Middle East Journal of Applied Sciences, 4(2): 318-325, 2014
ISSN: 2077-4613

Correlation coefficient analysis for quantitative and resistance characters with fingerprinting in ten soybeans *glycine max* (l.) Genotypes based on DNA polymorphism

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ABSTRACT

Ten soybean genotypes were evaluated for seed yield and its components, pubescence density, trypsin inhibitor content as resistant factor against defoliation %.Correlation coefficients analysis and classical selection index for the studied characters were estimated, DNA polymorphism and RAPD profiles for studied genotypes were performed. The studies indicated that out of 14 characters, correlations between them are of paramount importance. Higher (+ or -) correlation coefficients for almost all the character combinations suggested the inheritance association between various characters. The best genotype L105 was at the first rank showing highest classical selection value, with highest density of pubescence as physical characteristic and lowest rate of defoliation %, also had highest considering resist effect against insect attack. Short plant L105 had more branches and bearing larger number of pods /plant could increase seed yield /plant (g) and 100-seed weight (g). Yield production was clearly affected by increasing pubescence density as well as resistance genotype reflecting for defoliation % rate beside (TIU) contents. Giza 22 was at the last rank with lowest classical selection value including negative effect for earliness characters and high percentage of defoliation with lowest values of (TIU) /g DW content. Flowering date showed positive and high significant association with maturity date, avoiding genotypes of insect attack on the plant. RAPD – PCR profiles revealed high levels of polymorphism among the studied genotypes. Five primers successfully generated reproducible polymorphic products. These primers generated a total of 46 amplification products, among which 9 were monomorphic and 37 were polymorphic with an average of 9.2 bands/primer. Average polymorphic across all genotypes was 80.25%, Giza 22 and Giza 111 had maximum similarity value, and the genotypes were groped in two main clusters.

Key words: Soybean, Seed yield, Insect resistant, Correlation coefficients, Classical selection index, RAPD DNA

Introduction

Over the last century, soybean (*Glycine max* (L.) Merr.] has become the primary source of the world's vegetable oil and vegetable protein and conversed from a forage crop to a major seed crop. Soybean breeders have successfully developed hundreds of improved cultivars. Knowledge of diversity patterns will allow breeders to better understand the evolutionary relationships among accessions and to sample germplasm in a more systematic fashion and to develop strategies to incorporate useful diversity in their breeding programs Ude *et al*., 2003

AL-Saghir and Abdel-Salam 2011 conducted fingerprinting by using RAPD markers to evaluate the genetic diversity in twenty soybean accessions of the North American soybean (*Glycine max* L.). Twenty-seven RAPD primers produced 210 amplification products of which 78 (27.3%) were polymorphic, and demonstrated the usefulness of these markers in estimating the extent of genetic variation in *Soybean* germplasm.

Insects attacking soybeans cause leaf-feeding, and pod-feeding which may affect future growth, or pod-fill. Examples of defoliating insects include grasshoppers, bean leaf beetles, and several caterpillars' insects. Defoliation always appears worse than the resulting yield loss, to produce a good bean crop. Annually, chemical control of one or both of these pests is required to reduce economic losses. In the past, organophosphorous and carbamate compounds were routinely utilized to control pest outbreaks soybean defoliation damage. Moreover to high costs by repeated pesticide use in agricultural production, in addition to the detrimental environmental impacts created Boethel, 2004.

Thus, Biochemical and molecular characterization of insect-resistance or susceptible soybean cultivars as well as amount of genetic diversity among them are considered a central task for many purposes of soybean breeding Fahmy and Salama, 2002.

Soybean seed protein contains kinds of protein known trypsin inhibitor which produce antibiosis in susceptible insect, Attia 2003, Krishnan, 2001.

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Johnston *et al.,* 1993 implicated trypsin inhibitor as a soybean component that produces antibiosis in susceptible insects. The genetically controlled resistance factors in soybean are due to one or more chemicals that are found in plants.

Hill *et al.,* 2004 studied the effect of soybean pubescence on insect pests, such as reduced damage due to feeding by plant hoppers, and other pests and effects on the probing behavior of other aphid, also dense pubescence increased resistance to defoliation and reduced feeding leaf damage,

The development of DNA markers have been recently introduced in plant discriminations and being employed for the improvement of intractable traits such as, resistance to foliar–feeding insects and the combination of high protein/high yield Lefebvre *et al*., 2001; Lu & Myers 2002.

