# MOLECULAR AND BIOCHEMICAL MARKERS ASSOCIATED WITH TOLERANCE TO *Cassida vittata* VILL (COLEOPTERA: CHRYSOMELIDAE) INFESTATIONS IN SUGAR BEET

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Sugar beet, *Beta vulgaris*, L. (Family: Chenopodiaceae) ranks second as a source of sugar in Egypt and all over the world. In 2013 season, the total area cultivated with sugar beet reached 433303 feddans in Egypt (Anonymous, 2014).

Many investigations showed that sugar beet plants attracts numerous insect pests species that feed and damage plants causing partially or complete defoliation followed by great yield reductions beginning from seedling up to harvest (Bassyouni, 1998; Shalaby, 2001; Saleh *et al*., 2009; El-Mahalawy, 2011; Bazazo *et al*., 2012). *Cassida vittata* Vill proved to be one of the most destructive insects in sugar beet. Gurguis (1985) estimated the attacked sugar beet plants as 11-39%. El-Mahalawy (2011) measured the consumed area of sugar beet leaf as 23.5 $cm^2$ per one larva and one adult. Bazazo (2010) reported that this beetle appears in a high density (887 adults/130 plants) during April.

Strategies of insect pest control in sugar beet depend on applying integrated pest management (IPM) programs to avoid the use of insecticides since sugar beet is a food crop.

Resistant varieties integrate with other IPM elements to examine the effects of potential insect resistance genes in plant species through expression studies. Nechiporuk (1991) in Ukraine, indicated that a positive correlation was found between peroxidase activity and resistance of sugar beet to the black bean aphid, *Aphis fabae* L. Agrios (1997) indicated that higher plants have a broad range of mechanisms to protect themselves against various threats, including physical, chemical and biological stresses. Plant reactions to these threats are very complex, and involve the activation of set of genes, encoding different proteins. Quantitative trait loci studies as well as comparative studies with known resistance genes from

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other plants were done by McMullen and Simcox (1995); Dowd *et al*. (2005) and Miroslawa and Maria (2003) reported that eighteen varieties of sugar beet, originating from various European countries, were compared in respect of peroxidase variability level. They were cultivated in the same experimental plot. The cultivars differed in ploidy level: one variety was tetraploid, three were diploid and fourteen were triploid. The cathodic peroxidase system is controlled by four independent genes, of which only one is polymorphic. Consequently, the varieties were characterized by frequencies of 3 allozymes belonging to one locus. Only one variety proved to be fully monomorphic. Genetic similarities between the cultivars were illustrated by a dendrogram (UPGMA) and showed different groups of varieties not related to their ploidy level. Singh and Singh (2005) reported that with the advent of molecular biology, it is now possible to examine the effects of potential markers for insect resistance genes in plant species through gene expression studies. The current study was carried out to search for:

1. Presence of some markers of peroxidase and esterase enzymes in resistant variety.

2. Presence of protein bands associated with plant defense for *Cassida vittata* Vill.

3. Obtaining molecular markers associated with insect tolerance genes.

## MATERIALS AND METHODS

### *1. Monitoring populations of Cassida vittata on two sugar beet cultivars*

The experimental sugar beet field was at the experimental farm of Sakha Agricultural Research Station [(SARS) which lies at Sakha city (Kafr El-Sheikh Governorate), about 130 Km north of Cairo, 31° East longitude, and 31° North latitude, 6 m above the sea level)], sown during 2012/2013 and 2013/2014 seasons with Pyramids and Zinagri varieties on mid-October and received all recommended agronomic practices, but without any pesticides. Randomized complete block design with three replicates was used. The plot area 42 $m^2$ (6 x 7 m), each plot had twelve rows (50 cm apart and 7 m length). The population of *Cassida vittata* was monitored by direct visual examination, from 15 March to 25 April. Eggs, larvae and adults of the insects were recorded in the field on 30 plants from three replicates for each cultivar, every ten days in two seasons.

### *2. Biochemical and molecular analysis*

#### *SDS protein electrophoresis*

SDS-polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) was performed on water soluble protein fraction according to the method of Laemmli (1970).

***Extraction of water soluble protein fraction***

One gram of each leaf sample was frozen with liquid nitrogen and ground with one ml sample extraction buffer in a mortar. Samples were transferred to eppendorf tubes and then centrifuged for 20 min at 12000 rpm at 4°C. Supernatants, containing water soluble protein fractions were transferred to clean tubes and stored at -20°C until use.

