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Genetic variation in artificially selected strains of the egg parasitoid, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) using RAPD analysis*

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ABSTRACT: Artificial selection is extensively used to develop pesticide resistance in natural enemies. RAPD markers were used to estimate genetic relatedness between the parent and artificially selected hybrids of the egg parasitoid *Trichogramma chilonis* Ishii resistant to multiple insecticides (700ppm of endosulfan, 540ppm of monocrotophos and 20ppm of fenvalerate) and high temperature (≥ 40°C). Highly polymorphic markers could be identified through the primers OPF-1 and OPJ-20. RAPD marker from 15 oligomers clearly outgrouped the susceptible parent from artificially selected pesticide-resistant strains. The first group comprised the susceptible strain. The second group comprised the two subclusters, the first subcluster including the strain resistant to endosulfan and fenvalerate, multiple-insecticide resistant strain (MIRS) and high temperature resistant (HTR) strain while the second subcluster consisted of monocrotophos and HTR strains. The discriminating property of RAPD markers allowed differentiation of the resistant strain from the parental susceptible strain. Artificially selected resistant strains shared relatively high similarity (61-66%) with susceptible parent strain as per Jaccard's index.

KEY WORDS: *Trichogramma chilonis,* RAPD, pesticide resistance, high temperature, genetic relatedness

INTRODUCTION

The concept of genetic improvement is implied to enhance the potential of natural enemies towards better pest management in biological control programmes. Parasitoids with desirable traits can be produced in the laboratory using artificial selection. About 19 parasitoids and predators have been selected in the laboratory for enhanced fecundity, host synchrony, non-diapause, pesticide resistance, temperature tolerance or thelytoky (Whitten & Hoy, 1999). *Trichogramma chilonis*, the egg parasitoid that plays an important role in the suppression of lepidopterous pests is released in biological control programmes or in IPM on many crops in India (Jalali *et al*., 2003). Nevertheless, to achieve desirable control, released parasitoids have to overcome many problems like pesticides and high temperature conditions. In view of these limitations, an endosulfan resistant strain was developed in the laboratory (Jalali *et al*., 2006a), and later a multiple insecticide resistant strain was developed and evaluated against cotton bollworms throughout India (Jalali *et al*., 2006b). Assessment of laboratory selected natural enemies in the context of their ability to adapt to field conditions and provide efficient control of the pests needs to be addressed since very few genetically improved natural enemies have been deployed in pest management programmes. Plant breeders have exploited RAPD–PCR technique to study genetic diversity and population genetic structure in plants (Dawson *et al*., 1993; Huff *et al*., 1993; Martin *et al*., 1997; Buso *et al*., 1998; Hsiao and Lee 1999; Zhao *et al*., 2000).

In insects, RAPD-PCR technique has been widely used to study the genetic variation in pest populations collected from different geographical lacations (Kumar *et al.*, 2001; Subramanian and Mohan, 2006), to track the origin of introduced insect pests (William *et al*., 1994), and in differentiating ecotypes (Pornkulwat *et al*., 1998). In natural enemies RAPD–PCR markers have proved to be useful in distinguishing pesticide-resistant laboratory biotype and wild orchid biotype of *Trioxys pallidus,* a parasitoid of walnut aphid, *Chromaphis juglandicola* (Edwards *et al*., 1995). There are no studies on genetic variation in artificially selected natural enemies. The objective of the present study was to assess the genetic diversity in genetically improved hybrids of *T. chilonis.*

*A part of Ph. D. work of the first author

MATERIALS AND METHODS

Source of *T. chilonis*

All the strains of *T. chilonis* were maintained on *Corcyra cephalonica* (Stainton) eggs in the laboratory. The source strain was collected from parasitised eggs of *Helicoverpa armigera* infesting cotton crops from different places in India. A small batch of these parasitized eggs that was not exposed to any stress like pesticides and temperature was considered as susceptible and maintained in the laboratory for more than 20 years while another batch was used for artificial selection experiments. Descriptions of endosulfan tolerant strain (Jalali *et al*., 2006a) and multiple insecticide and high temperature tolerant strains (Ashok *et al*., 2008) of *T. chilonis* are given in Table 1.

