Potential of molecular marker techniques to identify the susceptibility of some Egyptian cotton varieties to bollworms and whitefly infestation

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Received 7 Nov 2025; Accepted 18 Dec 2025; Published 5 Jan 2026
DOI: https://doi.org/10.64171/JAE.6.1.01-08

Abstract

The current investigation was conducted to study the response of six cotton varieties Giza 45, Giza 87, Giza 92, Giza 94, Giza 96 and Giza 97 to infestation of the *Pectinophora gossypiella* (Saunders), *Earias insulana* and *Bemisia tabaci*, the genomic DNA extraction and purification were studied under field conditions at the experimental farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, through 2024 and 2025. Therefore, results showed that, Giza 94 variety was the most susceptibility to infested by *P. gossypiella*, *E. insulana* and *B. tabaci*, while Giza 97 variety was the opposite any the most resistant for the same pests and recorded the number during the two seasons. While, the other varieties of infections are mean. Results showed that the significant differences between Giza 94 and each of the other cotton varieties. Present study can be helpful in selecting resistant varieties in the development of IPM strategies against bollworms and whitefly for good quality production of cotton. Also, this study used ten SCoT primers to generate 130 DNA bands, averaging 13 per primer, with 37 polymorphic bands (41.16% polymorphism). Primers SCoT-1 and SCoT-9 produced the most bands, while SCoT-4 showed the lowest polymorphism, highlighting useful variations for cotton DNA identification.

Keywords: Cotton varieties, *Pectinophora gossypiella, Earias insulana. Bemisia tabaci*, Genomic DNA, SCoT markers

Introduction

Egyptian cotton, *Gossypium barbadense* L., has been known as an exporter of high-quality cotton and one of the important crops of the all over the world, mainly in Egypt (Abu Hatab, 2009) [2]. It is the most abundant natural fiber crop with over 25 million tons produced annually. Cotton production, consumption, and trade are the most successful works in the world (Dochia *et al*., 2012) [9]. Cotton plays an important role as a major source of revenue (Jody Campiche *et al*., 2016) [20]. According to General Organization of Export and Import Control (GOEIC), Egypt is one of the most important countries for textiles industry of yarns, fabrics, and ready-made garments in the Middle East. GOEIC also reported that 30% of the local employments are working in textiles and Ready-Made Garments (RMG) sector (GAFI, 2014) [11]. From the beginning of seedling emergence until harvesting plants are exposed to many piercing sulking and chewing insect pests (Abro *et al*., 2004) [1]. The spiny and pink bollworm (Gelechiidae: Lepidoptera) are among the most important insect pests that limit the production and quality of the cotton crop, due to great economic losses and damages they cause, through infesting and damaging the flower buds and preventing this from opening, as well as the dropping of the almont and damage to the cotton fluff, which leads to a significant decrease in the quality and quantity of the cotton yield production (Chaudhry *et al*., 1999) [8]. The sweet potato whitefly, *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae), which has be-come one of the most intractable and damaging pests of economic crops, vegetables and ornamentals world-wide (Perring, 2001; Bayhan *et al*., 2006) [28]. The adults and nymphs damage the infested plants either directly by sucking the phloem sap or indirectly by secretion of the honeydew causing severe problems to plant growth and productivity. Moreover, this insect can transmit more than 150 virus species mainly to vegetables (Polston *et al*., 2014) [29]. Cultivation of resistant varieties agricultural crops is an effective element in the estrategy of integrated insect pest management programs (IPM). It can reduce the incidence of insect pests without causing any damage to the natural environment (Khan *et al*., 2010) [21]. Producing pest-resistant cotton strains and varieties is a more economical and environmentally friendly control strategy, increasing crop productivity (Khan, 2011) [22]. Resistant plants control insect pests without the use of insecticides and without any pesticides contamination. Grouting resistant plant varieties helps control insect pest infestations (Hua and Hua 2000) [17]. However, resistance is governed by a combination of environmental and physical factors, not just a single factor. Genetically modified cotton plays a significant role in controlling the incidence of biting insect pests without polluting the environment with pesticides, and it increases yield per acre (Sunilkumar *et al*., 2006) [33]. SCoT markers are a valuable tool for population studies, assessing genetic diversity and structure, and identifying genotypes through unique fingerprinting (Baghizadeh and Dehghan, 2018) [6]. The main advantages of SCoT genotyping include its simplicity and cost-effectiveness (Guo *et al.,* 2012) [12]. These markers have been effectively utilized to evaluate genetic diversity in various crop species, including rice, cowpea, Plantago, and sesame (Igwe *et al.,* 2017; Rahimi *et al*., 2018; Mesfer Alshamrani *et al*., 2022) [19, 30, 26]. The present study was carried out to study the comparative tolerance of six cotton varieties against *P. gossypiella, E. insulana* and *B. tabaci* during two successive seasons, 2024 and 2025. As well as Genomic DNA extraction and purification were also studied.

Materials and Methods

Field treatments

This investigation was conducted at the experimental Farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt during the cotton growing seasons 2024 and 2025, to determine the susceptibility of six cotton varieties, Giza 45, Giza 87, Giza 92, Giza 94, Giza 96 and Giza 97 to infestation by the pink and spiny bollworms in an area of approximately 2100 m^2 for every variety was divided into 7 plots (300 m^2/ plots) and 3 replicated / plot (100 m^2/replicate). All recommended practices were followed without insecticidal applications in the field experiments. The inspection process is carried out weekly after two months planting, from the beginning of July to the end of August, by taking a specific number of green cotton bolls randomly from all tests cotton varieties. The bolls must be unform and of the same age, and are transferred to the laboratory to detergent the percentage bollworm infestation. In each check, cotton green bolls of the same age were randomly chosen from all verities to count the larvae of the bollworms. The cotton green bolls (100 bolls/variety, meaning 25 green bolls from each replicate)) were confined completely into a cloth bag. The samples were transferred to the laboratory for examination. Examination is performed using a sharp knife and then the infestation ratio was determined as green bolls containing larvae. weekly, numbers of *P. gossypiella* and *E. insulana* larvae were counted by dissecting cotton green bolls in the laboratory. While in the case of whitefly, *Bemisia tabaci* examination, every week samples were taken, as 100 random leaves (20 leaves from each replicate) per cotton variety, the adult insect is visually counted in the field, the remaining 100 leaves are transferred into a clear paper bag to the laboratory for nymph*, B. tabaci* examination using a binocular microscope. The mean population of all pests studies are recorded for all cotton varieties.

