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Genetic variability of populations of *Earias vittella* (Fab.) (Noctuidae: Lepidoptera) as revealed by Random Amplified Polymorphic DNA

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ABSTRACT

Ten populations of Earias vittella collected from different localities of Tamilnadu, India from the bhendi crop were subjected to RAPD analysis to study the genetic variability. Twenty random decameric primers were used, out of which, two primers – OPA07 and OPA12 – produced clear, consistent and reproducible RAPD bands for all the populations. Each primer produces a species-specific RAPD marker with the molecular weight of 1422bp (OPA07) and 615bp (OPA12). The UPGMA based dendrogram analysis grouped all the populations of E. vittella in two major clusters and the similarity coefficient values ranged from 0.27 to 0.90. The RAPD profiles of populations of E. vittella based on banding pattern, UPGMA analysis and genetic similarity index revealed genetic polymorphisms among the populations.

Keywords: Earias vittella, RAPD, DNA marker, Genetic variability, Dendrogram, Similarity Index

INTRODUCTION

The spotted bollworm Earias vittella (Fab.) is a polyphagus insect and damage the terminal buds of shoots, bolls, succulent internodes, young leaves, flower buds of cotton, bhendi etc. This damage finally leads to flowers and bolls shedding and fruit spoilage in horticultural crops. The pest is active almost throughout the year on its different host plants under field conditions (Arif and Attique, 1990). Different kinds of molecular markers can reveal different levels of genetic variation, making population genetics studies possible on a wide range of geographical scales. DNA markers are especially effective tools in making inference about movement between insect populations, because they present selectively neutral characters (Black et al., 2001). Among the available DNA marker studies, the technique of random amplified polymorphic DNA (RAPD) (Williams et al., 1990), also known as arbitrarily primed polymerase chain reaction (AP-PCR) (Welsh and McClelland, 1990), has been employed as a powerful tool for DNA fingerprintings and genetic variability analysis of insects (Haymer, 1994). Hence, in the present study, RAPD-PCR method was employed to analyze the genetic variability among the populations of E. vittella.

MATERIALS AND METHODS

The fifty instar larvae of Earias vittella from infested bhendi fruits were collected from ten different districts of Southern Tamilnadu, India. Genomic DNA was isolated using phenol-chloroform procedure (Sambrook and Russell, 2001). 20ng of DNA was dissolved in 20µl of PCR reaction buffer containing 10mM Tris-HCl (pH 9.0), 1.5mM MgCl2, 50mM KCl, 0.01% gelatin, 0.2 mM dNTPs, 20 pM of primer and 0.5 U of DNA polymerase. Twenty primers OPA01 to OPA20) obtained from Operon

Technologies, USA was used for RAPD-PCR studies. PCR was done according to the methods of Williams et al. (1990) with initial heat step (94ºC for 5 min), 40 cycles of denaturation (94ºC for 1 min), annealing (36ºC for 1 min) and extension (72ºC for 2 min), and final extension step (72ºC for 7 min). Application was performed using a programmable thermal cycler PTC-1148 MJ Mini Gradient Thermal Cycler (BIO RAD, USA). The products of PCR and DNA size markers [λ DNA double digested with E.CoR1 and Hind III (Bangalore Genei)] were loaded onto a 1.6% Tris-borate-EDTA (Sambrook et al., 1989) agarose gel and run for 4 h at 50 V. The gels were stained with ethidium bromide and photographed using DP-001.FDC Photo Documentation System (Vilber Lourmat, France). The RAPD products were analysed using the Bio 1D software (Vilber Lourmat,

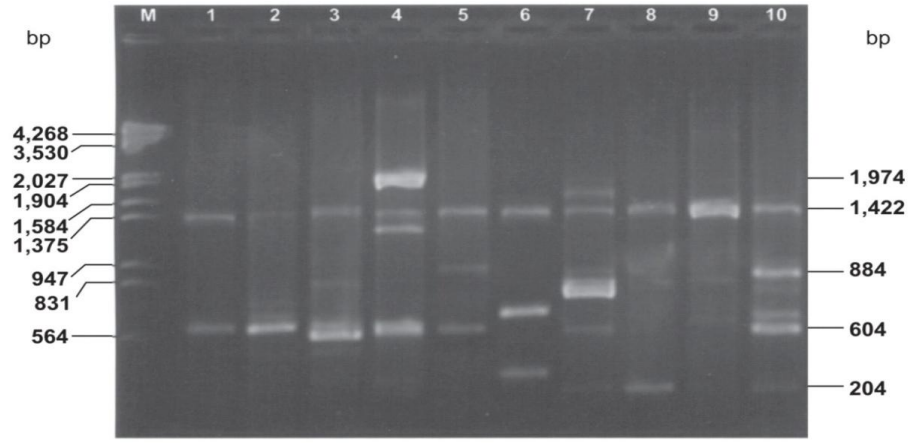


Figure-1a. Random amplified polymorphic DNAs of populations of *E. vittella* generated by the primer OPA07

