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Genetic Diversity of Chickpea Pod Borer, *Helicoverpa armigera* (Hubner)

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A B S T R A C T

Helicoverpa armigera (Hübner) is a polyphagous and cosmopolitan pest inflicting annual crop losses worth US $ 1 billion. Here, we aimed at studying the genetic diversity of pod borer using random primers. Thirteen random primers generated a total of total of 150 amplicons with an average of 11.53 bands per primer. There were a total of 138 polymorphic bands (average of 10.61 bands per primer) with polymorphism ranging from 66.6 to 100%. The similarity coefficient values ranged from 0.13 to 0.42. UPGMA based dendrogram revealed the presence of two principal clusters which were further subdivided into 12 sub-clusters. The most striking inference of our study was that larval populations collected in the presence of multiple hosts were hyper diverse forming a separate cluster clearly distinct from populations collected on sole chickpea.

Introduction

Helicoverpa armigera (Hübner) (Noctuideae: Lepidoptera) is a serious pest responsible for US$1 billion annual crop losses in India alone (Chandrashekar and Gujar, 2004). In India the pest is known to thrive on 182 species of host plants belonging to 47 families (Pawar, 1998). Differences in ecological behaviour of the pest are known to exist in populations collected from diverse hosts and geographic locations. Presence of strong genetic variability in the pest governing its behaviour makes it a serious pest on several crops (Scott *et al.,* 2005). Studies on genetic diversity of the pest provides a better insight in understanding the structure, behaviour and population dynamics of the pest, its response to selection pressure and management strategies.

Among the wide range of DNA marker techniques available for genetic analysis, RAPD-PCR is a simple approach to unravel the genetic structure of the organisms. It is

cheaper, rapid, non-radioactive, requiring only a small amount of DNA and does not require any prior knowledge of DNA sequence (Moorthy *et al.*, 2013). The added advantage of RAPD method is its ability to generate polymorphisms and genome analysis of any organism employing a universal set of primers (Jain *et al.*, 2010). In view of above aspects the present study was initiated to assess RAPD-based genetic diversity of *H. armigera* collected from chickpea.

Materials and Methods

The present study was conducted during *rabi* summer, 2017-18 at the College of Agriculture, Vijayapura (sample collection) and Microbial Genetics Laboratory, Department of Agricultural Microbiology, College of Agriculture, Dharwad. *Helicoverpa* larvae were collected weekly from experimental fields of chickpea, College of Agriculture, Vijayapura. Collections were continued for six weeks and larvae were preserved in 70% ethyl alcohol at -20 ºC for further analysis. Genomic DNA of the field collected larvae was isolated using CTAB (Cetyl Trimethyl Ammonium Bromide) method with slight modifications (Subramanian and Mohankumar, 2006). The quantification of genomic DNA was done using Nano Drop ND-1000 spectrophotometer and visualised on 0.8 % agarose with 1 ml of 0.5X TAE buffer and 5 µl of ethidium bromide and compared with a reference of 50 bp DNA ladder (and 1 kb ladder wherever necessary). OD260/280 was employed to assess the purity of DNA. A ratio less than 1.7 indicates protein contamination, whereas ratio greater than 1.8 indicates RNA contamination.

Thirteen primers (Table 1) were considered based on the band pattern repeatability. The PCR mixture consisted of 2 µl of template DNA, 0.3 µl Taq polymerase, 1 µl of Taq buffer, 1 µl of dNTPs and 5.7 µl of nuclease free water (M/S Bangalore Genei Pvt. Ltd., Bangalore and Fermentas Company, Bangalore). The PCR amplification was carried out in Eppendorf Master Cycler gradient (Hamburg, Germany and these reaction products were kept at 4 ºC until further use. RAPD-PCR products were then analysed on 2% agarose with 2 ml of 0.5X TAE buffer and 10 µl of ethidium bromide with 50 bp ladder as a reference (1 kb ladder wherever necessary). After electrophoresis, the gel was visualised on a UV trans-illuminator and photographed with gel documentation unit for further analysis. Amplification profiles for all the primers were observed carefully and the individual bands were scored as 1 or 0 based on presence or absence of bands with reference to the DNA ladder generating the 0, 1 matrices. The scored data matrix was subjected to analysis using the standard procedure in DARwin 6.0 CIRAD package. A dendrogram was constructed after the cluster analysis of similarity coefficients by using pair-group method (UPGMA) analysis using in DARwin 6.0 CIRAD.