Laboratory treatments

a) Genomic DNA deposition and cleaning

Following the manufacturer's instructions, DNA was extracted using the DNeasy Mini Kit (Qiagen Santa Clarita, CA). Nano-drop 2000 (Thermo Scientific, USA) and 1% agarose gel electrophoresis (ethidium bromide staining) were both used to detect the quality and quantity of DNA. Also, be discovered and deposition by the DNeasy Mini Kit (Qiagen Santa Clarita, CA) according to the source's instructions.

b) SCoT-PCR reactions

Ten SCoT primers were used in the detection of polymorphism Table (1). The amplification reaction was carried out in 20 µl reaction volume containing 10 µl Master Mix (sigma), 2 µl primer (10pcmol), 2 µl template DNA (10ng) and 6 µl dH2O, according to (Ibrahim *et al*., 2019) [18].

Table 1: Sequences of SCoT Primers and annealing temperature

Primer Name	Sequence
SCoT-1	5'-ACGACATGGCGACCACGC-3'
SCoT-2	5'-ACCATGGCTACCACCGGC-3'
SCoT-3	5'-ACGACATGGCGACCCACA-3'
SCoT-4	5'-ACCATGGCTACCACCGCA-3'
SCoT-5	5'-CAATGGCTACCACTAGCG-3'
SCoT-6	5'-CAATGGCTACCACTACAG-3'
SCoT-7	5'-ACAATGGCTACCACTGAC-3'
SCoT-8	5'-ACAATGGCTACCACTGCC-3'
SCoT-9	5'-ACAATGGCTACCACCAGC-3'
SCoT-10	5'-ACAATGGCTACCACTACC-3'

c) Interaction PCR

The polymerase chain reaction (PCR) assay was expanded in a Perkin-Elmer/GeneAmp® PCR System 9700 PE programmed system to complete 40 cycles after an initial five min analysis cycle at 94ºC. Each cycle consisted of an analysis phase at 94ºC for 45 seconds, an annealing step at 50ºC for 50 seconds, and an elongation step at 72ºC for 1min. In the final cycle, the first part was extended to 7 min at 72ºC.

d) Detection PCR

The final products of the amplification were resolved by centrifugation using 1.5% agar gel containing 0.5ug/ml ethidium bromide dissolved in in the final cycle 1X TBE solution - 4at 95 V. The PCR chain reaction was observed under UV light and imaged using an agar system (BIO-RAD 2000).

e) Findings analysis

For SCoT tests, a statistical binary matrix was created for all types detected chains, were visually scored as either visible (1) or no visible (0) and final findings recorded for both monomorphic and polymorphic chains. Dice's resemblance matrix structure was recorded amedist genotypes application the unweight pairs group technique with arithmetic means (UPGMA). To build an evolutionary tree (dendrogram), this matrix was applicated according to the regional similarity index application the PAST software Version 1.91 (Hammer *et al*., 2001) [15].

Statistical analysis

The current experiment's data showed that the mean and infestation percentage estimates were accurate. One-way analysis of variance for significance differences (ANOVA) was used to examine the results. Using the SAS Institute's PC-SAS software, the Tukey's approach (LSD, $p \leq 0.05$) of multiple comparison of the vulnerability of cotton varieties to infection by various pests of significant economic relevance was carried out among the tested verities.

Results and discussion

1. Sensitivity of six cotton varieties to infestation of *P. gossypiella* and *E. insulana* larvae

From the data presented in Table (2 and 3) mentioned that the infestation of cotton green bolls by the pink bollworm, *P. gossypiella* and the spiny bollworm, *E. insulana* larvae during 2024 and 2025 seasons. Thus, considered Giza 94 one of the most susceptible cotton varieties, was the percentage infestation of 7.15 %, followed by Giza 45 variety (5.40%). Therefore, the varieties G 87 and Giza 92 were the most resistant, have a mean percentage of infestation, 4.40 and 3.50 % by *P. gossypiella*, respectively. Also, Giza 94 is sensitive to infestation by *E.insulana* (8.20%) followed by Giza 45 (4.90%) and so on G 87, Giza 92, Giza 96, and Giza 97 with a percentage of infestation to 3.40, 3.50, 3.30 and 3.00 %, respectively, in the first season. In the 2025 season, data in same Table showed the dates of Giza 94 increased insects populations achieving a percentage infestation 7.40 and 8.60 % both *P. gossypiella* and *E. insulana* larvae, respectively comparing with the other varieties. Also, two varieties of bollworm insects in cotton boll G 45 and G 87 with a percentage of the infestation were 5.30 and 4.48 %, respectively by *P. gossypiella.* On the other hand, G 45 and G 87 were significantly resistant cotton to infestation of green bolls by the spiny bollworm, *E. insulana* with a percentage of infestation to 6.05 and 4.66 %, respectively. In the both seasons, the statistical analysis indicated that there were significant differences between Giza 94 and each of the other cotton varieties, while there were no significant differences between cotton varieties; G 45, G 87, G 92, G 96, and G 97. Therefore, these cotton varieties can be growing as a source of resistance to bollworms infestation. Supported by those indicated by many authors such as Saad *et al.* (2013) [31] showed that Giza 92 was significantly resistant genotypes to infestation of *H. armigera* El-Mezayyn (2004) [10] and Shawer (2000) [32] mentioned that G 45 was the most susceptible cotton varieties *P. gossypiella E. insulana*. Ahmed *et al*., (2005) [4] observed different levels of *Amrasca devastans* infestation among 18 cotton varieties and reported that two varieties (CRIS-467 and CRIS-134) were resistant, whereas two (CRIS-82 and MNH-536) were susceptible.

