MORPHOLOGICAL, QUNTITATIVE AND BIOCHEMICAL TRAITS OF TEN PROMISING SOYBEAN (*GLYCINE MAX*, L. MERR.) GENOTYPES

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Abstract

Morphological and biochemical traits were utilized to identify among ten promising soybean genotypes (H30, H32, Toano, DR101, H117, H127, H129, H132, H2 L12 and H15 L5). A field experiment was carried out at Gemmieza Agricultural Research Station in 2010 and 2011 summer seasons. Results revealed that, H30 genotype can be identified with shape of lateral leaflet (lanceolate), density of trichomes (dense) and seed hilum colour (brown). H132 was identified with seed hilum colour (brown). H129 was identified with flower color (Purple). H127 was identified with brown seed testa, pod color (very dark brown) and flower color (white). DR1Q1 was identified with shape of lateral leaflet (lanceolate to thermboidal) and pod color (dark brown). Leaflet color was green in H2 L12 genotypes. Concerning the onset of flowering and maturity, Toano and DR101 were the latest, while hybrid 30, H15 L5 and H2 L12 were the earliest compared with other genotypes. DR101 recorded the highest number of seeds/ plant and the heaviest 100-seed wight. H2 L12 gave the highest seed yield, seed protein and oil contents. The RAPD markers were used to determine the genetic differences among the ten soybean genotypes. Different sets of RAPD primers were used to study the polymorphism at molecular level. Six random primers were used for RAPD analysis generating 62 bands, 53 (85.4%) of them were polymorphic. The dendrogram divided these genotypes into two main clusters where H127 was in a separate cluster and all the other genotypes were in the second cluster. The highest similarity observed between H129 and H2 L12 while the lowest was observed between H30 and H127.

Key words: Soybean genotypes, identification, Morphological, biochemical and molecular characterization.

INTRODUCTION

Morphological, quantitative and biochemical studies were designed to find out definite distinguished characters of soybean (*Glycine max L. Merr.*) genotypes to furnish such information to breeders and field inspectors. The breeder requires these data in the evaluation course of his program. In addition, it serves as a turn witness in the course of breeders right implementation. Field inspectors require to have recognizable characters of the variety of crop under inspection so that study of purity might be worked out properly. In addition to determine the value of a genotypes to

be grown on a large scale, it is important to be easily recognized during seed multiplication. In order to achieve such requirements, the characteristics that distinguish a cultivar from another have to be established and easily observed. Stable morphological features of seed, seedling and adult plant are required so that the identity of a cultivar can not be eroded during seed multiplication from one generation to another. Furthermore, morphological description is a precondition for the protection and registration of varieties (UPOV 1984). Cultivars within a species are normally discriminated by morphological descriptors. In species with a narrow genetic base, novel varieties tend to be very similar and often indistinguishable by morphological traits Lanza *et. al.*, (2000). Many tools are now available for studying genetic variability among accessions including total seed protein, isozymes and various types of molecular markers. However, morphological characterization is the first step in the description and classification of the germplasm. The characterized material then helps the plant breeders to select the accessions to be utilized in hybridization programme. Addalla *et. al.*, (2004), identified twelve soybean genotypes based on morphological differences in seed, seedling and adult plant, agronomic characters such as days from planting to flowering and maturity, yield and its components, in addition to seed chemical components and biochemical variability of genomic fingerprinting. Sharma and Singh (2011), studied characterization of 62 soybean varieties. These varieties were evaluated for five morphological characters (flower color, pubescence color, seed coat color, hilum color and intensity of pubescence) and twelve agronomical characters among them (days to 50% flowering, days to maturity and 100-seed weight). DNA-based markers are a powerful tool for studies of genetic diversity. One of suitable DNA-based markers for genetic diversity studies is RAPD (Random Amplified Polymorphic DNA). This marker has a high potential in order to polymorphic evaluation in all races of plants and animals (Welsh *et al.,* 1991, Vierling and Nguyen 1992 and Abdelnoor *et al.*, 1995). DNA-based markers have received increased attention due to the almost unlimited number, to the easiness of DNA extraction and its real availability. Genetic distances based on molecular markers have been determined in both autogamous (common bean) and alyogamous (*Eucalyptus*) species, in cultures with broad as well as with narrow genetic bases (Paterson, 1998). However, in some cases, little or no attention has been paid to the suitability of the method used for genetic distance calculations. Several investigations have demonstrated the usefulness of RAPD for cultivar identification and genetic diversity determination when morphological characteristics were insufficient (Williams *et al.,* 1990). Therefore, the methods that evaluate and identify the genotypes more

precisely during the growing season, especially at early stage, are preferred by plant readers (Major, 2002).

