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Biochemical features of a resistant population of the rice stem borer, *Chilo suppressalis* (Walker)

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Abstract: Bioassay showed that the rice stem borer, *Chilo suppressalis* (Walker), collected from Cixi City, Zhejiang Province, possessed 37.7 and 52.7-fold resistance against monosultap and methamidophos respectively compared with a susceptible population collected from Taihu County, Anhui Province. The activities of microsomal O-demethylase and N-demethylase in the resistant population were 3.3 and 1.34-fold higher than those in the susceptible population. There was, however, little difference in the activities of esterase and glutathione S-transferase between the resistant and susceptible population. These findings suggest that the increased activities of microsomal O-demethylase and N-demethylase may be important mechanisms involved in the resistance of the rice stem borer to methamidophos and monosultap. In order to find the hypothesized monosultap-resistant related point mutation, cDNA fragments encoding *Chilo suppressalis* nAChR α1 (Csα1) subunit were amplified using the RT-PCR technique from susceptible and resistant individuals. Though no unique mutation was found in resistant individuals, the pattern of single nucleotide polymorphisms was described and discussed. There were a total of 33 single nucleotides polymorphisms, of which 14 resulted in amino acid polymorphisms. An insertion was also found in two of the nine cDNAs.

Key words: *Chilo suppressalis*; insecticide resistance; biochemical mechanism; nicotinic acetylcholine receptor (nAChR); α subunit; single nucleotide polymorphism (SNP)

1 Introduction

The rice stem borer *Chilo suppressalis* (Walker) is an economically important pest widespread in rice producing regions in China. This insect feeds on rice causing significant loss of yield and resultant economic losses to farmers. Since hybrid rice became the standard crop outbreaks of this pest have become serious (Ding, 1991). The major control measure for the rice stem borer is the application of chemicals which are known to cause resistance and control problems. Since the introduction of methamidophos, an organophosphorus insecticide, and monosultap, a nereistoxin insecticide in the early of 1980's, farmers have mostly depended on these pesticides to control rice stem borer because of their low price and satisfactory control efficiency. In 1980's and mid 1990's, only low or moderate resistance of rice stem borers was detected (Wang *et al*., 1987; Chu *et al*., 1987a, 1987b; Su *et al*., 1996). However, following the over application of and dependence on methamidophos and monosultap resistance to these pesticides developed rapidly, and surprisingly high levels of resistance have been recently reported in many areas of China (Li *et al*., 2001; Peng *et al*., 2001). More seriously, some instances of control failure happened in Zhejiang and Jiangsu provinces.

Nicotinic acetylcholine receptors (nAChRs), the target of nereistoxin insecticides, are members of a diverse super family of neurotransmitter-gated ion channels, which also include receptors for γ-aminobutyric acid (GABA), glycine, glutamate and 5-hydroxytryptamine (serotonin) (Millar, 1999), and play an important role in excitatory synaptic transmission in both vertebrates and invertebrates. Whereas glutamate is the principal excitatory neurotransmitter in the mammalian brain, acetylcholine (acting on nAChRs) appears to play this role in insects. nAChRs are confined to the insect central nervous system which is an extremely rich source of this excitatory neurotransmitter receptor (Sattele, 1980). In-

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sect nAChRs are also one of the most important targets for insecticides, especially the newly introduced chloronicotinyl compound imidacloprid (Elbert *et al*., 1996) and conventionally used nereistoxin insecticides such as cartap and monosultap. This has highlighted the need to examine the molecular characterization of insect and vertebrate nAChRs for better understanding of the action mode of such compounds.

In vertebrates, five muscle nAChR subunits (α, β, γ, δ and ε) and 11 neuronal nAChR subunits have been cloned (Lindstrom, 1995; Mcgehee and Role, 1995; McGehee and Role, 1995). Subunits of nAChR have also been cloned from some insects, such as ALS (Bossy *et al*., 1988), SAD/Dα2 (Baumann *et al*., 1990; Sawruk *et al*., 1990a), Dα3 (Schulz *et al*., 1998), Dα4 (Lansdell and Millar, 2000), ARD (Hermans-Borgmeyer *et al*., 1986) and SBD (Sawruk *et al*., 1990b) from *Drosophila*, Mpα1 – 5 (Sgard *et al*., 1993; Huang *et al*., 1999) and Mpβ (Huang *et al*., 1999) from *Myzus persicae*, Locα1 – 4 and Locβ1 from *Locusta migratoria* (Hermsen *et al*., 1998), αL1 from *Schistocerca gregaria* (Marshall *et al*., 1990) and MARA1 from *Manduca sexta* (Eastham *et al*., 1998).

