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Genetic polymorphism in the field populations of *Pectinophora gossypiella* (Saunders) by using RAPD-PCR

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Abstract
The populations of *Pectinophora gossypiella* obtained from different locations in Tamil Nadu were analyzed by RAPD-PCR. A total of twenty 10-mer oligonucleiotide primers (RAPD Kit A1 to Kit A20) were tested for their ability to provide suitable banding pattern for eight different populations of *P. gossypiella*. Of the primer set tested, RAPD KitA3, A9, A15, A18 and A20 yielded clear, consistent and discrete banding patterns for the populations of *P. gossypiella*. Dendrogram and similarity index were constructed based on the RAPD profiles generated by Kit A3, A9 and A15 primer using UPGMA. The three primers amplified a total of 115 scorable bands in the molecular weight range of approximately 267bp to 1352bp. The number of amplification products obtained was specific to each primer and ranged from thirty (Kit A9) to forty five (Kit A15). The UPGMA based dendrogram of all the primers grouped the populations of *P. gossypiella* in two clusters. The similarity coefficients values were ranged from 0.03 to 0.86, indicating that no two populations were 100% similar. The similarity matrix of all the primers indicated that most of the populations exhibited <50% similarity coefficient.

Keywords: cotton pest, pink bollworm, *Pectinophora gossypiella, RAPD-PCR*, genetic polymorphism

Introduction

The pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Insecta) is the most important cotton pest in the world (Dennehy *et al*., 2004) [7]. It is found in almost every cotton producing country and has caused lot of damage to the cotton bolls. The bollworm adult or moth lays its eggs on cotton bolls. The eggs hatch into larvae and eat the cottonseeds, damage and discolor the fiber. The pink bollworm, *Pectinophora gossypiella* (Saunders), was described by W.W. Saunders in 1843 as *Depressaria gossypiella* from specimens found to be damaging cotton in India in 1842 (Ingram, 1994) [10]. The insect has been taxonomically designated under several other generic names, and the complete synonymy was reported by Common (1958) [6]. The origin of pink bollworm remains unknown but the diversity of parasite species found in Pakistan (Cheema *et al*., 1980) appears to support an Indo-Pakistan origin (Ingram, 1994) [10]. It has also been suggested that its origin occurred in the area of the eastern Indian Ocean bordered on the east by northwestern Australia and on the west by the various islands of Indonesia-Malaysia (Common, 1958) [6].

In India a research team comprised of scientist from the Central Institute of Cotton Research (CICR, Nagpur), the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) at Patancheru, Andra Pradesh studied various geographical strains of major cotton pests, which are as follows: the cotton bollworm (*Helicoverpa armigera*), tobacco catterpiller (*Spodoptera litura*), white fly (*Bemisia tabaci*), pink bollworm (*Pectinophora gossypiella*) and spotted bollworm (*Earias vittella*). The damage caused by these five major cotton pests were calculated as 20 to 80% (Kranthi *et al*., 2005).

The pink bollworm, *Pectinophora gossypiella* is considered to be the most destructive pests of cotton bolls. It is worldwide in distribution and cotton is considered to be the preferred host for the insect. The pest can affect cotton seed and lint yield as quality and quantity (Temerak, 2003). Different kinds of molecular markers can reveal different levels of genetic variation, making population genetics studies possible on a wide range of geographical scales. DNA markers are especially effective tools in making inferences about movement between insect populations, because they present selectively neutral characters (Black *et al*., 2001). Randomly amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams *et al*., 1990) [23, 24] is a polymerase chain reaction (PCR) technique that allows detection of many polymorphisms within the genomic DNA in a short time. RAPD markers are generated by the amplification of random DNA segments with single primers of arbitrary nucleotide sequence. Polymorphisms most of which are inherited as dominant traits, are detected as the presence (or) absence of amplification products from a single locus (Williams *et al*., 1990) [24]. This technique has been used for many systematic and population genetic studies. RAPD-PCR requires very small amount of DNA and can be used with very small insects (Black *et al*., 1992) [3].

