

## Comparison of Insecticide Resistant and Susceptible Populations of *Spodoptera litura* Fab.

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Esterase activity of various populations of *Spodoptera litura*, collected from cotton fields of different regions of Tamil Nadu, was compared using  $\alpha$ - and  $\beta$ -naphthyl acetate as substrates for enzyme reaction. Among the ten populations analyzed, five populations showed elevated activities for esterases. The RAPD-PCR analysis of resistant and susceptible populations revealed the existence of polymorphism. Specific amplification for esterase gene using custom made primers produced amplified product in resistant populations.

**Keywords:** *Spodoptera litura*, esterase, RAPD-PCR

### Introduction

The Asian armyworm, *Spodoptera litura* Fab. is a polyphagous pest and major defoliator of several crop plants, viz. cotton, castor, tomato, pulses, groundnut, etc (David & Kumarasamy, 1998). Application of synthetic insecticides is in vogue to suppress this pest in the field. Instead, their continuous and indiscriminate use lead to the development of resistance (Zhai & Robinson, 1992). High degree of resistance to carbaryl and organophosphates was reported in *S. litura* from India (Park & Kamble, 1998). Among the mechanisms responsible for resistance to synthetic chemicals, detoxifying enzyme modifications in insects play a vital role (Georghiou & Pasteur, 1978). Earlier studies have reported the increase in activity of enzymes like carboxylesterases, phosphotriesterases, acetylcholineesterases, transferases and oxidases in insects exposed to synthetic insecticides (Valles *et al*, 1994; Prabhakaran & Kamble, 1995). Among them, esterases (as hydrolases) catalyse the hydrolysis of a wide range of aliphatic and aromatic esters, cholin esters and even organophosphorus compounds (Dauterman, 1985). Several researchers have suggested the overproduction of esterases to be responsible for sequestration of insecticidal compounds (Prabhakaran & Kamble, 1995; Chen & Sun, 1994; Small & Hemingway, 2000). Thus, elevation in the

level of esterases, produced as a result of insecticide application, could be used as biochemical markers for such resistance monitoring in insect pests (Byrne & Devonshire, 1991; Abdel-Aal *et al*, 1992). The present study was undertaken to explore such biochemical markers in various populations of *S. litura* collected from different parts of Tamil Nadu, India.

### Materials and Methods

#### Sample Collection

Ten populations of *S. litura* were collected from different cotton fields, six representing southern (two samples each from Madurai, Theni and Cumbum) and four representing northern regions (two samples each from Padappai and Thiruvannamalai) of Tamil Nadu, India between October and December 2001. Each population was considered as a sample.

#### Esterase Analyses

Larval midguts from each group (4<sup>th</sup> instar larvae) were dissected out in ice-cold potassium phosphate buffer (0.2 M; pH 7.8 with 1 mM EDTA) and the gut contents removed. The midguts were ground in a glass homogenizer and the homogenate was centrifuged at 10,000 g for 10 min at 4°C. The supernatant was used for carboxylesterase assay following the method of van asperon (1962) using two substrates, namely  $\alpha$ - and  $\beta$ -naphthyl acetates. Protein concentrations in the supernatant from various populations were determined by the Bradford (1976) assay using Bovine serum albumin as standard.

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### DNA Extraction and RAPD-PCR

The midgut (single individual) from the sample larvae showing high and low esterase activities was pooled separately. The genomic DNA was extracted from the tissues with Tris-EDTA phosphate buffer (50 mM Tris; 10 mM EDTA; pH 8.0) followed by phenol:chloroform extraction and absolute ethanol DNA precipitation (Ballinger-Crabtree *et al*, 1992). The DNA pellet was resuspended in 100 µl of Tris-EDTA buffer and 1 µl (50 ng genomic DNA) of the dilution was used for RAPD-PCR analysis. Random Amplified Polymorphic DNA (RAPD) analysis of genomic DNA of all the populations using 20 different random decamer primers (OPA01-OPA20; Operon Technologies Inc., USA) were carried out. For the purpose, 25 µl of PCR reaction mixture contained 20 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM of each dNTPs, 15-picomole primer, 0.3 unit of Taq DNA polymerase and 50 ng of template DNA. PCR was carried out in an Eppendorf thermal cycler with heat-enabled lid. The cycle conditions involved a denaturation at 94°C for 4 min, followed by 40 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 60 sec and extension at 72°C for 90 seconds with a final extension at 72°C for 7 min. The amplified DNA products were fractionated on 1.7% agarose gel in 1 X Tris Borate-EDTA buffer and visualised on UV transilluminator after staining with ethidium bromide.

