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Both Point Mutations and Reduced Expression of Acetylcholinesterase 2 (*ace2*) Contribute to Omethoate Resistance in *Myzus persicae*

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ABSTRACT

***Myzus persicae* (Sulzer) is a major agricultural pest responsible for significant crop losses worldwide, primarily through direct feeding and virus transmission. The extensive use of organophosphorus insecticides has led to the development of resistance in many field populations, posing a serious challenge for pest management. This study aimed to investigate the contribution of the S431F point mutation and the expression level of the acetylcholinesterase 2 gene (*ace2*) to omethoate resistance in *M. persicae*.**

Seven field populations and one susceptible laboratory strain were evaluated using bioassays, mutation screening, and gene expression analysis. Toxicity tests revealed a wide range of resistance levels, with resistance ratios (RR) varying from 3.69 to 207.73. The S431F mutation was detected in five populations, with frequencies ranging from 10% to 60%, and showed a positive association with resistance levels. In contrast, no mutation was detected in the two most susceptible populations.

Quantitative real-time PCR analysis indicated a significant down-regulation of *ace2* expression in resistant populations compared to the susceptible strain. The reduction in gene expression ranged from 38.2% to 69.0%, suggesting a potential role in resistance mechanisms.

The results demonstrate that both the S431F mutation and reduced *ace2* expression contribute to omethoate resistance in *M. persicae*. These findings provide valuable insights into the molecular basis of insecticide resistance and may support the development of more effective resistance management strategies.

Keywords: *Myzus persicae*, Acetylcholinesterase 2 (*ace2*), organophosphate (OP), mutation S431F, insecticide resistance.

INTRODUCTION

The peach-potato aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) as a versatile aphid, has a wide range of hosts (Sharma et al., 2024). It mainly spreads plant viruses indirectly by sucking on plant leaf juice, which leads to serious agricultural losses (Li et al., 2025). At present, the prevention and control ways are becoming more diversified. Although different strategies have been adopted to control peach aphids, their management mainly relies on insecticides (Kho et al., 2024; Sabra et al., 2025). This has also led to the formation and evolution of insecticide resistance becoming the biggest challenge affecting the sustainable development of insect pest control in many cases (Hawkins et al., 2019; Singh et al., 2021). In addition, the peach-potato aphids in field populations have developed different degrees of resistance to different insecticides, including organophosphate (OP) insecticides, bringing difficulties to manage (Umina et al., 2014; Tang et al., 2017). Omethoate (OMT) is an organophosphorus insecticide with strong systemic absorption and high toxicity, and is widely used to control pests on various crops. Many pest developed serious resistance as its extensively appliacation in field populations (Shang et al., 2012; Wang et al., 2016; Tang et al., 2017).

Insects have four primary resistance mechanisms, including metabolic, target-site, penetration, and behavioral resistance (Bass et al., 2014; Ingham et al., 2018). Increased metabolic detoxification is considered as a crucial mechanism of organophosphate resistance (Li et al., 2003; Zibaee, 2016). As the enhanced expression of cytochrome P450 (P450) in *Aphis gossypii* (Shang et al., 2012),

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glutathione S-transferases (GST) in *Periplaneta americana* (Sun et al., 2024), and carboxylesterase in mosquito are involved in organophosphorus insecticide resistance (Zhang et al., 2004).

It has been reported that change of *Ace1* and *Ace2* expression is often related to organophosphate resistance (Revuelta et al., 2009; Pan et al., 2010; Meng et al., 2015). It was found that *Ace1* expression downregulation significantly improved grain aphid resistance to omethoate in *A. gossypii* (Pan et al., 2010); However, It was also found that *AChE* expression upregulation significantly improved grain aphid resistance in *Schizaphis graminum* (Gao and Zhu, 2002), showing that it could be a possible resistance and/or tolerance mechanism to organophosphorus insecticides.

Decreased AChE sensitivity to insecticides resulting from gene mutation is a major factor contributing to the development of organophosphate resistance. Target gene mutations can cause insecticide resistance by affecting the ability of insecticides to bind to receptors (Zhu et al., 2000; Alon et al., 2008; Kakani and Mathiopoulos, 2008). Acetylcholinesterase (AChE) is a key enzyme involved in the termination of nerve transmission at cholinergic synapses by hydrolyzing acetylcholine (ACh) released from the presynaptic terminal, and thus it is an effective target for organophosphate (OP) and carbamate insecticides (Aroniadou-Anderjaska et al., 2023). The insensitive AChE was proved responsible for resistance to some other organophosphate and carbamate insecticides in *M. persicae* and cotton aphid, *A. gossypii* (Andrews et al., 2004; Srigiriraju et al., 2010; Cai et al., 2021).

