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Molecular basis of mutual benefits between Cucurbit chlorotic yellows virus (CCYV) transmission and imidacloprid resistance in *Bemisia tabaci*

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Abstract

Increased insecticide resistance among insect vectors and arboviruses results in significant economic losses to agricultural production and poses a continuous threat to food safety. To date, many studies have been conducted on how virus-infected host plants indirectly and plant virus directly change the physiological characteristics of insect vectors. However, the relationship between the insecticide resistance of the insect vector and the virulence of the viral pathogen has not received sufficient attention. Here, we investigated the effect of Cucurbit chlorotic yellows virus (CCYV), a plant virus transmitted by whitefly (*Bemisia tabaci*) in a semi-persistent manner, on the resistance of *B. tabaci* to the neonicotinoid imidacloprid. We found CCYV enhanced the resistance of *B. tabaci* to imidacloprid. The *CYP6CM1* gene was significantly up-regulated in viruliferous susceptible *B. tabaci* compared with non-viruliferous *B. tabaci* after an acquisition access period of 10 d, including its upstream regulators. Silencing the *CYP6CM1* gene by RNA interference reduced the ability of *B. tabaci* to acquire CCYV compared with the control. Additionally, imidacloprid-resistant *B. tabaci* outperformed sensitive adults in terms of virus transmission ability. These results suggest that CCYV enhances the ability of imidacloprid-sensitive *B. tabaci* to resist adverse external factors (insecticides). In turn, *B. tabaci* resistance affects virus transmission. Thus, to a certain extent, the plant virus and insect vector establish a mutually beneficial relationship that facilitates virus transmission.

Key message

- This research aims to reveal the correlation between the virus transmission ability and insecticide resistance of *Bemisia tabaci*.
- Cucurbit chlorotic yellows virus (CCYV) enhanced the resistance of *B. tabaci* to imidacloprid.
- Imidacloprid-resistant *B. tabaci* outperformed sensitive adults in terms of virus transmission ability.
- The plant virus and insect vector establish a mutually beneficial relationship that facilitates virus transmission.

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Introduction

Viral pathogen-induced plant diseases show a global distribution and severely affect crop yield and the global economy (He and Krainer,2020). Insect-borne viruses account for the majority of plant viruses and are efficiently transmitted by insect vectors (Eigenbrode et al., 2018). The current prevention and control strategies of plant viruses are mainly focused on controlling the corresponding vectors. Therefore, insecticides play a critical role in ensuring food security worldwide. However, the overuse of insecticides in the field has led to the emergence of insecticide resistance among insect vectors. The tobacco whitefly (*Bemisia tabaci*) is known to transmit more than 200 plant viruses and is thus considered a super pest worldwide (De Barro et al., 2011;

Polston et al., 2014). Synthetic insecticides used to control *B. tabaci* include organophosphorus, pyrethroids, insect growth regulators, nicotinoids, and neonicotinoids. Among them, neonicotinoids have been used continuously, which has led to the emergence of high-level resistance to this class of chemical insecticides in the whitefly, making whitefly management difficult (Ahmad et al., 2002; Horowitz et al., 2002; Nauen and Denholm, 2005). Recently, we showed that the ability of *B. tabaci* to transmit Cucurbit chlorotic yellows virus (CCYV) is closely and intrinsically linked to resistance to imidacloprid a neonicotinoid.

CCYV (genus Crinivirus) was first reported by Okuda et al. (2010) and is transmitted in a semi-persistent manner specifically by *B. tabaci* (Li et al., 2016). CCYV causes the fading and yellowing of the entire leaf, except the leaf veins (which remain green), resulting in serious yield losses. In recent years, CCYV has been reported in many countries in North America and East Asia (Kwak et al., 2021; Kavalappara et al., 2021; Chynoweth et al., 2021; Hernandez et al., 2021; Abrahamian et al., 2012), and the extent and scope of its damage is ever expanding.

