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BULKED SEGREGANT ANALYSIS FOR DEVELOPING GENETIC MARKERS OF DROUGHT TOLERANCE IN FABA BEAN (*Vicia faba* L.)

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A field experiment was conducted to study the response of two contrasting drought tolerant parents (L8 and L3), their F_1, F_2 and two comparative varieties (G716 and G461) to cultivation under natural rain conditions at Maryout region. Seed yield/plant and its components revealed significant differences among all genotypes for all traits recorded. The F_2 tolerant segregants group surpassed all other genotypes tested for seed yield/plant. This is attributed mainly to number of pods/plant and partially to seed index.

The results of protein markers indicated that parental drought tolerant line and F_2 tolerant segregant group had a specific band of 87KDa molecular weight which could be used to distinguish the drought tolerant genotypes among others, for instance F_1 cross (L3xL8) and Giza 461 gave the same band.

Acid phosphatase isozyme patterns didn't give clear cut markers for the discrimination between drought tolerant and drought sensitive genotypes. A positive band for drought sensitive genotypes as it was observed in F_2S and L8 sensitive parent. α-esterase (band No.6) and β-esterase (band No.5) isozyme patterns could be used to distinguish the drought sensitive genotypes among others.

Bulked- segregant analysis was used to analyze DNA extracts with RAPD-PCR technique. Of 42 random primers tested, only ten primers yielded informative data. Primers O2, O10, Z10 and Z20 gave a number of bands that could be used as positive or negative molecular markers. Primer O2 exhibited a band with 5905bp which appeared in the drought tolerant parental line (L3) and the tolerant F_2 population but absent in all other genotypes. Hence, it seemed to be a positive DNA-RAPD

marker for drought tolerance in faba bean. Primer O10 exhibited 3 bands with molecular size 1471bp, 1186bp and 977bp which were detected in the drought sensitive genotypes. Hence, they seemed to be negative DNA-RAPD markers for drought tolerance. Primer Z10 exhibited a band with molecular size of 1718bp only in L8, F_2S and G716 which seemed to be sensitive for the aimed abiotic stress but it was absent in all other genotypes studied. Consequently, it is considered as a negative molecular marker for drought tolerance.

The resulted dendrogram reveald three different genetic clusters. The first cluster includes the four genotypes;L3,F_1,G461 and F_2T. the second cluster includes the two drought sensitive genotypes; G716 and F_2S. The third cluster comprises the sensitive parental line L8.The results generated from SDS – protein and DNA-RAPD profiles employed in the present investigation were pooled for drawing the genetic relationships among the seven faba bean genotypes tested.

The use of RAPD markers appears to be a good choice for assessing genetic relationships than SDS – protein in faba bean with polymorphism levels sufficiently high to establish informative fingerprints. The primer OPZ3 was the most useful primer for identifying the tested genotypes.

Keywords: Faba bean, Drought tolerance, Bulked segregants, RAPD-PCR, Isozymes, SDS PAGE.

Faba bean, *Vicia faba* L. is the most important pulse crop cultivated in Egypt due to the high level of seed protein content. The importance of faba bean in Egypt lies not only in its multiple uses in preparing diverse local dishes but also in its important role in fixing atmospheric nitrogen. The crop is also used as animal feeding and green manure. Accordingly, there is a great need to increase its production by its expansion in newly reclaimed areas at Eastern and Western Coasts of Egypt.

The electrophoretic banding patterns of protein (SDS PAGE) have provided a good genetic marker that can be used in many genetic studies. Many reports accepted such method (Abdelsalam *et al.*, 1998; Afiah *et al.*, 1999-a and El-Rabey *et al.*, 2002 in barley; Afiah *et al.*, 1999-b in bread wheat, El-Saied and Afiah, 1998 in *Brassica sp.* and Afiah and Rashed, 2000; Hassan, 2001-b and El-Saied and Afiah, 2004 in legume crops.

Similarly, isozymes markers have greatly facilitated research in many biological branches such as taxonomy, phylogenetic relationships and biochemical genetics. Isozymes polymorphism have been used successfully

by many authors to identify genotypes of various crops (Pontikis *et al.*, 1980).

Nowadays, polymerase chain reaction (PCR) based molecular markers have developed into controllable tools to analyze genetic relationships and genetic diversity using random amplified polymorphic DNA-RAPD (Williams *et al.*,1990; Tinker *et al.*, 1993; Gonzaler and Ferrer,1993). So, RAPD analysis has been used for *Brassica* (Demeke *et. al.*, 1992), *Oryza* (Yu and Nguyen, 1994; Mackill, 1995), *Triticum* (Vierling and Nguyen, 1992; Chadrashekhar and Nguyen, 1992; Abdel-Tawab *et al.*, 2003) and cotton (El-Kady *et al.*, 2006). Moreover, RAPD analysis has been used earlier for genetic diversity analysis for Egyptian barley cultivars (Abdelsalam *et al.*, 1998 and El-Halfawy *et al.*, 2006). At the same time, technical simplicity and speed of RAPD methodology is a principal advantage (Gepts, 1993).

This investigation based on the study of genotypic performance of the tested varieties / lines under stress conditions and comparing them with genetic distance estimated from RAPD markers. The aim of this study is extended to find the relationships between some yield-related traits and drought tolerance in seven faba bean genotypes. Also to obtain reliable molecular markers for drought tolerance that can be used in breeding programs.

MATERIALS AND METHODS

Field experiment was conducted to study the response of two contrasting parents (L8 and L3), their F_1, F_2 and two comparative Egyptian varieties (G716 and G461) to cultivation under rainfed conditions at Maryout Agricultural Experiment Station of Desert Research Center (DRC) during 2004/2005 growing season. Name, origin and pedigree of the parental genotypes are illustrated in table (1). These genetic materials are obtained through the DRC faba bean breeding program (Afiah and Abdel-Aziz, 2003). Soil of Maryout research station is characterized as sandy loam, slightly saline (EC 3.3 dSm^{-1}), calcareous (34% $CaCO_3$) with pH 7.8 and 0.84% organic matter. The analysis of biochemical and molecular genetic markers were done in Ain Shams Center of Genetic Engineering and Biotechnology (ACGEB) belongs to Ain Shams University, Cairo, Egypt.

Faba bean genetic materials were grown under rainfed conditions with one supplemental irrigation at sowing date (25/10/2004). The total rainfall precipitation was 175 mm during the growing season with a good distribution (Table, 2). The experiment was laid out in randomized complete blocks design with three replicates. Each parental line, F_1, F_2 and each comparative variety were distributed in 2, 1, 5 and 2 rows, respectively. Each row was 4 m long, 60 cm width and 20 cm from plant to plant.

TABLE (1). Name, origin and pedigree of the two contrasting drought tolerant faba bean parental genotypes as well as the two comparative Egyptian varieties.