Nabeshima et al. (2003) discovered that the point mutation S431F in the *Ace2* gene confers pirimicarb resistance in *M. persicae*; While there were other reports that S431F mutation in acetylcholinesterase-1 of *A. gossypii* confers pirimicarb and omethoate resistance (Benting, and Nauen, 2004), and S431F mutation on AChE1 confers pirimicarb in *Sitobion miscanthi* (Wang et al., 2024). Most studies showed that insect *ace1* gene encoded the main synaptic AChE in agricultural pests (Nabeshima et al., 2003; Alout et al., 2007; Lee et al., 2007). It was well known that insect *ace1* had more important roles than *ace2* (Lee et al., 2006, 2007). However, *Bm-ace2* was expressed more highly than *Bm-ace1*, suggesting *ace2*, rather than *ace1*, is the major type of AChE in silkworm (*Bombyx mori*) (Chen et al., 2001). There was another study revealed that predominantly expressed *ace2* rather than *ace1* is as the major catalytic enzyme (Kim and Lee, 2013). As a typical case, AChE2 was confirmed to play the major role in synaptic transmission, whereas AChE1 has non-neuronal functions in the western honey bee (Kim et al., 2012).

So, determination of the S431F mutation mutations reported, omethoate resistance level and the relative expression levels of *Mpace2* by real-time quantitative reverse transcription polymerase chain rection (qPCR) in the peach-potato aphid from 7 populations were conducted. The results indicated that both mutations and down-regulation of *Mpace2* may be involved in organophosphorus insecticide resistance in *M. persicae*.

Despite these advances, the combined effects of *ace2* mutations and gene expression on omethoate resistance in *M. persicae* remain insufficiently understood, particularly in field populations.

Therefore, the objective of the present study was to investigate the role of the S431F mutation and ace2 gene expression in conferring resistance to omethoate in different populations of *M. persicae*. By integrating bioassays, mutation analysis, and gene expression profiling, this study aims to provide a better understanding of the molecular mechanisms underlying insecticide resistance and to support the development of improved pest management strategies.

MATERIAL AND METHODS

Insects

In 2016, a susceptible laboratory population of *M. persicae* strain was collected from a Chinese cabbage (*Brassica oleracea* var. capitata L.) field in Liudian Village, Qiliying Town, Xinxiang City, Henan Province, China (Site: N 35.119217°, E113.802246°) in 2016.

Subsequently, this population was continuously raised in the laboratory without insecticide exposure to establish a stable susceptible reference strain. 7 field populations of *M. persicae* were randomly collected according to geographical distribution and field insecticide application pressure from four provinces in China (Figure 1) in 2024. At least 30 *M. persicae* were isolated from the 7 populations, and each aphid was kept alone in a insect cage. Mutations in the offspring of these 30 peach-potato aphids were measured after 7 days. They were reared on radish (*Raphanus sativus*) seedlings without any insecticide exposure and kept in the greenhouse with a temperature of 23-26°C, relative humidity of 60-70%, and a 16:8 h light/dark photoperiod (Lu and Gao, 2009).

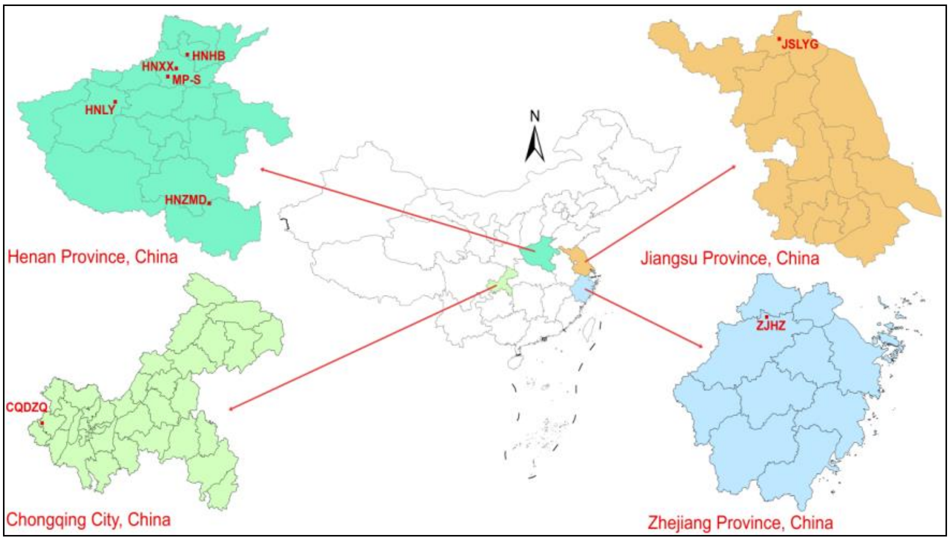


Figure 1. Collection sites of *M. persicae* in 7 different populations and MP-S of China

