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Safaa Z.Baker[1] *, Akeel H. Al- Assie[2], Rand Sh. Mahmood[1]

[1] *Department of Plant Protection- College of Agriculture-Tikrit University*

[2] *Department of Biology- College of Sciences- Tikrit University*

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Determination of Some Morphological Traits and Genetic Factor of Two Species of *Liriomyza spp* (Diptera: Agromyzidae) on the Climbing Beans and Tomato Crops

ABSTRACT

This study was carried out to study some morphological parts of adult male of *Liriomyza* spp, including the antenna, the femur and the middle vein (Cu Al), as well as the shape of mines that larvae made them on the leaves. Results showed that there were two species of *L.bryoinae* on tomato crop and *L.sativae* on climbing beans crop, this was agreed with the molecular study that it was conducted using the RAPD Random Amplification of Polymorphic DNA based on the PCR technique to determine genetic variability of DNA that it was extraction from adults and pupae and obtained a quantity of it ranged from (35-556.9) mg and purity ranged between (1.7-2).

The RAPD markers were studied using 19 random primers, 13 of which showed a bands and 6 of them didn't show any bands, the total number of bands loci were 53 loci, 23 of which were main bands and 30 were polymorphism and genetic analysis was performed based on these results. The genetic distance was found and lowest was (0.125) among adults and pupae of bean crop and higher distance was (0.323) the among the adults and the pupae of the tomato crop and the genetic distance between the tomato pupae and bean pupae was (0.185), according to this values the genetic relationship was found which was the tomato adult's species was independent and the genetic relationship between Adults and pupae have closest. Results showed that the pupae which were taken from the crops, there are two species of *Liriomyza* spp .

INTRODUCTION

The green climbing beans *Phaseolus vulgaris* L. and tomato *Lycopersicon esculentum* Mill were economically important vegetable crops, and green beans was of nutritional importance to humans and It had been successfully cultivated in Turkey and Iraq under the conditions of plastic houses. The tomato crop was one of the most consumed agricultural products in the world (Devinder *et al*., 2005) . Both the two crops were infected with many insect and non-insect pests , the *Liriomyza sativae* was polyphagous insects on many plant families of vegetables and flower crops such as kernels , legumes and peanut(Spencer,1973,1990), and causes losses of vegetable crops ranging from 80-100% depending on the type of crop and the level of infection (Chabi-Olaye *et al*.,2008) . As for the *Liriomyza bryoinae* of the tomato leaves are also polyphagous and is an important pest on tomatoes, cucumbers, eggplants and other vegetable crops in addition to the weeds and ornamental

* *Corresponding author: E-mail*: safaa_baker@tu.edu.iq

plants (Al-Mashhadany,2000).Both species caused significant damage to leaf tissue, where the larvae made mines on leaves, reduce green space and process photosynthesis and thus weaken the plant. If an early injury occurs, a significant loss occurs (Al-Mashhadany 1998). The resistance of the insect to pesticides as a result of its extensive and frequent use had shown that strains resistant to groups of insecticides (Leibee,,1984; Mason *et al*.,1987) , the species were belonging to the genus *Liriomyza* distinguished between them in adult stage through morphological characteristics were antenna , femur ,middle vein in fore wing (Spencer,1973,1976) and shape of mines (EPPO,2005). The phenotypic similarity between different species of *Liriomyza* made it difficult to identify the species, so the methods of detection of these species were used at the molecular level to distinguish between them (Firake *et al*.,2018). The study of the genetic variability of the species of this insect is important to know the extent of the genetic distance and genetic relationship between them to help in the put of effective strategies gradate within the control. Using the Random Amplified Polymorphic DNA (RAPD) based on the Polymerase Chain Reaction (PCR) technique. This marker relies on random primers that do not need to know the nucleotide sequence as they are related to the loci that comply with their rules (Jan *et al*., 2011).

MATERIALS AND METHODS

The collection of pupae and adults of Liriomyza

A plastic house was prepared in the plastic houses group of the Faculty of Agriculture - Tikrit University with a variety of tomato(yeste F-1), climbing beans (maselae variety) and cucumbers (Roni F -1) but because the cucumber crop was infected with a number of insect diseases in conjunction with the of *Liriomyza* spp . As a result of the lack of specialized pesticides and the fear of injury to leaf-cutters, the procedure was non-control, as these diseases led to eliminate it. . The adult insects were collected for phenotypic diagnosis through the laboratory breeding of the insect, where the leaves containing the larvae were collected from the field for each of the climbing green beans and tomato leaves , and the leaves of each crop were placed in a glass container containing a small amount of sand for the purpose of obtaining moisture , and cover White gauze and labeled with date of collection and the plant family that were collected, and transferred to the laboratory of postgraduate insects in the Department of Plant Protection in the Faculty of Agriculture and placed in the incubator at a temperature of 27 c^{o}, or the purpose of completing the insect life cycle and was a daily observation to follow the exit of adult insects and after 12 days emerged adults were getting out the incubator and put in capsules for conservation for the purpose of phenotypic diagnosis.

The pupae were taken from the leaves of the plant for each of the tomatoes and beans and took adults from the plant using aspirator and placed in screw glass cups were and they transferred to the Molecular Biological Laboratory for the purpose of isolating the DNA.

