Pesticide Biochemistry and Physiology 102 (2012) 142–145

Contents lists available at SciVerse ScienceDirect

Pesticide Biochemistry and Physiology

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

Frequencies of the M918I mutation in the sodium channel of the diamondback moth in China, Thailand and Japan and its association with pyrethroid resistance

Shoji Sonoda [a,*], Xueyan Shi [b], Dunlun Song [b], Youjun Zhang [c], Jianhong Li [d], Gang Wu [e], Yong Liu [f], Ming Li [g], Pei Liang [b], David Wari [a], Masaya Matsumura [h], Chieka Minakuchi [i], Toshiharu Tanaka [i], Tadashi Miyata [i], Xiwu Gao [b]

[a] Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama 710-0046, Japan
[b] Department of Entomology, China Agricultural University, Beijing 100193, China
[c] Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[d] Department of Plant Protection, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[e] Department of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China
[f] Hunan Plant Protection Institute, Hunan Academy of Agricultural Sciences, Changsha, Hunan 410125, China
[g] Crop Protection Institute, Guizhou University, Guiyang, Guizhou 550025, China
[h] National Agricultural Research Center for Kyushu Okinawa Region, Koshi, Kumamoto 861-1192, Japan
[i] Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi 464-8601, Japan

ARTICLE INFO

Article history:
Received 24 October 2011
Accepted 18 December 2011
Available online 30 December 2011

Keywords:
Diamondback moth
Insecticide resistance
Plutella xylostella
Pyrethroid
Sodium channel

ABSTRACT

In addition to the allele frequencies of the L1014F and T929I mutations which are involved in nerve-insensitive resistance to a pyrethroid, those of the M918I mutation were examined using field strains obtained in China, Thailand, and Japan during 2009–2011. Results show that the resistance allele frequencies at the L1014F site were 89–100%, 97–100% and 65–85%, respectively, for strains in China, Thailand, and Japan. The respective allele frequencies at the T929I site were 86–100%, 70–97% and 58–84% for Chinese, Thai, and Japanese strains. With low frequencies up to 27%, M918I was found in Japan and China, but not in Thailand. The strain homozygous for the M918I and L1014F mutations was established and its resistance level to a pyrethroid was examined. The strain lacks a portion of the sodium channel gene corresponding to the 3′ portion of exon 18a, intron 18, and the 5′ portion of exon 18b. Nevertheless, the strain showed a similar level of resistance to that which was homozygous for the T929I and L1014F mutations.

1. Introduction

The diamondback moth *Plutella xylostella* is a major pest of *Brassica* worldwide [1]. In Japan, resistance to various insecticides, including acephate, phenthoate, permethrin, chlorfluazuron, cartap, and *Bacillus thuringiensis* (BT) *kurstaki* has been reported for *P. xylostella* [2]. For that reason, *P. xylostella* has been recognized as a severe pest in the early 1990s. In the late 1990s, new insecticides such as chlorfenapyr, emamectin-benzoate, spinosad, pyridalyl and BT *aizawai* have been developed. Since then, the occurrence of *P. xylostella* has decreased in Japan [2]. However, *P. xylostella* is still regarded as a great threat of *Brassica* in many other countries.

Pyrethroids including permethrin are synthetic insecticides that are derived structurally from naturally occurring pyrethrins that are present in the pyrethrum extract of *Chrysanthemum* species [3]. Pyrethroids interact with the sodium channels of insects and modify their normal function by inhibiting channel deactivation and stabilizing the open configuration, resulting in repetitive discharges that quickly engender nervous exhaustion, paralysis, and death [4]. The major structural subunit of the channel, the α-subunit, comprises four homologous domains (I–IV), each of which contains six transmembrane segments (S1–S6) [5].

Intensive use of pyrethroids for insect pest control has engendered a worldwide emergence of resistant insects [6]. Nerve insensitivity associated with amino acid mutations in the sodium channel is a major mechanism of pyrethroid resistance along with increased metabolic detoxification by cytochrome P450s. The amino acid mutations in the sodium channel were first reported in *Musca domestica* and *Blattella germanica* [7,8]. The first mutation from Leu to Phe (L1014F) at domain IIS6 was found in both species. The second mutation (M918T) was identified within domains IIS4–IIS5 in *M. domestica* [8]. In addition to these mutations, several mutations outside of domain II have been reported in other insects [6,9].

The pyrethroid resistance in *P. xylostella* conferred by nerve insensitivity has been correlated with two amino acid mutations

* Corresponding author. Fax: +81 86 434 1249.
E-mail address: sonodas@rib.okayama-u.ac.jp (S. Sonoda).

0048-3575/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pestbp.2011.12.005

in domain II of the sodium channel [10,11]. The first mutation at domain IIS6 was exactly the same as that at L1014F. The second mutation (Thr to Ile), denoted as T929I, was located at domain IIS5. Sonoda et al. [12] reported the presence of insects having an amino acid mutation from Met to Ile (M918I) at exactly the same site of the M918T in *M. domestica*. Consequently, at least three amino acid mutations are presumably involved in nerve-insensitive pyrethroid resistance of *P. xylostella*. The allele frequencies for the L1014F and T929I in the field strains of *P. xylostella* were examined in Korea [13]. We also previously reported the allele frequencies of the three mutations using field strains obtained in Japan [12]. In this study, we examined the allele frequencies of the three mutations in field strains from China, Thailand, and Japan. Furthermore, we established the strain homozygous for the M918I and L1014F and examined its resistance level to a pyrethroid, because the strain lacks a portion of the sodium channel gene corresponding to the 3' portion of exon 18a, intron 18, and the 5′ portion of exon 18b.

