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AGRO-MORPHOLOGICAL CHARACTERIZATION AND EVALUATION OF GENETIC DIVERSITY USING MICROSATALLITE MARKERS FOR SOME SOYBEAN GENOTYPES

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ABSTRACT: Genetic diversity of cultivated soybean {*Glycine max* (L.) Merrill} 2n = 40 is very narrow, although includes diversity for many obvious morphological traits. A field experiment was conducted to study characterize of fifteen soybean genotypes on agro-morphological trait basis and to identify genetic diversity using SDS-PAGE, with resistance to defoliating insects, at Sakha Agricultural Research Station, Kafr ElShiekh, Egypt during 2017 and 2018 seasons,. Morphological description was performed with 10 qualitative and 7 quantitative traits and screening for defoliation by cotton leaf worm in the open field. The results exhibited significant differences among the tested fifteen genotypes for all studied characters. Defoliation of test plants by the cotton leaf worm, in the field screenings system showed that genotypes H_{14} L_8, H_1 L_{10S}, H_1 L_{10} and H_{10} L_{10} recorded the highest rating values of insect resistant to cotton leaf worm with defoliation rating (0.0 – 0.2), whereas, soybean genotype H_5 L_{21} showed the highest insect susceptible and had defoliation rating (3.8) indicating heavy insect feeding, over both seasons. Genotypes H_{11} L_{145} and $H_{14}L_8$ recorded the highest values of seed yield/fad., this attributed to the considerable increase in their number of branches/plant, number of pods/plant, and 100-seed weight, in both seasons. This indicates that such genotypes are the promising ones. The protein identification indicated that the pattern was uniform where each genotype was not affected by year or location. The soybean genotypes were different in their banding pattern and each one is characterized by certain proteins with different molecular weight. Cluster analysis based on qualitative morphological characters showed clear separation of genotypes on the basis of their plant growth habit.

Key words: Soybean, defoliation, genotypic variance UPOV description, morphological characters, electrophoresis, insect tolerance.

INTRODUCTION

Soybean {*Glycine max* (L.) Merrill} is an economically important leguminous crop for feed and food products where soybean is rich in seed protein (40%) and oil (20%) contents. The crop is ranked number one in the world production in the international trade markets among the major oil crops (**Singh *et al.*, 2007**). Also, crop supplies more than 61% of the global demand for vegetable oil and protein (**USDA, 2016**). Genetic diversity of cultivated soybean 2n =40 is very narrow (**Brown-Guedira *et al.*, 2000; Khatab and Morsy, 2012**), although it contains a great deal of diversity (**Carter *et al.*, 2004**). This includes diversity for many obvious morphological traits like flower, pubescence, seed and hilum color, and insect resistance traits, physiological and biochemical traits as well as content of protein, oil and carbohydrates and their constituents (**Boerma and Specht, 2004**). One of the pre-requirements for successful breeding strategies is the complete understanding of the genetic diversity of the crop plant. Several

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methods have been used to investigate the genetic variation in soybean. Morphological and agronomic traits have been employed by **Sneller *et al.*, (1997), Hassan (2001) and Morsy *et al* (2011).**

Under Egyptian condition, soybean is attacked by about twenty different major insect pests especially cotton leaf worm (*Spodoptera littoralis*), the main leaf feeding insect. The National Legume Research Program (NLRP) has been successful in releasing five high yielding soybean cultivars named Giza 21, Giza 22, Giza 35, Giza 83, and Giza 111 with acceptable resistance to cotton leaf worm. Also, some elite breeding lines were recently identified as resistance to cotton leaf worm, but unfortunately they are late maturing genotypes under Egyptian conditions. These elite breeding lines were initiated from crosses between Egyptian soybean cultivars and resistant introduced cultivars. The most economical way to deal with these insect-pests to avoid yield losses is to cultivate insect resistant/ tolerant varieties (**El-Garhy *et al.*, 2015**), in order to avoid using pesticides and to minimize environmental pollution as revealed by **Lucas (2012**). The development of soybean cultivar with insect resistance has been an objective of several breeding programs (**All *et al.*, 1999**). During the 1970s after the identification of three plant introductions [PI 171451(Kosamame), PI 229358 (Sodendaizu), and PI 227687 (Miyako White)] accessions with resistance to the Mexican bean beetle (MBB), Epilachnavarivestis Mulsant (**Van Duyn *et al.*, 1971 and 1972**), and provide resistance to other insect pests (**Lambert and Kilen, 1984a and 1984b**). Unfortunately, it is difficult to capture the full resistance levels of these accessions in progenies derived from crosses with adapted high-yielding cultivars or poor agronomic qualities (**Kilen and Lambert, 1986; Lambert and Tyler, 1999**).