In this study, we measured the detoxification enzyme activity of a resistant population and a susceptible population, analyzed the biochemical mechanism of resistance, and attempted to find resistance related point mutations by amplifying cDNA fragments containing the complete open reading frame (ORF) of nAChR α subunit from susceptible and monosultap-resistant individuals of *Chilo suppressalis*.

2 Materials and methods

2.1 Insects

Rice stem borers was reared on rice seedling as described by Shang *et al*. (1979). The resistant population was collected in June 2000 from Cixi, Zhejiang Province where monosultap and methamidophos had been used intensively and frequently for many years. The susceptible population was collected in 1996 from Taihu, Anhui Province where chemicals are seldom used to control rice stem borer, and did not subsequently come into contact with any insecticide. The larvae used for biochemical tests and molecular analysis were refrigerated in liquid nitrogen, and stored at −76℃. DNA fragments were cloned from susceptible larvae with the most intensive response to monosultap at a dosage of 0.045 μg/larva, and from the most active surviving resistant individuals after treatment with monosultap at a dose of 24 μg/larva.

2.2 Chemicals

94% 4-chloro-N-methlaniline (PCMA) was purchased from Aldrich Chem. Co.; NADPH-Na4 was from Sigma; 1, 2-dichloro-4-nitrobenzene (DCNB) was a product of Merck-Schuchard; Fast blue RR salt was bought from Fluka; 4-dimethlaminobenzaldehyde (PDAB) was produced by the Shanghai Chemical Plant; 4-chloroaniline (PCA), chemical grade, was produced by Shanghai Qunli Chemical Plant; 4-nitroanisole (PNA), 2, 4-dinitrochloro benzene (CDNB) and 1-naphthyl acetate, all chemical grade, were the products of the Shanghai Chemical Reagents Plant.

2.3 Toxicity test

The bioassay was performed using the topical application method developed by Tan (1987).

2.4 Esterase activity assay

The method was a modification of that described in Han *et al*. (1998). For each population, twenty 4th instar larvae were homogenized in 5 mL 0.02 mol/L sodium phosphate buffer (pH 7.6), and the homogenate was centrifuged at 5 000 × g 4℃ for 15 min. The supernatant served as the enzyme source. Enzyme activity was measured by a kinetic assay using a Bio-Rad model 550-microplate reader. In a single well of the plate, the total reaction incubation was 300 μL containing 50 μL enzyme, 1.5 mmol/L Fast Blue RR salt and series concentrations of 1-naphthyl acetate. All components were mixed quickly and the plate put into the reader immediately. Reactions were monitored for 5 min at a wavelength of 450 nm.

2.5 Glutathione S-transferase activity assay

See Kao *et al*. (1989) for the method. Twenty 4th instar were homogenized in 10 mL tri-HCl buffer (0.1 mol/L, pH 8.9, see below), containing 10 mmol/L reduced glutathione at 0℃ as a protectant, with a hand operated tissue grinder. The homogenate was filtered through 4 layers of cheesecloth and centrifuged at 10 000 × g and 4℃ for 15 min. The supernatant served as the enzyme source. Two substrates, 1, 2-dichloro-4-nitrobenzene (DCNB) and 1-chloro-2, 4-dinitrobenzene (CDNB) were used as substrate. When DCNB was used, 0.5 mL of enzyme source was added to 1.0 mL tris-HCl buffer (pH 8.0, 0.1 mol/L) and the mixture was incubated at 25℃ for 10 min. Then 20 μL of the substrate, 100 mmol/L DCNB in ethanol, was added. For CDNB, 0.1 mL of the enzyme source was added to 1.4 mL 0.1 mol/L tris-HCl buffer (pH 8.9). After preincubation at 25℃ for 10

min, 60 μL series concentrations of CDNB in ethanol was added. Reactions were followed for 2 min with a spectrophotometer at 344 nm (DCNB) or 340 nm (CDNB). The control was the same process without the substrate.

