Journal of Molecular Genetics and Gene Research

PRIME OPEN ACCESS

Volume 1, Issue 2

Research Article

Date of Submission: 05, Nov, 2025
Date of Acceptance: 05 Dec, 2025
Date of Publication: 10 Dec, 2025

Population Variation and Polymorphism of the Cotton Pink Bollworm Pectinophora Gossypiella (Saunders) (Lepidoptera: Gelechiidae) Using Inter Simple Sequence Repeats (ISSRS) as Molecular Markers

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Citation: Taha, H. S. E. D. (2025). Population Variation and Polymorphism of the Cotton Pink Bollworm Pectinophora Gossypiella (Saunders) (Lepidoptera: Gelechiidae) Using Inter Simple Sequence Repeats (ISSRS) as Molecular Markers. *J Mol Genet Gene Res*, 1(2), 01-13

Abstract

Background

The most dangerous pest infesting Egyptian cotton plants is the pink bollworm Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae). Substantial insecticide applications required for effective control in production season, resulting insecticide resistance problems. Some investigation ideas were floating for sponsored management. Thus, PCR based examination of pest population DNA genetic diversity using eight Inter-Simple Sequence Repeats (ISSRs) primers screening for population differentiation based on similarity in the pattern of SDS-page bands for larvae.

Results

We detected 117 loci with an average population polymorphism of 97.64%. Genetic distance and similarity between test groups were calculated and UPMGA phylogenetic tree were constructed. The average primer efficiency calculated value of PIC and other component were detected for Sharkia, Benisuef and Fayoum governorate. Number of identified alleles Na and the effective number of alleles Ne was attained. Principal component, correspondence analysis and AMOVA results revealed great variance between populations, in addition to moderate level of gene flow, and Infinite diversity distinguished by Fixation index Fst across the genotypes. Estimated Shannon's index (I) parameter as Nei's genetic diversity, eveness, estimated diversity, and species richness all results indicates a high level of genetic variation exists among the three geographical sample populations of the same pest.

Conclusions

All results statistics indicates a distinct level of genetic variation exists among the three geographical samples of the P.gossypiella field collections. And the PCR technology appear to be effective molecular marker for this investigations.

Keywords: ISSR-PCR, Primers, Polymorphism, Population Differentiation, Genetic Diversity, Similarity, Distance, Egyptian Governorates, Pectinophora Gossypiella

Background

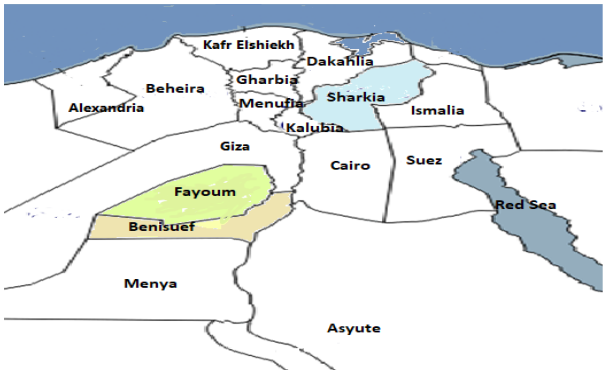
Cotton is the most significant fibre crop in Egypt and at most world countries. It grows in both tropical and sub-tropical regions throughout the world as well as the cultivated area of African regions, [1,2]. The production of Egyptian cotton are facing a much lepidopteran pest infestation, which cause more than 80% of damage. The pink bollworm Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), is known to be one of the major insect pests that causes severe damage and loss to Egyptian cotton production, [3]. The larvae were spend much time in diapause stage inside the cottonseed, after gather and before industries, and become well distributed in all cotton-growing season zones of Egypt. The failures to control this pest with insecticides is due to its larval stage hiding inside cotton bolls and insecticide

application exposure cannot reach except the eggs and first instar. In addition, repeated applications induce the development of insecticide resistance [4,5]. To limit resistance to insecticides some actions were bending depending on genetic variation differentiations such as monitoring the genetic diversity, conservation priority that enhance ecosystem stability of the pest and the genetic structure of its populations [6]. A variety of molecular markers developed for a variety of purposes based on the Polymerase Chain Reaction (PCR), including Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), Random Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and [7,8]. Phylogenetic manipulation to recognize the evolutionary relationships among organisms require some array data types, such as morphological traits and molecular sequence data that become more common, Minoarivelo et al., 2015 [9]. Moreover, exploitation and utilization of ISSR-PCR as a Deoxyribonucleic acid (DNA) marker technique for high polymorphism and high efficiency of the proper amplification due to the number of fragments and bands produces when using dissimilar templates and primer of random nucleotide sequence to attain results of genetic differentiation as a phenotypic measurement tools considered helpful in some argument including genetic diversity assessment, insect genome fingerprint in diploid or haploid genotypes, genetic map construction [10]. Furthermore higher annealing temperature processes of PCR thermal phases can differentiate between closely related individuals and the production of nucleotides sequence to identify orthologues of other species or homologs of the same species at the same geographical locations related to the proper traits [11,12]. The investigation of the genetic differentiation using ISSR technique were done in numerous agriculture pests genomes information such as blackflies, the planthopper Sogatella furcifera, the boll weevil, [13-16]. In addition to, bollworm species collection genetic diversities as [17]. Insect habitually occupying a relatively small geographic area and construction of populations occurs in very heterogeneous environments due to differences in average temperature and precipitation and some topography features [18]. Investigating genetic diversity within and among populations of a species, can lead to increase knowledge of the evolutionary processes underlying genetic variation, provide important basic information for insect climate conditions and suitable habitats to conserve the genetic resources [19]. Thus regional and local scales in the high environmental heterogeneity and a high potential for divergent selection can affect patterns of nucleotide variation at some loci. But also from demographic processes related to population size changes, genetic drift and the development of local adaptation will realize the spatial heterogeneity in the environment. Phylogenetic is used by evolutionary ecologists to explain the emergence and maintenance of diversity estimating the rate and time of phenotypic diversification or the rate of speciation or extinction within a set of species Minoarivelo et al., 2015 [9]. Then the magnitude of genetic differentiation between populations depends on several factors including demographic history of the pattern of natural selection that can lead to local adaptation, level of gene flow and heterogeneity [20]. Generally populations with low average densities may be particularly affected by such spatial variability compared to higher-density populations. Therefore monitoring the effects of these processes on population variation at the molecular scale are urgently needed [21]. Study of nucleotide diversity is known to assess genetic variation within and among insect populations from different environments across cultivated areas and build brief relationship through pairwise genetic distance and matrix to show dissimilarity between samples and to draw a phylogeny tree that can created from gel page of electrophoresis bands and lane of different samples or directly from the alignment of multiple sequences [22,23]. In this study within population sampling exploited to focus on genetic differentiation among P. gossypiella few populations at a definite scale, and to investigate the nature genetic variations in the three-governorate population of P. gossypiella that attack cotton crops in closely related geographical areas of Egyptian governorates without insecticide exposure through PCR-ISSR which considered the molecular procedure of ecological genetic diversity profile produces acquired in the same species and population level to show the environmental differences between sites.