Specific amplification of esterase gene in selected resistant populations and single least susceptible sample was carried out as follows. An earlier reported primer sequence for esterase gene (forward-5'-TGAATTGCTCGGTGACACCTC-3'; reverse-5'-ATCTCCTTCGACCGGCATTATTTTC-3') (Vontas *et al*, 2000) was synthesized and used for the purpose. Using the PCR reaction conditions as described above, the PCR cycle involved an initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min and extension at 72° for 1 min and a final extension at 72°C for 7 min. The amplified products were fractionated on 1.5% agarose gel in 1 X Tris Borate-EDTA buffer and visualised by staining with ethidium bromide.

### Results and Discussion

Activity of midgut carboxylesterase ( $\alpha$ - and  $\beta$ -naphthyl acetates as substrates) of the 4<sup>th</sup> instar larvae of *S. litura* from various populations are presented in Table 1, showing elevated activities of esterases in

Table 1—Activity of carboxylesterases from various populations of *S. litura*

No. Populations	$\alpha$ NA esterase activity*	$\beta$ NA esterase activity*
	nmol/min/mg protein	
1 Madurai I	141.43 ± 23.81	121.19 ± 26.58
2 Madurai II <sup>#</sup>	1460.00 ± 68.11	1241.66 ± 49.32
3 Theni I <sup>#</sup>	1820.48 ± 118.46	1336.28 ± 89.15
4 Theni II <sup>#</sup>	1740.14 ± 96.67	1382.62 ± 58.47
5 Cumbum I <sup>#</sup>	1346.82 ± 87.78	972.34 ± 29.34
6 Cumbum II	180.15 ± 41.19	210.37 ± 26.80
7 Padappai I <sup>#</sup>	997.56 ± 62.39	1058.41 ± 47.43
8 Padappai II	126.35 ± 18.15	240.52 ± 42.81
9 Thiruvannamalai I	131.38 ± 28.63	212.73 ± 29.72
10 Thiruvannamalai II	92.03 ± 33.64	150.23 ± 20.28

\* - Average of ten replicates

# - Resistant populations based on esterase activity

Critical Difference (CD) for  $\alpha$  NA esterase activity = 113.44

Critical Difference (CD) for  $\beta$  NA esterase activity = 81.80

The F coefficient for variation was significant at P>0.01 for  $\alpha$  &  $\beta$  NA esterase activities among populations

five different populations. On the basis of esterases as biochemical marker, the order of resistance showed by various populations was Theni I  $\geq$  Theni II > Madurai II > Cumbum I > Padappai I. The potential benefits of a marker (enzymes) assisted selection have been discussed earlier by various workers (Behura *et al*, 1999; Beebe & Saul, 1995; Black *et al*, 1992; Black & DuTeau, 1997.). For RAPD analysis, DNA from resistant and susceptible populations was used as template for the amplification. Among the 20 random decamer oligonucleotide primers (OPA01-OPA20) used, one primer OPA05 generated distinguishable amplification products (Fig. 1) showing the presence of a distinct and consistent 0.6 kb band in the resistant populations which was not found in susceptible populations. Repeated amplifications using individual resistant and susceptible populations also confirmed the existence of this polymorphism.

Further, to tag the esterase gene, specific amplification was carried out, using custom made primers and DNA samples derived from resistant populations, viz. Theni I, Theni II, Madurai II, Cumbam I and Padappai I. A sample from highly susceptible (Thiruvannamalai II) was used as control. Earlier studies have suggested that insecticide resistance is caused by synthesis of more copies of genes (DNA sequence per haploid genome above the level that is characteristic for an organism or amplification) that code for esterases. In these cases extra copies of structural genes apparently could cause increased production of enzymes that