G.	Name	Origin	Pedigree
L8	ILB 3879	Canada	ILB 3879
L3	L 82009-3	ICARDA*	A2/ILB1179
G 461	Giza 461	Egypt	G.3/ILB938
G.716	Giza 716	Egypt	461/842/83x50/455/83

* ICARDA: International Center for Agricultural Research in The Dry Areas.

TABLE (2). Rainfall precipitation (mm) in 10 days intervals during 2004/2005 growing season at Maryout station, Alexandria governorate.

Month	Oct. 2004	Nov. 2004	Dec. 2004	Jan. 2005	Feb. 2005	Mar. 2005	April 2005	May 2005	Grand total
1st ten days	0	0	1.5	26.5	9.5	21	0	0	58.5
2nd ten days	0	10	10	39.5	12.5	0	2	0	74
3rd ten days	0	33	2.5	1	0	0	6	0	42.5
Total	0	43	14	67	22	21	8	0	175

Before flowering stage (after 60 days from sowing) composite leaves sample from ten plants were taken from each parental line, F_1 and each comparative variety. While 240 F_2 plants were divided into ten groups on the base of growth parameters. From the two extreme groups, single plant leaves sample was taken and kept until harvest and seed yield/plant as well as its components were measured. The highest and lowest ten plants were chosen for mixing their leaves samples and shared the other five composite samples (2 parental lines + 1 F_1 + 2 comparative varieties) in lab. for RAPD analysis and total soluble protein SDS-PAGE.

At harvest, twelve guarded plants were randomly collected from each of the parental lines, their F_1 and comparative varieties together with ten plants of each of the two extremely F_2 bulked segregant groups from each replicate for recording pods/plant, seeds/pod, seed index and yield/plant.

Protein Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed in 10 % acrylamide slab gels following the system of Laemmli (1970). Gels were photographed, scanned and analyzed using Gel Doc 2000 BioRad system.

Isozymes electrophorasis

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted using three isozymes systems. Fresh young leaf samples were used for isozymes extraction. The studied isozymes are acid phosphatase (Acph) and α- and β-esterase (α- and β- *Est*). These isozymes were

separated in 10 % polyacrylamide gel electrophoresis according to Stegemann *et al.* (1985).

The gel was stained for esterase activity by incubation at 37 °C in a solution of 100 mg α-naphthyl acetate or β- naphthyl acetate (as a substrate) and 100 mg fast blue RR salt in 200 ml of 0.1 M phosphate buffer pH 6.5 (Scandalios, 1964).

DNA Extraction

From the field experimental site, young leaves of each genotype were randomly collected from ten plants and then one gram of ten leaves sample was treated with liquid nitrogen and transferred to ACGEB Lab. for DNA extraction according to the method of Welsh and Mc Cleland (1990).

DNA Amplification

The protocol for RAPD - PCR reaction was conducted using (10-base) oligonucleotide primers (Operon Technologies Inc., U.S.A) according to Williams *et al.* (1990) in the following sequences:

TABLE (3). List of operon primers and their nucleotide sequence:

Description	Primer sequence 5' → 3'
OP O 2	5-ACGTAGCGTC-3
OP O 4	5-AAGTCCGCTC-3
OP O 10	5-TCAGAGCGCC-3
OP O 15	5-TGGCGTCCTT-3
OP O 20	5-ACACACGCTG-3
OP Z 1	5-TCTGTGCCAC-3
OP Z 3	5-CAGCACCGCA-3
OP Z 4	5-AGGCTGTGCT-3
OP Z 10	5-CCGACAAACC-3
OP Z 20	5-ACTTTGGCGG-3

Gels were photographed under UV light and analyzed by gel documentation system. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

Statistical Analysis

The data collected from the growing season were subjected to the ordinary analysis of variance of the randomized complete blocks design on individual plant means basis. The effect of blocks and genotypes assumed to be fixed as outlined by Snedecor and Cochran (1981). Duncan's multiple range test (Duncan,1955) was used to verify the significance of mean performances for all traits recorded.

Genetic similarity was estimated according to Bardakci and Skibinski (1994). The banding patterns of bulked sampels were compared within and between genotypes tested. Bands were scored as 1 if present or 0 if absent. The index of similarity among genotypes was calculated using the formula.

$$S_{xy} = 2n_{xy} / (n_x + n_y)$$

Where: nxy is the number of bands shared by individuals x y, nx and ny are the number of detected bands scored for each genotype. According to Lynch (1990).

For constructing a dendrogram dealing with genetic relationships among genotypes tested, the data generated from RAPD markers were introduced to SPSS package program according to binary values (1 and 0). The output results involved both different unweighted pair-group method of analysis (UPGMA) and dendrogram was constructed according to Sokal and Sneath (1973).

Cluster analysis was based on a similarity matrix obtained with the un-weighed pair group method using arithmetic averages UPGMA (Rohlf, 1990) and relationships between accessions were illustrated as a dendrogram. All data were scored in the form of a binary matrix.

RESULTS AND DISCUSSION

Seed Yield/plant and its Components

Results on the mean performance of the studied faba bean genotypes are presented in table (4). Significant differences were detected among all genotypes for all traits recorded. The F_2 tolerant segregants group i.e. the most tolerant twelve F_2 plants surpassed all other genotypes in seed yield / plant. This superiority is attributed mainly to number of pods/plant and partially to seed index (100 seed weight) as shown in table (4). It is worthy noted that F_2 plants (240 individual plants) were classified into groups according to their vegetative behavior until flowering date under rainfed condition of the experimental site. Furthermore, 12 F_2 plants which representing the most drought tolerance (gave the best seed yield attributed to one or more of its components) and 12 F_2 sensitive one's were selected; means of all traits recorded among them are illustrated in table (4). Number of pods/plant ranged from 14.45 in L8 to 25.3 in F_2T. The later population (F_2T) exhibited 70.8g for seed index with insignificant difference under the highest value (71.55g) of F_1 hybrid. L3 and the F1 cross (L3xL8) showed the highest number of seeds/pod. These findings are in harmony with those previously obtained by Omar *et al.* (1998), El-Hosary *et al.* (2002), Omar (2003) and Afiah and Abdel-Aziz (2003).

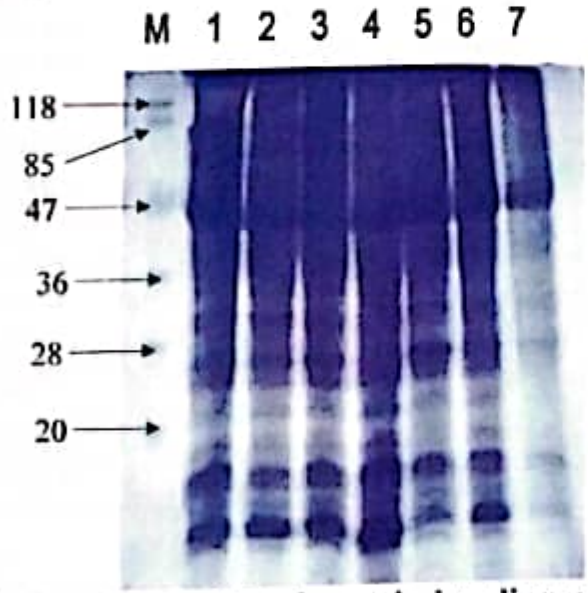
TABLE (4). Seed yield/plant and its components of the two parental lines, F_1, two bulked segregants of F_2 and two comparative varieties.

