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# BIOLOGICAL AND GENETIC OVERVIEW OF *Pectinophora gossypiella* AND ITS ASSOCIATED PARASITOID *Bracon brevicornis* GENOMES USING TWO MOLECULAR MARKERS TECHNIQUES

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**AUTHORS' CONTRIBUTIONS**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

**Bachground:** The comparative between the long-term autumn storage *Pectinophora gossypiella* diapauses larvae (field strain) (durations extended to 4 months) with active larvae (laboratory strain) and its associated field parasitoid *Bracon brevicornis* were investigated.
**Results:** The mean duration of the total immature stages of *B. brevicornis* was 12.5 and 10.0 days, respectively, when reared on *P. gossypiella* diapaused larvae (field strain) and active laboratory strain. The generation time of *B. brevicornis* was 19.2 when reared on field diapaused larvae and 14.9 days for laboratory strain. The Sex ration of *B. brevicornis* estimated by (0.6:0.4) in active larvae and (0.55:0.45) in diapause larvae. The percentage of produced females in progeny was significantly different from that of two cultured parasitoids. Also, results revealed that adult male numbers decreased than female which considerable necessary in the *B. brevicornis* progeny production of following generations. The total eggs number per *B. brevicornis* female was 158.0 when reared on diapaused larvae of *P. gossypiella* field strain, while it increased to 236.0 when reared on active larvae of *P. gossypiella* laboratory strain. Identification of *P. gossypiella* and *B. brevicornis* genomes using two different techniques; SCoT method which conjugate with genes for the first time in Egypt on the host and its parasitoid and ISSR method which conjugate with sequences.
**Conclusion:** using the two techniques had been recognized the most parts of *P. gossypiella* and *B. brevicornis* genomes, our findings may be helpful in genetic and genomic studies.

**Keywords:** *Pectinophora gossypiella*; diapauses; active larvae; *Bracon brevicornis*; biology; genetic profile; SCoT, ISSR.

## 1. INTRODUCTION

In Egypt, up till now, the pink bollworm (PBW), *Pectinophora gossypiella* (Saunder**s**) (Lepidoptera: Gelechiidae) considerable the most important pests attacking the cotton plants and infesting the cotton green bolls. The dry cotton bolls were stored on a low building roof between cotton sticks layers used for heating and cooking, which considerable the infestation source of the next year.

The ecto-gregarious larval parasitoid, *Bracon brevicornis* (wesmeal) (Hymenoptera: Braconidae) is an important biocontrol agent of lepidopterous pests.

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The changes in parasite behavior occur in developmental time, fecundity and sex ratio of parasitoid wasps were affected by some environmental factors such as the active host species or natural environment (temperature) and pesticide used during the control pests in fields during, larval development [1-4]. But a few studies were cared on characteristically and development of parasitoid on diapaused larvae [5].

Interest has been focused in recent years on the development of non-chemical strategies such as cultural, physical, biological and genetic control measures replace of conventional pesticides for the management of stored-product insects [6-7]. The biological control of insect pests' considerable eco-friendly economic and safest approach in integrated pest management.

Start Codon Targeted (SCoT) Polymorphism [8] was developed as a novel technique based on the short conserved region. SCoT markers suggested that primer length and annealing temperature are not the sole factors determining reproducibility, SCoT markers have been used to evaluate genetic polymorphism, identify genotypes, and DNA fingerprinting in various species where it conjugate with genes.

Inter simple sequence repeat (ISSR) markers are considered very useful in determined genetic diversity studies of genetic diversity, phylogeny, genomics and evolutionary biology [9] [10] it conjugate between sequences. Strain identification of *Spodoptera frugiperda* larvae is helpful in developing effective and specific pest management plans to control these insects [11].

This work aimed to study the biology of ecto-parasitoid *B. brevicornis* on field diapause larvae and active laboratory strains of *P. gossypiella*, also, identification of *P. gossypiella* and *B. brevicornis* genomes for the first time in Egypt using two different techniques; SCoT and ISSR analysis technique.

