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Relative amount of symbionts in *Bemisia tabaci* (Gennadius) Q changes with host plant and establishing the method of analyzing free amino acid in *B. tabaci*

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The impact of symbionts on their insect hosts depends on their infection density. In the current study, we investigated the effects of host plants (cucumber, cabbage and cotton) on the relative amount of symbionts *Portiera* and *Hamiltonella* in the whitefly *Bemisia tabaci* (Gennadius) Q. The relative amounts of symbionts in 3 host plant *B. tabaci* Q populations with the same genetic background were evaluated by quantitative PCR. The whiteflies of cabbage population harbored more *Portiera* than those of cucumber and cotton populations, and the relative amount of *Portiera* did not differ statistically between cotton and cucumber populations. The whiteflies of cucumber and cabbage populations harbored more *Hamiltonella* than that of cotton population, and the relative amount of *Hamiltonella* did not differ statistically between cabbage and cucumber populations, indicated that the relative amount of symbionts was significantly affected by host plant. In addition, the method of analyzing the composition of free amino acid in *B. tabaci* was established. Twenty-eight amino acids were detected in the *B. tabaci* Q population, the non-essential amino acids, such as glutamate, glutamine, alanine, proline and the essential amino acid arginine were the dominant amino acids in *B. tabaci* Q.

Introduction

The sweet potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a destructive pest of many field and protected crops worldwide. *B. tabaci* has been regarded as a species complex consisting of many biotypes that are morphologically indistinguishable but differ in host range, feeding behavior, virus transmission, insecticide resistance, or the symbionts that they harbor.[1-5] The two most invasive and destructive biotypes are biotype B (also known as Middle East-Minor Asia 1 genetic group, hereafter referred to as B) and biotype Q (also known as the Mediterranean genetic group, hereafter referred to as Q). During the past two decades, *B. tabaci* B and Q have spread from their native ranges to as many as 60 countries and resulted in serious economic losses to agricultural production worldwide.[6]

Bemisia tabaci was first recorded in China in the late 1940s,[7] but the crop damages and loses caused by this insect had not been serious until the introduction of *B. tabaci* B in the 1990s.[8] Since then, B rapidly invaded the entire country, and has led to serious yield losses in many crops.[9,10] Q was first found in Yunnan Province in 2003 and was considered a new, invasive whitefly in China.[10] During the past several years, Q has gradually displaced the well-established populations of B and has become the dominant form of *B. tabaci* in field agricultural systems in most parts of China.[5,11]

Like many other insects, *B. tabaci* hosts bacterial endosymbionts.[12] The symbionts of insects are generally divided into two groups: primary symbionts (referred to as P-symbionts) and secondary symbionts (referred to as S-symbionts).[12,13] To date, seven symbionts (*Portiera*, *Hamiltonella*, *Cardinium*, *Wolbachia*, *Fritschea*, *Arsenophonus* and *Rickettsia*) have been reported from *B. tabaci*.[14-18] The P-symbiont *Portiera* provides nutrients that supplement the insufficient nutrients that *B. tabaci* obtains from its restricted diet of plant phloem,[12,15] while S-symbionts play important roles in *B. tabaci* biology and ecology.[19-24] For example, high bacterial densities in *B. tabaci* are correlated with the insect's ability to detoxify insecticides and other toxic compounds.[22]

The amount of the symbiont (the number of symbionts per host whitefly) is important because it influences both the efficiency of transmission of the symbiont to the offspring and the

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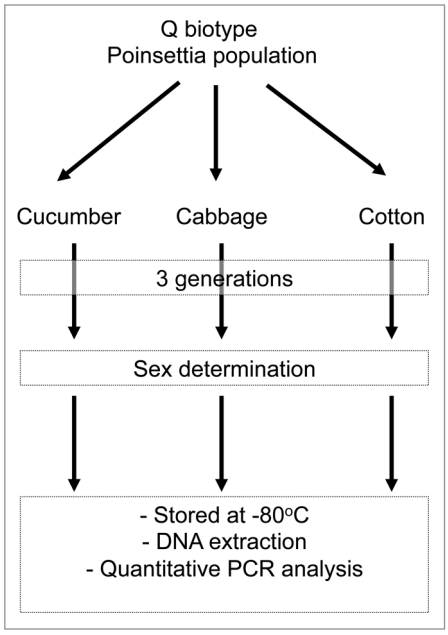

Figure 1. The three host plant *B. tabaci* Q populations (cotton, cucumber and cabbage) used in this study.

virulence of the symbiont.[25] Latest research from our laboratory has recently shown that the relative amount of symbionts in insect hosts *B. tabaci* B changes with host-plant adaptation and insecticide resistance.[26]