Barakat 2004 showed low levels of polymorphism which could not be used to discriminate completely among six studied soybean cultivars (Clark, Crawford, Giza 83, Giza 21, Giza 35 & Giza 111) and RAPD-PCR analyses were performed to establish fingerprints for studied soybean cultivars and to elucidate their genetic relationships. On the other hand, RAPD-PCR profiles revealed high levels of polymorphism among the studied cultivars.

Some investigations were carried out on soybean(*Glycine max* (L.) Merr.), (Komatsu *et al*., 2005; Komatsu *et al*., 2007 and Lambert *et al*., 1992), they reported that there was a relationship between the resistance of the soybean cultivar and pubescence density. They also genetically analyzed this resistance using DNA markers and recognized two quantitative trait loci (QTL), and concluded that although pubescence density is a possible factor, but the immediate cause remains unknown.

Komatsu *et al*., 2007 analysis of antibiosis resistance to common cutworm(*Spodoptera litura* Fabricius) in soybean(*Glycine max* (L.) Merr.), but the immediate cause remains unknown.

Showkat and Tyagi2010 estimated correlation and path coefficient analysis for seed yield and its components in 40 genotypes of soybean and indicated that, seed yield showed positive and significant correlation with biological yield, days to maturity, branches/per plant, harvest index, pod filling period, pods per plant, 100-seed weight and clusters per plant indicating that an intense selection for these characters improve seed yield in soybean. Their results indicated that biological yield is responsible for manipulation of seed yield in soybean.

Overall pubescence density and plant development stage with maturity date and trypsin inhibitor contents are assumed to be the immediate cause of resistance to several caterpillars insects as *Spodoptera littoralis*.

The present investigation was undertaken to estimate the classical selection index, correlations and their direct and indirect effects on seed yield to make the selection effective for seed yield, earliness and insect resistance with their fingerprint DNA RAPD markers in ten soybean genotypes.

Material and Methods

A –Filed experimental:

The experiment materials consisted of ten differing genotypes of soybean(*Glycin Max* L.), the genotype H88L1 was originated from a study by El-Shabory *et al.,* 2006, the rest provided from ARC, and NRC Giza, Egypt. Table (1) shows the genotype names, pedigree, maturity group, origin, growth habit, and flower color.

The field experiment was carried out to evaluate the genotypes during 2010 at a farm in EL –Salam village in EL- Nobaria region, El- Beheara Governorate, Egypt. The plants were sown in a randomized block design with three replications in a single row of 3m length the plants were spaced at 30x10cm in each plot. Common cultural practices for soybean were followed.

Table 1: Pedigree, Maturity group, flower color and origin of studied soybean genotypes.

No.	Genotype	Pedigree	Maturity group	Flower color	Origin
1	Giza 22	Crawford x Forrest	IV	Purple	Egypt
2	H 30	Crawford x L62-1686	III	Purple	Egypt
3	Giza 111	Crawford x Celest	IV	Purple	Egypt
4	Crawford	Williams x Columbus	IV	Purple	U. S.A.
5	H 129	D76-8070 x G35	III	White	Egypt
6	H 132	G35 x G82	III	Purple	Egypt
7	H88L1	L86-k-73 x G111	III	White	Egypt
8	L 113	G111x Major	II	Purple	Egypt
9	L105	G35 x lamer	V	White	Egypt
10	Line153	G38 x G111	III	White	Egypt

Flowering date was recorded at 50 % flowering of plants and maturity date was recorded at 95% pods maturity. At harvest, the following traits were recorded on ten guarded individual plants chosen at random from each plot: plant height (cm), number of branches / plant, number of pods / plant, number of seeds / pod, number of seeds / plant, number of seeds /pod , 100-seed weight (g) and seed yield /plant (g).

B-Laboratory experimental:

Laboratory experiment was conducted at Genetics and Cytology Department, NRC, Giza, Egypt.

B-1-Protein content % and oil content %:

Protein content % in the seeds of soybean was determined using the Micro-Kjeldahl method, and oil content (%) was determined according to the extraction method as described by A.O.A.C. (1975).

B-2-Trypsin inhibitor activity (TIA):

Trypsin inhibitor activity (TIA)was measured as trypsin inhibitor units (TIU) / gram of dry seed weight, and evaluated according to Filippetti *et al.*,1999 and the modify of method Attia (2003).