Bioassays

The toxicity of insecticides to the peach-potato aphid was determined using the leaf-dipping method (Hu et al., 2022). The omethoate (95.3%) were mixed with acetone to prepare the stocks (10000 μg·mL^{-1}), and then serially diluted with distilled water containing 0.05% Triton X-100 (Sigma). Fresh cabbage leaves were pressed into disks (2 cm in diameter) and immersed in the diluted solution (100, 50, 10, 1, and 0.1 μg·mL^{-1}) for 10 s. The treated leaf disks were allowed to dry at room temperature. The dried cabbage leaves were placed backside up in 12-well cell culture plates filled with 1.8 ml of 1.5% to 2% agar to maintain humidity. Healthy wingless adult aphids were placed on the leaf disks with at least 20 aphids per disk. The plates were covered with rice paper to prevent the aphids escaping and were maintained in the artificial climate where the aphids were reared. Each concentration was repeated three times, using the 0.05% Triton X-100 (Sigma) solution alone as the control, and the aphid mortality rate was examined after 24 h. The median lethal concentration (LC_{50}) values were calculated using PoloPlus 2.00 (LeOra Software Inc., Petaluma, CA). The resistance ratios were estimated at the LC_{50} level by dividing the LC_{50} of the resistant strain by the LC_{50} of the susceptible strain.

Extractions of aphid RNA and synthesis of cDNA

TRIzol (TaKaRa Bio) was used to extract total RNA from single adult aphids from the 8 populations; in total, RNA was extracted from more than 30 aphids. The concentration and quality of the total RNA were measured using a NAS-99 spectrophotometer (ACT Gene). First-strand complementary DNA (cDNA) was synthesized from the RNA (1.0 μg) using the PrimeScript II 1st Strand cDNA Synthesis Kit (TaKaRa Bio). Specific primers were used to amplify *M. persicae Mpace2* based on gDNA (GenBank: AY147797.1) (Table 1).

Quantitative real-time PCR (qPCR)

Total RNA from the same samples used for deep sequencing were used for the first-strand cDNA synthesis using PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara Biotechnology, Dalian, China) per the manufacturer's instructions. qPCR analysis was carried out using SYBR Premix Ex Taq (Takara Biotechnology, Dalian, China). Each reaction was performed on an ABI 7500 Real Time PCR system (Applied Biosystems) with three biological replicates. The relative expression levels of *Mpace2* were were normalized to *EF1α* reference gene. The expression of *Mpace2* was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Each sample was conducted for three biological replications. All primers used in this study are listed in Table 1.

Table 1. Primers used for the *Mpace2* amplification and qPCR

Primer name	Sequence (5′-3′)	Application
Mpace2-431-F	TTTCTGGGTCATTTGGGCTG	Mutation detection
Mpace2-431-R	AGGCGAATAGTTCAACAATG	Mutation detection
Mpace2-F	CTGGACAAAATGGTCGGC	qPCR
Mpace2-R	TCATCACCGTGCATCACC	qPCR
EF1*α*-F	CTGATTGTGCCGTGCTTATTG	qPCR
EF1*α*-R	TATGGTGGTTCAGTAGAGTCC	qPCR

Mutation survey of *M. persicae*

Total RNA was extracted from single adult aphids from the 8 populations, with a total of 240 aphids in all. The *Mpace2* was amplified by cDNA synthesis and the amplified product was recovered. The product was ligated into the pClone7 plasmid (Tsingke). After transfection into Escherichia coli DH5α cells (Tsingke), five to seven colonies were selected from each sample and cultured for sequencing. Sequences were compared with those of the sensitive strain to analyze the relative positions of the S431F mutation. In the mutation S431F was identified (Table 2).

Table 2. Codons of the variable positions in the *Mpace2*

	Polymorphic nucleotide position (codon) (from ATG)
Clone	1291
Susceptible population	TCA
Field population	TTT
Amino acid residue	S-F
	Ser-Phe
Position	431

Statistical analysis

Statistical analysis was performed using unpaired *t*-tests with GraphPad InStat 3.0 software (GraphPad Software). The relative expression levels across various developmental stages and tissues were compared through one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Spearman correlation analysis was performed to assess the correlations among the *ace2* mutation frequency, *ace2* relative expression level, and resistance ratio (RR) across seven field populations.

RESULTS AND DISCUSSION

The toxicity of omethoate to the peach-potato aphid of 7 different populations

The toxicity of omethoate to the peach-potato aphid of 7 different populations was determined using the leaf-dipping method. The results showed that resistance ratio in HNXX, HNHB, HNLY, HNZMD, JSLYG, CQDZQ, and ZJHZ was 207.730, 196.419, 140.142, 17.189, 6.0811, 4.466, and 3.696-fold, respectively (Table 3).