Table 2: Susceptibility of some cotton varieties of *Pectinophra gossypiella* larvae infestation during seasons 2024 and 2025.

Inspection date	Giza 45	Giza 87	Giza 92	Giza 94	Giza 96	Giza 97	Mean ±SE
2024							
July 18	3.50±0.43 ^{b}	2.75±0.50 ^{b}	2.50±0.43 ^{b}	4.25±0.41^{a}	2.00±0.35 ^{b}	2.50±0.56 ^{b}	2.95±0.41^{b}
July 25	4.50±0.55 ^{a}	4.00±0.60 ^{a}	3.25±0.41 ^{b}	5.50±0.55 ^{a}	3.50±1.36 ^{b}	3.50±0.25 ^{b}	4.05±0.42 ^{a}
Aug 1	5.25±0.11 ^{b}	4.50±0.89 ^{b}	3.25±0.21 ^{c}	7.25±0.64 ^{a}	3.25±0.51^{c}	3.25±0.22 ^{c}	4.45±0.80 ^{a}
Aug 8	6.75±0.21 ^{b}	5.50±0.90 ^{b}	4.00±0.35 ^{c}	9.50±0.41^{a}	4.00±0.35 ^{c}	2.25±0.22 ^{d}	5.30±1.27 ^{a}
Aug 15	7.00±0.35 ^{b}	5.26±0.88 ^{c}	4.50±0.55 ^{c}	9.25±0.45 ^{a}	4.00±0.35 ^{c}	4.50±0.25 ^{c}	5.85±0.99 ^{a}
Mean±SE	5.40±0.26 ^{b}	4.40±0.95 ^{b}	3.50±0.15 ^{c}	7.15±0.15 ^{a}	3.35±0.13 ^{c}	3.20±0.24 ^{c}	-
2025							
July 21	4.25±0.22 ^{a}	3.65±0.60 ^{a}	3.00±0.35 ^{b}	5.00±0.35^{a}	2.50±0.56 ^{b}	2.25±0.41 ^{b}	3.40±0.53 ^{b}
July 28	4.50±0.25 ^{b}	4.00±0.45 ^{b}	3.25±0.22 ^{b}	6.50±0.25 ^{a}	2.75±0.52 ^{c}	3.50±0.25 ^{c}	4.10±0.66 ^{a}
Aug4	5.75±0.52 ^{b}	4.50±0.53 ^{b}	4.00±0.35 ^{b}	8.25±0.22 ^{a}	4.50±0.43 ^{b}	3.75±0.52 ^{c}	5.25±0.83 ^{a}
Aug 11	5.75±0.52 ^{b}	5.25±0.60 ^{b}	5.25±0.22 ^{b}	8.25±0.22 ^{a}	4.50±0.25 ^{c}	4.25±0.22 ^{b}	5.60±0.71 ^{a}
Aug 18	6.25 ±0.41^{b}	5.00±0.25 ^{b}	5.50±0.25 ^{b}	9.00±0.00 ^{a}	5.25±0.22 ^{b}	4.25±0.22 ^{c}	6.05±0.80 ^{a}
Mean±SE	5.30±0.05 ^{b}	4.48±0.56 ^{b}	4.20±0.07 ^{b}	7.40±0.12 ^{a}	3.90±0.18 ^{b}	3.60±0.16 ^{b}	-

Means with the same letters are not significantly different ($p \leq 0.05$% level)

Table 3: Susceptibility of some cotton varieties of *Earias insulana* larvae infestation during seasons 2024 and 2025

Inspection date	Giza 45	Giza 87	Giza 92	Giza 94	Giza 96	Giza 97	Mean ±SE
2024							
July 18	3.25±0.41^{b}	1.25±0.67 ^{c}	1.75±0.65 ^{c}	5.75±0.22 ^{a}	1.75±0.52 ^{c}	1.25±0.22 ^{c}	2.75±0.82 ^{b}
July 25	4.75±0.52 ^{b}	4.00±0.56 ^{b}	4.25±0.22 ^{b}	8.25±0.41^{a}	3.50±0.52 ^{b}	2.00±0.50 ^{c}	4.55±1.04 ^{a}
Aug 1	4.75±0.52 ^{b}	3.50±0.50 ^{b}	4.00±0.50 ^{b}	8.25±0.22 ^{a}	3.75±0.25 ^{b}	3.25±0.22 ^{b}	4.80±0.89 ^{a}
Aug 8	5.75±0.52 ^{b}	5.00±0.78 ^{b}	3.25±0.22 ^{c}	9.25±0.22 ^{a}	4.25±0.22 ^{b}	4.25±0.22 ^{b}	5.35±1.05 ^{a}
Aug 15	6.00±0.35 ^{b}	3.25±0.45 ^{c}	4.25±0.22 ^{c}	9.50±0.25 ^{a}	3.25±0.22 ^{c}	4.25±0.22 ^{c}	5.45±1.11 ^{a}
Mean ±SE	4.90±0.04 ^{b}	3.40±0.90 ^{b}	3.50±0.08 ^{b}	8.20±.0.00 ^{a}	3.30±0.15 ^{b}	3.00±0.16 ^{b}	-
2025							
July 21	5.50±0.43 ^{a}	3.00±0.35 ^{b}	3.00±0.35 ^{b}	6.25±0.22 ^{a}	2.25±0.22 ^{b}	2.25±0.22 ^{b}	3.85±0.85 ^{b}
July 28	5.50±0.25 ^{b}	5.25±0.54 ^{b}	4.50±0.25 ^{b}	8.50±0.43 ^{a}	3.25±0.22 ^{b}	4.50±0.43 ^{b}	5.25±0.89 ^{a}
Aug4	6.00±0.61^{b}	5.05±0.60 ^{b}	4.75±0.22 ^{b}	9.25±0.22 ^{a}	3.25±0.22 ^{c}	4.22±0.22 ^{b}	5.49±1.04 ^{a}
Aug 11	6.75±0.65 ^{b}	4.00±0.76 ^{c}	5.25±0.22 ^{b}	9.25±0.22 ^{a}	4.25±0.65 ^{c}	4.25±0.25 ^{c}	5.95±0.94 ^{a}
Aug 18	6.50±0.22 ^{b}	6.00±0.53 ^{b}	5.00±0.22 ^{c}	9.75±0.22 ^{a}	5.25±0.22 ^{c}	4.25±0.25 ^{c}	6.15±0.97 ^{a}
Mean±SE	6.05±0.14 ^{b}	4.66±0.50 ^{c}	4.50±0.14 ^{c}	8.60±0.10 ^{a}	3.65±0.24 ^{c}	3.89±0.10 ^{c}	-