## 2. MATERIALS AND METHODS

To study the rate of developmental, survivor and mortality of *Bracon brevicornis*, the parasitoid were reared on (diapaused larvae of *P. gossypiella*) among diapause (resting) larvae hibernating in dry, unpickable bolls that were left on cotton sticks at end of cotton season compared to active laboratory strain of *P. gossypiella*.

### 2.1 Insect Used

**Collection and culture of *P. gossypiella* diapaused larvae and *B. brevicornis*:** Different stages of the ecto-parasitoid *B. brevicornis* and diapaused full grown larval of *Pectinophora gossypiella* field strain collected from dried cotton bolls, at late of season 2020 Sharkia, Governorate, Egypt, diapaused full grown larval of *Pectinophora gossypiella* kept in long periods under low environmental temperature in the laboratory room for used in parasitoid rearing.

Newly adult emergence (one day old) of the parasitoid resulted were sexed and fed on 10% honey solution and kept at 25± 1°C and 65±5% R.H. with ten (diapaused full grown larvae) of *P. gossypiella* and the same conditions for (laboratory active larvae). Parasitoid reared for two successive generations at 25±1°C and 65±5% RH before beginning the experiments.

**Laboratory strain:** Full-grown larvae of pink bollworm *P. gossypiella*, used in this study was obtained from laboratory colony of Bollworm Department, Plant Protection Research Institute; Agriculture Research Center (ARC), reared on an artificial diet (wheat, milk, yeast, sorbic acid, ascorbic acid and vitamin complex) described by Amer [12].

**Rearing of *B. brevicornis* field strain parasitoid on *P. gossypiella* diapaused and laboratory active larvae host:** To study some biological parameters of *B. brevicornis* field strain parasitoid on *P. gossypiella* field diapaused larvae, under room temperature or *P. gossypiella* active laboratory larvae which kept at 25±1°C and 65±5% RH; Ten individual of *P. gossypiella* larvae (both active laboratory and diapaused *P. gossypiella* host) carried parasitoid eggs (number from 6 to 12 eggs of parasitoid) were removed and kept individually in glass tube (2×7.5 cm) under laboratory condition. It was replicated 3 times to allow parasitism and observed daily to record and estimate the time required for eggs incubation period, larval and pupal duration, percentage of adults emergence and sex ratio were determined.

**DNA isolation procedure:** The bulked DNA extraction was performed using DNeasy insect Mini Kit (Biobasic). Larvae tissues were ground using liquid nitrogen to a fine powder, then, 400 µl of buffer AP1, 5 µl of proteinase k and 5 µl of Lysozyme were added to a maximum of 100 mg of extracted larvae then vortexed vigorously. Mixture was incubated for 10 min at 65$^{o}$C and mixed 2-3 times during incubation by inverting tube. PCR was performed in 30-µl volume tubes according to Williams *et al*. [13].

**Polymerase chain reaction (PCR) condition for ISSR and SCoT:** The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 57º C, and 2 min at 72º C. the reaction was finally stored at 72º C for 10 min and the used primers of ISSR and SCoT techniques are listed in Charts (1 and 2).

## 2.2 Data Analysis

The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by dendrograms were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among cultivars [14].

## 2.3 Statistical Analysis

All obtained data were subjected to one-way analysis of variance (ANOVA). Means were separated by using Least Significant Difference (LSD) test for windows. The biological parameters significance of *B. brevicornis* were observed at P = 0.05 values.

## 3. RESULTS AND DISCUSSION

Obtained data recorded in Table (1) exhibited that the parasitism eggs laid by *B. brevicornis* (field strain) females reared on diapaused (field strain) larvae of *P. gossypiella* required longed time for eggs development (3.6 days) compared to (2.3 days) when reared on *P. gossypiella* laboratory strain.