Genotype	No. of pods/plant	No. of seeds/pod	100 seed weight (g)	Seed yield/plant (g)
L8	14.45 f	2.38 d	66.15 d	23.8 f
L3	18.6c d	2.91 a	68.32 b	33.4 c
F_1	21.5 b	2.84 a	71.55 a	38.7 b
G 461	16.72 e	2.65 b	67.9b c	29.86 d
F_2T	25.3 a	2.6 b	70.8 a	43.75 a
F_2S	17.61 de	2.45 c	64.11 e	25.68 ef
G 716	16.58 e	2.41cd	66.89 cd	26.32 e

Means within columns followed by the same letter (s) are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

The SDS-PAGE for Total Protein in Fresh Leaves

The SDS-PAGE for total protein in leaves was carried out for the seven faba bean genotypes as illustrated in fig. (1). Bands with different molecular weights (MW) were detected in the different genotypes and ranged from about 5 KDa to 46 KDa. The total number of bands among genotypes ranged from 7 in Giza 716 to 9 in F2 drought sensitive segregant groups.



Lane M: is the standard SDS marker
Lanes 1-7: are F_1, L8, L3, F_2T, G.461, F_2S and G.716

Fig. (1). SDS-PAGE of protein banding patterns of the seven faba bean genotypes leaves tested under rainfed conditions.

TABLE (5). SDS-PAGE of protein banding patterns of the seven faba bean genotypes leaves tested under rainfed conditions.

Band No.	MW (KDa)	L8	L3	F_1	G.461	F_2T	F_2S	G.716	Rf
1	116	-	-	-	-	-	+	-	0.061
2	87	-	+	+	+	+	-	-	0.088
3	76	-	-	-	-	-	+	-	0.101
4	60	+	-	-	-	-	-	-	0.126
5	50	+	+	+	+	+	+	+	0.155
6	40	+	+	+	+	+	+	+	0.359
7	30	+	+	+	+	+	+	+	0.473
8	24	+	+	+	+	+	+	+	0.563
9	15	+	+	+	+	+	+	+	0.706
10	10	+	+	+	+	+	+	+	0.788
11	5	+	+	+	+	+	+	+	0.874
Total No. of bands		8	8	8	8	8	9	7	

MW: Molecular weight

The results showed three unique polymorphic bands which can differentiate the genotypes L8 by one band of a molecular weight (MW) of 60 KDa and F_2S by two bands of 76 KDa and 116 KDa MW. Also, the results indicate that there are seven monomorphic bands with a relatively low molecular weights ranging from 5 KDa to 50 KDa while the remainders are polymorphic bands with a percentage of 36.4%. The results also indicate that parental drought tolerant L3 and F_2 tolerant segregant group have a specific band of 87 KDa molecular weight which can be used to distinguish the drought tolerant genotypes among others. For instance, F1 cross (L3xL8) and Giza 461 gave the same band with about 87 KDa molecular weight. The former results are in line with those previously obtained by Afiah *et al.* (1999, a and b), Afiah and Rashed (2000), El-Saied and Afiah (2004) and Abou-Deif *et al.* (2005).

SDS-PAGE data of total protein (in leaves) were applied to the computer SPSS (Version 10) program to get dendrogram for genetic distances and similarity matrix as shown in fig. (2) and table (6). The results revealed that the highest estimate of similarity coefficient was 0.968 between Giza 461 and L3xL8 F_1 hybrid while, the lowest value was 0.815 between the parental line no.3 and F_2 sensitive plants. Therefore, protein system could discriminate between the seven faba bean genotypes tested. Similar results were earlier obtained by Abdel-Tawab *et al.* (2001) who reported that the protein electrophoretic pattern considered as a useful tool for identification of maize inbred. Also, Hassan (2001-b) reported that slight polymorphism was observed among protein patterns in mung bean cultivars.

A dendrogram was constructed from the combined data of protein SDS-PAGE (Fig. 2). The dendrogram was again separated the drought tolerant parental L3 on a genetic distance of 0.25 and the tolerant F_2 segregant group on 0.21 genetic distance. The dendrogtam further devided

the five remained genotypes into two main clusters, A and B where, the three drought sensitive genotypes (L8, G.716 and F2 sensitive population) fell in cluster B which further separate L8 in sub-cluster on 0.06 genetic distance. The remaining two drought tolerant genotypes (G.461 and F1 cross between L3xL8) fell in cluster A with a genetic distance of 0.01 (Fig. 2) and a very close similarity coefficient of 0.968 as shown in table (6).

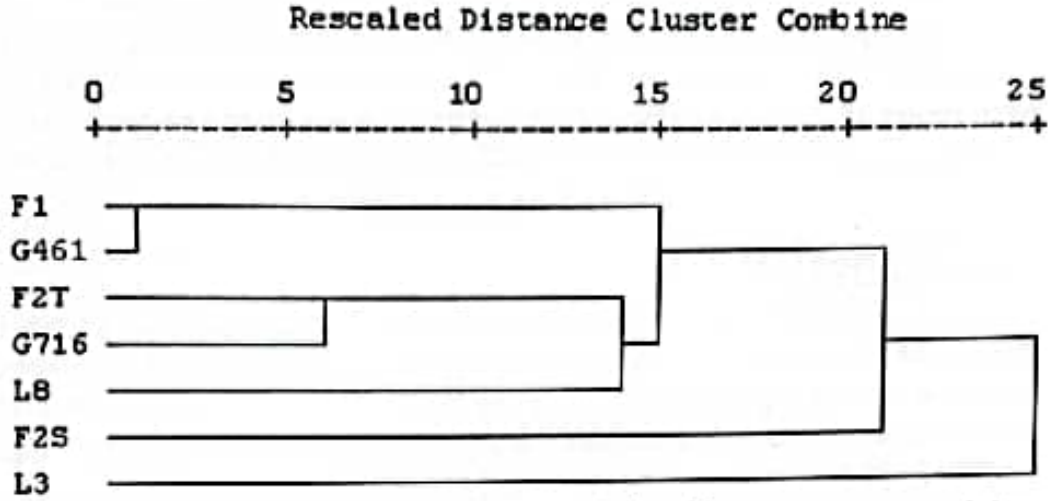


Fig. (2) Dendrogram demonstrated the relationship among seven faba bean genotypes under rainfed conditions of Maryout, North Western Coast of Egypt based on protein patterns.

