J. Plant Production, Mansoura Univ., Vol. 2 (4): 641 - 651, 2011

BIOCHEMICAL AND MOLECULAR IDENTIFICATION OF SOME SOYBEAN (*Glycine max L.*) CULTIVARS

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ABSTRACT

This study was carried out to distinguish among eight soybean (*Glycine max* L.) cultivars using biochemical and molecular marker. Therefore, SDS-storage proteins, some isozyme (esterase (EST), peroxidase (Prx) and glutamate oxaloacetate transaminase (GOT)) and PCR-RAPD (polymerase chain reaction-randomly amplified polymorphic DNA) markers were carried out.

The results of protein electrophoresis revealed that total number of bands ranged from 10 in cultivar G.21 to 14 in cultivar Crawford.. Nine common bands were found in all cultivars. Some cultivars showed specific bands which could be used to distinguish between others. Each of G.22, G.35, G.21, G.111 and G.83cultivars had only unique band, with molecular weight of 53.83, 44.84, 33.37, 21.66 and 16.73 kda, respectively.

Bands of three isozymes systems (Est, Prx and GOT) were determined for soybean cultivars identification based on polyacrylamide gel electrophoresis. Using the number of bands, Rf values and its densities can be used to identify these varieties. One polymorphic Est isozyme was found band in cultivar Giza 21 with RF about 0.579 this band considered as a marker for this cultivar. A total number of two Prx isozyme bands ware detected in two band was monomorphic RF 0.441 and 0.569 with different band densities in height densities in cultivars Giza35 and Giza 83. A total number of two GOT isozyme bands ware monomorphic RF 0.556 and 0.721 with different densities and intensities.

While molecular marker RAPD analysis utilizes six random primer the largest number of RAPD specific markers was generated by primer O02 (four markers) followed by O08 (three markers). While primers B04 and B12 generated two markers the lowest number of RAPD specific markers was generated by primers A12 and B06 (one marker). The largest number of RAPD specific markers were scored for cultivar Giza 21 (7 markers) followed by Giza 35 (3 markers).

INTRODUCTION

Soybean (*Glycine max* L.) is becoming an important oil and protein crop in the Egyptian Agriculture, soybean contain about 18-24% oil and 30-50% protein with considerable amounts of essential amino acid, especially lysine as well as phosphorous, calcium and vitamins. Soybean is one of the most economic and nutritious crop as it contains high protein and oil(Yaklich *et al.*, 2002).

Biochemical markers, especially the electrophoresis profiles of isozymes and proteins, have been widely used for identification of crop varieties. Electrophoresis methods have been standardized for a large number of crops and found useful for the purpose of variety identification and characterization (Patra and Chawla 2010).

Selim (1997) identified nine soy bean genotypes by using sodium dodecyle sulfate polyacrylmide gel electrophoresis (SDS-PAGE). The result

that soybean genotypes had a special one or more of protein that found in the seeds. These proteins differed in the molecular weight depending on the soy bean genotypes.

Isozyme techniques have been used in plant genetics and breeding. As biochemical markers, isozymes can be used for germplasm classification, gene mapping, selection, monitoring genetic segregation and recombination in distant crosses, variety/hybrid purity, and determination of phylogenic relationship in plants. Once isozyme genes are mapped, they can be utilized efficiently as biochemical markers to map other genes such as morphological and physiological genes or otherwise classical linkage methods are utilized (Murphy et al. 1990).

Molecular genetic technique (PCR-RAPD for analysis) is increasingly used to assess cultivar identification in many crop plant(chen *et al.*,1995). Most soybean cultivars are classified, solely, by their phenotypic characteristics, which are highly variable from on growing season to the next. This calls for the need to develop a more stable molecular screening technology to be used for germplasm identification (Harvey and Botha, 1996), the polymerase chain reaction (PCR) technology has been developed as a novel genetic assay based on selective DNA amplification (Saiki *et al.*, 1988; Dallaporta *et al.*, and Innis *et al.*,1990).

