MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION OF SOME SOYBEAN GENOTYPES

ABDALLA, SAFIA T.[1], N. A. NAGUIB[2], A. H. SELIM[2] AND M.S.A.MOHAMED[1]

1-Food Legumes Res. Sec., Field. Crops. Res. Institute., A.R.C.
2- Seed Tech. Res. Sec., Field. Crops. Res. Institute., A.R.C.

(Manuscript received 15 February 2004)

Abstract

Field and Laboratory experiments were carried out at Gemmiza Experimental Research Station during 2002 and 2003 growing seasons, with the objectives of identifying morphological and biochemical characteristics of commercially released soybean varieties namely Giza/21, Giza /35, Giza /111, Crawford, and some promising genotypes viz., Hybrid1/10, Hybrid 1/12, Hybrid /85, Hybrid 88/1, Hybrid 88/3 , Hybrid 93, Giza /30, Giza/32. Twelve soybean (*Glycine max* L. Merr) genotypes were identified 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 composition and biochemical variability of genomic fingerprinting. Field observations indicated that some genotypes were indistinguishable from each other by using phenotypic and agronomic characteristics. Furthermore, genotypic differences in agronomic and chemical characteristics (protein, Carbohydrate and fats) can be affected by environmental conditions prevailing in the field. DNA analysis was considered essential to distinguish among genotypes where protein banding patterns were effective in providing resolution for soybean genotypes. The results of this study will increasingly become important in variety registration and breeder's rights .

INTRODUCTION

In addition to determining the value of a cultivar to be grown on a large scale , it is important to be easily recognized during seed multiplication stages. 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). However, with increasing number of soybean varieties, identification of varieties become difficult and uncertain unless it relies on field or greenhouse examination of growing plants which is expensive and time-consuming

(Payne 1979) In 1990, the random amplified polymorphic DNA (RAPD) was introduced in genotypic identification. The technique uses a single arbitrarily chosen oligonucletide primer that hybridizes to the genomic DNA template at two different sites, one on each strand of the DNA complementary DNA. Under temperature alternations, a thermo stable DNA polymerase is able to synthesize discrete DNA products that can be resolved on an agarose gel following electrophoresis. Each primer has the capability to amplified of several using DNA fragments in the genome. Some of these amplified fragments (patterns) may be unique to a genotype and then they can be useful in varietal identification. Several workers have pointed out that the RAPD technique is a reliable approach for rapid identification and discrimination among soybean varieties when morphological characteristics were insufficient. (McDonald *et al.*,1994; Smith,1995; Williams *et al.*,1990 and Zhang *et al.*, 1996). However, the aim of the present investigation was to evaluate the most distinguished morphological and biochemical traits of some soybean genotypes cultivated under Egyptian conditions.

MATERIALS AND METHODS

A field experiment was carried out at Gemmiza Agric. Res. Station, Agric. Research Center, Giza, Egypt in 2002 and 2003 growing seasons. The experiment included 12 soybean genotypes that were arranged in a randomized complete block design with three replications. The experimental plot consisted of four ridges 6 m long and 60 cm apart. Seed and pedigree of the studied genotypes were received from Legume Research Dept., Field crops Research Institute, Agricultural Research Center.

Genotype	**Pedigree**
Giza/21	(Crawford x Celest)
Giza /35	(Crawford x Celest)
Giza /111	(Crawford x Celest)
Crawford	(Williams x Columbus)
Hybrid1/10	(Giza /21 x L86-k-73)
Hybrid 1/12	(Giza /21 x L86-k-73)
Hybrid /85	(PI416937 x L86-k-73)
Hybrid 88/1	(Giza /21 x L86-k-73)

Hybrid 88/3	(Giza /21 x L86-k-73)
Hybrid 93	(H5L21 x Giza 83)
Giza /30	(Crawford x L62-1686)
Giza/32	(Crawford x L62-1686)

Sowing took place on May 20th in both seasons. Seedlings were thinned 21 days after sowing to achieve 20 plants per meter of linear ridge. Other cultural practices were applied as recommended. Qualitative traits were visually recorded using scales reported by IBPGR (1984). These characters included seed coat colour identified as yellow or green; seed coat luster as dull or shiny; pubescence density as normal or dense; flower colour identified as white; purple and light purple; pod colour as brown, light brown or gray; stem determination as indeterminate; semi determinate or determinate; hypoctyl colour as purple or colourless; leaflet shape as lanceolate; elliptic, orbicular; growth habit as erect or half –erect; days from sowing day to 50% of plants with at least 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 , number of pods/plant, seed weight/plant, seed weight/plant. Yield/fed was calculated from the yield of the two inner ridges.