Table 3. Resistance of *M. persicae* to omethoate in different regions

Regions	Slope	LC_{50} (95 % Confidence limit) $\mu g \cdot mL^{-1}$	Degree of freedom	χ2	Resistance Ratio[a]
HNXX	0.709±0.092	30.744(21.701-43.237)	13	28.616	207.730
HNHB	0.720±0.197	29.07(12.331-137.142)	13	11.79	196.419
HNLY	1.169±0.122	20.741(16.679-26.156)	13	13.648	140.142
HNZMD	2.101±0.265	2.544 (1.996-3.222)	13	1.243	17.189
JSLYG	2.400±0.362	0.900(0.658-1.180)	13	21.877	6.0811
CQDZQ	0.491±0.128	0.661(0.112-1.741)	13	23.976	4.466
ZJHZ	0.652±0.163	0.547(0.035-1.365)	13	34.325	3.696

Notes: [a]RR (Resistance Ratios) = LC_{50} of the field population/LC_{50} of the MP-S strain; the LC_{50} of the MP-S strain was 0.148 $\mu g\ mL^{-1}$.

Analysis of *Mpace2* sequence

In the MP-S strain, the *Mpace2* was amplified using primers designed using specific primers based on *Mpace2* gene sequence in *M. persicae* (GenBank ID: AY147797.1). After sequence comparison, *Mpace2* sequence in the 7 populations was cloned and detected, among which, resulted in the S431F mutation (Figures S1-S3). The mutation frequencies of S431F in HNXX, HNHB, HNLY, HNZMD, JSLYG, CQDZQ, and ZJHZ were 60%, 46.7%, 26.7%, 10%, 10%, 0%, and 0% (Table 4). Spearman correlation analysis revealed a significant positive correlation between the *ace2* mutation rate and resistance ratio (RR) across 7 field populations of *M. persicae*. (Figure 2, $r = 0.982$, $P < 0.01$).

Table 4. Mutation frequency of acetylcholinesterase 2 (*Mpace2*) mutation site S431F in *M. persicae* from different regions

Region	Sample count	Mutation count	Mutation rate
HNXX	30	18	60%
HNHB	30	14	46.7%
HNLY	30	8	26.7%
HNZMD	30	3	10%
JSLYG	30	3	10%
CQDZQ	30	0	0%
ZJHZ	30	0	0%

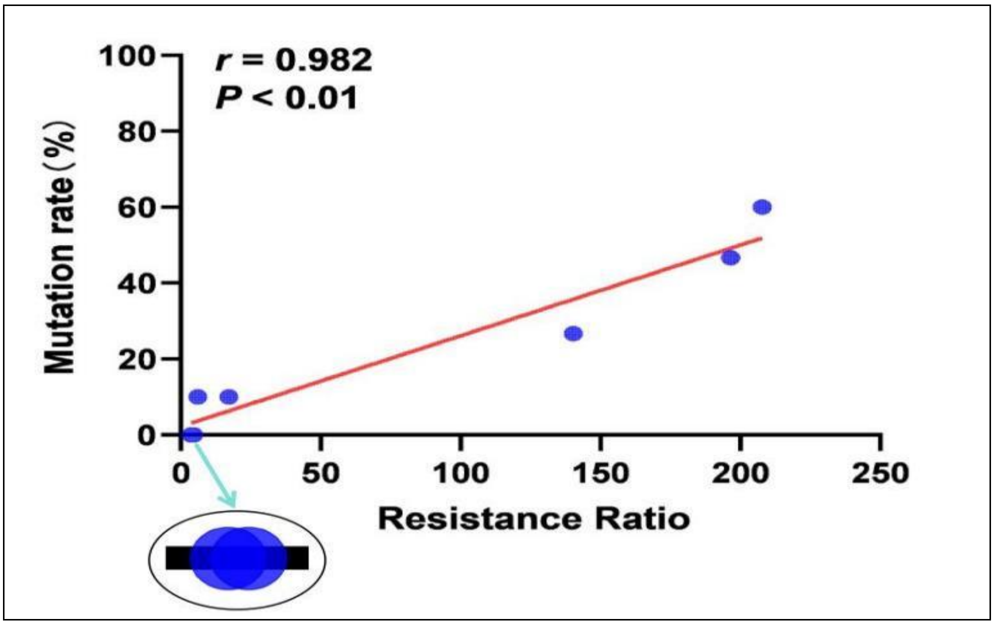
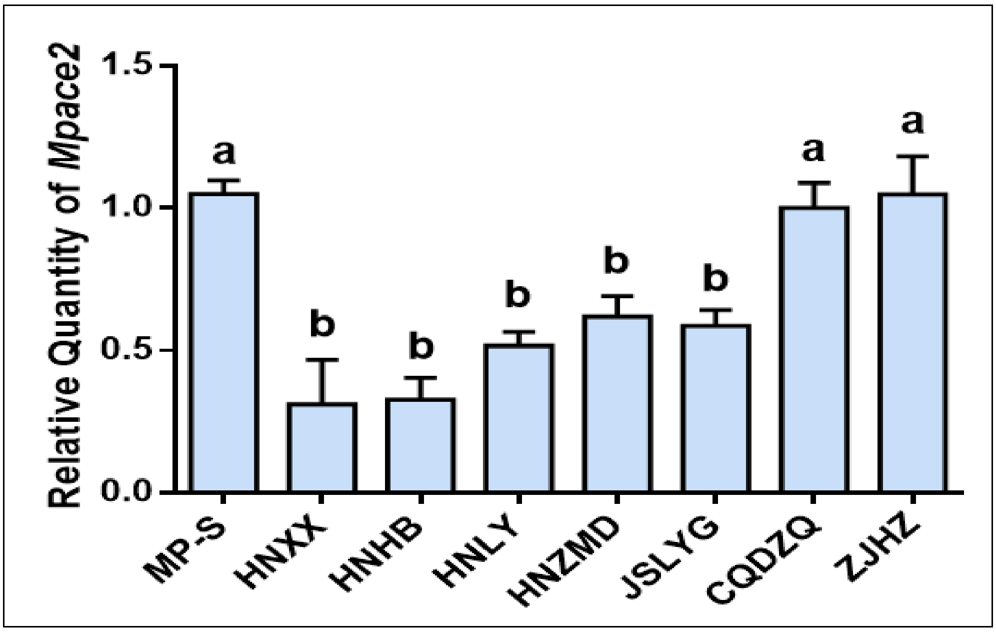