Soybean yield loss degree resulting from defoliation depends on both levels and stages of development at which defoliation occurs (**Gazzoni *et al.*, 1998**). Defoliation during the early stages has little effect on yield (**Board *et al.*, 1994**). Soybean becomes sensitive to defoliation at the beginning of bloom; but the most sensitive stage for defoliation is R5 when beans begin to develop with a fully developed leaf (**Fehr *et al.*, 1981**). Yield is affected by defoliation through reduced light interception resulting in decreased canopy photosynthesis, loss of leaf storage material, and/or shortening of the effective grain filling period (**Board, 2004**).

Different types of markers were used for assessing genetic variability of soybean genotypes *i.e.* agronomic, morphological, biochemical traits and molecular marker polymorphisms (**Goyal *et al.*, 2012**). All mentioned marker groups have limitations, but applied together they can provide reliable information about examined germplasm (**Sudaric *et al.*, 2008**).

Qualitative traits are usually controlled by a few genes, thus easily observable and suitable for cultivar differentiation and identification. On the other hand, quantitative traits have more limitations in cultivar description, since they are controlled by polygenes and highly affected by environmental effects.

In soybean with a narrow genetic base, morphological markers may not be sufficient for detection of differences between varieties; then, electrophoresis (SDS-PAGE) is widely used to describe seed protein diversity of crop germplasm (**Das and Mukarjee, 1995**). The SDS-PAGE is practical and reliable method for species identification because seed storage proteins are largely independent of environmental fluctuation (**Gepts, 1989**). Genetic diversity and the pattern of variation in the Asian soybean population have been evaluated with seed protein (**Han *et al.*, 1999; Hirata *et al.*, 1999**). **Bushehri *et al.* (2000)** evaluated twenty one soybean cultivars electrophoretically for the banding pattern of storage proteins and suggested that SDS-PAGE is more powerful tool to characterize soybean cultivars. **Dobhal (1995)** revealed significant variability among soybean accessions for yield component, all owing accessions to be grouped into 17 clusters. The study aimed to investigate characterize of fifteen soybean genotypes on agro-morphological trait basis and to identify genetic diversity using SDS-PAGE, with resistance to defoliating insects.

MATEREALS AND METHODS

A two-year field experiment was conducted at the Experimental Farm of Sakha Agricultural Research Station, Kafr El-Sheikh, and the

Laboratory of Department of Seed Technology, Field crops Research Institute, Agricultural Research Center, Giza, Egypt, during 2017 and 2018 summer seasons to evaluate the yielding ability of 15 different soybean genotypes. The tested genotypes (denoted as G1 to G15) comprised the Egyptian commercial cultivar Giza 111, in addition to 14 newly developed promising lines ($H_{14}L_8$, H_1 L_{10S}, H_{20} L_2, H_1 L_{10}, H_5 L_{21}, H_{19} L_{96}, H_2 L_3, H_{10} L_{10}, H_1 L_1, H_9 L_{113}, H_{11} L_{145}, H_1 L_3, H_5 L_6 and H_{29} L_{115}) selected from the soybean breeding program of Food Legume Research Section. A detailed description of the code, name, and pedigree of the tested genotypes are presented in Table 1.

These genotypes belong to different maturity groups according to the American classification, *i.e.* Giza 111, H_1 L_{10S}, H_{20} L_2, H_1 L_{10}, H_5 L_{21} and H_5 L_6 (Maturity group IV), and the others are Maturity group V. The experimental design was a randomized complete block design with three replications. The experimental plot area for both seasons was 16.80 m^2 with six ridges, each of 4.0 m in long and 0.70 m apart. Planting date was May 10^{th} and 13^{th} in 2017 and 2018 seasons, respectively. Phosphorus fertilizer was added during seed- bed preparation at level of 15.5 kg P_2O_5/faddan in the form of calcium superphosphate (15.5%). Nitrogen fertilizer was applied in the form of ammonium sulfate 20.6% at the level of 15 kg nitrogen/faddan 12 days after planting. Irrigation was scheduled at 15 day intervals after planting. The other cultural practices for growing soybean were conducted properly as recommended.

Seeds were inoculated with nitrogen fixing bacteria, *Rhizobium jabonicum*, at planting and sown in hills 20 cm apart on both sides of each ridge at a rate of 3-5 viable seeds per hill to achieve two seedlings per hill to give a plant population of 120,000 plants/faddan.

