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GENETIC DIVERSITY OF FRUIT BORER, *HELICOVERPA ARMIGERA* (LEPIDOPTERA: NOCTUIDAE) BASED ON RANDOM AMPLIFIED POLYMORPHIC DNA- POLYMERASE CHAIN REACTION

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Abstract

The genetic variability of *Helicoverpa armigera* (Hübner) at different agro-ecological zones of Bangladesh in comparison with Indian population was conducted in India during September 2008 to February 2009. A total of 12 *H. armigera* populations of which 10 populations collected from different agro-ecological zones of Bangladesh and two populations from India were tested for their genetic variability. Eight out of the ten primers produced scorable PCR products by amplifying the template DNA with taq polymerase and were subjected for analysis. Those eight primers got amplified to a total of 138 markers which produced polymorphic markers. The similarity coefficient based on 138 RAPD markers ranged from 0.000 to 0.777 of the pair-wise combination among twelve samples of *H. armigera*. An UPGMA dendrogram based on Jaccard's similarity coefficient was constructed for the 12 samples of *H. armigera*. The dendrogram showed that *H. armigera* population from Bangladesh had 25 to 45 percent similarity, and in its Indian population the similarity remained within this range.

Keywords: *Helicoverpa armigera*, genetic diversity, RAPD-PCR, primers.

Introduction

Fruit borer, *Helicoverpa armigera* (Hübner) is widely distributed in Asia, Africa, Australia, and the Mediterranean Europe, while *Heliothis virescens* and *Helicoverpa zea* are widely distributed in the Americas (Reed & Power, 1982). Being polyphagous, this pest feeds on more than 500 plant species, including economically important crops, such as cotton, maize, sorghum, chickpea, pigeon pea, sunflower, vegetables, and fruits. They cause an estimated loss of over US$ 5 billion annually despite application of pesticides costing over US$ 1 billion every year. (Sharma, 2005). In Bangladesh, *Helicoverpa armigera* is becoming an alarming pest in different vegetable crops. It was reported that infestation range of *H. armigera* on tomato was up to 46.85 percent at Jessore (Alam *et al*.,

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2007). In recent times, crop production has been severely affected in controlling the pests as they have developed high level of resistance to the commonly used insecticides worldwide. Fruit borer has developed a high level of resistance to many of the commonly used pesticides (Kranthi *et al.*, 2002). Insecticide resistance in *H. armigera* leads to heterogeneity in population.

In general, *Helicoverpa* species have been described as preferentially feeding on buds, flowers, and fruits. The preference of fruiting structures and the tendency to move from one fruit to another, often without consuming it completely, is the main reason why extensive damage often results to crops even when the number of large larvae is relatively low (Zalucki *et al*., 1986). Damaged flowers and young fruits may fall off. Damaged fruits have roughly spherical holes made by fully mature larvae through which they escape the host plant for pupation in soil. Older fruits rot or become deformed. A single caterpillar may eat and destroy 2 – 8 fruits. *H. armigera* is abundant throughout Bangladesh. Its damage severity varies from place to place. There might exist some genetically variation in *H. armigera* of different agro-ecological zones of Bangladesh.

The versatility of this species may be due to the presence of a strong genetic variability governing the behaviour of *H. armigera* (Zhou *et al.*, 2000; Scott *et al.*, 2003) making it a serious pest on several crops. In this regard, a better understanding of the genetic differences of polyphagous pest like *H. armigera* can be very useful to the structure and population dynamics, their behavior and response to various selection pressures. Therefore, an attempt was made to study the genetic variation of *H. armigera* using RAPD markers.

Materials and method

This study was conducted in the Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, India during September 2008 to February 2009.