Figure 2. Spearman correlation analysis between *ace2* mutation rate and insecticide resistance ratio (RR) in 7 field populations of *M. persicae* ($r = 0.982$, $P < 0.01$)

***Mpace2* expression in 7 populations**

The mRNA relative expression levels of the *Mpace2* showed no significant differences among MP-S, CQDZQ, and ZJHZ. However, the *Mpace2* was reduced by 69.0%, 67.4%, 48.5%, 38.2, and 41.4% in HNXX, HNHB, HNLY, HNZMD, and JSLYG compared with that of MP-S, respectively (Figure 3). Spearman correlation analysis revealed a significant negative correlation between the relative expression level of *Mpace2* and the resistance ratio (RR) to the tested insecticide across 7 field populations of *M. persicae* (Figure 4, r = -0.964, P < 0.01).



Error bars represent the standard deviation of the mean of three replicates. Significant differences among the different treatments are represented by different letters (one-way ANOVA followed by Tukey's multiple comparison tests, P < 0.05).

Figure 3. Relative mRNA expression level of *Mpace2* in apterous adult aphids of MP-S and 7 different populations

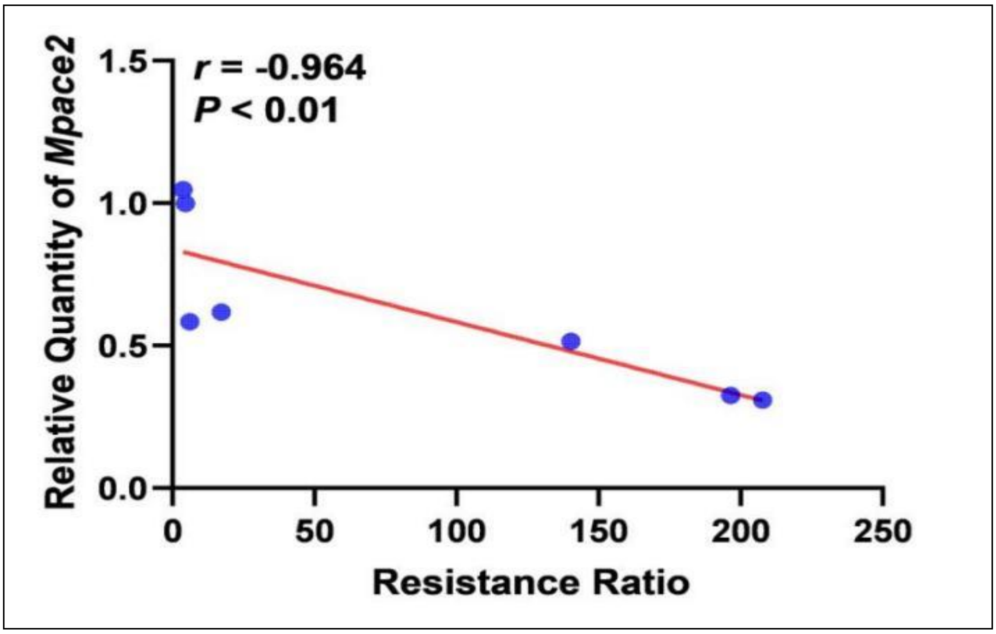


Figure 4. Spearman correlation analysis between *Mpace2* relative expression and insecticide resistance ratio (RR) in 7 field populations of *M. persicae*. (r = -0.964, P < 0.01)