The objective of this study was to identify some soybean genotypes by using some morphological, quantitative and biochemical traits.

MATERIALS AND METHODS

A field and laboratory experiments were conducted at Gemmeiza Agricultural Research Station, EL-Gharbia Governorate and Seed Technology Research Section, ARC, Egypt, in 2010 and 2011 summer seasons to distinguish among ten promising soybean genotypes through using some morphological, seed, seedling and adult plant, agronomic characters and biochemical variability of genomic fingerprinting. Seeds of the studied genotypes were received from Legumes Research Dep., Field Crops Research Institute, Agricultural Research Center. The experimental design was a Randomized Complete Block Design with three replicates. The texture of experimental soil was salty clay loam. The preceding crop was Egyptian Clover (*Trifollium alexandrine L.,*) in the two seasons. The soil was ploughed and calcium super phosphate (15% P2O5) was incorporated into the soil at a rate of 150 Kg fed^{-1} before sowing. Sowing dates were 20 and 30 May in the first and second seasons respectively. Each plot consisted of five ridges, 60 cm a part and 4 m long. Seeds were inoculated, with the specific rhizobic prior to planting. Nitrogen fertilizer was added as a starter dose before the first irrigation at a rate of 15 Kg N fed^{-1}. Hand hoeing was practiced twice to control weeds. All the agronomic practices were conducted as recommended and the studied traits were as follows:-

1- Morphological characters

Qualitative traits were visually recorded using scales reported by IBPGR (1984). These characters included hypoctyle color, color of hairs, density of trichomse, shape of lateral leaflet, leaflet color, flower color, pod color, seed shape, color of seed testa, seed coat luster and seed hilum luster.

2- Quantitative characters

These characters included days from sowing to 50% flowiring of plants with at lest one flower and days from sowing to maturity were recorded. At harvest, ten plants from the two central ridges were randomly taken to measure plant height, highest of the first pod (cm), No. of seed/ plant, 100-seed weigh and seed yield (t. fed-1).

3- Chemical composition

For seed chemical composition, air-dried seed samples (50g each) were randomly taken from each genotype to determine crude protein percentage (calculated by multiplying the total nitrogen by 6.25) and crude oil percentage (determined using soxcelt apparatus using n- hexane), according to (AOAC, 1990).

Collected data for each season were statistically analyzed was used to compare among them and the Least Significant Difference (L.S.D.) (Gomez and Gomez, 1984).

4- Genomic DNA extraction

Dneasy plant minikit (Quigen Inc., Cat.no.69104, and USA) was used for DNA extraction from the ten soybean genotypes.

RAPD- PCR analysis :

RAPD – PCR reactions were conducted using 6 arbitraty 10- mer primers with the 5′ → 3′ sequences as shown in Table 1.

Table 1. Names and sequences of primers used for RAPD- PCR analysis.

Primer name	Sequence 5→3
OP-A01	CAGGCCCTTC
OP-A06	GGTCCCTGAC
OP-B04	GGACTGGAGT
OP-B14	TCCGCTCTGG
OP- B15	GGAGGGTGTT
OP- B20	GGACCCTTAC

Polymerase chain reaction (PCR) conditions:

The reaction conditions were optimized, mixtures and prepared (30µl total volume) consisting of the following. DNTPs 2.4 µl, MgCl2 3.0 µl, 10 x buffer 3.0 µl, Primer (10 um) 2.0 µl, Taq (5u/µl) 0.2 µl,Template DNA (50 ng / µl)2.0 ul, H2O (dd) 17.4 ul. Amplification was carried out in a PTC- 200 thermal cycler (MJ Research, Watertown, USA) programmed as follows: Denaturation, 94 °C for 2 minutes, then for 40 cycles. Each cycle consisted of 1 minute at 94 °C , 1 minute at 37 °C, 2 minutes and 30 second at 72 °C, followed by a final extension time of 12 minutes at 72 °C and 4 °C (infinitive).