DNA Extraction

DNA from each strain was isolated from 40 adults arising from an isofemale line using Chelex-100® (Biorad industries, Hercules, USA) as described in Vanlergerghe-Masutti (1994). Two adult parasitoids were homogenized in 20µl of 5% chelex. The tubes were incubated at 56°C for 3h followed by 100°C for 5 minutes. The supernatant was collected by centrifugation at 10000rpm for 5 minutes. DNA from different strains was stored at –20°C till further use.

RAPD-PCR

Initially 45 primers (Operon Technology, USA) were tested for polymorphism in seven strains out of which 15 primers were selected (Table 2) based on their ability to produce polymorphic bands. These amplifications were confirmed twice before further analysis. PCR reactions were performed as described by Laurent *et al*. (1998) in a total volume of 25µl containing 2µl DNA, 75mM Tris HCL (pH 8.0), 20mM $(NH_4)_2 SO_4$, 3mM $MgCl_2$, 0.1% tween, 100µM of each dNTP, 100ng of primer and 1unit of Taq DNA polymerase (Dr. Taq Polymerase, Biogene). Amplification was performed on Biorad icycler programmed for 3 minutes at 94°C followed by 40 cycles at 94°C for 45 seconds, 36°C for 30 seconds, 72°C for 2 minutes and a final extension at 72°C for 10 minutes. A negative control without DNA was included to check contamination. PCR products were electrophoresed on 1.8% agarose in 0.5X Tris–borate buffer at 5-volts/cm. Bands were visualized by ethidium bromide staining (0.5 µg ml^{-1}).

Table 1. List of strains of *Trichogramma chilonis* used

Strain	Parent	Pesticide	Description
Susceptible strain (SuscS)	Collected from parasitised eggs of *Helicoverpa armigera* infesting cotton crops from different places in India		Maintained in the laboratory for past 20 years.
Endosulfan resistant strain (Endo)	SuscS	Endosulfan	Artificially selected for 341 generations resulting in tolerance to 700ppm of endosulfan
Monocrotophos resistant strain (Mono)	Endo	Monocrotophos	Artificially selected for 81 generations resulting in tolerance to 540ppm of monocrotophos
Fenvalerate resistant strain (Fen)	Endo	Fenvalerate	Artificially selected for 81 generations resulting in tolerance to 20ppm of fenvalerate
Multiple insecticide resistant strain (MIRS)	Endo, Mono, Fen	Endosulfan, Monocrotophos, Fenvalerate	Developed by hybridization technique by allowing all the three strains, i.e., Endo, Mono, Fen to mate among each other followed by selection to insecticides.
Temperature resistant strain (HTR)	SuscS		Artificially selected for high temperature resistance (> 40º C)
Multiple insecticide and temperature resistant strain (MIRS-HTRS)	MIRS	Endosulfan, Monocrotophos, Fenvalerate	Developed MIRS was subjected to variable temperature (32-38º C)

Data Analysis

RAPD bands were scored as present (1) or absent (0). The scored binary data was analyzed using NTSYSpc 2.02j version (Rohlf, 1998). The genetic distance was estimated by Jaccard's similarity coefficient using SIMQUAL module. Cluster analysis was performed according to unweighted pair group mean algorithm (UPGMA) within SAHN module of NTSYS programme. Principal Coordinate analysis (PCA) to construct a three-dimensional array of eigen vectors was performed using D-Center nodule of NTSYS programme.

RESULTS AND DISCUSSION

RAPD-PCR

Fifteen primers generated 88 bands of different sizes ranging from 150 to 2500bp out of which 50 bands were polymorphic resulting in 56.8% polymorphism. The number of bands per primer ranged from 3 to 10 with a mean of 5.86 whereas the number of polymorphic bands ranged between 1 and 10 with a mean of 3.3. Primers OPA-4, OPC-12 (Fig. 1), OPP-9 (Fig. 2) and OPL-4 (Fig. 3) were able to uniquely identify the Susc strain from the improved strain. Primers OPF-1 (Fig. 4) and OPJ-20 produced maximum polymorphic bands. Pair wise similarity among strains calculated based on Jaccard's similarity coefficient ranged from 0.61 to 0.91 (Table 3). MIRS and Fen showed high degree of similarity (0.91) as did MIRS and Endo (0.89). Contrastingly, Lab and MIRS shared the lowest similarity value (0.61).

