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GENETIC DIFFERENCES BETWEEN SUSCEPTIBLE, RESISTANT AND FIELD STRAINS OF THE COWPEA APHID, *Aphis craccivora* (Koch)

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ABSTRACT

Random Ampilified polymorphic.DNA Polymerase Chain Reaction (RAPD-PCR) method one from several approaches of assaying genetic variation in molecular biology science. This analysis was performed using a battery of five primers to evaluate the mutagenic effect among the cowpea aphid, *Aphis craccivora* resistant strain of dinotefuran insecticide (neonicotcnoid) compared with field and laboratory susceptible strains. The five primers (A-14,16,17,18 and 19) yielded 96 fragments, primer A -18 detected 57 % difference between genome of field and resistant aphids. The same primer gave 33% variation between susceptible and resistant aphids genome. The highest degree of difference (60%) between the field and susceptible populations DNA sequence was revealed by primer A-14. The high diversity in genome of field and dinotefuran resistant strains compared with the susceptible strain may be explain the activity of dinotefuran against field population of cowpea aphid and support the valuble application of this insecticide in control of *A.croccivora* (Koch).

Keywords: The cowpea aphid – dinotefuran (neonicotinoid insecticide) – RAPD – PCR.

INTRODUCTION

The cowpea aphids, *Aphis craccivora (*koch). has an extinsive host range. Host plants: lentils, alfalfa, colver, beans, cowpeas, dandelions, lambsquarters, mustard and peas. Damage is caused by large numbers sucking plant sap and weekening plants. This aphid produces a considerable amount of honet dew upon which sooty mold grows. The black sooty mold reduces photosynthesis and may take leaves unpalatable to live stock. Aphids are the primary vectors responsible for virus diseases transmission from infected to healthy plants (Basky and Nasser, 1989; Ferguson, 1994 and Berlandier *et al.*, 1997).

Atiri and ligan (1986) mentioned that the pyrethroids have the potential effect than organophosphate and carbamate on the behaviour of *A.craccivora* and on the transmission of cowpea aphid – borne mosaic virus (CAMV). This effect in the short term while reistance breeding is in progress. In the past more than 500 arthropod species have been resistant to the toxicological action of many insecticides and these included more than 20 resistant aphid species (Georghiou, 1990)

The neonicotinoids, the newest major class of inseticides have outstanding, potency and systemic action of crop prtection aginst piercing suking pests. They have low toxicity to mammals (acute and chronic), birds and fishs. Biotransformation involve some activation reactions but largely detoxification mechanisms (Corbel *et al.*, 2004; Nauen and Denholm, 2005 and Tomizawa and Casida, 2005). The nicotinic acetylcholine receptor (nAChR) is an agonist regruated ion channel complex responsible for rapid

neurotransmission. The insect nAChR is the primary target site for the neonicotinoid insecticides (Tomizawa and Casida , 2001) .

Prabhaker *et al.*, (2005) reported that, dinotefuran was the most toxic of the other neonicotinoids (acetamiprid, Imidacloprid and thiamethoxam) against field populotions of Bemisia tabaci. Also, dinotefuran was more toxic than either imidacloprid or thiamethoxam to two imidacloprid resistant strains (120 fold and 109 fold).

Recently, there has been a remarkable increase in application of the DNA analysis to problems in population genetics and systematic (Post *et al.*, 1992). The applications of molecular genetic analysis include: species and hybrid identification, establishing species and population phylogeny, determination of population structure, measuring level of genetic variation in wild and cultured population, conservation biology determination of breeding strategies, gene mapping and linkage analysis (Ferguson *et al.*, 1995 and Utter, 1995). The Polymerase Chain Reaction (PCR) has proven useful for detection and differentiation of a wide range of organisms (Mullis and Faloona, 1987). This method allows the exponential and selective amplification of a short fragment of template DNA, also, it requires prior knowledge of sequence information to desing primers (Saiki *et al.*, 1985 and 1988). Random Amplified polymorphic DNA . polymeras Chain Reaction (RAPD-PCR) is a highly sensitive method for discovering polymorphism randomly distributed throughout the genome (Williams *et al.*, 1990 and Black, 1993). Aronstein *et al.*, (1995) used PCR amplification of cyclodiene resistance alleles within Drosophila flies in the presence and absence of selection. Schlipalius *et al.*, (2002) detected high levels of inheritable resistance to phosphine in *Rhyzopertha dominica* in Australia. They were used random amplified DNA finger printing (RAF) to produce a genetic linkage map of insect. They were identified two genes that are responsible for resistance (50x & 12.5x) and in combination, the two genes act synergistically to produce a resistance level 250x greater than that of fully susceptible beetles.