Preparation of insect parts for microscopic examination

The insect was placed in a petri dish under the dissecting microscope with a magnification force (20X). The parts were separated by a thin needle and a glass slide was placed. A drop of Canada balsam was placed on it. Then the part was placed on the slide and covered with the lid. The host was taken from the insect and then the slides were examined under a microscope compound with a camera and took pictures of the insect parts studied(Malipatil and Ridland, 2008) with modulation.

Genomic DNA Extraction

DNA was isolated from pupae and adult insects *Liriomyza* spp in the manner described by (Stirling and Pearson, 2003).

Determination of the concentration and purity of extracted DNA

The concentration and purity of the extracted DNA was estimated using the Nano drop. This was done by taking 1 ml of the sample DNA samples after spin in Centrifuge for 5-7 second to complete the mixing of the sample components, The concentration and purity that appear on the computer screen has been taken.

RAPD reactions

RAPD reactions were based on (Williams *et al*., 1990) on (4) DNA samples of pupae and adults of *Liriomyza* species.

Table (1) contains the Premix PCR ingredients obtained from BIONEER company

Reaction size / Component	20ml
Taq DNA polymerase	One unite
Each:dATP(dATP,dCTP,dGTP,dTTP)	250mM
Tris-Hcl(PH9.0)	10mM
KCl	30mM
Mgcl2	1.5mM

Table (2) The 19 random primers using with their sequences Procedure of RAPD Technique

5-----3 of Sequence	Primers
5-CAGGCCCTTC -3	OP A-01
5-GGTCCCTGAC-3	OP A-06
5-GGACTGGAGT-3	OP B-04
5-CCTTGACGCA-3	OP B-12
5-TCCGCTCTCC-3	OP B-14
5-GGACCCTTAC-3	OP B-20
5-TGGACCGGTG-3	OP C-08
5-CACACTCCAG-3	OP C-16
5-TGTCTGGGTG-3	OP C-10
5-GTCGCCGTCA-3	OP D-03
5-GGTCTACACC-3	OP D-10
5-GAGAGCCAAC-3	OP D-18
5-GGCACTGAGG-3	OP G- 02
5-TCACGTCCAC-3	OP G-08
5-GGATGAGACC-3	OP G-14
5-TCTCAGCTGG- 3	OP H-16
5-CCGAACAGGG-3	OPJ-04
5-GTCTACGGCA-3	OPR-06
5-GGCTGCAATG-3	OPY-04

1. After amendment the concentration of the DNA in all the samples studied by dilution by sterile distilled water to obtain the concentration required for RAPD reactions was 25 ng / µl for each sample.

2. Prepare the reaction mixture by mixing the reaction components in 0.2 mL sterile premix tube.

As in the following table:

Table (3) represents the main reaction components of the RAPD marker

Size per sample	concentration	Component
2 ml	–	PCR Premix
16.5 ml	–	sterile distilled water
0.5 ml	10 picomol	Primer
1 ml	25 ng/ml	Genomic DNA sample
Final volume		20 ml

3.Apply the following program:

One cycle for 4 minutes at 94 ° C temperature for the initial denaturation of the DNA tape followed by 40 cycles. Each cycle includes 30 seconds at a temperature of 92 ° C for and 45 seconds at a temperature of 36 ° C for annealing with the template DNA and 45 seconds at 72 ° C to extension and then a final cycle of 7 min at 72 ° C to complete the extension phase.(Williams *et al*., 1990)

4. The tubes were removed from Thermocylces after the end of the reaction time. They were loaded into the 1.5% agarose which was prepared, and 5 microliters of amplicons . The samples were then run by electrophoresis for 55 minutes. The gel was then exposed to the UV-Transilluminator to record gel photography using a high-resolution digital camera.

The resulting bands were rely from random multiplication processes used in the RAPD marker that appeared on the agarose gel. The genetic distance of Adults and virgins of the *Liriomyza* spp has been calculated by similarity for Quantitative Data System (SIMQUAL). The calculations of this program are carried out according to(Nei and Lei , 1979) .

After the genetic distance was extracted, the combin analysis was performed using a unweighted pairing grop method for the arithmetic average (UPGMA) and all these genetic analyzes using Numerica Taxonomy and Multivariate Analysis System (NTSYS - PC). (Rohlf, 1993).

RESULTS AND DISCUSSION

The permanent microscopic slides were prepared of tomato adults *L. bryoniae* and bean adults *L. sativae* male. Which were prepared by the Natural History Museum of Baghdad University, where the same samples were taken and the slides were studied according to the method mentioned by (Ridland, and Malipatil ,2008) with modulation since sodium hydroxide, cold acetic acid and ethanol were not used.

The results obtained are as follows:

Antenna:

The results of this study show that the antenna was type arista which is a yellow both species. In *L. bryoniae*, the third parts (which represents the flagella) was the terminal round, its dimensions were 4X2,5 mm on magnification force (40X) . The arista was a lateral position on the third round yellow segment , its color was dark brown . Arista gradually tapering upward vertically and this description agrees with with (Spencer , 1973).