TABLE (6). Matrix of the genetic similarity estimates of total protein banding patterns among seven faba bean genotypes under rainfed conditions of Maryout, North Western Coast of Egypt.

Genotype	L8	L3	F_1	G.461	F_2T	F_2S
L3	.877					
F_1	.915	.933				
G.461	.915	.897	.968			
F_2T	.915	.897	.933	.933		
F_2S	.915	.815	.897	.897	.897	
G.716	.933	.915	.915	.915	.951	.915

Isozyme Electrophorasis

Acid phosphatase together with the two enzymes system (α and β esterases) were studied in leaves of faba bean genotypes.

1-Acid Phosphatase (Acph)

Table (7) and figs. (3 a and b) show the banding pattern of acid phosphatase for genotypes under study. The resulted Acph patterns of all studied sampls revealed a high polymorphism in band's number between the studied genotypes. Differences in band's intensity were also noticed between and within all the studied genotypes. A total of four bands were identified for the studied samples, where three bands were scored as polymorphic

bands and one monomorphic band. Bands no. 1 and no. 2 were detected in F_1 and F_2S (F_2 sensitive population). On the other hand band No.2 was detected in all genotypes exept G461 while, band No.3 was a monomorphic band. The two bands 2 and 3 were more intense in F_1 and F_2S. This may indicate that the activity of Acph isozymes increased in F_1 and F_2S compared with other genotyps.

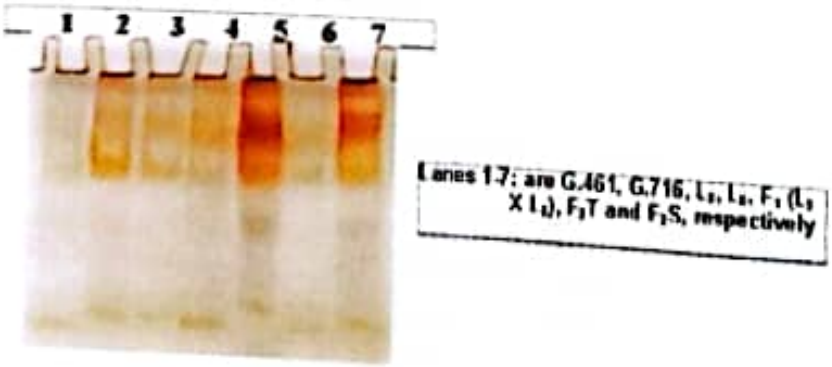
Acph isozymes patterns didn't give clear cut markers to allow discrimination between drought tolerant and drought sensitive genotypes in this study. These results are in agreement with Foolad and Jones (1993) who reported negative association between some markers loci and salt tolerance in tomato and Sayed (2004) who reported that Acph isozymes patterns didn't give clear-cut markers for the tolerant or sensitive genotypes of alfalfa.

2-Esterases (Est.)

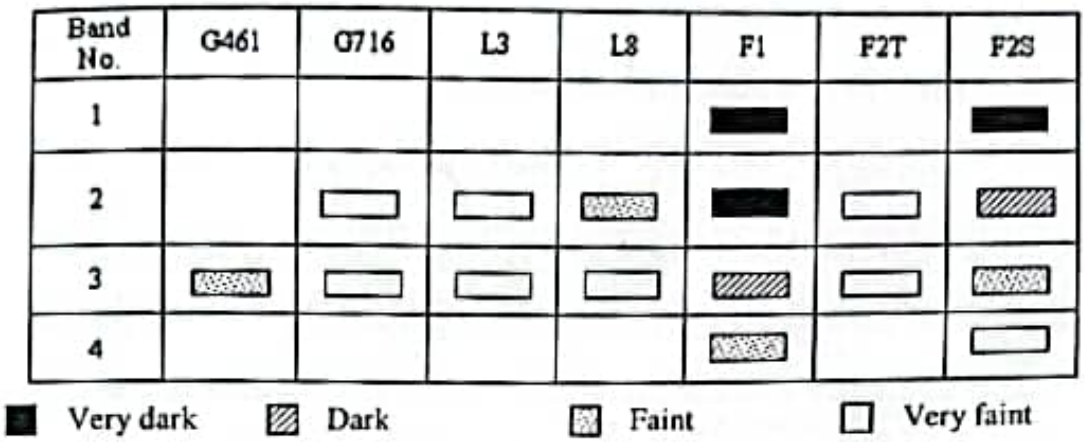
Est. is a gene family controling enzymes that hydrolyze ester bond in liped to produce plant energy for biochemical reactions. The data included in the present work were obtained by using two different substrates; α-naphthyl acetate and β- naphthyl acetate. Both α-est. and β-est. exerted highly polymorphic patterns among the studied genotypes.

2.1- α-esterase

studies on α-esterase patterns of the present genotypes reveald a high polymorphism among them. Differences in band's intensity were also noticed between and within the studied genotypes. Figs. (4 a and b) and table 8 show the α-esterase electrophoretic patterns of the two comparative Egyptian varieties (G.461 and G.716), two contrasting drought tolerant parents (L3 and L8), F1 cross and the two F_2 bulked segregants. A total of seven bands could be identified for the studied genotypes which were present in some samples and absent in others and were scored as polymorphic bands. The most important polymorphic band was band No.6 which was scored as a positive band for drought sensitive genotypes since it was observed in F_2S and L8 sensitive parent. For instance F_1 gave the same band.



(a)



(b)

Fig. (3). Electrophoretic pattern (a) and diagram (b) of acid phosphatase isozymes for the seven faba bean genotypes tested under rainfed conditions

TABLE (7). The presence (+) and absence (-) of bands in acid phosphatase isozyme profiles for the seven faba bean genotypes tested under rain fed conditions

Band No.	G 461	G 716	L3	L8	F_1	F_2T	F_2S
1	-	-	-	-	+	-	+
2	-	+	+	+	+	+	+
3	+	+	+	+	+	+	+
4	-	-	-	-	+	-	+

TABLE (8). The presence (+) and absence (-) of bands in α- esterase isozyme profiles for the seven faba bean genotypes tested under rainfed conditions

Band No.	G.461	G.716	L3	L8	F_1	F_2T	F_2S
1	-	-	+	+	+	-	+
2	-	-	+	+	-	-	+
3	+	-	+	-	-	+	+
4	-	+	+	+	+	+	-
5	+	-	-	-	-	-	+
6	-	-	-	+	+	-	+
7	-	-	-	-	+	+	+