Sampling of insect

H. armigera larvae collected from different locations of Bangladesh and were reared at IPM laboratory, Entomology Division, BARI separately using their respective hosts. Indian *H. armigera* populations were also reared separately in their respective hosts. The emerged female from each locality was preserved immediately in vials containing 70 percent alcohol. The samples were maintained at -20°C until DNA extraction. A total of 10 samples of *H. armigera* were collected from 10 different localities of Bangladesh on different hosts like tomato, chickpea, chili, and mungbean during March to May of 2008. For comparison, two populations of *H. armigera* were collected from two locations of Coimbatore, Tamil Nadu, India during October 2008. Hence, a total of 12

samples were collected from 12 different localities depicting a garden-land ecosystem with field and horticultural crops that are being grown round the year (Table 1).

Table 1. Sample collected from different locations for assessing diversity in *H. armigera* populations from different host plants.

Plant source	Date of sampling	Location	Geographic coordinate	Population code
Tomato	27/4/2007	Gazipur	22°78'N 90°20'E	TOGAZ
Tomato	30/4/2007	Mymensingh	24°45'N 90°24'E	TOMYM
Tomato	01/5/2007	Comilla	23°28'N 91°10'E	TOCOM
Tomato	25/4/2007	Norsingdhi	23°92'N 90°71'E	TONOR
Mungbean	10/5/2007	Pabna	24°01'N 89°18'E	MBPAB
Chili	1/5/2007	Rajbari	23°75' N 89°65'S	CHIRAJ
Chili	3/5/2007	Manikgonj	23° 51'N 90° 0' E	CHIMAN
Mungbean	12/5/2007	Ishurdi	24° 8' N 89° 5' E	MBISD
Mungbean	02/5/2007	Magura	24°46'N 89°15'E	MBMAG
Mungbean	15/5/2007	Jessore	23°10'N 89°10'E	MBJES
Chick pea	01/10/2008	Coimbatore	11°00 N 77°00 E	CPCO
Chick pea	03/10/2008	Trichy	10°48'8"N 78°1'7"'E	CPTRY

DNA extraction

Total nucleic acids were extracted from individual female of *H. armigera* using C-TAB (Hexadecyl trimethyl ammonium bromide) method (Doyle and Doyle, 1987) with some modifications. Modifications were made as follows: 1. Second wash of crude DNA with Chloroform and iso amyl alcohol is prevented. 2. Washing of crude DNA with sodium acetate 3. Washing with 70% ethanol for several times is limited based on the final crude DNA pellet obtained. Single washing of crude DNA with ethanol was maximum followed. Quantity of the isolated DNA was measured in NanoDrop® ND-1000 spectrophotometer and the quality were checked in 0.8% Agarose gel electrophoresis before using it as the template for polymerase chain reactions (PCR). The reagents were purchased from Bangalore Genei Ltd., Bangalore, India.

RAPD assays

Amplification reactions were performed in a 20 µL reaction mix, containing a final concentration of 2.5 mM dNTPs, 25 mM $MgCl_2$, dimethyl sulfoxide, Taq polymerase 3U/µL, 10x Taq buffer, sterile water, primer 100 nmol and DNA 20-

25 ng/uL. The RAPD analysis was performed with seventeen decamers supplied by Operon Technologies Inc., California, USA. Amplification was performed in thermocycler (BioRad, USA) programmed as one cycle of initial denaturation at 95°C for 2 min; one cycle of denaturation at 95°C for 1 min; 30 cycles each of 95°C for 1 min., annealing at 40°C for 1 min., extension at 72°C for 1 min. and final extension at 72°C for 7 min. The PCR products were separated in 1.5 % agarose gel electrophoresis.

Analysis of PCR amplification profiles

Data obtained by scoring the RAPD profiles of the eight primers individually were subjected to cluster analyses. PCR amplification products of the 12 samples were scored as presence (1) or absence (0) of bands. The data matrix was used to calculate Jaccard's similarity coefficient (Sneath and Sokal, 1973), which does not consider the joint absence of a marker as an indication of similarity. The similarity values were used for cluster analyses. Sequencial agglomerative hierarchial non-overlapping (SAHN) clustering was done using Unweighted Pair-Group method with arithmetic averages (UPGMA). This analysis was performed using NTSYS-PC software, version 2.0 (Rohlf, 1998).