The present work aimed to investigate the genetic variation in DNA of field and dinotefuran resistant strains compared with the laboratory susceptible strain of cowpea aphid, *Aphis craccivora* (Koch).

MATERIALS AND METHODS

1) Insect:

Laboratory susceptible strain

A Laboratory susceptible population of cowpea aphid *A. craccivora* was established from parthenogenic females collected form faba bean plants *Vicia faba* in Sharkyia Governorate in March 2000 . The population was maintained on faba bean plant grown in a laboratory (24 ± 2°C,65 ± 5% R.H and 12 hours photoperiod) without any exposure to insecticides.

Resistant strain

The leaf dip – bioassay (O'Brien *et al.* 1992) was used to evaluate the toxicity of dinotefuran insecticide (MTI-446 20% WP) to susceptible population of *A. craccivora* under constant laboratory condition. Aphid

mortality was recorded after 24 hours of treatment and corrected according to Abbott (1925). LC50 and slope value were determined by a computerized probit analysis program . Aphids were transferred form old faba bean plants to another plant treated with LC50 of dinotefuran insecticide The colony was raised under pressure of dinotefuran insecticide to twenty generations

Field strain

The fileld population of *A.craccivora* was collected from Sharkyia faba bean plants in February 2005 . Also, the effect of dinotefuran insecticide on field population was determined, and compared with susceptible and resistance populations.

2) Molecular biology assay

100 aphids of susceptible, resistant and field strains were grinded in liquid nitrogen. The genome of aphids was extracted by Qiagen kit.

Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR):

A single primer only used to detect polymprhic DNA (Williams *et al.*, 1990; Welsh and Mc Clelland, 1990). However, in practice primers ten nucleotides in length (10 – mers) have been adopted as a universal standard. A total of five adecamer oligonucleotides (Kit A) from Metabion International AG were used for the amplification of random DNA markers.

PCR mix :

dNTP	2ul	DNA	10ul
10x buffer	5ul	Primer	0.5ul
$MgCl_2$	5ul	Taq polymerase	0.5ul
		H_2O	27ul

The PCR program was as follows:

1- First denaturation at 94 °C for 5min .
2- Denaturation at 94 °C for 1min .
3- Annealing at 36 °C for 2min.
4- Extension at 72 °C for 3min.
5- From 2 to 4 repeated 40 cycle
6- The final extension at 72 °C for 10 min.
7- Reaction was stopped by chilling to 4 °C

The amplification product was visualized by electrophoresis on 1.8% agarose gel. The gel was photographed and scanned .

Statistical analysis:

$$\textbf{Similarity index} = \frac{2Nab}{(Na + Nb)}$$

$$\textbf{Commonality band ratio} = \frac{Nab}{(Na + Nb) - Nab}$$

Where: Nab = the number of bands common to individual a and b.
Na and Nb = the total number of bands in individual a and b, respectively (Nei and Li, 1979)

RESULTS AND DISCUSSION

The neonicotinoide dinotefuran insecticide proved a great toxic effect against susceptible *A. craccivora* strain (LC50 = 0.89 ppm and slope value 2.95) compared with the field strain (LC50 = 5.61 ppm and slope value = 1.66), the resistance ratio reached to 6.3 fold. Wiesner and Kayser (2000)reported that cowpea aphid *A.craccivora* was more susceptible to the insecticidal effect of thiamethoxam than *Myzus persicae* and *Locusta migratoria*. Thiamethoxam was more active on *Aphis* nicotinic acetylcholine receptors in radioligand binding assays. The continuous selection of susceptible strain for twenty generations with dinotefuran produced 42 fold of resistance ratio (LC50 = 37.38 ppm and slope value = 2.58). These results agree with Nauen and Denholm (2005). They mentioned that the neonicotinoids have proved relatively resilient to the development of resistance, especially when considering aphids such as Myzus persica and phorodon humuli.

The genomic DNA of susceptible, resistant and field population of *A.creccivora* was screened for DNA damage or sequence changes using five primers of arbitrary sequences. RAPD –PCR produced a series of discrete to 96 DNA fragments, which typically vary in intensity and range in size from 188 – 1219 bp. Primer A-14 produced ten faint bands. Three of them with field insects (F) (236,307 and 514bp), four bands with susceptible insects (S) (236 – 307,785 and 906 bp) and three bands with resistant (R) insects 236,307 and 785 bp). Two bands of 236 and 307 bp were common in the three samples of insect, another band (785bp) was shared in susceptible and resistant insects. The similarity index value between F & S, S & R and F & R were 0.57, 0.86 and 0.67, respectively. The commonality band ratio were 0.40, 0.75 and 0.5 between F & S , S & R and F & R resp. (Tables1&2 and Figure 1)

