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Genetic Diversity in the Vidharbha Region's Pink Bollworm *Pectinophora gossypiella* (Saunders) Adults Populations

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ABSTRACT: Pink bollworm is the most damaging and ubiquitous cotton pest. Recently "survival of pink bollworm" on bt cotton and causing up to 70-80% losses was reported. Genetic diversity is the result of recombination of genetic material during the inheritance process it shifts over time and space. Individuals' physical characteristics and ability to adapt to stress, diseases, and unfavourable environmental conditions are influenced by genetic diversity. Natural or anthropogenic environmental changes result in natural selection and survival of the fittest. As a result of genetic diversity, susceptible varieties die while those that can adapt to changes survive. In order to understand the variation at genetic level, present experiment "Molecular characterization of pink bollworm adults" has done at Department of Agricultural Entomology and Botany, Centre of Excellence in Plant Biotechnology, Dr PDKV Akola. During the study total of eight primers *i.e.* seven SSR and one ISSR were used for molecular characterization of pink bollworm adults, collected from different locations viz., Akola, Amravati, Washim, Nagpur, Yavatmal and Wardha of Vidarbha region. Results revealed that a total of 56 amplicons were produced from the molecular characterization of adult pink bollworms with seven SSR and an ISSR primer, of which 31 (55.35%) are polymorphic bands, showing substantial genetic variability in the *Pectinophora gossypiella* population. The pink bollworm samples were grouped into four major clusters the first group is named as cluster-A, which includes samples belonging to Washim, Nagpur and Yavatmal. Second group is named cluster-B, which includes sample belonging to Amravati. Third group is named cluster-C, which includes samples belonging to Wardha and the fourth group is named cluster-D includes sample belonging to Akola. In the phylogenetic tree, the Washim sample had a higher similarity coefficient (0.774), whereas the Akola sample had a lower similarity coefficient (0.354). It reveals that the Washim sample is highly similar to the Nagpur sample, whereas the Akola sample is the least similar to the Washim and Nagpur samples.

Keywords: Pink bollworm, SSR and ISSR Markers, Molecular characterization, Genetic polymorphism.

INTRODUCTION

Cotton is India's most important fibre and cash crop, and it is vital to the agricultural and industrial economies. It is utilised as basic raw material in the cotton textile industry, providing a direct income to approximately 6 million farmers and 40- 50 million people engage in the cotton trade.Many insect pests and pathogens harm cotton crops, reducing seed cotton production and lowering fibre quality. Globally, approximately 1326 insect species are recorded in cotton, of which approximately 162 species invade at various stages of crop growth, of which approximately 15 are major pests (Kannan *et al.,* 2004). Pink bollworm *Pectinophora gossypiella* (Saunders) activity has ramped up among cotton bollworms. It has emerged as one of the most damaging and dangerous cotton pests, producing locule damage to the tune of 55% and a drop in seed cotton production ranging from 35% to 90% (Naik *et al.,* 2011). The projected cost of the losses caused by this pest was 6525 metric tonnes of lint, worth of Rs. 1216 million (Agarwal and Katiyar 1979). Around the world, it is known to result in losses of 2.8 to 61.9% in seed cotton yield, 2.1 to 47.1% in oil content, and 10.7 to 59.2% in normal boll opening (Patil, 2003). The pink bollworm has returned seeking retribution. About 30 years ago, this pest was a significant concern for cotton in India. There have been very few reports of the pink bollworm causing substantial cotton damage in the nation since 1982. But suddenly everything changed. This year, large areas of Gujarat as well as parts of Andhra Pradesh, Telangana, and Maharashtra reported serious bollworm damage and yield losses in Bt cotton. Bollgard-II-Bt-cotton, which has two genes (cry1Ac+cry2Ab) that were meant to be very successful in controlling the pest, is being cheerfully chewed up by the pink bollworm, which is alarming (Kranthi, 2015). The pest originally appeared on Bt cotton occasionally in 2010, but by the 2015-16 season, vast tracts of the cotton crop were damaged,

lowering output by an estimated 7- 8%. According to surveys conducted by the state revenue and agriculture departments in November 2017 and February–March 2018, almost 80% of the 4.2 million hectares of cotton planted in Maharashtra were infested with pink bollworm. Each farmer reportedly lost between 33% and over 50% of the standing crop. The Maharashtra Department of Agriculture forecast a 40% decrease in cotton production and bales in January 2018.The present study molecular characterization of pink bollworm adult moths with SSR and ISSR molecular markers was conducted to know the present status, understand variation and design a breeding program for resistance management.

