GENETIC VARIABILITY OF GEOGRAPHICAL POPULATIONS OF THE BOLLWORM, *HELICOVERPA ARMIGERA* HÜBNER (LEPIDOPTERA: NOCTUIDAE), IN WEST AND NORTHWEST OF IRAN

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ABSTRACT: Old World or African bollworm, *Helicoverpa armigera* Hübner (Lep.: Noctuidae) is one of the key pests causing severe yield losses, infesting several crops such as cereals, pulses, cotton, vegetables and fruit crops as well as wild hosts. It is expected that the recognition of intraspecific variation, its nature and scope, may aid our understanding of the pest and also to predict its spatial and temporal occurrence, to devise effective management strategies, and also to characterize pod borer population responses to control measures. The purpose of this study is to estimate the relative importance of generic and environmental effects on characters of pod borer in Iran. Specimens were collected from several provinces in Iran from tomato. The genomic DNA from *H. armigera* larvae collected during summer 2006-2007 from five different places were subjected to polymerase chain reaction (PCR) using 10 different SSR primers. The highest numbers of 14 markers were produced by the primer HaSSR1, followed by 9 markers by HaSSR6 with high degree of polymorphism 75–100%. The primers HaSSR6, HaSSR4, HaC87 and HaD47 were found to be highly informative to differentiate populations with a polymorphism information content value of 100 percent. Ten tested SSR primers produced 46 bands in geographical populations. Within population genetic diversity based on Nie s gene index ranged from 0.188 to 0.250 Molecular variance analysis showed significant within and between population variance. The between and within geographical variance accounted for 13.88 and 86.12 percent of total molecular variance, respectively. Cluster analysis based on molecular data in geographical assigned the studied pod borer moth populations into two groups. In this grouping, group one consisted of Golestan population. The maximum and minimum genetic distances were observed between Gorgan- Mughan (0.21853) and Kermanshah- Shahindej (0.05789). Significant correction was not found between genetic and geographic matrices revealed by Mantel test.

KEY WORDS: Pod borer, *Helicoverpa armigera*, SSR, Molecular markers, Biodiversity, Geographic population.

Old World or African bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is one of the key pests causing severe yield losses, infesting several crops such as cereals, pulses, cotton, vegetables and fruit crops as well as wild hosts (Zaluki et al., 1986,1994; Fitt, 1989). The bollworm is a polyphagous pest of worldwide occurrence inflicting annual crop damage in India worth US $1 billion

(Sharma, 2001). Ecological and Physiological features like high fecundity, multi-voltinism, ability to migrate long distances and diapauses during unfavorable conditions contribute for it's severity in different situations.

Understanding the genetic variation among the *H. armigera* populations occurring on host plants and different geographical conditions has become essential to understand the variation in their susceptibility to different insecticides and suitable management. This species is migratory on all continents, and a key pest on all of them (Feng et al., 2005). It reported in the main agriculture area of Iran too. This pest is primarily controlled by using chemicals that has many unfavorable side effects such as weather, air, food and environmental pollutions. Observations on the pest behavior show interspecific variation in damage caused to several crops in different geographic locations. It is expected that the recognition of intraspecific variation.

Its nature and scope, may aid our understanding of the pest and also to predict its spatial and temporal occurrence, to devise effective management strategies, and also to characterize pod borer population responses to control measures. The purpose of this study is to estimate the relative importance of generic and environmental effects on the morphological characters of pod borer in Iran.

The genetic variation among geographic populations of *H. armigera* collected from the South Indian cotton ecosystem was analyzed using RAPD markers and 12 populations were classified into two distinct groups (Fakrudin et al., 2004). In the present study the genetic variability of *H. armigera* occurring on five different geographical regions were analyzed using simple sequence repeat (SSR) markers. The characteristics of SSR markers such as coverage of multiple loci, co-dominance and high polymorphism suit them better in the task of measuring genetic structure in *H. armigera* (Scott et al., 2003) than the RAPD markers used in the previous studies. The use of SSR markers for *H. armigera* was previously hampered by non-availability of the DNA sequence information. Recently, many SSR markers specific for *H. armigera* have been identified (Tan et al., 2001; Ji et al., 2003; Scott et al., 2004; Ji et al., 2005).