**Larval and pupal stages:** Data in Table (1) recorded that the times required for completed the mean duration of *B. brevicornis* field strain larval and pupal stages, when reared on *P. gossypiella* diapaused larvae was (7.2 and 5.3 days) significantly longer than when reared on active laboratory strain was (5.4 and 4.6 days), for larval and pupal stages of *B. brevicornis*, respectively. The analysis data recoded high significant differences between reared *B. brevicornis* larvae on two host strains.

**Total of immature stages:** The mean duration of the total immature stages of *B. brevicornis* was significantly different ($P<0.05$) 12.5 and 10.0 days, respectively, when reared on *P. gossypiella* field diapaused larvae and laboratory strain, Table (1).

**Life cycle:** According to data in table (1) showed the time required for complete a generation of *B. brevicornis* when reared on field diapaused larvae and laboratory active strain. Generation time elongated to 19.20 days of *B. brevicornis* reared on field diapaused larvae and it decreased to 14.90 days when reared on laboratory strain.

**Adult emergence and sex ratio:** Analysis of variance of the results arranged in Table (1) recorded high significant decrease of the adult emergence when reared on diapaused (78.0%) than active strain (97.0%).

Sex ration of *B. brevicornis* parasitoid varied when reared on diapaused than active laboratory strain of *P. gossypiella*. The Sex ration (the total production of adult progeny female: male) of *B. brevicornis* estimated by (0.6:0.40) in active larvae and (0.55:0.45) in diapause larvae of *P. gossypiella*. Significant differences between the two cultured parasitoids in the percentage of produced females in progeny. Also, results revealed that population of male decreased than female which considerable necessary in the produced progeny of *B. brevicornis* different generations, Table (1).

**Chart 1. List of the primer names and their nucleotide sequences used in the study for ISSR procedure**

| No | Name | Sequence | No | Name | Sequence |
|---|---|---|---|---|---|
| 1 | 49A | 5` CAC ACA CAC ACA AG 3` | 5 | HB-10 | 5` GAG AGA GAG AGA CC 3` |
| 2 | 44B | 5` CTC TCT CTC TCT CTC TGC 3` | 6 | HB-11 | 5` GTG TGT GTG TGT TGT CC 3` |
| 3 | HB-8 | 5´ GAG AGA GAG AGA GG 3` | 7 | HB-12 | 5`CAC CAC CAC GC 3` |
| 4 | HB-9 | 5`CAC CAC CAC GC 3` | 8 | HB13 | 5´ GAG GAG GAG C 3` |

**Chart 2. List of the primer names and their nucleotide sequences used in the study for SCoT procedure**

| No | Name | Sequence | No | Name | Sequence |
|---|---|---|---|---|---|
| 1 | SCoT 2 | ACC ATG GCT ACC ACC GGC | 4 | SCoT 9 | ACA ATG GCT ACC ACT GCC |
| 2 | SCoT 5 | CAA TGG CTA CCA CTA GCG | 5 | SCoT 10 | ACA ATG GCT ACC ACC AGC |
| 3 | SCoT 8 | ACA ATG GCT ACC ACT GAG | 6 | SCoT 11 | ACA ATG GCT ACC ACT ACC |

**Table 1. Effect of two strains of *P. gossypiella* on total immature stages and generation time of *B. brevicornis***

| Strain used | Different stages resulted from adults treated with three compounds ( Duration times in days) ( days ± SE) | | | | | | |
|---|---|---|---|---|---|---|---|
| | eggs Incubation | larval stage | Pupal stage | Total immature stage | Life cycle | Pre- ovi | Generation time |
| diapause | 3.60[a]±0.1 | 7.20[a]±0.5 | 5.30[a]±0.3 | 12.50[a]±0.7 | 16.10[a]±1.3 | 3.10[a]±0.1 | 19.20[a]±1.5 |
| active | 2.30[b]±0.2 | 5.40[b]±0.3 | 4.60[a]±0.4 | 10.00[a]±0.42 | 12.30[b]±1.1 | 2.60[a]±0.1 | 14.90[b]±0.9 |
| LSD | 0.585 | 0.883 | 0.628 | 1.959 | 1.608 | 1.203 | 1.783 |
| P | .0043[**] | .0099[**] | .1277[ns] | .0955[ns] | .0028[**] | .8852[ns] | .0021[**] |
| F | 34.22 | 21.27 | 3.673 | 4.723 | 43.033 | 0.024 | 49.85 |

