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Identification and Detection of CYP4G68 Overexpression Associated With Cyantraniliprole Resistance in *Bemisia tabaci* From China

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Bemisia tabaci, the tobacco whitefly, is one of the most notorious agricultural sucking insect pests that severely damage a series of crops worldwide. Throughout China, *B. tabaci* threatens agricultural production with increasing cases of resistance to commonly used insecticides, prompting the widespread use of cyantraniliprole as an alternative to control hemipteran pests. Here, we found overexpression of the *CYP4G68* gene conferring cyantraniliprole resistance using quantitative real-time PCR (qPCR) and RNA interference (RNAi) in one lab-selected resistant strain CYAN-R (to about 80-fold higher than control). Furthermore, we measured levels of resistance to cyantraniliprole in whiteflies with 18 field-sampled populations across China and then confirmed that, among them, 14 field-sampled populations showed low-to-high resistance to cyantraniliprole compared with the susceptible strain. We measured *CYP4G68* expression in the 14 field populations, and the results of qPCR and RNAi indicated that in two of these populations, Haikou and Wuhan, significant overexpression of *CYP4G68* contributed to the development of field-evolved resistance to cyantraniliprole. These results indicate the need to facilitate strategies of management to delay the evolution of resistance to cyantraniliprole and control of whiteflies more sustainably, and to prevent overuse of insecticides in the environment through rational application practices.

Keywords: *Bemisia tabaci*, cyantraniliprole, resistance management, field-evolved resistance, P450s, overexpression, RNA interference

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INTRODUCTION

Bemisia tabaci, the tobacco whitefly, is a destructive sucking pest that devastates the production of economically important and horticultural crops worldwide. The tobacco whitefly is invasive in many locations and reported to infect over 700 species of plants (Wang et al., 2017; Horowitz et al., 2020). In addition to damaging plants by sucking, *B. tabaci* transmits over 200 plant viruses in the process of feeding (Wei et al., 2017). In recent decades, *B. tabaci* has been controlled *via* the application of various widely used chemical agents including organophosphates, carbamates, pyrethroids, insect growth regulators (pyriproxyfen and buprofezin), and neonicotinoids. However, the management of whiteflies rests primarily on the extensive usage of chemical agents over the long-term, which has

caused *B. tabaci* to develop a high or exceedingly high level of resistance to various popular chemical agents (Horowitz et al., 2020). Therefore, widely and heavily applied insecticides will not be effective for controlling *B. tabaci* in China.

It has been shown that anthranilic diamides can be used against a variety of agricultural insect pests efficiently since they have been introduced to markets around the world (Jeanguenat, 2013). Apart from excellent insecticidal functions with lethal concentrations of anthranilic diamides, they also exert influences on target insect pests at sublethal concentrations and then result in biological and physiological alterations in the pests (Huang et al., 2016; Nozad-Bonab et al., 2017; Meng et al., 2020). Among commercialized insecticides of anthranilic diamide, cyantraniliprole is a second-generation product and targets a wide range of agricultural pests from various orders of insects by acting on their ryanodine receptors (Lahm et al., 2005; Sattelle et al., 2008; Jeanguenat, 2013). Considering that cyantraniliprole can be absorbed via roots and stems of the plants, this chemical agent displays significant insecticidal effects against various orders of insects such as sucking and chewing pests, in comparison with first-generation products like flubendiamide and chlorantraniliprole, which are largely useful for controlling caterpillars (Foster et al., 2012; Barry et al., 2015; Bielza and Guillén, 2015; Grávalos et al., 2015; Moreno et al., 2018). Moreover, it was demonstrated to successfully manage immature stages and adults of *B. tabaci* and reduce the efficiency of transmitting plant viruses (Portillo et al., 2009; Schuster et al., 2009; (Stansly et al., 2010).

Due to long-term and excessive applications of insecticides, it has been well indicated that among most conventional chemical agents, a growing number of them became inefficacious for controlling agricultural insect pests (Palumbo et al., 2001). In the field applications in China, the heavy dependence on chemical agents for controlling insect pests contributed to more and more resistance cases to various classes of chemical agents that were significantly effective against *B. tabaci*. In particular, neonicotinoids are highly effective for whitefly control, but resistance to neonicotinoids has been widely reported in whiteflies from several geographic regions across China, which has led to serious control failures (Wang et al., 2010; Yang et al., 2013; Zheng et al., 2017; Zheng et al., 2021). Based on this situation, resistance to neonicotinoids has been investigated, and mechanisms of resistance were demonstrated gradually in China (Yang et al., 2020; Yang et al., 2021; Du et al., 2021; Liang et al., 2022). Not surprisingly, although it has been shown that cyantraniliprole could be one powerful alternative to popular chemical agents, field-evolved cyantraniliprole resistance in *B. tabaci* has been reported in China (Wang et al., 2018), and it has been reported that in *Aphis gossypii*, UGTs and P450s are possibly related with resistance to cyantraniliprole (Zeng et al., 2021).

In our previous work, a baseline of susceptibility to cyantraniliprole in China was established and five field-collected populations with moderate cyantraniliprole resistance were detected (Wang et al., 2018). Based on the above results, high cyantraniliprole resistance was observed in the SX population (138.4-fold) after successive selection (Wang et al., 2019). By crossing and successive backcrossing between SX and the susceptible population, one near-isogenic line of the CYAN-R strain was developed that showed 63.317-fold cyantraniliprole resistance compared to the control (Wang et al., 2020a). In the current work, we carried out lab experiments to select the CYAN-R strains with cyantraniliprole, generation by generation, to obtain stable cyantraniliprole resistance (80.8-fold) and found overexpression of the *CYP4G68* gene conferring cyantraniliprole resistance in the use of qPCR and RNAi in the CYAN-R strain. Then, in 2021, we established the baseline of susceptibilities to cyantraniliprole in 18 field-sampled populations from China and demonstrated that most of the field-sampled populations of *B. tabaci* showed various levels of cyantraniliprole resistance. Furthermore, expression levels of *CYP4G68* were measured in 14 field populations, and the results of qPCR and RNAi indicated that in two of the populations, Haikou and Wuhan, significant overexpression of *CYP4G68* contributed to the development of field-evolved resistance to cyantraniliprole. These results provide new insights for the cognition of P450-associated insecticide resistance and are solid evidence for putting forward appropriate tactics for the sustainable management of whiteflies without the overuse of insecticides.

