electrophoresed directly using 15 g/L agarose gel at 8 V/cm for 2 h with 1× TAE, afterwhich the gel was dyed in 10 mg·mL^{-1} ethidium bromide for 30 min. A 100 bp DNA ladder (Sino-America Biotech, China) was used in each gel

Bands on the gels were made visible using a UV light and then photographed.

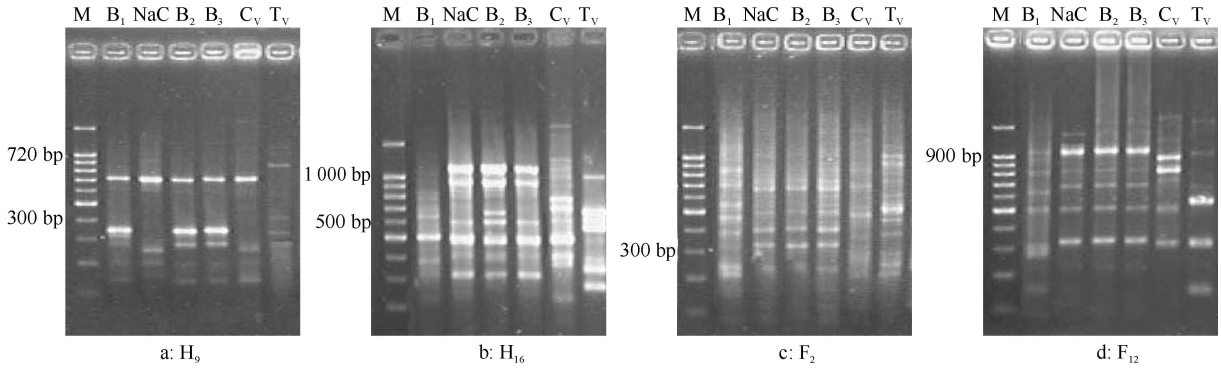
1.4 Data analysis

PCR amplification products that migrated at the same distance in agarose gel were scored as the same loci, and the presence or absence of bands was recorded as 1 or 0. Then these data were used to calculate the genetic distances (D). The genetic distances were calculated as[14-15]: $D = -\ln I$, $I = I_{ab} / (I_a I_b)^{1/2}$, $I_{ab} = \sum a_i b_i / n$, $I_a = \sum a_i^2 / n$, $I_b = \sum b_i^2 / n$, where n is the total number of loci; a_i, b_i is the gene frequency at i loci that observed in specimen A and B, respectively. Dendrogram was drawn according to the results of genetic distances matrixes with averaged linkage methods using the cluster program of SAS 6.12[16].

2 Results

2.1 RAPD-PCR amplification

The RAPD-PCR amplifications of the six whitefly populations with 4 primers are shown in Fig. 1. There are 183 generated bands with an averaged of 7.6 bands for each sample. The sizes of most of the amplified fragment ranged between 100 bp to 1 500 bp.



M: a 100 bp DNA ladder; NaC: native haplotype; B_1-B_3: B biotype; C_v: new haplotype; T_v: *T. vaporariorum*

Fig. 1　The RAPD-PCR patterns of six whitefly populations generated by 4 primers

The amplified result of RAPD-PCR showed that there was a clearly distinct difference between the two different haplotypes of whitefly populations. As shown in Fig. 1a, greenhouse whitefly (T_v) distinguished from the five *B. tabaci* populations with a band absent at about 720 bp. For *B. tabaci* B biotype populations (B_1, B_2 and B_3), they were distinguished from the native population, NaC haplotype, and the C_v haplotype by 2 bands located near 300 bp. Also in Fig. 1b, all the *B. tabaci* populations had a same band at about 500 bp, which was absent in the greenhouse whitefly. On the other hand, the three *B. tabaci* B biotype populations had 2 bright bands at about 950 bp and 1100 bp while the NaC and the C_v haplotypes had no bands at these loci. Such findings were also existed in the position of 300 bp and 900 bp in Fig. 1c and Fig. 1d, respectively. The amplification result indicated that *T. vaporariorum* population was obviously different from the five *B. tabaci* populations; the three *B. tabaci* B biotype populations were similar to each other and the NaC haplotype was to some extent similar to the C_v haplotype population.