2.6 Microsomal O-demethylase activity assay

The method was a modification of that described in Shang and Soderlund (1984); 50 4th instar larva were homogenized in 5 mL phosphate buffer (0.1 mol/L, pH 7.8). The enzyme source was obtained by filtering the homogenate through 4 layers of cheesecloth. The incubation mixture contained 1 mL filtered homogenate, 1 mL 0.5 mmol/L NADPH-Na4 (buffer prepared) and 0.1 mL 0.1 mmol/L PNA in methyl Cellosove. The mixture was shaken during incubation at 34℃ in normal (air) atmosphere for 30 min. The reaction was stopped with 1 mL of 1 N HCl, and 5 mL of chloroform was added with shaking. 3 mL of the chloroform layer was pipetted into a new tube, then 3 mL of 0.5 mol/L NaOH was added to abstract the reaction product, 4-nitrophenol, from the chloroform. 2 mL of the aqueous phase was pipetted out to determine the OD value at a wavelength of 400 nm with a spectrophotometer.

2.7 Microsomal N-demethylase activity assay

The method was described by Yu (1982). The enzyme source was obtained by homogenizing 6th instar larvae in 0.1 mol/L phosphate buffer at pH 7.5. 5 mL of the incubation mixture contained 4 mL of homogenate (equivalent to 4 larvae), 1.5 μmol of NADPH-Na4, 60 μL of 0.1 mol/L PCMA in methyl Cellosove and buffer. Incubation was carried out with shaking at 34℃ in a normal (air) atmosphere for 30 min. The reaction was stopped by the addition of 2 mL of 6% PDAB in 3 N H_2SO_4, and then centrifuged at 10 000 × g for 15 min. The N-demethylase product, PCA, was detected with a microplate reader of Bio-Rad (Model) at the wavelength of 450 nm by pipetting 200 μL of the supernatant into a plate well.

2.8 Protein determination

Protein determinations were performed using the method of Bradford (1976) with bovine serum albumin as the standard.

2.9 RT-PCR and DNA sequencing

Total RNA was isolated from single larva with an isolation kit (Gibcobrl Co.). cDNA was synthesized with an oligo (dT) 18 primer by SuperScript Ⅱ (Gibco BRL) at 42℃ for 52 min. The cDNAs of the nAChR α subunit were amplified by LA (long and accurate) polymerase (TaKaRa) system with 94℃ for 30 s, 55℃ for 30 s, and 72℃ for 2 min with the forward primer 5′-CAGTGAAAATGCGTTTAGGA-3′ and reverse primer 5′-CCCATATTTTGCTGGCGGAAG, both of which were based on the previously cloned cDNA sequence of Csα1 (Han and Han, 2002). PCR products were checked with agarose gel electrophoresis, and the target bands were purified using a Wizard PCR Preps kit DNA purification system purchased from Premega Chemical Co. The purified fragments were cloned with pGEM-T Easy Vector Systems and transformed into *E. coli* DH5α. Takara Chemical Co. sequenced the positive clones.

3 Results

3.1 Toxicity test

Table 1 shows the results of monosultap and methamidophos topical applications. LD_{50} of monosultap and methamidophos were 2.2725 and 0.5321 μg/larva in the Cixi population, and 0.0603 and 0.0101 μg/larva in the Taihu population. The Cixi population had developed high (37.7 and 52.7-fold) resistance against monosultap and methamidophos.

3.2 Esterase activity

The results of 1-naphthyl acetate hydrolysis activity determinations are shown in Table 2. The enzyme catalyzing reaction dynamic parameters, V_m and K_m, in the two population were almost the same. These results suggest that esterase is not involved in the resistance of Cixi population.