Methods

Insect Sources and Sampling

A large quantity of cotton plant bolls heavily infested with Pectinophora gossybiella larvae were collected from three localities of cotton cultivated lands in Egypt (Sharkia, BeniSuef and Fayoum) of Nile Delta governorates (Figure 1) and transferred to the laboratory directly used without any insecticide bioassay operations. The larvae extracted from bolls into petri dishes with a thin layer of pure cotton inside Petri dishes and transferred to a molecular biology lab for DNA extraction.



	latitude	longtude
Benisuef	29 ° 05 N	31 ° 06 E
Fayoum	29 ° 19 N	30 ° 50 E
Sharkia	30 ° 72 N	31 ° 79 E

Figure 1: Sits of P Gossypiella Collection were in Color of Egyptian Governorate Map. Longitude and Latitude Information were in Table Attached

Molecular Biology Techniques

Genomic DNA Extraction, Purification and Quantification

DNA extracted using modified CTAB method, and purified by phenol/chloroform extraction method [24,25]. For total DNA isolation, larvae weighing about 50 to 100 mg macerated with micro pestle in a 1.5 mL Eppendorf tube containing 500 µL of grinding homogenizing buffer, followed by 25µL of 20% SDS,20µL of 20 mg /mL proteinase K, 100µL of 0.5M EDTA and 50µL of 0.1M Tris (pH8.0). Then incubated at 55°C for 3 hours or overnight. Then 550µL of phenol added and shacked as vortex for 1 minute and centrifuged for 5-10 minutes. The supernatant placed in new tubes. The previous steps repeated 2-3 times, until the cloudy color of supernatant disappeared. Next, 500µL of chloroform added to samples, vortex for 1 minute and centrifuged for 5-10 minutes. Then, the supernatant placed in new-labeled tubes and repeated these steps 5-7 times. Hence, 750µL of cold 100% EtOH added to the supernatant kept for 2 hours at 20 °C overnight. Then, samples centrifuged for 10 minutes to pellet DNA and gently discarded the supernatant by slowly pouring it off, leaving only the pellet. The pellet washed by 70% ethanol dried and finally suspended in 300-500µL sterile distilled water. Quality of genomic DNA assessed by agarose gel (0.7% prepared in TAE buffer) that high molecular weight and DNA band near wells and no streaking or RNA band.

Amplification of DNA using ISSR:

The PCR reactions were performed in a final volume of 25 µl containing 50 ng total genomic DNA, 12.5µl 2× Deam Taq green master Mix kit (2X) and 50-pmole primer found in Table (1). Gently vortexed and centrifuged. Then placed a thin-walled PCR tube on ice. Some components added for each 25 µl volume of reaction: Dream taq green M.M (2X) equal 12.5µl, primer 2µl, template DNA50 ng, water, nuclease-free to 25 µl and total volume 25 µl, gently vortex and spin down. The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min followed by 40 cycles of denaturation at 95oC for 30 seconds, annealing at 54oC for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 10 min. The amplified PCR fragments separated on 1.5% agarose gel in Tris-acetic acid/EDTA buffer (1×TAE) and electrophoresis carried out with a constant voltage.

No	Name	Sequence	Frequency	NP	Fixed band	F/I	Tm	Length	MW bp	GC %
1	UBC818	(CA)8G	0.25	13	2	5.5	50.2	17	5148	52.9%
2	UBC834	GAGAGAGAGAGAGAGAGAGAT	0.86	16	0	4.6	50.5	21	6728	47.6%
3	TA-1	(AG)10C	1	11	4	7.7	51.9	12	3534	91.7%
4	UBC-823	TCTCTCTCTCTCTCTCC	0.26	13	0	5.1	46.3	17	5036	52.9%
5	UBC-835	AGAGAGAGAGAGAGAGYC	0.75	12	2	6.25	47.1	18	5725	52.8%
6	17898A	(CA)6AC	0.5	13	2	5.75	47.5	14	4217	50%
7	17898B	(CA)6GT	0.25	14	0	4.25	43.2	14	4403	50%
8	HB 11	(GT)6CC	0.25	14	1	5.0	56.8	14	4104	92.9%
		total		106	11			127		

Table 1: List of ISSR primes Used and Their Information

NP= number of polymorphic bands, F/I=frequency /individuals, Tm= melting temperature, MW= Molecular weight, and GC= guanine cytosine ratio

Data Analysis of the Gel Results

Fragment Analysis Preparations

Gel picture documents for each primer screened by gel analyzer software and get the results of gel Bands and Lanes. Only the distinct amplified DNA fragments were scoring by (1) as presence and (0) as absence constructing matrix of bands as binary data of distance and similarity matrixes, and ignoring the weak bands.