***Polyacrylamide gel electrophoresis (PAGE)***

Esterase (Est.) E.G.3.1.1.1 and peroxidase (Prx.) E.G. 1.111.1.7 were determined using polyacrylamide gel electrophoresis according to the method of Davis (1964). Slabs of 7.5% acrylamide for separation gel and 2.5% acrylamide for stacking gel were used.

***Sample extraction***

Samples of 200 mg of fresh leaves from the two genotypes were extracted. Each sample was homogenized in 1.0 ml of an ice cold 50 mM tris-HCl buffer (pH 6.8) containing 20% (w/v) sucrose and 3 mM Dithiotritol (DTT). The mixture was left in 4°C for one hour with stirring then centrifuged at 15000 rpm at 4°C for 5 min. The gel was washed with distilled water.

***Genomic DNA isolation, purification and quantification***

DNA isolation and purification was carried out using CTAB (Cetyl - tetramethyl ammonium bromide) method according to Murray and Thompson (1980).

***DNA quantification***

DNA was quantified using gel quantification method according to Dellaporta *et al*. (1983).

***Randomly Amplified Polymorphic DNA of the Polymerase Chain Reaction (RAPD-PCR) amplification***

A total volume of 20 µl PCR was used which is containing 1.0 µl (30 ng template DNA), 0.2 µl dNTP's (10 mM), 1.6 µl Mg $Cl_2$ (25 mM), 2.0 µl tris buffer (10 mM tris, pH 8.0, 50 mM KCl and 50 mM ammonium sulphate), 4.0 µl of each primer (15 pmole) (five primers were used as shown in Table (1), 0.1 µl *Taq* polymerase (10 ug/µl). The volume was brought up to 20 µl by autoclaved double distilled $H_2O$.

The PCR cycling condition involved initial denaturation at 94°C for 5 min. followed by 35 cycles of amplification under the following by 35 cycles parameters, template denaturation at 94°C for 1 min., primer annealing at 36°C for 1.5 min. and primer extension at 72°C for 2 min., final extension at 72°C for 7 min. was given, followed by storage at 4°C. PCR thermocycler machines from Biometra (Germany) T-Gradient thermoblock were used. Agarose (1.5%) was used for resolving the PCR products and 1 kb DNA marker as a standard DNA were used in the present study. The run was performed for 1 hour at 50 volt in

SDE-PLAS submarine (10 cm x 10 cm). Bands were detected on UV- trans-illuminator, photographed by Gel documentation system and according to analysis by Phoretix program ID gel analysis software version 4.01.

***ISSR analysis***

Five ISSR primers were used to amplify the DNA template in this investigation (Table 2). The procedures for ISSR PCR and for the separation of amplified products were carried out as described by Hou *et al*. (2005).

***Data analysis***

Quantity one software (Gel Doc, Bio-Rad Laboratory, Inc.) was used to estimate the length of amplification products and for capturing gel images.

***Statistical analysis***

The data were analyzed using 't' test.

## RESULTS AND DISCUSSION

***Monitoring populations of Cassida vittata on two sugar beet cultivars***

Data in Table (3) showed that there is a highly significant difference between the two sugar beet varieties in the total of individuals (eggs + larvae + adults), whereas they were 836 and 813/210 plants on Zinagri cultivar causing severe infestation, while were 20 and 16/210 plants in Pyramids cultivar in both seasons. The beetle infestations were higher in the 1st season than in 2nd one. These results demonstrated that the Pyramids variety is resistant to *Cassida vittata*, whereas the Zinagri variety is susceptible.

The acceptance or rejection of plants depends on the insect behavioral responses to plant features. These features may be physical or chemical products contained in plants and affect the insect's orientation. Many of plant chemicals are toxic to herbivorous act or feeding deterrents, stimulate feeding and oviposition stimuli in plant defense. Some of secondary compounds are found in epidermal tissue, vacuoles in xylem in the park, in cell walls or deserted in the waxes of plant surface.

Juniper and Jeffree (1983) stated that there are important factors influencing host choice by the insects include:

1. Variation in leaf shape among host plants.
2. Host plants phenology (Collinge and Louda, 1989).
3. Other physical plant defenses; toughness, hairs and waxes.
4. Optical stimuli characters which limit insect orientation to the oviposition sites and food plants.