Al-Saghir and Abdel-Salam (2011) mentioned that the RAPD-PCR was used to analyze the genetic diversity of twenty soybean cultivars and to asses their genetic relationships using similarity index and dendogram tree. Twenty seven random primers were used to determine RAPD polymorphism. Twenty soybean accessions were included in the study and were subjected to RAPD molecular markers analysis. Twenty seven RAPD primers produced 210 amplification product of which 78 (27%) were polymorphic. In conclusion, this study reported a successful fingerprinting of soybean accessions using RAPD markers and demonstrated the usefulness of these markers in estimating the extent of genetic variation in Soybean germplasm.

This investigation was carried out to characterize of eight soybean cultivars using biochemical and molecular marker.

MATERIALS AND METHODS

Seed samples.

Seed of eight varieties of soybean (*Glycine mex L.*) were used in this investigation namely, Gize 21, Gize 22, Gize 35, Gize 82, Gize 83, Gize 111, Clark and Crawford. Seed samples under study were obtained from the Leguminous Crops Department Research (LCDR), Field Crops Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Methods of Analysis:-

SDS- protein electrophoresis:-

Sodium dodecyl sulfate polyacrylamid gel electrophoresis (SDS-PAGE) technique was used to characterize the total protein of the different genotypes. Protein profiling was carried out according to Laemmli (1970) and modified by Studier (1973).

Isozymes electrophoresis:

Native polyacrylamide gel electrophoresis (Native-PAGE) technique was used to charactarize the isozymes finger print of soybean cultivars such as esterase (EST), peroxidase (Prx) and glutamate oxaloacetate transaminase (GOT). Isozymes fractionation ware performed on vertical slab (19.8cm x26.8cm x.02cm) using the gel laconic electrophoresis apparatus according to Jonathon and Wendel (1990).

Table (1): The ingredients of staining solutions.

Enzymes	Compounds and references	Amounts
Prx	A-sodium acetate (1M, pH4.7) Methanol	50 ml
	3,3,5,5tetramethyl benzidine (TMBZ)	50 ml
	B-0.30 % H2O2.	50 ml
	Graham *et al.*(1964)	
EST	Sodium phosphate (100mM, pH6.0)	50 mg
	α -naphthyl acetate	25 mg
	Fast blue RR salt	50 mg
	Jonathan and Wendel (1990)	
GOT	A-Tris (1M,pH 8.5)	50 ml
	A-Ketoglutaric acid	50 mg
	Aspartic	100 mg
	B-pyrodxal-5- phosphate	5 mg
	Fast blue BB salt	150 mg
	Jonathan and Wendel (1990)	

DNA Molecular studies.

The RAPD markers assays are based on the PCR amplification of random locations in the plant genome. The DNA amplification protocol was performed as described by Williams *et al.* (1990) with some modifications. The DNA isolation from plant tissues was conducted using DNneasy Plant Mini kit (QIAGEN Hilden, Germany). The RAPD-PCR reactions were conducted using 5 arbitrary 10-mer primers. Their names and sequences are shown in Table (2).

Table (2): primer names and sequences in 5 to 3 direction

Primer Name	Sequence (5 to 3)
A12	´5-CAGCACCCAC-3´
B04	´5-GGACTGGAGT-3´
B06	´5-TGCTCTGCCC-3´
B12	´5-CCTTGACGCA-3´
O 02	´5-GTGACGTAGG-3´
O 08	´5-GAGGACCCAC-3´

The reaction conditions according to Williams *et al.* (1990) were optimized and mixtures (30 µl total volume) consisted of the following were used: dNTPs (2.5 Mm) 0.2 mM, MgCl2 (25 Mm) 1.5 m M, 10× buffer 3.0 µl, Primer (10 µM) 0.2 µM, TemplateDNA (50 NG/ µL) 2.0 µL, Taq (5u/ µl) 0.3 µl, D H2O up to 30 µl .Amplification was carried out in a PTC-200 thermal cycles (MJ Research, Watertown, USA) programmed for 40 cycles as follows : 94

◦C/4 min (1 cycle) ; 94 ◦C/30 sec, 36 ◦C/1 min, 72 ◦C/2 min (40 cycles); 72 ◦C/10 min (1cycle) and 4 ◦C (infinitive) .