B- 3- Defoliation % of leaf feeding damage:

The defoliation % was estimated as leaf feeding damage in the laboratory against cotton leaf worm (*Spodopteralittoralis*) under forced feeding. The test plant were categorized as resistant (zero defoliation %), moderately resistant (5,10,15 defoliation %) and susceptible (20, 25, 30 defoliation %)according to Kumatsu *et al*., 2007 and Bhattachaeyya and Ram 2001.

B-4- Pubescence density:

Pubescence density was evaluated as the number of hairs per 10 mm^2 of the underside of the leaf. Four leaflets were collected from each plant and a 10 mm^2 area of the underside of each leaflet was evaluated under compound microscope and the hairs counted. The mean of four density evaluations was used as the index of pubescence density for soybean genotypes.

Statistical analysis:

Statistical analysis of the estimated collection data were performed as mean performance and classical selection index was estimated according to Smith (1936), as well as correlation coefficients analysis for studied characters in ten genotypes of soybean using the formula of Al-Jibouri *et al*., (1958).

B- 5- DNA Extraction and RAPD Amplification Conditions:

Molecular studies aimed to clarify genetic polymorphism between ten different genotypes of soybean.

B-5-1- DNA extraction:

Leaves were obtained from 14 days old plantlets from the ten soy bean genotypes and ground to a fine powder in liquid nitrogen. The genomic DNA was extracted using the Biobasic kit protocol. RAPD analysis was performed using ten 10-mer random primers and successful five primes (Table 2) produced from Operon Technologies (Metabion International AG).

Table 2: Code and sequence of the five DNA random primers used for identifying the soybean varieties.

No.	Primer	Sequence(5'- 3')
1	OPA-03	5'-AGTCAGCCAC-3'
2	OPC-19	5'-GTTGCCAGCC-3'
3	OPD-13	5'-GGGGTGACGA-3'
4	OPW-04	5'-CAGAAGCGGA-3'
5	OPX-17	5'-GACACGGACC-3'

RAPD assay was performed as described by Williams *et al.* (1990) with some modifications. The 20 µl PCR reaction mixture contained 1x PCR buffer, 2 mM MgCl2, 200 mMdNTPs, 0.25 mM of primer, 1 unit of *Taq*DNA polymerase (Promega Inc., USA) and 2 µ l (50ng) template DNA. PCR amplification was performed in PTC-100 PCR version 9.0 from M. J. Research- USA, programmed for 95°C for 5 min (denaturation), 36 cycles of [94°C for 1 min, 36°C for 1 min and 72°C for 1 min (annealing)] and a final extension of 2 min at 72°C. PCR products were analyzed using 1% agarose gel electrophoresis and visualized with ethidium bromide staining. The sizes of the fragments were estimated based on a DNA ladder of 100 bp (Fermentas).

B -5 - 2-RAPD Data Analysis:

Clear and distinct amplification products were scored for presence (1), absence (0). The genetic similarity coefficient (GS) between two genotypes i and j was estimated using Dice coefficient (Sneath and Sokal, 1973). Dendrogram was built using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering procedure, based on the dissimilarity matrices obtained from RAPD data**.**

Result and Discussions

Correlation coefficients:

Understanding correlation between traits is of paramount importance, as they serve as a guide for incorporating the characters of economic importance. Such information helps the breeder to select the best donor parents for use in breeding programs. The existence of a positive correlation between any two traits suggests that improvement of one character would automatically improve the other. The negative correlation indicates that simultaneous improvement of these traits is not possible.

The estimates of correlation coefficients data were tabulated in Table 3. The higher correlation coefficients for almost all the character combination, for all the pattern of correlation (+ or -) levels, suggested the inherent association between various characters. Therefore results have been discussed among the 14 character combinations. Plant height (cm) showed negative and significant association with defoliation rate % and protein % content, also showed positive and significant association with seed yield /plant (g), pubescence density and (TIU)/g DW content and high positive significance with 100-seed weight (g). Flowering date only was positive significant and highly significant with defoliation rate % and protein % content, respectively. Maturity date did not show significant association with all characters.

Branches/pant had higher correlation coefficients for all the character combinations of correlation (+ or -), which indicated the effect of this trait on plant productivity, except with number of seeds/pod was not significant.

Pods/plant had positive correlation with five characters, number of seeds/plant, 100-seed weight, and seed yield/plant, density of pubescence and content of (TIU)/g DW, and high negative correlation with defoliation affect%.