MATERIALS AND METHODS

Insects and Chemicals

The CYAN-R strain of *B. tabaci* with cyantraniliprole resistance and the MED-S susceptible strain were recorded previously (Wang et al., 2020a), and all field-collected populations used in this work were recorded before (Wang et al., 2022). All used populations were raised on a plant of cotton without exposure to insecticides in the chamber with a temperature of 26 ± 1°C, relative humidity of 55 ± 5%, and photoperiod of 16 h light: 8 h dark. About 300 adults of *B. tabaci* were collected at random from each of the lab-reared and field-collected populations for identification of cryptic species according to the reported approach (Luo et al., 2002), and all of the tested ones were confirmed as Mediterranean cryptic species. All chemical agents utilized were analytically standardized, and cyantraniliprole (Sigma Aldrich, CAS# 736994-63-1, catalog# 32372-25MG), triton X-100 (Sigma Aldrich, CAS# 9002-93-1, catalog# 93443-100 ML), and dimethyl sulfoxide (Sigma Aldrich, CAS# 67-68-5, catalog# D8418-500 ML) were bought from Sigma Aldrich, Shanghai, China.

Bioassays

Based on our previously reported approach (Wang et al., 2018), leaf-dipping bioassays were carried out with whitefly adults from each of the tested populations. Cotton discs with a 2-cm diameter were soaked for about 20 s in the water (control) or the specific working concentration, and after air-drying, they were moved into test tubes with plug caps, respectively. After that, 25–35 whitefly adults were sampled and moved into each of the test tubes at random, and all the test tubes were kept in the chamber for 48 h and then mortality was checked. The CYAN-R cyantraniliprole resistance strain was screened with cyantraniliprole for 15 successive generations, every generation

TABLE 1 | Selection of cyantraniliprole resistance in the CYAN-R strain of *Bemisia tabaci*.

G[a]	N[b]	LC_{50} (95%CL)[c] (mg L^{-1})	Slope (±SE)	X^2 (Df)	RR[d]
0	599	85.487 (70.805–101.269)	1.484 ± 0.138	1.985 (3)	55.0
1	601	73.004 (60.288–90.682)	1.288 ± 0.133	1.211 (3)	47.0
2	593	83.420 (69.848–97.928)	1.587 ± 0.142	2.282 (3)	53.7
3	588	80.207 (66.186–100.322)	1.321 ± 0.136	2.072 (3)	51.6
4	579	94.274 (80.982–108.744)	1.820 ± 0.149	2.446 (3)	48.5
5	594	83.251 (70.446–100.754)	1.559 ± 0.142	1.192 (3)	53.6
6	590	89.523 (72.782–109.232)	1.239 ± 0.133	1.614 (3)	57.6
7	582	92.885 (75.775–110.078)	1.668 ± 0.153	1.879 (3)	59.8
8	603	105.144 (84.521–126.335)	1.435 ± 0.138	1.089 (3)	67.7
9	593	109.064 (83.600–135.258)	1.199 ± 0.134	2.141 (3)	70.2
10	596	117.425 (90.397–145.688)	1.169 ± 0.132	2.050 (3)	75.6
11	594	128.392 (100.653–158.165)	1.187 ± 0.132	1.277 (3)	82.6
12	598	120.752 (94.639–148.269)	1.222 ± 0.132	1.473 (3)	77.7
13	597	126.814 (101.082–153.085)	1.419 ± 0.140	2.348 (3)	81.6
14	599	132.184 (97.337–167.897)	1.069 ± 0.131	2.567 (3)	85.1
15	599	125.566 (93.133–158.609)	1.112 ± 0.132	2.187 (3)	80.8

[a]*Generation of adults used in the bioassay.*
[b]*Number of tested adults.*
[c]*CL, confidence limits.*
[d]*RR (resistance ratio) = LC_{50} of selected generation/LC_{50} of MED-S (1.554 mg L^{-1}).*

of *B. tabaci* adults were put through the selection with a median lethal concentration of cyantraniliprole. F_1 offspring adults of 18 field-sampled populations from nine provinces of China were used for bioassay to monitor the levels of resistance to cyantraniliprole in China.

Expression Patterns of Detoxification-Related P450s

Based on previous publications concerning insecticide resistance associated with P450 genes in *B. tabaci* (Wang Q et al., 2020; Zhou et al., 2020), 12 candidate P450s (*CYP6DZ4*, *CYP6CM1*, *CYP4G68*, *CYP6CX4*, *CYP6DW2*, *CYP303A1*, *CYP4C64*, *CYP6DZ7*, *CYP6CX1v1*, *CYP6CX3*, *CYP6CX5*, and *CYP6DW3*) were picked out and set as candidates for the analysis of gene expression. For each tested population, total RNA was extracted from 100 adults of *B. tabaci* collected at random from each tested populations, and on the basis of our reported approach (Wang et al., 2020c), qPCR was carried out, and two reference genes, EF-1α and TUB1α, were selected for the normalization. All sequences of primers are listed in **Supplementary Table S1**.

Silencing of *CYP6DW2* and *CYP4G68*

Silencing of *CYP6DW2* and *CYP4G68* was conducted, respectively, to confirm the function of *CYP6DW2* and *CYP4G68* in whiteflies via RNA interference (RNAi) based on the method recorded before (Wei et al., 2018). The double-stranded RNA (dsRNA) was synthesized using a T7 RiboMAX Express RNAi kit (Promega, Madison, WI, United States), and the primer sequences are listed in **Supplementary Table S1**. Adult *B. tabaci* were fed dsRNAs targeting enhanced green fluorescent protein (dsEGFP), or *CYP6DW2* (dsCYP6DW2), or *CYP4G68* (dsCYP4G68) for 48 h, and concentrations of dsCYP6DW2, dsCYP4G68, and dsEGFP were 0.5 μg μL^{-1}, and the artificial diet solution contained 30% sucrose (w/v) and 5% yeast extract.