***Peroxidase and esterase isozymes electrophoresis***

The biochemical methods, especially isozyme polymorphism, have provided valuable tools for sugar beet breeders. Isozyme can serve as unique molecu-

lar genetic markers for biochemical characterization of genotypes (Tanksley and Orton, 1983). The variations of numbers and activities of peroxidase and esterase were assayed in the crude extract of leaves from the two sugar beet cultivars. Tables (4 & 5) and Figs (1 & 2) showed the variations of activity and number of bands. The band number five for esterase was present in the resistant variety (Pyramids) whereas, it was absent in the susceptible variety (Zinagri). The band number three for peroxidase was present in Pyramids variety and absent in Zinagri variety. The opposite was noticed with band number six.

These results may show markers for the tolerance of Pyramids to insects. The same result was reported by Aba and Tsuda (1987) who illustrated that peroxidase isozymes have proven to provide useful markers in establishing differences between natural beet species belonging to the *vulgares* section, which encompasses also sugar beet (*Beta vulgaris* L.). Nechiporuk (1991) estated that in the Ukraine, a positive correlation was found between peroxidase activity and chlorophyll and total-carotenoid contents and resistance of sugar beet to the black bean aphid *Aphis fabae* L. as measured by their values in seedlings of samples and strains, and also by the concentration of the pigments in leaves of biotypes, differing in their degree of infestation. Steinite and Levinsh (2003) indicated that increased strawberry resistance to *Tetranychus urticae* Koch has been described to be dependent on the presence and higher activity of polyphenol oxidase and peroxidase. El-Mahalawy (2011) studied protein and peroxidase isozyme banding patterns under insect attacks and normal plants without attacks. In addition, he used genomic DNA (ISSR technique) to find molecular markers associated to insect attacks tolerance in sugar beet. He also found that for peroxidase isozyme, the band number 2 can be considered as a marker associated with plant defense to insect pests. Osorio *et al*. (2011) reported that a partial demethylation of strawberry cell wall oligogalacturonides by the pectin methyl esterase gene required for eliciting defense responses in wild strawberry.

***SDS protein electrophoresis***

Water soluble leaf proteins extracted from the two sugar beet after subjected to the attack and damage by *Cassida vittata* were assessed by SDS-PAGE. Banding patterns of total soluble proteins were presented in Table (6) and Fig. (3). The bands had molecular weights (Mw) ranging from 6.5-212 KDa. Regarding bands number, Zinagri and Pyramids varieties showed 17 and 18 bands, respectively which ranged from 3.5 KDa to 255 KDa. The bands with MW 127 KDa and MW 17.5 KDa were present in the resistant variety (Pyramids), whereas they were absent in the susceptible variety (Zinagri). These bands can be considered as negative markers associated with plant defense for *Cassida vittata*. On the other hand, the band with Mw 80 KDa was present in the susceptible variety and absent in resistant. These bands can be consid-

ered as positive markers associated with plant defense for *Cassida vittata*. These results are in harmony with those reported by Torronen and Maatta (2002) who reported that plants make vitamins, polyphenolic and other antioxidants to protect them-selves from dangers such as pests and drought. Many of these compounds are also healthy compounds for human consumption as they can act as antioxidants and may protect human cells against different diseases. Parissa and Tarighi (2010) showed that plants exhibited a variety of responses during infection by pathogen, insects and abiotic stresses, many of which involve the activation of host defense genes. Activation of these genes leads to physical and biochemical changes in plant cells which are not favorable for damage progress in plant. Biosynthesis and the accumulation of inducible defense related proteins are among the major biochemical changes.

El-Mahalawy (2011) studied three genotypes of sugar beet (Farida, Kawemira and Montopianco), He illustrated that the bands with Mw 150 KDa can be considered as a negative marker associated with plant defense to insect pests, the band with Mw 11.5 KDa can be considered as a positive marker associated with plant defense to insect pests.