Gel electrophoresis:

Gel electrophoresis was applied according to Sambrook et al. (1989). Agarose (1.2 %) was used for resolving the PCR products. The run was performed for one hour at 80 volt in pharmacia submarine (20 x 20 cm). Bands were detected on UV– transilluminator and photographed by Gel documentation 2000, Bio- Rad. Fragment sizes of RAPD were estimated from the gel by comparison with the 1 kb ladder marker. Similarity, coefficients were calculated according to Dice matrix, (Nei

and Li 1979). Construction of the dendrogram tree was performed using the unweighted pair group method based on arithmetic mean (UPGMA) as implemented in the SPSS program version.

RESULTS AND DISCUSSION

Morphological characters of the different soybean genotypes are presented in Table (2). Hypoctyle color can divide the tested genotypes into two groups, colourless (H30, H32, H117, H127, H129, H132 and H2 L12) and dark violet (Toano, DR101 and H15 L5). Color of trichomse, can divide the

Table 2. Morphological characters of ten soybean genotypes (data of 2010 and 2011 growing seasons).

Characters / Genotypes	Hypoctyle color	Color of trichomse	Density of trichomse	Shape of lateral leaflet	Leaflet color
H_{30}	Colourless	Dark brown	Dense	Lanceolate	Green
H_{32}	Colourless	Grey	Medium	Rhemboidal	Dark green
Toano	Dark violet	Grey	Sparse	Ovoid	Dark green
DR_{101}	Dark violet	Dark brown	Medium	Lanceolate to rhemboidal	Dark green
H_{117}	Colourless	Dark brown	Sparse	Rhemboidal	Dark green
H_{127}	Colourless	Light brown	Sparse	Ovoid	Dark green
H_{129}	Colourless	Grey	Sparse	Ovoid	Dark green
H_{132}	Colourless	Llight brown	Medium	Rhemboidal	Light green
H_2 L_{12}	Colourless	Light brown	Medium	Ovoid	Green
H_{15} L_5	Dark violet	Grey	Sparse	Ovoid	Dark green

tested genotypes into three groups, dark brown (H_{30}, DR_{101} and H_{117}), light brown (H_{127}, H_{132} and H_2 L_{12}) and grey (H_{32}, Toano, H_{129} and H_{15} L_5). The studied genotypes are divided according to density of trichomse soybean to sparse (Toano, H_{127}, H_{129} and H_{15} L_5), medium (H_{32}, DR_{101}, H_{132} and H_2 L_{12}) and dense (H_{30} and H_{117}). H_{30} is identified with shape of lateral leaflet (lanceolate) and leaflet color (green). H_2 L_{12} identified with Leaflet color (green). Morphological description is a precondition for the protection and registration of varieties (UPOV 1984). These results agreed with those reported by Abdalla *et. al.* (2004), they indicated that some soybean genotypes were indistinguishable from each other by using phenotypic and agronomic characteristics.

Also Table 3, show the morphological characters of the different soybean genotypes included in this study. Hybrid 129 identified with flower color (Light violet). DR_{101} and H_{127} identified with pod color to (dark brown and very dark brown, respectively), while other soybean genotypes (Toano, H_{117} and H_2 L_{12}) have white pod color, (H_{32} and H_{132}) have light brown pod color and (H_{30}, H_{129} and H_{15} L_5) have brown

pod color. Regarding seed shape, genotypes (H_{30}, Toano, DR_{101} and H_{117}) have (spherical flattened), genotypes (H_{129}, H_{132} and H_2 L_{12}) have (elongated flattened) and genotypes (H_{32}, H_{127} and H_{15} L_5) have (spherical). H_{127} with seed coat color (brown), meanwhile other genotypes were yellow. Genotype Toano can identified with dull seed coat luster, whoever the other genotypes were shiny. Morphological description is a precondition for the protection and registration of varieties (UPOV 1984). Present results were similar to those previously reported by Sharma and Singh (2011), they found that the varieties differences in flower color, pubescence color, seed coat hilum color and intensity of pubescence.