Table 1　Bioassay of insecticide lethality against two population of the rice stem borer

Populations	Insecticides	(a + bx)	LD_{50} (95% CL) (μg/larva)	RR
Taihu	monosultap	6.8230 + 1.4943	0.0603 (0.0443 – 0.0821)	1
	methamidophos	9.8044 + 2.4074	0.0101 (0.0059 – 0.0172)	1
Cixi	monosultapmon	4.6153 + 1.0791	2.2725 (1.603 – 3.2215)	37.7
	methamidophos	5.5893 + 2.1505	0.5321 (0.4259 – 0.6647)	52.7

Table 2 Esterase activity in two populations of the rice stem borer

Populations	V_m[mOD/(min·μg)]	K_m (mmol/L)
Cixi-R	14.36±7.4 a	4.20±0.94 a
Taihu-S	14.53±7.2 a	4.24±0.85 a

Mean of three replicates ± *SD*. Means within columns followed by the same letter are not significantly different (*t* test, $P>0.05$)

3.3 Glutathione S-transferase (GSTs) activity

GSTs activity was assayed with two substrates, CDNB and DCNB (Table 3). When CDNB served as the substrate, V_ms were 5.40 and 4.56 OD/(min·mg) in the resistant and susceptible population respectively. In the resistant population, the V_m was 1.18-fold higher than in the susceptible population. The K_m, however, were markedly different between the two populations. K_m for DCNB, the enzyme activity of the resistant population was as much as 89% that of the susceptible population. These results suggest that there might be some modifications of GST protein in the Cixi population.

Table 3 GST activity in two populations of the in rice stem borer

Populations	Substrates				
	CDNB			DCNB	
	V_m[OD/(min·mg)]	R/S	K_m(mmol/L)	Enzyme activity[OD/(min·mg)]	R/S
Cixi-R	5.40±0.43 a	1.18	0.152±0.030 a	0.085±0.013 a	0.89
Taihu-S	4.56±0.61 a	1	0.102±0.027 b	0.095±0.014 a	1

Mean of three replicates ± *SD*. Means within columns followed by the same letter are not significantly different (*t* test, $P>0.05$)

3.4 Microsomal demethylase activity

Table 4 shows that microsomal O-demethylase activity was 1.036 and 0.318 nmol/(mg·min) respectively in the Cixi and Taihu populations, and N-demethylase activity in these populations was 0.197 and 0.147 nmol/(mg·min) respectively. It is clear that the activity of microsomal demethylase was significantly higher in the Cixi than in the Taihu population, suggesting that the increment of detoxification of MFO may be an important mechanism involved in the resistance of the Cixi population.

Table 4 Microsomal O-demethylase and N-demethylase activity in two populations of the rice stem borer

Populations	Microsomal O-demethylase		Microsomal N-demethylase	
	[nmol/(mg·min)]	R/S	[nmol/(mg·min)]	R/S
Cixi-R	1.036±0.207 a	3.3	0.197±0.043 a	1.34
Taihu-S	0.318±0.090 b	1	0.147±0.036 b	1

Mean of three replicates ± *SD*. Means within columns followed by the same letter are not significantly different (*t* test, $P>0.05$)

3.5 Single nucleotide polymorphism (SNP) in cDNAs of the nAChR α subunit form *Chilo suppressalis*

All of the amplified cDNAs contains an open reading frame (ORF) of 1 602 nucleotides encoding 533 amino acids. The deduced amino acid sequence of the precursor has typical features of the nAChR family: a signal peptide of 24 amino acids; a conserved disulfide-linked Cys loop (a structural motif present in all subunits of nAChR and other ligand-gated ion channels) formed by 15 amino acids from positions 152 to 166 (128 to 142 in the mature protein excluding the signal peptides); four hydrophobic putative transmembrane domains MT1-4; and a potential N-glycosylation site present at positions 48 (Asn^{48}, or Asn^{24} in the mature protein), which is conserved in all insect nAChR subunits reported to date and most of the vertebrate neuronal receptors (Marshall *et al*., 1990; Gundelfinger, 1992; Lindstrom, 1995; Huang *et al*., 1999). Two cysteins at positions 225 and 226 (201 and 202 in the mature protein) corresponding to the vicinal cysteines that are definitive of all known α subunits were found. All these characteristics confirmed that the cloned cDNA was from Csα1 (GenBank AF418987) as previously reported (Han and Han, 2002).