***ISSR and RAPD-PCR analysis***

Bulked analysis was carried, using DNA samples of each of the two sugar beet varieties samples with five inter-simple sequence repeats (ISSR) primers to obtain molecular markers associated with insect tolerance genes in plant. Results confirmed the presence of different numbers of amplified bands, all the five primers succeeded to give high polymorphism among the studied verities. The data presented in Table (7) and Fig. (4) showed that each ISSR primer gave nine bands which ranged from 165-1040 base pairs (bp). Bands with MW 165 bp, (UBC848), 605 and 390 bp (844A), 390 and 350 bp (17889A) and 350 and 180 bp (UBC836) were present in Pyramids variety and were absent in Zinagri variety. These bands can be considered as a positive markers associated with sugar beet resistance to *Cassida vittata*. Also, the results indicated that the bands which obtained by ISSR with 265 bp (UBC848 and HB12), 350 bp (844A), 1040 and 180 bp (17889A) and 740 bp (UBC836) were present in Zinagri variety and absent in Pyramids variety. These bands can be considered as a negative markers associated with sugar beet resistance to *Cassida vittata*. Also, Randomly Amplified Polymorphic DNA of the Polymerase Chain Reaction (RAPD- PCR) primers was used to obtain molecular markers associated with *Cassida vittata* tolerance genes. Data in Table (8) and Fig. (5) showed that each RAPD-PCR primer gave ten bands ranging from 65-295 base pairs (bp). Bands with MW 85 bp and 65 bp (OPA-5), MW 145 bp (OPA-1) and MW 85 (OPA-7, OPA-8 and OPA-9) were present in Pyramids variety while absent in Zinagri variety. These bands can be considered as positive markers associated with sugar beet resistance to *Cassida vittata*. Also, the results indicated that the bands were

with molecular size 130 bp (OPA-1) and 95 bp (OPA-9) were present in Zinagri variety and were absent in Pyramids variety. These bands can be considered as a negative markers associated with sugar beet resistance to *Cassida vittata*. These results are in agreement with those obtained by El-Mahalawy (2011) who reported that a high level of DNA polymorphism was detected by ISSR technique. For the three genotypes under study and showed some positive and negative markers associated with plant tolerance to insect attacks.

## SUMMARY

Insect infestation of sugar beet is one of the most important problems in sugar beet fields at Delta, Egypt. Plant defense against insects represents the most successful element in integrated pest management (IPM). So, the behavior of insect attack, biochemical and molecular analysis were utilized to study protein, peroxidase and esterase isozymes banding patterns under insect attacks to sugar beet varieties. In addition, protein electrophoresis, genomic DNA (ISSR technique) and RAPD-PCR were used to find molecular markers associated with insect attack tolerance in sugar beet plants. These studies were carried out during 2012/2013 and 2013/2014 seasons on two sugar beet varieties (Pyramids and Zinagri).

The insect attack behavior study showed that Pyramids variety is resistant to *Cassida vittata* and Zinagri variety is susceptible to *Cassida vittata*.

From biochemical and genetic studies some peroxidase and esterase enzymes markers were found in the resistant variety. Presence of bands number 3 for peroxidase and number 5 for esterase can be considered as a marker associated with plant defense to *Cassida vittata*. Also, the bands with Mw 127 and 17.5 KDa can be considered as negative markers associated with plant defense to *Cassida vittata*, the band with Mw 80 KDa can be considered as a positive marker associated with plant defense to *Cassida vittata.* A high level of DNA polymorphism was detected by ISSR and RAPD-PCR techniques for the two sugar beet varieties showing some positive and negative markers associated with plant tolerance to insect attack.

## REFERENCES

Aba, J. and C. H. Tsuda (1987). Genetic analysis for isozyme variation in section Vulgares, Genus Beta. Japan. J. Breeed., 37: 253-261.

Agrios, G. (1997). Plant Pathology, 4th ed. San Diego, Academic press, p. 93-114.

Anonymous (2014). Sugar crops council. Annual Report of 2013, Ministry of Agriculture and Land Reclamation, Arab Republic of Egypt.

Bassyouni, A. M. and F. A. Abo-Attia (1998). Effect of organic manures on sugar beet properties and the insect infestation. J. Agric. Sci. Mansoura Univ., 23: 1729-1737.

Bazazo, K. G. I. (2010). Studies on some insect pests and natural enemies in sugar beet fields at Kafr El-Sheikh region. Ph.D. Thesis, Fac. Agric., Tanta Univ., pp 139.

Bazazo, K. G. I. (2012). First isolation of the entomopathogenic fungi, Stachybotrys sp. from naturally infected tortoise beetle, *Cassida vittata* Vill (Coleoptera: Chrysomelidae in sugar beet fields in Egypt. J. Plant Prot. and Path., Mansoura Univ., 3: 601-609.

Collinge, S. K. and S. M. Louda (1989). Influence of plant phenology on the insect herbivore/bittercress interaction. Oecologia. 79: 111-116.