For seed chemical composition , air-dried seed samples (50g each) were randomly taken from each plot to determine crude protein percent (calculated by multiplying the total nitrogen by 6.25), total carbohydrates and crude oil percentages according to (AOAC, 1990). For determining cultivar finger printing, genomic DNA was isolated individually from a sample extracted from one week old seedling of each cultivar. Approximately, 100 mg leaf material was used to extract DNA using QIAGEN kit. The DNA purity and concentration were estimated using spectrophotometer A260/280 readings. The polymerase Chain Reaction (PCR) was carried out in 0.5 ml thin walled tubes in a final reaction volume of 30 µl. The reaction mix consisted of a final concentration of 1.5 mµ of $MgCl_2$, 200 mµ of dNTPs, 50 mg primer and 0.2 µl Tag DNA polymerase (Promega) and completed by adding distilled water up to 30 µl . Twenty 10- mer oligonucleotides were screened for use as primers to determine those which produce the greatest number of polymorphisms. Five out of those were selected as the most useful in discriminating soybean cultivars. The sequences of these primers are shown in table (1):

Table 1. The nucleotide sequences of 10 decamer primers used for RAPD- PCR analysis:

Primer code	Sequences (5□ ___3□)
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-15	TTCCGAACCC
OPB-10	CTGCTGGGAC

DNA amplification were carried out with a thermo cycler (PTC -200). Forty amplification cycles were employed. Each cycle consisted of preheating at 94ºC for 4 minutes and then denaturation at 94ºC for 30 second. Annealing at 35ºC for one minute, and extension at 72ºC for 2 min, followed by a one final extension cycle for 10 minutes at 72ºC . Amplification products were separated in a 1.2 g agarose gel together with DNA size standard. PCR products were visualized by ethidium bromide.

Data were subjected to statistical analysis of variance using Mstatc computer program (1994). Multiple range test was used for comparison between means of soybean genotypes. Different alphabetical letters in the column are significantly differed at 5% level of significance according to (Snedecor and Cochran 1980)

RESULTS AND DISCUSSION

Table (2) shows the morphological characteristics of the different soybean genotypes included in this study. Pubescence colour can divide the tested genotypes into two groups; either gray (Giza 32 hybrid 1/10, hybrid 1/12, hybrid 88/3 and hybrid 93) with gray pubescences, (Crawford, Giza 21, Giza 35, Giza 111, Giza 30 , hybrid 85 and hybrid 88/1) or with brown pubescence . The first group (with gray pubescence), all genotypes have colourless hypoctyle and white flower except hybrid 88/3 which have purple hypoctyle and purple flower. All genotypes of the second group (with light brown or brown pubescence) have light purple or purple hypoctyle and light purple or purple flower except hybrid 88/1 which have colourless hypoctyle and white flower. Giza /21 and Giza 35 have unique light green seed coat which distinguish them from all genotypes that had yellow seed coat. Both Giza 21 and Giza 35 were similar in all the recorded morphological characteristics except for seed coat luster, where Giza 21 have shiny seed coat while Giza 35 have dull seed coat.

Results of the leaflet shape indicated that genotypes can be divided into three groups; . Hybrid 1/10, hybrid 1/12, Hybrid 88/1, Hybrid 88/3 and Giza32 genotypes have lanceolate shape, (Hybrid /85, Hybrid /93, Giza /21 Giza /35 and Crawford) have orbicular shape , while Giza /30 and Giza /111 have elliptic leaflet shape. Pod colour ranged between light brown for (Hybrid 1/10, Hybrid 1/12, Hybrid 88/1, Hybrid /93,Giza /32, Giza /21, Giza /111, Giza /35 and Crawford) to brown (Hybrid /85 and Giza /30). Regarding hilum colour, four soybean genotypes (Hybrid 1/10, Hybrid 1/12, Hybrid 88/1, and Giza /32) have yellow hilum colour, while Hybrid /93 has brown colour. Other genotypes, viz. Hybrid /85, Hybrid 88/3, Giza /30, Giza /21, Giza /111, Giza /35 and Crawford) have black hilum.