cDNA fragments of Csα1 were sequenced from five susceptible and four resistant individuals and nucleotides and deduced amino acids were compared (Table 5 and 6). There were a total of 33 nucleotide variations among the compared sequences, of which 14 resulted in amino acid substitution. Seven amino acids substitutions were found in the susceptible strain and 9 in the resistant strain. No substitution occurred in the signal peptides, but 8 occurred in the extra cellular N terminal, where the structure was mostly conserved among the mature proteins of the insect nAChR α subunits reported previously. An insertion, TTCTTATTGCAG, which encodes the four amino acids SYCS inserted between S_{384} and R_{385}, was found in two of the nine-cDNA sequences (Fig. 1).

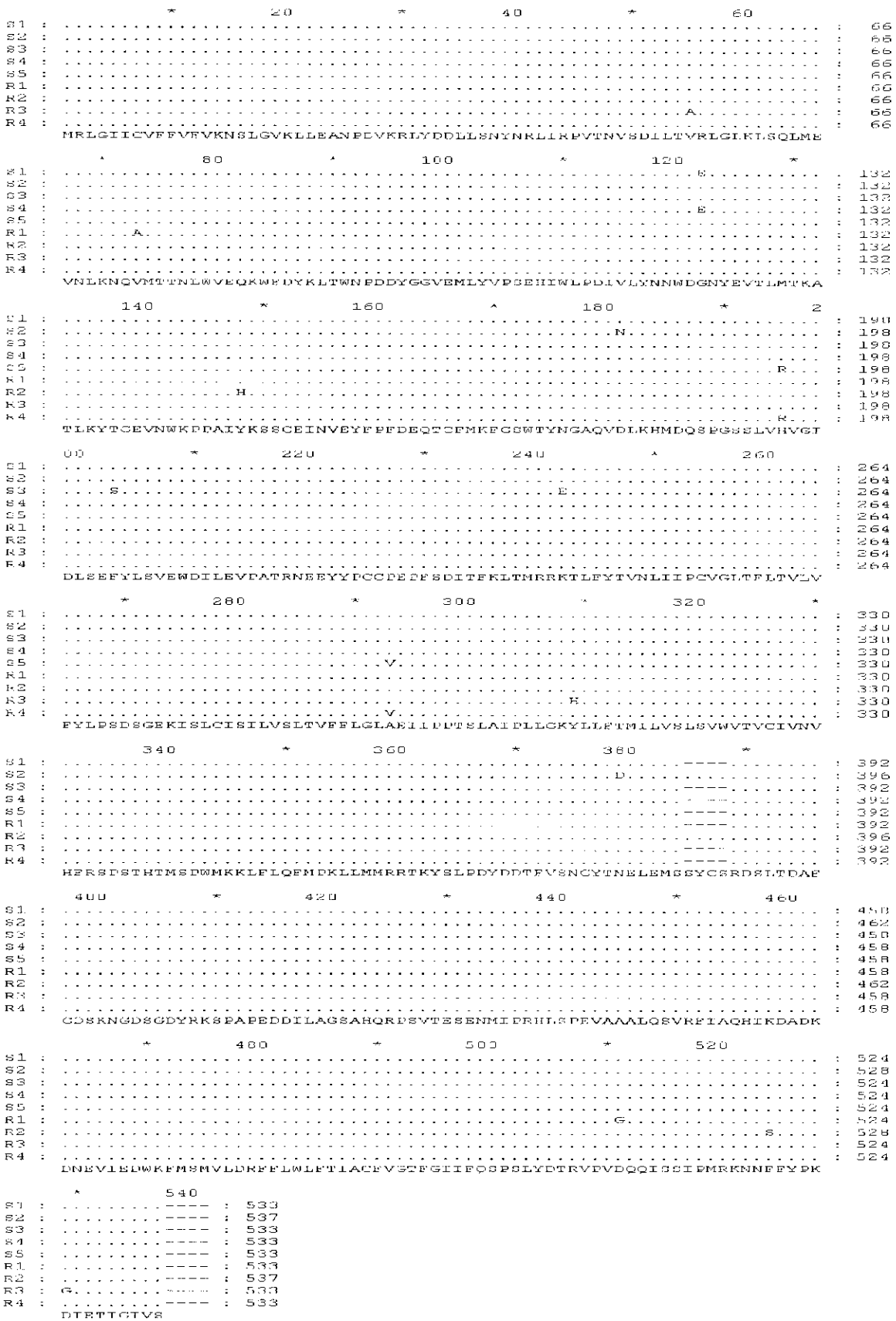

Fig. 1 Amino acid polymorphirism of Csα1 in *Chilo suppressalis*

Table 5 Single nucleotide polymorphisms in Csα1 cDNA in monosultap susceptible and resistant strains of *Chilo suppressalis*

Strains	Codon (base #)													
	* GC (52)	A * T (143)	G * C (218)	GT * (240)	G * G (365)	A * C (368)	* AC (442)	* AA (463)	TA * (474)	CC * (480)	* AC (541)	C * C (584)	T * C (608)	* AG (724)
S	G4/5	A4/5	T5/5	G4/5	G4/5	A4/5	T5/5	C4/5	T4/5	T3/5	G4/5	A4/5	T4/5	A4/5
	A1/5	G1/5		A1/5	A1/5	G1/5		T1/5	C1/5	G2/5	A1/5	G1/5	C1/5	G1/5
R	G4/4	A4/4	T3/4	G2/4	G4/4	A4/4	T3/4	C4/4	T3/4	T4/4	G4/4	G4/4	T4/4	A4/4
			C1/4	A2/4			C1/4		C1/4					

Strains	Codon (base #)													
	AA * (747)	G * C (878)	G * G (905)	GG * (921)	* AC (925)	TT * (936)	GT * (962)	A * G (1070)	* AC (1135)	TG * (1237)	C * G (1271)	GC * (1338)	CT * (1341)	GA * (1377)
S	T4/5	C4/5	C4/5	A3/5	T5/5	T5/5	A4/5	T4/5	A4/5	A4/5	A4/5	C5/5	G5/5	C4/5
	C1/5	T1/5	T1/5	G2/5			G1/5	C1/5	G1/5	G1/4	C1/5			T1/5