Davis, R. J. (1964). Disc electrophoresis 2-method of application to human serum protein. Ann. N. Y. Acad. Sci., 121: 404-427.

Dellaporta, S. L., J. Wood and J. B. Hicks (1983). Plant molecular biology. Reporter, 1: 19-21.

Dowd, P. F., E. T. Johnson and W. P. Williams (2005). Strategies for insect management targeted toward mycotoxin management. In: Abbas, H. K. (Ed.), Aflatoxin and food safety. New York, CRC Press, Boca Raton, FL, 517-542.

El-Mahalawy, N. A. (2011). Ecological and biological studies on some sugar beet insects. M.Sc. Thesis, Fac. Agric., Tanta Univ., pp 135.

Gurguis, G. Z. (1985). Studies on certain insects attacking sugar beet in Western Desert, Egypt. Ph.D. Thesis, Fac., Menufiya Univ., pp 150.

Hou, Y. C, H. Y. Ze, M. W. Yu and L. Z. You (2005). Genetic diversity in barley from west China based on RAPD and ISSR analysis. Barley Genetics Newsletter, 35: 9-22.

Juniper, B. E. and C. E. Jeffree (1983). Plant Surfaces. Edward Arnold, London, pp 347.

Laemmli, U. K. (1970). Cleavage of structure proteins during assembly of head bacteriophage T4. Nature, 227: 680-686.

McMullen, M. D. and K. D. Simcox (1995). Genomic organization of disease and insect resistance genes in maize. MPMI, 8: 811-815

Miroslawa, K. and K. Maria (2003). Variability of cathodic peroxidases in sugar beet (*Beta vulgaris* L.) cultivars. J. Appl. Genetics, 44: 55-62.

Murray, M. G. and W. F. Thompson (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, 8: 4321-4325.

Nechiporuk, T. (1991). Biochemical characteristics of the resistance of sugar beet to leaf aphid. J. Sel'skokhozyaĭstvennaya *Biologiya*, 3: 120-122. [C.F. (CD Rom computer system)]

Osorio, S., B. Stephens and C. Araguez (2011). Demethylation of oligogalacturonides by FaPEI in the fruits of the wild strawberry triggers metabolic and transcriptional changes associated with defense and development of the fruit. J. Exp. Bot., 62: 2855-2873

Parissa, T. and S. Tarighi (2010). The role of peroxidase in basal resistance of sugar beet against the *Rhizoctonia* root rot diseases. 6th Australasian Soil Borne Diseases Symposium, 2010.

Saleh, M. M., K. A. Draz, M. A. Mansour, Mona A. Hussein and M. F. Zawrah (2009). Controlling the sugar beet beetle, *Cassida vittata* with entomopathogenic nematodes. J. Pest. Sci., 82: 289-294.

Shalaby, G. M. (2001). Ecological studies on some important sugar beet pests and natural enemies and their control. Ph. D. Thesis, Fac. Agric., Kafr El-Sheikh, Tanta Univ., pp 141.

Singh, D. and A. Singh (2005). Disease and insect resistance in plants. Science Publishers, Enfield, New Hampshire, pp 428.

Steinite, I. and G. Levinsh (2003). Possible role of trichomes in resistance of strawberry cultivars against spider mite. Acta Univ., Latviensis, 662: 59-65.

Tanksley, S. O. and T. J. Orton (1983). Isozymes in plant genetic and breeding. Part B. Elsevier, Amsterdam, pp 472.

Torronen, R. and K. Maatta (2002). Bioactive substance and health benefits of strawberries. Acta Hort., 567: 797-803.

Table (1): The sequence of the five RAPD primers used.

| Primer No. | Primer name | Sequence 5' → 3' |
|---|---|---|
| 1 | OPA-5 | AGG GGT CTT G |
| 2 | OPA-1 | CAG GCC CTT C |
| 3 | OPA-7 | GAA ACG GGT G |
| 4 | OPA-8 | GTG ACG TAG G |
| 5 | OPA-9 | GGG TAA CGC C |

Table (2): ISSR primer sequences used in the present study.

| Primer No. | Primer | Sequence 5' → 3' |
|---|---|---|
| 1 | UBC 848 | (CA) 8R*G |
| 2 | HB12 | (CAG) 3GC |
| 3 | 844A | (CT) SAC |
| 4 | 17889A | (CA)6AC |
| 5 | UBC 836 | (AG) 8Y*A |

*Y: (C, T) nucleotide bases and R (A, G) nucleotide bases.