In the type *L..sativae* the third segment was more rotated , its dimensions were 3X3mm on magnification force (40X) and the arista has a lateral position on the third noudle and the color is dark brown, but the base part of the oversized Aristatae is shorter than in the type *L. bryoniae*. This description is consistent with (Amin *et al*.,2014) The flagella is yellow in type *L. bryoniae*, and we conclude that they are two different types.

Figure (1) third antenna segment species *L.bryoinae*(left 40x,right 20x)

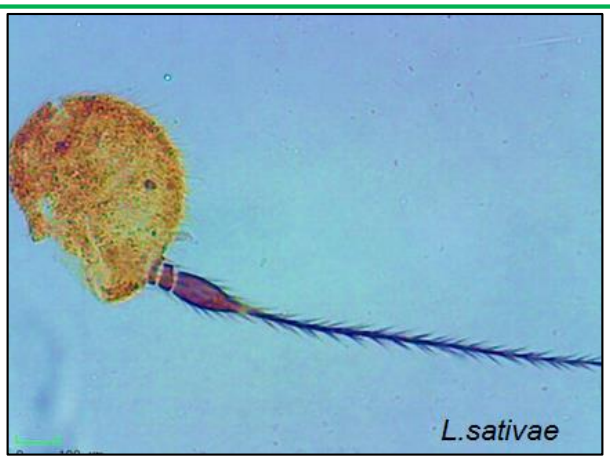
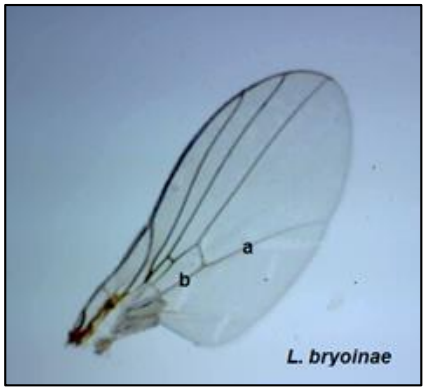


Figure (2) third antenna segment species *L .sativae* (40X)

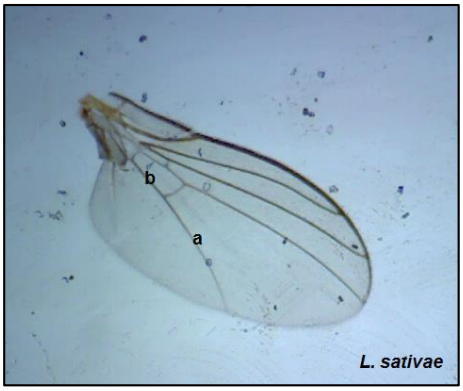
The Wing:

The results showed that type *L. bryoniae* differed from the other type that the length of the final part of the middle vein (Cu lA a) was twice the length of the final part of the middle vein(Cu lA b) This was agreed with (Spencer, 1973).

The type *L.sativae* the length of the final part of the central vein (Cu lA a) was 2.5 times twice the length of the intermediate part of the middle vein (Cu lA b) this was not agreed with (Amin *et al*., 2014). These percentages were within the range described by (Sampanukhro *et al*., 2010) and the ratio of (a to b) in the parts of the central vein (Cu lA) of the genus *Liriomyza* ranged between 1.95 and 4.2, which indicated that they were two different species.



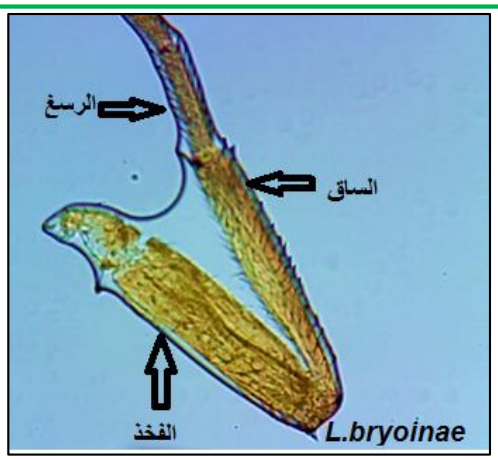
Figure(3)fore wing *L.bryoinae*



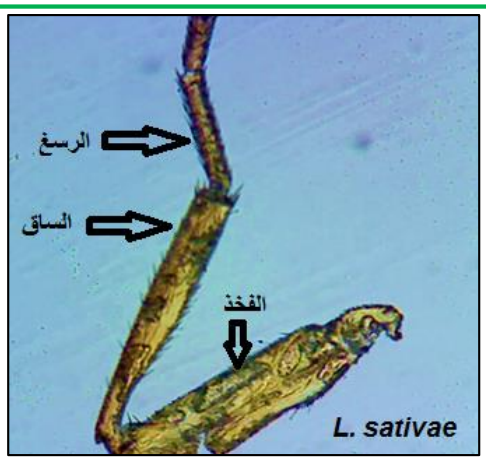
Figure(4)fore wing *L.sativae*

Legs:

The results showed that in *L. bryoniae* femur was yellow, with brown notch , tibia, and tarsus also brown. This description was identical to (Spencer 1973, Hayani, 2016). The type *L..sativae* femur is bright yellow, the tibia and the tarsus brown color and this was agreed with (Amin *et al*.,2014 ;Mazumdar and Bhuiya,2014) . This indicates that they are two different species.