2. Sensitivity of six cotton varieties to infestation of *B. tabaci,* adults and nymphs

Data on the mean population of *B. tabaci* on cotton plants during seasons 2024 and 2025 is presented in Table (4 and 5). During season 2024, the infestation of *B. tabaci* adults gradually increased during inspection period on Giza 94 variety, it reached its peak during on August 1st, also in all varieties. Giza 94 variety, considered the most sensitive to whitefly (33.90 adult and 22.00 nymph per 100 cotton leaves)., followed by the Giza 96 variety (26.15 and 15.70 adult and nymph per 100 cotton leaves). While, Giza 97 variety was the most resistant for whitefly (16.60 adult and 14.65 nymphs). Also, the other varieties of infection are moderate of 15.20, 15.15 and 14.48 adult with G 45, G 92 and G 87, respectively. Similar results were noticed in the second season 2025, on July 21st, July 28th and August 4, with highly population densities. Results indicated that G 94 variety was significantly the most susceptible cotton varieties to cotton plant infestation by whitefly adult and nymphs (*B. tabaci*) 34.45 adult and 16.15 nymph). While Giza 97 variety was the most resistant (15.65 adult and 8.80 nymph) and the other varieties of infection are moderate. Metwally, *et al.* (2005) showed that G 89 variety had the highest susceptibility cotton varieties of *B. tabaci* adult and immature stages while G 45 indicated that the least infested.

Table 4: Susceptibility of cotton varieties to *Bemisia tabaci* infestation adults infestation during seasons 2024 and 2025

Inspection date	Giza 45	Giza 87	Giza 92	Giza 94	Giza 96	Giza 97	Mean ±SE
2024							
July 18	8.50±0.75 ^{b}	5.00±0.55 ^{c}	14.25±1.14^{a}	3.25±0.54 ^{c}	4.25±0.41^{c}	8.00±0.35^{b}	7.65±1.94 ^{d}
July 25	39.00±1.61 ^{a}	12.00±0.90 ^{b}	15.25±1.43 ^{b}	13.00±0.35 ^{b}	34.75±1.29 ^{a}	9.00±0.61^{c}	22.20±6.11 ^{c}
Aug 1	40.75±1.71 ^{c}	25.30±0.86 ^{d}	23.25±1.29 ^{d}	66.25±3.66 ^{a}	47.50±2.38 ^{b}	47.50±1.44 ^{b}	45.05±6.91 ^{a}
Aug 8	39.75±1.14 ^{a}	24.25±0.95 ^{b}	22.75±0.41^{b}	43.00±1.54 ^{a}	23.25±0.41^{b}	8.25±0.54 ^{c}	27.40±6.33 ^{b}
Aug 15	7.25±0.54 ^{c}	18.30±0.98 ^{b}	23.00±0.79 ^{b}	44.00±2.26 ^{a}	21.00±0.79 ^{b}	10.25±0.82 ^{c}	21.10±6.47 ^{c}
Mean ±SE	27.05±0.62 ^{b}	16.97±0.65 ^{d}	19.70±3.04 ^{c}	33.90±0.62 ^{a}	26.15±0.35 ^{b}	16.60±0.33 ^{d}	-
2025							
July 21	3.75±0.43 ^{c}	9.00±0.92 ^{b}	2.25±0.43 ^{c}	18.25±0.41^{a}	18.00±1.84 ^{a}	11.00±0.94^{b}	10.75±1.94 ^{c}
July 28	18.50±3.01 ^{c}	8.50±0.87 ^{d}	21.50±1.52 ^{c}	26.75±0.74 ^{b}	34.75±4.02 ^{a}	16.75±1.30 ^{d}	23.65±6.11^{b}
Aug4	44.50±1.60 ^{b}	35.50±0.86 ^{c}	34.00±0.94 ^{c}	62.00±2.79 ^{a}	59.25±2.70 ^{a}	20.00±0.61^{d}	43.95±6.91 ^{a}
Aug 11	13.00±1.87 ^{d}	20.65±0.94 ^{c}	23.75±1.85 ^{b}	45.75±3.49 ^{a}	23.00±1.27 ^{b}	18.50±1.60 ^{c}	24.80±6.33 ^{b}
Aug 18	12.75±0.57 ^{c}	15.20±0.53 ^{c}	10.50±1.25 ^{d}	19.50±0.56 ^{b}	23.50±0.43 ^{a}	12.00±0.25^{c}	13.65±6.47 ^{c}
Mean ±SE	16.50±0.47 ^{b}	17.77±0.87 ^{b}	18.50±1.92 ^{b}	34.45±0.43 ^{a}	31.70±0.81 ^{a}	15.65±0.23 ^{b}	-