Table (3): Mean numbers of *Cassida vittata* attacking sugar beet cultivars, Zinagri and Pyramids, during 2012/13 and 2013/14 seasons, using direct visual examination.

| Date of examination | Mean number of individuals (eggs + larvae + adults)/plant | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012/2013 | | | | | | 2013/2014 | | | | | |
| | Zinagri | | | Pyramids | | | Zinagri | | | Pyramids | | |
| | E | L+A | Total | E | L+A | Total | E | L+A | Total | E | L+A | Total |
| 25 Feb. | 30 | 0 | 30 | 2 | 0 | 2 | 26 | 0 | 26 | 1 | 0 | 1 |
| 5 Mar. | 40 | 2 | 42 | 3 | 0 | 3 | 38 | 2 | 40 | 1 | 0 | 1 |
| 15 Mar. | 100 | 10 | 110 | 3 | 1 | 4 | 90 | 8 | 98 | 2 | 0 | 2 |
| 25 Mar. | 102 | 20 | 122 | 0 | 1 | 1 | 110 | 20 | 130 | 1 | 2 | 3 |
| 5 Apr. | 40 | 127 | 167 | 1 | 4 | 5 | 22 | 130 | 152 | 1 | 3 | 4 |
| 15 Apr. | 21 | 160 | 181 | 0 | 3 | 3 | 20 | 157 | 177 | 0 | 2 | 2 |
| 25 Apr. | 20 | 164 | 184 | 0 | 2 | 2 | 15 | 175 | 190 | 0 | 3 | 3 |
| Total 210 plants | 836 | | 836 | | | 20 | | | 813 | | | 16 |
| Mean/plant | | | 3.98 | | | 0.09 | | | 3.87 | | | 0.07 |
| 't' calculated | $4.865^{**}$ | | | | | | $4.738^{**}$ | | | | | |

E: Egg L: Larvae and A: Adult. ** highly significant

Table (4): Presence (1) versus absence (0) of esterase zymogram.

| Band No. | Zinagri | Pyramids |
|---|---|---|
| 1 | 1 | 1 |
| 2 | 1 | 1 |
| 3 | 1 | 1 |
| 4 | 1 | 1 |
| 5 | 0 | 1 |
| 6 | 1 | 1 |
| 7 | 1 | 1 |
| 8 | 1 | 1 |
| 9 | 1 | 1 |

Table (5): Presence (1) versus absence (0) of peroxidase zymogram.

| Band No. | Zinagri | Pyramids |
|---|---|---|
| 1 | 1 | 1 |
| 2 | 1 | 1 |
| 3 | 0 | 1 |
| 4 | 1 | 1 |
| 5 | 1 | 1 |
| 6 | 1 | 0 |
| 7 | 1 | 1 |
| 8 | 1 | 1 |

Table (6): Presence versus absence for protein patterns of two sugar beet varieties.

| MW KDa | Sugar beet varieties | |
|---|---|---|
| | Zinagri | Pyramids |
| 255.0 | 1 | 1 |
| 158.0 | 1 | 1 |
| 127.0 | 0 | 1 |
| 80.0 | 1 | 0 |
| 70.0 | 1 | 1 |
| 59.5 | 1 | 1 |
| 55.6 | 1 | 1 |
| 40.5 | 1 | 1 |
| 34.6 | 1 | 1 |
| 26.7 | 1 | 1 |
| 25.5 | 1 | 1 |
| 22.7 | 1 | 1 |
| 21.7 | 1 | 1 |
| 20.6 | 1 | 1 |
| 17.5 | 0 | 1 |
| 17.0 | 1 | 1 |
| 12.5 | 1 | 1 |
| 3.8 | 1 | 1 |
| 3.5 | 1 | 1 |

Table (7): Presence (1) versus absence (0) of DNA polymorphism for two sugar beet genotypes with five ISSR primers.

| Marker bp | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Zinagri | | | | | Pyramids | | | | |
| | ISSR primers | | | | | ISSR primers | | | | |
| | UBC 848 | HB12 | 844A | 17889A | UBC 836 | UBC 848 | HB12 | 844A | 17889A | UBC 836 |
| | Polymorphic bands No. | | | | | Polymorphic bands No. | | | | |
| | 1 | 3 | 5 | 7 | 9 | 2 | 4 | 6 | 8 | 10 |
| 1040 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 740 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 605 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| 545 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| 390 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| 350 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| 265 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 180 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| 165 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