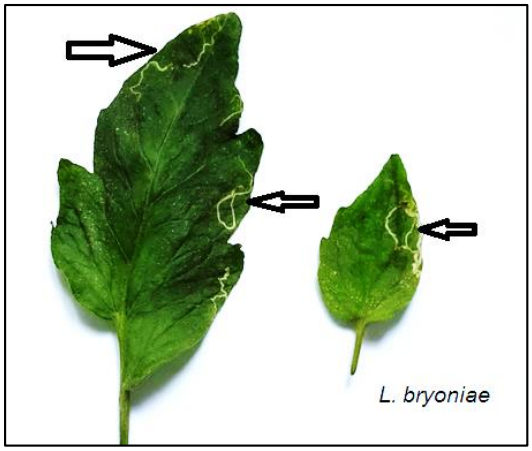
Figure(5)med leg *L.bryoinae* 40X



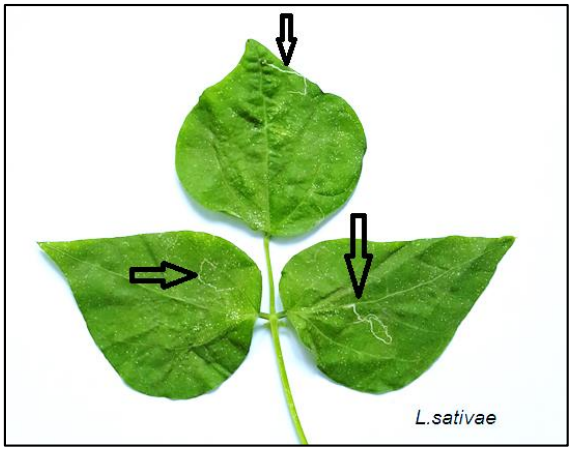
Figure(6)med leg *L.sativae* 40X

The shape of mines on the leaves

In general, the mines of both species appeared in a loose, irregular zigzag shape ,in species *L.bryoinae* the location of the mines on extremity tomato leaves But the end of the *L..sativae* mines on the bean leaves appeared in a downward motion and the location of the mines was near to the central vein . It was agreed with (EPPO , 2005) that it often ends of the mines is made by species larvae *L.sativae* like a spot. Collins (1996) and Jiao *et al*. (1998) note that the form of mines is influenced by the type of host plant, the physiological and physical state of the leaves and the number of larvae that make the mines on the leave itself.



Figure(7) the shape of mines on tomato



Figure(8) the shape of mines on climbing beans

The genomic DNA was isolated from adults and the offspring of *L. sativae* and *L. bryoniae*, according to the method described by (Pearson and Stirling , 2003) with some modification. Liquid nitrogen was not used in the extraction process and because of the small size of the insect. And the amount of DNA resulting from this method ranged from (35 - 556.9) and the purity ranged between (1.7-2) and the amount of DNA extracted and purity based on the device of Nanodrop and was set on 25 ng / ml stratified with sterile distilled water and then moved on 1% agarose gel

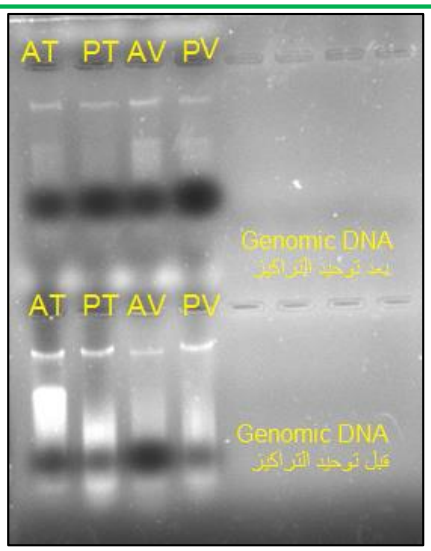


Figure (9) show the genomic DNA of the four samples of the *Liriomyza* spp *species* of adults and pupae and the stage on the agarose gel is a 1% AT represents the adult of *Liriomyza bryoniae* and PT represent the pupae of the *Liriomyza bryoniae* and AV represents the adults of *Liriomyza sativae* and PV represent the pupae of the *Liriomyza sativae insect* before and after unification of concentrations.

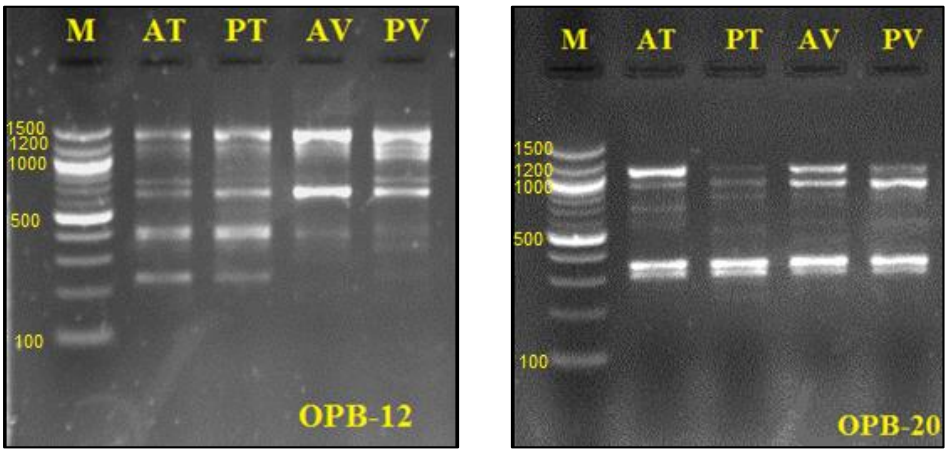
The differences recorded between adults and offspring of *L. sativae* and *L.bryoiane* were in four forms

1.The presence of DNA bands or absent.