*Values are mean ± SE of three replicates. Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05)*

**Table 2. Effect of *P. gossypiella* strains on different stages of *B. brevicornis***

| Strain used | adult emergence | | | Duration times in days (longevity ± SE) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Ovipostional periods | | Longevity | |
| | % adult emergence | Sex ratio female | male | Oviposition | Post-oviposition | Female | Male |
| diapause | 78.00[b] | 0.60[a] | 0.40 | 9.60[b]±0.30 | 3.30[b]±0.20 | 16.0[b]±1.2 | 11.0[b]±0.30 |
| active | 97.00[a] | 0.55[a] | 0.45 | 11.50[a]±0.60 | 4.30[a]±0.30 | 18.4[a]±1.4 | 13.5[a]±0.90 |
| LSD | 14.066 | 0.115 | 0.065 | 1.540 | 0.925 | 2.182 | 1.823 |
| P | .0143[*] | .4255[ns] | .1161[ns] | .0252[*] | .0126[*] | .0412[*] | .0174[*] |
| F | 17.182 | 0.786 | 4.00 | 12.144 | 18.49 | 8.813 | 15.281 |

*Values are mean ± SE of three replicates. Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05)*

**Oviposition period:** As shown in Table (2) the oviposition period required for adult female laid the eggs. The oviposition period was considerable shorter when *B. brevicornis* reared on diapause larvae (9.6 days) compared with (11.6 days) when reared on active larvae, statistical analysis indicated significant differences between the two ovipositional periods.

As noticed from Table (2) statistical analysis showed that the pre-oviposition period for parasitoid female was insignificant ($P$<0.05) when reared on diapause larvae field strain and laboratory strain of *P. gossypiella*. It estimated by 3.10 days on diapause larvae field strain and 2.60 days on laboratory strain. The post-oviposition period was 3.3 days when reared *B. brevicornis* on field diapause larvae and increased to 4.3 days on active laboratory strain of *P. gossypiella*.

Based on data recorded in Table (2), it was noticed that the longevity of parasitoid female was (16.0 days when reared on field strain and 18.4 days on laboratory strain). On the other hand, the correspondent male longevity was (11.0 and 13.5 days) resulted from rearing *B. brevicornis* on diapaused field strain and laboratory strain of *P. gossypiella* respectively.

Data in Fig. (1) Showed that total eggs number laid per *B. brevicornis* female was high decreased to 158.0 when reared on diapause field strain of *P. gossypiella*, while it reached to 236.0 when reared on active laboratory strain of *P. gossypiella.* At the same time, the eggs fertility was decreased to 80% when reared on diapause field strain of *P. gossypiella*, whereas, it increased to 93.0% when reared on active laboratory strain of *P. gossypiella.*

**Analysis of *P. gossypiella* genome using ISSR and SCoT techiique**s: Data illustrated in Table (3) and Fig. (2) Shows six ISSR primers (49A, 49B, HB-9, HB-10, HB-11 and HB-12) used to identification the *P. gossypiella* genome profile, these six primers resulted in different bands between 2 and 5, varying from 200 to 830 base pairs for each primer. There were five bands in primer 49A by molecular weight ranged between 290 to 830 bp. while, primer 49B had four bands with molecular weight ranged between 290 to 540bp. whereas, primer HB-9 ranged between 290 to 640bp, primer HB-10 had four bands varied from

345 to 700 bp. And, primer HB-11 had four bands varied from 200 to 500 bp. Finally, primer HB-12 had two bands 415 and 500bp.

Ten bands appeared in all primers Between 2 and 5 different bands, varying from 200 to 830 base pairs, were obtained by six primers screened of ISSR technique presented in Table (3) and Fig. (2). A unique band appeared in ISSR (49A) primer with band number 1 and 4 by 830 and 600bp, also, in HB-10 with band number 2 and 8 by 700bp and 345 while, 640bp band number 3. Whereas, polymorphic band with all primers by 415 bp in band number 7.