Results and Discussion

Eight out of the ten primers screened produced scorable PCR products by amplifying the template DNA with taq polymerase and were subjected for analysis. These eight primers (Table 2) got amplified to a total of 138 markers and all the primers produced amplified polymorphic markers. The total number of clear bands obtained from each primer ranged from 34 (OPA 02) to 6 (OPE 15) with an average of 17.25 bands per primer. The size of the amplicons ranged from 100 bp to more than 1000 bp, while clearly resolved bands were seen only below 800 bp in case of OPA 13 primer. RAPD amplification pattern is shown in Fig.1 and 2 for illustration. Genetic relationships between populations are shown in Table 3.

The similarity coefficient based on 138 RAPD markers ranged from 0.000 to 0.777. Of the pair-wise combination among 12 samples of *H. armigera*, CPCO (Chick pea from Coimbatore) location got the minimum similarity index (0.000) and the maximum index (0.777) was seen in TOMYM (Tomato from Mymensingh region). An UPGMA dendrogram based on Jaccard's similarity coefficient was constructed for the twelve samples of *H. armigera*. Two major clusters *viz.,* A and B are evident from the dendrogram (Fig. 2). PCR screening demarcates the *H. armigera* population based on the locality. The major cluster A is again divided into minor groups *viz.,* A_1, A_2, A_3 and A4. The minor cluster A_1 is comprised population from TOGAZ (Tomato host plants from Gazipur),

TOMYM (Tomato from Mymensingh region) and TONOR (Tomato from Norshindhi region), while A_2 comprised TOCOM (Tomato from Comilla region), MBPAB (Mung bean from Pabna region), CHIRAJ (Chili from Rajbari region), CHIMAN (Chili from Manikgonj region) and MBISD (Mung bean from Ishurdi region), while A_3 comprised MBMAG (Mung bean from Magura region), and A_4 comprised MBJES (Mung bean from Jessore region). The major cluster B is further divided into B_1 and B_2. The minor cluster B_1 represented the population of *H. armigera* collected from Coimbatore and B_2 represented chickpea from Trichy. The dendrogram deduced that *H. armigera* population from Bangladesh had 25 to 45 percent similarity, while 30 percent similarity was observed for population from India.

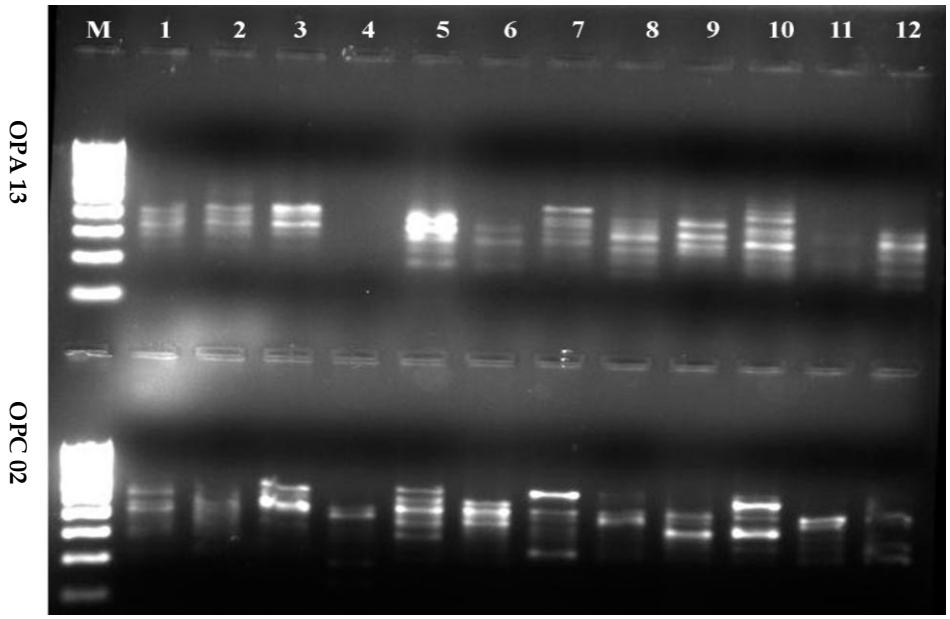

Fig. 1. Agarose gel electrophoretic profile from twelve populations of *H. armigera*: 1. Gazipur tomato; 2. Mymensingh tomato; 3. Comilla tomato; 4. Norshindi tomato; 5. Manikgonj Chili; 6. Rajbari Chili; 7. Ishurdi mungbean; 8. Pabna mungbean; 9. Magura mungbean; 10. Jessore mungbean; 11. Coimbatore chickpea; 12. Trichy chickpea, M= 100 bp ladder

