Pesticide Biochemistry and Physiology 136 (2017) 23–28

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Metabolic resistance in *Nilaparvata lugens* to etofenprox, a non-ester pyrethroid insecticide

Huahua Sun [a], Baojun Yang [a,b], Yixi Zhang [a], Zewen Liu [a,*]

[a] Key Laboratory of Integrated Management of Crop Diseases and Pests (Ministry of Education), College of Plant Protection, Nanjing Agricultural University, Weigang 1, Nanjing 210095, China
[b] Rice Technology Research and Development Center, China National Rice Research Institute, Hangzhou 310006, China

ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form 26 August 2016
Accepted 31 August 2016
Available online 31 August 2016

Keywords:
Nilaparvata lugens
Etofenprox
P450 gene
Resistance mechanism

ABSTRACT

Etofenprox, a non-ester pyrethroid insecticide, will be registered to control rice pests such as the brown planthopper (BPH, *Nilaparvata lugens* Stål) in mainland China. Insecticide resistance is always a problem to the effective control of *N. lugens* by chemical insecticides. An etofenprox resistance selection of *N. lugens* was performed in order to understand the related mechanisms. Through successive selection by etofenprox for 16 generations in the laboratory, an etofenprox-resistant strain (G16) with the resistance ratio (RR) of 422.3-fold was obtained. The resistance was partly synergised (2.68-fold) with the metabolic inhibitor PBO, suggesting a role for P450 monooxygenases. In this study, 11 P450 genes were significantly up-regulated in G16, among which eight genes was above 2.0-fold higher than that in US16, a population with the same origin of G16 but without contacting any insecticide in the laboratory. The expression level of four genes (CYP6AY1, CYP6FU1 and CYP408A1 from Clade 3, and CYP425A1 from Clade 4) were above 4.0-fold when compared to US16. RNA interference (RNAi) was performed to evaluate the importance of the selected P450s in etofenprox resistance. When CYP6FU1, CYP425A1 or CYP6AY1 was interfered, the susceptibility was significantly recovered in both G16 and US16, while the knockdown of CYP408A1 or CYP353D1 did not cause significant changes in etofenprox susceptibility. We supposed that CYP6FU1 was the most important P450 member for etofenprox resistance because of the highest expression level and the most noticeable effects on resistance ratios following RNAi.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Nilaparvata lugens Stål (brown planthopper, BPH), the primary insect pest of rice crops, is well known as a notorious pest throughout Asia because of its frequent outbreak [1]. In addition to directly extracting nourishment from plants, oviposition and virus disease transmission by *N. lugens* result in more severe damage [2,3]. Many insecticides including organophosphates, carbamates and neonicotinoids, have been used against *N. lugens*, nevertheless, *N. lugens* have developed high level of resistance to many insecticides in most areas in China, resulting in severe yield reduction and significant economic loss [2].

Etofenprox is a non-ester pyrethroid insecticide with an ether linkage instead of an ester linkage in traditional pyrethroid insecticides [4]. Because of its excellent effects on rice planthoppers and low toxicities to fish and honeybee [5,6], etofenprox has been used frequently to control rice planthoppers in many areas, such as Taiwan (China). With the forthcoming registration of etofenprox to control rice planthoppers in mainland China, it is significant to reveal the resistance mechanisms of etofenprox in advance, especially in *N. lugens*, the most important rice planthoppers in China.