Data were recorded on number of days to 50% flowering (flowering date), number of days to 95% maturity (maturity date). At harvest, a sample of ten guarded plants were randomly taken from each plot to measure plant height from the soil surface to the top of the main stem (cm), number of branches/plant, number of pods/plant and 100-seed weight were counted as an average of the sample. However seed yield was determined on plot basis from the central four ridges then transformed to kilograms per faddan. In addition a seed sample of 50 g from each plot was randomly taken to determine 100-seed weight.

Morphological Characters

Qualitative traits were visually recorded using scales reported by International Union forthe Protection of New Varieties of Plants (**UPOV, 2003**) (98-04-01). The Morphological characterization was performed using 10 qualitative characters including:

1. Hypocotyl anthocyanin coloration: present and absent.
2. Plant growth type: Indeterminate, semi-determinate and determinate.
3. Plant growth habit: erect, semi-erect, medium, semi-prostrate and prostrate.
4. Flower color: Violet or white.
5. Pubescence color (PC‡‡) :Tawny, light tawny, or gray.
6. Pubescence type (PT): Normal, sparse apprised, or semi- apprised.
7. Seed coat color: Yellow, green, gray, black, brown, reddish brown.
8. Seed coat luster: shiny, inter or medium and dull.
9. Seed size: small, medium and large.
10. Seed hilum color: gray, yellow, brown, dark brown and black.

Assessment of Defoliation Damage

In soybean, field scouting to assess insect populations is based on the number of insects per foot of row, insects per plant, sweep net samples, or the level of defoliation (**Boyd and Bailery, 2000**). The percent of defoliation is determined by estimating the amount of leaf tissue loss based on visual inspection of randomly selected plants. Examples provided (Fig. 1) are guidelines for estimating loss for individual leaflets. Actual defoliation estimates made for pest management decisions are based on estimated leaf area lost over the entire plant as described by **McCarville *et al*. (2010)**.

The growth stage of the soybean plant is important when making pest management decisions. Under most conditions, moderate defoliation early in the season has little effect on final soybean yield. As plants reach the flowering and pod-filling stages, defoliation poses a greater threat to yield.

Table 1. The name and pedigree of the tested soybean genotypes

Code No.	Genotype	Pedigree
G1	H_{14} L_8	Holladay x H_2 L_{12}
G2	H_1 L_{10S}	Giza 83 X H_2 L_{20}
G 3	H_{20} L_2	Giza 83 X H_5 L_{23}
G 4	H_1 L_{10}	Giza 21 x L_{86} K-73
G5	H_5 L_{21}	H_2 L_{24} x Giza 83
G6	H $_{19}$ L_{96}	H 73z x Hartwig
G7	H_2 L_3	Clark x Ware
G8	H_{10} L_{10}	Ware x Holladay
G9	H_1 L_1	DR 101 x Giza 22
G10	H_9 L_{113}	PI 416937 x H_2 L_{20}
G11	H_{11} L_{145}	Giza 111 x L75-6648
G12	H_1 L_3	H_{20} L_3 xGassoy 17
G13	H_5 L_6	H_2 L_{12} xClark
G14	H_{29} L_{115}	H73z x H_5 L_{23}
G15	Giza 111	Crawford x Celest

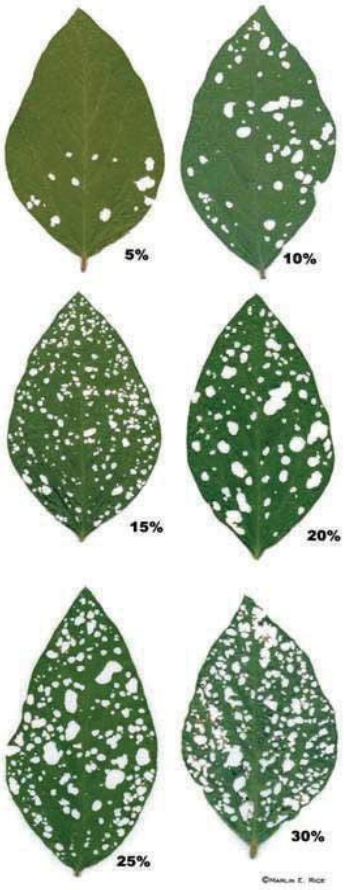

Fig. 1.Estimations of percent defoliation in soybean Reprinted from McCarville *et al*. (2010)

An early "trap" variety of Crwaford, was planted next to the soybean genotypes to attract and encourage the build up of a resident cotton leaf worm population. Field screening was accomplished by allowing a natural cotton leaf worm population to feed without disturbance.