The nutritional physiology of whiteflies is governed by two linked factors; the phloem sap on which whiteflies feed and the presence of P-symbiont. Phloem sap contains low concentrations of nitrogen in the form of free amino acids that are dominated by non-essential amino acids. The main nitrogen sources of whiteflies are free amino acids found in very low and unbalanced concentrations in the phloem sap and it is well known that whiteflies, like other animals, cannot synthesize all the amino acids.[12] *Portiera* supplement this diet through the provision of essential amino acids to the whitefly.[12] However, so far, only one study has studied the amino acids composition in *B. tabaci* using the high performance liquid chromatography (HPLC).[27]

In the current study we: (1) measured the effect of the host plant on the relative amount of P- and S-symbionts in *B. tabaci* Q populations; and (2) established the method of analyzing the composition of free amino acid in *B. tabaci*.

Results

Effects of host plant on symbionts. The whiteflies of cabbage population harbored more *Portiera* than those of cucumber and cotton populations ($F_{2, 6}$ = 15.893, p = 0.004) and the relative amount of *Portiera* did not differ statistically between cotton and cucumber populations (**Fig. 2**). The whiteflies of cucumber and cabbage populations harbored more *Hamiltonella* than that of cotton population ($F_{2, 6}$ = 31.765, p = 0.001), and the relative amount of *Hamiltonella* did not differ statistically between cabbage and cucumber populations (**Fig. 2**).

The composition and percentage of free amino acid in the *B. tabaci* Q population. Twenty eight amino acids were detected in the *B. tabaci* Q population (**Fig. 3**). The non-essential amino acids, such as glutamate, glutamine, alanine, proline and the essential amino acid arginine were the dominant amino acids. The percentage of these five amino acids was all above 8.0% (**Fig. 3**). In addition, the percentage of the essential amino acids histidine and tyrosine was between 4.0–8.0% (**Fig. 3**). The percentage of the rest amino acids was all below 4.0%.

Discussion

In the current study, our survey data of the 3 host plant sub-populations derived from the same parental population of *B. tabaci* Q indicate that host plants substantially affects the amount of the S-symbionts in *B. tabaci*. This is because the amounts of P- and S-symbionts were all significantly different among the 3 host plant sub-populations. This result almost agrees with that of Pan et al. (2013)[26] which reports that significant variations were exhibited in the amounts of P- and S-symbionts among different host plant-adapted *B. tabaci* B sub-populations with the same genetic background.[26] Host plants exhibited marked impacts on the amount of symbionts in Q whiteflies (**Fig. 2**). However, as discussed in our latest study,[26] possible founder effects might result in the S-symbiont-host plant association pattern from this experiment. Additionally, other factors such as the genetic background, population inertia and stochasticity also might confound the impacts of host plants on the amount of S-symbionts and cause the discrepancies.

Previous studies showed that multiple infections, i.e., infections by different symbionts within the same individuals, are common among diverse insect taxa.[5,29-34] Although all 3 host-plant populations contained higher densities of P-symbiont *Portiera* than of S-symbionts *Hamiltonella*, the amount of each symbiont often differed among the three host populations. In particular, the amount of the *Portiera* was significantly affected by co-infection with the *Hamiltonella* in the cucumber population of *B. tabaci* Q. This result is consistent with that of Pan et al. (2013)[26] who report the amount of *Portiera* and S-symbionts influence each other in *B. tabaci* B cabbage and cucumber populations, respectively.[26] However, the amount of *Portiera* was not influenced by co-infection with S-symbionts in both *B. tabaci* B[26] and Q cabbage populations (**Fig. 2**). This disparity may be because of the fact that different plant species harbor different phytotoxin or secondary metabolites that have antibiotic effects on herbivores.

More generally, this study illustrates the useful method to analyze the amino acid composition in the small insect whitefly. Wang et al. (2012)[27] used the method of HPLC to analyze the free amino acids in *B. tabaci*, 20 free amino acids was detected in *B. tabaci*, however, 28 free amino acids was detected in this study. It looks like our method was easier to operate and much cheaper (**Fig. 3**), this method should be taken to evaluate the free amino acids in small insect in future. Latest study showed that *Portiera* was able to synthesize the essential amino acids threonine and tryptophan and the nonessential serine and lack the last gene of phenylalanine, valine, leucine and isoleucine pathways.[35] Further

research is needed to determine the composition of free amino acid in the three *B. tabaci* Q (cotton, cucumber and cabbage) populations. If so, we can know better the relationship between the composition of acids, the amount of symbionts in *B. tabaci* and the host plant.