Correlation coefficients for number of seeds/plant were positive and highly significant with 100-seed weight, seed yield /plant (g), density of pubescence and (TIU) contents, while it was negative with defoliation %. Number of seeds/pod did not show any significance with all studied characters.

Positive correlations were found for 100-seed weight (g) with yield /plant (g), pubescence density and (TIU) /g DW, while defoliation % rate and protein % content were negative correlated. This is obvious when the leas trisk of defoliation % rate showed an increase in yield and its component.

Pubescence density was correlated positively with (TIU) /g DW and significant negative with defoliation % rate and protein content.

Defoliation % rate indicated highly positive significant correlation with protein content and highly negative significant correlation with (TIU) /g DW. Protein content showed negative significant correlation with (TUI) /g DW.

Pods/plant and density of pubescence were exhibited the maximum positive effect on seed yield/per plant, where as density of pubescence exhibited the maximum negative direct effect to avoid defoliation damage which affected on magnitude of seed yield/ plant. Seed yield is influenced by the interaction of different factors including environment. Type and nature of association is usually determined by studying correlation coefficients. It is an established fact that seed yield is a complex character for which direct selection is not much effective and correlation studies alone would be misleading Singh and Chaudhyany (1985). Therefore, the present result are in confirmation with the previous studies carried out by Bastawisy *et al*. (1997), Rajanna *et al*. (2000); Singh &Yadav (2000); Jyoti and Tyagi (2005); Showkat and Tyagi (2010).

Classical Selection index:

As regard to Table 4 it is clear that those studied characters were tightly positive correlated with seed yield, plant breeders need to know what are the most important characters in selection for high yield. Selection criterion depends mainly on phenotypic and genotypic covariance matrices, which was in agreement with correlations among the studied characters. The performance mean of soybean genotypes arranged descendly according to selection criterion for yield and its components with their corresponding density of pubescence, defoliation % rate, protein and oil contents as well as (TIU) /g DW contents.

The best genotype L105 was at the first rank showed highest classical selection value, with highest density of pubescence as physical characteristic and lowest rate of defoliation %, also had highest considering resist effecting against insect attack.

The above results suggested that the short genotype L105 had more branches and bearing larger number of pods /plant could increase seed yield/plant (g) and 100-seed weight (g), also it worth mention that yield production clearly affected by increasing pubescence density and reflect resistance genotype for defoliation % rate beside (TIU) contents.

On the other hand the variety Giza 22 was at the last rank with lowest classical selection value including negative effect for earliness characters and high percentage of defoliation with lowest values of (TIU) /g DW content.

Similarly, flowering date showed positive and high significant association with maturity date, this means avoiding genotypes of insect attack on the plant, however Komatsu *et al*., 2007 mentioned that this studies concept is still unclear.

In all, due to highest product of yield and its components in soybean genotypes, studies explain the relationships among pubescence density, defoliation % rate and (TIU) /g DW as the resistant factors effect indicating by classical selection values. A perusal of overall results, indicated that genotypes (L105, L113) were superior in seed yield and insect-resistance than resulted by Barakat 2004, who characterized the cultivars Giza 21, Giza35 and Giza 111 as improved quality and insect-resistance cultivars.

Table 3: Correlation coefficient among studied characters in ten genotypes of soybean.

Char-acters	(1) Ph (cm)	(2) Flow. date	(3) Mat. date	(4) Bra/pl.	(5) Pod/pl.	(6)Seeds /pl.	(7)Seeds/ pod	(8) 100-S(g)	(9) Yield/pl (g)	(10) Pub.den.	(11) Defo. %	(12) PC %	(13) Oil C %	(14) TIU/g
(1)	1	-0.194	-0.160	0.275	0.224	0.241	0.273	0.445**	0.308*	0.175	-0.331*	-0.353*	0.271	0.31*
(2)		1	0.942**	-0.156	-0.234	-0.254	0.083	-0.171	-0.216	-0.69**	0.238	0.041	0.031	-0.175
(3)			1	-0.04	-0.05	-0.106	-0.001	-0.041	-0.049	-0.92**	0.053	0.053	0.006	-0.041
(4)				1	0.768**	0.600**	-0.054	0.623**	0.795**	0.751**	-0.72**	-0.48**	0.408**	0.664**
(5)					1	0.839**	0.014	0.801**	0.978**	0.944**	-0.94**	-0.244	0.041	0.824**
(6)						1	-0.071	0.626**	0.828**	0.838**	-0.80**	-0.173	0.098	0.785**
(7)							1	0.194	0.029	0.021	-0.022	0.072	0.025	-0.001
(8)								1	0.869**	0.785**	-0.86**	-0.41**	0.106	0.704**
(9)									1	0.936**	-0.96**	-0.346*	0.058	0.835**
(10)										1	-0.94**	-0.230	0.065	0.819**
(11)											1	0.423**	-0.09	-0.77**
(12)												1	-0.53**	-0.177
(13)													1	0.038
(14)														1