Table 5: Susceptibility of cotton varieties to *Bemisia tabaci* infestation nymphs infestation during 2024 and 2025 seasons

Inspection date	Giza 45	Giza 87	Giza 92	Giza 94	Giza 96	Giza 97	Mean ±SE
2024							
July 18	12.25±0.40 ^{a}	5.43±0.86 ^{b}	6.25±0.42 ^{b}	15.75±1.19 ^{a}	2.25±0.41 ^{c}	3.75±0.22 ^{c}	8.05±2.57 ^{c}
July 25	13.00±0.94 ^{d}	17.35.45 ^{b}	16.75±0.67 ^{c}	24.25±1.14 ^{a}	18.00±0.35 ^{b}	19.50±0.56 ^{b}	18.30±1.84 ^{a}
Aug 1	19.50±0.75 ^{d}	18.50±0.75 ^{d}	22.50±0.75 ^{c}	29.00±2.15 ^{a}	24.50±2.36 ^{b}	19.25±0.73^{d}	22.95±1.80 ^{a}
Aug 8	12.75±1.19 ^{b}	15.15±0.76 ^{a}	12.75±0.89 ^{b}	18.75±1.04 ^{a}	16.00±2.10 ^{a}	17.00±1.00 ^{a}	15.45±1.19 ^{b}
Aug 15	18.50±1.60 ^{b}	16.00±0.87 ^{b}	17.50±1.46 ^{b}	22.25 0.96 ^{a}	17.75±0.98 ^{b}	13.75±1.51 ^{c}	17.95±1.35 ^{b}
Mean ±SE	15.20±0.72 ^{b}	14.48±0.45 ^{b}	15.15±0.28 ^{b}	22.00±1.48 ^{a}	15.70±0.80 ^{b}	14.65±0.47 ^{b}	-
2025							
July 21	7.75±1.24 ^{a}	4.75±0.96 ^{b}	4.50±0.56 ^{b}	9.00±0.61^{a}	3.50±0.43 ^{b}	2.25±0.11^{b}	5.40±1.28 ^{d}
July 28	15.50±1.09 ^{a}	14.76±0.65 ^{b}	12.25±0.41 ^{c}	18.00±1.84 ^{a}	13.50±1.14 ^{b}	13.75±1.29 ^{b}	14.60±0.99 ^{b}
Aug4	24.25±0.41 ^{a}	17.22±0.92 ^{b}	16.50±0.83 ^{b}	22.00±1.54 ^{a}	17.25±1.14 ^{b}	14.75±1.43 ^{b}	18.95±1.79 ^{a}
Aug 11	9.75±0.22 ^{b}	5.00±0.28 ^{c}	7.00±0.61 ^{b}	17.50±1.03 ^{a}	10.50±1.03 ^{b}	5.00±0.35 ^{c}	9.95±2.13 ^{c}
Aug 18	6.00±0.35 ^{b}	7.50±0.97 ^{b}	8.00±0.35 ^{b}	14.25±0.41 ^{a}	13.00±0.35 ^{a}	7.75±0.65 ^{b}	9.80±1.61 ^{c}
Mean ±SE	12.65±0.43 ^{b}	9.84±0.76 ^{c}	9.65±0.44 ^{c}	16.15±0.47 ^{a}	11.55±0.65 ^{b}	8.80±0.69 ^{c}	-

Means with the same letters are not significantly different ($P \leq 0.05$% level).

Molecular marker techniques

The amplification results of the ten SCoT primers used in this investigation are presented in Table (6). The band size and number data of monomorphic, polymorphic bands and the percentage of polymorphism obtained per each SCoT-primer. were used to amplify DNA fragments and produced a total of 130 bands with an average of 13 bands per primer amplified, ranging from approximately 150-1400 bp. Of them, 37 bands were polymorphic across the accessions with an average of approximately 3.7 bands. The average percentage of polymorphism was 41.16%. The oligonucleotide SCoT-1 and SCoT-09 amplified the highest no. of SCoT (16 and 17 bands) loci. The primer SCoT-4 and SCoT-10 (9 and 10 bands respectively) showed the lowest polymorphic (9 bands). While SCoT-4 was given the lowest percentage of polymorphism (11%). These differences in polymorphic bands may be useful in cotton DNA identification, as shown (Fig.1, 2 and 3). Table (7) shows the similarity matrices according to the Jaccard Coefficient among sesame based on start codon targeted (SCoT) analysis.

Table 6: The total number of amplicons, monomorphic, polymorphic fragments, percentage of polymorphism and Frequency as revealed by SCoT markers in cotton plant

Molecular Markers	Molecular Wight	Total No. of. Amplicons	Monomorphic Amplicons	Polymorphic Amplicons	Unique Amplicons	Polymorphism %	Frequency
SCoT-1	170-1400bp	16	11	4	1	31	0.79
SCoT-2	170-1300bp	14	9	1	4	36	0.73
SCoT-3	180-890bp	11	6	4	1	45	0.89
SCoT-4	190-1500bp	9	8	1	0	11	0.98
SCoT-5	196-856bp	11	4	4	3	63	0.62
SCoT-6	210-1400bp	14	7	4	3	50	0.68
SCoT-7	150-950bp	13	6	6	1	54	0.71
SCoT-8	190-1100bp	15	8	6	1	47	0.79
SCoT-9	210- 1400bp	17	12	4	1	29	0.84
SCoT-10	200-600bp	10	5	3	2	50	0.67
Total	-	130	76	37	17	416	7.7
Average	-	13	7.6	3.7	1.7	41.16%	0.77