For many years, insecticides have been used to combat peach-potato aphid. However, over time, the gradual formation of resistance has resulted in insecticides that were originally effective becoming less effective in controlling *M. persicae* than before. The emergence of this effect has gradually led to the population developing resistance to several insecticides. Omethoate resistance has been reported in field populations of peach-potato aphids, which has a remarkable killing effect on peach-potato aphids. However, with its continuous use, many pests including peach-potato aphids have developed significant resistance (Shang et al., 2012; Tang et al., 2017; Cai et al., 2021). In this study, the mutations, S431F with the selection pressure exerted by the omethoate insecticides were conducted in 7 populations. The S431F (TCA→TTT) mutation was detected in the 5 *M. persicae* populations, indicating that the S431F mutation play a important role in the resistance to organophosphate insecticides.

The mutation frequencies of S431F in HNXX, HNHB, HNLY, HNZMD, JSLYG, CQDZQ, and ZJHZ were 60%, 46.7%, 26.7%, 10%, 10%, 0%, and 0%, respectively. According to our results, S431F mutation might be very important to response for the high resistance to omethoate as well as other organophosphate insecticides. The S431F mutation may play a major role in a high level of resistance, especially for continuous insecticide pressure. The formation of insect resistance is the evolutionary result of adapting to insecticide stress. However, the formation of resistance insecticides has also led to changes in various aspects of the insects themselves, they often incur fitness costs, such as changes in body shape, weight, and fertility (Abbas et al., 2016; Wang et al., 2018). When the selection pressure of insecticides gradually subsides, the rules of their biological disadvantage become prominent, and resistant individuals may gradually be eliminated, ultimately leading to a decrease in population resistance (Zhu et al., 2016).

The resistance of the peach-potato aphid of 7 different populations to omethoate was determined using the leaf-dipping method, showing that resistance ratio in HNXX, HNHB, HNLY, HNZMD, JSLYG, CQDZQ, and ZJHZ was 207.730, 196.419, 140.142, 17.189, 6.0811, 4.466, and 3.696, respectively. Compared with the CQDZQ, and ZJHZ populations indicating a low resistance level, the S431F heterozygous mutantion in other 5 populations was more resistant to omethoate. The high resistance of HNXX, and HNLY to omethoate was from 207.730 to 140.142 -fold. This indicates that the S431F heterozygous mutation confer a high level of resistance to omethoate in the peach-potato aphid. It has been reported that the S431F heterozygous mutation confer a high level of resistance to organophosphate insecticides in the peach-potato aphid (Liu et al., 2017; Tang et al., 2017).

The resistance level to omethoate was higher and the S(431)F mutation frequency was also higher. This indicates that the S431F mutation plays an important role in conferring greater resistance to omethoate. The resistance of the HNXX to omethoate was the highest at 207.730-fold.; However, organophosphate insecticides is the most widely used insecticides to control peach-potato aphid (Li et al., 2016; Hu et al., 2023). Many studies have proved that the change of the *Mpace2* may be associated with organophosphate resistance in pests (Revuelta et al., 2009; Pan, 2010; Meng et al., 2015). In our current study, the relative expression levels of *Mpace2* in the 7 populations were also determined. The mRNA relative expression levels of the *Mpace2* showed no significant differences among MP-S, CQDZQ, and ZJHZ. However, the *Mpace2* was reduced by 69.0%, 67.4%, 48.5%, 38.2, and 41.4% in HNXX, HNHB, HNLY, HNZMD, and JSLYG compared with that of MP-S, respectively, possibly resulting from the selection pressure of organophosphate insecticides.

The down-regulation of the *Mpace2* could be a possible resistance mechanism of peach-potato aphid to organophosphate insecticides. This indicates that the S431F mutation and the resistance of different populations to organophosphate insecticides were not linked to the *Mpace2* expression level. The expression of *Mpace2* in the 2 populations was not altered, indicating that other factors mediate the resistance of peach-potato aphid to organophosphate insecticides. In addition to target resistance, increased resistance to pyrethroids caused by enhanced detoxification enzyme activity has also been repeatedly reported, especially increases in cytochrome P450 monooxygenase activity (Johnson et al., 2006; Ibrahim et al., 2016; Hu et al., 2024).

CONCLUSIONS

The resistance of *M. persicae* to organophosphate insecticides mediated by the S431F mutation has been widely reported. A strong positive correlation was observed between the frequency of the S431F mutation and resistance levels, indicating that this target-site modification plays a major role in conferring high resistance. In parallel, a significant down-regulation of *ace2* expression was detected in resistant populations, suggesting that transcriptional modulation may represent an additional mechanism contributing to resistance. However, the absence of expression changes in some low-resistance populations indicates that multiple mechanisms may be involved, including other target-site mutations or enhanced metabolic detoxification. These findings highlight the complexity of insecticide resistance in *M. persicae*, where both genetic mutations and gene expression changes interact to determine resistance phenotypes. Understanding these mechanisms is essential for the development of more effective and sustainable pest management strategies.