Recently, we found that the ability of *B. tabaci* to transmit CCYV is associated with overexpression of *CYP6CM1*, a gene belonging to the cytochrome P450 family, which is closely related to imidacloprid resistance (Karunker et al., 2008; Yang et al., 2013; Li et al., 2015). The *cAMP response element-binding protein* (CREB) transcription factor regulates the expression of *CYP6CM1* via the mitogen-activated protein kinase (MAPK) signaling pathway (Yang et al., 2020) (Fig. 1). In this study, we investigated whether CCYV changes the resistance of *B. tabaci* to imidacloprid at the genetic level, thus facilitating its transmission. This research aims to reveal the correlation between the virus transmission ability and insecticide resistance of *B. tabaci*, which may provide a new hypothesis for the further investigation of insect-virus coevolution.
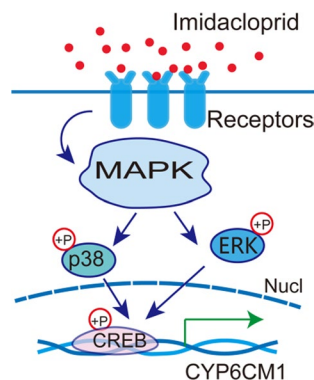

Fig. 1 MAPK signaling-mediated the overexpression pattern of the neonicotinoid resistance-related gene *CYP6CM1* (Yang et al., 2020)

Materials and methods

Plants, insects, and virus

Cucumber (*Cucumis sativus* L. cv. Bojie-107) and cotton (*Gossypium hirsutum* L. cv. Zhongmian-49) plants were grown in pots (diameter = 10 cm; height = 12 cm) in separate insect-proof cages (60 cm × 40 cm × 80 cm), which were placed in a greenhouse under controlled conditions: long-day photoperiod (16-h light/8-h dark); 28 ± 0.5 °C temperature; and 60–70% relative humidity. The colony of *B. tabaci* Mediterranean (MED; formerly biotype Q) cryptic species was initially collected from the cucumber plants in a vegetable plantation field in the suburban Zhengzhou City, China, in 2018 and maintained on cucumber plants in the greenhouse. The population not exposed to any insecticide during the culture period was served as the sensitive strain in this experiment. Meanwhile, *B. tabaci* MED population reared in the presence of imidacloprid (spray with LC_{50} concentration of imidacloprid in each generation) for > 20 generations was used as the resistant strain. The purity of *B. tabaci* MED was monitored every 2–3 generations using reverse transcription PCR (RT-PCR) and sequencing of *mtCOI* gene (Khasdan et al., 2005; Shatters et al., 2009) with sequence-specific primers (Tab. S1). The CCYV-infected cucumber plants were maintained in the laboratory for two years. Cucumber plants were used for virus clone maintenance and transmission assays. Cotton is a suitable host for *B. tabaci*, but not for CCYV; therefore, it was used for the retention time experiment in transmission assays. Viruliferous *B. tabaci* adults were collected from CCYV-infected plants and transferred to virus-free cucumber plants for inoculation.

Imidacloprid bioassay of non-viruliferous and viruliferous *Bemisia tabaci*

Three-day-old non-viruliferous *B. tabaci* adults were collected from virus-free plants and placed on CCYV-infected plants. After feeding for 10 d, a total of 20 *B. tabaci* individuals were chosen for the imidacloprid bioassay, which was performed using the leaf dip method (Houndété et al., 2010). The cucumber leaf dics (35 mm in diameter) were dipped for 10 s in 50 mg/L imidacloprid (pesticide registration no.: PD20131915; product standard no.: GB/T28143-2011; dosage form: emulsifiable oil; imidacloprid 5%). Subsequently, the leaf dics were air-dried and placed in a Petri dish (diameter = 35 mm) containing 1% agar. The mortality rate of *B. tabaci* was determined after 48 h by counting the number of dead insects (i.e., insects that showed no movement or abnormal movement). Experiments were repeated 12 times.

Resistant *Bemisia tabaci* strains

The LC_{50} of imidacloprid was determined using the leaf dip method, as described above. The calculated LC_{50} value was then used to screen the resistant strains of *B. tabaci*. Total RNA was extracted using the TaKaRa RNAiso Plus Kit (Cat# 9109, Lot# AL42064A), according to the manufacturer's instructions. Then, cDNA was synthesized using the TaKaRa Reverse Transcription Kit (Cat# RR047A, Lot# AL21113A). To detect the expression of the resistance gene *CYP6CM1*, quantitative real-time PCR (qRT-PCR) was performed using TaKaRa TB GreenR Premix Ex Taq™ II (Cat# RR820A, Lot# AJF2612A), according to the manufacturer's instructions.