Defoliation ratings were taken at beginning maturity [growth stage RT, (**Fehr and Caviness, 1977**)] of the earliest maturing line (Beeson) on a scale of 0 = no feeding to 4 = heavy defoliation (> 30%) as outlined by **Rufenerii *et al*. (1987)**. Ratings were analyzed with analysis of variance, with least significant difference ($LSD_{0.05}$) techniques used to separate defoliation rating means when significance was indicated by F test.

A combined analyses of variance was computed over the two seasons to estimate the genotypic variances. The analysis of variance was done according to **Snedecor and Cochran (1980)**. On the other hand, Levene test was used to satisfy the assumption of homogeneity of variances before running the combined analyses (**Levene, 1960**).

Protein electrophoresis (SDS-PAGE)

Samples taken from seeds of various genotypes were identified by sodium dodecylesulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to **Laemmli (1970)**. Protein bands were visualized by staining the gel with 0.25% coomassie brilliant blue R-250. Protein band sizes were determined by comparisons with the high molecular weight protein marker.

RESULTS AND DISCUSSIONS

Field Screening for Defoliation by Cotton Leaf Worm

Significant differences among soybean genotypes were obtained in the field screening for defoliation by cotton leaf worm (Table 2). The high susceptible genotype insect to had defoliation ratings of 3.8 was H_5 L_{21}, indicating heavy insect feeding. The five high resistant promising soybean genotypes, had defoliation rating from 0.0 to 0.3 (H_{14} L_8, H_1 L_{10S}, H_{10} L_{10}, H_2 L_3, H_1 L_{10} and H_{20} L_2), indicating extremely light feeding by cotton leaf worm. Whereas, H_{14} L_8 derived from cross (Holladay x H2L12), has exhibited high resistance level of defoliation, and derives its resistante genes from the H_2 L_{12} which was a resistance commercial cultivar in Egypt (unpublished results).

The commercial cultivar in Egypt Giza 111 and four promising genotypes (H_9 L_{113}, H_5 L_6 and H_1 L_3), had defoliation rating from 1.4 to 1.7, indicating extremely feeding by cotton leaf worm. The other soybean genotypes had intermediate susceptible defoliation ratings ranging from 1.7 (H_1 L_1, H_{11} L_{145} and H_{29} L_{115}) to 2.7 (H_{19} L_{96}).

Mean Performance

Results given in Table 3 show mean performance of 15 soybean genotypes and the check Giza 111 cultivar based on quantitative and qualitative traits. The results exhibited significant differences among the tested genotypes for all studied characters. This provides an evidence for the possibility to carry out a sufficient selection program on the basis of these traits using the studied genotypes. The results clearly indicated that the tested genotypes differed significantly in plant height. Genotypes; H_{11} L_{145} and H_{14} L_8 were the tallest plants (122.5, and 120.5 cm) while H_1 L_3 was the shortest (74.5 cm) one. Similar results were obtained by **Eisa *et al*. (1998), Hassan *et al.* (2001 and 2002) and Morsy *et al*. (2011)**.

Regarding number of branches/plant, H_{14} L_8, H_{11} L_{145}, H_{20} L_2 and H_{29} L_{115} genotypes produced the largest number of branches /plant being 5.0, 4.5, 4.5 and 4.5 respectively compared with H_1 L_{10} which recorded the lowest number of branches/plant (2.0). On the other hand, H_{11} L_{145}, H_{14} L_8, and Giza 111 produced the greatest number of pods/plant valued (160.0, 142.0 and 149.0, respectively), while H_1 L_1 and H_1 $L1_{0S}$ recorded the lowest number of pods/plant (86.5 and 60.5) over both seasons. Similar results were obtained by **Eisa *et al*. (1998), Hassan *et al.* (2001 and 2002) and Morsy *et al*. (2011)**. However, the heaviest weights of 100-seeds (20.22 and 19.49 g) were produced by H_{14} L_8 and H_{11} L_{145} genotypes compared to H_5 L_{21}

Table 2. Field defoliation ratings from cotton leaf worm resistance screening of soybean genotypes

No.	Soybean genotype	Field defoliation rate †
1	H_{14} L_8	0.0
2	H_1 $L_{10\,S}$	0.1
3	H_{20} L_2	0.3
4	H_1 L_{10}	0.2
5	H_5 L_{21}	3.8
6	H_{19} L_{96}	2.7
7	H_2 L_3	0.2
8	H_{10} L_{10}	0.1
9	H_1 L_1	1.7
10	H_9 L_{113}	1.2
11	H_{11} L_{145}	1.8
12	H_1 L_3	1.5
13	H_5 L_6	1.4
14	H_{29} L_{115}	1.8
15	Giza 111	1.4

† Field defoliation rating: zero= no noticeable feeding to 4 = >30%.