The RAPD technique (Williams *et al*., 1990) [24] has been widely used to elucidate the geographical origin of and gene flow among insect populations (Ayres *et al*., 2003) [2]. The properly performed RAPD analysis is a useful and reliable tool for studying the ecology and genetic structuring of populations (Armstrong and Wratten, 1996; Vaughn and

Antolin, 1998; Pearson *et al*., 2002) [1, 22, 18]. Finger printing genomes with arbitrary primers is a versatile method, for detecting genetic polymorphisms useful for genetic mapping, phylogenetics and population biology (McClelland and Welsh, 1995). Most RAPD bands are dominant traits (Rafalski and Tingey, 1993) and their presence reflects priming sites flanking a segment of DNA suitable for amplification (William *et al*., 1990; Black, 1993) [24]. The RAPD-PCR based method has proved to be valuable in identifying large numbers of genetic polymorphisms in several insect species refractory to (or) little used for classical genetic analysis (Haymer, 1994) [9]. This technique is also applied for the identification of species and subspecies of various insects. Scataglini *et al*. (2000) [20] used random amplification of polymorphic DNA (RAPD) markers to analyse genetic structuring and gene flow among populations of *Anthonomus grandis* in South America collected from native plants and cultivated cotton. Kim and Sappington (2004) [12] performed randomly amplified polymorphic DNA analysis to study the magnitude and pattern of genetic differentiation among boll weevil populations from eighteen locations across eight US states and northeast Mexico using.

Polymorphic DNAs are frequently detected in genomic DNAs randomly amplified by polymerase chain reaction using random primers (RAPD-PCR) (Williams *et al*., 1990) [24]. This method has provided a useful tool to rapidly distinguish insects by the polymorphic patterns of their DNA. One of the most prominent features of RAPD-PCR is that DNA amplification can be achieved by using small amounts of DNA in relevant combinations with random primers, irrespective of target DNA sequences. RAPDs are viewed as having several advantages over other molecular markers and DNA fingerprints as the technique randomly samples the genome and hence multiple amplifiable fragments are present for each primer (Lynch and Milligan, 1994) [16]. In the present investigation, the field populations of *P. gossypiella* collected from different localities of Tamil Nadu were analyzed for their genetic variation based on their RAPD banding pattern.

Material and Methods

Experimental insect

***Pectinophora gossypiella* (Saunders)**

Systematic position

Phylum: Arthropoda
Class: Insecta
Subclass: Pterygota
Division: Endopterygota
Order: Lepidoptera
Family: Gelechiidae
Genus: *Pectinophora*
Species: *gossypiella*

The infested cotton bolls were collected from the cotton growing areas of Tamil Nadu districts namely Madurai, Sivaganga, Virudhunagar, Theni, Dindigul, Ramanathapuram, Tirunelveli and Tuticori. In the laboratory the bolls were cut opened and the fifth instar larvae of *Pectinophora gossypiella* were collected and used for the RAPD-PCR studies.

DNA isolation

A single whole fifth instar larva of *P. gossypiella* was ground with 300µl of CTAB (cetyltrimethyl ammonium bromide) DNA extraction buffer (1% W/V CTAB; 1.4M NaCl; 10mM EDTA, pH 8.0; 100mM Tris-HCl, pH 8.0; 0.2% V/V ß-mercaptoethanol) in a glass homogenizer. The mixture was emulsified with equal volume of phenol: chloroform (1:1). It was centrifuged at 10,000rpm for 5min. at room temperature. The aqueous phase was collected and mixed with equal volume of chloroform: isoamyl alcohol (24:1). The mixture was then centrifuged at 10,000rpm for 5min. at room temperature. The aqueous phase collected was then added with equal volume of cold absolute ethanol and the DNA was allowed to precipitate by keeping the tubes in -20ºC for overnight. DNA pellets were obtained by centrifugation at 10,000rpm for 5min. and the ethanol was air-dried. The pellet was dissolved in 50µl of TE buffer (Tris 10mM, pH 8.0 and EDTA 1mM, pH 8.0). The quality of the isolated DNA was tested by agarose gel electrophoresis.