Expression analysis of *CYP6CM1* and its upstream regulators in *Bemisia tabaci*

Non-viruliferous *B. tabaci* adults were collected from healthy plants and placed on CCYV-infected and healthy plants for 1, 5, 10, 15, and 20 d. Then, 80 *B. tabaci* adults were selected for each experiment. To determine the expression level of *CYP6CM1*, total RNA extraction and qRT-PCR were performed as described above, with five replicates. According to Yang et al. (2020), the effect of CCYV on the upstream regulatory factors was investigated. Imidacloprid-resistant and imidacloprid-sensitive non-viruliferous *B. tabaci* adults were placed on healthy and CCYV-infected cucumber plants for 1 and 10 d, respectively. Experimental methods and total RNA extraction were performed as described above. Four replicates were performed for this experiment.

RNA interference (RNAi) and qRT-PCR

The cDNA templates of *B. tabaci* samples were amplified by PCR, cloned into the pMDTM18-T vector (TaKaRa, Code NO.6011), and confirmed by sequencing. Double-stranded RNAs (*dsCYP6CM1*, *dsGFP*) were synthesized by in vitro transcription using the T7 RNAi Transcription Kit (TR102) (Vazyme) and fed to *B. tabaci* at a concentration of 40 μg/mL using the membrane feeding method, as described by Li et al. (2015). The effects of RNAi on the expression of the target gene in *B. tabaci* were analyzed by qRT-PCR after feeding for 3 d. This experiment was performed in triplicate. Then, the silenced *B. tabaci* were fed on CCYV-infected cucumber plants for 1 and 3 h. CCYV mRNA molecules were quantified by qRT-PCR. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with *actin* as the internal reference (Li et al., 2015; Wang et al., 2020). Eight replicates were performed for each group.

Virus transmission assays

To determine the acquisition access period (AAP), non-viruliferous resistant and sensitive *Bemisia tabaci* adults were starved for 2 h and placed on CCYV-infected cucumber plants in an insect-proof cage. The insects were collected after 1, 2, 6, 24, and 48 h of feeding.

To determine the retention time, resistant and sensitive *B. tabaci* adults were placed on CCYV-infected cucumber plants and collected after 3 d. Then, after 2 h of starvation, the *B. tabaci* adults were released onto cotton plants (non-host of CCYV) and collected after 1, 3, 6, 9, and 15 d of feeding.

Sixteen replicates were performed for each experiment. A mixed sampling of male and female *B. tabaci* adults was used in both experiments, with 20 adults per tube (Li et al., 2016; Lei et al., 2021). Then, CCYV mRNA in insects was detected by qRT-PCR (Fig. S1).

Statistical analyses

Data were statistically analyzed using Student's *t*-test. Statistical analyses were performed in IBM SPSS Statistics 20.0, and graphs were generated using GraphPad Prism 8.0.2. The LC_{50} value (of imidacloprid), toxicity regression equation, and R^2 value were calculated using the Probit function. Data were expressed as mean ± standard error (SE).

Results

3.1 Bioassays

The imidacloprid resistance of *Bemisia tabaci* was measured by the leaf dip method after 10 d of AAP (Fig. 2a). Viruliferous *B. tabaci* exhibited a significantly lower corrected mortality rate than non-viruliferous *B. tabaci* (t = 2.131, df = 22, P = 0.045) (Fig. 2b).

Screening of resistant *Bemisia tabaci*

Under long-term imidacloprid treatment, the resistance of *B. tabaci* to imidacloprid gradually increased. The expression level of *CYP6CM1* was approximately 30-fold higher in resistant *B. tabaci* than in sensitive *B. tabaci* (Fig. 3a). According to the classification of resistance classes (Yan et al., 2012), the adults of *B. tabaci* screened in this study showed moderate resistance to imidacloprid, with a resistance ratio of 11.30 compared with the LC_{50} value of sensitive *B. tabaci* (Table 1).
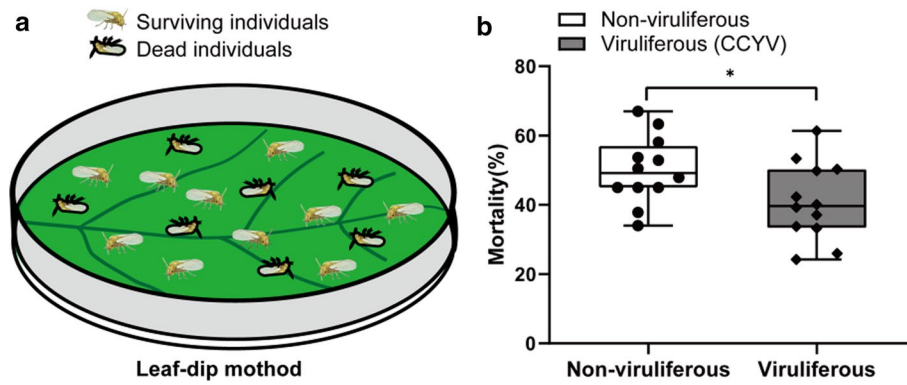
Fig. 2 Determination of the resistance of viruliferous *B. tabaci* to imidacloprid. (a) Schematic drawing of the dip method. Black outline represents dead *B. tabaci* adults. (b) Resistance of viruliferous and non-viruliferous *B. tabaci* adults to imidacloprid. Data represent mean $\pm$ SE ($n = 12$). Asterisk indicates significant difference between treatment (viruliferous) and control (non-viruliferous) groups (Student's *t*-test; *$P < 0.05$)

