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Molecular Diversity of Cotton Bollworm (*Helicoverpa armigera* Hübner) Using Rapd Markers

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Abstract: The bollworm, *Helicoverpa armigera* Hübner (Lepidoptera : Noctuidae) is a ploy phagous pest of worldwide occurrence inflicting annual crop damage in India US dollar 1 billion. In India this insect occurs as a major pest in many economically important crops, including cotton, pigeoonpea, chickpea, tomato, okra and blackgram. Understanding the genetic variation among the *H. armigera* populations occurring on cotton plants has become essential to understand the variation in their susceptibility to different insecticides, including *Bacillus thuringiensis*. In this preliminary study diversity among 5 cotton bollworm, *Helicoverpa armigera* (Hübner) populations from different geographic regions of North Karnataka, Indian state was done using RAPD markers. Nineteen selected RAPD markers generated a total of 58 PCR amplicons, of which 26 were polymorphic across all 5 populations. An average of 6.44 amplicons per primer was noted. All populations could be differentiated from one another using specific primers; specific band(s) could be potentially used to differentiate individual populations. Genetic relationships between the populations were evaluated by generating a similarity matrix using NTYSS PC2 package and a phenetic dendrogram was generated by UPGMA method. Principal component analysis separated the 5 populations into different groups based on band sharing data. Populations showed varied degrees of genetic similarity within a range of 0.84 to.00. Also, the populations appeared to be more dispersed on the principal component plot indicating a wide genetic base. On a larger scale, genetic differences among populations appear to result from low dispersal rates between populations. The level of genetic variation detected between the *H. armigera* populations with RAPD–PCR analysis suggests that it is an efficient marker technology for delineating genetic relationships amongst populations and estimating genetic diversity, thereby gaining insight into genetic structure of populations and its further use in formulation of appropriate area wide management strategies for this pest.

Key words: *Helicoverpa armigera* · RAPD markers · Cotton bollworm · Karnataka

INTRODUCTION

Assessing the nature of molecular diversity, extent and spread is essential for understanding the behaviour, response to selection pressure, structure and dynamics of different insect populations and their management. Availability of reliable polymorphic markers often restricts the exact estimation of molecular diversity between individuals or different populations. Elucidation of molecular diversity in geographical populations can be an important aspect to study the pest populations and their management [1, 2, 3,]. Within an ecosystem, the extent of molecular diversity between geographical populations depends on many factors including gene flow between populations, host range and time since separation [3]. Molecular differences within and between geographic populations of an ecosystem are likely to be defined by the population fluxing patterns as influenced by various ecological factors in the immediate past and the historical pressures on the genome [4, 5].

PCR-RAPD approach has been handy and convenient technique for studies of molecular diversity and genome mapping [6] when compared to RFLP markers. Being simple and non radioactive, the technique is quite sensitive and used to detect molecular diversity in many organisms [7, 8].

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Cotton bollworm, *Helicoverpa armigera* (Hübner) is a key pest of cotton and other crops in India and elsewhere in general and in particular in north Karnataka cotton growing area resulting in huge crop loss regularly. Looking at its versatility in rapidly evolving resistance to almost all classes of insecticide molecules and its ability to thrive on several hosts, there must be a strong molecular basis regulating the behaviour of *H. armigera* in making it a serious pest on several crops [9]. Hence, the understanding of molecular diversity within and between geographical populations of *H. armigera* in the north Karnataka cotton growing areas would help in identifying the causes for outbreaks and versatility in evolving resistance to insecticides at a faster rate [10].