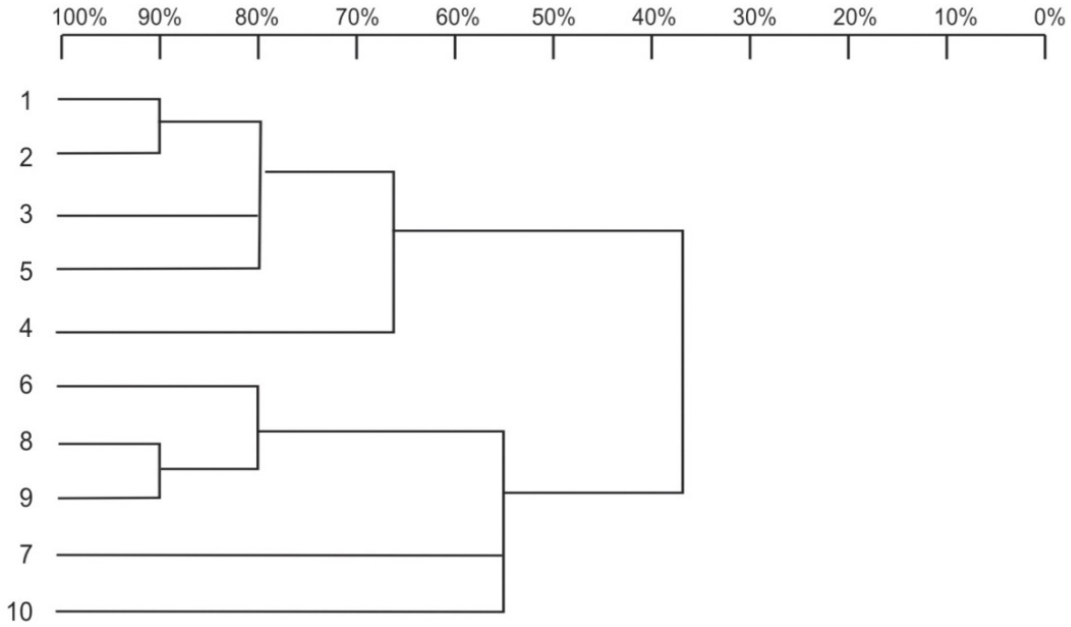
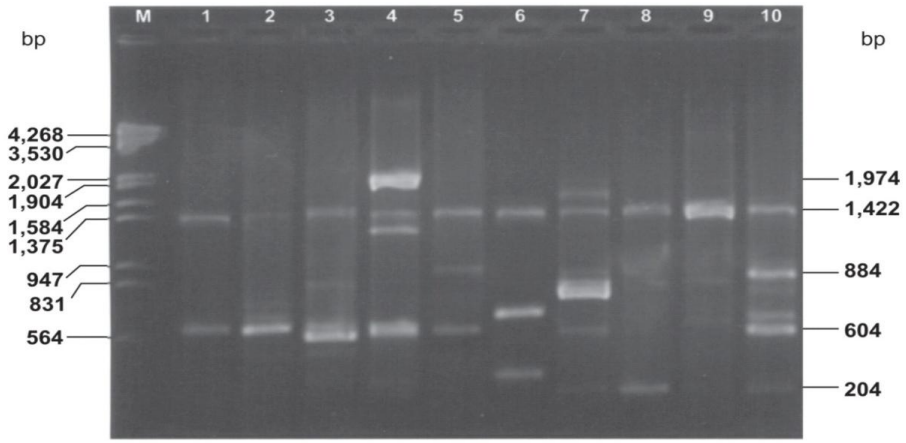


Figure-1b. Dendrogram constructed based on the RAPD profile of OPA07
1 Madurai population; 2. Theni population; 3. Dindigul population; 4. Virudhunagar population; 5. Sivaganga population; 6. Trichy population; 7. Ramanathapuram population; 8. Karur population; 9. Pudukkottai population; 10. Coimbatore population; M. Molecular weight marker

France). Cluster analysis was performed and dendrogram was plotted based on pair wise genetic distance estimated using the unweighted pair group method with arithmetic mean (UPGMA) based on Nei (1978).

RESULTS & DISCUSSION

RAPD-PCR analysis was conducted to study the genetic variability among ten populations of Earias vittella. Twenty RAPD decameric primers, OPA01 to OPA20, were tested for their ability to study the DNA polymorphism of different populations of E. vittella. Of all the primers tested, OPA07 and OPA12 yielded clear amplification pattern for different populations of E. vittella. Dendrogram and similarity index were constructed based on the RAPD profiles generated by primers using UPGMA (Bioprofile 1D software).