Results and Discussion

A total of 150 amplicon levels (average of 11.53 bands per primer) with 138 polymorphic bands (average of 10.61 bands per primer) were generated with polymorphism ranging from 66.6 to 100% (Table 1). The amplicon size ranged from 100 bp to more than 1000 bp.

The gel electrophoresis profile of *H. armigera* larvae using OPB-13 and OPAT-3 primers is shown in Fig. 1. Even a small sample size of *H. armigera* collected at different time intervals in the present study provided a great insight into the variations in the population. Earlier studies (Rahman *et al.*, 2014; Yenagi *et al.*, 2012) documenting high degree of polymorphisms generated by RAPD markers involved extensive sampling from different host plants and geographical locations.

The highest number of amplicons were produced by OPF-3 primer (19 amplicons) followed by OPB-13 (18 amplicons). The lowest numbers of amplicon levels are produced by OPA-15 primer (3 amplicons). The data matrix prepared by visual observation for presence (1) or absence (0) of bands for each primer was used to construct genetic similarity distance table (DARwin 6.0 CIRAD-similarity coefficient). The similarity coefficient values ranged from 0.13 (between tenth and eleventh samples) to 0.42 (first and fourteenth sample) (Table 2).

The RAPD polymorphisms were analysed with the help of similarity coefficient values from which an Unweighted Paired Group Method with Arithmetic Mean (UPGMA) based dendrogram was constructed. The dendrogram (Fig. 2) revealed the presence of two principal clusters X and Y where the cluster Y is composed of only two samples (1 and 3) which belong to the first week collection. The principal cluster X is further divided into two clusters i.e., X_1 and X_2 at similarity coefficient of 0.015. Cluster X_1 includes seven samples (sample no. 2, 4, 5, 6, 7, 8, 9) which belong to third and fourth week collection except second sample which belongs to first week. Cluster X_2 included all the remaining seven samples (10, 11-week 4; 12, 13-week 5; 14, 15, 15- week 6).

Table.1 Detailed summary of RAPD-PCR analysis

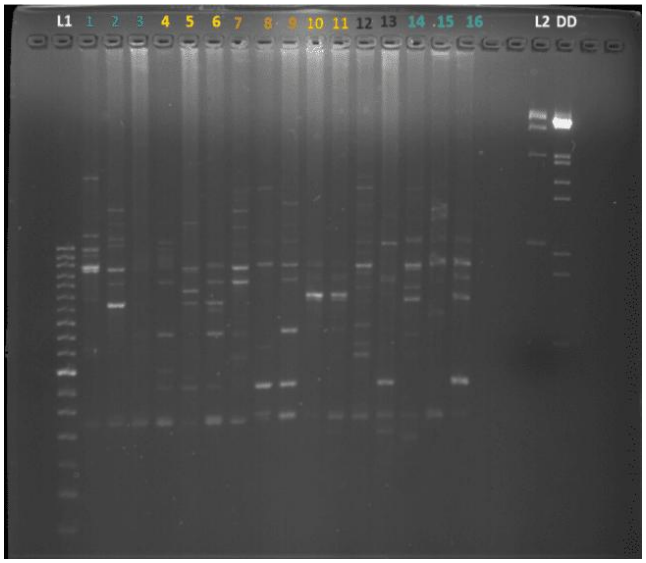
Sl. No.	OP series	Primer sequence (5´→ 3´)	Total no. of amplified bands	Total no. of polymorphic bands	Per cent polymorphism	T_m
1.	A-15	TTCCGAACCC	3	3	100	35 ºC
2.	A-16	AGCCAGCGAA	12	9	75	37 ºC
3.	A-20	GTTGCGATCC	11	11	100	38 ºC
4.	B-12	CCTTGACGCT	11	11	100	38 ºC
5.	B-13	TTCGCTCGCT	18	18	100	35 ºC
6.	C-9	CTCACCGTCC	7	6	85.7	36 ºC
7.	F-03	CCAAGCTTCC	19	19	100	35.5 ºC
8.	F-04	GGTGATCAGG	12	11	91.6	35.5 ºC
9.	K-18	CCTAGTCGAG	7	6	85.7	36 ºC
10.	AT-01	CAGTGGTTCC	15	14	93.3	36 ºC
11.	AT-03	GACTGGGAGG	14	14	100	36 ºC
12.	AT-04	TTGCCTCGCC	12	8	66.6	36 ºC
13.	AT-08	TCCTCGTGGG	9	8	88.8	36 ºC
	Total		150	138	1186.7	
	Average		11.53	10.61	91.28	