Fig. 3 Culture of resistant *B. tabaci* (a, $n = 4$) and the effect of CCYV on the expression of the susceptible *B. tabaci* (b, $n = 5$) and resistant *B. tabaci* (c, $n = 4$) about resistance-related gene *CYP6CM1*. Sensitive represents susceptible *B. tabaci*. Resistant represents imidacloprid-treated resistant *B. tabaci*. Non-viruliferous is CCYV-free *B. tabaci*, Viruliferous is CCYV-viruliferous *B. tabaci*. S + non-viruliferous, sensitive non-viruliferous *B. tabaci*; S + viruliferous, sensitive viruliferous *B. tabaci*; R + non-viruliferous, resistant non-viruliferous *B. tabaci*; R + viruliferous, resistant viruliferous *B. tabaci*. Data represent mean $\pm$ SE. Asterisks indicate significant differences between treatment and control groups (Student's *t*-test; ***$P < 0.001$)
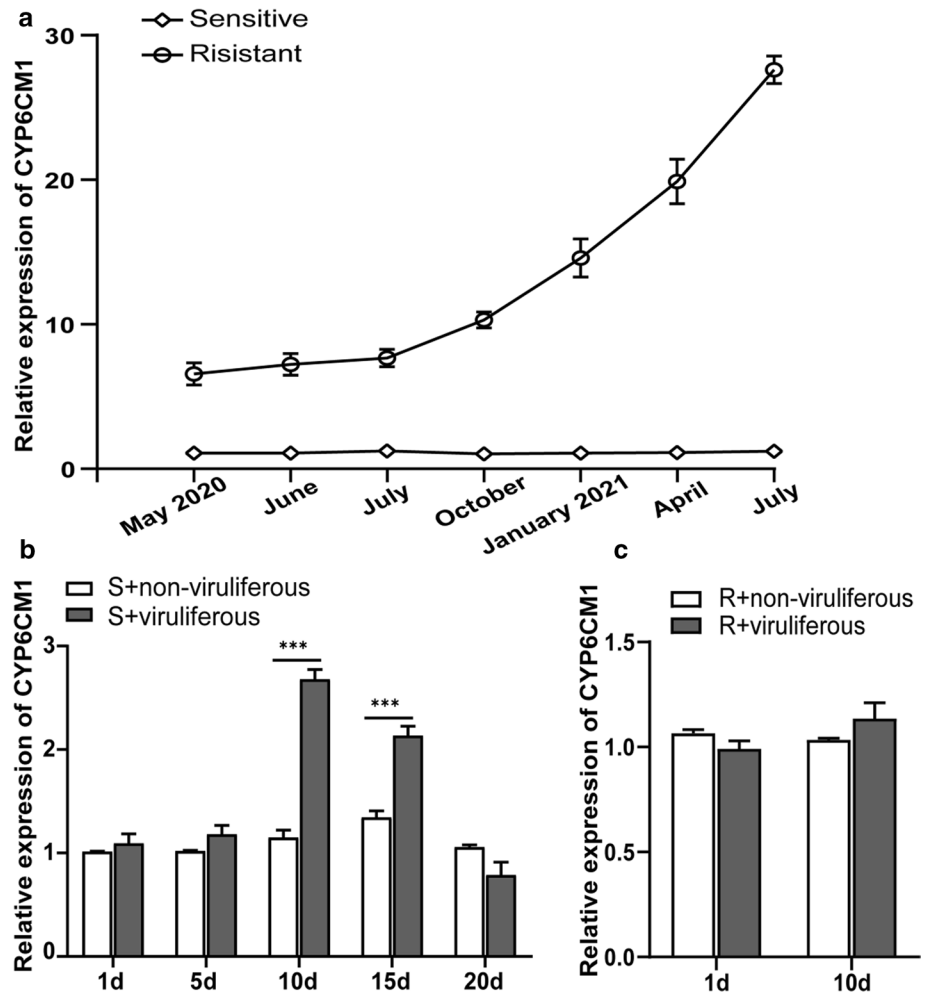
Table 1 Toxicity regression equations and resistance ratios