Materials and Methods

Plant cultures. Three crop species from 3 families, which have been widely cultivated in China, were used in the experiments: cabbage (*Brassica oleracea* L., cv Jingfeng 1), cucumber (*Cucumis sativus* L., cv Zhongnong 12) and cotton (*Gossypium herbaceum* L., cv DP99B). All the 3 field crops were grown in a potting mix (a mixture of peat moss, vermiculite, organic fertilizer, perlite in a 10:10:10:1 ratio by volume) in 1.5 L pots (1 plant/pot) and enclosed in whitefly-proof screen cages under natural light and controlled temperature (26 ± 2°C) in a glasshouse. When these plants grew to the 5–7 true leaf stage, they were used to maintain the corresponding *B. tabaci* laboratory populations (see next section).

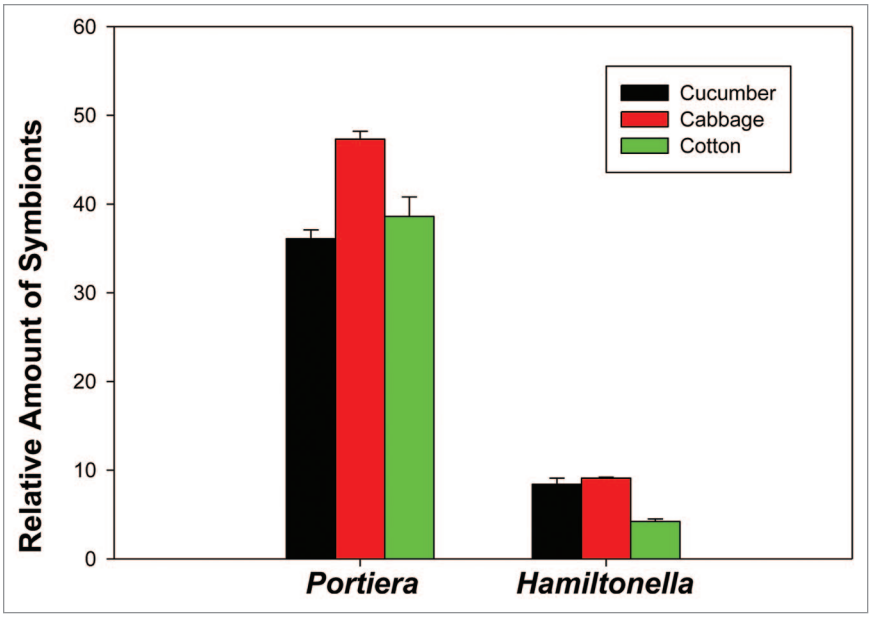

Figure 2. Relative amount of *Portiera* and *Hamiltonella* in three *B. tabaci* Q populations (cucumber, cabbage and cotton) as determined by quantitative PCR (normalized according to the amount of actin gene). Values for relative amount of symbionts are means ± SEM of three replicates for each kind of plant. The data were analyzed with one-way analysis of variance. For each kind of symbiont, different letters above the bars indicate significant differences among the three populations (Tukey test, $P < 0.05$).

***Bemisia tabaci* Q host populations.** The three host populations of *B. tabaci* Q (cotton, cucumber and cabbage) used in the present study were derived from the same parental population (**Fig. 1**). The parental Q whitefly population used has been maintained on poinsettia (*Euphorbia pulcherrima* Wild. ex. Klotz.) in isolated whitefly-proof screen cages under natural lighting and controlled temperature (26 ± 2°C) in a glasshouse.[5] Only the P-symbiont *Portiera* and S-symbiont *Hamiltonella* were detected from the parental population using the method of PCR and fluorescence in situ hybridizations (FISH) (Qi Su, unpublished data). The purity of biotype has been monitored and determined by the cleavage amplified polymorphic sequence (CAPS) and mitochondrial cytochrome oxidase I genes (*mtCOI*) for 15 adults per generation.[28] After three successive generations, the three host populations were used.

Quantitative real-time PCR (qPCR). Females from each of the three populations (cabbage, cucumber and cotton) of *B. tabaci* Q were collected (giving 3 samples for each population with 20 females per sample), stored at -80°C and then subjected to DNA extraction with a TIANamp Genomic DNA Kit (Tiangen Biotech Co., Ltd). The purified DNA from each sample was eluted with 200 μL of AE buffer supplied in the kit. The symbionts in the samples were quantified by qPCR according to the method of Pan et al. (2013).[26] The gene names, amplicon sizes and primers are listed in **Table 1**.