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SUPPLEMENTARY MATERIAL

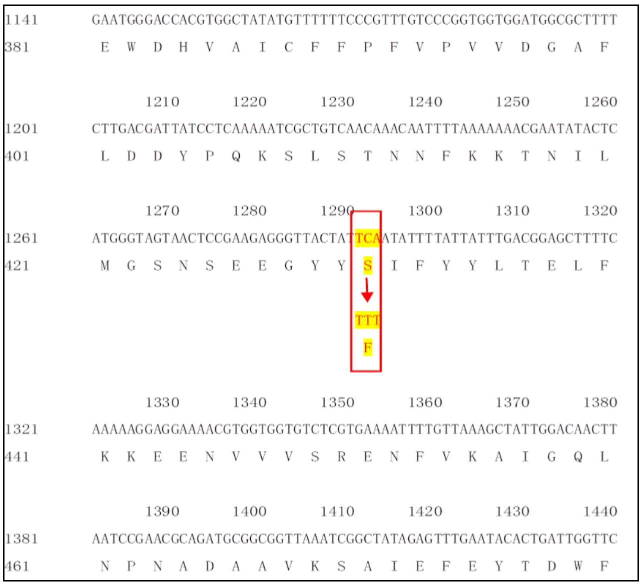
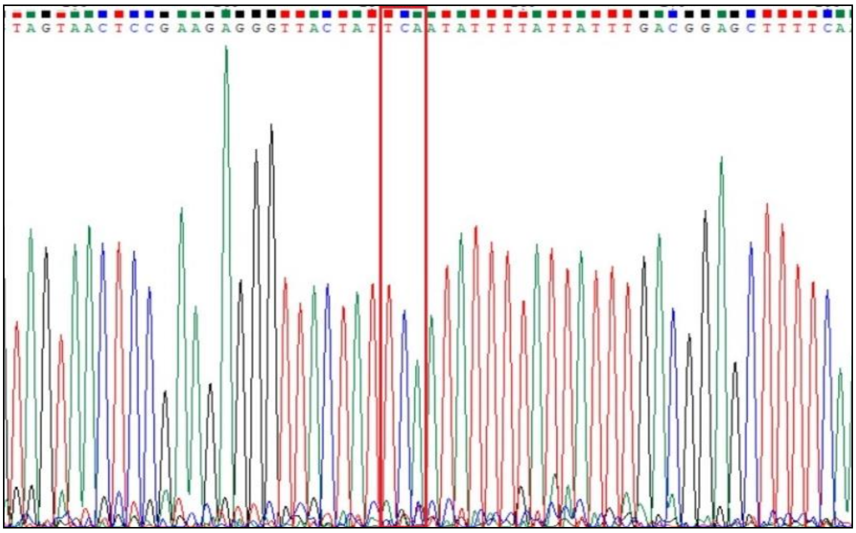
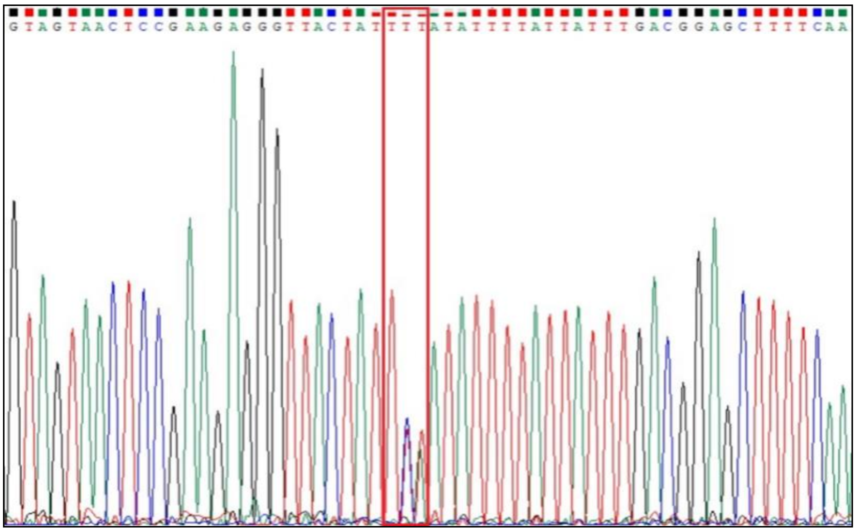


Figure S1. Schematic diagram of *M. persicae* acetylcholinesterase gene 2 (Mpace2) mutation site S431F from 7 populations