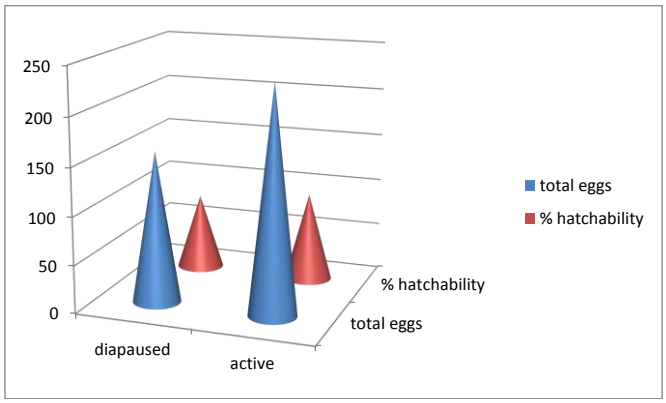


**Fig. 1. Effect of host *P. gossypiella* strains on fecundity and fertility females stages of *B. brevicornis***

**Table 3. Numbers and DNA bands molecular weight by six ISSR primers for *P. gossypiella* larvae**

| Band No | M.W bp | *ISSR Primers* 49A | 49B | HB-9 | HB-10 | HB-11 | HB-12 |
|---|---|---|---|---|---|---|---|
| 1 | 830 | 1 | - | - | - | - | - |
| 2 | 700 | - | - | - | 1 | - | - |
| 3 | 640 | - | - | 1 | - | - | - |
| 4 | 600 | 1 | - | - | - | - | - |
| 5 | 540 | - | 1 | 1 | 1 | - | - |
| 6 | 500 | 1 | 1 | 1 | - | 1 | 1 |
| 7 | 415 | 1 | 1 | 1 | 1 | 1 | 1 |
| 8 | 345 | - | - | - | 1 | - | - |
| 9 | 290 | 1 | 1 | 1 | - | 1 | - |
| 10 | 200 | - | - | - | - | 1 | - |
| Total | | 5 | 4 | 5 | 4 | 4 | 2 |

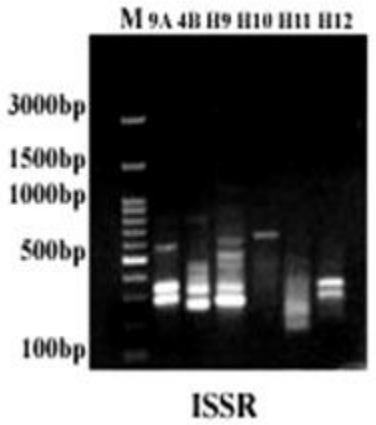


**Fig. 2. ISSR patterns of the *P. gossypiella* larvae revealed by seven primers**

Six SCoT primers appeared in this technique to identify *P. gossypiella* genome profile resulted in different bands between 3 and 8 varying from 200 to 935 base pairs for each primer shown in Table (4) and Fig.(3).

In primer SCoT2 and SCoT5 there were four bands by molecular weight ranged between 200 to 530 bp for SCoT2 and between 220 to 460 bp SCoT5. Primers SCoT8 and SCoT11 had three bands with molecular weight ranged between 230 to 380 bp in SCoT8 and from 720 to 420 bp for SCoT11. Whereas, SCoT9 had eight bands varied from 290 to 935 bp. while, SCoT10 had five bands with molecular weight varied between 720 to 290bp. A unique band appeared in SCoT1, SCoT10, SCoT11 and SCoT12 primers with 200, 220, 230 and 935bp, while, five polymorphic bands between SCoT2 to SCoT primers with 290bp.