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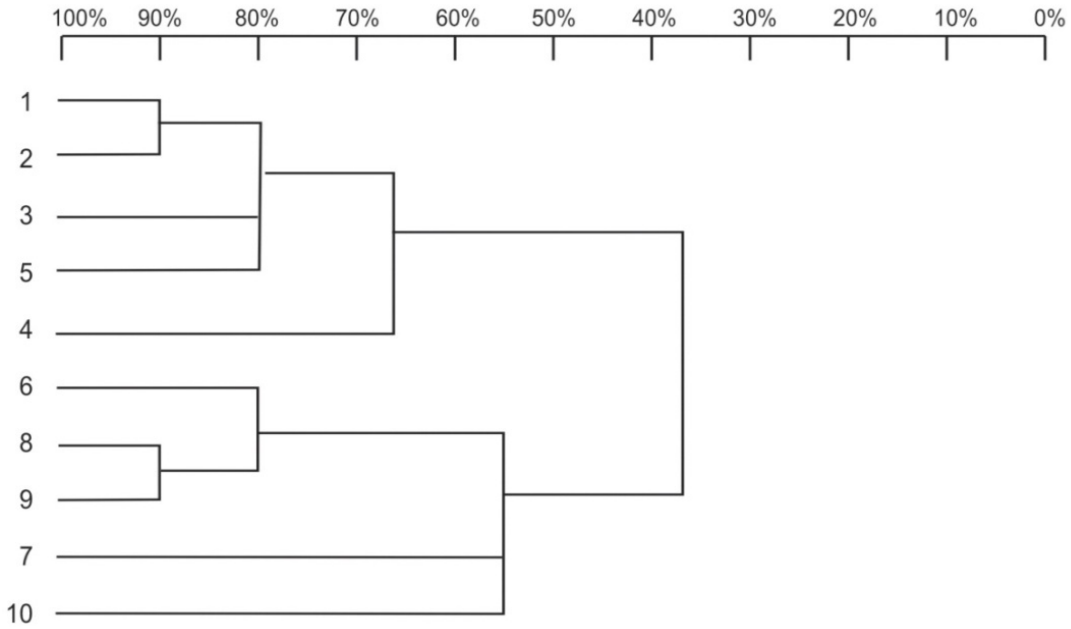

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The RAPD profile of *E. vittella* generated by the primer OPA07 produced amplified fragments of DNA in the molecular weight ranging 204bp to 1974bp (Figure 1a). A maximum of five numbers of amplified fragments was recorded in the Coimbatore population and only two fragments were recorded in Madurai and Theni populations. The UPGMA based dendrogram (Figure 1b) of the RAPD profile of OPA07 clearly indicated two major clusters that comprised populations of Madurai, Theni, Dindigul, Virudhunagar and Sivaganga in one group and Trichy, Ramanathapuram, Karur, Pudukkottai and Coimbatore in another group.

Figure 2a depicts the RAPD profile of E. vittella generated by the primer OPA12. The scorable fragments produced by this primer were ranged from 1718bp to 220bp. The RAPD profile showed a maximum of seven fragments in virudhunagar population and a minimum of two in Dindigul population. The UPGMA based dendrogram tree showed two major clusters, one comprised populations of Madurai, Theni, Karur, Sivaganga, Coimbatore and Trichy and the rest of the populations in another cluster (Figure 2b). Each primer recorded one species-specific RAPD marker with the molecular weight of 1422bp (OPA07) and 615bp (OPA12). A total of eighty one RAPD fragments were generated by both the primers with the similarity coefficient value ranged from 0.27 to 0.90, which indicates that no two populations were 100% genetically similar.

RAPD markers are playing an important role in the analysis of genetic diversity of a large number of insect species. The simplicity and reproducibility of the PCR-based assays, added to their higher multiplex ratio and capacity to detect higher levels of polymorphisms, makes them a lucid method to obtain intraspecific genetic variation in the insect species, where no prior sequence information is available (Kumar et al., 2001). In the present study, RAPD markers generated from two random primers revealed sufficient polymorphism to characterize genetic variation within the E. vittella populations. The genetic variability among the populations of E. vittella could be attributed to varied ecology and geography of the collection sites. And also, the insect species undergoes and experiences different pattern of stresses and strains caused by our farming practices.

REFERENCES

1. Arif, M.I. and Attique, M.R. 1990. Alternative hosts in carryover of Earias insulana (Boisd.) and Earias vittella (F.) in Punjab, Pakistan. The Pak. Cotton. 34: 91-96.
2. Black, W.C.I.V., Baer, C.F., Antolin, M.F. and Duteau, N.M. 2001. Population genomics: genome-wide sampling of insect populations. Annu. Rev. Entomol. 46: 441-469.
3. Haymer, D. S. 1994. Arbitrary (RAPD) primer sequences used in insect studies. Insect Mol. Biol. 3: 191-194.
4. Kumar, L.S., Sawant, S.A., Gupta, V.S. and Ranjekar, P.K. 2001. Genetic variation in Indian populations of Scirpophaga incertulas as revealed by RAPD-PCR analysis. Biochem. Genet. 39: 43-47.
5. Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89: 583-590.
6. Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold spring harbor laboratory press, cold spring harbor, New York.
7. Sambrook, J. and Russell D.W. 2001. Molecular Cloning: A Laboratory Manual. 3rd Ed. Cold Spring Harbor Laboratory Press, New York. pp. 1723–1726.
8. Welsh, J. and McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213-7218.
9. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

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