Data Analysis

Data of bioassays were analyzed using PoloPlus software (2003). Resistance ratio (RR) of each of the tested chemical agents was determined by dividing the median lethal concentration of the tested field-sampled population by the median lethal concentration of the susceptible population. Values of RR were utilized to display grades of insecticide resistance, and Student's t-test and one-way ANOVA followed by Tukey's HSD for multiple comparisons were performed to analyze statistical significance ($p < 0.05$) in SPSS software (SPSS Inc., Chicago, IL, United States).

RESULTS

Cyantraniliprole Resistance Selection

Cyantraniliprole resistance in the resistant CYAN-R strain of *B. tabaci* was continuously selected for 15 generations. According to the values of LC_{50}, there was no considerable increase from G_0 to G_{15} (**Table 1**), but the resistance ratio (RR) was increased from 50.0-fold at G_0 to 80.8-fold at G_{15}. Specifically, the resistant strain of *B. tabaci* developed resistance rapidly from G_0 to G_9 (RR from 55.0- to 70.2-fold) and then remained steady after G_9, with RRs around 80-fold.

Monitoring Resistance to Cyantraniliprole in China

Baseline of susceptibilities to cyantraniliprole was constructed in the basis of 18 field-sampled populations from nine provinces across China in the year of 2021 (**Table 2**). Compared to the susceptible population MED-S, 14 of the 18 field-collected populations displayed low-to-high levels of resistance to cyantraniliprole with RRs ranging from 5.0- to 59.6-fold (LC_{50} from 8.521 to 101.474 mg L^{-1}).

Expression Profiles of the Selected P450s and RNA Interference

Compared to the susceptible ones, expression patterns of the 12 candidates (*CYP6DZ4*, *CYP6CM1*, *CYP4G68*, *CYP6CX4*, *CYP6DW2*, *CYP303A1*, *CYP4C64*, *CYP6DZ7*, *CYP6CX1v1*, *CYP6CX3*, *CYP6CX5*, and *CYP6DW3*) in CYAN-R were measured using qPCR. Expressions of *CYP6DW2* (increased 4.50-fold) and *CYP4G68* (increased 6.52-fold) in CYAN-R were significantly elevated in comparison with the susceptible ones (**Figure 1**). To explore the functions of *CYP6DW2* and *CYP4G68* further, dsCYP6DW2 and dsCYP4G68 were made and fed to adult *B. tabaci* from the CYAN-R strain to knockdown the expression of *CYP6DW2* and *CYP4G68*, respectively. After 48 h of feeding on dsCYP6DW2 and dsCYP4G68, the expression of *CYP6DW2* and *CYP4G68* in adult *B. tabaci* decreased by 41% (**Figure 2A**) and 47% (**Figure 2C**), respectively. After

TABLE 2 | Bioassays of 18 field populations and one susceptible strain of *B. tabaci* to cyantraniliprole.

Population	N[a]	Slope ±SE	LC_{50} (95%FL)[b] (mg L^{-1})	X^2 (Df)	RR[b]
MED-S	590	1.337 ± 0.137	1.703 (1.381–2.051)	2.559 (3)	—
LY	558	1.421 ± 0.142	7.070 (5.771–8.478)	1.986 (3)	4.2
CY	558	1.295 ± 0.139	4.849 (3.939–6.273)	1.617 (3)	2.8
HD	607	1.913 ± 0.148	4.706 (4.105–5.387)	2.555 (3)	2.8
TZ	600	1.048 ± 0.134	38.052 (25.899–50.023)	1.038 (3)	22.4
WQ	574	1.047 ± 0.133	15.871 (12.521–21.105)	1.207 (3)	9.3
JH	569	1.451 ± 0.142	29.108 (23.397–35.013)	1.600 (3)	17.1
ZJK	566	1.152 ± 0.137	4.848 (3.576–6.135)	1.248 (3)	2.8
BD	582	1.427 ± 0.138	30.835 (25.865–37.205)	2.487 (3)	18.1
ZZ	602	1.261 ± 0.135	22.026 (17.194–27.004)	2.196 (3)	12.9
XZ	596	1.194 ± 0.134	9.034 (7.292–11.750)	1.058 (3)	5.3
JN	584	1.409 ± 0.140	16.186 (13.197–19.381)	1.930 (3)	9.5
TA	593	1.782 ± 0.146	8.521 (7.345–9.844)	1.471 (3)	5.0
WH	592	1.378 ± 0.137	76.642 (63.818–94.437)	1.935 (3)	45.0
XY	583	1.082 ± 0.131	62.890 (50.203–80.686)	1.157 (3)	36.9
CS	573	1.218 ± 0.133	52.089 (42.353–64.292)	1.374 (3)	30.6
YY	593	1.140 ± 0.131	70.357 (56.804–89.825)	1.225 (3)	41.3
HK	597	1.335 ± 0.134	87.995 (72.632–105.840)	2.169 (3)	51.7
SY	577	1.922 ± 0.155	101.474 (87.203–116.694)	1.640 (3)	59.6

[a]*Number of insects used.*

[b]*RR (resistance ratio) = LC_{50} (field-collected population)/LC_{50} (MED-S).*

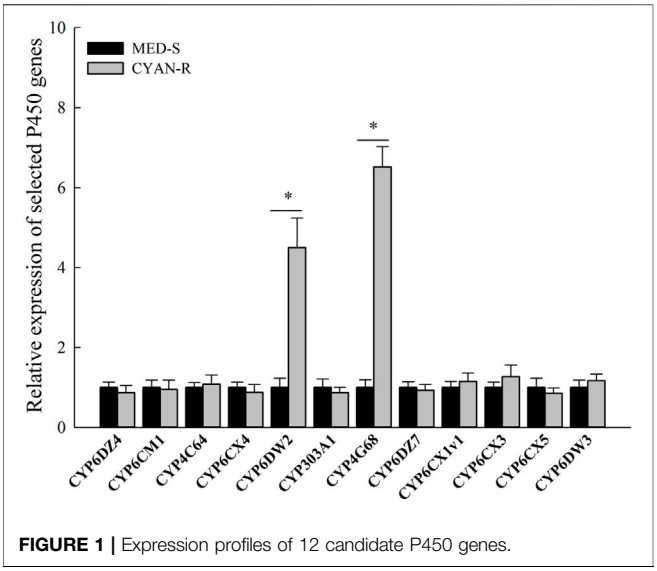

FIGURE 1 | Expression profiles of 12 candidate P450 genes.