MATERIAL AND METHODS

Cotton bollworms were collected during peak incidence from each of the five locations of the North Karnataka cotton growing areas: Dharwad (September), Haveri (September), Belgaum (September), Bijapur (October) and Raichur (October) (Fig. 1). About 20 larvae for each location were randomly picked for isolation of genomic DNA separately. The larvae were desensitized using formalin swab, each larva was dissected and the gut contents were completely removed to avoid any contamination of plant DNA and resulting skin and legs were used to prepare genomic DNA following modified CTAB method. DNA was further purified by phenol-chloroform treatment. In order to make a better representation of each location, equal amount of DNA from each of 20 larvae for each location was pooled and the resulting 5 bulked DNA samples were used for PCR-RAPD analysis. Bulked DNA was diluted to 20 to 40 ng/µl before used in PCR reactions. A set of 19 random decamer primers randomly selected from 'A', 'D', 'G', 'H', 'T', 'L' and 'M' commercial kits obtained from Operon Technologies Inc. (Alamedas, USA) were used. PCR was carried out for each primer in 25 µl mixture consisting of 10 ng of template DNA, 0.2 mM of each primer, 3.5 mM $MgCl_2$, 1X Bangalore Genie buffer, 0.6 U *Taq* polymerase (Bangalore Genie Pvt Ltd) and 200 mM of dNTPase (Bangalore Genie Pvt Ltd). PCR amplification was carried out on an Eppendorf Master Gradient Cycler (Eppendorf AG. Germany) in 2 min initial denaturation at 94°C followed by 40 cycles of 1 min at 94°C, 1 min at 37°C, 2 min at 72°C for extension and ended with a final 10 min extension at 72°C. These reaction products were kept overnight at 4°C prior to electrophoresis on 1.2% agarose gels, which resolved DNA fragments ranging from 100 bp to 3 kb. All the 19 primers were tested at least twice for the reproducibility of banding pattern. Of the 19 primers(obtained from Operon Technologies Inc. Alamedas, USA) ,nine showed amplification (AC-4, AC-10, AT-5, AT-21, G-10, G-11, H-11, A-14 and L-12) in the present study. All the bands, in the range of resolution were scored, except for very faint and ghost bands. The gel pictures acquired through a U.V. gel documentation system (Uvitec. Cambridge, England) fed to computer were processed and scored to get binary data.

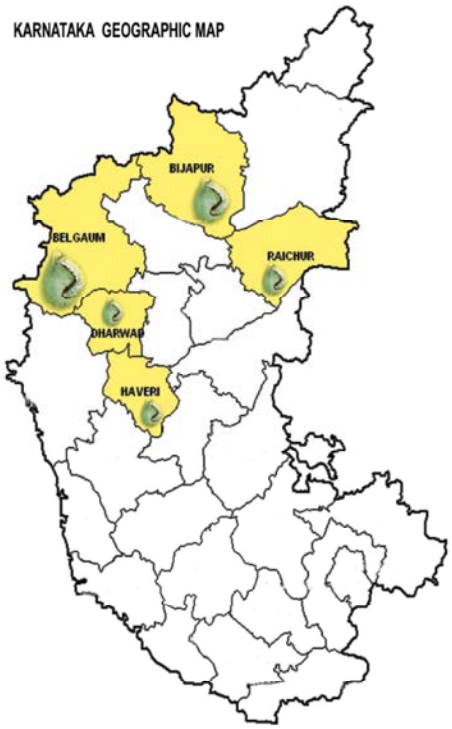

Fig. 1: Geographical distribution of cotton bollworm populations in North Karnataka cotton growing areas used in the present investigation

The presence / absence data (1, 0) matrix was analyzed using the standard procedure in NTYSS PC2 package. Similarity matrix was computed for each individual population and used to generate UPGMA dendrogram (Unweighed pair group method with arithmetic average) using the software NTSYSpc version 2.0 [11].

RESULTS AND DISCUSSION

RAPD profiles of all the five geographical populations of cotton bollworm (*H. armigera*) with respect to each of the 19 random primers revealed that, of the 19 primers, nine showed amplification (Table 2 & 3).

Table 1: Genetic distance (%) based on RAPDs pooled over nine primers in five geographical populations of cotton bollworm (*H. armigera*)

	Dharwad	Haveri	Belgaum	Bijapur	Raichur
Dharwad	00.0				
Haveri	00.0	00.0			
Belgaum	00.0	00.0	00.0		
Bijapur	17.0	17.0	17.0	00.0	
Raichur	11.3	11.3	11.3	25.8	00.0

Table 2: List of random decamer primers used for RAPD

Sl. No.	Primer	Sequence (5' x 3')
1.	AC-4	ACGGGACCTG
2.	AC-10	AFCAGCGAGG
3.	AT-5	ACACCTGCCA
4.	AT-21	ACATCAGCCC
5.	DH-2	GTAAGCCGAG
6.	G-9	CTGACCTCAC
7.	G-10	AGGGCCGTCT
8.	G-11	TGCCCGTCGT
9.	H-1	GGTCGGAGAA
10.	H-8	GAAACACCCC
11.	H-11	CTTCCGCAGT
12.	H-12	ACGCGCATGT
13.	H-14	ACCAGGTTGG
14.	I-18	TTTGCCCGGT
15.	L-11	ACGATGAGCC
16.	L-12	GGGCGGTACT
17.	L-13	ACCGCCTGCT
18.	L-14	GTGACAGGCT
19.	M-3	GGGGGATGAG