MATERIALS AND METHODS

Specimens were collected from several provinces in Iran on tomato. From the provinces of Kermanshah: City of Ravansar. From the province of Ardabil: City of Mogan (MT). From the province of East Azarbyjan: City of Shabestar (SBT). From the province of West Azarbyjan: City of Shahindeg (SHT). From the province of Golestan: City of Gorgan (GT) (Table 1).

Larvae of *H.armigera* were collected during summer 2006-2007 from Tomato. from first generation of each population 15 individuals of each sex were collected for preliminary analysis. larvaes were randomly selected for the isolation of genomic DNA and stored at –70° C.

DNA extraction

The larvae were washed thoroughly in double distilled water and the genomic DNA was prepared from the 2th instar larvae using by modified protocol (Zimmerman et al., 2000). Briefly, the cleaned larvae were ground in liquid nitrogen and then 500 microlitre buffer containing 100 mM Tris-HCI (pH 8.0), 0.1 M sodium chloride, 20 mM EDTA, 0.1% of SDS and suspended in the same buffer. The suspension was incubated at 60° C for 3 hours and then equal volume of chloroform: isoamylalcohol (24:1) was added. The suspension was centrifuged

at 13000 g for 5 minutes at 4° C. The upper aqueous layer was transferred to a fresh micro centrifuge tube taking care not to disturb the middle protein interface. Then added 15 microllitre Nacl 5M and shacked by hand slowly. DNA was precipitated by adding equal volume of ice-cold isopropanol. The tube was kept for 20 min in -20°C. The precipitated DNA was spun at 13000 g for 5 minutes and the Supernatant was discarded and the DNA pellete was finally washed twice using ethanol 70% and dissolved in 200 µl TE (Tris EDTA, 100 mM). Extracted DNA was further purified free of RNA contaminants by addition of 10 µl/100 µl of RNase. The intact genomic DNA was visualized in a 1% agarose gel. Depending upon the concentration, the DNA samples were diluted with sterile water to get a working solution of 20–25 ng/µl.

PCR amplification

The genomic DNA from *H. armigera* larvae collected from five different places were subjected to polymerase chain reaction (PCR) using 10 different SSR primers (Tan et al., 2001; Ji et al., 2003) (Table 2) obtained from Sigma-Aldrich, (www.sigmaaldrich.com). PCR was carried out in 20 µl reaction mixture containing 50 ng DNA as the template. Genomic DNA 2.0 µl (25 ng), dNTPs 0.8 µl (2.5 mM), assay buffer 1 µl (10X), SSR forward primer 2 µl (20 µM), SSR reverse primer 2 µl (20 µM), Taq polymerase 0.15 µl (3 units), magnesium chloride 0.15 µl (25mM), sterile distilled water 3.7 µl, were added and PCR was performed in a DNA thermal cycler programmed for 5 min at 94° C for initial denaturation. Following the initial denaturation the thermal cycler was programmed for 35 cycles of 1 min at 94° C for denaturation , 1 min for annealing depend on primers and 50 second at 72° C for extension and additional cycle of 10 min at 72° C for final extension.

Electrophoresis of PCR products

PCR products were analyzed by electrophoresis in 6 percent polyacrylamide gel electrophoresis run at 70 W for 30 min in 1x TBE buffer. The banding pattern was visualized using the silver staining method (Panaud et al. 1996). The silver stained gel plates were allowed to dry and photographed. The molecular size of the amplified products was estimated using a 100 bp DNA marker (Fermentas Inc., www.fermentas.com.) The samples were analyzed all 12 primers to test the reproducibility of bands.

Scoring of bands and statistical analysis

Based on log molecular weight of the co-migrating 100 bp DNA marker (Fermentas Inc., USA) and their migration distances scatter plots were established and trend lines with best fit was fitted. Based on the mathematical expression of the trend lines the molecular weight of the fragment corresponding to their migration distances was calculated. The individual DNA bands were scored as present or absent (1/0) in the amplification profile of each sample. Only clear bands with good resolution were scored. The scored marker data matrix was analyzed using the standard procedure in NTSYS pc-2.0 package (Rohlf, 1998).

The percentage of polymorphism was calculated as the proportion of the polymorphic markers to the total number of markers. A dendrogram was constructed after cluster analysis of the similarity coefficients by the un-weighted pair-group method analysis, UPGMA (Sneath and Sockal, 1973) using NTSYS Pc-2.0.