Data analysis.

The similarity matrices were done using gel works advanced software UVP-England Program. The relationships among varieties as revealed by dendograms were done using SPSS Windows (version 10) Program.

RESULTS AND DISCUSSION

SDS- protein electrophoresis:-

SDS-Polyacrylamide gel electrophoresis of proteins provides good information for the identification and characterization of different genotypes. Eight soybean genotypes were fingerprinted by SDS-PAGE for total storage grain proteins.

Protein banding patterns of the studied soybean cultivars as revealed by SDS-PAGE for the total seed protein are shown in Tables 3 and 4 . Regarding the presented data, the total numbers of bands in all of the studied cultivars were 20 bands with molecular weight (mw) ranged from 197.55-14.55 KDa. There was clear variation in the number of the obtained bands. The obtained total number of bands among cultivars was ranged from 10 in cultivar G.21 to 14 in Crawford cultivar.

Table (3): SDS-PAGE of total proteins extracted from the eight soybean cultivars.

Mw	G21	G22	G35	G82	G83	G111	Clark	Crawford
197.55	-	+	+	-	-	-	-	+
176.77	-	+	+	-	-	-	-	+
161.4	+	+	+	+	+	+	+	+
146.26	+	+	+	+	+	+	+	+
126.64	+	+	+	+	+	+	+	+
103.99	+	+	+	+	+	+	+	+
91.42	-	-	-	+	+	-	-	+
82.85	-	-	-	-	-	+	+	+
73.39	+	+	+	+	+	+	+	+
62.12	-	-	-	+	+	+	+	+
53.38	-	+	-	-	-	-	-	-
44.84	-	-	+	-	-	-	-	-
43.17	+	+	+	+	+	+	+	+
33.37	+	-	-	-	-	-	-	-
28.46	+	+	+	+	+	+	+	+
24.64	+	+	+	+	+	+	+	+
21.66	-	-	-	-	-	+	-	-
20.08	-	-	-	-	-	+	-	-
16.37	-	-	-	-	+	-	-	-
14.55	+	+	+	+	+	+	+	+

(+) presence (-) absence

From results, there are nine common bands that were found in all cultivar which nine of them ware monomorphic and the rest were polymorphic band was molecular weight about 53.38KDa prsesnt in cultivar

Giza 22 and absent of all cultivars under study and band with mw about 44.84KDa considered as a marker for cultivar Giza 35 while the band with mw about 33.37KDa considered as a marker for cultivar Giza21.On the other hand, found two marker in mw about 21.66 and 20.08KDa can be considered as a marker for cultivar Giza 111. But band with mw about 16.37KDa considered as a marker for cultivar Giza83. In conclusion, this study recorded five marker for different cultivars Giza22, Giza35, Giza21, Giza83 and Giza111. This obtained results could be considered as positive unique marker

Table (4): Total number of bands and Molecular weight (MW) of the lowest and the highest bands for storage seed protein.

Genotypes	Maximum MW (kda)	Minimum Mw (kda)	Total band
G. 21	161.4	14.55	10
G. 22	197.55	14.55	12
G. 35	197.55	14.55	12
G. 82	161.40	14.55	11
G. 83	161.40	14.55	12
G. 111	161.40	14.55	13
Clark	161.4	14.55	11
Crawford	197.55	14.55	14