RAPD-PCR analysis

20ng of DNA was dissolved in 20µl PCR reaction buffer containing 10mM Tris-HCl (pH 9.0), 1.5mM $MgCl_2$, 50mM KCl, 0.01% gelatin, 0.2mM dNTPs, 21 pM of primer and 0.5 U of DNA polymerase. Twenty primers (RAPD Kit A1 to RAPD Kit A20) obtained from IDT were used for RAPD-PCR studies. PCR was conducted according to the methods of Williams *et al*. (1990): initial heat step (94ºC for 5min.), 40 cycles of denaturation (94ºC for 1min.), annealing (36ºC for 1min.) and extension (72ºC for 2min.) and a finial extension step (72ºC for 7min.). Amplification was performed using a programmable thermal Cycler PTC-150 (MJ Research, USA). The products of PCR and DNA size markers [λ DNA digested with EcoRI and HindIII (Bangalore Genei)] were loaded onto a 1.6% tris-borate-EDTA (Sambrook *et al*., 1989) agarose gel and run for 4hrs. at 50V. The gels were stained with ethidium bromide and photographed. Each lane of RAPD profiles was subjected to gel documentation system (Vilbert-Lourmat, France). The dendrogram analysis was carried out using Bioprofile 1D software.

Table 1: Sequence of the Primers

Primer code	Sequence
RAPD Kit A1	5' CAGGCCCTTC 3'
RAPD Kit A2	5' TGCCGAGCTG 3'
RAPD Kit A3	5' AGTCAGCCAC 3'
RAPD Kit A4	5' AATCGGGCTG 3'
RAPD Kit A5	5'AGGGGTCTTG 3'
RAPD Kit A6	5' GGTCCCTGAC 3'
RAPD Kit A7	5' GAAACGGGTG 3'
RAPD Kit A8	5' GTGACGTAGG 3'
RAPD Kit A9	5' GGGTAACGCC 3'
RAPD Kit A10	5' GTGATCGCAG 3'
RAPD Kit A11	5' CAATCGCCGT 3'
RAPD Kit A12	5' TCGGCGATAG 3'
RAPD Kit A13	5' CAGCACCCAC 3'
RAPD Kit A14	5' TCTGTGCTGG 3'
RAPD Kit A15	5' TTCCGAACCC 3'
RAPD Kit A16	5' AGCCAGCGAA 3'
RAPD Kit A17	5' GACCGCTTGT 3'
RAPD Kit A18	5' AGGTGACCGT 3'
RAPD Kit A19	5' CAAACGTCGG 3'
RAPD Kit A20	5' GTTGCGATCC 3'

Results & Discussion

The pink bollworm, *Pectinophora gossypiella* are widely distributed pest of cotton, which are most active from August to November with 4-6 generations in a year. The female moth lays whitish, flat eggs single on the underside of young leaves, shoots, flowers buds and on young green fruits. The egg stage ranges from 4-25 days and the larval stage lasts for 8-41 days. On emergence the larvae are white in color and later turn pink. The larvae pupate in fallen fruits and debris. Within a week the moths emerge and starts laying eggs. The larvae of last generation hibernate and responsible for the infestation of the following crop. At early instar stage the larva enters the fruit during fruit formation, remains inside and feeding on immature seed. The flowers infested with larvae do not open fully.

The populations of *Pectinophora gossypiella* obtained from different locations in Tamil Nadu were analyzed by RAPD-PCR. A total of twenty 10-mer oligonucleiotide primers (RAPD Kit A1 to Kit A20) were tested for their ability to provide suitable banding pattern for eight different populations of *P. gossypiella*. Of the primer set tested, RAPD KitA3, A9, A15, A18 and A20 yielded clear, consistent and discrete banding patterns for the populations of *P. gossypiella*. Dendrogram and similarity index were constructed based on the RAPD profiles generated by Kit A3, A9 and A15 primer using UPGMA (Bioprofile 1D software).