Performance of Each Primers

Measured by the online program of marker efficiency calculations using absence and presence of gel band data and this iMEC site was. Results attained by calculating different parameters was the content of polymorphism information where pi is the allele frequency and expected heterozygosity (H), by serial formula of calculations began from H=1-∑pi and continued to the final of the online test according to [26,27]. Generally, all calculation of all analysis in this study was by using such recommended software according to Labate (2000) [28].

Genetic Differentiation

Completed by similarity indices calculations to compare patterns between populations according to [29]. Dendrogram constructed by Past v.3 software, using average linkage UPGMA tree (Unweighted Pair Group Arithmetic Mean) under different Bootstrap 1,000 replicates of analysis [30-32]. Certainly Genetic dissimilarity coefficients among the P.gossypiella population individuals were estimated using Dice method from FAMD v.1.3 software [33]. and the phylogenetic tree was obtained from unweighted pair group matrices by method with arithmetical averages of linkage (UPGMA) [34].

Genetic Relationship Achieved by Statistical Analyses

Firstly, cluster analysis performed to determine the relative genetic distance and similarity between pairwise populations and to check the consistency of the genetic differentiation. Secondly, Principle Component Analysis (PCA) based on Euclidian measures. In addition to corresponding analysis and AMOVA (Analysis of Molecular Variance and diversity) calculated using the FAMD and Past software and for comparing results of data analysis and visualization.

Genetic Diversity Analysis

Quantifying Genetic Diversity in Relation to Population Structure

Completed according to Hedrick (2005) and Chesnokov, and Artemyeva (2015), from estimated (Gst) of Nei's genetic differentiation index among populations [27,36]. Where genetic divergence attained using, FST is the coefficient of gene differentiation (Nei 1973). And GST= DST/Ht= (Ht-Hs) /Ht' where DST is the average gene diversity between subpopulations, DST= (Ht-Hs) and Ht = (1–Σpi 2), where pi is the frequency of allele at a locus in a population and Σ is the summation of all alleles representing gene diversities can be defined as expected heterozygosity under Hardy-Weinberg equilibrium and averaged among sub populations (Hs) and of total population (Ht). In addition, gene flow (Nm) = 0.5 (1-GST)/GST was calculated using Popgene version 1.32 [36,37].

Quantifying Genetic Diversity using Shannon (1948) Index

Formula is: H'= -Σ [ni/N] ln [ni/N]. Where: ni is the number of individuals in each species, N is the total number of individuals in the sample, and Σ is the total number of species in the sample [38]. Evenness index, e = H'/log S Where: H is diversity index and S is number of species and index of similarity S % = (2C / A+B) X 100 [39]. Where, A is number of species in one study site, B is number of species in another study site and C number of species common to both sites. S = species richness H = Shannon's index of diversity, E = Shannon's evenness index [40,41]. The power of the base e (e = 2.718281828) must be raised to obtain the natural logarithm (ln) number.

Results

Polymorphism Quantification and Fingerprinting by ISSR Primers

PCR amplification of P.gossypiella DNA from three distinct geographical area of Egyptian governorate samples detected by eight ISSR primers and analytically performed in Cairo University molecular biology laboratories considered as genotype were showed the percentage of polymorphism data found in (Table 2). Formerly, banding pattern of specific gel images detected for each primer was very distinct and repeatable, images found in (Figure. 2). The range of the amplified band noted from 100 to 2000 bp (Figure 2). However, all primers generated multiple band patterns spanned from 16 to 17 band with a mean of 15.93 alleles per loci. However, when speak about individual places (Samples); the total number of bands was 134,136 and 128 for Sharkia, Benisuef and Fayoum, respectively. Number of polymorphic bands of all primer was 24, 26 and 18, respectively, and representing percentage of polymorphism was 17.4, 16.25 and 13.89 % for the same arrangement respectively. Results of all primer together showed total number of bands were 468 in average 117 per all sample (geographical area was Sharkia, Benisuef and Fayoum) as sample and total 106 polymorphic band representing averaged 90.6% polymorphism. ISSR profiles with some amplified DNA fragments ranged from a total 30, 36 and 37 polymorphic band for all primer used and mean about 3.7 to 4.6 polymorphic band for each primer (standard deviation calculated was 1874.9 and standard error =43.3.

	Sharkia				Benisuef				Fayoum			
Primer	a	b	c	Freq	a	b	c	Freq	a	b	c	Freq
UBC818	17	3	17.6	0.20	17	3	17.6	0.20	16	7	12.5	0.13
UBC834	16	4	40	0.0625	17	4	23.5	0.125	16	5	31.25	0.0625
TA-1	20	5	25	0.4	20	5	25	0.4	18	3	16.6	0.26
UBC-823	15	5	33	0.076	19	6	13.5	0.38	16	3	18.7	0.15
UBC-835	17	5	29.4	0.35	16	4	25	0.28	16	4	25	0.28
17898A	15	2	13.3	0.26	14	5	7.1	0.13	14	5	35.7	0.13
17898B	18	4	22	0.14	16	2	12.5	0.071	16	2	12.5	0.071
HB 11	16	2	12.5	0.13	17	6	17.6	0.20	16	7	12.5	0.13
Total	134	30	139.3	1.618	136	36	130	1.786	128	37	111.15	1.213
Mean	16.75	3.75	17.4	0.20	17	4.12	16.25	0.223	16	4.6	13.89	0.151

a=Total No, of band b=No. of polymorphic bands c=% polymorphism

Table 2: Information of Polymorphism of Each Primer Used in ISSR Procedure Represented by Number of Polymorphic Band and Percentage of Polymorphism Produced by Gel Scanning