Table 3. Morphological characters of ten soybean genotypes (data of 2010 and 2011 growing seasons).

Characters / Genotypes	Flower color	Pod color	Seed shape	Seed coat color	Seed coat luster	Seed hilum color
H_{30}	Violet	Brown	Spherical flattened	Yellow	Shiny	Brown
H_{32}	White	Light brown	Spherical	Yellow	Shiny	Black
Toano	Violet	White	Spherical flattened	Yellow	Dull	Grey
DR_{101}	Violet	Dark brown	Spherical flattened	Yellow	Shiny	Dark brown
H_{117}	White	White	Spherical flattened	Yellow	Shiny	Yellow
H_{127}	White	Very dark brown	Spherical	Brown	Shiny	Black
H_{129}	Light violet	Brown	Elongated flattend	Yellow	Shiny	Dark brown
H_{132}	White	Light brown	Elongated flattend	Yellow	Shiny	Brown
H_2 L_{12}	Violet	White	Elongated flattend	Yellow	Shiny	Grey
H_{15} L_5	Violet	Brown	Spherical	Yellow	Shiny	Dark brown

Combined data of quantitative and chemical characters of the studied genotypes are given in Table 4. It is clear that, Hybrid 129 have the highest first pod (17.9 cm) and plant high (103.8 cm). Concerning time of beginning of flowering and maturity, Toano and DR_{101} were the latest among all genotypes, while hybrid 30, H_2 L_{12} and H_{15} L_5 were the earliest compared with the other genotypes. DR_{101} recorded highest number of seeds/ plant (153), heaviest wight of seed/ plant (25.7 g) and heaviest 100-seed weight (43.6 g). H_2 L_{12} gave the highest seed yield (2.38 t. fed^{-1}), seed protein content (58.4 %) and seed oil content (21.4 %). Similar results were obtained by Kumar *et al.* (2005), Deshmukh *et. al.* (2006), El-Kalla *et al.* (2007) and Hamdi *et. al.* (2008), they revealed that, soybean genotypes significantly differed in

seed yield, seed index, days to flowering and maturity, plant height, number of seeds/ plant and seed protein and oil content.

Table 4. Quantitative and chemical characters of some soybean genotypes (combined data of 2010 and 2011 growing seasons).

Characters / Genotypes	Time of beginning of flowering	Time of maturity	Highest of the first pod (cm)	Plant highest (cm)	No. of seed/ plant	Wight of seed/ plant	100-seed weight	Seed yield (t. fed^{-1})	Seed protein (%)	Seed oil (%)
H_{30}	40	120	11.5	85.6	80	15.5	35.3	1.99	51.3	19.6
H_{32}	45	127	11.3	88.5	114	20.6	36.6	1.93	51.2	20.3
Toano	55	135	14.8	69.4	139	22.6	43.3	2.11	52.3	19.9
DR_{101}	53	131	12.8	73.9	153	25.7	43.6	1.90	52.7	19.7
H_{117}	44	128	16.2	90.6	112	16.6	33.4	1.98	50.2	17.3
H_{127}	43	125	12.7	85.8	123	20.9	37.0	1.46	49.9	17.8
H_{129}	48	134	17.9	103.8	115	16.1	38.9	1.50	49.0	17.1
H_{132}	53	130	15.3	93.3	121	19.7	24.8	1.62	48.7	17.8
$H_2 L_{12}$	40	125	10.8	85.6	126	20.3	33.8	2.38	58.4	21.4
$H_{15} L_5$	41	127	13.5	84.6	111	20.4	38.4	2.29	53.9	21.4
L.S.D. at 5%	1.3	2.3	1.2	5.2	4.4	0.8	1.2	0.06	4.5	0.1

Molecular marker

Polymorphisms among genotypes were detected by 6 out of 20 random primers. Figure 1 shows the amplification patterns obtained with these primers. Out of a total of 62 amplification products (56 to 549 base pairs) using six primers, 53 (85.4%) were polymorphic and 9 products were shared among all genotypes (not polymorphic). Each primer amplified 7 to 15 bands.