2.Differences in molecular weight between bands.

3.Differences in the number of bands.

4.Differences in the density of the bands. (Bardakci, 2001; Betancor *et al*., 2004)



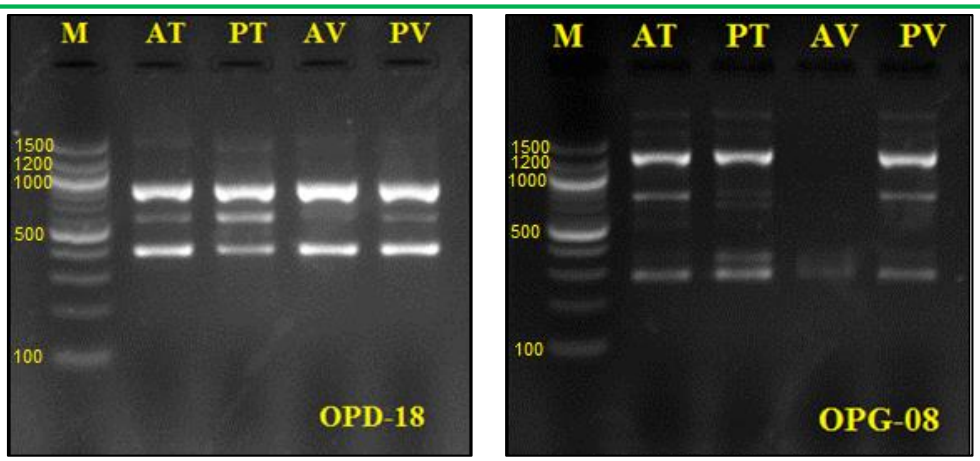


Figure (10) show primers multiplication products of the RAPD marker the four samples of the *Liriomyza* spp *species* of adults and pupae and the stage on the agarose gel is a 1.5% M represents DNA Ladder 100 pb AT represents the adults of *Liriomyza bryoniae* and PT represent the pupae of the *Liriomyza bryoniae* and AV represents the adults of *Liriomyza sativae* and PV represent the pupae of the *Liriomyza sativae*.

The results obtained from RAPD markers are as follows:
(19) random primers were using, (13) primers show a bands, and 6 did not show it on agarose gel and because of their inability to bind and replication because there is no complementary sequences for primres in DNA strand (Devos and Gale, 1992). The results showed a difference in the number of DNA bands as well as variations in their molecular wight according to the primers used, ranging in wight between 100 - 1500 pb, very light bands were neglected. The emergence of the main band in (10) primers indicates the link of these species with certain characteristics and this made the marker of RAPD more DNA indicators suitable to study the genetic relationship that depends on the emergence of these bands, because of the importance of being often represented by a shared site for All genus and species individuals (Penner *et al*., 1995). The intensity of the bands is used as a guide for variation when the concentration of the template DNA is accurate, and the appearance of these bands is evidence that there is more than one bundle produced at the same molecular weight and appears as a thick, high density bands on the gel.

The emergence of the unique band in 6 primers is one of the most important diagnostic characteristics, and its appearance in a particular species markers that it exists in the genomic DNA of that species without the other species under study (Williams *et al*., 1990) thus the Finger print DNA can be obtained through its presence in a species and its absence in the other , and this agree with (Bettaibi *et al*., 2012) (Khatib *et al*., 2017). The Absent band appeared in (11) primers and the absence of this band in the species without the other is an indication of the existence of large genetic differences. The 13 primers showed the loci of different bands, the total of these loci were (53), of which (23) were main bands and (30) were different loci for adults and pupae of both species and showed unique band and absent bands of adults and pupae of both species which were in this study (9) unique bands and (21) absent bands.
he genetic distance was estimated based on the results of the RAPD markers for adults and pupae of the main bands between the two species of the studied *Liriomyza* strain, which is based on the Nei and Li (1979) equation and the table (showing the values of the genetic distance of adults and pupae of *Liriomyza* spp).

Table (4) represents the values of the genetic distance of adult species and pupae of *Liriomyza bryoniae* and *Liriomyza sativae* insect.