Table 3. Mean performance of some yield traits for the tested soybean genotypes (combined over 2017 and 2018 seasons)

No.	Soybean genotype	Plant height (cm)	No. of branches/plant	No. pods/plant	100-Seed weight (g)	Seed yield (ton/fad.)
1	H_{14} L_8	120.50	5.0	142.0	20.22	2.16
2	H_1 $L_{10\,S}$	87.50	3.0	60.5	17.63	1.26
3	$H_{20}L_2$	112.50	4.5	109.0	17.85	1.57
4	H_1L_{10}	102.50	2.0	96.0	18.47	1.57
5	H_5 L_{21}	107.50	3.5	102.0	17.22	1.25
6	H_{19} L_{96}	82.50	3.0	87.5	17.50	1.55
7	H_2 L_3	107.50	3.5	140.5	17.39	1.76
8	H_{10} L_{10}	84.50	3.0	100.5	18.74	1.94
9	H_1 L_1	92.50	2.5	86.5	18.52	1.43
10	H_9 L_{113}	82.50	2.5	90.0	17.61	1.96
11	H_{11} L_{145}	122.50	4.5	160.0	19.49	2.21
12	H_1 L_3	74.50	2.5	109.5	18.27	2.10
13	H_5L_6	107.50	2.5	124.0	17.67	2.13
14	H_{29} L_{115}	102.50	4.5	99.5	17.89	1.59
15	Giza 111	112.50	2.5	149.0	18.13	1.85
LSD0.05		8.51	1.36	18.69	0.46	0.147

Table 4. Qualitative characteristics and maturity group of 15 tested soybean genotypes

No.	Genotype	Seed coat color	Seed coat luster	Hilum color	Seed size	Maturity group[+]	Growth habit	Growth type[+†]	Flower color[+‡]	Pubescence color	Pubescence type	Anthocyanin
1	H_{14} L_8	Yellow	shiny	Yellow	Medium	V	Erect	I	W	T	H	-
2	H_1 $L_{10\,S}$	Yellow	inter.	Yellow	Medium	IV	Semi-Erect	I	W	G	H	-
3	$H_{20}L_2$	Yellow	shiny	Black	Medium	IV	Erect	I	P	T	N	√
4	H_1L_{10}	Yellow	shiny	Black	Medium	IV	Medium	I	P	G	H	√
5	H_5 L_{21}	Yellow	shiny	Gray	Medium	IV	Erect	I	P	G	N	√
6	H_{19} L_{96}	Yellow	shiny	Black	Medium	V	Semi-Erect	D	P	G	H	√
7	H_2 L_3	Yellow	dull	Yellow	Medium	V	Semi-Erect	I	P	G	H	√
8	H_{10} L_{10}	Yellow	inter.	Gray	Medium	V	Erect	D	P	G	H	√
9	H_1 L_1	Yellow	shiny	Black	Medium	V	Medium	I	W	L	H	√
10	H_9 L_{113}	Yellow	inter.	Black	Medium	V	Semi-Erect	D	P	G	H	-
11	H_{11} L_{145}	Yellow	inter.	Gray	Medium	V	Erect	I	P	T	H	√
12	H_1 L_3	Yellow	inter.	Black	Medium	V	Medium	D	P	G	H	√
13	H_5L_6	Yellow	shiny	Black	Medium	IV	Erect	I	P	T	H	-
14	H_{29} L_{115}	Yellow	shiny	Black	Medium	V	Erect	I	P	G	H	√
15	Giza 111	Yellow	inter.	Black	Medium	IV	Erect	I	P	T	H	√

genotype which gave the lightest weight of 100-seeds (17.22 g). The results showed that H_{11} L_{145} and H_{14} L_8 genotypes surpassed the other tested genotypes for seed yield/fad., recording 2.21and 2.16 ton/fad., respectively, while H_1 L_{10S} and H_5 L_{21} genotypes were inferior to the mentioned genotypes recording 1.26 and 1.25 ton/fad., respectively. Therefore, the promising genotypes H_{11} L_{145} and H_{14} L_8 could be recommended to be involved in soybean breeding programs aiming to improve seed yield. This finding is in agreement with those reported by **Hassan *et al.* (2001 and 2002), Mohamed and Morsy (2005), Hamdi *et al.* (2008) and Morsy *et al.* (2011)**.

Furthermore, soybean genotype H_{14} L_8 combines the utmost characters as it exhibited the highest seed yield (2.16 ton/fad.) with resistance to cotton leaf worm (zero feeding).