The primer KitA3 amplified a total of 40 scorable bands and the size of the amplified products varied from 267bp to 1,119bp (Fig. 1a). The amplification pattern showed a maximum of six bands in the populations of Madurai and Tirunelveli and a minimum of four bands in Sivaganga and Ramanathapuram populations (Fig. 1a: lane 2 & lane 6) for the primer Kit A3. The dendrogram analysis grouped the populations of *P. gossypiella* in two clusters (Fig. 1b). The populations Madurai, Theni, Dindigul and Tuticorin are grouped in one cluster, and Sivaganga, Virudhunagar, Tirunelveli and Ramanathapuram populations in another. The similarity coefficient values were varied from 0.13 to 0.86 with maximum similarity of 86% among populations of Sivaganga and Virudhunagar and minimum of 13% among Madurai and Ramanathapuram populations (Fig. 1c).

A total of 30 scorable fragments were produced by KitA9 primer with the molecular weight range of 306bp to 962bp (Fig. 2a). The RAPD profile showed a maximum of five fragments in Sivaganga population and a minimum of two fragments in Virudhunagar population. The UPGMA dendrogram showed two major clusters, one comprised populations of Madurai, Dindigul, Sivaganga and Tuticorin, and the other included Virudhunagar, Theni, Tirunelveli and Ramanathapuram populations (Fig. 2b). The similarity index revealed a maximum of 86% similarity among Sivaganga and Tuticorin populaions (Fig. 2c).

The KitA15 primer produced 45 scorable fragments of the range 266bp to 1,352bp (Fig. 3a). Maximum of eight fragments were exhibited by Virudhunagar and Theni populations (Fig. 3a: Lane 3&4) and minimum of four in Madurai and Sivaganga populations (Fig. 5a: Lane 1&2). The dendrogram analysis grouped the populations of *P. gossypiella* in two clusters, comprised of Madurai, Tuticorine and Theni in one cluster, and Sivaganga, Ramanathapuram, Virudhunagar, Dindigul and Tirunelveli in another cluster (Fig. 3c). The similarity coefficients were ranged from 0.21 to 0.80 (Fig. 3a).

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) [25] have reported geographical distribution of Argentine stem weevils, *Listronotus banariensis* (Kushcel), by RAPD-PCR using genomic DNAs. Haymer and McInnis (1994) [9] have also pointed out a distinctive variation in PCR-amplified DNA patterns between laboratory-cultured and wild populations of Mediterranean fruit flies, *Ceratitis capitata* (Weidemann). RAPD evidence of introgression in two closely related sympatric species of *Choristoneura* in Atlantic Canada (Deverno *et al*., 1998) [8]; RAPD as a tool to identify genetic variation in ecotypes of the European corn borer (Pornkulwat *et al*., 1998); population structure of *Helicoverpa armigera* using RAPD analysis (Zhou *et al*., 2000) [20]; RAPD markers linked to *Nilaparvatha lugens* (Amudha *et al*., 2000); genetic diversity of Iberian populations of *Bemisia tabaci* based on RAPD-PCR (Moya *et al*., 2001) [17] were some known literature indicating that RAPD-PCR has promise for displaying intraspecific genetic variations in insect species. Skoda *et al*. (2002) [21] and Kawamura *et al*. (2002) [11] have attempted to clarify PCR-based DNA patterns in *Cochliomyia* sp. and sweet potato weevils, *Cylas formicarius* collected from various ecotypes.