cyantraniliprole selection, feeding on dsCYP4G68 by adults resulted in considerably higher death rate compared to that of the dsEGFP control (**Figure 2D**), and little remarkable increase in death rate was found with the feeding on dsCYP6DW2 compared with the control (**Figure 2B**).

Expression Patterns of *CYP4G68* in the Fourteen Field-Resistant Populations

Relative expression profiles of *CYP4G68* in 14 field-collected cyantraniliprole-resistant populations were established and compared to MED-S (**Figure 3**). Among the field-collected population and the susceptible strain, significant overexpression of *CYP4G68* was observed in WH (3.52-fold), CS (3.41-fold), HK (4.55-fold), and SY (3.98-fold). In other field-resistant populations, significant overexpression of *CYP4G68* was not observed.

Confirmation of the Role of *CYP4G68* in the Four Field-Resistant Populations

Based on the above results, to further investigate the functions of *CYP4G68* in the four field-resistant populations, dsCYP4G68 was synthesized and fed to the WH, CS, HK, and SY populations of adults to silence *CYP4G68* in each of the populations. After 48 h of ingestion of dsCYP4G68, adult *B. tabaci* displayed decreased expression of *CYP4G68* from 42 to 49% in the four tested populations (**Figures 4A–D**). After the cyantraniliprole treatment, feeding on dsCYP4G68 by adults resulted in a considerably elevated death rate compared to the dsEGFP control in WH and HK populations (**Figure 4E** and **Figure 4G**), but little remarkable increase in death rate was found with the feeding on dsCYP4G68 compared to that of the control in CS and SY populations (**Figure 4F** and **Figure 4H**).

DISCUSSION

Cyantraniliprole has shown excellent efficacy against sucking insect pests worldwide, but field-evolved cyantraniliprole resistance in whiteflies has been recorded in China after several years of extensive application (Wang et al., 2019). Previously, we established the CYAN-R strain of *B. tabaci* on the basis of a field-developed cyantraniliprole-resistant

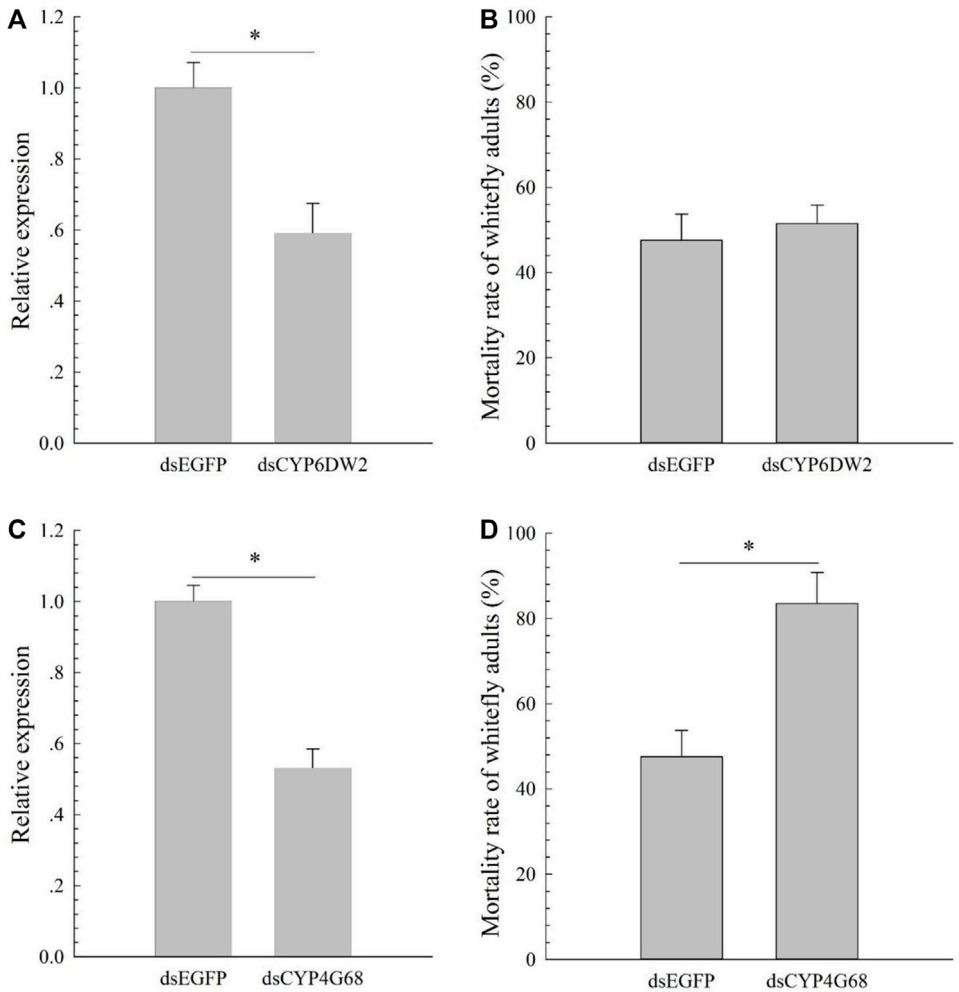

FIGURE 2 | Effects of dsCYP6DW2 on the expression of *CYP6DW2* **(A)** and effects of silencing *CYP6DW2* **(B)** on resistance to cyantraniliprole, and effects of dsCYP4G68 on the expression of *CYP4G68* **(C)** and effects of silencing *CYP4G68* **(D)** on resistance to cyantraniliprole.

population. After that, a series of biochemical assays were performed, and the results demonstrated that enhanced P450 activities functioned directly in the cyantraniliprole resistance of the CYAN-R strain (Wang et al., 2020a). Insect pests have been demonstrated to develop some resistance to other classes of chemical agents under chronic exposure of selection, prompting studies to explore mechanisms of resistance (Wang et al., 2020b; Wang et al., 2020d; Yang et al., 2020; Zeng et al., 2021). In the current work, continuous cyantraniliprole selections for 15 generations increased resistance from 55.0-fold to 80.9-fold in the CYAN-R strain.