$B.\ tabaci$ strain[†]	Toxicity regression equation	R^2	LC_{50} (mg/L)	95% CI (mg/L)[‡]	Resistance ratio
Resistant (R_n)	$Y = -9.621 + 3.521X$	0.933	540.398	490.845–629.279	11.300
Resistant (R_1)	$Y = -4.427 + 1.921X$	0.878	198.579	164.053–233.764	4.160
Sensitive (S_0)	$Y = -3.805 + 2.266X$	0.913	47.769	32.813–58.680	–

[†]S_0, susceptible $B.\ tabaci$ strain; R_1, first-generation resistant strain; R_n, resistant strain cultured for multiple generations. [‡]CI, confidence interval
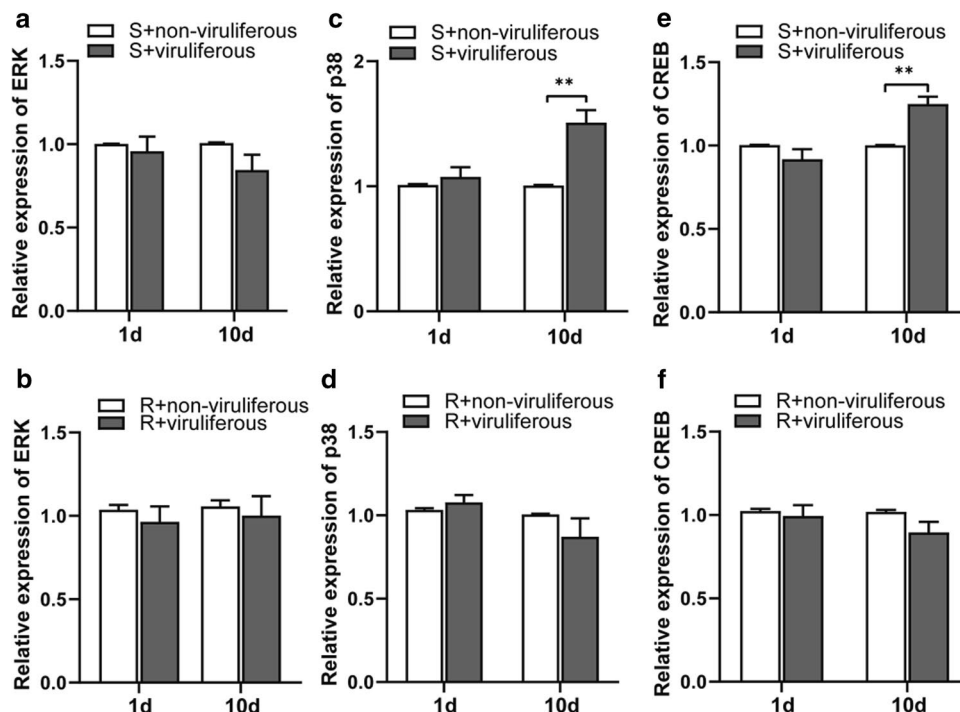
3.3 Effects of CCYV on the expression of *CYP6CM1* in *Bemisia tabaci*

The *CYP6CM1* gene was first up-regulated and then down-regulated in viruliferous *B. tabaci* (Fig. 3b). No significant difference was observed in the expression of *CYP6CM1* between viruliferous and non-viruliferous *B. tabaci* after 1 d of feeding ($t_{1d} = 0.873$, df = 8, $P = 0.247$) and 5 d of feeding ($t_{5d} = 1.862$, df = 8, $P = 0.076$). At 10 and 15 d, the *CYP6CM1* gene was significantly up-regulated in viruliferous *B. tabaci* compared with the non-viruliferous group ($t_{10d} = 12.870$, df = 8, $P < 0.001$; $t_{15d} = 7.243$, df = 8, $P < 0.001$). However, *CYP6CM1* expression was down-regulated in viruliferous *B. tabaci* at 20 d, and the difference in its expression between the two groups was not significant ($t_{20d} = 2.175$, df = 8, $P = 0.062$). In the meantime, no significant difference was observed in the expression of *CYP6CM1* between viruliferous and non-viruliferous resistant *B. tabaci* at 1 and 10 d (Fig. 3c).