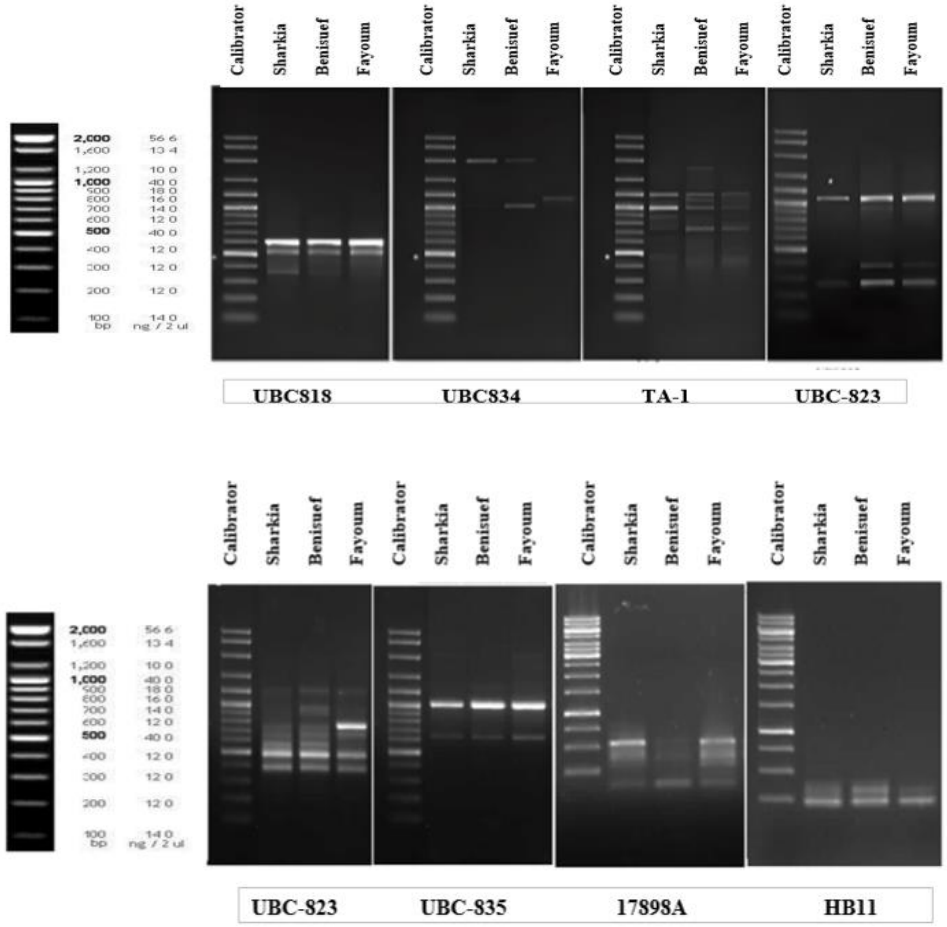


Figure 2: ISSR-PCR Image Profile by Eight Primers, Amplification of Three Geographical Field Region of Cotton Plant Infested with P Gossipiella Larvae Sampled Plus Molecular Weight Ruler on SDS-page (Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis)

Marker Efficiency Analysis (MEA)

The polymorphic efficiency of each ISSR primers iMEC details found in table (3). PIC is the polymorphic information content considered as an important likelihood index of variations, diversity and number of allele frequency distribution, where the higher PIC was 0.379 recorded for Sharkia followed by 0.286 Benisuef larvae and the lowest was 0.226 for Fayoum larvae. The estimated heterozygosity (H) is the second informative genomic marker indices of polymorphism its values varied from 0.346 for Benisuef and 0.326 for Sharkia and 0.260 for Fayoum. The arithmetic means of heterozygosity (Havp) ranged between 0.00296 for Benisuef and 0.0278 for Sharkia. The effective multiplex ratio (EMR) is the publicity of primer polymorphism was 26, 24 and 18. Also marker index (MI) values was 40.768, 0.669 and 0.04 for Benisuef, Sharkia, and Fayoum respectively. Discriminative power (D) gain similar values for the three insect collections, values ranged between about 0.977 to 0.952. Moreover, note the Rp is 0 for the governorate analysis but in the primer analysis RP ranged from 90 to 97.

	H	EMR	H.avp	PIC	D	Mi	Rp
Calibrator	1.27	1.09	1.088	1.92	1.32	1.86	0.0
Sharkia	0.326	24.0	0.00278	0.379	0.960	0.0669	0.0
Benisuef	0.346	26.0	0.00296	0.286	0.952	0.0768	0.0
Fayoum	0.260	18.0	0.0022	0.226	0.977	0.040	0.0

Table 3: Marker Efficiency Analysis (MEA)

PIC= Polymorphic Information Content. Rp= Resolving Power. D= Discriminating Power. H= Expected heterozygosity. Hayp= Arithmetic mean heterozygosity. MI= Marker index. EMR: Effective multiplex ratio, TNB= Total number of bands; NPB: Number of polymorphic bands

Genetic differentiation (distance and similarity) among P. gossypiella populations

The genetic relationships among the three species genotype were determined, the gel band intensity scoring data were completed the calculation of the similarity matrices in the cluster analysis and dendrogram generated using UPGMA method (Figure 4). Distance and similarity matrices and statistical stability of the clusters was estimated by Bootstrap analysis was about 1000 replication using the mentioned software packages, data in table (4 and 5). This data revealed

that distance between the three populations ranged from 9.849 to 4 means that there is 98.4 % variability related to the different geographic distance between them (Table 4). In addition, data showed such similarity indices ranged from 0.89 to 0.12 (Table 5).