RESULTS AND DISCUSSION

All ten primers listed produced score able markers in each DNA sample. Sample gels resulting from the HaSSR1, 2, 4, 9 and 10 primers across the populations collected from different places are presented in Figure 1.

A total of 46 markers from 10 primers were available for analysis across the different populations. The highest numbers of 14 markers were produced by the primer HaSSR1, followed by 8 markers by HaSSR9 with high degree of polymorphism 75–100%. Analysis of molecular of variance among and within populations showed significant variation (Table 3).

Fixation Index FST : 0.50542

Computed FST values for these comparisons were low to moderate, from 0.05789 to 0.2185 with a mean of 0.13797 (Table 4).

Generalized distances values were then utilized to cluster the data using the un-weighted pair-group method analysis of Sneath & Sokal (1973). Since test geographic populations were all associated with the same host (Tomato), population differences may have been mainly the result of geographic barriers that are defined as any terrain that prevents gene flow between populations (Mayr & Ashlock 1991). Sometimes distance alone can function as a barrier to genetic exchange among populations (Ruggiero et al., 2004). The Mantel test did not show correlations between geographic and genetic distances in test populations. The dendrogram (Figure 2) revealed the existence of two principle clusters and a single sub-cluster. The population occurring on GT stood out in a single cluster (A), while the population occurring in MT, SHT, KT and SBT grouped together in cluster B. The population occurring from Gorgan was found in a single sub-cluster.

The authors suggested that this difference might be due to the variation in environmental and ecological factors. Because Gorgan situated in near of Caspian sea and has the least elevation 13.3 M and maximium annually rainfall 599.6 mm. Scott et al. 2003 found genetic shifts in *H. armigera* collections over monthly intervals and collection in any month was genetically distinct from all previous monthly collection. The author suggested that this might be due to the migration of populations from different locations.

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Table 1. List and code of collecting sites of *Helicoverpa armigera* on tomato.

Province	**Population**	**code**
Ardabil	Mogan	MT
Golestan	Gorgan	GT
Kermanshah	Kermanshah	KT
East Azarbyjan	Shabestar	SBT
West Azarbyjan	Shahindej	SHT

Table 2. SSR markers utilized in the study, no. of markers generated for populations collected.

Primer	Sequence (5′ – 3′)	Repeat type	Number of markers generated	Polymorphism(%)
HaC14	TCCACACAGTTTGCATTATGA CGCCATAATCCTATTGATTC	(attt)5	5	80
HaD47	TCAAACACACATACTTGACTA TCCAGCAGTGGAATGCGA	(ca)5(tca)4	5	100
HaC87	ACGCGAGCACCAACTGTAA GAGACCAATAGCAGTAGTTC	(tc)5	3	100
HaSSR1	TAGGTGATTGTGGCTCAGTTTT CAAACCCATCAGCAAATGCAAC	(ttgc)2gat(tgy)4gat(tgy)35 (tga)2agc(tgy)8	14	75
HaSSR4	TGTTACTTGGGTTTCCTGAATA ACCACCGACACGTGCCGACTTC	(gyt)25	3	100
HaSSR5	GATAAGTTATTTCGGTTTAGTATT AAGTACCTAATCCGTTTTTATTC	[t(t)aa]6	4	50
HaSSR6	CATAGGAAGTGGTGAAGGGT CACATTCGTCTTTCATCGAC	(tttga)19	6	100
HaSSR7	ACGTCGATGAAAGACGAATGA AAGCTGGTCTGTGCTGCCAT	(taaa)2(taaat)4	4	66.67
HaSSR9	TAGTCTGGGAATTTTGTCTGGTGT CGTGCCATTGAAATAGTAAGCCAT	(t)n(g)n	8	66.67
HaSSR10	TAAGTATGCCCTCGACTGTCGT CACTTTCCAATTAGCCTCGATGCT	(gat)2tt(gat)2tt... .....(aata)5	6	83.3

Table 3. AMOVA of 5 tested geographic populations of *Helicoverpa armigera* using 10 SSR primers.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among Populations	**3**	**134.821**	**5.63265Va**	**50.54**
Within populations	**24**	**132.286**	**5.51190Vb**	**49.46**
Total	**27**	**267.107**	**11.14456**	

Fixation Index FST : 0.50542

Table 4: Population pair wise FST of 5 tested geographic population comparisons using 10 SSR primers.

