Detection of genetic polymorphism in the populations of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenee)

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In the present study six different populations of *L. orbonalis* were collected and subjected to analysis of genetic variability in terms of carboxylesterase isozyme pattern and DNA polymorphism using RAPD-PCR. Pattern of carboxylesterase revealed a similar isozyme cluster in the populations namely, sivaganga (population-3), dindigal (population-4), virudhunagar (population-5) and coimbatore (population-6). Similarly, the populations of *L. orbonalis* recorded 3 distinct randomly amplified polymorphic DNA markers in all populations grouped above. This pattern of genetic variability in the populations was also supported by the analysis of the similarity indices and UPGMA dendrogram.

**Keywords:** Carboxylesterase, *Leucinodes orbonalis*, RAPD-PCR

*Leucinodes orbonalis* (Guenee) is the most injurious and a ubiquitous pest of brinjal, *Solanum melongena* L., which is found to feed on the shoots and fruits and occur throughout the year with varying degrees of infestation intensity. The pest is known to cause about 16 and 70 per cent damage to shoots and fruits, respectively. The larva confines its feeding activities on shoots in the early stages of crop and later on, on fruits, which become unfit for human consumption. Varied control measures including the chemicals and other non-chemical approaches applied against the control of *L. orbonalis* have modified and resulted in heterogeneity among the populations. In response to the stresses, the populations of *L. orbonalis* have changed to adapt the ill-effects by changing their eco-behavioural pattern, feeding physiology and reproduction, in addition to the changes in their molecular machineries. Hence, it is of practical importance to precisely trace the genetic variability in the expanding geographical distribution of the pest. Genetic characterization of populations of insect pests plays a pivotal role in determining management strategies of the insect pest.

Use of isozymes as biochemical marker to study the insect populations began in the late 1950s and continues to be widely applied. Within the last few years, techniques using the polymerase chain reaction (PCR) to amplify specific genomic regions have become widespread. Williams et al. developed the technique that uses a single decamer primer to amplify arbitrary regions of a genome. They demonstrated that RAPD-PCR process reveals polymorphisms in the genomes of a wide variety of insect species. In this paper, we describe carboxylesterase and RAPD-PCR patterns to illustrate their use as molecular markers in six different populations of *L. orbonalis*.

**Materials and Methods**

Populations of *L. orbonalis* were collected from six different localities (population-1: Madurai; population-2: Theni, population-3: Sivaganga, population-4: Dindigal, population-5: Virudhunagar, population-6: Coimbatore) of southern parts of Tamil Nadu, India. The fifth instar larvae were used for both carboxylesterase and RAPD-PCR analysis. The larval tissues were dissected out and used for the enzyme extraction and gel electrophoresis by non-denatured polyacrylamide gel as described by Georgiou and Pasteur. DNA was also isolated from the tissues using genomic DNA purification kit supplied by MBF Fermentas, USA. DNA (20 ng) was dissolved in 20 μl of PCR reaction buffer containing 10 mM Tris-HCl (pH 8.8); 1.5 mM MgCl₂; 50 mM KCl; 0.1%, Triton X-100; 0.2 mM, dNTPs; 5 μM of all the primers and 0.5 U of DNA polymerase. Ten primers (OPI-11 to OPI-20) obtained from Operon Technologies (USA) were used for RAPD-PCR studies. PCR was
conducted according to the methods of Williams et al.\(^7\): initial heat step (94°C for 4 min.), 40 cycles of denaturation (94°C for 45 sec.), annealing (37°C for 45 sec.) and extension (72°C for 90 sec.) and a final extension step (72°C for 7 min.). Amplification was performed using a programmable Thermal Cycler PTC-150 (MJ Research, USA). The products of PCR and DNA size markers (λ DNA digested with EcoRI and HindIII (MBI Fermentas, USA)) were loaded onto a 1.7% tris-borate-EDTA agarose gel and run for 4 hr at 50 V. The gels were stained with ethidium bromide and photographed. Each lane of enzyme and RAPD profiles were subjected to gel documentation system (Vilbert-Lourmat, France). The dendrogram analysis was carried out using Bioprofil 1D software.