	Pairwise Individual Distance of Governorate				Population features		
Population	Calibrator	Sharkia	Benisuef	Fayoum	pi	Allele freq freq	N.allele
Calibrator	0	0	0	0	0.809	0.51	110
Sharkia	9.849	0	0	0	0.79	0.502	25
Benisuef	9.849	3.464	0	0	0.72	0.52	27
Fayoum	9.644	4.243	4	0	0.75	0.523	19

Table 4: Pairwise Genetic Distance Values Between P Gossypiella Populations Quantifying Genetic Relationships

Primer	Calibrator	Sharkia	Beni-suef	Fayoum	Primer	Calibrator	Sharkia	Beni-suef	Fayoum
UBC818	1	0.24	0.24	0.25	UBC-835	1	0.35	0.38	0.5
	0.24	1	1	0.8		0.35	1	0.89	0.44
	0.24	1	1	0.8		0.38	0.89	1	0.5
	0.25	0.8	0.8	1		0.5	0.44	0.5	1
UBC834	1	0	0.12	0.13	17898A	1	0.27	0.14	0.14
	0	1	0.67	0		0.27	1	0.67	0.67
	0.12	0.67	1	0		0.14	0.67	1	1
	0.13	0	0	1		0.14	0.67	1	1
TA-1	1	0.5	0.5	0.33	17898B	1	0.33	0.13	0.25
	0.5	1	0.8	0.75		0.33	1	0.67	0.67
	0.5	0.8	1	0.75		0.13	0.67	1	0.5
	0.33	0.75	0.75	1		0.25	0.67	0.5	1
UBC-823	1	0.27	0.53	0.38	HB 11	1	0.13	0.12	0.13
	0.27	1	0.5	0.4		0.13	1	0.8	0.5
	0.53	0.5	1	0.67		0.12	0.8	1	0.8
	0.38	0.4	0.67	1		0.13	0.5	0.8	1

Table 5: Similarity Index of Three Pink Boll Worm p Gossipiella Population Each for 8 Primer Individually

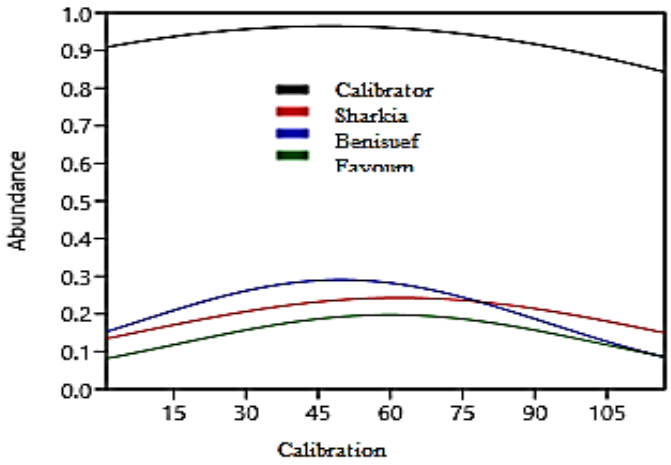
Standard genetic distances (DS) =-In [Jxy/JxJy root (Nei and Li, 1972)

Inter population genetic diversity

Quantifying genetic diversity concluded dimension of inter and Intra population genetic diversity, using analysis of molecular variance (AMOVA) calculated from FAMD Software, performed using absence and presence of bands of ISSR markers, where constructing molecular distance matrix scores to attain the total variance among and within population group component. Degrees of freedom and P-values at different hierarchical levels, between pairs (Euclidean) of multi locus ISSR phenotypes was calculated and results found in table (6). The data showed variance of within population was 0.1298, whereas variance among population was -0.00126 and percentage of variance was 100.98 of within population and -0.984 of among populations. The within population variance in facts expressed that the populations are homogenous in nature, whereas the higher genetic variability (100.98%) among the populations indicated to populations different between each. Data showed the total variation in P. gossyoiella three populations was mostly attributable to diversity within populations (141.47%), whereas variation of diversity among populations (77.9%). while the total observed phenotypic diversity among all population was highly significant ($P < 0.001$). Diversity profile and clusters showing similarity found in Figure (3, 4), data showed high levels of variation within populations in each taxon and the phenotypic diversity among subpopulations was highly significant ($P < 0.001$). Figures shows, distance was 24, 36.3 and 71.8 and Similarity was 76, 36.6 and 28.1 for Sharkia, Benisuef and Fayoum.

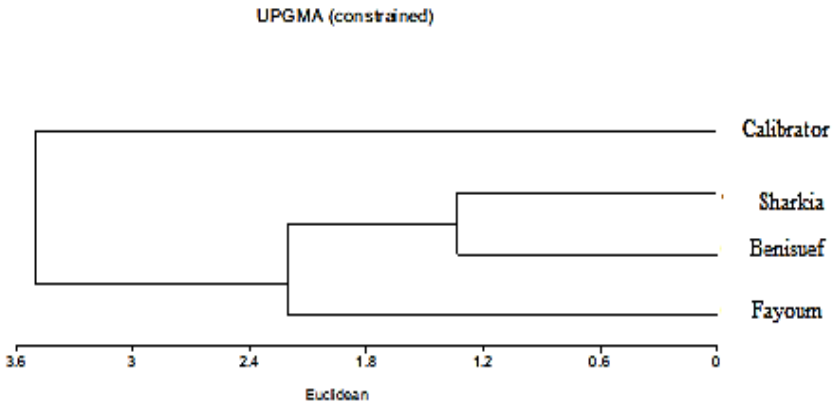
AMOVA	Sum of squares	Statistics	Variance components	% Variance	df	P	PhiST
Among populations	(SSD)AP	0.779	0.00126	-0.984	7	0.001	-0.00984
Within populations	(SSD)WP	14.147	0.1298	100.98	109	0.001	
Total	(SSD)T	14.925	0.1285	100	116	0.001	

Table 6: Analysis of Molecular Variance (AMOVA) for Insecticide Treated Populations of P Gossypiella using Random Amplified Polymorphic DNA Phenotypes



Species packing of the three governoarte samples calculated by Past software

Figure 3: Species Of P Gossypiella Abundancies in the Three Governorate Showed the Exact Similarities Between Governorates Each Other and the Calibrator



UBGMA cluster analysis of the three samples by Past software

Figure 4: Phenogram Generated by Famd Software Expressing Amova, Showing Grouping of Three Populations of P Gossypiella Displaying Relationships Using Upgma Method Based on Dice Dissimilarity Matrix to Determine the Genetic Variability Among Population. Distance Detected was 24, 36.3 and 71.8 and Similarity was 76, 36.6 and 28.1 for Sharkia, Benisuef and Fayoum Appear in the Dendogram

Intra population genetic diversity

The hierarchal cluster analysis completed based on unweighted pair group method to produce a cluster tree dendogram by ordinal weight kneibuor joining (Figure 5) and the phenotypic relationship among P. gossypiella populations of Egyptian governorate samples this was using past software were attained. The figure showed that governorate samples distributed in line connected of the constructed nodes attached to each other where the length of any branch refer to the genetic distance. The data scores divided into two main groups according to genetic similarity reached 97%. Then those groups divided into two sub-cluster with genetic similarity reached 45% and 48%, bootstrap values given at each group was 100.

