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GENETIC VARIATIONS AMONG HOST-ASSOCIATED POPULATIONS OF *BEMISIA TABACI* (GENN.) IN EGYPT (HEMIPTERA: STERNORRHYNCHA: ALEYRODIDAE)

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ABSTRACT

Genetic variations among six different host-associated populations of *Bemisia tabaci* (Genn.) were determined using two isozymes electrophoresis (esterase and malate dehydrogenase) as well as RAPD-PCR molecular technique using seven different arbitrary primers. The two different techniques showed highly polymorphic variations among the studied populations. These results indicated that *B. tabaci* may have different biotypes on adaptations to certain host plant species in Egypt.

Key Words: Electrophoresis, RAPD-PCR, Isozymes, Molecular, *Bemisia tabaci*, Host-plants, Esterases, Malate-dehydrogenase, Biotypes.

INTRODUCTION

Bemisia tabaci (Genn.) has become a prominent pest in worldwide bases especially in tropical and sub-tropical agroecosystems. Millions of dollars have been lost as a result of direct feeding damage and plant diseases caused by whitefly transmitted geminiviruses (**Costa & Brown, 1991; Brown *et al* 1992 and Costa *et al* 1993**). The existence of biotypes or host races of *B. tabaci* was proposed in the 1950s after the discovery that morphologically indistinguishable populations of *B. tabaci* exhibited measurably different biological traits with respect to host range, host-plant adaptability and plant virus transmission capabilities (**Muniz & Nombela, 2001; Muniz *et al* 2002; Jiang *et al* 2004; Muniz *et al* 2004 and Pascual & Callejas, 2004**).

Electrophoresis techniques have been used extensively for genetic surveys of natural populations, so several authors used electrophoretic patterns of isozymes especially esterase to determine host associated or/and geographical biotypes of *B. tabaci* (**Costa & Brown, 1991; Burban *et al* 1992; Liu *et al* 1992; Brown *et al* 1995 and Homam, 2000**). Also in recent years, the development of molecular markers based on nucleic acids technology has provided new insights in the study of *B. tabaci* variations (**Perring *et al* 1993; Guirao *et al* 1997; Cervera *et al* 2000; Moya *et al* 2001; Horowitz *et***

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***al* 2003; Delatte *et al* 2005 and Sseruwagi *et al* 2005).**

This work aims at detecting biochemical and molecular genetic diversity in *B. tabaci* populations according to host plants in Egypt.

MATERIAL AND METHODS

1. Collecting & Preserving Materials

To detect variations among host plants associated populations of *B. tabaci* adults samples of infested hosts were collected in the field as pupae from six host plants; Egg-plant, *Solanum melongena*; Lantana, *Lantana camara*; Cotton, *Gossypium barbadense*; Watermelon, *Citrullus vulgaris*; Squash, *Cucurbita pepo ovifera* and okra, *Hibiscus esculantus* at Qalyubiya Governorate. These samples were brought to the laboratory. The adults emerged from the six hosts were aspirated and collected. Samples were deep-frezed and stored at -20°C until analysis.

2. Isozymes Electrophoresis

Thirty adults of each population were homogenized in Eppendorf tubes containing 50 µl of extraction buffer (0.1 M Tris-Borate EDTA buffer pH 7.0 containing 10% sucrose) by aid of a handle plastic homogenizer. Tubes were centrifuged for 10 min at 10.000 rpm at 5°C. The supernatants were transferred to new eppendorf tubes and kept at deep- freeze until use for electrophoretic analysis. Vertical polyacrylamide gel 8% was used, the gels were completely covered with electrode buffer. The electrodes were connected to power supply and adjusted at 200V for two hours. The gels were stained after electrophoresis according to its system, staining for esterase was in 100 mM Na-phosphate buffer, pH 6.0, using α-β naphthyl acetate as substrates in the ratio α: β= 9:1, while staining for Malate dehydrogenase was in 50 mM Tris-HCl, pH 8.5, using Malic acid as a substrate with NAD, MTT and PMS. Gels were incubated at 37 °C in dark for complete staining, after that gels were fixed in a 1:5:5 mixture of acetic acid, ethanol and water for 24 hours and rinsed with distilled water two times (**Stegemann *et al* 1980**). All gels were photographed and scanned using Bio-Rad GelDoc2000.