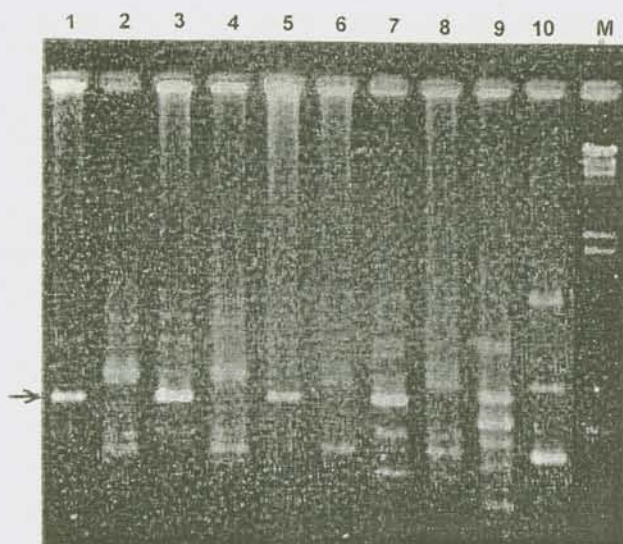


Fig. 1—RAPD-PCR products generated with primer OPA-05 and genomic DNA from ten *S. litura* populations. 1) Madurai II, 2) Madurai I, 3) Cumbum I, 4) Cumbum II, 5) Padappai I, 6) Padappai II, 7) Theni I, 8) Thiruvannamali I, 9) Theni II, 10) Thiruvannamalai II. M-  $\lambda$  DNA double digested with EcoRI & HIND III. Arrowhead indicates the distinct amplified product at 0.6 kb region in lanes 1, 3, 5, 7 & 9

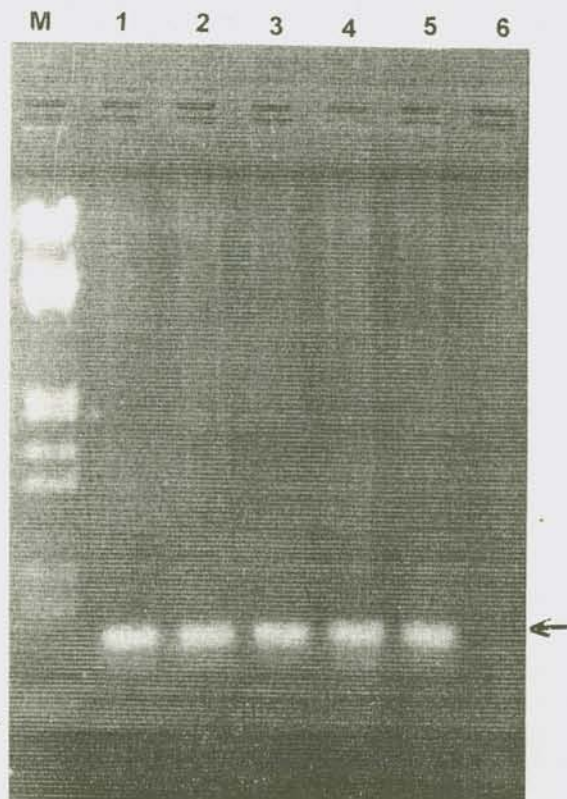


Fig. 2—PCR analysis using esterase gene sequence based primers. M-  $\lambda$  DNA double digested with EcoRI & HIND III. Arrowhead indicates the specific amplified product at 0.6 kb region in resistant populations (lanes 1-5); lane-6-highly susceptible

metabolize or sequester insecticides, thereby increasing the insect ability to insecticide resistance (Tabashnik, 1990; Field *et al*, 1988; Devonshire & Sawicki, 1979). The present study shows an amplification product at around 0.6 kb region (Fig. 2), indicating the presence of esterase gene in the resistant populations. Amplification of esterase gene in the resistant populations in general appears to be mediated by functional regulation or availability of more copies of the gene. It could also be a characteristic that non-amplification of gene, a phenomenon due to low copy number of gene, is responsible for susceptibility associated with other populations of *S. litura*. Scharf *et al* (2001) reported similar results while identifying insecticide resistant and susceptible genes of cytochrome P450 in western corn rootworms. In case of *Myzus persicae*, however, unstable expression of esterase genes was found in the susceptible clones when selection by insecticides was absent (French-Constant *et al*, 1988). As reported in the present study, the absence of esterase gene in susceptible populations could be either due to instability of genes or their non-availability in high copies for amplification.