Data in Table (2) reveal that the phenotypic traits can not be effectively used to distinguish among hybrid 1/10, hybrid 1/12 and Giza 32 as they have the same characteristics. Furthermore, it is worth noting that pubescence density may not be taken as a morphological trait for characterization of any of the studied genotypes, as all have dense or normal pubescence. Stem determination and growth habit of all studied soybean were indeterminate and erect, respectively.

Table 2. Morphological characters of different soybean genotypes. (data of 2002 and 2003 growing seasons).

Genotype	Hypoctyle colour	Leaflet shape	Flower colour	Pod colour	Pubescence density	Pubescence colour	Seed coat colour	Seed coat luster
Hybrid 1/10	Colourless	Lanccolate	White	Light Brown	Dense	Gray	Yellow	Shiny
Hybrid 1/12	Colourless	Lanccolate	White	Light Brown	Dense	Gray	Yellow	Shiny
Hybrid /85	Purple	Orbicular	Purple	brown	Normal	Brown	Yellow	Dull
Hybrid 88/1	Colourless	Lanccolate	White	Light Brown	Dense	Brown	Yellow	Dull
Hybrid 88/3	Purple	Lanccolate	Purple	Brown	Dense	Gray	Yellow	Shiny
Hybrid /93	Colourless	Orbicular	White	Light Brown	Normal	Gray	Yellow	Dull
Giza/30	Light purple	Elliptic	Light Purple	Brown	Dense	Light brown	Yellow	Dull
Giza/32	Colourless	Lanccolate	White	Light Brown	Dense	Gray	Yellow	Shiny
Giza/21	Purple	Orbicular	Purple	Light Brown	Dense	Light brown	Green	Shiny
Giza/111	Light purple	Elliptic	Light purple	Light Brown	Dense	Light brown	Yellow	Shiny
Giza/35	Purple	Orbicular	Purple	Light Brown	Dense	Light brown	Green	Dull
Crawford	Purple	Orbicular	Purple	Light Brown	Normal	Light brown	Yellow	Dull

Combined data of crop yield and characteristics of the studied genotypes are given in Table (3). It is clear that Giza 111, followed by Giza 21 and Giza 32 have greater pod and seed number per plant, heavier 100 seed- weight and seed weight/plant. Seed yield per feddan followed the same trend. Hybrid 85,hybrid 93 and hybrid 88/1 recorded lower number of pods and seeds/plant, lighter weight of both 100 seeds and seeds /plant, as a result, these genotypes produced lower seed yield /fed (1.214, 1.304 and 1.334 ton, respectively) .

Giza 30, Giza 35 and hybrids 1/10, 1/12 and 88/3 exhibited intermediate values for most of the studied traits.Giza/111 had the tallest plants (114.7 cm), while Hybrid /85 had the shortest ones (79.5 cm). Concerning the period to 50% flowering and 95% maturity, Hybrid 88/3, Giza/30 and Giza /35, were the earliest among all genotypes, while Hybrid /85 was the latest one.

Giza /111 had the heaviest 100-seed weight (18.47 g) followed by Giza /21 (18.1g) surpassing other genotypes. On the other hand Hybrid /85 had the lowest value of 100-seed weight (13.65g) followed by Hybrid 88/1 (13.88 g).

Table 3. Quantitative characters of different soybean genotypes.(Combined data of 2002 and 2003 growing seasons).