3. Randomly Amplified Polymorphic DNA of the Polymerase Chain Reaction (RAPD-PCR)

PCR was conducted for thirty adults of each population that were crushed in 1.5 ml Eppendorf tubes to extract the genomic DNA according to a protocol described by **Cenis *et al* 1993**.

All populations were tested against seven 10-mer random primers. All primers were synthesized by Operon Technology (USA) as follows:

OPA-9: 5'GGGTAACGCC'3
OPB-7: 5'GGTGACGCAG'3
OPA-12: 5'TCGGCGATAG'3
OPB-3: 5'CATCCCCCTG'3
OPA-15: 5'TTCCGAACCC'3
OPA-19: 5'CAAACGTCGG'3
OPA-18: 5'AGGTGACCGT'3

All gels resulted from both isozymes and RAPD-PCR techniques were scanned using Bio-Rad GelDoc2000.

RESULTS & DISCUSSION

1. Isozymes Electrophoresis

1.1. α-β naphthyl acetate Esterases (Est)

Electrophoretic patterns of esterase isozymes (α-β) showed highly polymorphism across the six host plant-associated populations (**Fig. 1, A and Table, 1**). A total number of 10 enzymatic bands were detected. The least number of bands was four bands found in population of *B. tabaci* on okra, while the highest number of bands was eight bands found in populations of cotton. Six bands were detected in egg-plant *B. tabaci* population only. Five bands were found in both lantana and squash populations. While population from watermelon showed seven esterase bands. Band no. 3 was specific for lantana population. Also bands no. 1, 2 and 4 were specific bands for two *B. tabaci* populations on both cotton and watermelon. While band no. 8 was specific band for populations of both eggplant and squash. Also band no. 10 was specific band for populations of both eggplant and cotton. Bands number 5, 6, 7 and 9 were detected to be common bands for all the six host-associated populations.

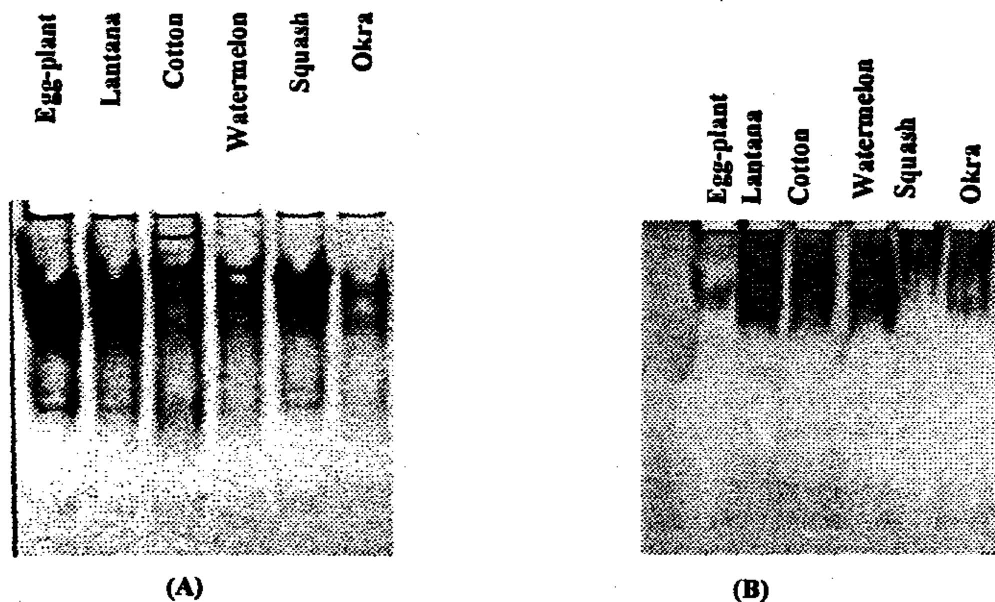

Fig 1. Electrophoretic patterns of two isozymes (A) esterase isozymes (Est) and (B) malate dehydrogenase isozymes (Mdh) for six host plant-associated populations of *B. tabaci*.

Table 1. Two isozymes (Est &Mdh) patterns extracted from six host-associated populations of *B. tabaci* in Qalyubiya, Egypt.