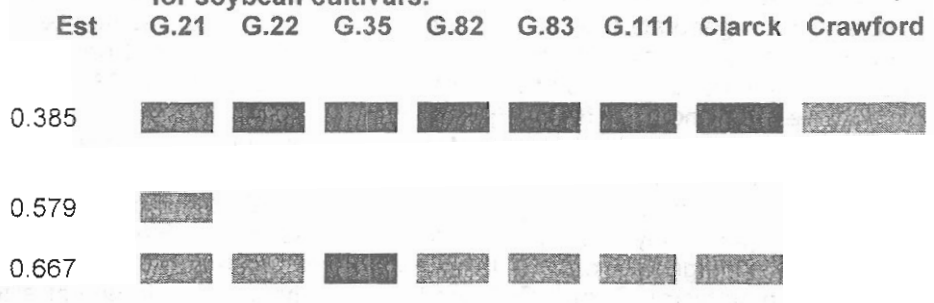
Isozymes electrophoresis

The target of this investigation is to use three different isozymes systems esterase (Est), peroxides (Prx) and glutamate oxaloacetae transamiase (GOT) for the identification of eight soybean cultivars under study based on polyacrylamide gel electrophoresis profiles .

Estrease (Est).

Zymogame esterase of eight soybean cultivars are illustrated in Table (5) the result of electrophoresis showed a maximum number of three bands in which two of them monomorphic RF 0.385 and 0.667 with different band densities and intensities. One polymorphic band in cultivar Giza 21 with RF about 0.579 this band considered as a marker for this cultivar

Table (5): Electrophoretic patterns of esterase isozymes and diagram for soybean cultivars.

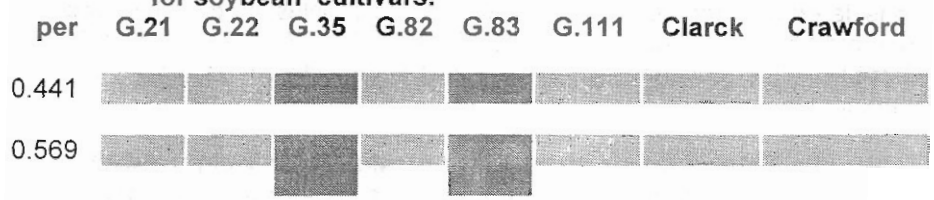


Peroxidase (Prx).

Peroxidase is isozyme electrophoresis was carried out as shown in Table (6). A total number of two isozyme bands was detected in two band

was monomorphic RF 0.441 and 0.569 with different band densities in height densities in cultivars Giza35 and Giza 83.

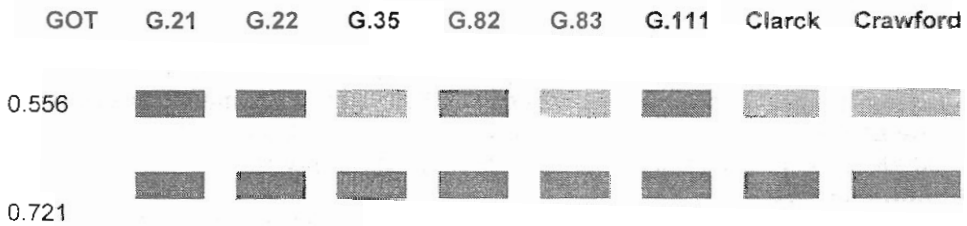
Table (6): Electrophoretic patterns of peroxidase isozymes and diagram for soybean cultivars.



Glutamate Oxaloacetae Transamiase (GOT).

The result of GOT isozymes for the eight soybean cultivars are shown in Table (7). A total number of two bands was monomorphic RF 0.556 and 0.721 with different densities and intensities.

Table (7): Electrophoretic patterns of got isozymes and diagram for soybean cultivars.



Molecular analysis of soybean genotypes

RAPD analysis DNA of the eight soybean genotypes were subjected to PCR against 6 random 10-mer primer (A12, B04,B06, B12, O02 and O08) as described in Table (8) and illustrated in Figure (1). The number of amplicons per primer varied from three (A12) to nine (B12) the size of the amplified fragment ranged from 285 bp (A12) to 1300 bp (B12). Two primers B12 and O02 generated no monomorphic bands. The highest number of monomorphic bands two of four ware scored for primer B06. Primer A12 produced three bands in which fragment sizes ranged from 435 to 285 bp two of them were polymorphic (66% polymorphism).