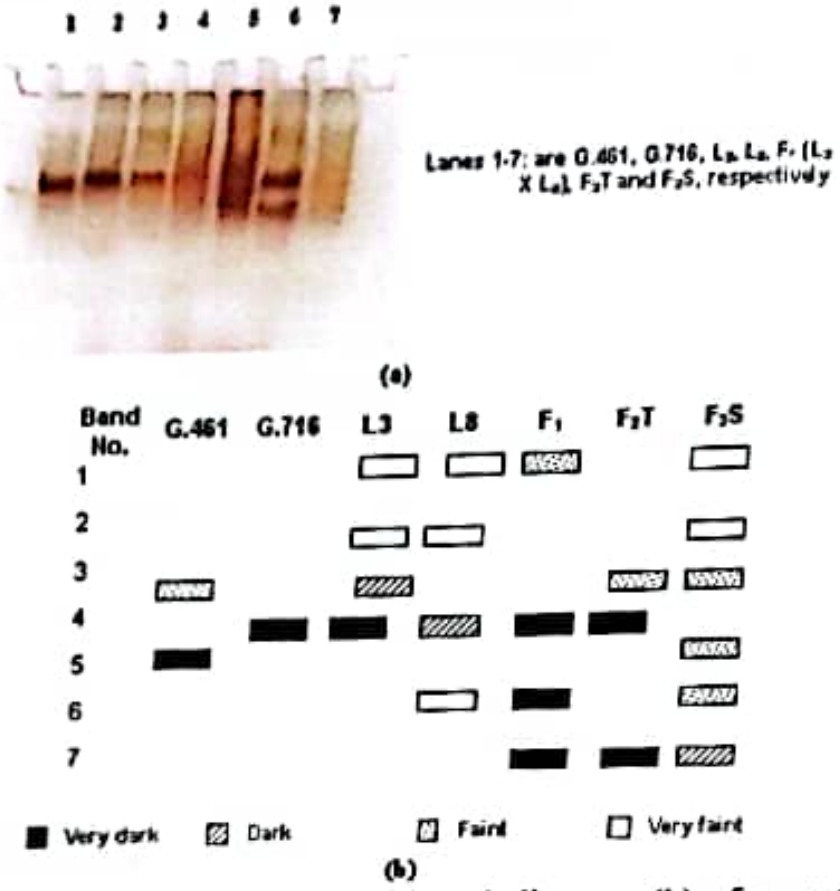


Fig. (4). Electrophoretic pattern (a) and diagram (b) of α- esterase isozymes for the seven faba bean genotypes tested under rainfed conditions.

2.2- β-esterase

Figs. (5 a and b) and table (9) show β-esterase electrophoretic patterns of the two comparative Egyptian varieties (G.461 and G.716), two contrasting drought tolerant parents (L3 and L8), F_1 cross and the two F_2 bulked segregants. Generally, β-naphthyl acetate exerted highly polymorphic patterns among the studied genotypes. The patterns have varied in both band's number and intensity among the studied genotypes. The obtained patterns exhibited a maximum no. of 10 bands, all of which were polymorphic. Bands No. 2, 4, 6 and 9 were scored as unique bands appeared in F_1, G.716, F_2T and F_2S, respectivly. Band No. 5 was detected in the L8 sensitive parent and in F_2S population and thus could be used to distinguish the drought tolerant genotypes among others. For instance, F1 cross (L3xL8) and Giza 716 gave the same band. However, Abbott *et al.* (1992) and Weising *et al.* (1995) detected genetic diversity for esterases in Legumenaceae members, which agreed with the obtained results. On the other hand, Sayed (2004) reported that esterase isozyme patterns didn't give

cosistent markers to rely on for the discrimination between tolerant and sensitive F_2 under stresses.

TABLE (9). The presence (+) and absence (-) of bands in β - esterase isozyme profiles for the seven faba bean genotypes tested under rainfed conditions

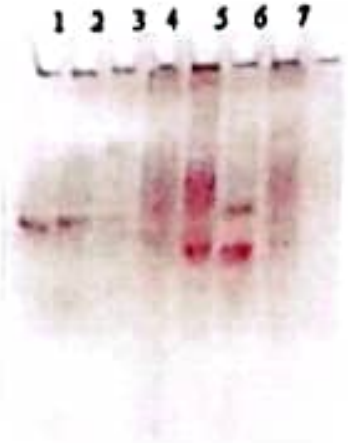
Band No.	G.461	G.716	L3	L8	F_1	F_2T	F_2S
1	-	-	-	+	+	-	+
2	-	-	-	-	+	-	-
3	-	-	+	-	-	+	+
4	-	+	-	-	-	-	-
5	-	+	-	+	+	-	+
6	-	-	-	-	-	+	-
7	+	+	+	+	-	-	-
8	-	-	-	+	+	+	+
9	-	-	-	-	-	-	+
10	-	-	-	-	+	+	-

RAPD Molecular Markers for Drought Tolerance via Bulked Segregant Analysis

In this regard , DNA isolated from the two contrasting parental lines (ten individual plants) of the tolerant parent (L3) and the sensitive one (L8), subsequent F_1, bulks of the two F_2 segregant groups (composite sample of 12 plants for each) as well as the two comparative Egyptian varieties. Of 42 random primers tested, only ten primers yielded informative data. DNA's of the tested genotypes were amplified against five 10-mer O random primers (OP O2, 4, 10, 15 and 20) as well as five Z one's (OPZ1, 3, 4, 10 and 20) as illustrated in table (10) and Fig.s (6-a and b). bands of Banding pattern for each primer were scored as present (1) or absent (0). The total numbers of bands exhibited by each of the five OPO PCR reactions were 11, 19, 10,14, and 16 for O2, O4, O10, O15 and O20, respectively.

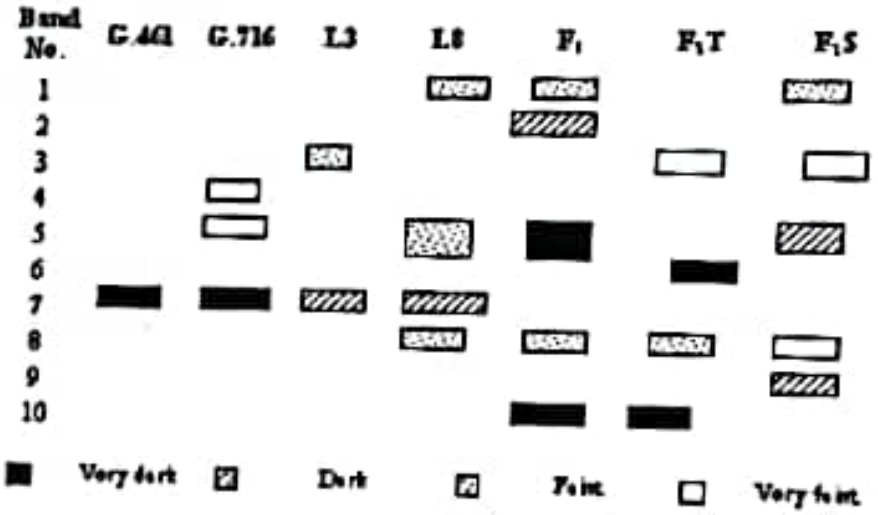
TABLE (10). Molecular size in base pairs (bp) of the amplified polymorphic (unique) DNA bands generated by the ten DNA random primers used.