Isozyme	Band	eggplant	lantana	cotton	watermelon	squash	okra
Est	1	0	0	1	1	0	0
	2	0	0	1	1	0	0
	3	0	1	0	0	0	0
	4	0	0	1	1	0	0
	5	1	1	1	1	1	1
	6	1	1	1	1	1	1
	7	1	1	1	1	1	1
	8	1	0	0	0	1	0
	9	1	1	1	1	1	1
	10	1	0	1	0	0	0
Mdh	1	0	1	1	1	1	1
	2	1	1	1	1	1	1
	3	0	0	1	0	0	1
	4	0	1	0	1	0	0

(1= present, 0= absent)

1.2. Malate dehydrogenase (Mdh)

Electrophoretic patterns of (Mdh) showed less polymorphism across the different six *B. tabaci* populations than those detected by α-β naphthyl acetate Esterases (**Fig. 1, B and Table 1**). Only four polymorphic enzymatic bands were detected with relative migration (Rm) ranged from 0.072 to 0.553 μm. The highest number was three bands which detected in four *B. tabaci* populations (lantana, cotton, watermelon and okra). While two bands were detected in squash population only. Only one band was detected in *B. tabaci* as specific band for eggplant population.

Band no. 3 was detected as specific for both cotton and okra populations. While Band no. 4 was detected as specific for both lantana and watermelon *B. tabaci* populations. Band no. 2 was established as a common band for the all six host-associated populations of *B. tabaci*.

These results indicate that isozymes electrophoresis specially for general esterases and newly for Malate-dehydrogenase could be used successfully to determine variations among different *B. tabaci* populations that morphologically indistinguishable.

These results are in agreement with those obtained by several authors used isozyme electrophoresis especially esterases to detect geographical or/and host-associated biotypes of *B. tabaci* (**Costa & Brown 1991; Liu *et al* 1992; Legg *et al* 1994; Brown *et al* 1995 and Guirao *et al* 1997**). Also several authors used this technique to distinguish differences among different known whitefly species as well as identify new ones (**Bellows *et al* 1994; Oliveira *et al* 1997 and Helmi, 2003**).

2. Randomly Amplified Polymorphic DNA of the Polymerase Chain Reaction (RAPD-PCR)

Seven 10-mer arbitrary primers were used in this study which amplified a total number of 232 DNA fragments 224 of these fragments were polymorphic (Fig. 2 & Table, 2) while the other eight fragments detected as common among the six tested host-associated populations.

2.1. Primer OPA-09

The total number of PCR products generated by this primer was 29 bands ranged from 4 to 7 bands with molecular weight (MW) ranged from 60 to 1148 bp. The lowest number of bands (4 bands) was detected in Lantana *B. tabaci* population, while the highest number (7 bands) was found in okra population. Six bands were detected in three *B. tabaci* populations (eggplant, watermelon and squash). While no PCR fragments found in cotton *B. tabaci* population. Three specific bands with MW of 799, 464 and 188 bp were determined for eggplant *B. tabaci* population. Also three specific bands with MW of 387, 343 and 157 bp were found for watermelon population. Two specific bands with MW of 1148 and 628 bp were found for okra population. Only one specific band with MW of 411 bp was detected for lantana *B. tabaci* population. No common bands were found among the six host-associated populations.

2.2. Primer OPA-12

The total of PCR fragments amplified by this primer were 30 fragments ranged from 2 to 7 bands with MW ranged from 27 to 923 bp. The lowest number of bands (2 bands) was found in population of *B. tabaci* on squash. While the highest number (7 bands) was detected in three *B. tabaci* populations (eggplant, cotton and watermelon). Three *B. tabaci* populations (eggplant, lantana and okra) had specific bands with MW of 369, 55 and 157 bp for the three populations, respectively. Two common bands with MW of 121 and 27 bp were distinguished for the six tested host-associated populations.

2.3. Primer OPA-15

The total PCR fragments generated by this primer were 24 fragments with MW ranged from 244 to 3277 bp. The lowest number of bands (2 bands) was found in two *B. tabaci* populations (eggplant and cotton). While the highest number (9 bands) was detected in watermelon *B. tabaci* population. Four bands were found in both squash and okra *B. tabaci* populations. Five specific bands with MW of 3277, 2033, 1645, 1330 and 1196 bp were detected for the watermelon population. While one specific band only with MW of 414 bp was detected for the lantana population. Two common bands with MW of 1020 and 870 bp were distinguished for the six tested host-associated populations.