**Analysis of *B. brevicornis* genome using ISSR and SCoT techiques:** Data illustrated in Table (5) and Fig. (4) showed six ISSR primers (HB-8, HB-10, HB-11, HB-12 and HB-13) used to identification the *B. brevicornis* genome profile, five primers resulted in different bands between 1 to 6 varying from 240 to 1320 base pairs for each primer. There were four bands in primer HB-8 by molecular weight ranged between 335 to 920 bp. while, primer HB-10 had six bands with molecular weight ranged between 335 to 800bp. whereas, primer HB-11 had only one primer at 240bp, two bands in primer HB-12 at 240 and 280 bp and HB-13 at 425 and 335bp.

Nine bands appeared in all primers varied from 240 to 1320 base pairs, were obtained by five primers screened of ISSR technique presented in table (5). A unique band appeared in ISSR (HB-8 at band number 2 by 920bp, also, HB-10 at band number 1, 3 and 4 by 1320, 800 and 615 and HB-12 at band number 8 at 280bp), while, polymorphic bands with (HB-8, HB-10 and HB-13) primers at bands number 6 and 7by 425 and 335 bp.

**Table 4. Numbers and DNA bands molecular weight by six SCoT primers for *P. gossypiella* larvae**

| Band No | M.W bp | *SCoT Primers* | | | | | |
|---|---|---|---|---|---|---|---|
| | | **SCoT2** | **SCoT5** | **SCoT8** | **SCoT9** | **SCoT10** | **SCoT11** |
| 1 | 935 | - | - | - | 1 | - | - |
| 2 | 720 | - | - | - | 1 | 1 | 1 |
| 3 | 640 | - | - | - | 1 | - | - |
| 4 | 530 | 1 | - | - | 1 | - | - |
| 5 | 500 | - | - | - | 1 | 1 | 1 |
| 6 | 460 | - | 1 | - | 1 | 1 | - |
| 7 | 420 | - | - | - | 1 | 1 | 1 |
| 8 | 380 | 1 | 1 | 1 | - | - | - |
| 9 | 290 | 1 | 1 | 1 | 1 | 1 | - |
| 10 | 230 | - | - | 1 | - | - | - |
| 11 | 220 | - | 1 | - | - | - | - |
| 12 | 200 | 1 | - | - | - | - | - |
| Total | | 4 | 4 | 3 | 8 | 5 | 3 |

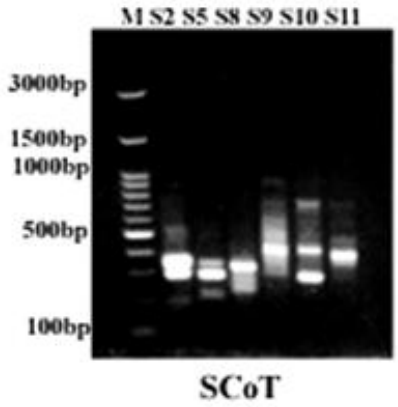


**Fig. 3. SCOT patterns of the *P. gossypiella* larvae revealed by six primers**

**Table 5. Numbers and DNA bands molecular weight by five ISSR primers for *B. brevicornis* larvae**

| Band No | M.W bp | *ISSR Primers* | | | | |
|---|---|---|---|---|---|---|
| | | HB-8 | HB-10 | HB-11 | HB-12 | HB-13 |
| 1 | 1320 | - | 1 | - | - | - |
| 2 | 920 | 1 | - | - | - | - |
| 3 | 800 | - | 1 | - | - | - |
| 4 | 615 | - | 1 | - | - | - |
| 5 | 500 | 1 | 1 | - | - | - |
| 6 | 425 | 1 | 1 | - | - | 1 |
| 7 | 335 | 1 | 1 | - | - | 1 |
| 8 | 280 | - | - | - | 1 | - |
| 9 | 240 | - | - | 1 | 1 | |
| Total | | 4 | 6 | 1 | 2 | 2 |