2. Materials and methods

2.1. Insects

A total of 13 field collections (strains) were obtained from four locations in China (Beijing, BJ; Fuzhou, FZ; Wuhan, WH; Changsha, CS), three in Thailand (Sai Noi, SN; Bang Bua Thong, BBT; Lad Lum Kaew, LLK) and two in Japan (Kumamoto, KM; Shimane, SMN) during 2009–2011 (Table 1). Insects were maintained with radish seedlings or cabbage leaves at 25 °C under a long photoperiod (16L: 8D).

2.2. Bioassay

Permethrin (Adion 20% suspension concentrate) was purchased from Sankyo Agro Co. Ltd., Tokyo, Japan. The leaf-dipping method using newly molted fourth-instar larvae was adopted for bioassay. Serial dilutions of the insecticide with more than three replicates were used for bioassay. Ten larvae were used in each replicate. Larvae were allowed to feed for 48 h before mortality assessment. The LC_{50} value was estimated using probit analysis [14].

2.3. DNA extraction

Each individual was homogenized with 500 μl of extraction buffer (50 mM Tris·HCl pH 8.5, 10 mM EDTA, 100 mM NaCl, 2% SDS). For DNA extraction, 400 μl of the homogenate was used. DNA was extracted twice with phenol:chloroform:isoamylalcohol (25:24:1) and precipitated using 99.5% ethanol in the presence of 3 M sodium acetate (pH 5.2). The DNA pellet was washed with 70% ethanol and dissolved in TE. After treatment with RNase A, the DNA solution was purified once with phenol:chloroform:isoamylalcohol (25:24:1) and once with chloroform:isoamylalcohol (24:1) and precipitated using 99.5% ethanol in the presence of 3 M sodium acetate (pH 5.2). The DNA pellet was washed with 70% ethanol and dissolved in H_2O. The remaining 100 μl of the homogenate was used for RNA extraction in another study.

2.4. Genomic DNA PCR

The genomic DNA fragments covering the amino acid positions of 918 and 929 in the sodium channel were amplified using PCR with primer A (5′-caaacataacaagtrgtcccaag) and primer B (5′-caccaatgaacatgcaacactcc) [12]. The PCR conditions were 40 cycles of 15 s at 94 °C, 30 s at 60 °C, and 1 min at 72 °C, with subsequent final extension of 72 °C for 7 min. The purified DNA fragments, having lengths of ca. 1.1 kb and ca. 0.3 kb, were respectively used for direct sequencing with primer C (5′-gtgattgtggcaaggagtac) and primer D (5′-caccaatttcacttacccac). The DNA fragments covering the amino acid position of 1014 of the sodium channel were amplified using PCR with primer E (5′-ctgtgtgtgtgtagatcacg) and primer F (5′-gtgaaaccagggcaaacac). The PCR conditions were as described above. The purified DNA fragments, having a length of ca. 0.3 kb, were used for direct sequencing with primer G (5′-catgcacagcttcatgatcg).

2.5. Nucleotide sequencing

The nucleotide sequence was determined using a dye terminator cycle sequencing kit (Applied Biosystems, Carlsbad, CA, USA) and a DNA sequencer (3130xl; Applied Biosystems). The nucleotide and deduced amino acid sequences were analyzed using Genetyx ver. 10 (Genetyx Corp., Tokyo, Japan).

2.6. Crossing

To establish the strain homozygous for the M918I and L1014F or the one which is homozygous for the T929I and L1014F, males and females from the WH strain were separated at the fourth instar. Then emerged adults were paired. Paired females were allowed to lay eggs for two days in the presence of males. Then DNA was extracted individually from both to confirm their zygosities related to the M918I, T929I, and L1014F using PCR and direct sequencing according to the method described above.

3. Results

3.1. Allele frequencies for the L1014F, T929I, and M918I

Xu et al. [15,16] reported that posttranscriptional regulation through RNA allelic variation and RNA editing is involved in allelic

Table 1
Insects used in this study.

Strain	Country	Location (Latitude/Longitude)	Month/Year
BJ1-09	China	Zhongguancun South Road, Beijing (N39°96′/E116°32′)	Jun. 2009
BJ2-09	China	Xiaotangshan Town, Beijing (N40°18′/E116°39′)	Jun. 2009
FZ	China	Fuzhou, Fujian Province (N26°05′/E119°18′)	Oct. 2009
WH	China	Wu Xue, Hubei Province	May 2010
BJ1-10	China	Zhongguancun South Road, Beijing (N39°96′/E116°32′)	Sep. 2010
BJ2-10	China	Xiaotangshan Town, Beijing (N40°18′/E116°39′)	Sep. 2010
BJ3	China	Yuanmingyuan West Road, Beijing (N40°02′/E116°28′)	Sep. 2010
CS	China	Yue Yang, Hunan Province (N29°52′/E112°91′)	Jul. 2011
SN	Thailand	Sai Noi, Nonthaburi Province	Mar. 2010
BBT	Thailand	Bang Bua Thong, Nonthaburi Province	Mar. 2010
LLK	Thailand	Lad Lum Kaew, Pathumthani Province	Mar. 2010
KM	Japan	Koshi, Kumamoto	Apr. 2010
SMN	Japan	Izumo, Shimane	May. 2010