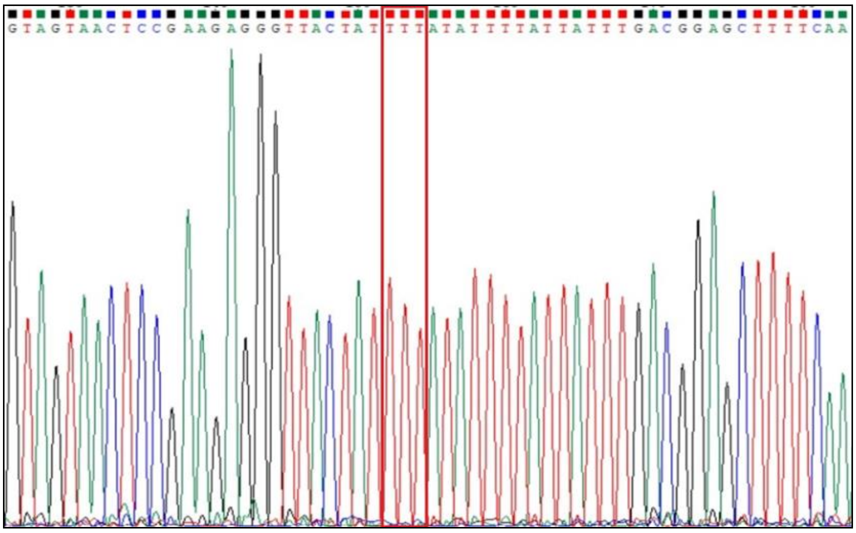
Sequencing chromatograms of S431F



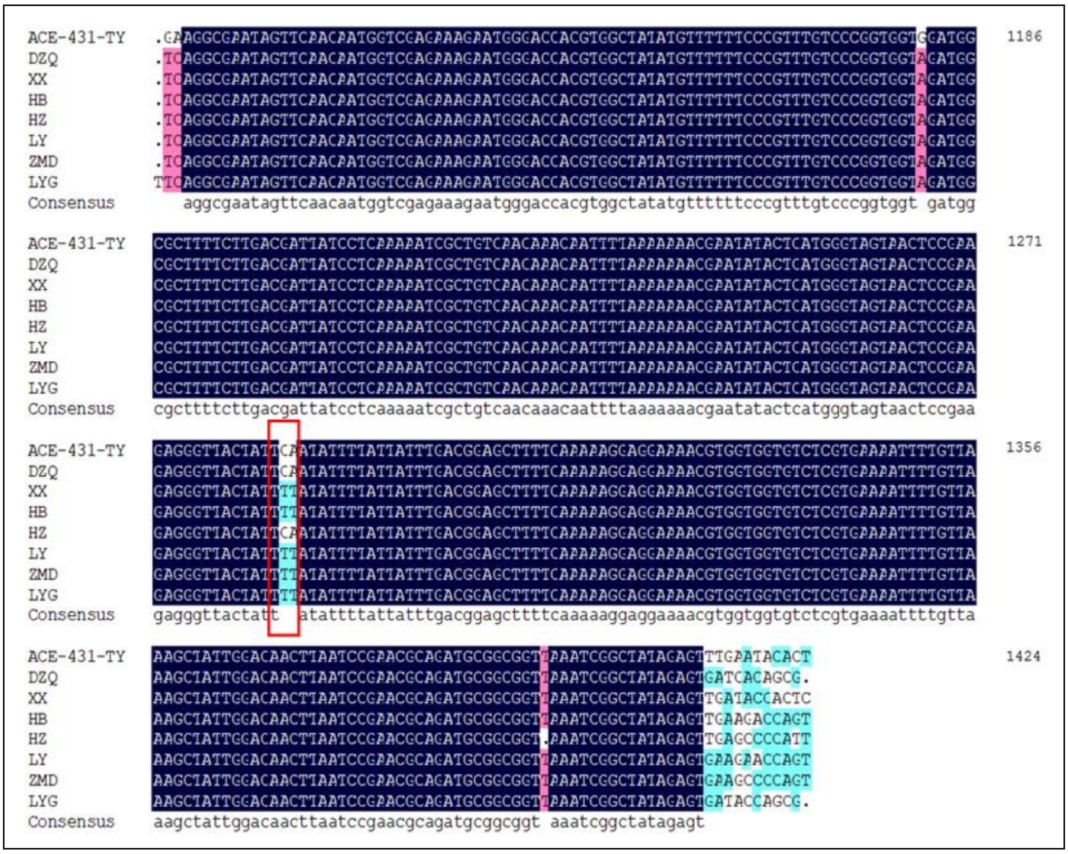
A1. Non-mutation of R81T



A2. Heterozygous mutation of S431F



A3. Homozygous mutation of S431F



B mRNA sequences

Figure S2. Comparison of mRNA sequences of *M. persicae* Acetylcholinesterase gene 2 (Mpace2) fragments from 7 populations

Figure S3. Comparison of amino acid sequence of *M. persicae* Acetylcholinesterase 2 (Mpace2) fragments from 7 populations