The overexpression of P450s mediating metabolic resistance to various insecticides has been indicated in many species of insect pests worldwide (Nauen et al., 2022). In recent reports of insecticide resistance in *B. tabaci*, P450-mediated resistance was one of the most reported mechanisms underlying resistance to several insecticides including imidacloprid, thiamethoxam, acetamiprid, and flupyradifurone (Wang et al., 2020c; Wang Q et al., 2020; Yang et al., 2020; Zhou et al., 2020; Liang et al., 2022). In the current study, overexpression was detected for two P450 genes, *CYP6DW2* and *CYP4G68*, in the CYAN-R strain compared with the susceptible strain. Similarly, a previous report showed that various levels of resistance to imidacloprid in field populations of *B. tabaci* resulted from the overexpression of *CYP4C64* and *CYP6CM1*, two P450 genes (Yang et al., 2013). Furthermore, we found that silencing *CYP4G68* resulted in a considerably increased death rate in adults treated with cyantraniliprole in the CYAN-R strain, yet silencing *CYP6DW2* did not significantly increase the death rate of adults treated with cyantraniliprole in the CYAN-R strain. All the findings demonstrated that *CYP4G68* can contribute to increased cyantraniliprole resistance in whiteflies. In addition to P450-mediated cyantraniliprole resistance, it has been demonstrated that the expression of calmodulin and 1,4,5-trisphosphate receptor can be associated with changed susceptibility to cyantraniliprole (Guo et al., 2017; Guo

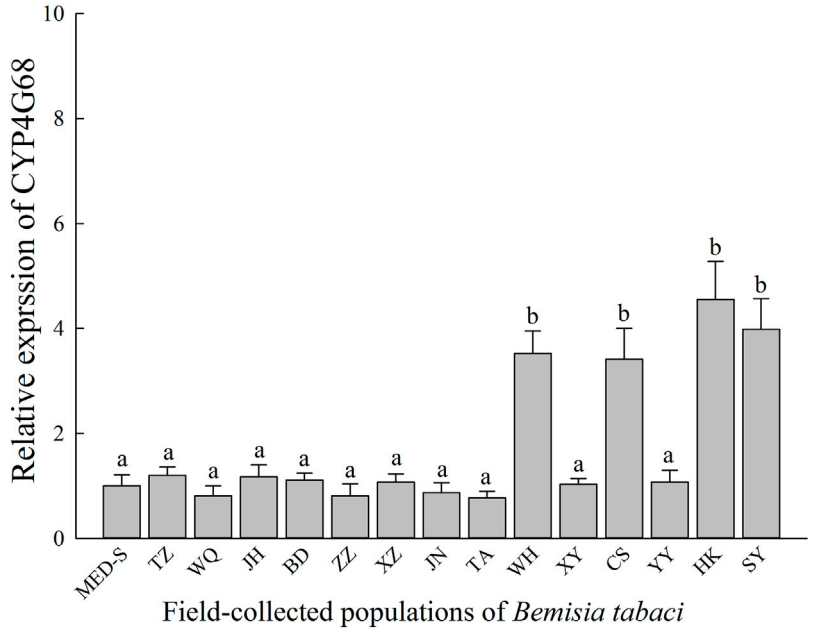

FIGURE 3 | Expression profiles of *CYP4G68* in 14 field-evolved cyantraniliprole-resistant *B. tabaci* populations from China.