Primer A-16 generated 37 fragments, nine of them (193, 218, 286, 317, 376, 525, 688, 874 and 105 7bp) were common in the three samples of insect, one fragment (1104 bp) was found in both F and S, another fragment (252bp) was presented in both S and R. Four fragments (128, 400, 600, and 656 bp) were specific to F insects and two fragments (575 and 1219 bp) were specific to R insects. The similarity index and commonality band ratios between F & S, S& R and F&R were 0.8 & 0.67 , 0.87 & 0.77 and 0.70 & 0.53 resp.(Tables 1&2 and Fig. 1) .

Primer A- 17 yielded 23 fragments, five common bands of 172, 243,302,486 and 529 bp was found in the three samples. Two bands of 723 and 1153 bp were found in both S and R, one band of 252 bp was detected in F & S, one band (346 bp) was specific to S , and one band (287 bp) was specific to R. The similarity and commonality band ratios were 0.80 & 0.67, 0.82 & 0.70 and 0.71 &0.56 between F& S ,S & R and F& R resp.(Tables 1& 2 and Fig. 1)

Primer A-18 produced 14 bands, three bands of 188, 247 and 480 bp were shared in F, S and R insects. One band (354bp) was detected in S and R, one band (397bp) was found in F only and two bands (672 and 880 bp) were specific to in R. The similarity and commonality of bands were 0.75 &

0.60, 0.80 &0.67 and 0.60 & 0.43 between F & S, S & R and F&R resp.(Tables 1& 2 and Fig. 1)

Primer A-19 produced 12 bands, four bands of 193, 247, 341, and 393 bp were shared in the three samples. All samples gave the same profile and no difference between them.

RAPD- PCR patterns produced from amplification of DNA with five primers revealed that the highest degree of difference (60%) between the field and susceptible populations was detected by primer A -14 . Primers A - 18 and 14 recorded 57 and 50% changes in genome of field and resistant populations . Primers A – 18 and 17 gave 33 and 30% difference between DNA sequence of susceptible and resistant insects.

The present results run in common with Bardakci and Skibinski (1994), they found that the patterns of similarities and differences between populations showed broad agreement across primers but that the overall level of similarity varied between primers. Therefore, the choice of a primer is of major importance for discriminatory power of the technique.

The results have proven that the arbitary primers A - 14,18 and 17 are strong tool to investigate changes in the cowpea aphids, *A.craccivora* DNA than other primers, probably due to changes in sequences of the primer. Usually the sequence of the primer is the key factor for evaluation a primer to be used as a tool for screening DNA polymorphism. The same finding were reported by Soliman (1997) who found that primers OPA- 1,2 and 3 exhibited a degree of difference which reached 66.7 -100% between the genomic DNA of untreated and cholrofluzuran treated Med Fly *Ceratitis capitata*. Radwan (2001) stated that primers OPA -14 , 18 and 20 revealed the highest degree of difference (57- 83%) in DNA sequence between the control and treated larvae (with abamectin and esfenvalerate) of the spiny bollworm *Earias insulana*. Kubota *et al.*,(1992) mentioned that AP- PCR techniques detected not only DNA damage causing dominant lethal mutation but also, those causing other phenotypes.

Therefore, the present work suggested that the field population of *A. craccivora* has severity of abnormality randomly distributed in genome due to their mult resistance to several groups of insecticides.

Table (1) : The nucleotide sequence of the primers and the total number of RAPD–RCR fragments of field (F), susceptible (S) and resistant (R) strains of *A. craccivora* (Koch).

Primers	Sequence from 5̀ to 3̀	No. of fragments in different sampies			Total No. Of DNA fragment
		F	S	R	
A – 14	TCTGTGCTGG	3	4	3	10
A – 16	AGCCAGCGAA	14	11	12	37
A – 17	GACCGCTTGT	6	9	8	23
A – 18	AGGTGACCGT	4	4	6	14
A – 19	CAAACGTCGG	4	4	4	12
Total		31	32	33	96

The high diversity in genome of field and dinotefuran resistant strains compared with the laboratory susceptible strain may be demonstrate the toxic activity of dinotefuran on field population and support the application of this insecticide in control of *A .craccivora*. Koch .

Table (2): The similarity index (S.I.) and commonality band ratio (Co.B.R.) between field (F) ,susceptible (S) and resistant (R) strains of *A.craccivora* (Koch).