Primers B04 yielded five bands with the fragment size ranged from 1220 to 305 bp four of them were polymorphic (80% polymorphism). Primer B06 revealed four bands with fragment sizes 480 to 225 bp two of them were polymorphic (50% polymorphism). Primer B12 resulted in nine bands with fragment size ranged from 1300 to 265 bp all of which were polymorphic (100% polymorphism). Primer O02 produced six bands with fragment size ranged from 1195 to 335 bp all of which were polymorphic (100% polymorphism). Primer O08 yield four bands with fragment size 1260 to 605 bp three of them were polymorphic (75% polymorphism). All specific markers

bands on RAPD analysis were scored for the presence (positive marker) or absence (negative marker) of bands for a given genotype as a show in table (2).

Table (8): DNA polymorphism in eight soybean genotypes using PCR with 6 random primers.

Bp		1	2	3	4	5	6	7	8
435	A12	+	+	+	+	+	+	+	+
330		+	-	+	+	+	-	+	-
285		+	-	-	-	-	-	-	-
1220	B04	-	-	-	-	-	-	-	+
715		+	+	+	+	+	+	+	+
480		-	+	-	-	+	-	-	-
350		+	+	+	-	-	-	+	-
305		-	-	-	+	-	-	-	-
480	B06	+	+	+	+	+	+	+	+
355		+	+	+	+	+	+	+	+
300		-	-	-	-	-	-	+	+
225		+	+	+	+	-	+	+	+
1300	B12	-	-	+	-	-	-	-	-
1005		-	-	+	-	-	-	-	-
800		+	-	+	-	-	-	-	-
755		+	-	-	-	+	-	-	-
630		+	+	+	-	-	-	-	-
515		-	-	+	+	-	+	-	+
410		+	-	-	-	+	-	-	-
315		+	+	-	-	+	-	+	+
265		+	+	-	-	-	-	-	-
1195	O02	+	+	+	-	-	+	-	-
980		+	-	-	-	-	-	-	-
830		+	-	-	-	-	-	-	-
660		+	-	-	-	-	-	-	-
420		+	+	-	+	+	+	+	+
335		+	-	-	+	+	-	-	+
1260	O08	-	+	+	+	+	+	+	+
985		+	-	-	-	-	-	-	-
720		+	-	-	-	-	-	-	-
605		+	+	+	+	+	+	+	+

The largest number of RAPD specific markers was generated by primer O02 (four markers) followed by O08 (three markers). While primers B04 and B12 generated two markers the lowest number of RAPD specific markers ware generated by primers A12 and B06 (one marker). The largest number of RAPD specific markers were scored for cultivar Giza 21 (7 markers) followed by Giza 35 (3 markers).

The soybean Giza 21 was characterized by one positive marker against primer A12 with MW about 285 bp, three positive markers of primer O02 with MW about 980, 830 and 660 bp. While, primer O08 produced two of them positive markers in MW about 985, 720 bp and one of them negative

marker in MW about 1260 bp. The genotype Giza 35 showed two positive markers (1300 and 1005 bp of primer B12) and one negative markers (420 bp of primer O02). The genotype Giza 83 exhibited one negative marker (225 bp of primer B06). The genotype Giza 82 showed one positive marker (302 bp of primer B04). The genotype Crawford was distinguished by one positive marker (1220 bp of primer B04).

The four specific RAPD markers for Crawford can also be tested for their linkage with genes for high yielding.