Effects of CCYV on the upstream regulators of *CYP6CM1*

No difference was detected in the expression of the *ERK* gene between non-viruliferous and viruliferous *Bemisia tabaci*, regardless of their resistance to imidacloprid, at both 1 and 10 d after feeding (Fig. 4a and 4b) ($St_{1d} = 0.502$, df = 6, $P = 0.633$; $St_{10d} = 1.758$, df = 6, $P = 0.261$). Although the *p38* gene showed no difference in expression between resistant and sensitive *B. tabaci* at 1 d ($St_{1d} = 0.847$, df = 6, $P = 0.429$), it was significantly up-regulated in viruliferous adults compared with non-viruliferous *B. tabaci* at 10 d (Fig. 4c and 4d) ($St_{10d} = 4.993$, df = 6, $P = 0.002$). The transcription factor genes *CREB* showed similar expression profiles, with significant up-regulation at 10 d in sensitive viruliferous *B. tabaci* (Fig. 4e and 4f) ($St_{1d} = 1.431$, df = 6, $P = 0.202$; $St_{10d} = 5.587$, df = 6, $P = 0.001$).

Fig. 4 Effects of CCYV on the upstream regulators of the *CYP6CM1* gene. (a, b) Relative expression levels of the *ERK* gene. (c, d) Relative expression levels of the *p38* gene. (e, f) Relative expression levels of the *CREB* gene. S + non-viruliferous, sensitive non-viruliferous *B. tabaci* (control group); S + viruliferous, sensitive viruliferous *B. tabaci* (treatment group); R + non-viruliferous, resistant non-viruliferous *B. tabaci* (control group); R + viruliferous, resistant viruliferous *B. tabaci* (treatment group). Data represent mean ± SE ($n = 4$). Asterisks indicate significant differences between treatment and control groups (Student's *t*-test; **$P < 0.01$)
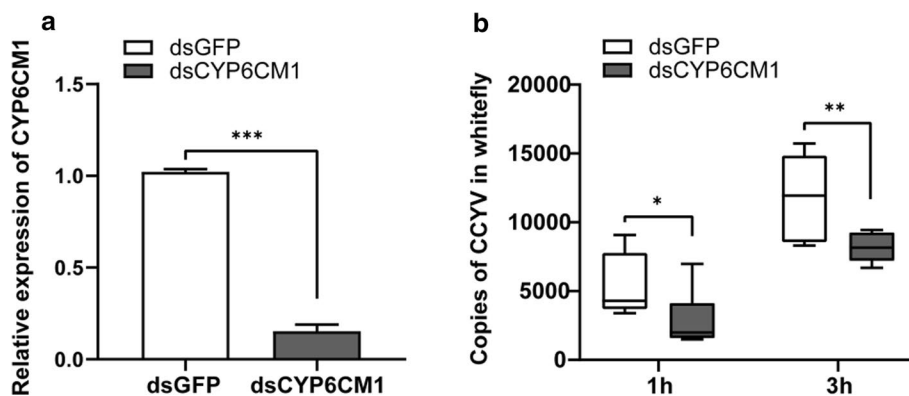
Fig. 5 Effect of *CYP6CM1* knockdown on the acquisition of CCYV by *B. tabaci*. (a) Comparison of the effects of the silencing of *GFP* (control) and *CYP6CM1* on the expression of *CYP6CM1* in *B. tabaci*. (b) CCYV acquisition after *CYP6CM1* gene silencing. Data repre-

sent mean $\pm$ SE; $n = 3$ in (a), 8 in (b). Asterisks indicate significant differences between treatment and control groups (Student's *t*-test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Effects of silencing *CYP6CM1* on the acquisition of CCYV by *Bemisia tabaci*

When *dsCYP6CM1* and *dsGFP* were fed to *B. tabaci* at a concentration of 40 µg/mL by membrane feeding, the effect of *dsCYP6CM1* on *CYP6CM1* expression was significantly higher than that of *dsGFP* (t = 38.850, df = 4, $P < 0.001$) (Fig. 5a). The silenced *B. tabaci* were placed on CCYV-infected cucumber plants to acquire the virus and were collected after 1 and 3 h of feeding. We found that the silencing of *CYP6CM1* significantly decreased the amount of virus acquired by *B. tabaci* (t$_{1h}$ = 2.299, df = 14, $P = 0.037$; t$_{3h}$ = 3.110, df = 14, $P = 0.0076$) (Fig. 5b).