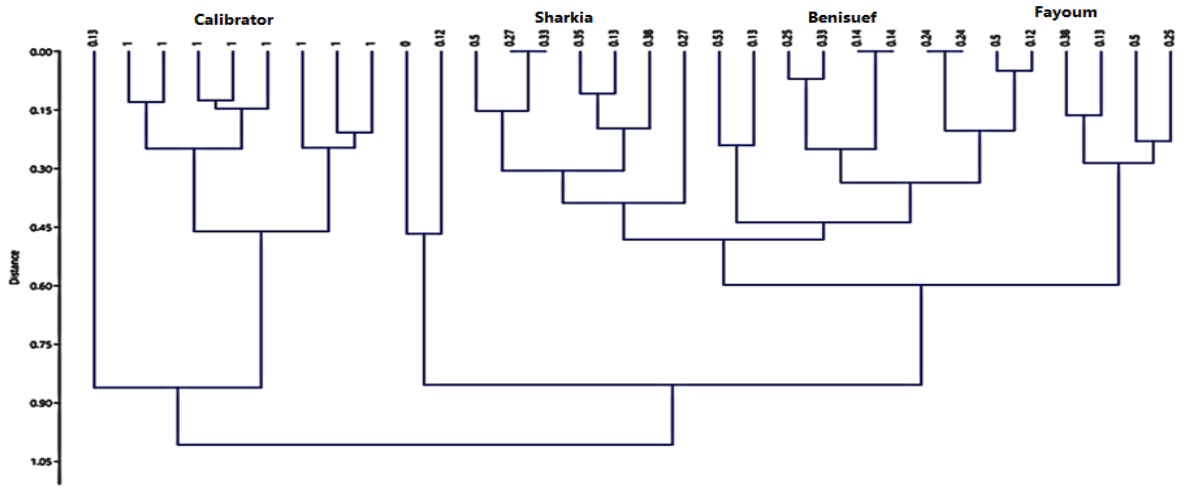


Figure 5: Dendrogram Showing Kneibour Joining Tree Grouping of Individual Bands Distribution Displayed the Existence Significant of Genetic Divergence Among Three Populations of P Gossypilla Based on the Genetic Distance

Genetic relationship achieved by statistical analyses

The study data used to calculate the PCA scores and completed the statistical technique for plot dataset in few dimensions and identify clusters of closely related data points to describe clusters of genetically related samples that linearly uncorrelated. Scores of the nods in addition to bootstrap and eigenvalues of the covariance matrix found in table (6), (Figure 6) and proved that geographical places was different in each other of scores and nods with minimum dissimilarity was 0.2 and the maximum was 1. Nucleotide diversity was pi = 0.809, 0.79, 0.72 and 0.75 at number of segregating site average was 14. In addition PCA and correspondence analysis creates orthogonal components like PCA 1,2,3 depending on the scores used but it preserves chi-square distance (Figure 6 and 7). Variance values refer to the distance between samples like 2.79, 3.27, 0.59 for PCA and 18.0, 4.33, 6.5 for correspondence analysis respectively for Sharkia and for Benisuef and Fayoum that refer to the geographical distance detected between each was 24, 36.3, 71.8 and similarity was 76.0, 63.6, 28.1 between each respectively.

	Principle component analysis					Corresponding analysis				
Scores	PC1	PC2	PC3	Eigenvalue	Variance	PC1	PC2	PC3	Eigenvalue	Variance
Sharkia	0.604	-0.515	0.607	34.6	2.79	-1.34	1.37	-1.6	40.1	18.0
Benisuef	0.648	-0.124	-0.75	7.5	3.27	1.34	0.31	1.9	8.7	4.33
Fayoum	0.462	0.847	0.259	4.9	0.595	-1.0	-2.6	-0.79	5.7	6.5

Table 6: Principal Component and Corresponding Analysis Scores and Eigenvalue

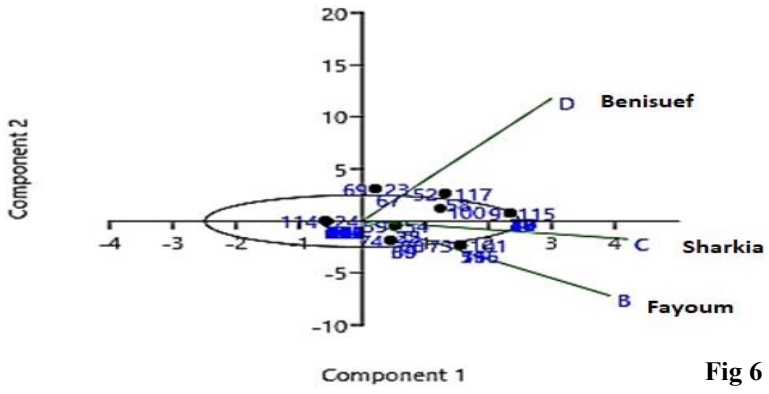


Fig 6

Figure 6: Principle Component Analysis of the Three Governorate of P Gossypiella Samples, Showed the Spaces and Similarity Between Cluster Nods and Axes