Table 7: Similarity matrix among eight sesame plants based on SCoT-PCR analysis

Samples	48	87	92	94	96	97
48	100					
87	86.9	100				
92	86.9	91.6	100			
94	87.2	90.0	88.2	100		
96	87.4	90.2	89.3	86.9	100	
97	86.8	86.8	85.9	86.3	90.3	100

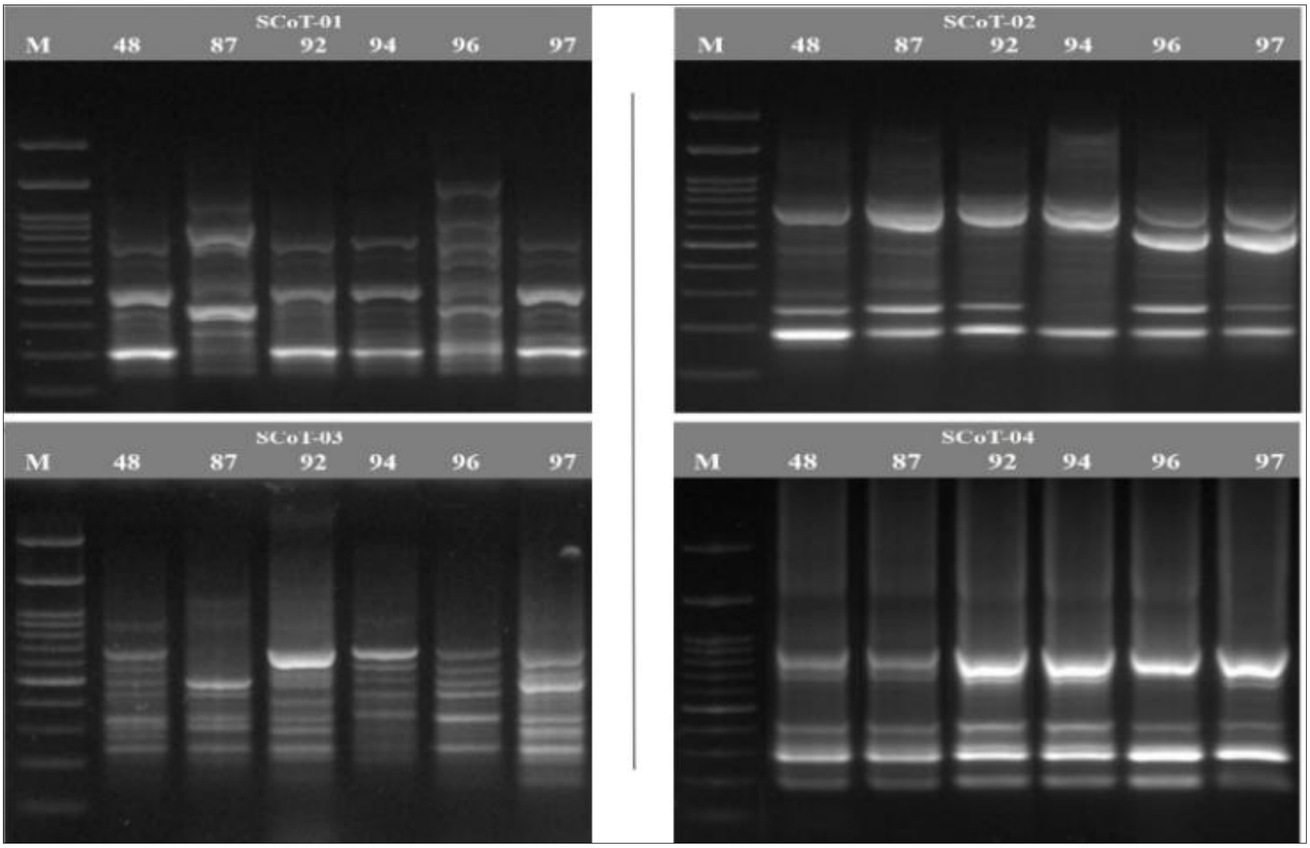

Fig 1: SCoT products on 1% agarose gel using different primers: SCoT 1, SCoT 2, SCoT 3 and SCoT4

