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Short communication

Lack of cross resistance to non-*Bt* insecticides in a mode-1, Cry1Ac-resistant population of *Plutella xylostella* (Lepidoptera: Plutellidae)

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Abstract

Bacillus thuringiensis transgenic plants substantially reduce the use of conventional insecticides for insect pests. Despite sufficient evidences of cross resistance between the *Bt* toxins, studies on crosses resistance between the *Bt* toxins and non-*Bt* insecticide are rare. In the present study, similar cross-resistance mechanism was investigated in a *Plutella xylostella* population possessing single-gene, recessive mode of inheritance but lacking toxin-binding mechanism. Bioassays on unselected (Unsel-Karak) and Cry1Ac-selected (Sel-Karak) populations of *P. xylostella* revealed that deltamethrin, chlorpyrifos and spinosad were significantly more toxic than Cry1Ac. The resistance ratio against Cry1Ac in Sel-Karak population was more than 660-fold compared with the susceptible population (Lab-UK). However, compared to Unsel-Karak the resistance ratio against Cry1Ac in Sel-Karak population was less than10-fold. In the present study, it was found that a population with mode 1 resistance (the most common type of lepidopteran resistance to *Bt* toxins) and single factor (*i.e.*, Sel-Karak) is unlikely to show a common resistance mechanism to both the conventional insecticides and *Bt* toxin; this might be mainly due to highly different mode of action of the insecticides.

Key words: Cry1Ac, resistance, *Plutella xylostella*, cross-resistance, *Bacillus thuringiensis*

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چکیده

گیاهان تراریخته با *Bacillus thuringiensis* بطور اساسی کـاربرد حـشره‌کـش‌هـای متـداول جهت کنترل حشرات آفت را کاهش می‌دهند. علـی‌رغـم مـستندات کـافی در مـورد مقاومـت تقاطعی بین توکسین‌های *Bt*، تحقیقات نادری در مورد مقاومت تقاطعی بین توکسین‌هـای *Bt* و دیگر حشره‌کش‌ها انجام شده است. در مطالعه حاضر مکانیسم مقاومت تقاطعی در جمعیتـی از بید کلم (*Plutella xylostella*) با نحوه وراثت تک‌ژنی مغلوب و فاقد مکانیسم اتصال به توکسین بررسـی گردیـد. آزمـون‌هـا ی‌زیـست‌سـنجی بـر روی جمعیـت‌هـای بیـد کلـم غیرانتخـابی (Unsel-Karak) و انتخابی با Cry1Ac (Sel-Karak) نشان داد که سمیت دلتـامترین، کلرپـایریفوس و اسپینوزاد بطور معنی‌داری بیـشتر از Cry1Ac بـود. نـسبت مقاومـت بـه Cry1Ac در جمعیـت انتخابی بیش از ٦٦٠ برابر جمعیت حـساس (Lab-UK) بـود. اگرچـه، در مقایـسه بـا جمعیـت غیرانتخابی نسبت مقاومت به Cry1Ac در جمعیت انتخـابی کمتـر از ١٠ برابـر بـود. در تحقیـق حاضر مشخص گردید که غیرمحتمل است که جمعیتی با نحوه ١ مقاومت (عمومی‌تـرین نـوع مقاومت به توکسین‌های *Bt* در بال‌پولکداران) و تک عامل، مکانیسم مـشترک مقاومـت بـه هـر دوی حشره‌کش‌های متداول و توکسین‌های *Bt* نشان دهد. این امر بطور عمده می‌تواند به علت نحوه اثر بسیار متفاوت حشره‌کش‌ها باشد*.

واژه‌های کلیدی: Cry1Ac، مقاومت، *Plutella xylostella*، مقاومت تقاطعی، *Bacillus thuringiensis*.

Introduction

Bacillus thuringiensis (*Bt*) irregular sprays on vegetable crops has resulted in development of resistance against *Bt* toxins in field populations of *Plutella xylostella* (L.) (Lepidoptera, Plutellidae) (Tabashnik *et al.*, 1990; Sayyed *et al.*, 2000); such resistance is largely due to mutation in midgut receptor (Pigott and Ellar, 2007). Cross-resistance may occur as a result of non-specific enzymes, mutation at an insecticidal target site and delayed cuticular penetration. Resistance to Cry1Ac has conferred cross-resistance to pyrethroids and vice versa in a population of *P. xylostella* (i.e., SERD4), which has indicated a high level of

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resistance to Cry1Ac and Cry1Ab, such that the mode of inheritance of resistance has been an incompletely dominant, being controlled by more than one genes (Sayyed and Wright, 2001; Sayyed *et al.*, 2008).