Free amino acid analysis. Newly emerged (0–8 h post-emergence) Q whitefly adults were collected from poinsettia. Twenty mg whiteflies as one replicate that kept in 1.5 ml tube were fully homogenate, shaked for 2 min on the vortex shaker (QL-866, Qilinbeier). The sample was then centrifuged at 14,000 rpm for 10 min, 1 ml supernatant was got and was mixed with an equal volume of n-hexane and then centrifuged at 10,000 rpm for 10 min (wipe off fat), the supernatant was discarded, 0.5 ml of underlayer was got, and was mixed with an equal volume of 8% sulfosalicylic acid and then centrifuged at 10,000 rpm for 10 min (wipe off protein), 0.5 ml of the supernatant concentrated to dryness and 0.75 ml sample buffer was used to redissolved, and then filtrated through 0.45 μm membrane. Free amino acid in whiteflies was analyzed by amino acids analyzer (S433, sykam)

Data analysis. The free amino acids were determined by automatic amino acid analyzer. A total of 28 different peaks were detected, the peak area represents the respective amino acid content. The percentage of the various amino acids was calculated by

Table 1. Oligonucleotide primers used in quantitative PCR

Gene	Amplicon size (bp)	Primer sequence (5' to 3')
Portiera 16S rDNA★	229	TAG TCC ACG CTG TAA ACG
		AGG CAC CCT TCC ATC T
Hamiltonella 16S rDNA▲	243	GCA TCG AGT GAG CAC AGT TT
		TAT CCT CTC AGA CCC GCT AGA
Actin●	130	TCT TCC AGC CAT CCT TCT TG
		CGG TGA TTT CCT TCT GCA TT

★Sequences obtained from Pan et al. (2013). ▲Sequences obtained from Brumin et al. (2011). ● Sequences obtained from Ghanim and Kontsedalov (2009).

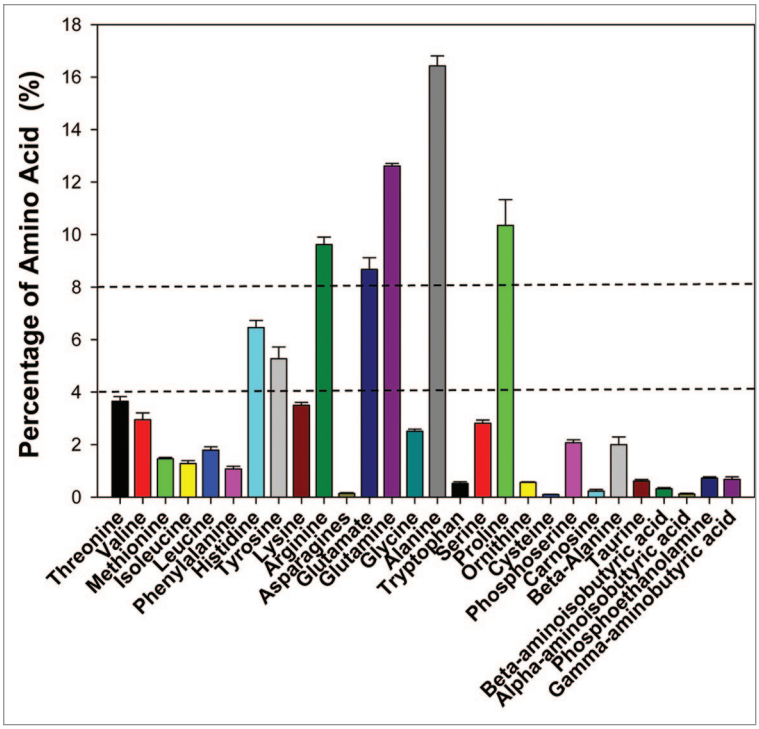

Figure 3. Free amino acids composition in the *B. tabaci* Q poinsettia population. Free amino acid in whiteflies was analyzed by amino acids analyzer (S433, sykam, Germany). A total of 28 different peaks were detected, the peak area represents the respective amino acid content. The percentage of the various amino acids was calculated by the formula: the respective amino acid content / the total amino acids content × 100%.

the formula: the respective amino acid content / the total amino acids content × 100%. The differences in relative amount of symbionts in *B. tabaci* Q reared on three host plants were analyzed using one-way analysis of variance (ANOVA), and the means were compared by the Tukey test at $p < 0.05$. All statistical analyses were performed with SPSS version 17.0 (SPSS Inc.).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Author Contributions

Y.J.Z., H.P.P., W.X., S.L.W., Q.J.W. and BYX designed the experiments. H.P.P., Q.S., L.Z. and B.M.L. performed the experiments. H.P.P., L.Z. and X.G.J. analyzed the data. H.P.P., X.G.J., Q.S. and Y.J.Z. wrote the paper.

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