Table.2 Similarity distance matrix table for collected samples of *Helicoverpa armigera* larvae

Week	Sample no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1	0														
	2	0.35														
	3	0.29	0.32													
2	4	0.32	0.21	0.28												
	5	0.37	0.31	0.34	0.27											
	6	0.36	0.30	0.32	0.26	0.25										
3	7	0.35	0.30	0.31	0.26	0.32	0.30									
	8	0.38	0.32	0.34	0.28	0.30	0.28	0.32								
	9	0.35	0.29	0.32	0.26	0.27	0.26	0.30	0.24							
4	10	0.33	0.27	0.30	0.24	0.25	0.24	0.28	0.25	0.22						
	11	0.33	0.27	0.30	0.24	0.25	0.24	0.28	0.25	0.22	0.13					
5	12	0.38	0.32	0.35	0.28	0.30	0.29	0.33	0.29	0.27	0.20	0.20				
	13	0.41	0.34	0.37	0.31	0.32	0.31	0.35	0.32	0.29	0.23	0.23	0.26			
6	14	0.42	0.36	0.38	0.32	0.34	0.32	0.36	0.33	0.30	0.24	0.24	0.26	0.30		
	15	0.41	0.35	0.38	0.31	0.33	0.32	0.36	0.32	0.30	0.24	0.24	0.25	0.29	0.25	
	16	0.36	0.30	0.33	0.27	0.28	0.27	0.31	0.28	0.25	0.19	0.19	0.22	0.23	0.25	0.25

Fig.1 Gel electrophoresis profile of collected samples of *Helicoverpa armigera* larvae using OPB-13 and OPAT-3 primers. Sample 1, 2 and 3: Week 1; Sample 4, 5, 6: Week 2; Sample 7, 8, 9: Week 3; Sample 10, 11: Week 4; Sample 12, 13: Week 5; Sample 14, 15, 16: Week 6; L and L_1: 50 bp ladder; L_2: 1kb ladder, DD: Double digest