Table 2. List of RAPD primers used

No	Primer	Sequence
1	OPF-1	ACGGATCCTG
2	OPAA-17	GAGCCCGACT
3	OPC-12	TGTCATCCCC
4	OPJ-4	CCGAACACGG
5	OPE-11	GAGTCTCAGG
6	OPP-9	GTGGTCCGCA
7	OPP-14	CCAGCCGAAC
8	OPA-4	AATCGGGCTG
9	OPE-2	GGTGCGGGAA
10	OPL-15	AAGAGAGGGG
11	OPJ-20	AAGCGGCCTC
12	OPI-10	ACAACTGGGG
13	OPC-5	GATGACCGCC
14	OPL-4	GACTGCACAC
15	OPN-4	GACCGACCCA

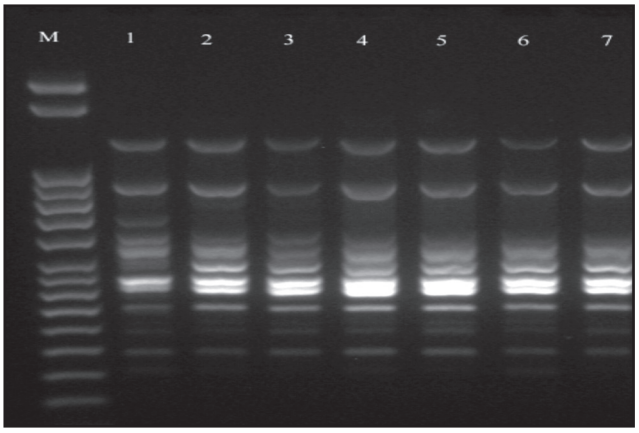

Fig. 1. RAPD profile obtained from primer OPC-12 (Lanes: M, 50bp DNA perfect ladder (Novagen); 1,SuscS strain; 2,Endo; 3,Mono; 4,Fen; 5,MIRS; 6,HTR; 7, MIR-HTRS)

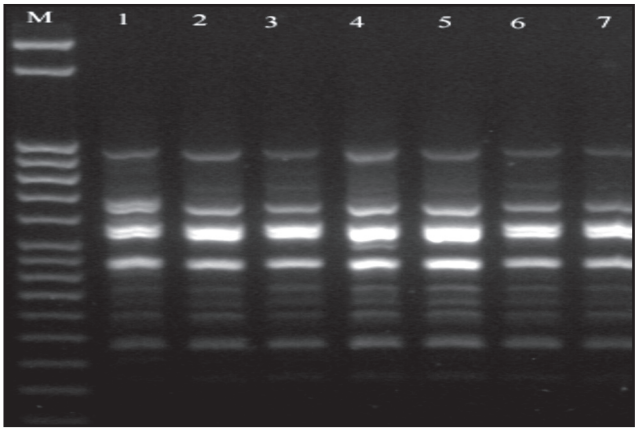

Fig. 2. RAPD profile obtained from primer OPP-9 (Lanes: M, 50bp DNA perfect ladder (Novagen); 1,SuscS strain; 2,Endo; 3,Mono; 4,Fen; 5,MIRS; 6,HTR; 7, MIR-HTRS)

Cluster analysis separated the seven strains of *T. chilonis* in two groups (Fig. 5). The first group consisted of only the parental strain while the second group comprised strains. The second group consisted of two subgroups six (B & C). Subgroup B consisted of Endo, Fen, MIRS, and MIR–HTRS while subgroup C consisted of Mono and HTRS. Mono was grouped along with HTRS in subgroup C separated from the hybrid MIRS strain. Mono strain was selected for resistance to monocrotophos, a potent inhibitor

of acetylcholine esterase. Owing to the different mode of action of the pesticide, Mono most likely would have adopted a unique resistance mechanism dissimilar to the other strains. PCA clearly indicated that the genetically the improved strain was closely related and separated from the parental strain (Fig. 6).

The outcome of the study clearly suggests that artificial selection of *T. chilonis* for pesticide and high temperature tolerance has resulted in genetic variation. During artificial selection it is expected that candidates with advantage of genetic bias will be selected and this overtime of selection would result in a homozygous population imbibing the desired characters. Homogeneity (61-66%) among the parental and artificially selected strains can be considered significant in such an extensively carried out artificial selection and hybridization programme, a part of genetic improvement strategy.