SDS-PAGE of Seed Storage Protein (Protein Fingerprint)

The seed storage proteins of the fifteen genotypes of soybean were analyzed by SDS-PAGE and the electrophoresis pattern of the different genotypes is presented in Table 5, and illustrated in (Fig. 2). On the basis of the relative mobility of seed proteins on the gel, 19 bands were detected in this study with molecular weights ranging from 166.966 to 276.611 KDa, (Table 5). Three major bands were recorded out of total 19 bands detected, while five from total bands were polymorphic. The banding pattern revealed variations among accessions. Three major monomorphic bands were identified in one genotype. The three bands at molecular weight of 239.953, 236.295 and 200.211KDa, respectively were detected in genotype $H_{14}L_8$.

Table 5. Molecular weight of soluble protein bands extracted from Soybean varieties by polyacrylamide gel electrophoresis

MW (KDa)	H_{14} L_8	H_1L_{10S}	$H_{20}L_2$	H_1 L_{10}	H_5 L_{21}	$H_{19}L_{96}$	H_2 L_3	$H_{10}L_{10}$	H_1 L_1	H_9L_{113}	$H_{11}L_{145}$	H_1L_3	H_5 L_6	$H_{29}L_{115}$	G_{111}
276.611	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-
264.545	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+
259.337	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
256.087	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+
250.751	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
247.352	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
243.098	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
239.953	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
236.295	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
234.203	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
228.456	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
222.187	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
217.368	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-
205.242	-	+	-	+	-	+	+	+	-	-	-	-	-	-	-
200.211	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
193.382	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-
189.538	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
180.956	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
166.966	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total	**11**	**9**	**7**	**9**	**6**	**8**	**9**	**9**	**8**	**7**	**8**	**8**	**8**	**8**	**8**

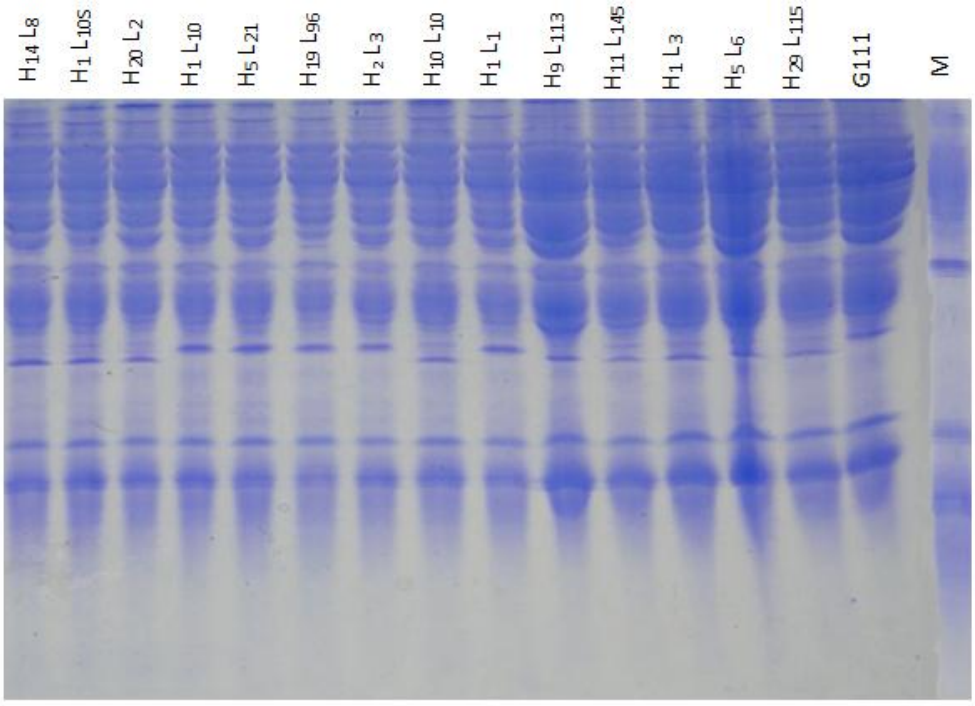

Fig. 2. SDS-PAGE of total protein extracted from the seed of fifteen soybean genotypes

These obtained results could be considered as positive unique markers (PUM) for this genotype of soybean, reinforcing its superiority in insect resistance and seed yield.

With regard to seed protein banding patterns, five polymorphic (common bands) have been identified in all genotypes which have MW of 250.751, 243.098, 222.187, 180.956 and 166.966kDa.