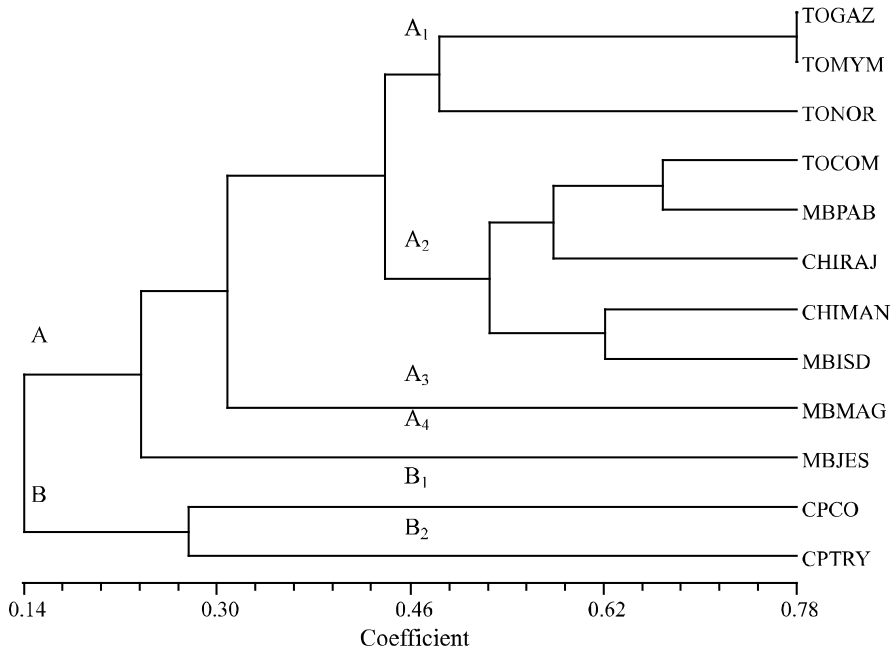

Fig. 2. Dendrogram based on Jaccard's similarity coefficient showing relationship among *H. armigera* populations from different host plants and regions of Bangladesh and India using RAPD markers

TOGAZ =Tomato from Gazipur region, TOMYM= Tomato from Mymensingh region, TONOR= Tomato from Norsindi region, TOCOM= Tomato from Comilla region, MBPAB= Mung bean from Pabna region, CHIRAJ = Chili from Rajbari region, CHIMAN= Chili from Manikgonj region, MBISD= Mung bean from Ishurdi region, MBMAG= Mung bean from Magura region, MBJES= Mung bean from Jessore region, CPCO= Chick pea from Coimbatote, Tamil Nadu, CPTRY= Chick pea from Trichi, Coimbatore, Tamil Nadu

This finding is supported by Gujar *et al.* (2007) who showed that six out of eight pairs of molecular markers were able to amplify the cadherin gene in eighteen insect populations of *H. armigera* collected from different locations in India and produces a total of 218 amplicons. The maximum similarity (96%) was found for three pairs of insect populations from Rajkot and Sirsa, Akola and Bhatinda, and Faridkot and Karnal; while the minimum similarity (82%) was for the pair of insect populations from Nanded and Hyderabad. The study showed 4–18% genetic diversity in cadherin-specific gene of *H. armigera* populations which differed at least 100-fold in their susceptibility to Cry1Ac. Chen *et al.* (2000) reported that genetic variations of *H. armigera* (Hubner) over different major cotton growing regions were analyzed by DNA polymorphism amplified with four simple repetitive sequence primers. The results showed that the laboratory population had lower genetic variation than natural populations. The

genetic variation between natural populations was not significant and genetic variation existed in the same location from different years, indicating frequent migration among natural cotton bollworm populations.