2.4. Primer OPA-18

The total PCR products generated by this primer were 44 bands with MW ranged from 644 to 79. The lowest number of bands (4 bands) was found in okra *B. tabaci* population. While the highest number (9 bands) was detected in lantana *B. tabaci* population. Seven bands were found in two *B. tabaci* populations

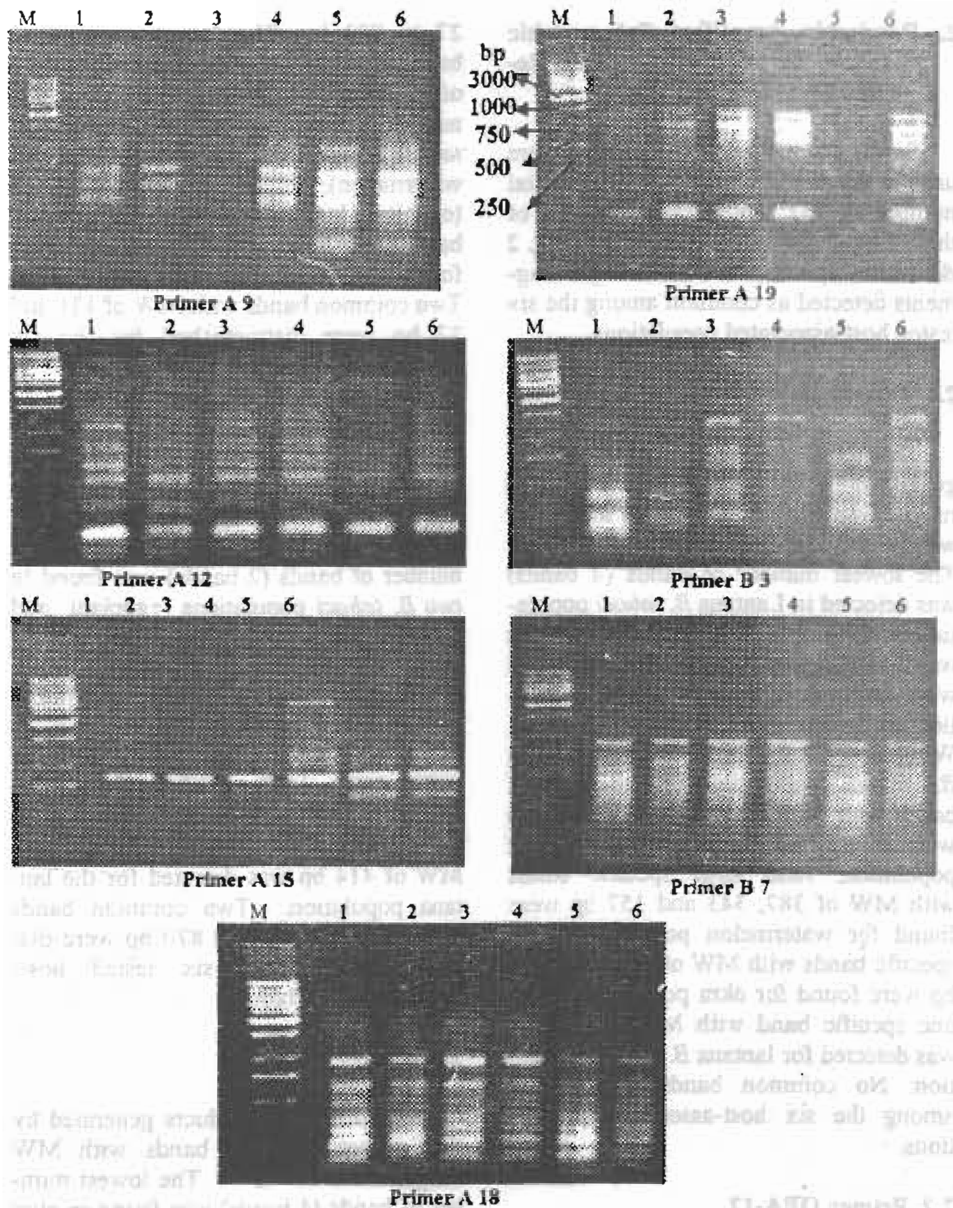

Fig 2. RAPD-PCR banding patterns of six *B. tabaci* host-associated populations by using seven random primers.
M, DNA marker; **bp,** base pair **1,** eggplant; **2,** lantana; **3,** cotton; **4,** watermelon; **5,** squash; **6,** okra.