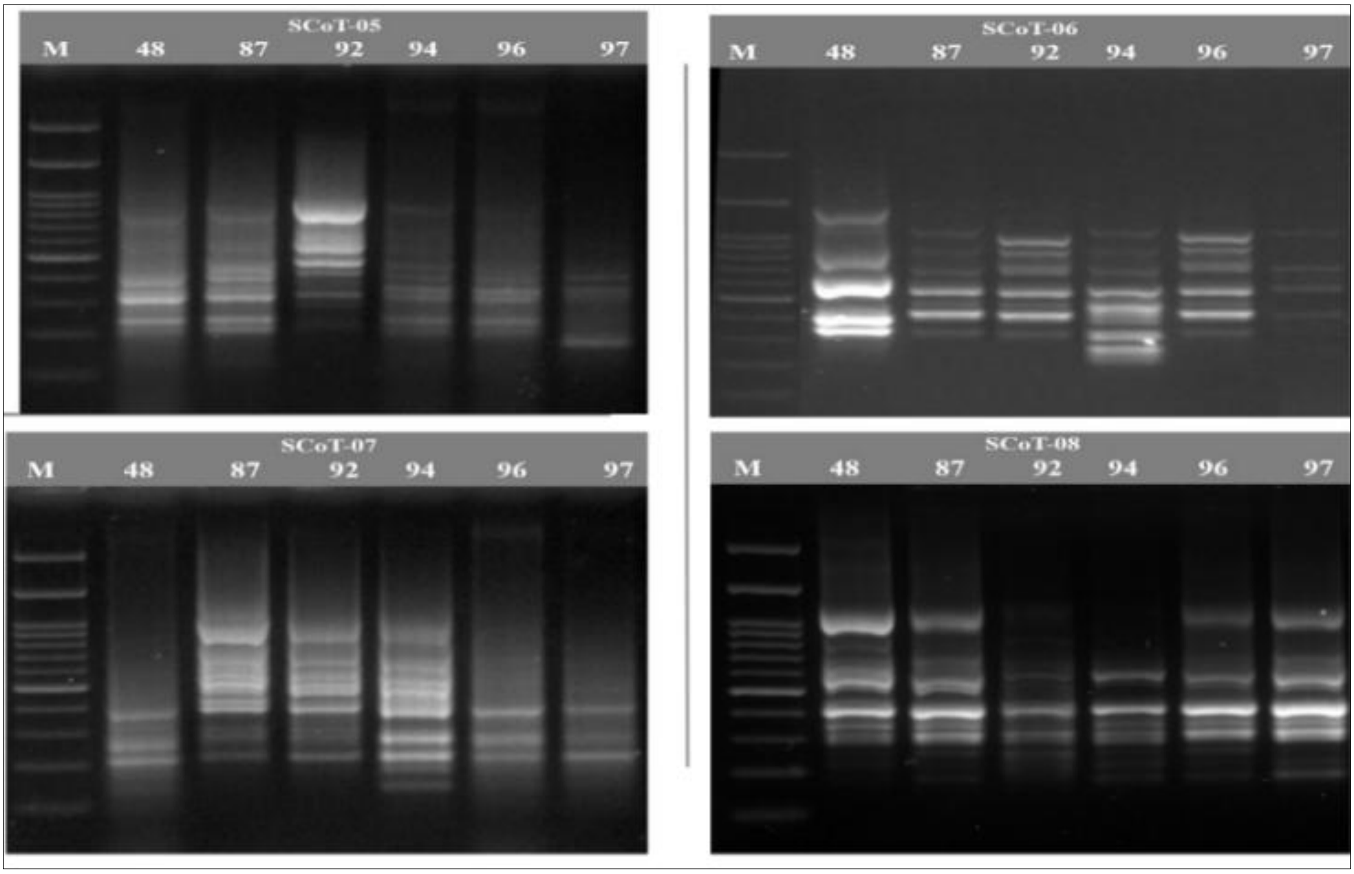

Fig 2: SCoT products on 1% agarose gel using different primers: SCoT 5, SCoT 6, SCoT and SCoT8

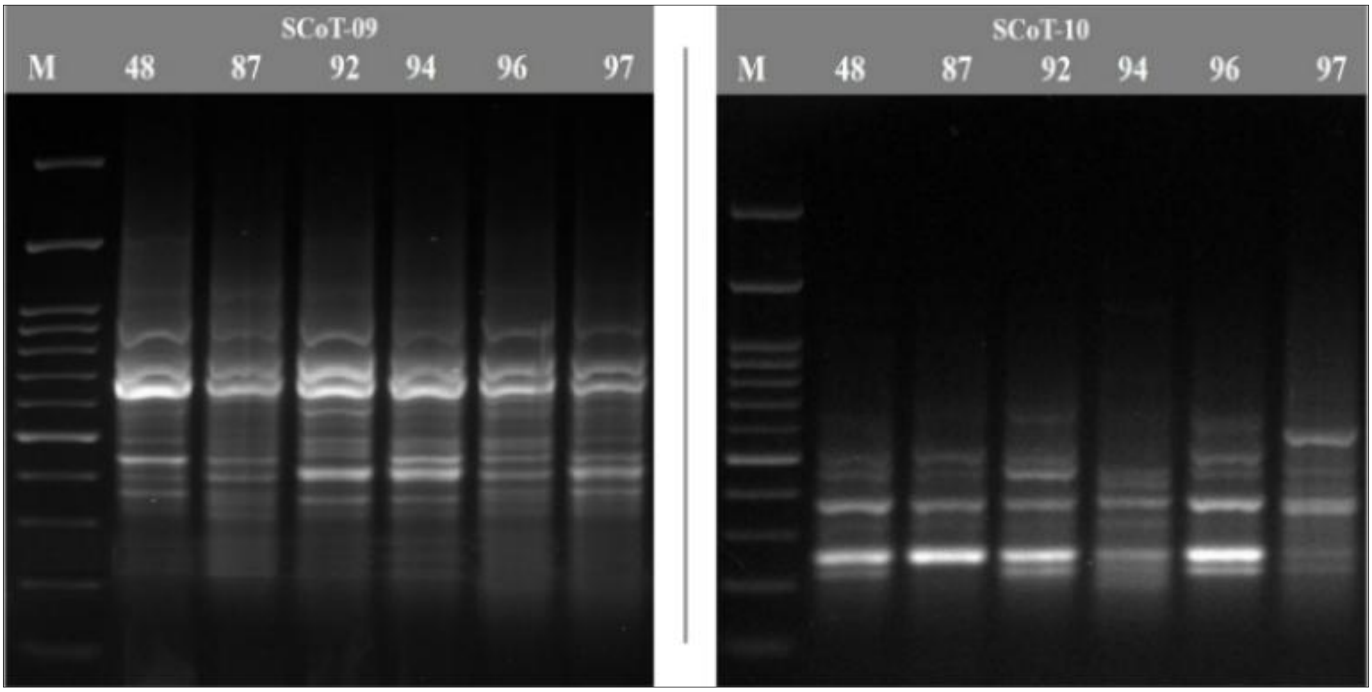

Fig 3: SCoT products on 1% agarose gel using different primers: SCoT 9, and SCoT 10