Genotype	No. of pods/ plant	No. of seed/ Plant	50% flowering (days)	95% maturity (days)	Plant height (cm)	100 seed weight (g)	Seed weight/ plant (g)	Yield/fed (ton)
Hybrid 1/10	51.17 d	119.7 d	37.50 c	113.5 d	106.2 bc	16.65 b	19.33 e	1.463 cd
Hybrid 1/12	46.00 def	105.3 e	34.50 de	109.0 e	102.3 cd	15.60 cd	16.15 g	1.437 de
Hybrid /85	34.17 g	73.50 g	41.50 a	125.7 a	79.50 f	13.65 h	9.900 I	1.214 h
Hybrid 88/1	37.50 g	84.50 f	32.33 f	110.0 e	96.50 de	13.88 gh	12.28 h	1.334 fg
Hybrid 88/3	45.17 f	107.3 e	30.67 g	105.3 f	103.7 c	14.90 de	16.32 g	1.388 ef
Hybrid /93	37.67 g	80.17 fg	39.67 b	122.3 b	85.33 f	14.07 fgh	11.50 h	1.304 g
Giza/30	60.83 c	133.8 c	30.67 g	105.3 f	95.33 e	15.92 c	20.70 d	1.525 c
Giza/32	73.17 b	161.7 b	33.67 ef	107.7 ef	105.7 c	17.12 b	26.98 c	1.616 b
Giza/21	78.17 ab	169.7 ab	38.00 c	118.7 c	112.2 ab	18.10 a	30.58 b	1.660 b
Giza/111	82.67 a	178.0 a	40.00 ab	121.5 b	114.7 a	18.47 a	32.13 a	1.745 a
Giza/35	50.67 de	116.7 d	30.67 g	105.7 f	96.33 de	14.68 ef	17.78 f	1.422 de
Crawford	45.50 ef	103.2 e	35.67 d	118.8 c	100.7 cde	14.50 efg	15.85 g	1.262 gh

Table (4) shows that crude protein content ranged between 32.74 and 38.99%,, while Giza 21 had the highest crude protein content (38.99%) followed by hybrid 88/3 and Giza /30, the lowest crude protein content was that of hybrid 1/10 (32.74% and hybrid 1/12 33.41%). Other genotypes had crude protein content values between those of the two groups . Crude oil content ranged between 22.84 and 29.00 %. Only Giza 111 and hybrid 88/1 had crude oil content of > 28% (29.00 and 28.36, respectively). The lowest crude oil content was that of Crawford (22.84%) and Giza 30(23.51%), while other genotypes had values in between. The variation in total carbohydrates content was not as large as that of crude protein or crude oil. Total carbohydrates content ranged between 15.12% for Crawford and 16.39% for Giza 35., while most genotypes had close values that did not differ significantly from each other.

Table 4. Seed chemical composition of twelve soybean genotypes .

(Combined data of 2002 and 2003 growing seasons).

Genotype	Crude protein (%)	Crude oil (%)	Total Carbohydrates (%)
Hybrid 1/10	32.74 f	27.67 bc	15.94 abcd
Hybrid1/12	33.41 ef	27.55 c	16.15 ab
Hybrid/85	36.36 bed	25.03 e	16.09 abc
Hybrid88/1	35.45 bcde	28.36 ab	15.81 bcde
Hybrid88/3	37.68 ab	27.14 c	15.63 cde
Hybrid/93	34.86 cdef	26.32 d	15.56 def
Giza/30	37.03 abc	23.51 fg	16.00 abcd
Giza/32	36.57 abcd	24.15 f	16.06 abcd
Giza/21	38.99 a	25.51 e	16.05 abcd
Giza/111	34.60 abcdf	29.0 a	15.42 ef
Giza/35	34.14 def	27.10 c	16.39 a
Crawford	35.52 bcde	22.84 g	15.12 f

A total of fifteen RAPD primers were tested against the twelve soybean genotypes , however five of them A08,A09, A10, A15 and B10 gave clear and scorable profiles. The profiles are reproducible with sufficient polymorphism. The profiles are reproducible with sufficient polymorphism. The sequences of these primers are listed in Table (1). The total number of bands and the percent of polymorphism revealed by each primer is given in Table (5). A total of 26 amplified bands were generated across the studied genotypes with 15 bands. The levels of

polymorphism ranged from 50.0% to 75.0% with a mean 65.8%. The size of amplified bands ranged from 50 bp to 1400 bp.

Table 5. Code and total bands of five DNA random primers used for identifying twelve soybean genotypes.

Primer Code	Total bands	Monomorphic bands	Unique bands	Polymorphic bands	Polymorphism %
OPA-08	4	1	1	2	75%
OPA-09	7	2	0	5	71.4%
OPA10	7	1	1	2	75%
OPA15	7	3	0	4	57.1%
OPB10	4	2	0	2	50%
Total	26	9	2	15	65.83%

The RAPD profiles of the amplification products are shown in Fig.(1). The results showed that two unique bands were specific for genotype Hybrid 1/10 at 600 bp and the second band were specific for genotype Hybrid 88/1 at 1000 bp.