Table 2. DNA polymorphism among six host-associated populations of *B. tabaci* using RAPD-PCR against seven different primers.

primer	MW (bp)	eggplant	lantana	cotton	watermelon	squash	okra
OpA-09	1148	0	0	0	0	0	1
	799	1	0	0	0	0	0
	667	0	0	0	1	1	0
	628	0	0	0	0	0	1
	464	1	0	0	0	0	0
	411	0	1	0	0	0	0
	387	0	0	0	1	0	0
	365	1	0	0	0	1	1
	343	0	0	0	1	0	0
	270	1	1	0	0	0	0
	254	0	0	0	1	1	1
	199	0	1	0	1	0	0
	188	1	0	0	0	0	0
	166	0	0	0	0	1	1
	157	0	0	0	1	0	0
	109	0	0	0	0	1	1
	67	1	1	0	0	0	0
	60	0	0	0	0	1	1
OpA-12	923	0	0	1	1	0	0
	623	1	0	1	1	0	0
	449	0	0	1	1	0	0
	369	1	0	0	0	0	0
	233	1	1	1	1	0	0
	179	1	0	1	1	0	0
	157	0	0	0	0	0	1
	121	1	1	1	1	1	1
	59	1	0	0	0	0	0
	55	0	1	0	0	0	0
	27	1	1	1	1	1	1

Table 2. Cont.

primer	MW (bp)	eggplant	lantana	cotton	watermelon	squash	okra
OpA-15	**3277**	0	0	0	1	0	0
	2033	0	0	0	1	0	0
	1645	0	0	0	1	0	0
	1330	0	0	0	1	0	0
	1196	0	0	0	1	0	0
	1020	1	1	1	1	1	1
	870	1	1	1	1	1	1
	437	0	0	0	1	1	1
	414	0	1	0	0	0	0
	244	0	0	0	1	1	1
OpA-18	**644**	1	1	1	1	1	1
	548	0	1	0	0	0	0
	519	0	0	1	0	0	0
	376	1	1	1	1	1	1
	287	1	0	0	0	0	0
	272	0	1	1	1	1	0
	186	0	1	1	1	1	0
	150	0	0	1	1	1	0
	142	1	1	0	0	0	1
	121	1	1	0	0	0	0
	115	0	0	1	1	1	1
	109	1	1	0	0	0	0
	92	1	0	0	0	0	0
	79	1	1	1	1	1	0
OpA-19	**1467**	0	0	1	1	0	0
	1055	1	1	0	0	0	0
	945	0	0	1	1	0	1
	802	1	1	0	0	0	0
	759	0	0	1	1	0	1
	644	1	0	0	0	0	0
	609	0	1	1	1	0	1
	463	0	0	1	1	0	1
	352	0	0	0	0	0	1
	154	0	1	1	1	0	1
	88	1	1	1	1	0	1

Table 2. Cont.

primer	MW (bp)	eggplant	lantana	cotton	watermelon	squash	okra
OpB-03	**1877**	0	0	1	1	0	1
	1255	1	0	1	1	1	1
	833	0	0	1	0	0	0
	798	0	0	0	0	1	0
	687	0	0	0	0	0	1
	591	0	0	1	1	0	0
	562	0	0	0	0	0	1
	195	0	1	1	0	0	0
	186	1	0	0	1	0	0
	145	0	0	0	0	0	1
	137	0	0	0	0	1	0
	131	0	1	1	0	0	0
	124	1	0	0	0	0	0
	118	0	0	0	1	0	0
	102	0	1	0	0	0	0
	97	1	0	0	0	0	0
OpB-07	**627**	1	1	1	1	1	1
	401	1	1	1	1	1	1
	359	0	1	0	0	0	0
	339	0	0	1	1	1	1
	321	1	0	0	0	0	0
	272	1	0	0	0	1	0
	257	0	0	1	1	0	1
	230	0	0	0	1	1	0
	205	0	1	0	0	0	0
	147	0	0	0	0	0	1
	139	1	1	1	1	1	0
	105	1	0	0	0	1	1
	99	0	0	1	0	0	0
	64	0	0	0	0	1	0

(watermelon and squash). While nine bands were determined for lantana population. Two specific bands with MW of 287 and 92 bp were detected for the eggplant population. While the band with MW of 548 bp was considered as a specific band for the lantana population. The two bands with MW of 644 and 376 bp were considered as common bands for the six tested host-associated populations.