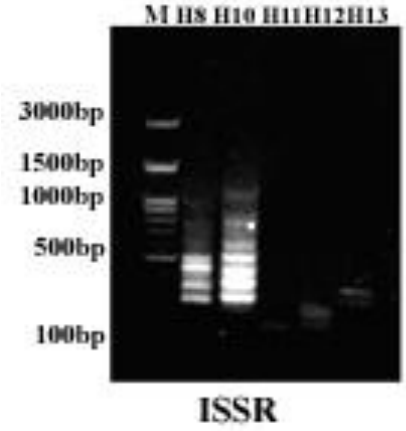


**Fig. 4. ISSR patterns of the *B. brevicornis* larvae revealed by five primers**

Five SCoT primers were used in this technique to identify *B. brevicornis* genome profile resulted in detection of different bands between 2 and 6 bands in each primer, varying from 210 to 1040 base pairs for each primer shown in Table (6) and Fig. (5).

In primer SCoT2 and SCoT8 there were two bands by molecular weight ranged between 330 and 370 bp for SCoT2 and between 210 and 370 bp SCoT5. SCoT9 had four bands with molecular weight ranged between 400 to 645 bp, whereas, SCoT10 had six bands varied from 330 to 1040 bp, while, SCoT11 had three bands with molecular weight varied between 400 to 700bp. A unique band appeared in SCoT8, SCoT9 primers with 210, 645bp and also, unique primer in SCoT10 primers with 910 and 1040bp, while, three polymorphic bands between SCoT9, SCoT10 and SCoT11 primers with 400bp.

## 4. DISCUSSION

*Bracon brevicornis* is a cosmopolitan, gregarious, ectoparasitoid that attacks the active or diapaused larval stage of several lepidopteran species. Results obtained in the present study assure that the host strains two types of *P. gossypiella* had great impact on all immature stages and longevity of parasitoid adults. Because, like other insects, *B. brevicornis* or *P. gossypiella* also require continuous supply of balanced food and optimum condition to perform their activities.

Several studies have shown that the survival, duration, male and female longevity, sex ratio, fecundity and fertility of *B. brevicornis* largely depend on active or laboratory reared hosts, but a few authors have reported these studies on field diapaused hosts, it is very important for use of parasitic wasps like *B. brevicornis* in agricultural and cold environmental systems. In this studies significant variation was observed in (development time) of *B. brevicornis* from eggs to adult stage when reared on diapaused larvae compared with active laboratory larvae of *P. gossypiella* because the laboratory strain larvae reared on artificial diet contents (wheat, milk, yeast, sorbic

**Table 6. Numbers and DNA bands molecular weight by five SCoT primers for *B. brevicornis* adults**

| Band No | M.W bp | *SCoT Primers* | | | | |
|---|---|---|---|---|---|---|
| | | SCoT2 | SCoT8 | SCoT9 | SCoT10 | SCoT11 |
| 1 | 1040 | - | - | - | 1 | - |
| 2 | 910 | - | - | - | 1 | - |
| 3 | 700 | - | - | - | 1 | 1 |
| 4 | 645 | - | - | 1 | - | - |
| 5 | 530 | - | - | 1 | - | 1 |
| 6 | 475 | - | - | 1 | 1 | - |
| 7 | 400 | - | - | 1 | 1 | 1 |
| 8 | 370 | 1 | 1 | - | - | - |
| 9 | 330 | 1 | - | - | 1 | - |
| 10 | 210 | - | 1 | - | - | - |
| Total | | 2 | 2 | 4 | 6 | 3 |