	AT	PT	AV	PV
AT	0.000			
PT	0.323	0.000		
AV	0.305	0.232	0.000	
PV	0.152	0.185	0.125	0.000

In the case of a genetic material that is identical between the two species, the genetic distance between them should be equal to zero. The total genetic similarity ratio is equal to 1(100%) (Esselman *et al*., 2000). The proximity or genetic distance is determined between the two species according to the number of shared bands, the greater the number of bands, the less the genetic distance and vice versa. In this study, the values of the genetic distance ranged between (0.323-0.125). The lowest value of the genetic dimension was (0.125.) Among the adults of the beans (PT) and the pupae of beans (PV), this indicates that the proportion of the similarity in the genetic material is large between them depending on the random primers used to emphasized that they are the same species, the pupae taken from the bean crop was identical to the adult insects of the same crop. The highest genetic distance (0.323) was among the adult Tomato (AT) with the tomato pupae (PT) and this means that the proportion of the similarity in the genetic material is few and show that the adults taken from this crop was of another species is different from the species of pupae , this may be due to the existence of another type of insect *Liriomyza* non-species which affected the crops of tomatoes and beans, which was Diagnosis by the Museum of Natural History University of Baghdad was the result of the agriculture of cucumber crop at the beginning of this study before the agriculture of tomato crop and beans and the his leaves infection of the with mines of this insect was severe, but the result of the cucumber crop infection a number of plant diseases, and the inability to carry out the control of these diseases because of the absence of specific pesticides for these diseases; so the procedure is not to carry out the control and then this led to these diseases to eliminate it when the adult insects appear and did not find a it host plant its preferred the tomato crop as a host on the crop of beans for the purpose of feeding before the emergence of adult insects of the species that was infected tomato crop, and when the samples were taken to extract the DNA was taken for this species of tomato crop because if it was the adult species that affected the crop of tomatoes, will not take adults and pupae of tomato the value highest of the genetic distance. There was study conducted on varieties of potato crop in Korea has found that *L.huidobrensis* preferred specific species, which is the result of the insect's response to olfactory signals from host plants, suggesting that smell is an important way to attract the insect into the host (Kwon *et al*., 2017). Another study found that tissue composition, leaf moisture content, and the thickness epidermis layer influences the host's selection by the insect (Wei *et al*., 2000). The genetic distance among the adults of tomato crop and the bean crop (0.305) this indicates that they were two different species, and the distance between the virgins of the tomato crop and the pupae of bean crops (0.185) were also shown to be different species.

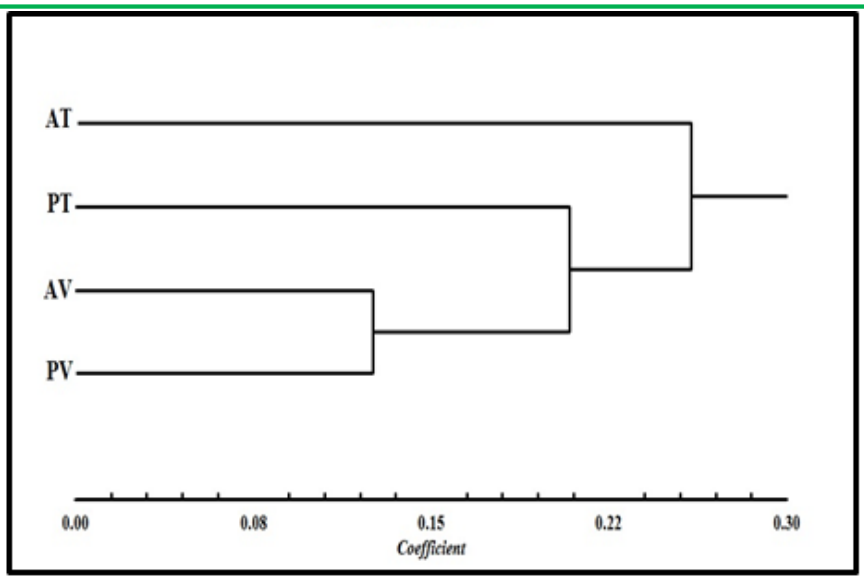


Figure (11) represents the genetic relationship of the adult and pupae species of *Liriomyza bryoniae* and *Liriomyza sativae* depending on the genetic distance of RAPD.

Synthesis analysis of both adults and pupae of both species was established to find the genetic relationship between them using cluster analysis:
The adults of tomato (AT) are appeared distinguished from the pupae of the tomatoes (PV), the adults of beans (AV) and the bean pupae (PV), for the least number of binding loci with the rest depending on the random primers used. the adults of beans (AV) and bean pupae (PV) were the closest genetic relationship between them. The importance of determining the genetic relationship to determine the degree of kinship between the two species. Depending on the pupae are shown to be two different two species, and markers of RAPD in the distinction between the species of insect *Liriomyza* spp in determining the degree of proximity or genetic distance between them, which can be used in the field of control.

REFERENCES

AL-Hayani ,L.M.A.(2016). Biology of Leafminers *Liriomyza* spp(Diptera:Agromyzidae) on tomato and climbing bean under plastic house conditions . Master thesis. College of agriculture. Tikrit University.

AL-Mashhadany ,W.A. ;Ali, A.A. and AL-Jboory ,I.J.(2000). Studies on some aspects of biology, food preference and spread of tomato leafminer *Liriomyza bryoniae* (Diptera: Agromyzidae). |Journal of Agricultural Research .10(1):126-142.

Al-Mashhadany,W.A.A. (1998). Ecological and biological studies and its response to some control practices of *Liriomyza bryoniae* (kalt.)(Diptera:Agromyzidae). Master thesis. College of agric.Baghdad University.