	GT	MT	SHT	SBT	KT
GT	**0.00000**				
MT	**0.21853**	**0.00000**			
SHT	**0.16852**	**0.05827**	**0.00000**		
SBT	**0.13958**	**0.14425**	**0.11072**	**0.00000**	
KT	**0.15175**	**0.16703**	**0.05789**	**0.16315**	**0.00000**

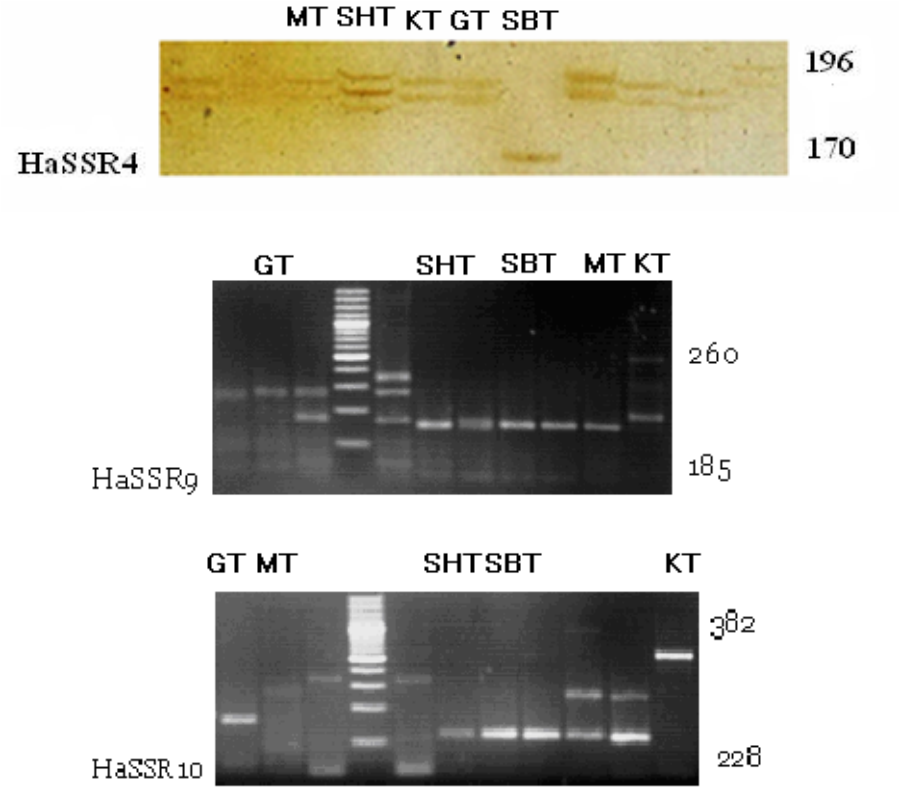

Figure 1. Simple sequence repeat (SSR) fragments from *Helicoverpa armigera* obtained from different geographical places.

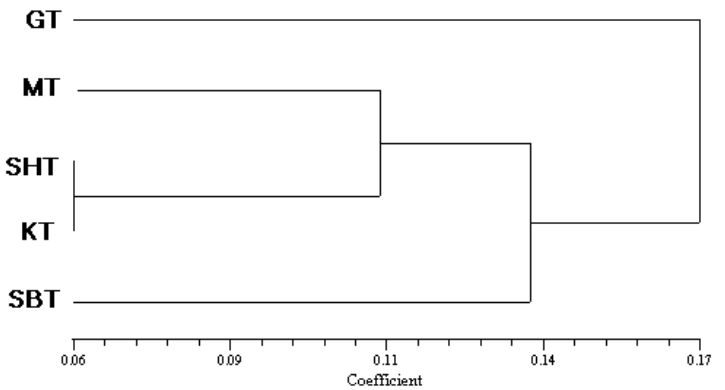

Figure 2. Dendrogram deduced from matrix of pairwise distances in SSR analysis between five populations of *Helicoverpa armigera* using the un-weighted pair-group method analysis, UPGMA.