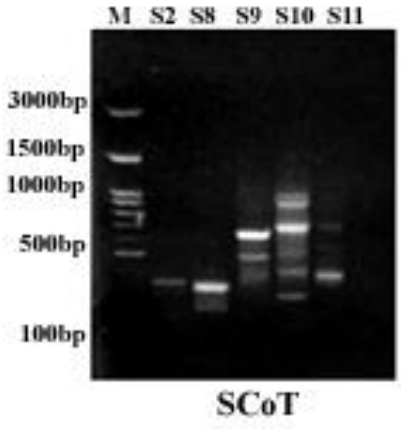


**Fig. 5. SCoT patterns of the *B. brevicornis* larvae revealed by five primers**

acid, ascorbic acid and vitamin complex). But, the diapause larvae might be dependent upon lipids of cotton seeds Farag and Omar [15]. In the present studies, the parasitoid female percentage resulted was significantly affected by the diapaused host than which reared on active larvae of *P. gossypiella*. Highest percentage of adult survivorship was significant when *B. brevicornis* reared on laboratory larvae of *P. gossypiella*. The final progeny of *B. brevicornis* life cycle was less when reared on field diapaused larvae because of adult female still in searching for the suitable place to deposited her eggs and after egg hatchability, the parasitoid larvae search for host larvae to complete the larval period and facing difficulties in find *P. gossypiella* larvae because it hides inside cotton seed before diapause.

The present results are mostly in accordance with the above reported findings as mentioned, Thanavendan and Jeyarani [16] recorded that the egg-adult development time was 7.2–15.13 days, male and female longevity were 12.55–20.21 and 10.78–17.57 days of *B. brevicornis* parasitizing chickpea diet fed *H. armigera* were resembles to *B. hebetor* egg-adult development time and male, female longevity. Also, [17] [18] [3] recorded that food consumption, especially from sugar rich sources, enhance the reproductive potential and insect parasitoid longevity. In addition, cold storage could improve genetic stability as few generations are reared [19]]. Also, the parasitoid may result in a probable loss of its major fitness especially mobility, survival rate, fecundity, longevity and sex ratio [20].

In addition, the present results show that the diapaused strain host of *P. gossypiella* which collected from field and kept in long periods under low environmental temperature may affect the parasitism rate as well as the total progeny of the parasitoid resulted and sex ratio. Same authors' studied that the cold storage is regarded as an important tool for storage of mass reared biocontrol agents [21]. Storage of the natural enemies at low temperature also permits coordinated field releases of

natural enemies during the crucial stages of pest occurrences [22] [23] After comparison study between rearing a field *B. brevicornis* strain on field strain diapaused larvae and active laboratory strain of *P.gossypiella* using primers of the two techniques of ISSR and SCoT had been recognized the most parts of the genome of *P. gossypiella* and so *B. brevicornis*. In this respect, many authors showed different genetic studies to identify differentiations between insect genotypes or to evaluate genetic polymorphism, Levy *et al*. [11] used fragment length polymorphism (RFLP) marker to analyze the two morphologically host associated strains of *Spodoptera frugiperda*, and found few nanograms of total DNA are needed to yield clear and accurate strain identification of individual insects. Whereas, in the population genetic study of *H. armigera* populations, Endersby *et al*. [24] excluded 3 pairs of SSR markers with the greatest null allele frequencies (i.e., 19.2%, 31.6% and 47.4%). While, Luque *et al.* [25] shown that some ISSR amplifications are possible and demonstrate their applicability in studying intra- and inter-specific variation in some Noctuid populations they found primer gives the most informative profiles. And, Fang *et al* [26] provides the genetic characterization of the gypsy moth using five polymorphic Inter simple sequence repeat markers which produced reproducible banding patterns and revealed that ISSR markers are a highly informative and efficient tool for estimating the genetic structure of the insect. Also, Moustafa *et al*., [27] used the SCoT and ISSR techniques for *E. insulana* genome profile characterization.

## 5. CONCLUSIONS

This study concluded that the food consumption had high effect on *P. gossypiella* host and its parasitoid *B. brevicornis*. Identification of *P. gossypiella* and *B. brevicornis* genomes by SCoT analysis technique used for the first time in Egypt and ISSR analysis technique, using of these two techniques had been recognized the most parts of genome in both *P. gossypiella* and *B. brevicornis*.

## DECLARATIONS

| **Ethics approval and consent to participate** | | **Not applicable** |
|---|---|---|
| Consent for publication | Applicable | |
| Availability of data and material | All data in the tables are so clear and these numbers are the raw data | |
| Competing interests | | Not applicable |
| Funding | | Not applicable |
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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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