Resistance to most pyrethroid insecticides commonly arises by the enhanced detoxification by metabolic enzymes, target-site insensitivity, delayed cuticular penetration and behavioral change [7]. Insensitivity of the voltage-gated sodium channels (VGSC) is the main reason of the target resistance mechanism of the pyrethroid and many knockdown resistance (kdr-type resistance) mutations have been found in many insects throughout the world, such as L1014F [6]. In addition, cytochrome P450 monooxygenases, including a multigenic superfamily of enzymes, are the most important detoxification enzymes to biosynthesize endogenous compounds and detoxify xenobiotic compounds [8]. In previous studies, the overexpression of several P450 genes has been proved to be associated with the increased metabolism of pyrethroid insecticides in resistant populations, including CYP9M10 from *Culex pipiens quinquefasciatus*, CYP6F1 from *Culex pipiens pallens*, CYP6P3 and CYP6M2 from *Anopheles gambiae*, CYP6P4 from *Anopheles arabiensis*, CYP6P7 from *Anopheles minimus* and CYP6D1 from *Musca domestica* [9–13]. Thus, the overexpression of P450 genes is a common metabolic mechanism for pyrethroid resistance.

Therefore, the etofenprox resistance in *N. lugens* might be also associated with the constitutive overexpression of multiple P450 genes and

* Corresponding author at: College of Plant Protection, Nanjing Agricultural University, Weigang 1, Nanjing 210095, China.
E-mail address: liuzewen@njau.edu.cn (Z. Liu).

http://dx.doi.org/10.1016/j.pestbp.2016.08.009
0048-3575/© 2016 Elsevier Inc. All rights reserved.

decreased sensitivity of the VGSC. The present study focused mainly on the roles of P450s in etofenprox resistance. With the whole-genome sequencing and gene annotation of *N. lugens*, the availability of all P450 sequences allows the global analysis of the importance of each P450 in etofenprox resistance [14]. In this study, we attempted to determine the putative P450 genes important in etofenprox resistance, which will give a full view on the metabolic resistance to etofenprox in *N. lugens*.

2. Materials and methods

2.1. Insecticides and synergists

Etofenprox (CAS 80844-07-1), piperonyl butoxide (PBO, CAS 51–03-6), triphenyl phosphate (TPP, CAS 115-86-6), diethyl maleate (DEM, CAS 141–05-9) and acetone (CAS 67-64-1) were purchased from Sigma-Aldrich (analytical standard, St. Louis, MO, USA).

2.2. Brown planthopper (N. lugens)

The susceptible strain (Sus) of *N. lugens* was a laboratory strain, obtained from the China National Rice Research Institute in September 2001, and had been reared as a laboratory strain without any contact of insecticides for 12 years. The unselected strain (US16) was originally collected from paddy field in Chiayi (Taiwan, China) in August 2013 and successively selected by etofenprox for 16 generations in the laboratory to obtain the resistance strain (G16). All test insects were reared in the laboratory at 25 ± 1 °C, humidity 70%–80% and 16 h light/8 h dark photoperiod.

2.3. Toxicity bioassay and resistance selection

The bioassay and synergism experiments were conducted as previously described [15,16]. Etofenprox was diluted to six concentrations with acetone and the 4th instar nymphs were used as test insects. After carbon dioxide anaesthesia, a droplet (0.04 μL) of etofenprox solution was applied topically to the prothorax notum of *N. lugens* with a hand microapplicator (Burkard Manufacturing Co Ltd., Rickmansworth, UK). Thirty insects were treated at each concentration and every treatment was repeated for three times. Insects treated with acetone (0.04 μL) alone were used as the control. The treated insects were reared on seedlings cultured soilless in the laboratory cages at 25 ± 1 °C, humidity 70%–80% and 16 h light/8 h dark photoperiod. The mortality was assessed after 24 h. The data obtained were analyzed to determine the LD_{50} values using Polo software (LeOra Software Inc., Cary, NC). In the synergism analysis, 2 μg of synergist (PBO, TPP or DEM) in 0.04 μL acetone was topically applied on the prothorax notum of *N. lugens* 1 h before the etofenprox application.

Resistance selection was performed by the similar procedure as described in bioassay above. The dose of etofenprox used in each generation was the LD_{50} determined in the parent generation and the mortality of each generation was kept at about 40–60%.