The mechanism of cross-resistance between Cry1Ac and non-*Bt* insecticides proved to be esterase mediated. Non-specific esterases are involved in resistance mechanism against broad-spectrum insecticides; these enzymes are found in the insect haemolymph and gut, where they hydrolyze insecticidal esters and sequester insecticides (Gunning *et al.*, 1999). Cross resistance between the *Bt* toxins is a well-documented phenomenon (Siqueira *et al.*, 2004) but little is known about cross resistance between the *Bt* toxins and non-*Bt* insecticides. The present study aimed to test the hypothesis that a *P. xylostella* population possessing single-gene, recessive mode of inheritance but lacking toxin-binding mechanism (Sayyed *et al.*, 2004) would exhibit cross-resistance mechanism observed in SERD4.

Materials and Methods

A field population of *P. xylostella* (Karak) was collected from the Karak area (Kuala Lumpur, Malaysia, November 2001). Two different cultures of Karak population were maintained in the laboratory as unselected (Unsel-Karak) or selected (Sel-Karak). After laboratory selection with Cry1Ac, Sel-Karak showed highly resistant to Cry1Ac and cross-resistance to Cry1Ab (Sayyed *et al.*, 2004). Sel-Karak possesses recessive mode of inheritance and loss of binding (as the only mechanism of resistance to Cry1Ac; Sayyed *et al.*, 2004). An insecticide-susceptible population of *P. xylostella* (Lab-UK) was obtained from Rothamsted Research (Harpenden, U.K.). Insect larvae were reared and tested on 4-6-week-old organically-grown Chinese cabbage (*Brassica pekinensis*) cv. Tip Top under constant environmental conditions (25±2 °C; 65±5% RH; L:D 16:8 h; Karimzadeh *et al.*, 2004).

Three different insecticides, Tracer (Spinosad; Dow AgroSciences, U.K.), Decis (deltamethrin 50 g/liter EC; Syngenta, Switzerland) and Lorsban 40 EC (chlorpyrifos; Dow AgroSciences, U.K.), and two synergists, PBO ***(piperonyl butoxide***; Sigma Ltd, UK) and DEF (*S,S,S*-Tributyl phosphorotrithioate; Sigma Ltd, UK), were stored at 4 ^{o}C. *Bacillus thuringiensis* crystal protein Cry1Ac was prepared as previously described (Sayyed *et al.*, 2005). Bioassays were conducted with the 3rd instar larvae of *P. xylostella* (Sayyed *et al.*, 2000). Test solutions were prepared in distilled water with Triton X-100 (50 µg/ml). Leaf discs (4.8 cm dia.) were immersed in a test solution for 10 s, allowed to dry at room temperature, and placed in Petri dishes (5 cm dia.) containing a moistened filter paper. Five larvae were placed in each dish, and each treatment was replicated 5-7 times. The mortality was assessed after 120 h for *Bt* toxin or 48 h for other insecticides. Further bioassays were performed to test synergistic effects of DEF (the specific inhibitor of esterases) and PBO (an inhibitor of cytochrome P450 monooxygenases and esterases) on the activity of Cry1Ac in Cry1Ac-SEL, UNSEL and Lab-UK populations. A test solution of the each inhibitor in acetone with control (acetone alone) was applied topically to the dorsal side of the 3rd instar larva an hour before exposing to Cry1Ac. Treated larvae were then transferred to the leaves treated with the Cry1Ac or distilled water with Triton X-100. The mortality was scored after 120 h.

Results and Discussion

Bioassays on Unsel-Karak and Sel-Karak populations of *P. xylostella* revealed that delatmethrin, chlorpyrifos and spinosad were significantly more toxic than Cry1Ac ($P < 0.01$; non-overlapping 95% CI; Table 1). In case of Lab-UK, only deltamethrin was more toxic than Cry1Ac ($P < 0.01$; non-overlapping 95% CI). When different populations were compared, there was no significant difference for the toxicity of chlorpyrifos to both the Lab-UK and Sel-Karak populations ($P > 0.05$; overlapping 95% CI; Table 1). On the contrary, other pesticides showed different toxicity between populations; deltamethrin, spinosad and Cry1Ac were more toxic to Lab-UK population compared with Sel-Karak population ($P < 0.01$; non-overlapping 95% CI). When it was compared with Lab-UK, the resistance ratio against Cry1Ac in Sel-Karak population was more than 660-fold.

However, compared to Unsel-Karak the resistance ratio against Cry1Ac in Sel-Karak population was less than10-fold (Table 1). There was no significant difference between Sel-Karak and Unsel-Karak populations for the slopes of deltamethrin, chlorpyrifos and spinosad (Table 1). The similarity in logit mortality slopes suggests that selection with Cry1Ac did not change heterogeneity in Sel-Karak population for deltamethrin, chlorpyrifos and spinosad. On the contrary, the significant difference between the Cry1Ac-mortality slopes of Sel-Karak and Unsel-Karak populations indicate that a loss of heterogeneity has been occurred; an increase in the LC_{50} value of selected population (Sel-Karak) compared with unselected population (Unsel-Karak) may support the idea of heterogeneity loss. Insects resistance to pyrethroids and organophosphates are commonly associated with metabolic mechanism of resistance, the insecticides are either sequestered or metabolized by an enzyme such as an esterase or P450 monooxygenase (Gunning *et al.*, 1999).