MATERIALS AND METHODS

The pink bollworm infested mature green bolls were collected at random from the farmer's cotton fields of Akola, Amravati, Washim, Nagpur, Yavatmal and Wardha districts/ locations, during February- March 2021, representing all the major cotton growing zones of Vidarbha region of Maharashtra (India). Infested cotton bolls were cut opened, larvae were taken out and reared to adult moths in the laboratory condition and these moths were used for genomic DNA extraction. The Cetyl trimethyl ammonium bromide (CTAB) technique reported by Murray and Thompson (1980) was adopted (with some modifications) to extract genomic DNA. It included all the necessary reagents, apparatus, software, and tools (Table 1). 2 ul of 10x PCR buffer, 0.5 ul of dNTPs, 1ul of genomic DNA, 0.3 ul of Taq DNA polymerase (M/S Bangalore Genei Pvt. Ltd., Bangalore), 1 ul of each primer, and 14.2 ul of molecular graded water were used in PCR reactions with a total volume of 20 ul.

Table 1: Different Chemicals and equipment used for the molecular characterization of pink bollworm adults.

Sr. No.	Name of chemicals/ equipment/ software
1. DNA Extraction	
1.	Cetyl trimethyl ammonium bromide(CTAB) (4%)
2.	Chloroform: Isoamyl alcohol (24:1)
3.	Boricacid
4.	ß-mercapto ethanol
5.	Phenol
6.	Ethanol (70%)
7.	Tris-HCl
8.	100% Isopropanol (Ice-cold)
9.	Sodium chloride
10.	Ethyline Diamine Tetra Acetic Disodium Salt (EDTANa2)
11.	Tris-EDTA (TE) buffer (10 mMTris and 1 mM EDTA, pH 8.0)
2. PCR Amplification	
12.	10X buffer
13.	dNTP Mix (10mM)
14.	Taq DNA polymerase
15.	Template DNA
16.	Oligo nucleotide SSR and ISSR primers
17.	Molecular graded water
3. Agarose gel electrophoresis	
18.	Tris buffer
19.	Tris- HCL
20.	Boric acid
21.	EDTA Na_2
22.	Agarose
23.	Ethydium bromide
24.	6X Gel loading dye
25.	DNAladder100 kb
4. Equipment used	
26.	Mortar and pestle
27.	Micropipettes
28.	Refrigerated centrifuges (Beckman GPR, Beckman G2-21)
29.	Centrifuge tubes 50 ml
30.	Micro centrifuge tubes 1.5 ml
31.	Thermostat 37°C
31.	Water bath 60°C
32.	- 20° C freezer
33.	PCR tube (Tarson make)
34.	PCR machine (Bio-rad)
35.	Gel electrophoresis unit
36.	Conical flask
37.	Autoclave
38.	Electronic balance
39.	Centrifuge (Eppendorf)
40.	Microwave oven
41.	Gel documentation unit
5. Software's used	
42.	NTSYS2.02

The samples were mixed by centrifugation to bring down the content of the tube. The PCR was programmed with an initial denaturation at 94°C for 3 minutes, followed by 39 cycles of denaturation at 94 °C for 30 seconds, annealing at 50°C to 60°C for 45 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes (the temperature varies depending on the primer). In the gel documentation system, the gel picture was collected and visualized under light. For each sample, the data were scored as the presence (1) or absence (0) of each specific band. Using Jaccard's coefficient, this binary data was used to compute the similarity coefficient. The similarity matrix was used to create the dendrogram using the SAHN (Sequential Agglomerative Hierarchal Nested) cluster analysis module and the Unweighted pair group technique of arithmetic average (UPGMA) (Rohlf, 2000).

Percent polymorphism =

$$\frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

RESULTS AND DISCUSSION

The similarity between genotypes can be predicted using the derived similarity matrix. The genotypes with a similarity index of "1" are considered to be 100% genetically identical, whereas those with a similarity value of "0" are considered to be 100% genetically dissimilar. The similarity coefficient value in the present study ranged from 0.354 to 0.774 among 6 samples of pink bollworm adults, demonstrating a high degree of variance in genetic similarity (Table 3). Genetic similarity (Jaccard's coefficient) was established based on SSR and ISSR banding patterns used for cluster analysis to depict genetic link in the form of a dendrogram shown in Fig. 1. Molecular analysis of pink bollworm adults using seven SSR and an ISSR primer yielded 56 amplicons, 31 (55.35%) of which are polymorphic bands, revealing substantial genetic variation in the *Pectinophora gossypiella* population.