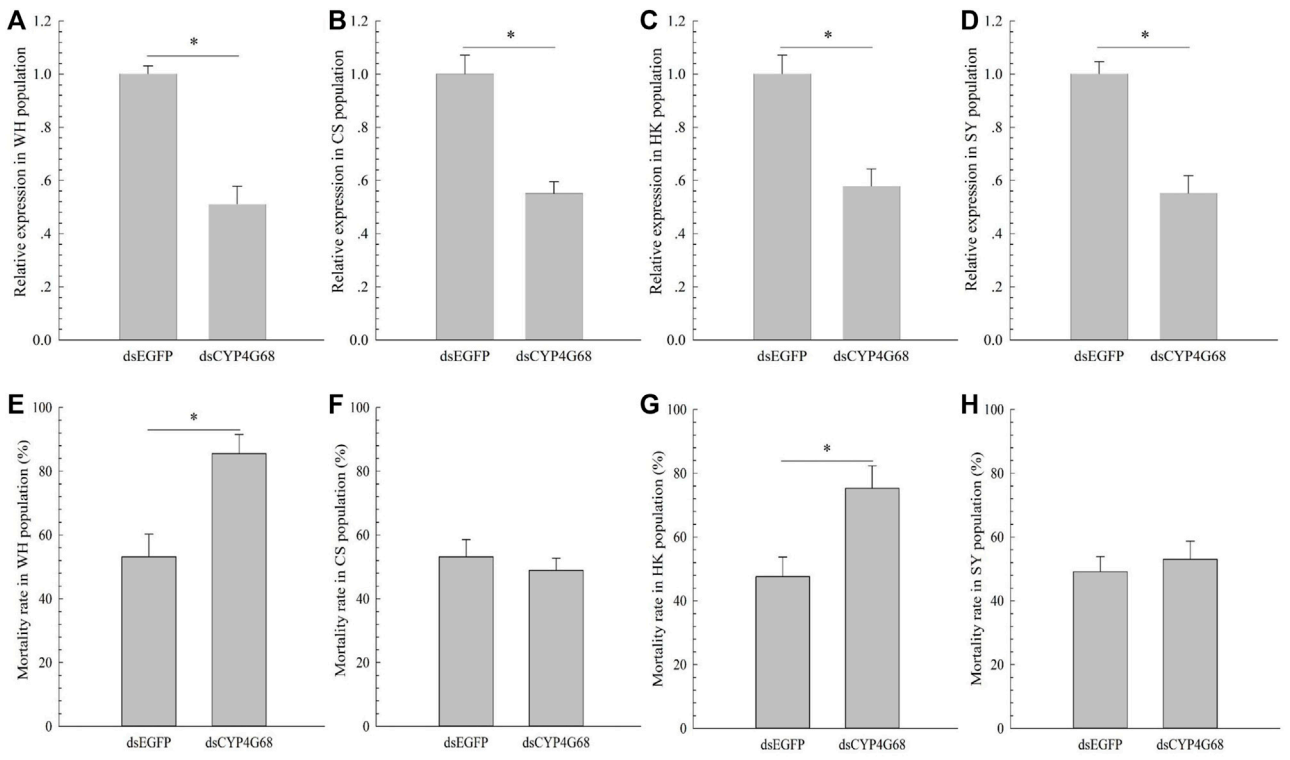

FIGURE 4 | Effects of dsCYP4G68 on the expression of *CYP4G68* in populations WH **(A)**, CS **(B)**, HK **(C)**, and SY **(D)**. Effects of silencing *CYP4G68* on resistance to cyantraniliprole in populations WH **(E)**, CS **(F)**, HK **(G)**, and SY **(H)**.

et al., 2021) and Ca^{2+}-binding protein function in response to cyantraniliprole exposure through the stabilization of Ca^{2+} concentration (Guo et al., 2019).

Considering that the use of cyantraniliprole across China has facilitated reasonable solutions for solving underlying problems of resistance to popular chemical agent against *B. tabaci*, it is essential to understand whether whiteflies have already developed resistance to cyantraniliprole in the field. Previously, we monitored the resistance levels of cyantraniliprole against whiteflies throughout China from 2015 to 2016 and found only a few field-collected populations showing low resistance (Wang et al., 2018). In our current work, we monitored the levels of cyantraniliprole resistance in 18 field-sampled populations from nine provinces across China in 2021, and found that 14 of the populations showed low-to-high resistance to cyantraniliprole, which means field-evolved cyantraniliprole resistance has escalated in China. Furthermore, we found the overexpression of *CYP4G68* in the cyantraniliprole-resistant populations, WH, CS, HK, and SY, and confirmed that this overexpression contributed to resistance in the WH and HK populations but not in the CS and SY populations. Considering that *CYP4G68* functions in thiamethoxam and imidacloprid resistance in *B. tabaci* (Wang Q et al., 2020; Liang et al., 2022), we surmise that overuse and the long-term application of neonicotinoids and cyantraniliprole in China may give rise to the rapid development of resistance associated with overexpression of *CYP4G68*. Hence, *CYP4G68* can be utilized for monitoring and managing cyantraniliprole resistance in field-developed resistant populations. Our current findings supply novel opinions and understandings concerning possible functions of P450 genes in cyantraniliprole resistance and provide more evidence for the further studies of P450s-associated resistance. Besides, our results could be instrumental in formulating strategies of pest management for controlling insect pests sustainably with more environment-friendly approaches.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RW, WC, and CL conceived and designed the study. RW and WC performed the experiments and analyzed the data with the help of CQ, JW, and CL. RW wrote the first draft of the manuscript. RW, WC, CQ, JW, and CL participated in manuscript drafting and modification.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.914636/full#supplementary-material

REFERENCES

Barry, J. D., Portillo, H. E., Annan, I. B., Cameron, R. A., Clagg, D. G., Dietrich, R. F., et al. (2015). Movement of Cyantraniliprole in Plants after Foliar Applications and its Impact on the Control of Sucking and Chewing Insects. *Pest. Manag. Sci.* 71 (3), 395–403. doi:10.1002/ps.3816

Bielza, P., and Guillén, J. (2015). Cyantraniliprole: A Valuable Tool for *Frankliniella occidentalis*(Pergande) Management. *Pest. Manag. Sci.* 71 (8), 1068–1074. doi:10.1002/ps.3886

Du, T., Fu, B., Wei, X., Yin, C., Yang, J., Huang, M., et al. (2021). Knockdown of UGT352A5 Decreases the Thiamethoxam Resistance in *Bemisia tabaci* (Hemiptera: Gennadius). *Int. J. Biol. Macromol.* 186, 100–108. doi:10.1016/j.ijbiomac.2021.07.040

Foster, S. P., Denholm, I., Rison, J. L., Portillo, H. E., Margaritopoulis, J., and Slater, R. (2012). Susceptibility of Standard Clones and European Field Populations of the Green Peach Aphid, *Myzus persicae*, and the Cotton Aphid, *Aphis gossypii* (Hemiptera: Aphididae), to the Novel Anthranilic Diamide Insecticide Cyantraniliprole. *Pest. Manag. Sci.* 68 (4), 629–633. doi:10.1002/ps.2306

Grávalos, C., Fernández, E., Belando, A., Moreno, I., Ros, C., and Bielza, P. (2015). Cross-resistance and Baseline Susceptibility of Mediterranean Strains of *Bemisia tabaci* Cyantraniliprole. *Pest. Manag. Sci.* 71 (7), 1030–1036. doi:10.1002/ps.3885

Guo, L., Liang, P., Fang, K., and Chu, D. (2017). Silence of Inositol 1,4,5-trisphosphate Receptor Expression Decreases Cyantraniliprole Susceptibility in *Bemisia tabaci*. *Pesticide Biochem. Physiol.* 142, 162–169. doi:10.1016/j.pestbp.2017.07.005

Guo, L., Li, C., Liang, P., and Chu, D. (2019). Cloning and Functional Analysis of Two Ca^{2+}-Binding Proteins (CaBPs) in Response to Cyantraniliprole Exposure in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *J. Agric. Food Chem.* 67 (40), 11035–11043. doi:10.1021/acs.jafc.9b04028