Table 2. Total number of markers and percentage of polymorphism developed among *H. armigera* population from different host plants and different regions by the RAPD primers.

Primer name	No.of markers generated	Polymorphic markers	Monomorphic markers	Percent Polymorphism
OPA 13	25	25	-	100.00
OPC 02	22	22	-	100.00
OPA 02	34	34	-	100.00
OPA 07	18	18	-	100.00
OPC 08	17	17	-	100.00
OPE 15	6	6	-	100.00
OPA 01	9	9	-	100.00
OPI 12	7	7	-	100.00
	138			

Table 3. Genetic similarity matrix among *H. armigera* populations.

Case	1	2	3	4	5	6	7	8	9	10	11	12
1	1.000											
2	0.777	1.000										
3	0.466	0.466	1.000									
4	0.416	0.545	0.437	1.000								
5	0.400	0.400	0.545	0.450	1.000							
6	0.277	0.277	0.526	0.333	0.521	1.000						
7	0.571	0.571	0.647	0.533	0.619	0.380	1.000					
8	0.357	0.461	0.666	0.538	0.476	0.625	0.562	1.000				
9	0.176	0.250	0.238	0.166	0.280	0.227	0.238	0.277	1.000			
10	0.105	0.166	0.350	0.222	0.434	0.400	0.350	0.411	0.250	1.000		
11	0.083	0.083	0.187	0.076	0.190	0.176	0.117	0.142	0.000	0.200	1.000	
12	0.062	0.062	0.150	0.125	0.208	0.200	0.277	0.176	0.050	0.157	0.272	1.000

1-TOGAZ =Tomato from Gazipur region, 2-TOMYM= Tomato from Mymensingh region, 3- TOCOM= Tomato from Comilla region 4-TONOR= Tomato from Norsindi Region, 5- CHIMAN= Chili from Manikgonj region 6-CHIRAJ = Chili from Rajbari region 7-MBISD= Mung bean from Ishurdi region, 8-MBPAB= Mung bean from Pabna region, , 9-MBJES= Mung bean from Jessore region 10-MBMAG= Mung bean from Magura region, , 11-CPCO= Chick pea from Coimbatore, Tamil Nadu, 12-CPTRY= Chick pea from Trichi, Coimbatore, Tamil Nadu

Subramanian and Mohankumar (2006) showed in their preliminary study using 10 microsatellite simple sequence repeat (SSR) markers the genetic variability of *H. armigera* populations from six different host plants with nine SSR primers indicating high variability across the different host associated populations with polymorphism ranging from 75 to 100 percent and high similarity among populations collected from vegetable crops and more variable while the population collected from the cotton crop. This phenomenon indicates a strong genetic variability among *H. armigera* populations collected from different host plants. Shravankumar and Jagdaishwar (2004) found differences in susceptibility to different insecticides among *H. armigera* populations collected from three hosts, chickpea, tomato, and grapes. The authors suggested that this difference might be due to the variation in plant factors. The results of the present study also suggest that genetic variation among populations collected from different host plants might be due to host characteristics.

Moya *et al.* (2001) and Maruthi *et al.* (2001) studied genetic diversity on whitefly, *Bemisia tabaci* genomic DNAs using RAPD-PCR. Molecular characterization of insects has been frequently conducted on the basis of existence of polymorphic DNA fragments amplified by PCR (Caterino *et al.,* 2000). Williams *et al.* (1994) have reported geographical distribution of Argentine stem weevils, *Listronotus bonariensis* (Kuschel), by RAPD-PCR using genomic DNAs. Within the last few years, techniques using the polymerase chain reaction (PCR) to amplify genomic regions have become widespread (Saiki *et al.,* 1988). Williams *et al.* (1993) reported that RAPD PCR process reveals polymorphism in the genomes of a wide variety of insect species.

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