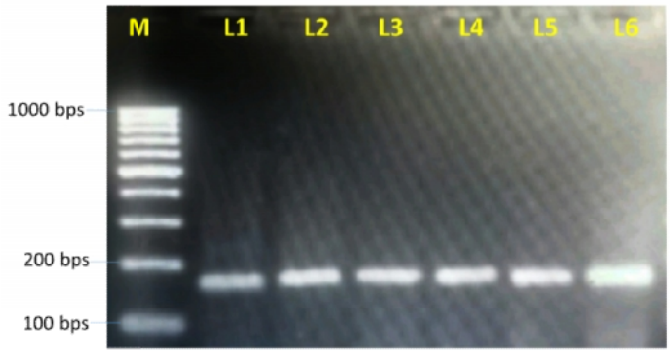
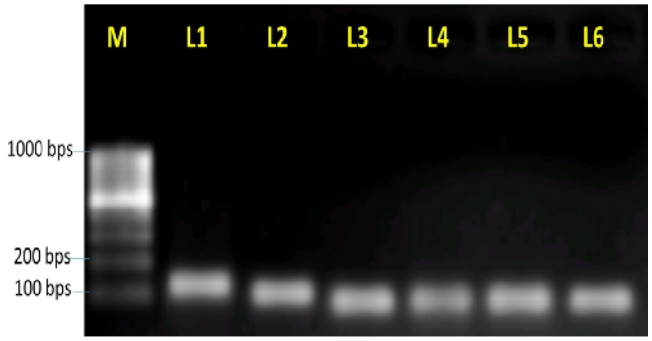


Plate 1: Banding pattern observed in primer SSR PBW-1.



M- Known DNA marker/Ladder
L-1 – Akola location L-2 – Amravati location L-3 – Washim location
L-4 – Yavatmal location L-5 – Nagpur location L-6 – Wardha location

Plate 2: Banding pattern observed in primer SSR PBW-3.

Table 3: Binary similarity matrix for SSR and ISSR analysis.

	AKOLA	AMRAVATI	WASHIM	NAGPUR	YAVATMAL	WARDHA
AKOLA	1.000					
AMRAVATI	0.516	1.000				
WASHIM	0.354	0.645	1.000			
NAGPUR	0.387	0.612	0.774	1.000		
YAVATMAL	0.483	0.580	0.677	0.709	1.000	
WARDHA	0.548	0.387	0.548	0.645	0.677	1.000

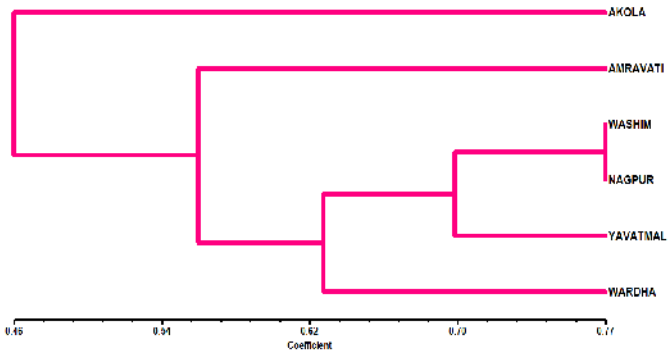


Fig. 1. UPGMA dendrogram based on Jaccard similarity coefficient obtained from SSR and ISSR study of six pink bollworm isolates.

In the dendrogram, the Washim sample had a greater similarity coefficient (0.774), whereas the Akola sample had a lower similarity coefficient (0.354). It reveals that the Washim sample is significantly related to the Nagpur sample, but the Akola sample is the least related to the Washim and Nagpur samples.

The polymorphism information content for SSR primers ranged from 0.727 to 0.935 in Nandini and Mohan's (2016) experiment. Amplification products ranged in size from 112 to 312 bp. Madurai and Srivilliputhur shared the greatest similarity index (0.875). The least similarity index (0.666) was found between Perambular, Salem, and Orissa to Coimbatore, as well as between Orissa and Delhi to Kovilpatti. As a result, the findings of their investigations support the current findings. Thus, the results of their studies have supported the present findings. Shahanaz and Vinay (2019) used RAPD-PCR to evaluate the genetic diversity of *Pectinophora gossypiella* (Saunders) larvae collected from different cotton-growing regions in India. OPI-11 to OPI-20 RAPD decamer primers were utilized, with four (OPI-11-OPI-14) producing amplicons with acceptable resolution and significant variety. A total of 153 amplicons were generated, with 118 (92%) of them being polymorphic bands, demonstrating substantial genetic variability among *Pectinophora gossypiella* populations.

CONCLUSION

Molecular characterization of Vidharbha region's Pink bollworm *Pectinophora gossypiella* (Saunders) adults populations concludes that at the genetic level Washim sample were highly similar to Nagpur sample whereas, the Akola sample where least similar to Washim and Nagpur sample.

FUTURE SCOPE

The current study on the molecular characterization of pink bollworm aids in development of breeding program for its resistance management.

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Conflict of interest. None.

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