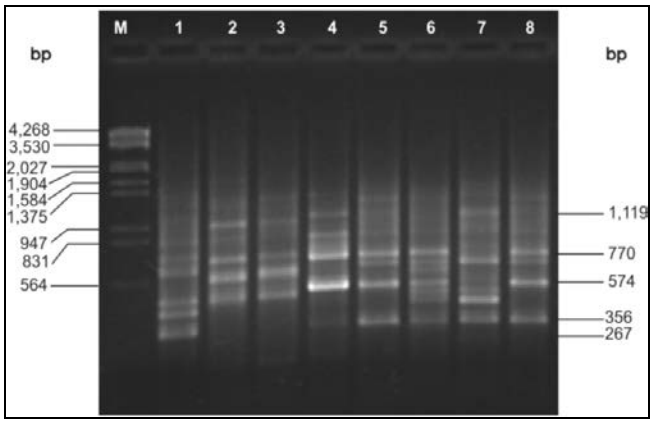

Fig 1a: Genetic population in the field populations of Pectinophora gossypiella generated by the primer RAPD KIT A3

	1	2	3	4	5	6	7	8
1	1.00							
2	0.33	1.00						
3	0.29	0.86	1.00					
4	0.29	0.29	0.25	1.00				
5	0.29	0.29	0.25	0.75	1.00			
6	0.13	0.33	0.29	0.29	0.57	1.00		
7	0.29	0.57	0.50	0.25	0.25	0.29	1.00	
8	0.29	0.29	0.25	0.50	0.75	0.29	0.25	1.00

Fig 1b: Similarity index based on RAPD profiles

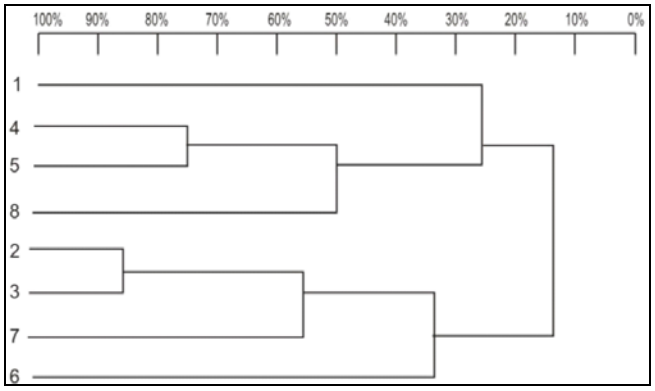

Fig 1c: Dendogram with Homology Coefficient % 0.0 (UPGMA)

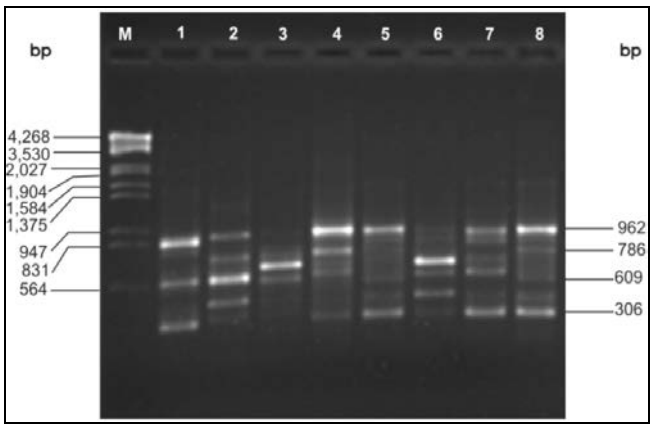

Fig 2a: Genetic population in the field populations of Pectinophora gossypiella generated by the primer RAPD KIT A9

	1	2	3	4	5	6	7	8
1	1.00							
2	0.80	1.00						
3	0.50	0.40	1.00					
4	0.40	0.33	0.29	1.00				
5	0.80	0.67	0.40	0.67	1.00			
6	0.33	0.57	0.29	0.57	0.29	1.00		
7	0.40	0.67	0.29	0.67	0.33	0.57	1.00	
8	0.67	0.86	0.33	0.57	0.86	0.50	0.29	1.00