Comparison of virus acquisition and retention

The ability of insect vectors to transmit the virus could be determined by measuring virulence acquisition and retention. We compared the differences in virulence acquisition and retention between resistant and sensitive *B. tabaci*. Resistant *B. tabaci* acquired significantly more copies of the virus than sensitive *B. tabaci* after non-viruliferous vectors were placed on CCYV-infected plants for 2 h and 6 h (t$_{2h}$ = 2.365, df = 26, $P = 0.026$; t$_{6h}$ = 2.940, df = 26, $P = 0.030$). We found that acquired virus copies no longer changed at 24 and 48 h for either resistant or sensitive *B. tabaci* (Fig. 6a). This indicated that, for the semi-persistent virus like CCYV, complete virus acquisition was possible from 24 to 48 h for *B. tabaci*. We investigated the virulence retention capacity of resistant and sensitive *B. tabaci*
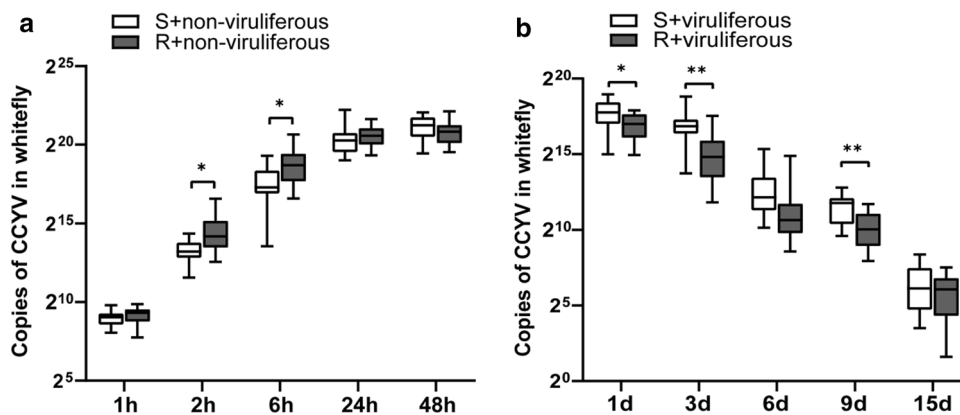

Fig. 6 Comparison of CCYV acquisition and retention between resistant and sensitive *B. tabaci*. (a) Comparison of CCYV acquisition. S + non-viruliferous, sensitive non-viruliferous *B. tabaci*; R + non-viruliferous, resistant non-viruliferous *B. tabaci*. (b) Comparison of CCYV retention. S + viruliferous, sensitive viruliferous

B. tabaci; R + viruliferous, resistant viruliferous *B. tabaci*. Data represent mean $\pm$ SE ($n = 16$). Asterisks indicate significant differences between resistant and sensitive groups (Student's *t*-test; *$P < 0.05$, **$P < 0.01$)

on cotton (a non-host of CCYV). When viruliferous *B. tabaci* were fed on cotton for 1 and 3 d, the resistant *B. tabaci* lost the virus more readily than sensitive adults, as indicated by a significant decrease in virulence retention capacity ($t_{1d} = 2.716$, df = 30, $P = 0.011$; $t_{3d} = 3.240$, df = 30, $P = 0.003$). At 9 d, the virus titer in resistant *B. tabaci* decreased significantly compared with that in sensitive adults ($t_{9d} = 3.646$, df = 30, $P = 0.001$) (Fig. 6b).

Discussion

Insecticide resistance is a common problem that prevents effective pest control; however, little attention has been given to the relationship between insect vector-mediated transmission of plant viruses and insecticide resistance (Rivero et al., 2010). In recent years, related studies have gradually emerged. Wan et al. (2020) found that spinosad-resistant western flower thrips (*Frankliniella occidentalis*) showed a significant increase in Tomato spotted wilt orthotospovirus (TSWV) acquisition and replication, thus promoting the spread of the virus. There have also been reports of microbe-mediated insecticide sensitivity. For example, the symbiotic bacteria or gut microbiota of wheat aphid *Sitobion miscanthi* and *Rhagoletis pomonella* are reportedly associated with detoxification (Li et al., 2021; Lauzon et al., 2003).