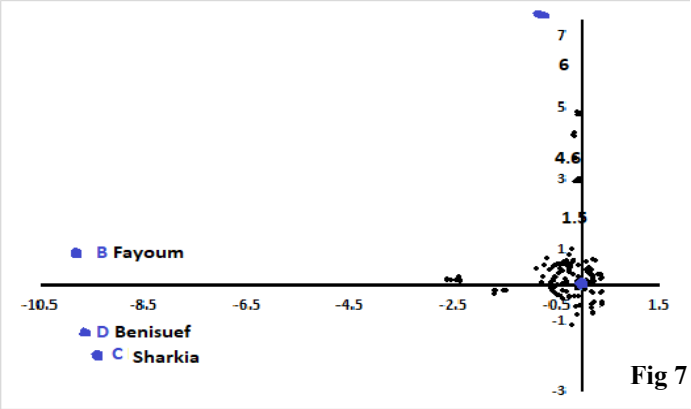


Figure 7: Corresponding Analysis of the Tested Species P Gossypiella Showed Spaces and Points of Diversity

Population Polymorphism information content and FST values

Genetic diversity data analyzed according to some gene structure parameters of within and among population variation as Na, Ne, (Fst values), (Nm), Gst and Dst, average distances He and Ho (Expected and observed heterozygosity) and fixation index F between individuals cluster recorded using Structure software program, results found in table (8). Variation within and among populations recorded mean of Fst value was 0.674, 0.673 and 0.676 for Sharkia, Benisuef and Fayoum. When FST is between 0 to 0.05 it small, 0.05 to 0.15 moderate, 0.15 to 0.25 large and >0.25 very large then large differentiation between the three P.Gossypiella population tested and support the fixation for alternate alleles in different subpopulations. The high amount of gene flow between populations Nm was (-0.474) for Benisuef and not different from other was 0.472, -0.462 for Sharkia and Fayoum and looks as similar in phenotypic traits and genetic similarity also represent near geographically and climate condition is the same. However, the random mating in subpopulations and gene diversities stand on observed and expected heterozygosity Ho and He under Hardy-Weinberg equilibrium averaged among sub populations gene diversity (Hs) and of the total genetic diversity in species population (Ht) attained in (Table 8). Gst is the mean of genetic diversity among population and represent the genetic differentiation = Dst/HT. Where Dst is the total genetic diversity distributed among populations or inter population diversity, Dst=Ht-Hs. Data found in table (8) explain Gst, Dst and where Hs is intra-within population genetic diversity and Ht is the total genetic diversity Ht = Hs + Dst. All items proved that samples near to each other and little differences are etected. The highest level of genetic diversity was (Hs=6.4, He=0.75) for Benisuef. Moreover, the lowest level of genetic diversity was (Hs= 4.4, He= 0.75) was for Fayoum. The genetic parameters including allele's frequency was 0.5 and showed observed number of alleles Na was 1.92, 2.11, 2.03 and number of effective allele was 1.524, 1.602, 1.929 and 1.778 for Sharkia, Benisuef and Fayoum respectively. Moreover, the genetic diversity Ht was 18.1, 19.6 and 13.6, for the same governorate respectively. Thus, the populations evaluated not differ in their phenotypic traits. The term heterozygosity (He) was represent 75% of the tested populations and the homozygosity was 25%.

Population	Ho	He	Hs	Ht	Na	Ne	DST	GST	F	Fis	Fst	NM
Calibrator	1.0	0.75	27.1	81.9	1.88	1.52	54.8	0.6691	-0.333	0.669	0.669	-0.493
Sharkia	1.0	0.76	5.9	18.1	1.929	1.60	12.2	0.6740	-0.316	0.674	0.674	-0.472
Benisuef	1.0	0.75	6.4	19.6	2.11	1.93	13.2	0.6735	-0.333	0.674	0.674	-0.474
Fayoum	1.0	0.76	4.4	13.6	2.03	1.78	9.2	0.6765	-0.317	0.677	0.677	-0.462

Table 8: Polymorphism Information Content of the Three Populations of P Gossypiella

He= 1 – Σpi2, F= Fixation Index= (He – Ho)/He, Fst = (H T – HS)/ H T, Fis= (Mean He – Mean Ho)/Mean He), Nm= 1/Fst/4Fst

Population	N	S	D	RA	ln N	ln S	H	ln E	LnE/ Lns	Distance	Diversity	Eveness
Calibrator	109	109	0.009174	109	4.6913	4.6913	4.6913	0	0	0.096	1.105	1.01
Sharkia	133	115	0.04167	24	4.8903	4.7449	4.7027	-0.042	-0.0089	0.225	1.245	1.09
Benisuef	159	117	0.03846	26	5.0689	4.7622	4.6566	-0.105	-0.022	0.229	1.23	1.089
Fayoum	177	117	0.05556	18	5.1761	4.7622	4.6023	-0.159	-0.0336	0.381	1.28	1.079

Table 9: Shannon Diversity Index Analysis (SHE) Results by Past Software

N= species counts, S= Species richness, RA=Relative abundance, D=Simpson diversity index or dominance, H=Shannon and E= Evenness

Diversity Monitoring

Data results of Shannon's index (I) was 6.62, variance 3.37 and standard deviation was 1.84, and unbiased expected heterozygosity (uHe = (2N/(2N − 1)) × He) were estimated for each cluster, where N is group size. The larger the Simpson index value, the lower the population diversity (Simpson 1949). The species dominance (the opposite of diversity) in a community and low evenness values indicates that one or few species dominate the community [42]. Data of P. gossypiella species diversification were found in table (9). The value of evenness were about 1. Means higher levels of evenness. In addition, relative abundance, species richness (S) values were looked similar to each other about 115-117 and 117 for Sharkia, Benisuef and Fayoum respectively.