OPB-13



OPAT-3

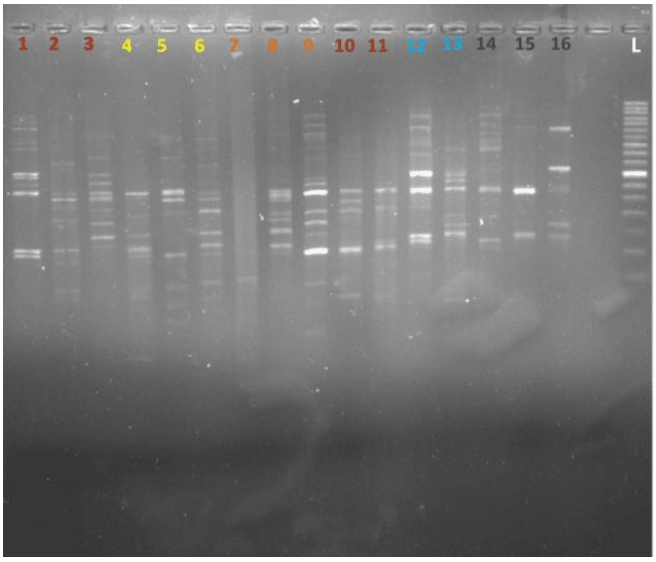
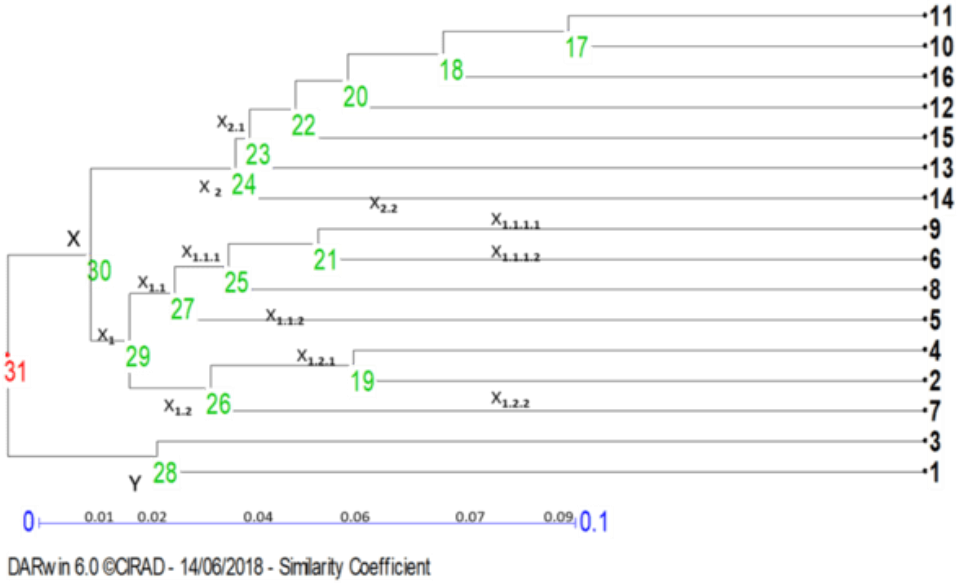


Fig.2 Dendrogram showing genetic relatedness of *Helicoverpa armigera* collected at different time intervals. Sample 1, 2, 3: Week 1; Sample 4, 5, 6: Week 2; Sample 7, 8, 9: Week 3; Sample 10, 11: Week 4; Sample 12, 13: Week 5; Sample 14, 15, 16: Week 6



Closer genetic structure in Y and X_1 separated from X_2 was due to the fact that populations in cluster Y and X_1 were sampled during the first three weeks where alternate hosts like pigeon pea, cotton etc. were available in the vicinity. It is possible that the pod borer being highly polyphagous might have migrated from hosts other than chickpea (pigeon pea or cotton) where they fed on them resulting in genetic structure of cluster X_1 and Y being different from that of X_2.

The hypothesis is that as the pest doesn't undergo any diapause, it will be continuously searching for a suitable host for its survival. This phenomenon is supported by the fact that the pest is well known for its long distance high migratory potential (Farrow, 1994; Pedgley, 1985). It has been reported that this pest can travel more than 2000 km (Nibouche *et al.,* 1998).

Lesser genetic variation in X_2 (in contrary to greater variation in populations of X_1 and Y) might be due to feeding on sole chickpea and also due to interbreeding among the populations of the X_2 and Y that caused the gene flow reducing the variability between them. The present results are in line with previous studies reporting the influence of host plants on the variability of pod borer (Subramanian and Mohankumar, 2004; Firempong and Zalucki, 1990). The greatest variation observed between the samples of *H. armigera* (1 and 14) can be explained by the possibility of migration from different geographic locations or from different host plants and also by different sampling periods. The present study also confirms the earlier reports (Scott *et al.,* 2005) on genetic shifts in *H. armigera* over monthly intervals.

Studying the genetic structure of *Helicoverpa* helps us in developing better management strategies to control the pest as high genetic variability results in faster development of resistance to insecticides, greater speciation as pest population varies in their susceptibility to various insecticides.

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