The highest level of polymorphism could be observed in primer OP-B04 which showed 91.6 % polymorphism, while the lowest level of polymorphism was 57.1 % in primer OP-B15 (Table 5). The highest number of markers was observed in $H_2 L_{12}$, which recorded three specific bands, two bands at 680 and 272 bp of primer OP-A06 and the band at 123 bp

Table 5. Levels of polymorphism and specific bands based on RAPD analysis.

Bands / Primer	Total bands	Polymorphic bands	Monomorphic bands	Polymorphism %	Specific bands	
					MS	Genotypes
OP-A01	15	13	2	86.6	158 bp -123 bp	H127
OP-A06	11	10	1	90.9	680 bp - 272 bp	$H_2 L_{12}$
OP-B04	12	11	1	91.6	123 bp - 241 bp	$H_2 L_{12}$ - H_{132}
OP-B14	9	8	1	88.8	-	-
OP- B15	7	4	3	57.1	-	-
OP- B20	8	7	1	87.5	-	-
Total	62	53	9	85.4	-	-

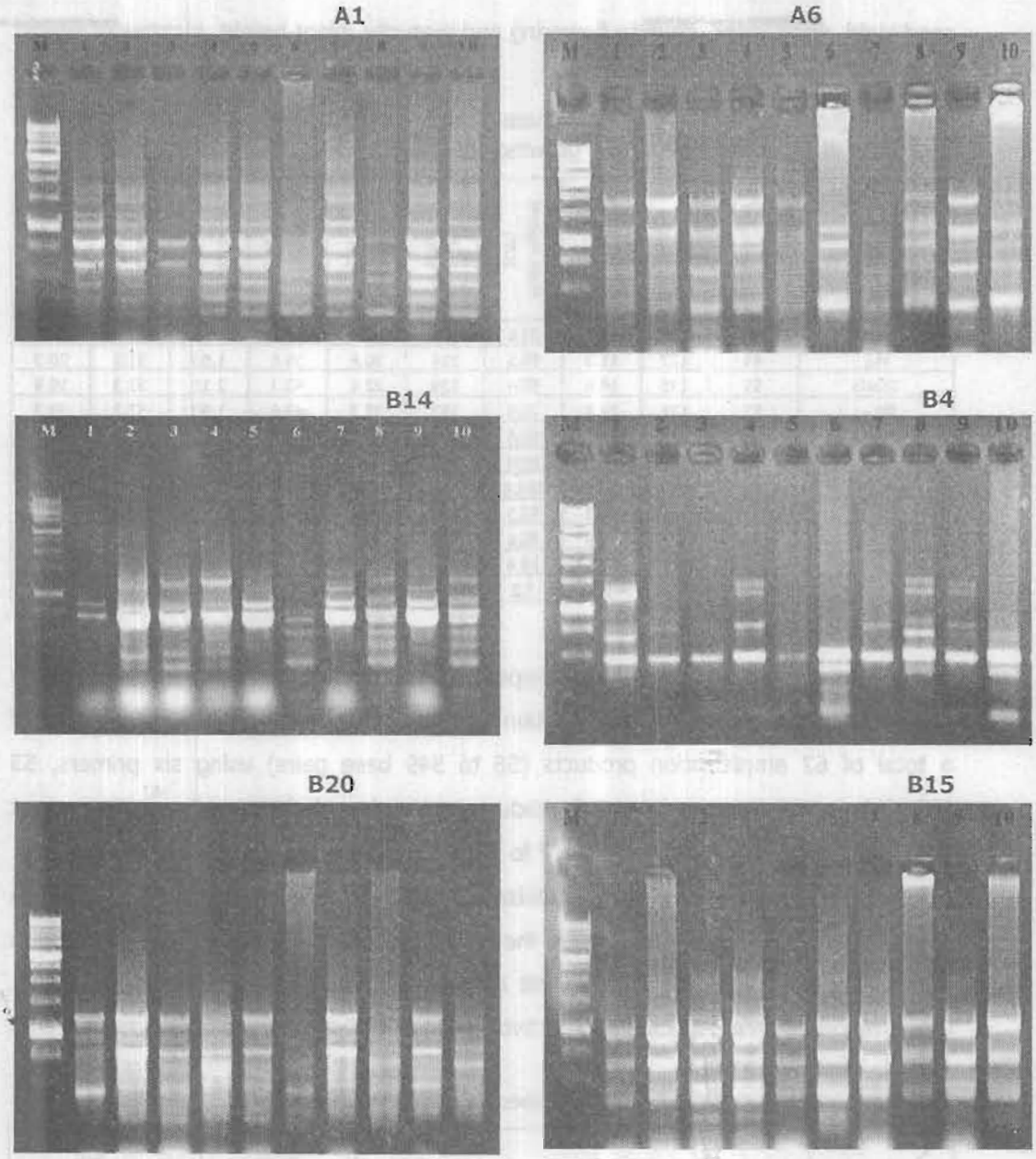

Fig 1. RAPD banding patterns amplified with seven primers. Lines from left to right: M= Marker, Lines from 1to 10 are: H_{30}, H_{92}, Toano, DR_{103}, H_{117}, H_{127}, H_{129}, H_{132}, H_2 L_{12} and H_{15} L_5.

of primer OP- B04. Also, H127 has two specific bands at 158 bp and 123 bp of primer OP-A01 and there is one specific band for H132 at 241 bp of primer OP- 804, as shown in Table 5.These results are in agreement with those Abdalla, Safia, *et. al.*, (2004), they found that DNA analysis was considered essential to distinguish among genotypes while, protein banding patterns were effective in providing resolution for soybean genotypes.

Cluster analysis