Discussions

Polymorphism and Fingerprinting by ISSR Primers

Results concluded that detecting high levels of polymorphism is one of the most important features of the ISSR technique, and it useful for this study. Similar results were attained by Liu et al. (2009), found highly polymorphic 13 microsatellite loci from 527 sample of P. gossypiella from 14 sites of three countries, have allele frequency distributions proved that Chinese P. gossypiella derived from the Pakistani and American populations [43]. And detected PCR-ISSR markers among mutant silkworm strains of Bombyx mori were 73.45% were polymorphic [44]. Al-Senosy et al. 2018, determine the malatox resistance in peach fruit fly (Bactrocera zonata) by (ISSR) by five primers, detected 176 total bands, and 35.2 bands per primer and percentage of polymorphism was 66% (P13) to 100% (C16). Moustafa et al. 2021, genetic profile of Earias insulana (Boisd) [16,17]. (Lepidoptera: Noctuidae) larvae was identified using five ISSR primers resulted in 15 bands with molecular weight between 1630 and 175 bp.

Marker Efficiency Analysis (MEA)

Generally, all primers used in this study gained appropriate results of polymorphism. Similar results were detected by some scientist of plant and animals proved the primer name is the limit as, who set of 32 ISSRs were screened 96 primers which detected a total of 510 loci of 44 Bambara groundnut (Vigna subterranea L. verdc.) genotypes averaged of 97.64% polymorphism using marker efficiency analysis [45].

Genetic Differentiation (Distance and Similarity) Among Populations

Calculation of distance, or dissimilarity, follows one of two models: first, the equilibrium model that mean distance remains constant over time, equilibrium exists between migration, genetic drift. The second is the disequilibrium model that means distance changes with time through species overlap, habitat suitability through climate changes, insect adaptations or environmental acclimations, population composition, population structure, densities, abundances, similarities and heterogeneity, dispersal processes under neutrality and [46,47]. In contrast find the genetic diversity of chinese populations of P. gossypiella using mitochondrial COII was extremely low of genetic variability between two regions among nine populations that not significantly different from each other [13]. Similar results was from, with Samia cynthia ricini (Lepidoptera: Saturniidae), the Indian Eri silkworm, using twenty ISSR primers produced 87% of inter population variability among the six populations [22].

Intra Population Genetic Diversity

The molecular variance measured using AMOVA sustained F-statistics testing for differentiation among populations groups and the genetic structure based on the method of determining population structure through a K-means clustering analysis incorporated in calculation of F-statistics using AMOVA were more useful, Meirmans and Liu (2018) [48]. The phonetic concept measures in many organisms requires identification of clusters by multivariate statistics, has sufficient similarity between two different groups then called a species. Based on specific degree there is a similarity between any two similar groups of the same species objects then called Phenotype. The members of the same species can be significantly different and individuals of various species may look more related to each other than of members of the same species [49]. From literature, the population samples originating from the various geographical regions of India clustering pattern and genetic distance showed genetic relatedness with geographically close populations and significant variation with distant populations achieved by clustering pattern of the kniebour joining tree and the PCA [50]. Also Wang et al. (2010) identify five haplotypes of P.gossypiella different geographical populations, from Australia and China compared to other global populations [19]. Naik et al. (2020), proved that two populations of pink bollworm, occurring early in the season are genetically close to the late season populations using partial Cytochrome Oxydase1 gene [51].

Population Fixation Index

This study results were similar to, said that genetic variability in natural populations of Aedes aegypti (Linnaeus) from Colombia indicated about 94% homozygous for the wild allele and 6% were heterozygous [52]. In addition, quantifying genetic diversity must be based on rate of polymorphism (Pj), proportion of polymorphic loci, and allelic richness and average number of alleles per locus, effective number of alleles (Ne), average expected heterozygosity (He) of Nei's formulas was effective ways [53]. Also similar as, PCR-ISSR markers of Bombyx mori mutant genetic stocks, found average number of observed allele was 1.7080, effective alleles 1.5194 and genetic diversity (Ht) was 0.2901 [43]. In fact, geographical isolation, mutation and selection are the most likely forces of population genetic differentiation like that observed in natural populations of the drosophila [54]. In fact the S. cynthia ricini silk moths discovered hadn't genetic mixing among geographically close populations as gene flow and prevents local adaptation that results from fixation of alleles favoured by local climatic conditions [22]. Generally, gene flow generates new polymorphisms and

gene combinations in which selection can act Khan et al., 2021 [45].

Diversity

In ecosystem ecology the diversity is measured by two main components: species richness is the number of species present in the insect community, species evenness is the relative abundant of the number of the specie presents, and the species composition expressed as which particular species are present [55]. Biodiversity index is a quantitative measure that reflects how many different types of insect species are in a dataset of a habitat populations, represented by (Richness, Evenness, and Dominance) that are useful for comparing data of different regions. Then for identifying locations with high native species abundant, where the larger the Shannon value, the higher the community diversity [39,56]. Diversity analysis utilization on any organism communities that being under stress or that unexposed, to identify the quantity of changes on population density or how natural habitat effect on insect population structure, as Torres et al 2012 investigate species richness in vegetation and the seed bank among microhabitats of the cork oak Quercus suber much of the regeneration after fire [57].

Conclusion

The genetic differentiation using ISSR primers for amplification of some similar populations of P.gossypiella, appear to be effective molecular marker for this investigations. The variation of genetic relationships exhibited by (UPGMA, PCA), among the evaluated insecticide treatments and divergence between them are observed, where the low diversity refer to low genetic variation due to environmental stresses, and experimentally detected differ from wild genetic resources (Calibrator). The analysis of genetic diversity and structure of populations involves the quantification of diversity and the relationships within and between populations are noticeable. In addition to choosing the correct selection of distance or similarity calculation and clustering methods to assess relationships in the sample of interest are clearly suitable tools.

Abbreviations

DNA Deoxyribonucleic acid
PCR Polymerase Chain Reaction
ISSR Inter Simple Sequence Repeate
SDS Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
MEA Marker Efficiency Analysis
UPGMA Unweighted Pair Group Arithmetic Mean
AMOVA analysis of molecular variance
PCA= Principle Component Analysis
SHE= Shannon Diversity Index

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