2.2 The genetic distances of the six whitefly populations

The results of the genetic distances indicated that the genetic distances among the greenhouse whitefly population and the *B. tabaci* populations were the largest with an average of 1.129 9 (ranged from 1.039 1 to 1.201 4). Meanwhile, the genetic distances among the three populations of *B. tabaci* B biotype from Beijing, Jiangsu and Guangdong Provinces were the smallest with an average of 0.091 2 (ranged from 0.086 0 to 0.094 5); on the other hand, the genetic distance between the NaC and the C_v biotypes (0.494 0) is smaller than that between the NaC and B biotypes (0.845 3-0.893 8)(Tab. 2).

2.3 Cluster analysis of the whitefly populations with genetic distances

Data of the genetic distances of the six whitefly populations are depicted in Fig. 2. In Fig. 2, the greenhouse

Tab. 2 Genetic distances matrix of six whitefly populations

code	NaC	B_1	B_2	B_3	C_v	T_v
NaC	0					
B_1	0.8453	0				
B_2	0.8672	0.0945	0			
B_3	0.8938	0.0860	0.0930	0		
C_v	0.4940	0.7554	0.7982	0.7079	0	
T_v	1.0452	1.2014	1.1895	1.1742	1.0391	0

whitefly was first separated from the five *B. tabaci* populations when $\lambda = 1.1299$. The five *B. tabaci* populations were clustered into two groups: one group contained three populations of *B. tabaci* B biotype, in which the *B. tabaci* B biotype B_1 population and B_3 population clustered together first and then grouped with the *B. tabaci* B biotype B_2; the second group of *B. tabaci* branch included the NaC haplotype and the C_v haplotype, which indicated that the NaC haplotype is more closed to the C_v haplotype than the others.

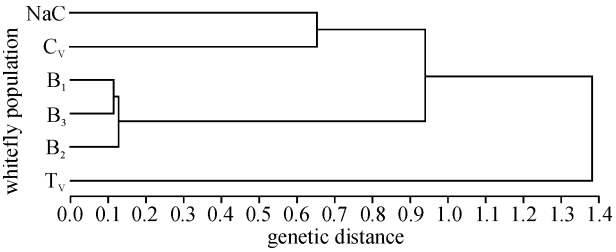


Fig 2 The cluster dendrograms of six whitefly populations with genetic distances

3 Discussion

Biotypes identification is important and necessary to evaluate a *B. tabaci* population. According to our investigations, there are at least 3 kinds of biotypes or haplotypes of *B. tabaci* in China with distinct differences in the biological and morphological characteristics among them.

The results obtained in this study indicated that the genetic distances indicating that the 3 B biotype populations belong to one group (B biotype group). This finding is consistent with the earlier results of Luo et al[8] and Qiu et al.[9]. Also these findings further indicated that the genetic and evolutional relationships between the NaC haplotype and the C_v haplotype were the closest followed by the relationship between the two non-B haplotypes and the B biotype. The NaC haplotype was thought to be the biotype "A", but the COI sequences analysis indicated there much differences between the A biotype from Arizona, USA and the NaC haplotype of China.

The C_v haplotype is a new undefined biotype found in the southern China on some ornamental plants recently, which bioassay study showed that it is a very serious and dangerous haplotype on ornamental plants, and both the NaC haplotype and the C_v haplotype belong to an undefined group of Asian biotype, which also includes the *B. tabaci* populations from Nepal, Nauru and Taiwan.

Due to the high ability of the *B* biotype in damage, great attention has been focused on identifying the biotype and finding the origin of *B. tabaci* using silverleaf reaction, esterase, RAPD-PCR, electromorph amplification and DNA fragment sequences[5-6,17-21]. All their results demonstrated that there were at least two different *B. tabaci* biotypes in each country, of which, the B biotype is one of them. *B. tabaci* B biotype has been a cosmopolitan pest since the last decade[3].

The result in this study showed that the C_v haplotype was more close to the native NaC haplotype, which is not considered as a serious haplotype in China, but the damage due to this haplotype is higher than the NaC populations, and it need further study on this puzzled area.

RAPD-PCR analysis in this paper showed that the relationships of the three populations of *B. tabaci* B biotype in China were intraspecific, and the finding that the C_v haplotype is very close to the indigenous haplotype (NaC haplotype) indicated this new haplotype might existed in China for long time, it maybe originated in China or from the neighboring countries. These findings are in accordance with those reported by Wu et al[11]. More work should be done in the future to study the genetic and evolutional relationship among the new haplotype C_v and other *B. tabaci* haplotypes in China.

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