OP	L8	L3	F1	G.461	F_2T	F_2S	G.716	Total No.
O2	5000	-	5228	7006	-	6788	5455	5
O4	5709-3140	-	-	-	-	-	-	2
O10	-	-	-	-	-	-	-	0
O15	1842	-	-	-	1177	-	-	2
O20	3422	-	-	-	-	1478-1414	-	3
Z1	5778	-	-	-	3112	-	-	2
Z3		-	-	-	3045-1013	-	3487	3
Z4	1504	-	-	-	-	-	-	1
Z10		-	-	-	-	-	-	0
Z20	3122	1429	881	3579-382	4048	3812-1576-531	5236-195	11



Lanes 1-7 are G.461, G.716, L_3, L_8, F_1 (L_3 X L_8), F_1T and F_1S, respectively

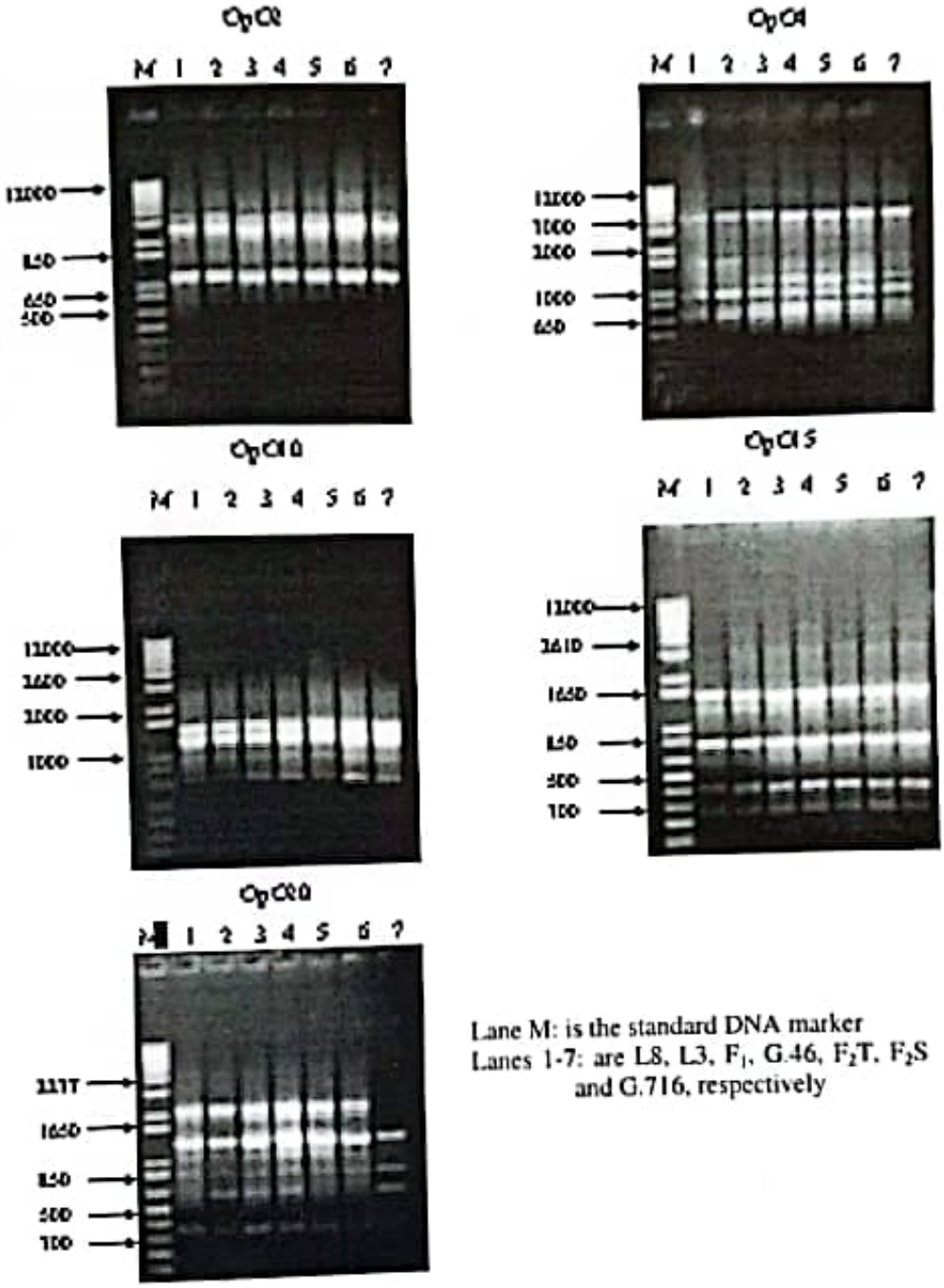
(a)



(b)

Fig. (5). Electrophoretic pattern (a) and diagram (b) of β - esterase isozymes for the seven faba bean genotypes tested under rainfed conditions.

A total of 29 polymorphic bands were scored as unique one's (Table 9). The number of unique bands varied depending on the primer employed .Such numbers were 5, 2, 1, 3, 2, 3, 1 and 11 across the primers O2, O4, O15, O20, Z1, Z3, Z4 and Z20, respectively. The size of amplified fragments ranged from 195 to 7006 bp across the operon primers used. This finding agreed with those previously reported by Abdelsalam *et al.* (1998), Hassan (2001-a) and Abdel-Tawab *et al.* (2003).



Lane M: is the standard DNA marker
Lanes 1-7: are L8, L3, F_1, G.46, F_2T, F_2S and G.716, respectively

Fig. (6-a). RAPD fingerprints of seven faba been genotypes using five random primers (O2, O4, O10, O15 and O20)