R	T4/4	C4/4	C4/4	A4/4	T3/4	T3/4	A4/4	T4/4	A4/4	A4/4	A4/4	C1/4	G2/4	C1/4
					C1/4	C1/4						A3/4	T2/4	T3/4

Strains	Codon (base #)				
	CT * (1509)	G * C (1532)	CA * (1536)	T * C (1571)	G * C (1574)
S	T5/5	A5/5	G3/5	T5/5	A5/5
			A2/5		
R	T2/4	A3/4	G4/4	T3/4	A3/4
	C2/4	G1/4		C1/4	G1/4

Notes: T4/5 and C1/5 mean four of five nucleotides are T and one is C.

Table 6 Amino acid polymorphisms in the cDNA encoding the AChR α subunit in susceptible and monosultap resistant strains of *Chilo suppressalis* and deduced amino acid residues

Strains	Amino acid residue												
	55	73	122	148	181	195	203	293	309	379	511	524	529
S	V5/5	V5/5	G3/5	Y5/5	D4/5	H4/5	F4/5	A4/5	Y5/5	N4/5	D5/5	F5/5	D5/5
			E2/5		N1/5	R1/5	S1/5	V1/5		D1/5			
R	V3/4	V3/4	G4/4	Y3/4	D4/4	H3/4	F4/4	A3/4	Y3/4	N4/4	D3/4	F3/4	D3/4
	A1/4	A1/4		H1/4		R1/4		V1/4	H1/4		G1/4	S1/4	G1/4

4 Discussion

Some studies on the resistance in the rice stem borer have been published (Konno *et al*., 1985, 1996, 1987, 1989; Han *et al*., 1995), but most of focused on resistance to organophosphorus (Ops) insecticides. Few reports on other kinds of insecticide resistance are available. In recent years, high levels of resistance in the rice stem borer to monosultap and methamidophos, the most widely applied pesticides in China for years, have been reported (Li *et al*., 2001; Peng *et al*., 2001). Overcoming such resistance is a big task, however, no studies on the mechanism of this resistance had been conducted. In our study, the activities of the major detoxification enzymes usually thought to be involved in insecticide resistance in insects were measured. We hoped to find some relationship between biochemical changes and the marked monosultap and methamidophos resistance of the Cixi population of rice stem borer.