Results
The pattern of carboxylesterase isozyme and RAPD profile of \textit{L. orbonalis} in this study showed characteristics of genetic variability of each population. Figure 1a shows native PAGE analysis of carboxylesterase isozymes of tissue samples of \textit{L. orbonalis}. The isozyme pattern showed one slow moving esterase fraction at Rm 0.46 and three fast moving esterase fractions at Rm 0.64, 0.68 and 0.81. The slow moving esterase fraction was observed in all the populations except the population-2 (Theni). The fast moving esterase fractions, Rm 0.68 and Rm 0.81, were found in all the populations with differential staining activity. The fraction electrophoresed at Rm 0.64 was recorded to be unique to population-1 (Madurai). The similarity index analysis of all the carboxylesterase fractions among various populations resulted in the generation of 100% similarity among the population-4 to population-6 (populations of Sivaganga, Dindigal, Virudhunagar and Coimbatore; Fig. 1b). UPGMA dendrogram also revealed these three populations in one cluster, and Theni (population-2) and Madurai (population-1) were separated from the cluster (Fig. 1c).

Ten different (OPI-11 to OPI-20) random primers were tested with DNA samples isolated from various populations. Of the primers used OPI-13 revealed a polymorphic pattern that enabled to distinguish various populations of \textit{L. orbonalis}. Similarity indices and dendrogram analysis were also computed and presented (Fig. 2a, b, c). All the scorable fragments ranged from 1450 to 480 bp. Like isozyme analysis, RAPD results were also revealed the close relationship of population-3 to population-6 (populations of Sivaganga, Dindigal, Virudhunagar and Coimbatore).

Fig. 1.-(a) Native PAGE analysis of esterase isozymes of tissue samples of \textit{L. orbonalis}; (b) Similarity index for the electrophoretic profile of esterases; (c) Dendrogram with Homology Coefficient %: 0.0 (UPGMA) (Lane 1-Madurai population; Lane 2-Theni population; Lane 3-Sivaganga population; Lane 4-Dindigal population; Lane 5-Virudhunagar population; and Lane 6-Coimbatore population)
Discussion

Molecular characterization of insects has been frequently conducted on the basis of existence of polymorphic DNA fragments amplified by PCR. Williams et al.\textsuperscript{10} have reported geographical distribution of Argentine stem weevils, \textit{Listronotus bonariensis} (Kuschel), by RAPD-PCR using genomic DNAs. Haymer and McInnis\textsuperscript{11} have also pointed out a distinctive variation in PCR-amplified DNA patterns between laboratory-cultured and wild populations of Mediterranean fruit flies, \textit{Ceratitis capitata} (Weidemann). RAPD evidence of introgression in two closely related sympatric species of \textit{Charistoneura} in Atlantic Canada\textsuperscript{12}; RAPD as a tool to identify genetic variation in ecotypes of the European corn borer\textsuperscript{13}; population structure of \textit{Helicoverpa armigera} using RAPD analysis\textsuperscript{14}; RAPD markers linked to \textit{Nilaparvata lugens}\textsuperscript{15}; Genetic diversity of Iberian populations of \textit{Bemisia tabaci} based on RAPD-PCR\textsuperscript{16}; were some known literature indicating that RAPD-PCR has promise for displaying intraspecific genetic variations in insect species. Skoda et al.\textsuperscript{17} and Kawamura et al.\textsuperscript{18} have attempted to clarify PCR-based DNA patterns in \textit{Cochliomyia} sp. and sweet potato weevils, \textit{Cylas formicarius} collected from various ecotypes. Results from our study also confirm the value of isozyme as a biochemical marker and the RAPD as genetic marker in distinguishing \textit{L. orbonalis} populations. Identification and discrimination of pest populations in terms of genetic characterization would be much useful in choosing precise management strategy concerning their susceptibility.

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References


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