Table (8): Presence (1) versus absence (0) of DNA polymorphism for two sugar beet genotypes with five RAPD primers.

| Marker bp | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Zinagri | | | | | Pyramids | | | | |
| | RAPD primers | | | | | RAPD primers | | | | |
| | OPA-5 | OPA-1 | OPA-7 | OPA-8 | OPA-9 | OPA-5 | OPA-1 | OPA-7 | OPA-8 | OPA-9 |
| | Polymorphic bands No. | | | | | Polymorphic bands No. | | | | |
| | 1 | 3 | 5 | 7 | 9 | 2 | 4 | 6 | 8 | 10 |
| 295 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| 275 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 235 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 215 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 185 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 145 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| 130 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 |
| 95 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 85 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| 65 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

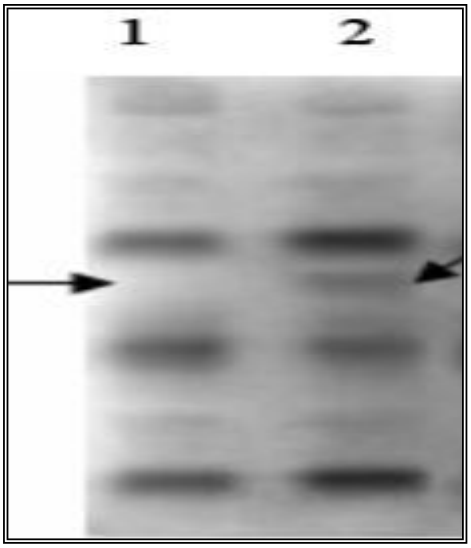


Fig (1): Polyacrylamide gel 7.5 % electrophoresis shows esterase enzyme patterns two sugar beet varieties (1-Zinagri, 2-Pyramids).

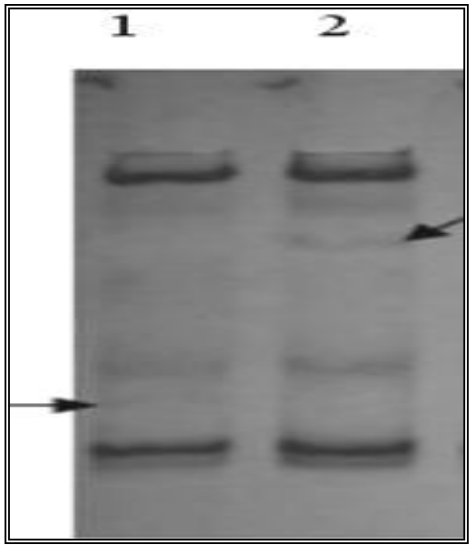


Fig. (2): Polyacrylamide gel 7.5% electrophoresis shows peroxidase enzyme patterns two sugar beet varieties (1- Zinagri, 2-Pyramids).

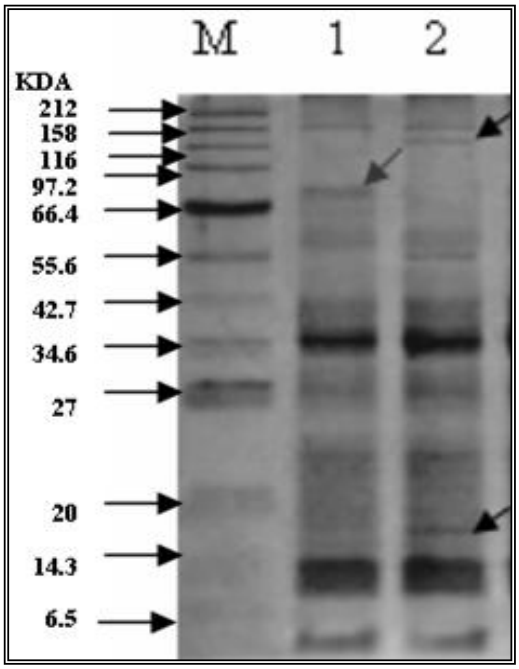


Fig (3): SDS-PAGE of protein banding patterns for two sugar beet genotypes (1-Zinagri, 2- Pyramids).