2.4. Total RNA isolation and cDNA synthesis

Total RNA was isolated from three pools of 20 nymphs (4th instar) using Trizol® reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA contaminations were removed by treating RNA extraction products with RNase-free DNase (Takara, Kyoto, Japan). The quality and quantity of RNA were checked by agarose gel electrophoresis (1%) and spectrophotometery (Thermo Scientific, Waltham, MA, USA). A sample containing 1 μg total RNA was used to synthesize first-strand cDNAs with PrimeScript II RTase (Takara, Kyoto, Japan) and random 6 mers, using the following cycling parameters: 30 °C for 10 min, and then 95 °C for 5 min.

2.5. Detection of P450 gene mRNA levels

According to the genome of *N. lugens* (GenBank accession number AOSB00000000, BioProject PRJNA177647), we obtained the information of 54 P450 genes, which provided by Prof. Zhang Chuanxi (Zhejiang University, Hangzhou, China). The expression levels of P450 genes were determined with quantitative real-time PCR (qRT-PCR) using the One Step SYBR PrimeScript RT-PCR Kit (Takara, Kyoto, Japan) following the manufacturer's protocol on a 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) with gene-specific primers (Table S1). Each reaction had three technical replicates and three biological replicates. The level of 54 P450 gene transcripts were normalized to the β-actin and GAPDH expression for each generation and treatment, the relative quantification was obtained according to the $2^{-\Delta\Delta CT}$ method [17] and data was expressed as fold change in the expression level relative to the calibrator.

2.6. RNA interference

The target P450 genes and bacterial Lac-Z (GenBank accession number AJ308295, as a control) were amplified using specific primers (Table S2) conjugated with 23 bases of the T7 RNA polymerase promoter sequence. The PCR products were used as templates for dsRNA synthesis using T7 Ribomax Express RNAi System (Promega, Madison, WI, USA) following the manufacturer's instructions. The dsRNA was isopropanol precipitated and resuspended in RNase-free water. The integrity and quantity of dsRNA were checked by agarose gel electrophoresis (1%) and spectrophotometery (Thermo Scientific, Waltham, MA, USA). The final concentration of dsRNA was adjusted to 1.5 μg/μL. The dsRNA injection was exactly conducted using the 4th instar nymphs as described previously [18]. The mRNA levels of P450 genes were determined 96 h after the injection by qRT-PCR.

2.7. Data analysis

The mortality and mRNA levels in different generations and treatments were analyzed using unpaired Student's *t*-test and one-way ANOVA with at least three repeats. An LSD pair wise comparison of means were used to analyzed the difference between values. The level of significance of results was set at $p < 0.05$ or 0.01.

3. Results

3.1. Etofenprox resistance selection

Through the successive selection by etofenprox for 16 generations in the laboratory, *N. lugens* developed etofenprox resistance to a high level. Although LD_{50} values increased in each selection generation, the increment was different among generations. In the first six generations, the increase was relatively small, with LD_{50} values from 2.13 to 14.76 ng/insect (Fig. 1). Then the LD_{50} values increased steeply from G7 to G13, with 82.13 ng/insect in G13. From G13 on, the LD_{50} values remained almost stable in the following generations. In order to avoid the influence of different insect origins on resistance mechanism study, the original field population G1 was reared in the laboratory without any selection for 16 generations to provide a control strain US16 ($LD_{50} = 0.76$ ng/insect), with a resistance ratio of 3.72 compared with the Sus strain ($LD_{50} = 0.20$ ng/insect). After 16 generations of selection, the resistance ratio (RR) reached 422.32- and 112.80-fold when compared to Sus and US16 strain, respectively.

3.2. Synergistic effect evaluation

The synergism analysis showed that there were not significant synergistic effects of PBO, TPP or DEM on etofenprox toxicities in Sus and

Download English Version:

https://daneshyari.com/en/article/5514953

Download Persian Version:

https://daneshyari.com/article/5514953

Daneshyari.com