Amin, S ., Scheffer,S.J.; Lewis,M.L. ; Pash,M.K and Bhuiya,B.A. (2014). DNA barcoding of the vegtable leafminer , *Liriomyza sativae* Blanchard (Diptera:Agromyzidae) in Bangladesh .DNA Barcodes 2:29-33.

Bardakci , F.(2001). Random amplified polymorphic DNA (RAPD) markers . Turh. Journal . Biol. 25 : 185-196.

Betancor, L.; Schelotto, F.; Martinez, A.; Pereira, M.; Algorta, G.; Rodriquez, M. A.; Rignoli, R. and Chabalqoity, J. A. (2004). Random amplified polymorphic DNA and phenotyping analysis of *Salmonella enteritidis* isolated collected from humans and poultry in uruguay from 1995 to2002. J. Clin. Microbiol. 42 (3) : 1155-1162.

Bettaibi,A. ; Khemakhem ,M. ; Bouktila,D. ; Makni,H. and Makni ,M.(2012). Genetic variability of the tomato leaf miner (*Tuta* absoluta Meyrick ,Lepidoptera :Gelechiidae), in Tunisia,inferred from RAPD-PCR.J.of agric.research 72(2):212-216.

Chabi-Olaye, A. ; Mujica, N., Lohr , B and Kroschel, J. (2008). Role of agroecosystems in the abundance and diversity of *Liriomyza* leafmining flies and their natural enemies.*In*: Abstracts of the XXlll International congress of Entomology, Durban, South Africa, 6-12,JuIy 2008.

Collins, D.W. (1996). Protocol for the diagnosis of quarantine organisms: *Liriomyza* spp. (*L. bryoniae, L. huidobrensis, L. sativae, L. trifolii*) [online] Available: www.csl.gov.uk/ specialInterest/liriomyza.pdf [access on March 13, 2009].

Devinder ,k.;soqia,D.S.; Garg,S.K., and Bawa, A.S.(2005): Flotion –cum-sedimentation system for skin and seed separation from tomato pomace .J.Food eng., 71(4):341-344.

Devos , K. M. and Gale, M. D. (1992). The use of random amplified polymorphic DNA markers in wheat. Theo. And Appl.Genet. 84:567-572.

EPPO.(2005). European and Mediterranean Plant Protection Organization . *Liriomyza* spp. PM 7/53(1). *EPPO Bulletin*, 35: 335–344.

Esselman, E. J. ; Crawford, D. J. ; Brauner, S. ; Stuessy, T. F. ; Anderson, G. J. and Mariosilva, O. (2000). RAPD markers diversity within and divergence among species of Dendroseris(Asteraceae : lactuceae). Am. J. Bot. 87: 591-609.

Firake,D.M. ; Sankarganesh,E. and Sharma,B.(2018).DNA barcoding confirmed the occurrence of invasive vegetable leaf miner,*Liriomyza sativae* Blanchard (Diptera:Agromyzidae) in Northeast India.J.of Asia-Pacific Biodiversity11(2018):56-60.

Jan,H.U. ;Rabbani,M.A. and Shinwari, Z.K.(2011). Assessment of genetic diversity of indigenous turmeric (Curcuma longa L.)germplasm from Pakistan using RAPD markers.J.Med.Plants Res.,5:823-830.

Jiao, W.J. ; Ling, Z. ; Wen, L.G. and Qiu, Z.W. (1998). The leafmining behavior and instars of *Liriomyza sativae*(Diptera: Agromyzidae). J. South Chin. Agric. Univ. 19: 27-31.

Khatib,F;Trissi,A.N.;Aleisa,Z. and El-Bouhssini.M.(2017). Genetic diversity of tomato leaf miner using RAPD molecular markers .Arab Journal of Plant Protection ,35(2):49-57.

Kwon,M. ; Kim,J. and Maharjan,R. (2017). Different potato (*Solanum tuberosum* L.) cultivars mediated life-history variables of the potato leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera:Agromyzidae). J.of Asia-Pacific Entomology 20(2017):705-712.

Leibee , G. L. (1984). Influence of Temperature on Development and Fecundity of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) on Celery. Environ. Entomol. 13: 497–501.

Malipatil, M. and Ridland, P.(2008). *Polyphagous agromyzid leafminers: Identifying polyphagous agromyzid leafminers (Diptera: Agromyzidae) threatening Australian primary industries.* Canberra, Department of Agriculture, Fisheries and Forestry, Australian Government.

Mason, G. A. ; Johnson, M.W. and Tabashnik ,B.E..(**1987**). Susceptibility of *Liriomyza sativae* and *L. trifolii* (Diptera: Agromyzidae) to Permethrin and Fenvalerate. J. Econ. Entomol. 80: 1262–1266

Mazumdar,S. and Bhuiya,B.(2014). Vegetable Leafminers (Diptera:Agromyzidae) and their Plant hosts in Bangladesh. J.of Threatened Taxa /www.threatenedtaxa.org.6(6):5894-5899.

Nei, M. and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction Endonucleases. Proceeding of the National Academy of Science, USA. 74, 5269-5273. Cited by Henry, R.J. (1997).