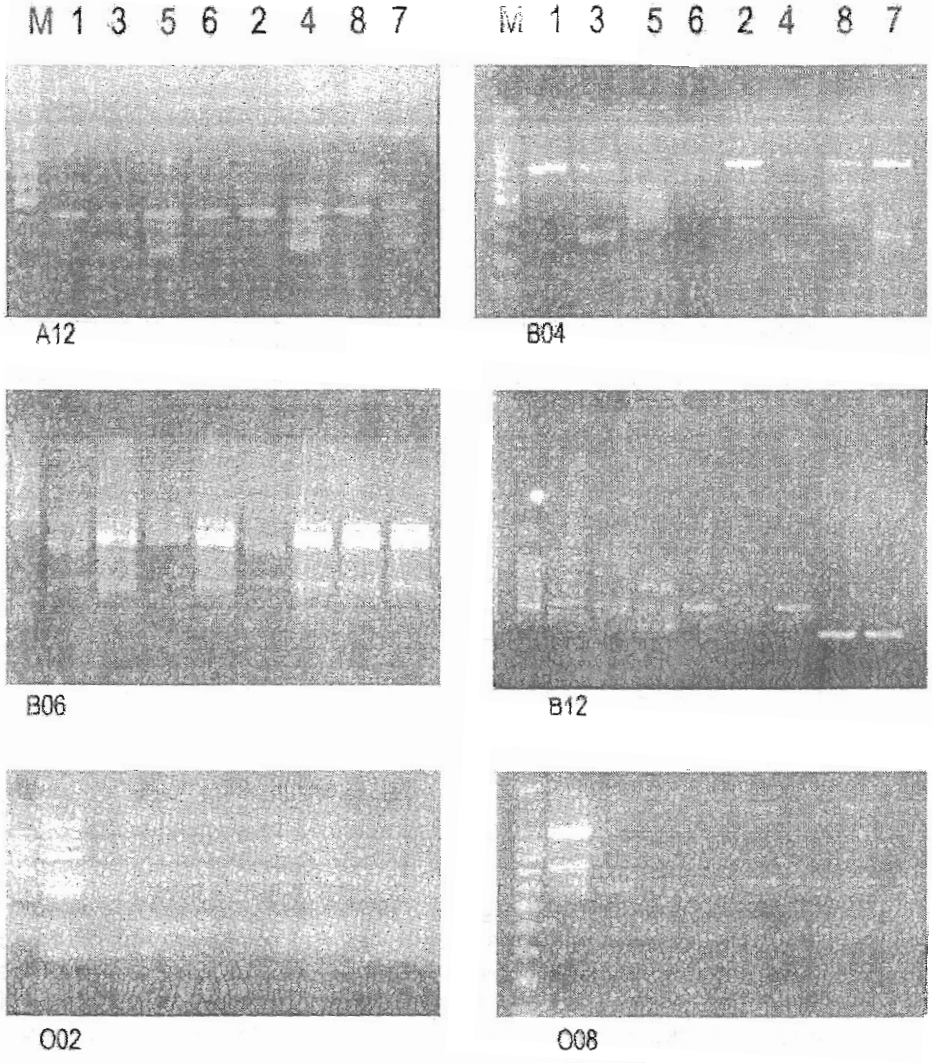


Figure (1): DNA polymorphism of eight soybean genotypes using 6 random primers.

SDS-storage proteins, some isozyme (esterase (EST), peroxidase (Prx) and glutamate oxaloacetate transaminase (GOT)) and were carried out.

Such recoded result of Protein electrophoresis of seed storage protein and isozymes in addition to use PCR-RAPD (polymerase chain reaction-randomly amplified polymorphic DNA) markers are in agreement with Patra and Chawla(2010), Selim (1997), Murphy *et al.* (1990), Hu and Wang (1997), Wang *et al.* (1997), Zhang *et al.* (1999) and Liu *et al.* (2000).In general, the present study calls for the search for more RAPD markers to initiate the genome map of soybean that involves molecular genetic markers linked to several genes of interest (i.e., QTLs) obtained from contrasting soybean genotype.