Guo, L., Li, C., Coupland, G., Liang, P., and Chu, D. (2021). Up-regulation of Calmodulin Involved in the Stress Response to Cyantraniliprole in the Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Sci.* 28 (6), 1745–1755. doi:10.1111/1744-7917.12887

Horowitz, A. R., Ghanim, M., Roditakis, E., Nauen, R., and Ishaaya, I. (2020). Insecticide Resistance and its Management in *Bemisia tabaci* Species. *J. Pest Sci.* 93, 893–910. doi:10.1007/s10340-020-01210-0

Huang, L., Lu, M., Han, G., Du, Y., and Wang, J. (2016). Sublethal Effects of Chlorantraniliprole on Development, Reproduction and Vitellogenin Gene (CsVg) Expression in the Rice Stem borer, *Chilo suppressalis*. *Pest. Manag. Sci.* 72 (12), 2280–2286. doi:10.1002/ps.4271

Jeanguenat, A. (2013). The Story of a New Insecticidal Chemistry Class: the Diamides. *Pest. Manag. Sci.* 69 (1), 7–14. doi:10.1002/ps.3406

Lahm, G. P., Selby, T. P., Freudenberger, J. H., Stevenson, T. M., Myers, B. J., Seburyamo, G., et al. (2005). Insecticidal Anthranilic Diamides: A New Class of Potent Ryanodine Receptor Activators. *Bioorg. Med. Chem. Lett.* 15 (22), 4898–4906. doi:10.1016/j.bmcl.2005.08.034

Liang, J., Yang, J., Hu, J., Fu, B., Gong, P., Du, T., et al. (2022). Cytpchrome P450 CYP4G68 is Associated with Imidacloprid and Thiamethoxam Resistance in Field Whitefly, *Bemisia tabaci* (Hemiptera: Gennadius). *Agriculture* 12 (4), 473. doi:10.3390/agriculture12040473

Luo, C., Yao, Y., Wang, R. J., Yan, F. M., Hu, D. X., and Zhang, Z. L. (2002). The Use of Mitochondrial Cytochrome Oxidase mt COI Gene Sequences for the Identification of Biotypes of *Bemisia tabaci* (Gennadius) in China. *Acta Entomol. Sin.* 45, 759–763.

Meng, X., Zhang, N., Yang, X., Miao, L., Jiang, H., Ji, C., et al. (2020). Sublethal Effects of Chlorantraniliprole on Molting Hormone Levels and mRNA Expressions of Three Halloween Genes in the Rice Stem Borer, *Chilo suppressalis*. *Chemosphere* 238, 124676. doi:10.1016/j.chemosphere.2019.124676

Moreno, I., Belando, A., Grávalos, C., and Bielza, P. (2018). Baseline Susceptibility of Mediterranean Strains of *Trialeurodes vaporariorum* (Westwood) to Cyantraniliprole. *Pest. Manag. Sci.* 74 (7), 1552–1557. doi:10.1002/ps.4869

Nauen, R., Bass, C., Feyereisen, R., and Vontas, J. (2022). The Role of Cytochrome P450s in Insect Toxicology and Resistance. *Annu. Rev. Entomol.* 67, 105–124. doi:10.1146/annurev-ento-070621-061328

Nozad-Bonab, Z., Hejazi, M. J., Iranipour, S., and Arzanlou, M. (2017). Lethal and Sublethal Effects of Some Chemical and Biological Insecticides on *Tuta absoluta* (Lepidoptera: Gelechiidae) Eggs and Neonates. *J. Econ. Entomol.* 110 (3), 1138–1144. doi:10.1093/jee/tox079

Palumbo, J. C., Horowitz, A. R., and Prabhaker, N. (2001). Insecticidal Control and Resistance Management for *Bemisia tabaci*. *Crop Prot.* 20 (9), 739–765. doi:10.1016/S0261-2194(01)00117-X

Portillo, H. E., Annan, I. B., and Marçon, G. Z. (2009). "Cyazypyr (DPX-HGW86, Cyantraniliprole): A Novel Anthranilic Diamide Insecticide for Control of Whiteflies and Other Important Arthropod Pests," in 5th International Bemisia Workshop Abstracts, 60, Guangzhou, China. Available at: http://invasivespecies.org.cn/uploadfile/20091207/ 20091207104910715.pdf (Accessed September 8, 2011).

Sattelle, D. B., Cordova, D., and Cheek, T. R. (2008). Insect Ryanodine Receptors: Molecular Targets for Novel Pest Control Chemicals. *Invert. Neurosci.* 8 (3), 107–119. doi:10.1007/s10158-008-0076-4

Schuster, D. J., Perez, N. A., Portillo, H. E., Marçon, G. Z., and Annan, I. B. (2009). "Cyazypyr (DPX6HGW86, Cyantraniliprole): A Novel Anthranilic Diamide Insecticide for Managing *Bemisia tabaci* and Interfering With Transmission of Tomato Yellow Leaf Curl Virus on Tomato Transplants," in 5th International Bemisia Workshop Abstracts, 59, Guangzhou, China. Available at: http://invasivespecies.org.cn/uploadfile/20091207/ 20091207104910715.pdf (Accessed September 8, 2011).

Stansly, P. A., Kostyk, B., and Riefer, R. (2010). Effect of Rate and Application Method of Cyazypyr (HGW86) on Control of Silverleaf Whitefly and Southern Armyworm in Staked Tomato. *Arthropod. Manag. Tests* 35, 1–3.