Mixed function oxidase (MFO) is the most important detoxification enzyme involved in the metabolization of all kinds of insecticides including organochlorine, organophosphorus, carbamate, and synthetic pyrethroids (Leng, 1996). The key component of this broad-spectrum oxidation system is a family of hemoproteins called cytochrome P450 (Fumio, 1985). Another component of this oxidase system is NADPH-cytochrome-c-reductase. Insect cytochrome P450 is a single polypeptide. This protein exists in multiple forms, and differences in substrate specificity, positional specificity and stereospecificity of the various cytochrome P450 forms appear to be involved in the type of mono-oxygenase activity toward a given chemical shown by *in vivo* and *in vitro* systems. Low

levels of microsomal mono-oxygenase activity are associated with susceptibility to insecticides, whereas high mono-oxygenase activity correlates with varying degree of resistance (Moises, 1985). Our study suggests that the enhancement of the activities of microsomal demethylase, O-demethylase and N-demethylase, which in Cixi population were 3.3 and 1.43-fold higher than in the Taihu population, is an important biochemical mechanism responsible for the monosultap and methamidophos resistance developed by the Cixi population. This is consistent with the synergism assay, which showed that in the Cixi population the synergism of Pbo to monosultap was 1.2-fold higher than in the Taihu population (Li *et al*., 2001). The increment of MFO activity has also been confirmed as playing an important role in the resistance of the diamond back moth to dimehypo (the other form of monosultap) and cartap (both dimehypo and cartap belong to the nereistoxin group of insecticides (Chen *et al*., 1993, 1994). In the resistance of insects against OPs, MFO has been proven to play a major role (Konno and Shishido, 1985; Leng, 1996; Tang, 1993). Thus it seems that MFO is involved in the resistance of the Cixi population against both monosultap and methamidophos. The roles of O-demethylase and N-demethylase are, however, not the same. O-demethylase is mainly responsible for the detoxification of methamidophos because both methamidophos and the model substrate, 4-nitroanisole (PNA) have the same chemical group, O-CH_3. On the other hand, N-demethylase detoxifies monosultap since the substrate, 4-chloro-N-methlaniline and monosultap have the same chemical group, N-CH_3. It remains unknown whether the increased activity of microsomal demethylase correlates with the enhancement of P450 content, which has been reported to be higher in pyrethoid resistant *Helicoverpa armigera* (Wu *et al*., 1997), or the modification in the protein.

Esterase activity changed little in Cixi population compared with Taihu population. This agrees with the previous report of Li (2001), which indicated that TPP, a specific inhibitor against esterases, had no synergism with respect to monosultap in the Cixi population. This suggests that esterase is not involved in resistance against monosultap. In the resistance of the diamondback moth to dimehypo and cartap, esterase was believed to be unimportant (Chen *et al*., 1993). The Cixi population was simultaneously resistant to monosultap and methamidophos. This suggests that esterase is not associated with the methamidophos resistance of this population either.

GSTs have been confirmed to be very important in the metabolization of, and development of resistance to, OPs (Tang, 1993; Oppenoorth *et al*., 1979). There was not much difference in the activity of glutathione S-transferase in the Cixi and Taihu populations, but the K_m value, which is a parameter that evaluates the affinity of an enzyme with a particular substrate, changed significantly when CDNB was used as the substrate. K_{ms} were 0.152 and 0.102 mmol/L in resistant and suceptible populations respectively, which indicates that GSTs of the resistant Cixi population had reduced affinity for CDNB. This suggests that modification of GSTs had occurred in the Cixi population. This raises the question of whether this modification is responsible for methamidophos resistance in Cixi population. Would the affinity be different if the substrate were OPs insecticides instead of CDNB?