Pearson, H . and Stirling, D.(2003). PCR protocols: method in molecular biology. 2nd edi. Vol 226. Human Press.

Penner, G. A. ; Clarke, J. ; Bezte, L. J. and Leisle, D. (1995). Identification of RAP markers linked to a gene governing cadmium uptake in durum wheat. Genome. 38: 543-547.

Rohlf,F.J.(1993) Numerical taxonomy and multivariate analysis system. Version 1.80 Exeter software. Setauket. N. Y.

Sappanukhro, P. ; Petcharat,J. ; Nualsri,J. and Permkam,S. (2011) . Identification of *Liriomyza* spp. (Diptera: Agromyzidae) on yardlong bean and cucumber in Songkhla province: II. Using Random Ampliflied Polymorphic DNA (RAPD) and male distiphallus technique. Journal of Agricultural Technology 7(2): 349-368**.**

Sappanukhro, P. ; Petcharat.J ; Nualsri .J and Permkam S.(2010). *Identification of Liriomyza spp. (Diptera: Agromyzidae) on yardlong bean and cucumber in Songkhla province : I. Feeding tunnel patterns, external morphology and male distipallus morphology.* Journal of Agricultural Technology , 6(2): 257-267.

Spencer, K.A. (1973) . Agromyzidae (Diptera) of economic importance (Series Entomologica No.9), 418 PP. Junk, The Hague, Netherlands.

Spencer, K.A. (1976). The Agromyzidae (Diptera) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavica*, 5: parts 1 and 2.

Spencer,K.A. (1990). Host specialization in the word Agromyzidae (Diptera). Kluwer Academic Publishers, London, UK.

Wei,J. ; Zou, L. ; Kuang,R. and He,L.(2000). Influence of leaf tissue structure on host feeding selection by pea leafminer *Liriomyza huidobrensis* (Diptera :Agromyzidae). Zool.Stud. 39,295-300.

Williams , J. G. ; Kubelic , A. R. ; Livak , K. J. ; Rafalski , J. A. and Tingey ,S. V. (1990). DNA polymorphisms amplified by aribitary primers are useful asgenetic markers . nucleic acids Res. 18 : 6531-6535.

Zakaria,H.S.(2013). Effect of planting date and variety on the growth and quantitative and qualitative distribution of climbing beans *Phaseolus vulgaris* L in the non-heated plastic house. . Master thesis. College of agriculture. Tikrit University.

تحديد لبعض الصفات المظهرية والعامل الوراثي لنوعين من صانعات انفاق الاوراق Liriomyza spp (: Diptera Agromyzidae) على محصولي الفاصوليا المتسلقة و الطماطة .

صفاء زكريا بكر[1] وعقيل حسين العاصي[2] ورند شاكر محمود[1]

[1] قسم وقاية النبات – كلية الزراعة – جامعة تكريت

[2] قسم علوم الحياة – كلية العلوم – جامعة تكريت

المستخلص

بينت نتائج الدراسة المظهرية والذي تم فيها اخذ اجزاء معينة من الحشرات البالغة الذكور لصانعات أنفاق الاوراق وهي قرون الاستشعار، الفخذ والعرق الوسطي Cu Al للجناح، بالإضافة الى شكل الانفاق التي تصنعها اليرقات على الاوراق. وتبين وجود نوعين هما نوع *L.bryoniae* على محصول الطماطة والنوع الاخر *L.sativae* على محصول الفاصوليا المتسلقة , وهذا اتفق مع الدراسة الجزيئية التي اجريت باستخدام مؤشر التضاعف العشوائي متعدد الاشكال لسلسلة ال DNA Random (RAPD) Amplification of Polymorphic DNA المعتمد على تقانة ال (PCR) لتحديد التباين الوراثي عزل ال DNA من البالغات والعذارى وتم الحصول على كمية منه تراوحت بين (556.9 -35) مايكرو غرام ونقاوة تراوحت بين (1.7-2) . درست تفاعلات (RAPD) المعتمد على تقنية (PCR) باستخدام 19 بادئا عشوائيا منها 13 بادئا اظهر حزم و6 بادئات لم تظهر اي حزمة وكان مجموع مواقع الحزم (53) موقع كان منها (23) موقع عام و(30) موقع متباين واجري التحليل الوراثي استنادا الى هذه النتائج وتم ايجاد البعد الوراثي كان اقل بعد (0.125) بين بالغات وعذارى محصول الفاصوليا واعلى بعد وراثي (0.323) كان بين بالغات وعذارى محصول الطماطة ،والبعد الوراثي بين عذارى الطماطة وعذارى الفاصوليا (0.185) وبالاستناد الى هذه القيم تم ايجاد العلاقة الوراثية حيث ظهر نوع بالغات الطماطة مستقلا وكانت بالغات وعذارى الفاصوليا العلاقة الوراثية اقرب بينهما وتبين من خلال نتائج التي ظهرت للعذارى التي اخذت من المحصولين وجود نوعين من صانعات انفاق الاوراق .

الكلمات المفتاحية: المظهر الخارجي ، التباين الوراثي ، *Liriomyza* spp ، الفاصوليا المتسلقة ، الطماطة