However many investigations have used seed storage protein variability for the identification and characterization of species and cultivars **(Badr *et al*., 2000; Bushehri *et al*., 2000; Alipour *et al*., 2002; Ibrahim 2003).**

Seed protein of soybean is known to contain two kinds of protein lipoxygenase and trypsin inhibitor which produces antibiosis in susceptible insects **(Johnston *et al*., 1993)**. Trypsin inhibitor protein has approximately molecular weight of 21500kDa **(Krishnan, 2001)**. In this investigation, the protein bands at molecular weights of 239.953, 236.295 and 200.211KDa could be that of trypsin inhibitor (Fig. 1), found only in the genotype $H_{14}L_8$ **(Stejskal and Griga, 1995)**. This genotype was more resistant than other genotypes. Researchers have estimated that as few as two **(Kenty *et al*., 1996)** and many as six genes **(Rufener *et al*., 1989)** could be responsible for the partial insect resistance.

The previous results indicated that SDS-PAGE of storage seed protein used in this study give clear identification of the tested genotypes. Each genotype has different number and/or position of bands. Protein marker confirmed the use electrophoretic analysis of seed storage protein of soybean as an aid to cultivar identification as reported by **El-Danasoury (2003)**, who reported that SDS-PAGE was widely used to separate proteins which are directly related to genetic background and can be used to certify the genetic makeup of wild cultivars or newly developed cereal plants.

The low level of protein polymorphism in this study could be attributed to conservation nature of the seed protein **(Bonfitto *et al*., 1999)**. Low level of protein polymorphism was also reported in early ripening peach of Sinai **(Mansour *et al*., 1998)** and in mung bean cultivars **(Hassan, 2001)**.

Cluster Analysis

The cluster analysis for the qualitative traits using the presence (1) or absence (0) of polypeptide bands was entered in a binary data matrix for use in cluster analysis. The polymorphic bands were analyzed for the level of polymorphism by counting the number of polymorphic bands and generating summary statistics on the band frequencies. Cluster analysis was also performed using the computer software statistical method classified genotypes into two groups. First group (I) was divided in two clusters (A and B). Cluster A consisted of varieties No. 3 (H_{20} L_2) and 5 (H_5 L_{21}), identical in maturity group, growth type erect, growth habit, flower color, seed coat color, seed size, pubescence type, hypocotyl anthocyanin coloration was present and seed coat luster. Cluster B encompassed two subclusters (B1and B2). Subcluster B1 was further divided in two subclusters (b1 and b2). Subcluster b1 comprised of one cultivar No. 15 (Giza 111) and one genotype No. 13 (H_5 L_6) identical in the maturity group, indeterminate growth type, erect growth habit, flower color was violet, seed coat color, seed size, hilum color, pubescence color and pubescence type. Subcluster b2 encompassed four genotypes 9 (H_1 L_1), 11 (H_{11} L_{145}), 12 (H_1 L_3) and 14 (H_{29} L_{115}) included genotypes of the same maturity group, seed coat color, seed size, hypocotyl anthocyanin coloration was present and pubescence type. Subcluster B2 was further divided in two subclusters (b1 and b2). Subcluster b1 encompassed three genotypes No. 4 (H_1 L_{10}), No. 6 (H_{19} L_{96}) and No. 8 ($H_{10}L_{10}$), identical in the seed coat color, seed size, flower color, pubescence color, pubescence type and hypocotyl anthocyanin coloration was present. Subcluster b2 comprised of two genotypes No. 2 (H_1 L_{10S}) and No. 7 (H_2 L_3) having the same growth type, growth habit, seed size, seed coat color, seed hilum color, pubescence type and pubescence color. Group II encompasses two genotypes No. 1 (H_{14} L_8) and No.10 (H_9 L_{113}) identical in maturity group, seed coat color, seed size, pubescence type and hypocotyl anthocyanin coloration was absent or very weak (Fig. 3).