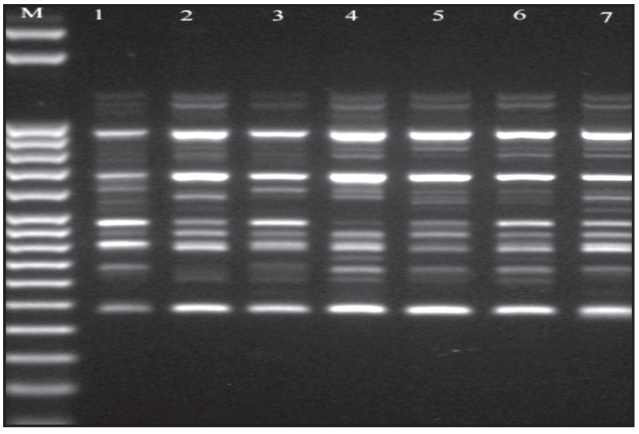

Fig. 3. RAPD profile obtained from primer OPL-4 (Lanes: M, 50bp DNA perfect ladder (Novagen); 1,SuscS strain; 2,Endo; 3,Mono; 4,Fen; 5,MIRS; 6,HTR; 7, MIR-HTRS)

Low polymorphism observed in field populations of *Meloidogyne* nematode was due to parthenogenesis (Cenis 1993). Similar type of genetic divergence was observed in populations of the egg parasitoid *Telenomus podisi* collected from soybean field in Brazil (Aljnabi *et al*., 1998). Low variation in RAPD–PCR pattern was associated to

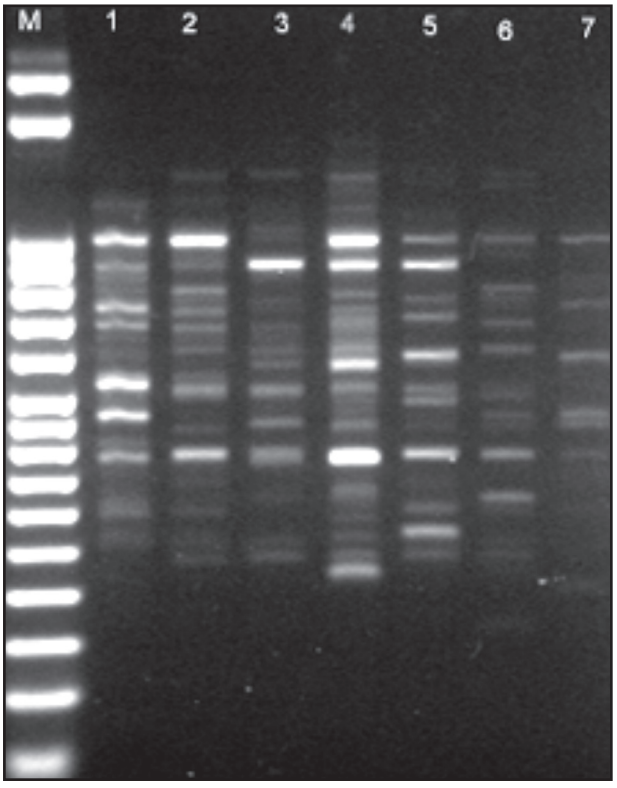

Fig. 4. RAPD profile obtained from primer OPF-1 (Lanes: M, 50bp DNA perfect ladder (Novagen); 1,SuscS strain; 2,Endo; 3,Mono; 4,Fen; 5,MIRS; 6,HTR; 7, MIR-HTRS)

Table 3. Jaccard's similarity coefficient showing variation among 7 strains of *T. chilonis*

Strains	SuscS	Endo	Mono	Fen	MIRS	HTR	MIR - HTRS
SuscS	1						
Endo	0.6625	1					
Mono	0.6410256	0.7763158	1				
Fen	0.6511628	0.8860759	0.8	1			
MIRS	0.6117647	0.8947368	0.8051948	0.9125	1		
HTR	0.6363636	0.75	0.8028169	0.775	0.75641	1	
MIR-HTRS	0.626506	0.8441558	0.7792208	0.8641975	0.8481	0.8	1