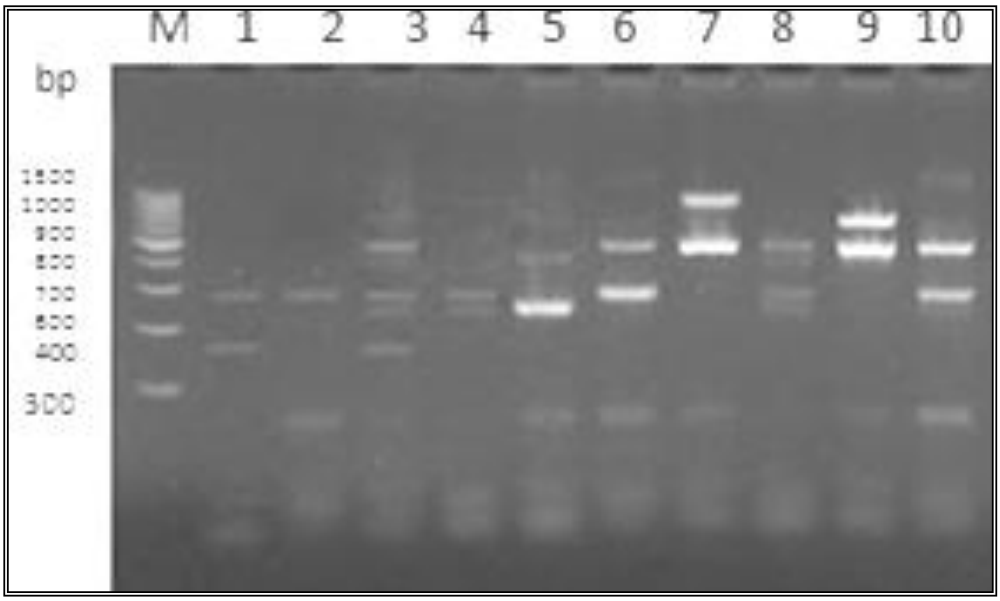


Fig (4): ISSR primers amplified polymorphic bands as plant defense of *Cassida vittata* for the two sugar beet cultivars.

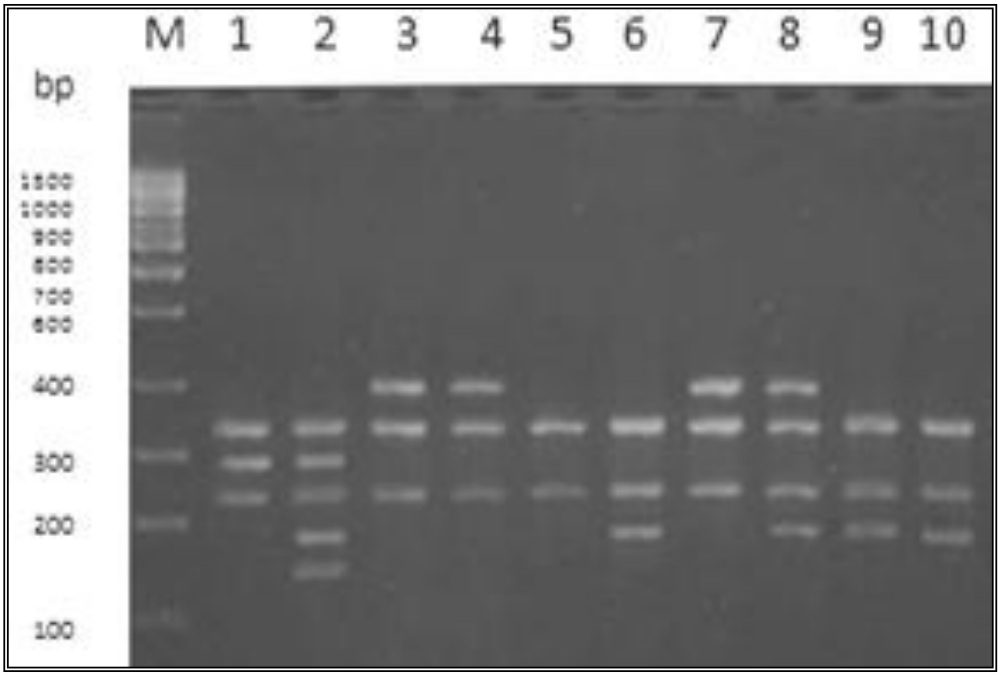


Fig (5): RAPD-PCR primers amplified polymorphic bands as plant defense of *Cassida vittata* for the two sugar beet cultivars.