Table 3: Random primers with sequences which have resulted in polymorphism in cotton bollworm (*H. armigera*)

Primer	Sequence (5' to 3')
AC-4	ACGGGACCTG
AC-10	ATCAGCGAGG
AT-5	ACACCTGCCA
AT-21	ACATCAGCCC
G-10	AGGGCCGTCT
G-11	TGCCCGTCGT
H-11	CTTCCGCAGT
H-14	ACCAGGTTGG
L-12	GGGCGGTACT

Table 4: Summary statistics of RAPD analysis for cotton bollworm (*H. armigera*)

Sl. No.	Primer	Total number of bands	Total number of polymorphic bands	Per cent polymorphism
1	AC-4	7	5	71.43
2	AC-10	6	1	16.67
3	AT-5	6	2	33.33
4	AT-21	5	2	40.00
5	G-10	8	3	37.50
6	G-11	7	4	57.14
7	H-11	5	2	40.00
8	H-14	5	1	20.00
9	L-12	9	6	66.67
	Total	58	26	382.74
	Average	6.44	2.89	42.53

The RAPD profiles of five geographical populations of cotton bollworm (*H. armigera*) were compared individually for each primer and were used for further analysis. The RAPD profiles of five geographical populations of cotton bollworm (*H. armigera*) were separately compared to find out the differences among them by the occurrence of polymorphic bands. In total, 58 scorable bands were produced in five geographical populations of cotton bollworm (*H.armigera*) with 9 primers (Table 4). This data was utilized for further computations. The number of bands produced per primer varied from one for AC-10 and H-14 to as many as 6 for L-12. The Mean number of bands per primer was 6.44. Out of 58 bands, 26 were polymorphic (42.53 %). The Mean number of polymorphic RAPD bands was 2.89 per primer. Primers AC-4 and L-12 produced 71.43 and 66.67 per cent polymorphism, respectively. Other primers namely AC-10, AT-5, AT-21, G-10, G-11, H-11 and H-14 produced 16.67, 33.33, 40.00, 37.50, 57.14, 40.00 and 20.00 per cent polymorphism, respectively.

The genetic distance was computed considering all the five geographical populations and dendrogram was constructed. The distance matrix is presented in Table 1. The maximum genetic distance of 25.8 per cent was observed between Raichur and Bijapur populations. The least genetic distance of zero per cent was found between Dharwad and Haveri populations, Dharwad and Belgaum populations and Haveri and Belgaum populations. The dendrogram for pooled data (Fig. 3) showed two major clusters. All the geographical populations of cotton bollworm (*H.armigera*) were found in one cluster except Bijapur population, which was found in the other cluster. The geographic populations which were found in one cluster were further divided into two sub clusters. Dharwad, Haveri and Belgaum populations were found in one sub cluster and Raichur population was found in another sub cluster.

The values of discrimination power (%) calculated for each primer are presented in Table 4. The values showed that the primers AC-4, G-10, G-11 and L-12 had discrimination power of 40 per cent. These primers produced distinct RAPD banding patterns for two geographical populations. Primers AC-10, AT-5, AT-21, H-11 and H-14 had a discrimination power of 20 per cent. These primers produced distinct patterns for only one population and the RAPD profiles for remaining four populations were identical.