The resistance mechanisms involved in insecticide resistance include incremental detoxification, decreased sensitivity of the target species, delayed penetration ratio and increased protein sequestration. It is clear that increased activity of microsomal O-demethylase and N-demethylase are involved in the resistance of the Cixi population. Is this sufficient to explain this population's high 37.7 and 52.7-fold levels of resistance to monosultap and methamidophos? Further studies on other mechanisms are necessary to gain a comprehensive understanding of the resistance of the Cixi rice stem borer population to monosultap and methamidophos.

Nicotinic acetylcholine receptor (nAChR) is an important insecticide target. So far, there has been no evidence to indicate that changes in its sensitivity are involved in insecticide resistance. However, the development of insensitivity in other physiological targets such as acetylcholinesterase, the sodium channel and the GABA receptor by amino acids replacement have been confirmed as important mechanisms for insecticide resistance (Zhu *et al*., 1996; Huang *et al*., 1997; Ffrench-Constent *et al*., 2000; Williamson *et al*., 1996; Martrez-Torres *et al*., 1999). Reasons for this difference are that insecticides acting on nAChR are not applied as widely or intensively as others, or that resistant insects are not available for research. However, *Chilo suppressalis* recently developed high resistance against monosultap (Li *et al*., 2001; Peng *et al*., 2001), a nereistoxin insecticide that acts on nAChR. We suspect that a point mutation may have occurred leading to target insensitivity. Unfortunately, the comparison between susceptible and resistant individuals didn't show any stable amino acid substitution, nor is there evidence

of geographic polymorphism. Kozaki *et al*. (2001) reported polymorphisms in the acetylcholinesterase gene of the housefly. Leu 260 was found only in organophosphate resistant strains, which can be a practical tool for characterization of the cinsecticide resistance of houseflies. However, locating the resistance related point mutation in nAChR is more complicated. Since subtypes of the snAChR α subunit commonly exist in both vertebrates and insects, and the accurate chemical formation of nAChR has not yet been established, this raises the following questions: How many other subtypes of the nAChR α subunit exist in *Chilo suppressalis* besides the full length of Csα1 cloned by Han and Han (2002) and a fragment reported by Sgard *et al* .(1993)? Which subtype plays the most important role in synaptic excitatory transmission? Besides the functional subunit (α subunit), would a mutation in the non-functional subunit (β) effect the functional performance of nAChR?

The insertion between S 384 and R 385 may have resulted from mRNA alternative splicing, which leads to different protein generation. According to the sequencing result, the insertion is not rare in the *Chilo suppresalis* α subunit gene of nAChR. Alternative splicing of mRNA has also been found in other insecticide target genes, such as those controlling the sodium channel in *Haematobia irritans* (Guerrero *et al*., 1997) and *Anopheles gambiaes* (Martinez-Torres *et al*., 1998). Further study of protein expression and function detection are needed to clarify whether the insertion causes functional change. Since the insertion is in the most variable region, the loop between TM3 and TM4, we suspect that it may have little effect.

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二化螟抗杀虫单和甲胺磷品系的生化特性

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摘要: 二化螟抗性和敏感品系分别采自浙江和安徽太湖。毒力测定结果表明, 抗性品系对杀虫单和甲胺磷分别产生了37.7和52.7倍的抗性。代谢酶活性的测定结果显示, 抗性品系多功能氧化酶氧脱甲基活性、氮脱甲基活性分别是敏感品系的3.3和1.34倍, 而羧酸酯酶活性和谷胱甘肽转移酶活性两个品系之间没有显著差异。说明多功能氧化酶活性提高可能是二化螟对甲胺磷、杀虫单抗性的一个重要机制。为了研究二化螟可能存在的对沙蚕毒素杀虫剂靶标不敏感机制, 采用RT – PCR等分子生物学技术, 分别对5个敏感个体和4抗性个体中克隆杀虫单作用的靶标烟碱型乙酰胆碱受体α1亚基 (nAChR α subunit 1) cDNA序列进行了分子克隆。序列比较发现一共存在33个单核苷酸的多态性, 其中14个引起了编码氨基酸的改变。但是没有发现与抗性有关的特有的碱基突变。

关键词: 二化螟; 抗药性; 生化机制; 烟碱型乙酰胆碱受体亚基 (nAChR α1); 单核苷酸多态性 (SNP)

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