Wang, Z., Yan, H., Yang, Y., and Wu, Y. (2010). Biotype and Insecticide Resistance Status of the Whitefly *Bemisia tabaci* from China. *Pest. Manag. Sci.* 66 (12), 1360–1366. doi:10.1002/ps.2023

Wang, X. W., Li, P., and Liu, S. S. (2017). Whitefly Interactions with Plants. *Curr. Opin. Insect Sci.* 19, 70–75. doi:10.1016/j.cois.2017.02.001

Wang, R., Wang, J. D., Che, W. N., and Luo, C. (2018). First Report of Field Resistance to Cyantraniliprole, a New Anthranilic Diamide Insecticide, on *Bemisia tabaci* MED in China. *J. Integr. Agric.* 17 (1), 158–163. doi:10.1016/S2095-3119(16)61613-1

Wang, R., Wang, J. D., Che, W. N., Sun, Y., Li, W. X., and Luo, C. (2019). Characterization of Field-Evolved Resistance to Cyantraniliprole in *Bemisia tabaci* MED from China. *J. Integr. Agric.* 18 (11), 2571–2578. doi:10.1016/S2095-3119(19)62557-8

Wang, R., Che, W., Wang, J., Qu, C., and Luo, C. (2020a). Cross-resistance and Biochemical Mechanism of Resistance to Cyantraniliprole in a Near-Isogenic Line of Whitefly *Bemisia tabaci* Mediterranean (Q Biotype). *Pesticide Biochem. Physiol.* 167, 104590. doi:10.1016/j.pestbp.2020.104590

Wang, R., Qu, C., Wang, Z., and Yang, G. (2020b). Cross-resistance, Biochemical Mechanism and Fitness Costs of Laboratory-Selected Resistance to Pyridalyl in Diamondback Moth, *Plutella xylostella*. *Pesticide Biochem. Physiol.* 163, 8–13. doi:10.1016/j.pestbp.2019.10.008

Wang, R., Wang, J., Zhang, J., Che, W., Feng, H., and Luo, C. (2020c). Characterization of Flupyradifurone Resistance in the Whitefly *Bemisia tabaci* Mediterranean (Q Biotype). *Pest Manag. Sci.* 76 (12), 4286–4292. doi:10.1002/ps.5995

Wang, R., Wang, Z., Luo, C., and Yang, G. (2020d). Characterization of Pyridalyl Resistance in a Laboratory-Selected Strain of *Frankliniella occidentalis*. *Pesticide Biochem. Physiol.* 166, 104564. doi:10.1016/j.pestbp.2020.104564

Wang, R., Gao, B., Che, W., Qu, C., Zhou, X., and Luo, C. (2022). First Report of Field Resistance to Afidopyropen, the Novel Pyropene Insecticide, on *Bemisia tabaci* Mediterranean (Q Biotype) from China. *Agronomy* 12 (3), 724. doi:10.3390/agronomy12030724

Wang, Q., Wang, M. N., Jia, Z. Z., Ahmat, T., Xie, L. J., and Jiang, W. H. (2020). Resistance to Neonicotinoid Insecticides and Expression Changes of Eighteen Cytochrome P450 Genes in Field Populations of *Bemisia tabaci* from Xinjiang, China. *Entomol. Res.* 50 (4), 205–211. doi:10.1111/1748-5967.12427

Wei, J., He, Y. Z., Guo, Q., Guo, T., Liu, Y. Q., Zhou, X. P., et al. (2017). Vector Development and Vitellogenin Determine the Transovarial Transmission of Begomoviruses. *Proc. Natl. Acad. Sci. U.S.A.* 114 (26), 6746–6751. doi:10.1073/pnas.1701720114

Wei, P., Che, W., Wang, J., Xiao, D., Wang, R., and Luo, C. (2018). RNA Interference of Glutamate-Gated Chloride Channel Decreases Abamectin Susceptibility in *Bemisia tabaci*. *Pesticide Biochem. Physiol.* 145, 1–7. doi:10.1016/j.pestbp.2017.12.004

Yang, X., Xie, W., Wang, S. L., Wu, Q. J., Pan, H. P., Li, R. M., et al. (2013). Two Cytochrome P450 Genes are Involved in Imidacloprid Resistance in Field Populations of the Whitefly, *Bemisia tabaci*, in China. *Pesticide Biochem. Physiol.* 107 (3), 343–350. doi:10.1016/j.pestbp.2013.10.002

Yang, X., Deng, S., Wei, X., Yang, J., Zhao, Q., Yin, C., et al. (2020). MAPK-directed Activation of the Whitefly Transcription Factor CREB Leads to P450-Mediated Imidacloprid Resistance. *Proc. Natl. Acad. Sci. U.S.A.* 117 (19), 10246–10253. doi:10.1073/pnas.1913603117

Yang, X., Wei, X., Yang, J., Du, T., Yin, C., Fu, B., et al. (2021). Epitranscriptomic Regulation of Insecticide Resistance. *Sci. Adv.* 7 (19), eabe5903. doi:10.1126/sciadv.abe5903

Zeng, X., Pan, Y., Tian, F., Li, J., Xu, H., Liu, X., et al. (2021). Functional Validation of Key Cytochrome P450 Monooxygenase and UDP-Glycosyltransferase Genes Conferring Cyantraniliprole Resistance in *Aphis gossypii* Glover. *Pesticide Biochem. Physiol.* 176, 104879. doi:10.1016/j.pestbp.2021.104879

Zheng, H., Xie, W., Wang, S., Wu, Q., Zhou, X., and Zhang, Y. (2017). Dynamic Monitoring (B versus Q) and Further Resistance Status of Q-type *Bemisia tabaci* in China. *Crop Prot.* 94, 115–122. doi:10.1016/j.cropro.2016.11.035

Zheng, H., Xie, W., Fu, B., Xiao, S., Tan, X., Ji, Y., et al. (2021). Annual Analysis of Field-evolved Insecticide Resistance in *Bemisia tabaci* across China. *Pest Manag. Sci.* 77 (6), 2990–3001. doi:10.1002/ps.6338

Zhou, C. S., Cao, Q., Li, G. Z., and Ma, D. Y. (2020). Role of Several Cytochrome P450s in the Resistance and Cross-Resistance against Imidacloprid and Acetamiprid of *Bemisia tabaci* (Hemiptera: Aleyrodidae) MEAM1 Cryptic Species in Xinjiang, China. *Pesticide Biochem. Physiol.* 163, 209–215. doi:10.1016/j.pestbp.2019.11.017

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.