SuscS =susceptible strain; Endo = endosulfan resistant strain; Mono = monocrotophos resistant strain; Fen = fenvalerate resistant strain; MIRS = multiple insecticide resistant strain; HTR = temperature resistant strain; MIR-HTRS = multiple insecticide and temperature resistant strain

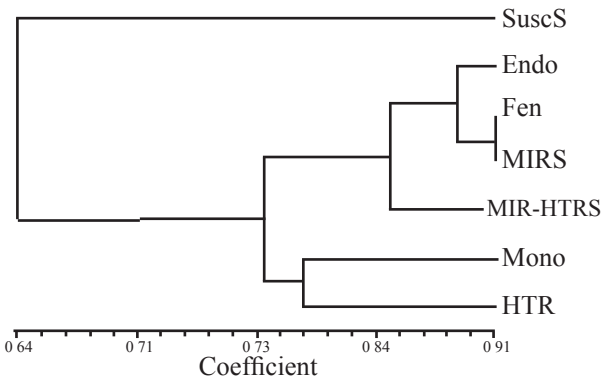

Fig. 5. UPGMA dendrogram based on Jaccard's similarity coefficient

the pseudo arrhenotoky nature in populations of *Euseius finlandicus* (Yli-Mattila *et al*., 2000). In our study, the Endo strain, which was selected for 341 generations, resulted in 15-fold resistance governed by incomplete dominance (Jalali *et al*., 2006a). This suggests the involvement of few alleles for tolerance to endosulfan. During artificial selection these alleles would have been selected and continuous inbreeding would have resulted in a more homogeneous population.

Limited amount of genetic variation in the present study could be due to gene flow in a small population. Laboratory rearing of natural enemies also contributes to low genetic variation.

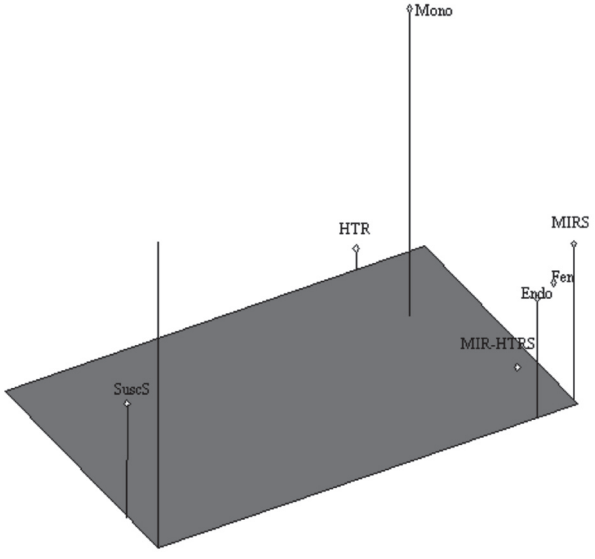

Fig. 6. Distribution of seven strains of *T. chilonis* along first three principal coordinate axes

The strains derived from Endo showed high homogeneity (75-89%) when compared to SuscS strain (61-66%). This can be explained by shorter duration of selection pressure (81 generations) imposed on the Endo strain. Interestingly, the HTR strain also showed high homogeneity though the parental strain was SuscS. RAPD–PCR is an appropriate tool to study small insects for which very less genetic information is available requiring less amount of DNA (Hoy 1994). RAPD-PCR tool is rather unexploited in studying genetic variation in natural enemies. RAPD markers are dominant and heterozygous individuals are not detected usually. Hence, in the present study genetic variability between the parent and artificially selected strains of *T. chilonis* was analyzed using phenetic distance measure (Jaccard's coefficient). RAPD marker was useful in assessing the variation in the population of *T. evanescens* (Vanlergerghe-Masutti, 1994). In studies elsewhere, RAPD-PCR was used as a diagnostic tool to differentiate the susceptible and propargite-resistant strains of *Tetranychus urticae* (Shah *et al*., 2002). The RAPD-PCR technique can also be extended to develop a more reliable strain-specific marker, which will be helpful in monitoring the dispersal rates of the artificially selected strains of *T. chilonis.*

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