## References

- Abdel-Aal Y A I *et al*, 1992. Diagnostic esterases and insecticide resistance in the tobacco aphid, *Myzus nicotianae* Blackman (Homoptera:Aphididae). *Pestic Biochem Physiol.* **43**, 123-133.
- Ballinger-Crabtree M E *et al*, 1992. Use of genetic polymorphism detected by the random polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegyptii* subspecies and populations. *Am J Trop Med Hyg.* **47**, 893-901.
- Beebe N W & Saul A, 1995. Discrimination of all members of the *Anopheles punctulatus* complex by polymerase chain reaction-restriction length polymorphism analysis. *Am J Trop Med Hyg.* **53**, 478-481.
- Behura S K *et al*, 1999. Differentiation of Asian rice gall midge, *Orseolia oryzae* (Wood-Mason), biotypes by sequence characterised amplified regions (SCARs). *Insect Mol Biol.* **8**, 391-397.
- Black W C IV & DuTeau N M, 1997. RAPD-PCR and SSCP analysis for insect population genetic studies. *in* The molecular biology of insect disease vectors: A methods manual, edited by J Crampton, C B Beard & C Louis. Chapman and Hall, New York. Pp 361-373.
- Black W C IV *et al*, 1992. Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect polymorphisms in aphids (Homoptera:Aphididae). *Bull Entomol Res.* **82**, 151-159.
- Bradford M M, 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem.* **72**, 248-254.
- Byrne F J & Devonshire A L, 1991. *In vivo* inhibition of esterase and acetylcholine esterase activities by profenofos treatments



- in the tobacco white fly, *Bemisia tabaci* (Genn.): Implications for routine biochemical monitoring of these enzymes. *Pestic Biochem Physiol*, **40**, 198-204.
- Chen W L & Sun C N, 1994. Purification and characterization of carboxylesterase of rice brown planthopper, *Nilaparvatha lugens* Stal. *Insect Biochem Mol Biol*, **24**, 347-355.
- Dauterman W C, 1985. Insect metabolism: Extra microsomal. in *Comprehensive insect physiology, biochemistry and pharmacology*, edited by G A Kerkut & L I Gilbert, Vol. 12. Pergamon Press, Oxford. Pp 713-730.
- David B V & Kumarasamy T, 1998. Elements of Economic Entomology. Popular Book Depot, Chennai.
- Devonshire A L & Sawicki R M, 1979. Insecticide resistant *Myzus persicae* as an example of evolution by gene duplication. *Nature (Lond)*, **280**, 140-141.
- French-Constant R H *et al*, 1988. Spontaneous loss and reselection of resistance in extremely resistance *Myzus persicae* (Sulzer). *Pestic Biochem Physiol*, **30**, 1-10.
- Field L M *et al*, 1988. Molecular evidence that insecticide resistance in peach potato aphids (*Myzus persicae* Sulz) results from amplification of an esterase gene. *Biochem J*, **251**, 309-312.
- Georghiou G P & Pasteur N, 1978. Electrophoretic esterase pattern in insecticide resistant and susceptible mosquitoes. *Entomol Soc Am*, **71**, 201-205.
- Park N J & Kamble S T, 1998. Comparison of esterases between lifestages and sexes of resistant and susceptible strains of German cockroach, (Dictyoptera:Blatellidae). *Entomol Soc Am*, **98**, 1051-1057.
- Prabhakaran S K & Kamble S T, 1995. Purification and characterization of an esterase isozyme from insecticide resistant and susceptible strains of German cockroach *Blatella germanica* (L.). *Insect Biochem Mol Biol*, **25**, 519-524.
- Scharf M E *et al*, 2001. Expression and induction of three family 4 cytochrome P450 (CYP4) genes identified from insecticide resistant and susceptible western corn rootworms, *Diabrotica virgifera*. *Insect Mol Biol*, **10**, 139-146.
- Small G J & Hemingway J, 2000. Molecular characterization of the amplified carboxylesterase gene associated with organophosphorus insecticide resistance in the brown planthopper, *Nilaparvatha lugens*. *Insect Mol Biol*, **9**, 647-653.
- Tabashnik B E, 1990. Implications of gene amplification for evolution and management of insecticide resistance. *Entomol Soc Am*, **90**, 1170-1176.
- Valles S M *et al*, 1994. Detoxifying enzymes in adults and nymphs of the German cockroaches: Evidence for different microsomal monooxygenase systems. *Pestic Biochem Physiol*, **49**, 183-190.
- van Asper en K, 1962. A study of housefly esterases by means of sensitive calorimetric method. *J Insect Physiol*, **8**, 401-416.
- Vontas J G *et al*, 2000. Comparison of esterase gene amplification, gene expression and esterase activity in insecticide susceptible and resistant strains of brown plant hopper, *Nilaparvatha lugens* (Stal.). *Insect Mol Biol*, **9**, 655-660.
- Zhai J & Robinson W H, 1992. Measuring cypermethrin resistance in German cockroach. *J Econ Entomol*, **85**, 348-351.