There are two major clusters resulted, the first cluster included only H127 and the second cluster included the rest of the genotypes. This second cluster was subdivided into two sub clusters, one included H132 and the other included the rest of the genotypes (Fig.2).

cluster analysis showed that genotype H129 and H2L12 were genetically close(they showed 92.1% similarity). However, the lowest similarity was observed between H30 and H127(54.5%) Table (6).

DNA marker systems are useful tools for assessing genetic diversity levels among germplasm (Lee 1995 and Karp et al 1996). This study reported a successful fingerprinting of soybean genotypes using RAPD and demonstrated the usefulness of these markers in estimating the extent of genetic variation in soybean germplasm. These results are in agreement with Ude et al., (2003).

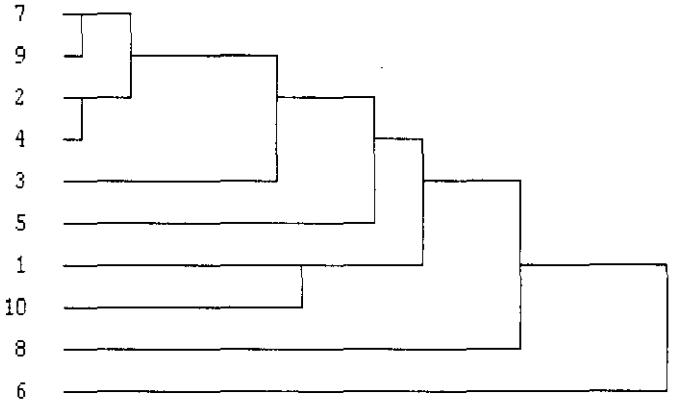

Figure 2. Dendrogram of the genetic distances between the Ten soybean genotypes based on RAPDs analysis.

Table 6. Similarity matrix among the ten soybean genotypes based on RAPD analysis.

Genotypes	H30	H32	Toano	DR101	H117	H127	H129	H132	H2L12
H30									
H32	.757								
Toano	.667	.865							
DR101	.800	.892	.827						
H117	.706	.789	.647	.753					
H127	.545	.649	.636	.640	.559				
H129	.720	.867	.773	.881	.857	.613			
H132	.704	.709	.676	.700	.603	.648	.675		
H2L12	.756	.844	.756	.901	.786	.585	.921	.690	
H15L5	.795	.815	.685	.805	.720	.712	.756	.769	.742

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دراسات مورفولوجية وبيوكيميائية على عشرة تراكيب وراثية مبشرة من فول الصويا

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قسم بحوث تكنولوجيا البذور – معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية.