This has raised the question whether insect vector-borne viruses and insect vectors exist in a mutually beneficial relationship over the long term. It is clear that there are correlations between virus transmission and insecticide resistance, and probably, plant viruses and their vectors with different transmission modes (nonpersistent, semipersistent, and persistent) may have different types and/or degrees of such mutual relationships. This is worthwhile to conduct parallel comparison among combinations of plant viruses and their vectors at different transmission modes. In this study, we found that CCYV, a semi-persistently transmitted virus, reduced the susceptibility of *B. tabaci* to imidacloprid (Fig. 2). And Liu et al. (2020) showed that the infection of *B. tabaci* with tomato yellow leaf curl virus (TYLCV), a persistently transmitted virus, increased its tolerance to the insecticide Flupyradifurone, while viruliferous *B. tabaci* exhibited significantly higher LC_{50} to this insecticide. The reduced susceptibility of *B. tabaci* to imidacloprid is probably caused by CCYV promoting the expression of the imidacloprid resistance gene *CYP6CM1* (Fig. 3b). Further investigation revealed that CCYV also affects the expression of the upstream regulators of *CYP6CM1* in sensitive populations (Fig. 4). Genes including *p38* (which encodes a MAPK family protein) and *CREB* (which encodes a transcription factor that regulates *CYP6CM1* expression) were significantly up-regulated when *B. tabaci* acquired CCYV for 10 d.

However, no difference was observed in the expression of *CYP6CM1* between the treatment and control groups in the resistant populations (Fig. 4). Therefore, we speculated that the effect of *B. tabaci* resistance on its regulatory factors is much more than that of CCYV.

In addition, "trade-off" between virus transmission and insecticide resistance in a vector is an interesting topic in the context of coevolution. Viruses are the fastest evolving organisms, and virus-vector interactions are thought to be the strong force driving for the diversification of cellular organisms (Rey, 2011). We propose that such mutual relationship between virus transmission and insecticide resistance in the vectors is developed for better spread of plant viruses, because resistant vectors have more chances to transmit viruses than susceptible ones under insecticide spray conditions in the fields, and in turn, transmission of plant viruses provides higher probability for vectors' survival resulting from increased insecticide resistance levels. We silenced *CYP6CM1* to investigate the effect of the resistance gene on the virulence acquisition of *B. tabaci*. Interference of *CYP6CM1* decreased the virulence acquisition (Fig. 5). This indicates that the resistance gene *CYP6CM1* is related to the virulence acquisition of the *B. tabaci*; however, the underlying mechanism needs further investigation. We also compared the virulence acquisition and retention capacity of resistant and sensitive *B. tabaci*. The results showed that resistant *B. tabaci* acquired a higher amount of the virus RNA than sensitive *B. tabaci* within the same time period (Fig. 6a). This is probably because resistant *B. tabaci* requires more energy to replenish the energy spent on enhancing insecticide resistance and feeding. However, the ability of resistant *B. tabaci* to retain virulence was weaker than that of sensitive *B. tabaci* (Fig. 6b). Considering the semi-persistent transmission of CCYV, resistant *B. tabaci* could spread the virus to other hosts faster than sensitive *B. tabaci*.

In conclusion, our results suggest that there is a mutually beneficial relationship between CCYV and its vectors, as indicated by two main findings: (1) CCYV up-regulated the expression of the resistance gene *CYP6CM1* and enhanced the resistance of *B. tabaci* to imidacloprid and (2) resistant *B. tabaci* outperformed sensitive *B. tabaci* in terms of virulence transmission ability. Nonetheless, it remains unclear how CCYV regulates the expression of resistance genes in *B. tabaci*. Further research is needed to address this limitation. The yearly increase in insecticide resistance and the widespread outbreaks of viral diseases also need to be discussed from an evolutionary perspective. An in-depth understanding of the interactions between vector insects and plant viruses can help model the synergistic evolution of insecticide resistance, which will assist in better pest control, a matter that requires constant attention in future.

Author contributions

FY, JL, and MY conceived and designed the experiment; MY, HH, ZZ, BZ, CZ (Chaoqiang Zhu), and WY performed the investigation; MY and ZZ analyzed the data; MY and FY wrote the original draft; and FY, JL, and CZ (Chenchen Zhao) reviewed and edited the document. All authors contributed to the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

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