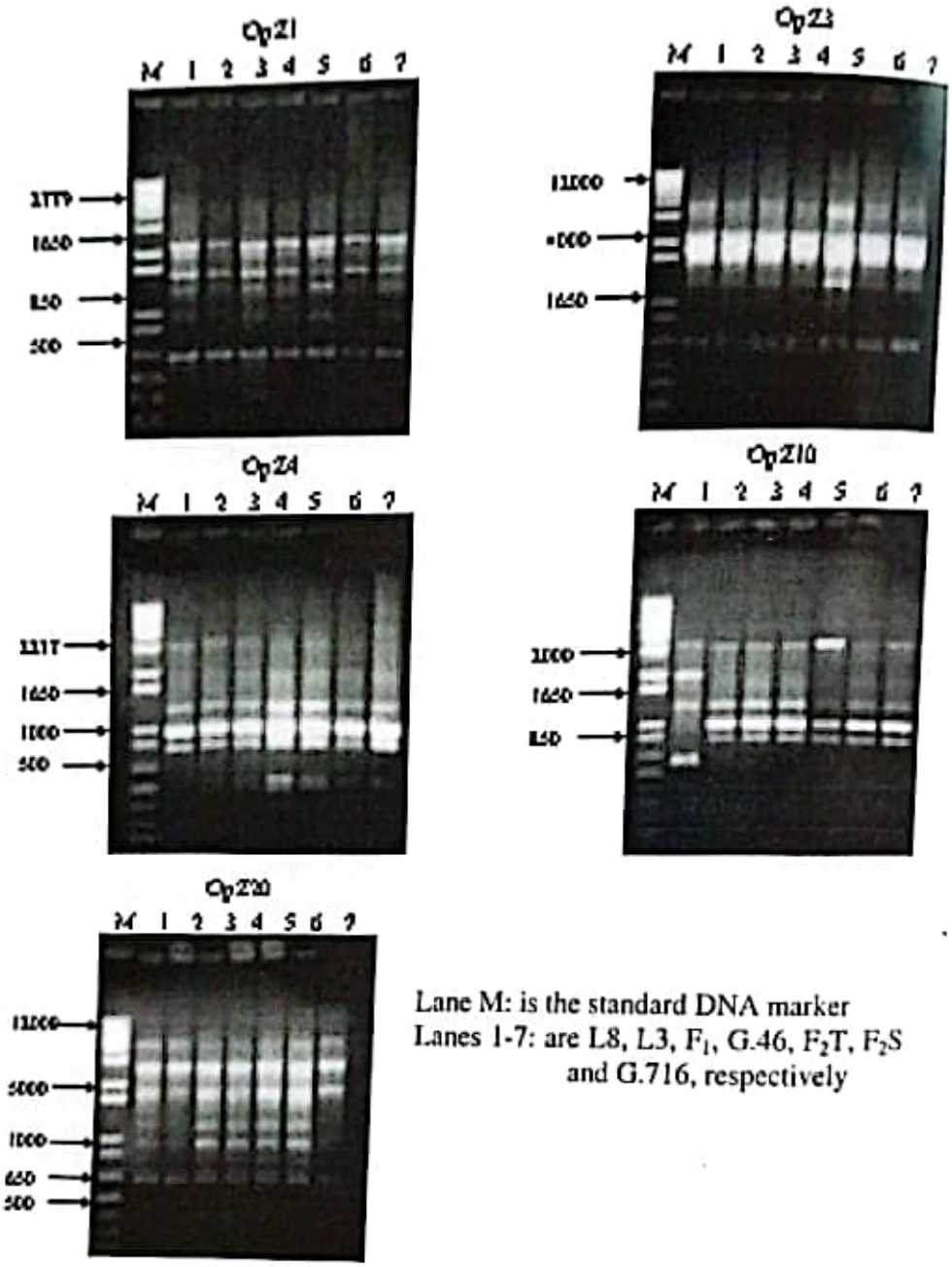


Fig. (6-b). RAPD fingerprints of seven faba been genotypes using five random primers (Z1, Z3, Z4, Z10 and Z20)

Dendrogram Based on RAPD-PCR

Genetic similarity matrix based on RABD-PCR data among the seven faba bean genotypes tested under rainfed conditions are presented in table (11). A maximum similarity of 0.618 and 0.518 were observed between F_1 plants and the tolerant comparative variety Giza 461 and between F_2S and Giza 716. Meanwhile, the lowest genetic similarity coefficient (0.294) was observed between the sensitive parental line (L8) and F_2 tolerant group which agrees with the diversity of their genetic makeup. Pair-wise similarities ranged from 0.294 to 0.618 with the mean value of 0.456 among *vicia faba* genotypes tested indicating the high level of polymorphism existing at their DNA level. These results agree with those previously obtained by Ajmone-Marsan *et al.* (1998), Yuan *et al.* (2000), Wouw *et al.* (2001) and Paris *et al.* (2003).

Cluster analysis based on similarity matrices using the un-weighed pair group method of arithmetic average (UPGMA) from RAPD-PCR data was performed. The relationships between the seven accessions were visualized as a dendrogram from each combination separately and finally a combined dendrogram was derived which summed up the final results as shown in fig. (7). Cluster analysis separated L8 from the seven genotypes tested with a genetic distance of 0.25 between them. It is warthy to note that, the dendrogram rescales the actual distances to numbers between 0 and 25, preserving the ratio of the distances between steps. The other six accessions were resolved into two main clusters with a genetic distance of 0.20 between them. The first cluster grouped the four drought tolerant genotypes (L3, F2T, F_1 and Giza 461) and was further devided into two subclusters; L3 and the three other genotypes which contain two sub sub-clusters i.e. F_2T and G.461 with F_1 in a genetic distance of 0.01 (Fig. 6). This means that Giza 461 (the drought tolerant comparative variety) and F_1 cross between (L8xL3) were closer in their similarity than the rest faba bean genotypes tested. The other cluster contained the two sensitive genotypes (Giza 716 and the F_2 sensitive population with a genetic distance of only 0.09. this would agree with the observed high estimate of genetic similarity coefficient 0.618 between the two genotypes (Table, 11).

TABLE (11). Matrix of the genetic similarity estimates of DNA banding patterns among the seven faba bean genotypes under rainfed conditions of Maryout, North Western Coast of Egypt.

Genotype	L8	L3	F_1	G.461	F_2T	F_2S
L3	.405					
F_1	.347	.487				
G.461	.347	.446	.618			
F_2T	.294	.426	.480	.480		
F_2S	.305	.377	.432	.432	.354	
G.716	.297	.405	.385	.385	.404	.518

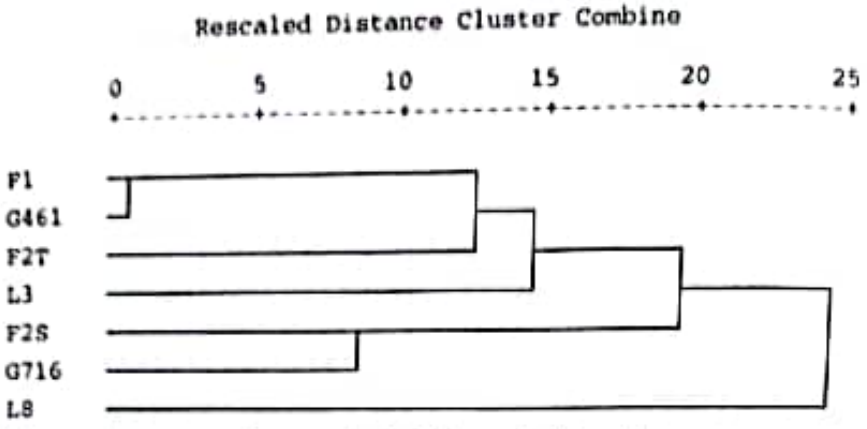


Fig. (7). Dendrogram demonstrated the relationship among seven faba bean genotypes under rainfed conditions of Maryout, North Western Coast of Egypt based on RAPD-PCR

The use of RAPD markers appears to be a good choice for assessing genetic relationships than SDS – protein in faba bean with polymorphism levels sufficiently high to establish informative fingerprints. The primer OPZ3 was the most useful primer for identifying the tested genotypes as shown in table (12).