*, **= significant at p=0.05 and 0.01 level respectively

(1)Plant height(cm) = Ph(cm), (2) Flowering date = Flow. date , ((3)Maturity date = Mat.date, (4)Branches /plant = Bra/pl.,(5)Pods/plant =Pod/pl., (6) No. seeds/plant = Seeds/pl. , (7) No. seeds/pod =Seeds/pod , (8)100-seed weight(g) = 100-S(g), (9) Seed yield/plant(g) = Yield/pl (g), (10)Pubescence density = Pub. den., (11)Defoliation% = Defo. % , (12) Protein content % = PC %, (13)Oil content % = Oil C% and (14) TIU/g DW= TIU/g.

Table 4: The genotypes arranged descendly according to selection index values for yield and its component performance as means,as well as their corresponding contents of protein content %,oil content% , trypsin inhibitor (TIU/gDW) , defoliation % and pubescence density.

Rank No.	Geno-types No.	Ph (cm)	Flow. date	Mat. date	Bra/pl.	Pod/pl.	Seeds/ pl.	Seeds / pod	100-S(g)	Yield/ pl(g)	Pub. den.	Defo %	PC %	Oil C %	TIU/g	Classica selection values
1	L105	90	30	105	4.32	271.12	661.54	2.44	14.7	97.32	24.2	0	44.13	19.67	4902	6196.44
2	L113	112	30	105	4.14	237.02	591.18	2.49	15.62	93.21	21.4	0	41.13	18.4	4899	6100.59
3	H88L1	105	33	115	4.05	210.26	525.64	2.49	15.73	88.47	18.5	5	40.1	19.5	4788	5874.74
4	H30	99	35	115	3.45	214.3	506.6	2.36	14.44	75.91	15.9	5	40	20.01	4604	5650.97
5	H129	96	35	115	3.6	215.47	571	2.65	14.54	81.91	17.5	5	39.61	19.83	3899	5016.11
6	Line153	94	34	114	4	192.71	449.3	2.33	14.07	70.1	14.9	10	40.57	19.55	3640	4603.53
7	H132	97	35	115	3.46	138.64	346.72	2.5	12.58	45.26	14.5	15	39.63	22.17	3700	4487.46
8	Crawford	91	45	130	3.56	121.97	297.6	2.44	10.9	36.08	8.5	30	42.2	21.2	3781	4471.45
9	Giza111	100	40	120	2.73	127.71	333.33	2.61	12.07	40.63	11.6	20	42.83	17.9	3674	4425.41
10	Giza22	102	40	120	2.5	131.14	301.9	2.3	11.28	36.93	10.6	25	47.9	15.87	3598	4325.42

(1)Plant height (cm) = Ph (cm), (2) Flowering date = Flow. date , ((3) Maturity date = Mat. date , (4) Branches /plant = Bra/pl. , (5) Pods/plant = Pod/pl. , (6) No. seeds/plant = Seeds/pl. , (7) No. seeds/pod =Seeds/pod , (8)100-seed weight(g) = 100- S (g), (9) Seed yield/plant(g) = Yield/pl (g), (10) Pubescence density = Pub. den., (11) Defoliation% = Defo. % , (12) Protein content % = PC % , (13) Oil content % = Oil C% and (14) TIU/g DW= TIU/g.

RAPD Analysis:

The use of RAPD –DNA markers to study the genetic diversity and genetic relationships among ten soybean genotypes. Ten random primers were used, five of these produced informative banding patterns Fig 1. The fingerprints generated by these primers revealed characteristic profiles for each genotype in terms of number and position of RAPD band.