Fig 2b: Similarity index based on RAPD profiles

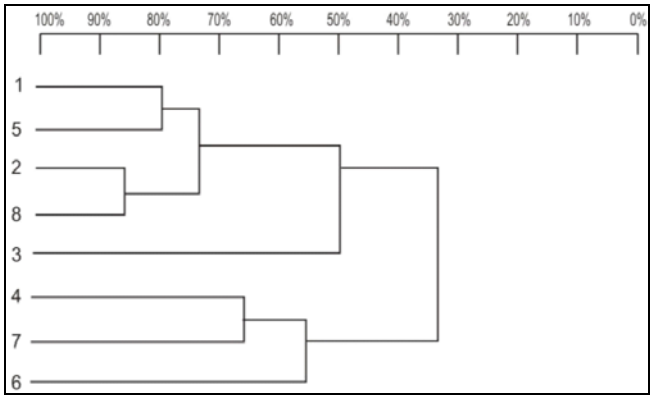

Fig 2c: Dendogram with Homology Coefficient % 0.0 (UPGMA)

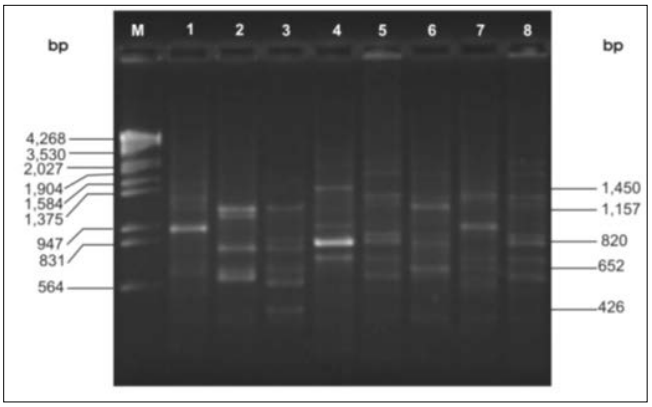

Fig 3a: Genetic population in the field populations of Pectinophora gossypiella generated by the primer RAPD KIT A15

	1	2	3	4	5	6	7	8
1	1.00							
2	0.03	1.00						
3	0.03	0.57	1.00					
4	0.03	0.03	0.03	1.00				
5	0.03	0.03	0.03	0.44	1.00			
6	0.33	0.33	0.57	0.29	0.25	1.00		
7	0.29	0.03	0.03	0.50	0.22	0.03	1.00	
8	0.03	0.40	0.18	0.18	0.50	0.40	0.36	1.00

Fig 3b: Similarity index based on RAPD profiles

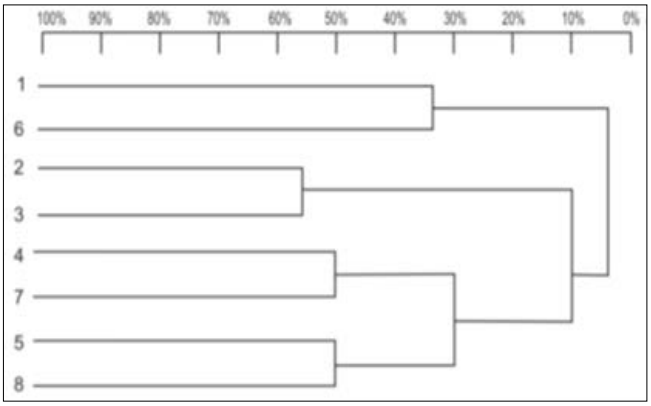

Fig 3c: Dendogram with Homology Coefficient % 0.0 (UPGMA)

Conclusion

The three primers amplified a total of 115 scorable bands in the molecular weight range of approximately 267bp to 1352bp. The number of amplification products obtained was specific to each primer and ranged from thirty (Kit A9) to forty five (Kit A15). The UPGMA based dendrogram of all the primers grouped the populations of *P. gossypiella* in two clusters. The similarity coefficients values were ranged from 0.03 to 0.86, indicating that no two populations were 100% similar. The similarity matrix of all the primers indicated that most of the populations exhibited <50% similarity coefficient. Thus RAPD banding patters suggested that all the populations of *P. gossypiella* exhibit a great degree of intraspecies variation, and this could be attributed to the

varied agronomical practices, stress on the insect, selection pressure etc.

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