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التعريف البيوكيميائى والجزيئى لبعض أصناف فول الصويا

رحاب تودى بحيرى و رشا يوسف عبدالخالق
قسم بحوث تكنولوجيا البذور-معهد المحاصيل الحقلية-مركز البحوث الزراعية

أجريت هذه الدراسه على ثمانية أصناف من فول الصويا وهى جيزه ٢١،جيزه ٢٢،وجيزه ٣٥،جيزه ٨٢،جيزه ٨٣،جيزه ١١١،كلارك،وكراوفورد.
والهدف هو توصيف وتمييز هذه الأصناف بإستخدام التفريد الكهربى لبروتين البذور وثلاثة من المشابهات الإنزيمية وهى (الاستيريز- البيروكسيديز- جلوتامات أكسالو اسيتات ترانس امينيز) وكذلك البصمه الوراثيه بإستخدام تحليل الـ RAPD من خلال تقنية تفاعل البلمرة المتسلسل PCR بإستخدام ستة من البادئات المحتوية على عشرة قواعد عشوائية التتابع

وكانت اهم النتائج كما يلى:-

بينت نتائج التفريد الكهربى لبروتين البذور ان إجمالى عدد الحزم يتراوح ١٠ حزم فى الصنف جيزه ٢١ الى ١٤ حزمه فى الصنف كراوفورد. كما وجدت تسعة حزم مشتركة فى جميع الأصناف تحت الدراسة. كما امكن الحصول على دلائل موجبة فى صورة حزمة مميزه لكل صنف من الاصناف الاتية عن الاخرى كما فى (جيزه ٢٢، جيزه ٣٥، جيزه٢١، جيزه ١١١، جيزه ٨٣)

عند الحزم ذات الوزن الجزيئى (٥٣.٨٣، ٤٤.٨٤، ٣٣.٣٧، ٢١.٦٦، ١٦.٧٣)كيلو دالتون على التوالى.

أما على مستوى المشابهات الإنزيمية فقد دلت النتائج أنــه باســتخدام عــدد المكونــات البروتينية وقيم الــ R_F لبعض المشابهات تحت الدراسة يمكن توصيف وتمييز هــذه الأصــناف. حيث دلت نتائج الاستيريز ان الصنف جيزه ٢١ امكن تمييزه عند قيمة Rf ٠.٥٧٩.ومــن نتــائج الاستيريز فقد دلت أنه أعطى حزمتان ذات قيم Rf ٠.٤٤١، ٠.٥٦٩ للأصناف مختلفه فى الكثافة وأمكن تمييزأصناف جيزه ٣٥، جيزه ٨٣. وتبين النتائج الخاصة بالجلوتامات أكسالواسيتات ترانس أمينيز أن كل الأصناف تحتوى على حزمتان بروتينية مختلفة فى درجة الكثافة.

ومن نتائج تحليل الــ RAPD من خلال تقنية تفاعــل البلمــرة المتسلــسل PCR أظهرت النتائج مايلى:-

تراوح عدد الحزم التى تم الحصول عليهــا لكــل بــادئ مــن ثلاثــة (A 12) إلــى ٩ (B 12).تراوح الحجم الجزيئى للحزم التى تم الحصول عليها لكل بادئ من 225 زوج قاعدة (B 06) إلى 1300 زوج قاعدة(B 12).أعطى البادئ (A 12) عدد 3 حزم بأحجــام جزيئيــة تراوحت بين 285 - 435 زوج قاعدة. و توجد حزمة مشتركة بين جميع الأصناف. وقد أمكــن تمييز الصنف جيزه ٢١ بوجود حزمة مميزة بحجم جزيئي ٢٨٥ زوج قاعدة. وقد أمكــن تمييــز الصنف جيزة ٨٢ بوجود حزمة مميزة بحجم جزيئي ٣٠٥ زوج قاعدة بالبــادئ (B04).ومــن النتائج أيضا أمكن الحصول على دلائل سالبة في صورة حزمة واحدة ذات حجــم جزيئــي 225 زوج قاعدة مميزة للصنف جيزة35.

قام بتحكيم البحث

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