The dendrogram generated by the UPGMA cluster analysis of CAPS markers based on the Nei and Li coefficient shows the clustering of ten samples (Fig. 4) under study. The investigation is divided into two groups at 0.864. The first one includes accession 48 only. The second group is divided into two subgroups at 0.884. The first subgroup has accessories, 96 and 97, only. Whereas the second subgroup is further divided into sub-subgroups at 0.912, one of them consists of accessions 96 and 87 only. Molecular markers are used to characterize phylogenetic relationships at the genomic DNA level (Madhumati, 2014). SCoT marker design is straightforward depending on a conserved sequence such as ATG surrounding the start codon of translation (Xiong *et al.*, 2011) [34]. Besides these markers are multilocus, which helps obtain high genetic polymorphism. The number of amplicons observed in cotton from the present study is comparable to the results obtained in other studies such as in groundnut (Xiong *et al*., 2011) [34], mango (Luo *et al*., 2010) [23], and Dendrobium Nobile (Bhattachryya *et al*., 2013). The genetic polymorphism generated by SCoT markers can be used for tracing and reconstruction of the breeding history of the domesticated genotypes. Because of their great resolving capacity, SCoT is useful in genetic diversity research (Hamidi *et al*., 2014 and Cao *et al*., 2006) [7]. These markers are effective and low-cost analytical tools for evaluating genetic variants in a wide range of plants.

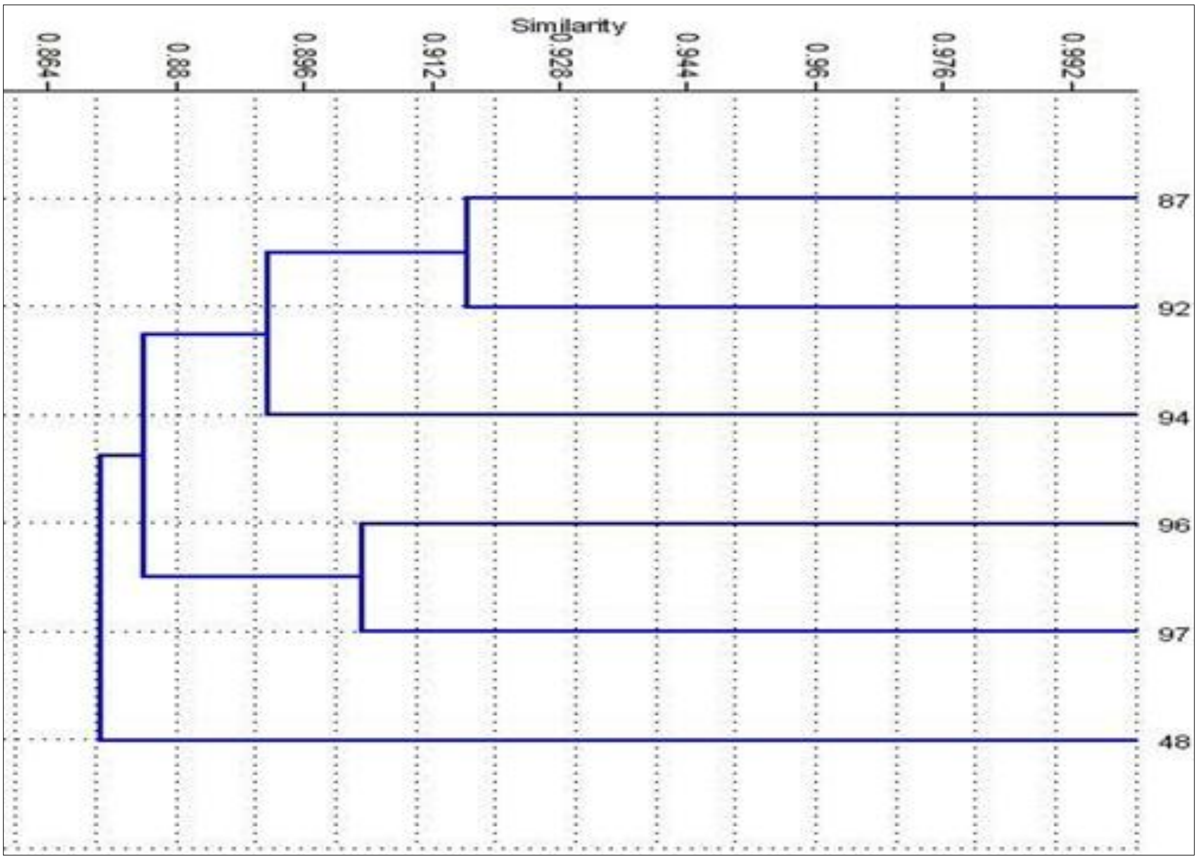

Fig 4: Phenogram obtained by UPGMA cluster analysis based on Nei and Li coefficient generated by SCoT-Markers showing clustering of cotton plants

Conclusion

These experiments were contacted to determine the susceptibility of certain cotton varieties to bollworms infestation; pink and spiny with 7.15 and 7.40, 8.20 and 8.60 % in both seasons, respectively, and it was found that the Giza 94 variety. Maximum infestation of whitefly, adult and nymphs on green leaves (22.00 and 16.15), (33.90 and 34.45) were noted at Giza 94 variety in both seasons, respectively. Expirmentes varieties, Giza 94 was found to be susceptible under ecological conditions and Giza 97 was found to resistant against pink, spiny bollworms and whitefly infestation. This study demonstrated clear differences in performance among the tested six cotton varieties and assessed their resistances to pink, spiny bollworms and whitefly infestation**.** Variations in infestation among varieties were found to be polymorphism and were due to differences in trachoma density, morphological features, and biochemical content, which influenced infestation levels. The SCoT primer analysis successfully demonstrated the utility of this molecular marker system for genetic diversity assessment. With an overall rate of 41.16% and successful amplification of 130 bands, the primer set provides adequate resolution for genetic studies. The identification of high-performing primers (SCoT-09, SCoT-1) and low-performing ones (SCoT-4) will inform future primer selection strategies for similar investigations.

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