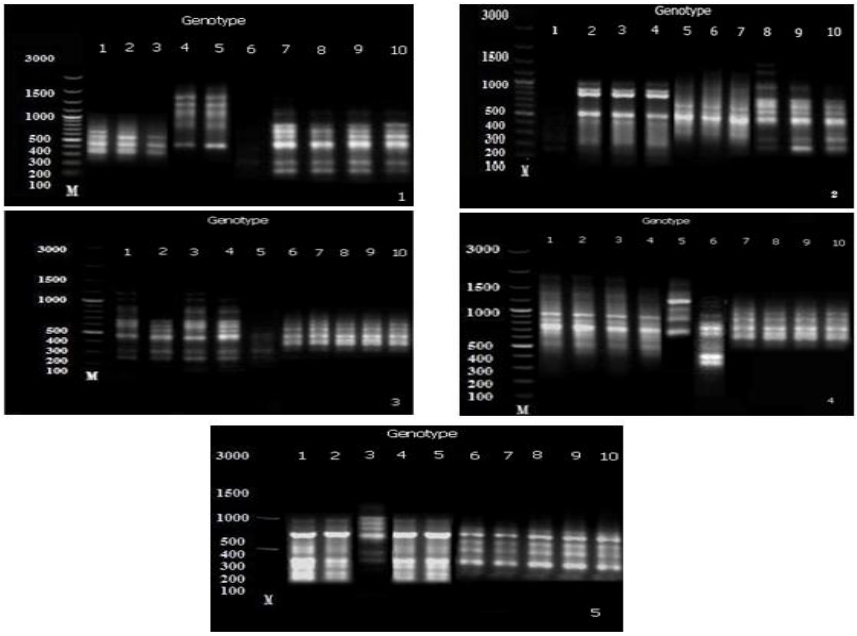

Fig. 1: RAPD fingerprints of ten soybean genotypes using five primers.

Table 5 summarizes the banding pattern obtained with these primers. RAPD bands produced were between 1808 to about 140 bp size produced. These primers generated a total of 46 amplification products, among which 9 were monomorphic and 37 were polymorphic with an average of 9.2 bands/primer.

The number of polymorphic bands varied from eight bands for primers OPW-04 to eleven bands for primer OPD-13. Average polymorphic across all genotypes in our study was 80.25, this average was less than reported (85.71%) by Barakat 2004 and higher than AL-Saghir and Abdel-Salam 2011 results.

The genetic relationships among the studied genotypes were analyzed using Jaccard's coefficient to get the genetic similarity matrices shown in Table 6. A maximum similarity value of 0.997 was observed between the two cultivars Giza22 and Giza111, the followed similarity value of 0.900 was observed between H30 and Giza111, another similarity was 0.890 between L113 and Line 153 indicating that these genotypes were closely related to each other.

Table 5: The total number of bands , monomorphic bands, polymorphic bands, mean of band frequency and polymorphism% of the five DNA random primer using RAPD –PCR.

Primer Nno.	Monom-orphic bands	Poylmo-rphic bands without unique	Uniqe bands	Polymo-rphic bands with unique	Total bands	Polymor-phism%	Mean of band frequnce	One -zero	Band size
1	0	9	0	9	9	100.00%	.422	9Polymorphic	1808- 154
2	2	7	0	7	9	77.78%	.511	7Polymorphic	859- 165
								2monomorphic	397-363
3	2	9	0	9	11	81.82%	.555	9 Polymorphic	1628- 188
								2 monomorphic	439-379
4	2	3	3	6	8	75.00%	.438	3Unique	1154-353-306
								3 Polymorphic	842-621
								2 monomorphic	686-518-428
5	3	6	0	6	9	66.67%	.611	6 Polymorphic	591- 140
								3 monomorphic	234-304-488
Total	9	34	3	37	46	80.25%	-	-	-

The similarity between Giza 111 and Crawford was 0.833, but Barakat 2005 found it 0.525. Several relations were found to be genetically most diverse (0.001) between the genotypes i.e. Giza 22 and H 129; Giza 111 and H 129; H 129 and L105.

As shown in Fig. 2, the Cluster analysis by the un-weighted pair group method of arithmetic means (UPGMA) showed that the soybean genotypes can be clustered in two distinct groups, first main cluster was also divided into two sub-clusters, first sub cluster separated 5(H129), while the second sub cluster, contains 1, 3, 2 and 4 (Giza 22, Giza 111, H30 and Crawford). The second main cluster was divided in two sub clusters, first sub-cluster separated 6(H 132) genotype, while the second sub-cluster contains 8, 10, 7 and 9 (L113, Line 153, H88L1 and L105)). In this grope the highest similarity values 0.890 was recorded between genotypes (L113) and (Line153), this most related than the value (0.636) with between the genotypes (H88L1) and (L105).