2.5. Primer OPA-19

The total number of PCR fragments amplified by this primer were 30 bands with MW ranged from 1467 to 88 bp for five populations only, while the squash *B. tabaci* population showed no bands with this primer. The lowest number of bands (4 bands) was found in eggplant population. While the highest number (7 bands) was detected in three *B. tabaci* populations (cotton, watermelon and okra). Five bands were found in lantana population. The bands with MW of 1467 were specific for cotton and watermelon. The bands with MW of 945, 759 and 463 bp were considered as specific bands for cotton, watermelon and okra *B. tabaci* populations. The bands with MW 352 and 644 were considered as specific bands for two *B. tabaci* populations (okra and eggplant, respectively). The band with MW of 88 bp was considered as a common band for the five populations with exception of population of squash.

2.6. Primer OPB-03

The total number of PCR fragments amplified by this primer was 26 bands with MW ranged from 1877 to 97 bp. The lowest number of bands (3 bands) was found in lantana *B. tabaci* population. While the highest number (6 bands) was detected in cotton population. Five bands were found in three *B. tabaci* populations (watermelon and okra). While three bands were determined for squash population. This primer showed specific bands for the all tested populations. Three specific bands with MW of 687, 562 and 145 bp were found for the okra population. While two specific bands with MW of 124 and 97 bp were detected for the eggplant population. Two specific bands with MW of 798 and 137 bp were found for squash population. One specific band were found for cotton population (833 bp) and one specific band for watermelon population (118 bp). One specific band only with MW of 102 bp was found for lantana *B. tabaci* population. No common bands were distinguished among the six tested populations.

2.7. Primer OPB-07

The total number of PCR fragments amplified by this primer was 37 bands with MW ranged from 627 to 64 bp and with Rm ranged from 0.48 to 0.89µm. The lowest number of bands (5 bands) was found in lantana *B. tabaci* population. While the highest number (8 bands) was detected in squash population. Six bands were found in the other four *B. tabaci* populations (eggplant, watermelon, cotton and okra). Bands with MW of 321, 99 and 64 bp were considered specific bands for populations of eggplant, cotton and squash, respectively. One specific band was found for okra *B. tabaci* population with MW 147 bp. Also, two specific bands were detected for lantana *B. tabaci* population with MW 205 & 359. Two common bands with MW of 627 and 401 bp were distinguished

among the six tested host-associated populations.

These results conclude that RAPD-PCR technique could be used as an efficient tool to distinguish variations in nucleic acids among different *B. tabaci* populations, and to identify different host-associated biotypes of this insect pest.

These results are in agreement with those obtained by (**Perring *et al* 1993; Guirao *et al* 1994; Barro *et al* 1997; Beitia *et al* 1997 Guirao *et al* 1997; Moya *et al* 2001; Horowitz *et al* 2003; Delatte *et al* 2005 and Sseruwagi *et al* 2005**) they used molecular techniques to differentiate between different host and/or geographical associated biotypes of *Bemisia tabaci*.

From the above results it could be concluded that *B. tabaci* may have different biotypes on adaptations to certain host plant species in Egypt according to different genetic techniques. Therefore, these results may be useful initially to understand the behavior variability of *B. tabaci* populations against different host plant species.

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الاختلافات الوراثية بين العشائر المرتبطة بالعائل النباتي لذبابة القطن البيضاء بيميزيا تباسى (هيمبترا: استرنورينكا: اليروديدى) في مصر

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تم في هذه الدراسة تحليل الاختلافات الوراثية لستة عشائر مرتبطة بالعائل النباتي لذبابة القطن البيضاء بيميزيا تباسى في مصر وذلك على مستوى الكيمياء الحيوية باستخدام تقنية التفريد الكهربي لنوعين من المشابهات الإنزيمية (ألفا & بيتا نفثيل اسيتات استيريز وماليت ديهيدروجينيز) وكذلك باستخدام تقنية التضاعف العشوائي المتنوع للحامض النووي (د ن ا) باستخدام سبعة بادئات عشوائية قصيرة النيكلوتيدا. وقد أظهرت النتائج وجود اختلافات كبيرة بين هذه العشائر تحت الدراسة مما يشير إلى احتمال وجود أنماط حيوية مختلفة لهذه الحشرة وفقا لتكيفها مع العائل النباتي.

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