The results generated from SDS – protein and DNA-RAPD profiles employed in the present investigation were pooled for drawing the genetic relationships among the seven faba bean genotypes tested. The similarity indices among the studied genotypes were estimated for each pair-wise group using SPSS version 10 computer program and the results are given in table (13).The constructed dendrogram tree is presented in fig. (8).

TABLE (12). Number, types and percentage of the total polymorphism generated by each of RAPD-PCR and total protein among the seven faba bean genotypes under rainfed conditions of Maryout, North Western Coast of Egypt.

Case		Monomorphic bands	Polymorphic bands		Total No. of bands	Polymorphism %
			unique	Shared		
RAPD-PCR	OP O 2	1	5	5	11	90.9
	OP O 4	2	2	15	19	89.5
	OP O 10	1	0	9	10	90.0
	OP O 15	4	2	8	14	71.4
	OP O 20	3	3	10	16	81.3
	OP Z 1	4	2	6	12	66.7
	OP Z 3	1	3	9	13	92.3
	OP Z 4	2	1	5	8	75.0
	OP Z 10	1	0	7	8	87.5
	OP Z 20	3	11	7	21	85.7
Protein		7	3	1	11	36.4

The resulted dendrogram reveald three different genetic clusters. The first cluster includes the four genotypes; L3,F_1,G.461 and F_2T. The second cluster includes the two drought sensitive genotypes; G.716 and F_2S. The third cluster comprises the sensitive parental line L8.

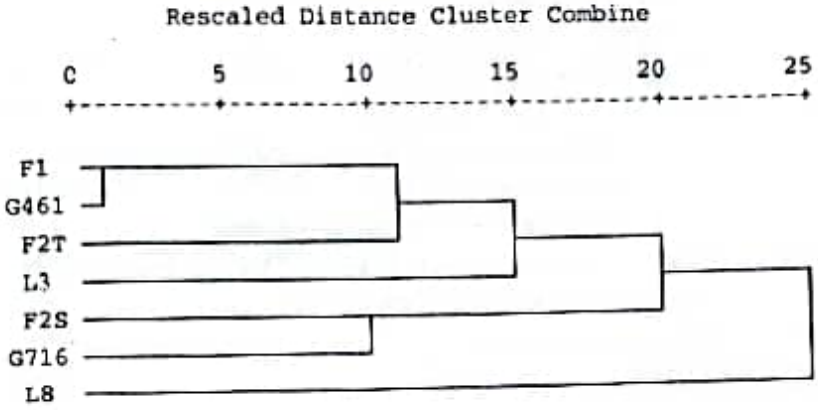


Fig. (8). Dendrogram demonstrated the relationship among seven faba bean genotypes under rainfed conditions of Maryout, North Western Coast of Egypt based on RAPD-PCR and protein banding patterns.

TABLE (13). Matrix of the genetic similarity estimates of total protein and RAPD-PCR banding patterns among seven faba bean genotypes under rainfed conditions of Maryout, North Western Coast of Egypt.

Genotype	L8	L3	F_1	G.461	F_2T	F_2S
L3	.799					
F_1	.781	.845				
G.461	.781	.820	.899			
F_2T	.753	.812	.845	.845		
F_2S	.758	.772	.816	.816	.781	
G.716	.758	.807	.799	.799	.816	.853

The highest similarity value (0.899) was observed between F_1 and G461 indicated that such two genotypes were closely related to each other. On the other hand, the lowest similarity value (0.753) was scored between the two contrasting genotypes L8 and F_2T. Emam *et al.* (2000) and Hassan (2001-b) reached more or less similar results in mungbean (*Vigna radiata* L.).

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تحليل ضم الانعزالات المتفارقة لاستنباط كاشفات وراثية لتحمل الجفاف فى الفول

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- أجريت تجربة حقلية لدراسة استجابة السلالتان الأبويتان L8 و L3 والجيل الأول والانعزالات المتفارقة للجيل الثاني وصنفين للمقارنة (جيزة ٧١٦ وجيزة ٤٦١) للزراعة تحت ظروف الزراعة المطرية السائدة في منطقة مريوط كما تم إجراء تحليل الكاشفات الوراثية البيوكيميائية والجزيئية بمركز الهندسة والتكنولوجيا الحيوية، كلية الزراعة، جامعة عين شمس.
- أظهر محصول البذور ومكوناته فروقا معنويه بين التراكيب الوراثية المستخدمة لكل الصفات المدروسة حيث فاقت مجموعة نباتات الجيل الثاني المتحملة للجفاف كل الأصناف الأخري في محصول البذور ويرجع ذلك لعدد القرون على النبات ودليل البذرة.
- أظهرت نتائج التفريد الكهربي للبروتين وجود الحزمة (87KDa) في L3 و F_2T وعلى ذلك يمكن استخدامها لتمييز التراكيب الوراثية المتحملة للجفاف.
- لم يظهر التفريد الكهربي لمشابهات إنزيم الفوسفاتيز الحامضي كاشفات محددة للتمييز بين التراكيب الوراثية المتحملة والحساسة للجفاف.
- أظهر نمط مشابهات الإنزيم α -esterase أن الحزمة رقم ٦ يمكن إعتبارها كاشفا موجبا للتراكيب الوراثية الحساسة للجفاف.
- وأظهر نمط مشابهات الإنزيم β- Esterase الحزمة رقم ٥ في L8 (الأب الحساس) و F_2S وبذلك يمكن استخدامها لفصل الأصناف الحساسة للجفاف عن غيرها.
- من تحليل الكاشفات الوراثية الجزيئية بإستخدام تقنية RAPD-PCR للتراكيب الوراثية المختبرة. ظهر أن عشرة فقط من مجموع ٤٢ بادئ عشوائي (خمسة بادئات OP Z Kit (Z1،Z3،Z4،Z10،Z20)، وخمسه OP, O Kit (O2،O4،O10،O15،O20) أعطت دلائل جزيئية هامة خاصة البادئات Z20،Z10،O4،O2 ويمكن استخدام نتائج تحليلها ككاشفات جزيئية موجبة أو سالبة.
- أظهر البادئ O2 حزمة (5905bp) والتي ظهرت في الأب المتحمل للجفاف (L3) ومجموعة نباتات الجيل الثاني المتحملة للجفاف ولكنها غابت في باقي الأصناف ولذلك يمكن استخدامها ككاشف جزيئي موجب.