Table 1- The response of the susceptible, unselected and Cry1Ac-selected populations of *Plutella xylostella* to different insecticides

Population	Insecticides	LC_{50} (95% CI)			Slope ± SE	No.[1]	Resistance Ratio[2] to Lab-UK	Resistance Ratio[2] to Unsel-Karak
Lab-UK (susceptible)	Cry1Ac	0.017 (0.012-0.066)	a[3]	EF[4]	1.50 ± 0.29	180		
	Spinosad	0.013 (0.006-0.023)	ab	FG	1.69 ± 0.32	180		
	Chlorpyrifos	0.008 (0.002-0.015)	ab	FG	1.39 ± 0.30	180		
	Deltamethrin	0.007 (0.003-0.011)	b	G	1.95 ± 0.37	180		
Unsel-Karak (unselected)	Cry1Ac	1.19 (0.76-1.68)	a	B	2.55 ± 0.47	180	70.0	
	Spinosad	0.21 (0.13-0.30)	b	C	2.71 ± 0.50	180	16.2	
	Deltamethrin	0.16 (0.09-0.20)	b	C	2.19 ± 0.42	180	22.9	
	Chlorpyrifos	0.09 (0.06-0.13)	b	CDE	2.72 ± 0.50	180	11.2	
Sel-Karak (Cry1Ac-selected)	Cry1Ac	11.27 (8.67-14.55)	a	A	3.77 ± 0.59	180	662.9	9.5
	Spinosad	0.11 (0.07-0.15)	b	CD	2.88 ± 0.54	180	8.5	0.5
	Deltamethrin	0.06 (0.04-0.08)	b	DE	2.42 ± 0.49	180	8.6	0.4
	Chlorpyrifos	0.013 (0.001-0.03)	c	FG	2.14 ± 0.53	180	1.6	0.1

[1]The number of larvae exposed to insecticides.

[2]The ratio of two populations' LC_{50}s.

[3] Different letters show a significant ($P < 0.05$) difference between insecticides but within a population.

[4] Different capital letters show a significant ($P < 0.05$) difference between insecticides and populations.

Table 2- The effects of synergists on the response of the susceptible and Cry1Ac-selected populations of *Plutella xylostella* to Cry1Ac

Population	Insecticides	LC_{50} (95% CI)		Slope ± SE	No.[1]	SR[2]
Lab-UK (susceptible)	Cry1Ac	0.017 (0.012-0.066)	a	1.50 ± 0.29	180	-
	Cry1Ac + PBO	0.013 (0.006-0.023)	a	1.75 ± 0.32	180	1.30
	Cry1Ac + DEF	0.015 (0.007-0.110)	a	1.82 ± 0.33	180	1.13
Sel-Karak(Cry1Ac-selected)	Cry1Ac	11.27 (8.67-14.55)	a	3.77 ± 0.59	180	-
	Cry1Ac + PBO	9.27 (7.44-11.51)	a	4.65 ± 0.73	180	1.21
	Cry1Ac + DEF	9.43 (7.24-12.16)	a	3.65 ± 0.61	180	1.19

[1]The number of larvae exposed to insecticides.

[2]Synergistic ratio: the ratio of "the LC_{50} of the insecticide alone" to "the LC_{50} of the mixture of the insecticide and the synergist"

The resistance to spinosad has also been shown to be associated with esterases (Wang *et al.*, 2007). Inhibitors of esterases have been shown to synergize the activity of pyrethroids, chlorpyrifos, spinosad and Cry1Ac against certain classes of resistant insect (Gunning *et al.*, 1999; Young *et al.*, 2006; Sayyed *et al.*, 2008). Previously studies have shown a common resistance mechanism for Cry toxin and conventional insecticide in a *P. xylostella* population (the presence of a common genetic locus or loci that control resistance to both insecticides; Sayyed *et al.*, 2008). In present study, however, the synergists (DEF or PBO) did not synergize the activity of Cry1Ac in both the Lab-UK and Sel-Karak populations (Table 2).

The most common type of lepidopteran resistance to *Bt* toxins is known as mode 1, which is characterized by extremely high resistance (over 500-fold) to at least one Cry1A toxin, recessive inheritance, little or no cross-resistance to Cry1C, and reduced binding of at least one Cry1A toxin (Tabashnik *et al.*, 1998). Sel-Karak is a single factor population with high level of resistance to Cry1A and no cross resistance to Cry1Ca, which are typical characteristics of mode 1 resistance (Tabashnik *et al.*, 1998; Sayyed *et al.*, 2004).

In the present study, it was found that a population with mode 1 resistance and single factor (*i.e.*, Sel-Karak) is unlikely to show a common resistance mechanism to both the conventional insecticides and *Bt* toxin; this might be mainly due to highly different mode of action of the insecticides.

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