تهدف هذه الدراسة إلى استخدام بعض الصفات المورفولوجية والبيوكيميائية لتمييز عشرة تراكيب وراثية مبشرة لنبات فول الصويا وهي: هجين ٣٠، هجين ٣٢، تونو، دى ار ١٠١، هجين ١١٧، هجين ١٢٧، هجين ١٢٩، هجين ١٣٢، هجين ٢ عائلة ١٢، هجين ١٥ عائلة ٥. لذلك أجريت تجارب حقلية ومعملية بمحطة البحوث الزراعية بالجميزة وقسم بحوث تكنولوجيا البذور بالجيزة– مركز البحوث الزراعية ، خلال موسمي ٢٠١٠، ٢٠١١م.

ويمكن تلخيص أهم نتائج هذه الدراسة فيما يلي:-

إمكانية استخدام الصفات المورفولوجية والمحصولية والبيكيميائية في تمييز بعض التراكيب الوراثية تحت الدراسة حيث أمكن تمييز.

- نباتات هجين ٣٠ عن باقي التراكيب الو راثية المدروسة بشكل الوريقة الوسطي (رمحيه) وكثافة الزغب (كثيف). أمكن تمييز نباتات هجين ١٣٢ بلون الورقة (أخضر فاتح). أمكن تمييز نباتات هجين ١٢٩ بلون الزهرة (بنفسجي فاتح). أمكن تمييز نباتات هجين ١٢٧ بلون القصرة (بني) ولون القرن (بني غامق جدا). أمكن تمييز نباتات الصنف تونو بأن غطاء البذرة معتم في حين باقي التراكيب الو راثية غطاء البذرة لامع. أمكن تمييز نباتات الصنف دي ار ١٠١ بشكل الوريقة الوسطي (رمحيه إلي معينة) ولون القرن (بني غامق). أيضا تشير النتائج الي أن الصنفين تونو، دى ار ١٠١ متأخرين في التزهير والنضج في حين هجين ٣٠، هجين ٢ عائلة ١٢، هجين ١٥ عائلة ٥ مبكرين في التزهير والنضج. كذلك سجل الصنف دي ار ١٠١ أعلي التراكيب الو راثية في عدد ووزن البذور/النبات وأقل وزن ١٠٠ بذرة بينما سجل هجين ٢ عائلة ١٢ أعلي محصول بذور وأعلي نسبة للبروتين والزيت.
- كما تم استخدام تكنيك RAPD-PCR لتمييز الاختلافات الوراثية بين هذة التراكيب علي المستوي الجزيئي وذلك باستخدام العديد من البادئات العشوائية. حيث تم استخدام ستة بادئات عشوائية أظهرت ٦٢ حزمة، منها ٥٣ حزمة أظهرت اختلافات بين هذة التراكيب الوراثية أي أن نسبة الاختلافات كانت ٨٥,٤%. قسمت الشجرة التطورية هذه التراكيب الوراثية إلي مجموعتين رئيسيتين حيث ظهر الهجين ١٢٧ في مجموعة مستقلة بينما تجمعت باقي التراكيب الوراثية في المجموعة الأخري. أظهر التحليل أيضا أن أعلي درجة تشابه كانت بين هجين ١٢٩ وهجين ٢ عائله ١٢ (٩٢،١%) بينما أقل درجة تشابه كانت بين هجين ٣٠ وهجين ١٢٧ (٥٤,٥%). أيضا أظهر التحليل عدد من الحزم المميزة التي تميز هذة التراكيب الوراثية.
- تعتبر النتائج المتحصل عليها من هذه الدراسة ذات أهمية كبيرة في حفظ حقوق مربو النباتات عند تسجيل التراكيب الوراثية كأصناف تجارية جديدة إلا أنة علي مربي النبات الانتخاب من قاعدة وراثية عريضة حتى يمكن الحصول علي صفات مورفولوجية مميزة للسلالات الجديدة عن الأصناف المنزرعة المسجلة وذلك عند تسجيلها كأصناف جديدة مما يسهل التحقق من نقاوة الصنف الجديد أثناء مراحل اكثارة المختلفة.