Primers	F & S		S & R		F & R	
	S.I.	Co.B.R.	S.I.	Co.B.R.	S.I.	Co.B.R.
A – 14	0.57	0.40	0.86	0.75	0.67	0.50
A – 16	0.80	0.67	0.87	0.77	0.70	0.53
A – 17	0.80	0.67	0.82	0.70	0.71	0.56
A – 18	0.75	0.60	0.80	0.67	0.60	0.43
A – 19	1	1	1	1	1	1

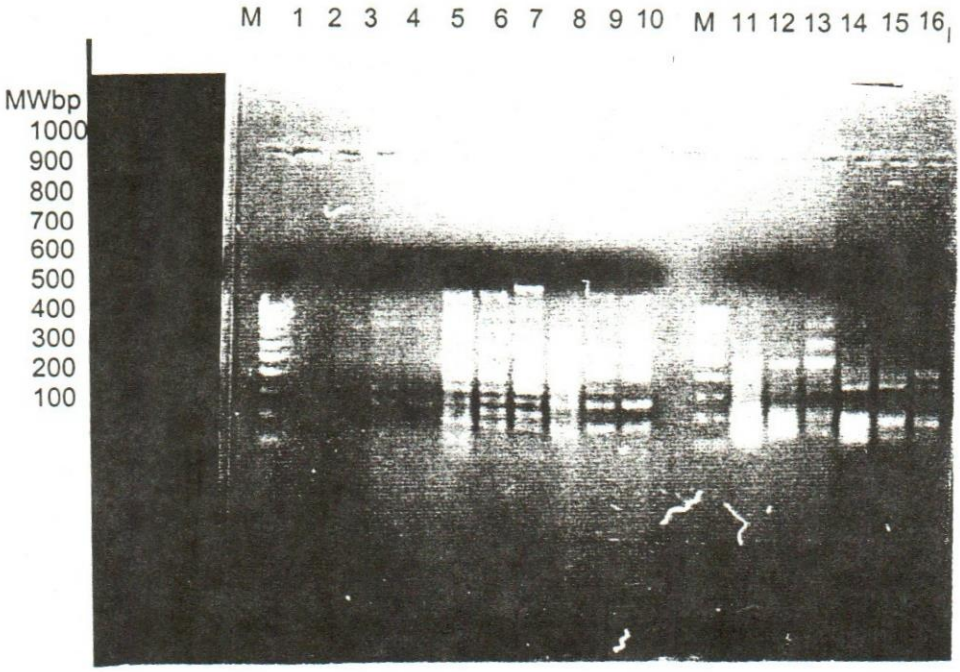

Fig.1: RAPD – PCR product for field, susceptible and resistant *A. craccivora* using primers A- 14 , 16, 17, 18 and 19. lanes M : moleculare size marker . lane 1 : control without DNA . lanes 2, 3 and 4 for F, S and R with primer A-14 . lanes 5,6 and 7 for F, S and R with primer A -16 . lanes 8, 9 and 10 for F,S and R with primer A-17 . lanes 11,12,and 13 for F, S and R with primer A-18 . lanes 14,15 and 16 for F,S and R with primer A-19 .

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الاختلافات الوراثية بين سلالات حساسة ومقاومة وحقلية لحشرة من البقوليات

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طريقة التكبير العشوائي المتباين للدنا باستخدام جهاز تفاعل البلمرة المتسلسل (RAPD-PCR) هي أحد الاتجاهات العديدة لاختبار الاختلافات الوراثية في علم البيولوجيا الجزيئية . وقد استخدم في هذا التكنيك خمسة بادئات عشوائية لتقييم الطفرات الجينية في سلالة حشرة من البقوليات المقاومة لمبيد الدانوتيفران (نيونيكوتينود) بالمقارنة بسلالة حساسة وأخري حقلية . وقد أنتجت الخمسة بادئات (١٤،١٦،١٧،١٨،١٩) المستخدمة ٩٦ حزمة وسجل البادئ العشوائي (١٨) ٥٧ % نسبة اختلاف في المادة الوراثية بين سلالتى المن المقاوم والحقلى بينما سجل نفس البادئ نسبة ٣٣% إختلاف بين جينات سلالتى المن المقاوم والحساس . وقد أعطي البادئ العشوائي (١٤) أعلي درجة اختلاف (٦٠%) بين تتابع دنا (DNA) فى السلالتين الحقلية و الحساسة . والاحتلاف الكبير في المادة الوراثية بين السلالتين الحلقية والمقاومة بالمقارنة بالسلالة الحساسة ربما يفسر كفاءة مبيد الدانوتيفران علي العشيرة الحقلية ويدعم أهمية تطبيق هذا المبيد في مكافحة حشرة من البقوليات.