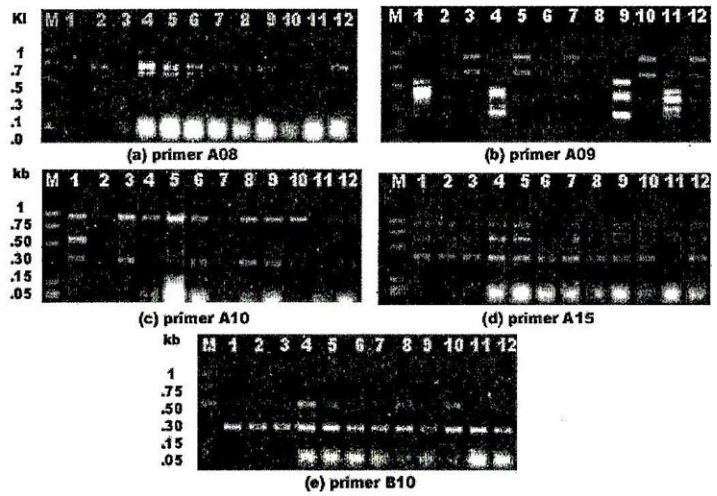

Fig 1. RAPD Fingerprints of the twelve soybean genotypes generated by the five primers (Ao8, A09, A10, A15 and B10) respectively.

Lines from left to right: M= molecular weight marker(BPL 50bp Ladder); Lanes from 1 through 12 are: Hybrid 1/10, Hybrid 1/12, Hybrid/85, Hybrid 88/1, Hybrid88/3, Hybrid /93, Giza 30, Crawford, Giza /21, Giza /111, Giza /35, Giza /32 respectively)

The dendogram as shown in Fig. (2) separated the genotypes into two clusters, i.e. Hybrid 88/3, Giza 21, Hybrid 85, Giza 32, Hybrid 30, Hybrid 93 and Crawford belong to the first cluster, while other genotypes belong to the second cluster. The first cluster can be divided into three sub-cluster. One of them was between Hybrid 88/3 and Giza 111, the second between Hybrid 85 and Giza 30, while the third was between Hybrid 93 and Crawford. The second cluster can be divided into two sub-cluster, one of them was between Hybrid 1/10 and Hybrid 1/12 while the second was between Giza 21 and Giza 30. The results of RAPD analysis were useful and rapid tools for identifying, detecting polymorphism and could discriminate among soybean genotypes. These results agreed with those reported by (Jianhua *et al.*, 1996) who found that RAPDs was useful for identifying and discriminating soybean cultivars.

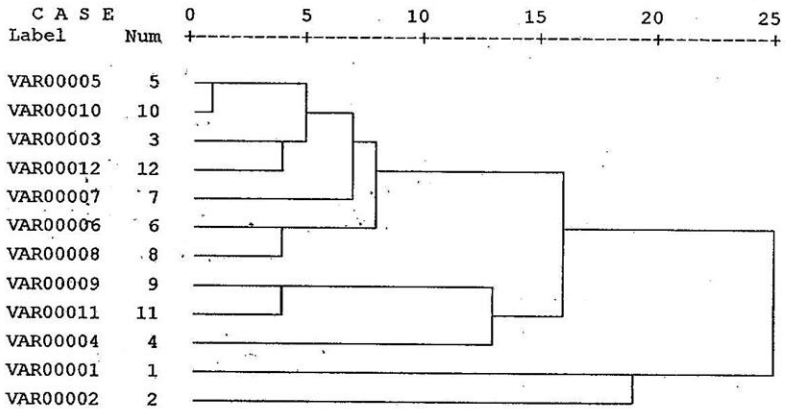

Fig.2. Dendrogram illustrating genetic distance between the twelve soybean genotypes based on RAPDs data.

REFERENCES

1. A.O.A.C. 1990. Official Methods of Analysis, of the Association of Official Analytical Agricultural Chemists, 15 th ed. Washington, D.C, U.S.A.