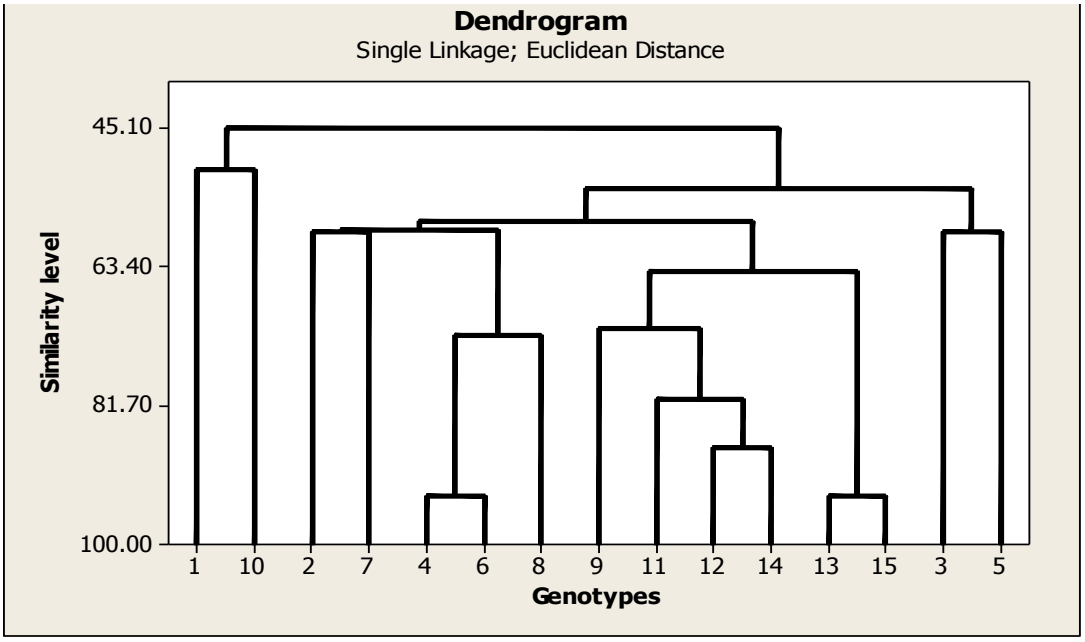

Fig. 3. Similarity levels for 15 soybean genotypes calculated by cluster analysis

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التوصيف المورفولوجي وتقييم التنوع الوراثي باستخدام علامات مجهرية لبعض التراكيب الوراثية لفول الصويا

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على الرغم من أن فول الصويا *Glycine max* (L.) Merrill{2n = 40} يشمل العديد من السمات المورفولوجية الواضحة إلا إنه ضيق في القاعدة الوراثية، لذا فقد أجريت هذه الدراسة في محطة البحوث الزراعية بسخا، كفر الشيخ، مصر، خلال موسمي الزراعة الصيفي ٢٠١٧، ٢٠١٨م لدراسة الصفات المورفولوجية الزراعية للتفرقة والتمييز بين ١٥تركيباً وراثياً مختلفاً من فول الصويا والكشف عن التنوع الجيني باستخدام الواسمات الجزيئية لصفة المقاومة للحشرات تم اجراء وصف مورفولوجي لعشر صفات من الصفات الوصفية وهي (لون السويقة الجنينية ووجود صبغة الأنثوسيانين، طبيعة نمو الساق الأصلي، طبيعة نمو النبات، لون الزهرة، لون الزغب، كثافة الزغب، لون القصرة، درجة لمعان البذرة، حجم البذرة و لون السرة) بالإضافة إلى سبع صفات كمية وهي (عدد الأيام حتى التزهير، عدد الأيام حتى النضج، طول النبات، عدد الأفرع على النبات، عدد القرون على النبات، وزن١٠٠ بذرة ومحصول البذور للفدان)، وتقدير نسبة تأكل الأوراق بواسطة حشرة دودة ورق القطن تحت ظروف الحقل المفتوح، أظهرت النتائج وجود اختلافات معنوية بين التراكيب الوراثية تحت الدراسة لجميع الصفات المدروسة، وأظهر التحليل القائم على الشكل المورفولوجي إمكانية تمييز التراكيب الوراثية بوضوح على أساس طبيعه نمو النبات، أظهرت الإصابة بدودة ورق القطن أن التراكيب الوراثية H_{14} L_8 و H_1 L_{10S} و H_1 L_{10} و H_{10} L_{10} كانت أعلى مقاومة لحشرة دودة ورق القطن ولم تتجاوز نسبه تآكل الأوراق بها ٢٠% بينما كان التركيب الوراثي H_5 L_{21} أقل مقاومه حيث بلغت نسبه التآكل (٣٨%) مما يدل على تغذية الحشرات على هذا التركيب الوراثي، على مدار الموسمين، سجلت التركيب الوراثية H_{11} L_{145} و $H_{14}L_8$ أعلى محصول البذور/الفدان، ويعزى ذلك إلى الزيادة الكبيرة في عدد الأفرع/النباتات، وعدد القرون/النبات، ووزن ١٠٠ بذرة في كلا الموسمين، مما يشير إلى تفوق هذه التراكيب الوراثية، كما أمكن تمييز مجموعه من البروتينات المختلفة في بذور التراكيب الوراثية تحت الدراسة باستخدام التحليل الكروموتوجرافي الكهربي. كما وجد اختلاف في الوزن الجزيئي للبروتينات الموجودة في بذور التراكيب الوراثية تحت الدراسة لذلك فهي وسيله هامه للتمييز بين الأصناف المختلفة.

المحكمـــــون:

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