Table 6: Genetic similarity matrices computed according to Dice Coffeiecient from RAPD of the ten soybean genotypes origins.

Genotypes	Giza 22	H 30	Giza 111	Crawford	H 129	H 132	H88L1	L 113	L105	Line153
Giza 22	1.0									
H 30	.807	1.0								
Giza 111	.997	.900	1.0							
Crawford	.749	.715	.833	1.0						
H 129	.001	.101	.001	.325	1.0					
H 132	.108	.224	.108	.108	.316	1.0				
H88L1	.337	.358	.237	.138	.197	.470	1.0			
L 113	.410	.436	.320	.230	.192	.430	.867	1.0		
L105	330	.347	.330	.163	.001	.217	.636	.769	1.0	
Line153	230	.237	.140	.050	.192	.430	.867	.890	.859	1.0

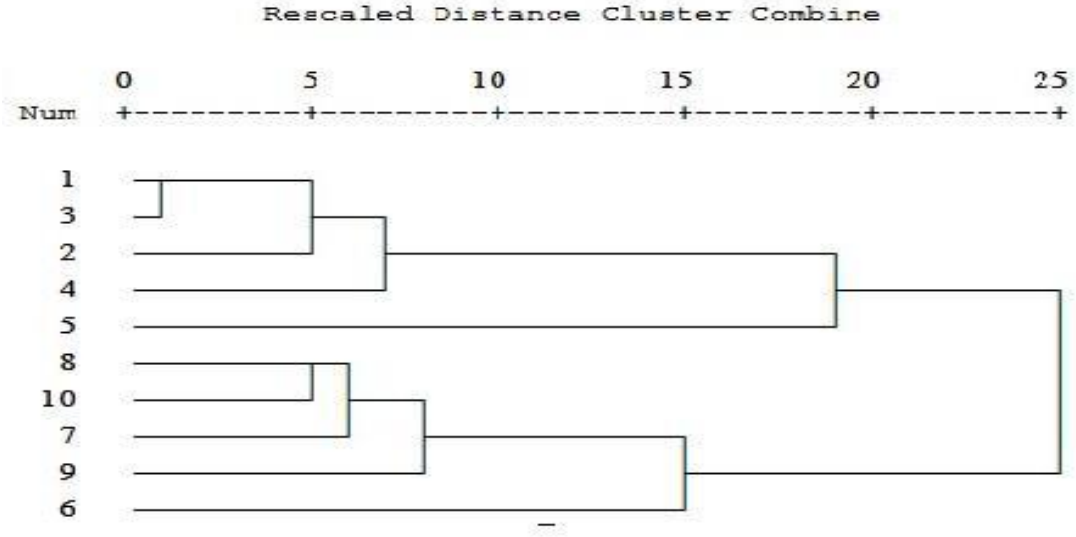

Fig. 2: Dendogram showing relative genetic distances among soybean genotypes, constructed using UPGMA based on 5 RAPD primers, where (1= Giza 22, 2= H30, 3= Giza 111, 4= Crawford, 5= H 129, 6= H 132, 7= H88L1, 8= L 113, 9= L105and10= Line153).

The noticeable unique bands generated by primer OPW-04 was identified at 1154 bp with genotypes H129 and two unique bands 306 and 353 bp with genotype H132 , this result are typical as their pedigree and indicate the relative genetic distance as shown in Table 5, Table 6, Fig.1 and Fig.2 .

Many investigators recommended the use and application of RAPD analysis as rapid and more powerful method to identify and characterize different plant species and cultivars. RAPD markers were used to detect genetic differences between soybean cultivars and to map major genes and quantitative traits loci Liu *et al*., 2000; Csanadi *et al*., 2001andFahmy&Shadia, 2002.

It can be concluded that RAPD markers are suitable method to assess genetic diversity between the ten soybean genotypes. Their genetic distance determined by RAPD markers may help to identify suitable germplasm for introgression into breeding stocks. These results agree with pedigree genotypes and demonstrated the usefulness of these markers in estimating the extent of genetic variation in *Soybean* germplasm. The study was successful to achieve the more genotypes for seed yield and resistant insect and help the breeder for improving soybean breeding programme.

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