2. IBPGR 1984. International Board of Plant Genetic Resources .Crop Genetic Resources Center, pp. 19-12, Rome.

3. Jianhua,Z., M.B. McDonald, and P.M. Sweeney. 1996. RAPDs from seeds of different soybean and maize genotypes. Seed Sci. & techno., 24,513-522.

4. McDonald, M.B.;L.J. . Elliot, and P.M. Sweeney. 1994. DNA extraction from dry seeds for RAPD analysis in varietal identification studies. Seed Sci. & Techno., 22, 171 – 176.

5. Smith, J.S.C. 1995. Identification of cultivated varieties by nucleotide analysis. P. 131-151. In c.w. Wrigley (ed), Identification of food –grain varieties, American Association of cereal chemists, St. Paul, MN.

6. Snedecor, G.W. and W. G. Cochran 1980. Statistical Methods. Seventh Ed., Iowa state Univ. Press, Ames, Iowa, USA, pp, 255-269.

7. Williams, J. G.K.; A.R. Kubelik; K.J. Livak; J. A. Rafalski and S.V. Tingey 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18, 6531-6535.

8. Payne, R.C.1979. Some new tests and procedures for determining variety J.Seed Tech.,3:61-76.

9. UPOV. 1984. The International Union for the Protection of New Varieties of Plants. Guidelines for the conduct of tests for distinctness, uniformity and stability for soybean Descriptor no. TG/ 12.

10. Zhang, J.; M.B. McDonald, and P.M. Sweeney. 1996. Soybean cultivar identification using RAPD. Seed Sci. & Technol., 24, 589 -592.

التمييز المورفولوجى والبيوكيماوى لبعض التراكيب الوراثية من فول الصويا

صفية تمام عبد الله [1]، نعمت عدلى نجيب[2]، أمال حسن سليم[2]، محمد سيد على محمد[1]

١-قسم بحوث المحاصيل البقولية- معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية .
٢- قسم بحوث تكنولوجيا الـبـذور- معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية .

أجريت تجارب حقلية ومعملية خلال موسمي ٢٠٠٢ ، ٢٠٠٣ بهدف تمييز بعض الأصناف والتراكيب الوراثية المبشرة من محصول فول الصويا وهى جيزة /٢١ ، جيزة /٣٥ ، جيزة /١١١ ، كروفورد ، هجين ١٠/١ ، هجين ١٢/١، هجين /٨٥، هجين ١/٨٨ ، هجين ٣/٨٨، هجين /٩٣ ، جيزة /٣٠ ، جيزة /٣٢ . وذلك من الناحية المورفولوجية والكيميائية والبيوكيميائية والتى تعتبر من المتطلبات الأساسية لتسجيل الأصناف النباتية وحماية حقوق مربو النباتات.

أشـارت نـتائج الـتجارب الحقلـية الـى أمكانـية استخدام بعض الصفات المورفولوجية والمحصولية فى تمييز بعض الأصناف والتراكيب الوراثية المبشرة، فى حين لم يفي استخدام هـذه الصفات فى تمييز حالات أخرى.

كـذلك دلت نتائج التجارب المعملية على وجود فروق معنوية بين بعض التراكيب الوراثية مـن حيث محتوى الـبذور من البروتين والكربوهيدرات والدهون والتى تتأثر عادة بالظروف البيئية الـتى تحيط بالنبات خلال موسم النمو. ومن خلال استخدام طريقة ال RAPDs فى تحديد البصمة الوراثية المميزة لكل تركيب وراثى أمكن التمييز تماما بين التراكيب الوراثية التى شملتها الدراسة.

تعتـبر النـتائج المتحصل عليها من هـذه الدراسة ذات أهمية كبيرة فى حفظ حقوق مربو النـباتات عـند تسجيل التراكيب الوراثية المبشرة كأصناف تجارية جديدة الا انه على مربى النباتات الانـتخاب من قاعدة وراثية عريضة حتى يمكن الحصول على صفات مورفولوجية مميزة للسلالات الجديـدة عن الأصناف المنزرعة المسجلة عند تسجيلها كأصناف جديدة مما يسهل التحقق من نقاوة الصنف الجديد أثناء مراحل إكثاره المختلفة.