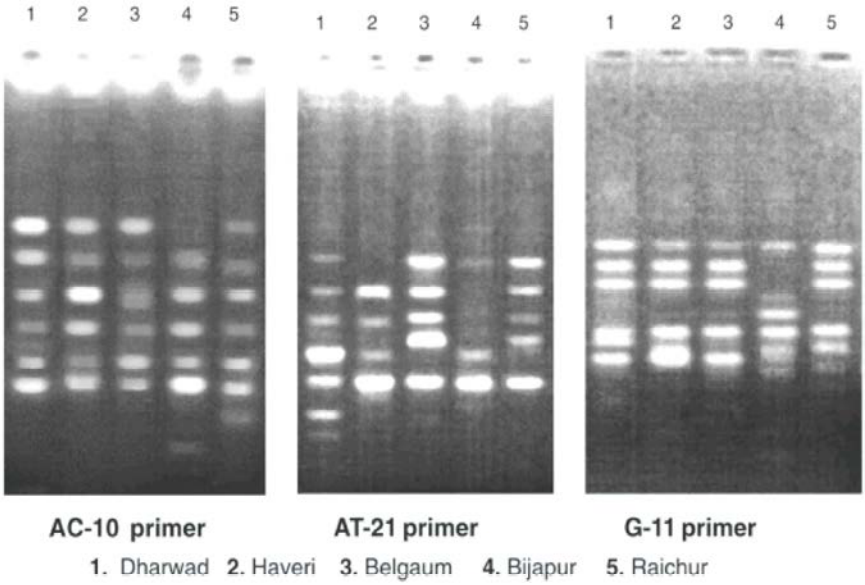

Fig. 2: PCR amplified genomic DNA of cotton bollworm using AC-10, AT-21 and G-11 random primer

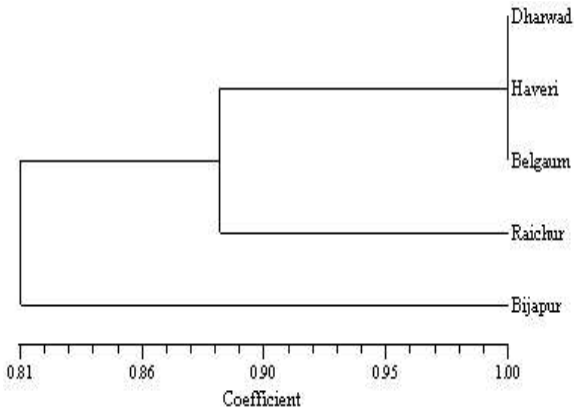

Fig. 3: Dendrogram showing diversity of geographical populations of *H. armigera* in North Karnataka cotton growing areas

Selected gels which were showing distinctness were presented in this paper. Hence, gels resulting from AC-10, AT-21 and G-11 random primers across individually pooled genomic DNA of all the five geographical populations were presented in Fig. (2). A total of 58 amplicon products resulting for nine primers were available for analysis. On an average, there were 6.44 amplicon levels per primer, of which 26 were polymorphic, indicating variability among *H. armigera* populations. All the nine primers selected for the study produced unique banding patterns that could differentiate all the five geographical populations. The polymorphism revealed by RAPD serves as dominant Mendel' Ian marker. Since heterozygotes are not normally detectable, results are not readily usable for computing Hardy-Weinberg gene frequencies. Hence in the present study, RAPD polymorphisms were analyzed to construct dendrogram, providing an indication of the diversity present within *H. armigera* in north Karnataka. Dendrogram clearly showed two major groups (Fig. 3). The first group comprised four populations while the distinct second group consisted of only Bijapur population. The first group is subdivided into two whereas Raichur is distinct from Dharwad, Haveri and Belgaum.

Clustering of the five geographic populations of *H. armigera* with 19 primers clearly indicated that a population from Bijapur formed a totally different cluster to be diverse from other populations with a genetic distance of 25.8% from Raichur population and with a genetic distance of 17% from Dharwad, Haveri and Belgaum populations (Table 1). This may be attributed to the fact that Bijapur was isolated from the rest by a vast stretch of dry land having low intensity cropping pattern with cotton/legumes. It is observed that the population from Dharwad, Haveri and Belgaum were similar having genetic distance of zero per cent indicating large scale migration and inbreeding of *H. armigera* resulting in larger genetic dilution and low genetic differentiation. The RAPD analysis of Turkish and Israeli populations of *H. armigera* revealed low level of genetic distance suggesting high level of gene flow [12, 13] which is in line with the present study in north Karnataka cotton ecosystem. Present/absence of topological barriers due to weather / environmental factors and temporal barriers due

to cropping pattern may play a key role in either high or low amount of genetic variability among geographical populations in north Karnataka cotton ecosystem. *H. armigera* being migrative in nature would result in high genetic variability within a population, when separated. Higher the genetic variability, faster is the rate of species evolution and adaption to different environments resulting in rapid development of resistance to insecticides or *vice-versa*.

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