J. Agric. Sci. Mansoura Univ., 29 (6): 3197 - 3207, 2004

GENETIC VERIFICATION OF SOME FABA BEAN DROUGHT TOLERANT GENOTYPES USING ISOZYMES

El-Saied, Fareida M. and S. A. Omar
Plant Genetic Resources Dept., Desert Research Center Cairo, Egypt.

ABESTRACT

Nine *faba bean* genotypes have been selected under different drought stress conditions throughout *faba bean* breeding program at F_5 generation. These genotypes were evaluated comparing with the commercial cultivar Giza-461 at F_6 generation for the performance of yield and its components and genetically verify for drought tolerance .

The obtained results from the green house experiment indicated that genotypes no.,4 , 5 and 7 gave the highest values for yield and its components. Moreover, the biochemecal genetic results verify that the genotypes no.4,5,7,8 and 9 gave the highest values for isozyme activities, either for isozymes accompanied with yield and its components or those associated with drought tolerance. Also, the results of isozyme banding patterns showed that genotypes no., 4 and 5 gave the highest gene expression for the genes associated with both drought or yield and its components.

Generally, it could be concluded that genotypes no., 4 and 5 were the best genotypes of faba bean breeding program, where gave the maximal productivity under drought conditions.

Keywords: Faba bean, Genotypes, Isozymes activity, Isozyme Electrophoresis , Drought condition.

INTRODUCTION

Faba bean (*Vicia faba L.*) is one of the most important leguminous crop in Egypt because its seeds have a valubale protein-rich food that has sustained large human populations, Okata and Okata (1998). The cultivated area of faba bean in Egypt reached about 270 thousand feddan Anonymous (2000). This area is now declined as a result of both biotic and abiotic stresses. Therefore, several efforts have been focused on developing high yielding faba bean genotypes with imporved tolerance to different stress conditions either traditional or biogenetical by using biochemical techniques.

Isozyme activity levels and isozyme banding patterns for the detection of genetic diversity among cultivers were successfully used. In this concern, many authors have been reported the potential value of isozymes as markers for genotype identification and characterization. Among these authors; Hussein and Salam (1984) Prestamo and Manzano (1993) and Hussein *et al.*,(2000 and 2001).

The objective of this study is to use isozyme activity levels and electrophoresis banding patterns to identify nine genotypes of faba bean and to compare them with the commercial cultivar as well as to search for isozyme markers which could be useful for genotype characterization in breeding schemes.

MATERIALS AND METHODS

PLANT MATERIALS

The genetic used in this investigation were selected from F_5 generations at faba bean breeding program which traced back to a dialle cross set involving seven parents of wide divergent origins of faba bean i.e.Giza-461, Giza Blanka, Giza-402, Giza-2 (as commercial cultivars belong to Agricultural Research Center (ARC), M-102, M-103 and M-127 (produced from faba bean breeding program at Dept.Agron.Fac.Agric.Moshtohor, Zagazig Univ., Egypt (El-Hosary,1989).

Nine faba bean genotypes have been selected under different drought stress conditions throughout faba bean breeding program at F_5 generations Table (1). These genotypes were evaluated comparing with the commercial cultivar Giza-461 at F_6 generations for the performance of yield and its components and to verify among them for drought tolerance.

Table (1): Pedigree of the used faba bean genotypes (Omar *et al.*, 1998).

Code number of genotypes	Cross and Pedigree
1	Giza-461 (commecial cultivar)
2	Moshtohor-127 (M-127)
3	Giza blanka x Giza -2
4	M-103 x M-127
5	Giza-461 x Giza-2
6	Giza-461 x M-103
7	Giza-461 x Giza blanka
8	Giza-402 x Giza-2
9	Giza -2 x M-127
10	Giza-461 x M-102

Experimental design:

The selected genotypes from the last F5 generations as well as the commercial cultivar Giza-461 were sown at 25 th November 2002 in a wire green house at plant Genetic Resources Dept. (DRC), using a randomized complete blocks design with three replications.Each polt consisted of four ridges (4 x 0.6 meter),hills were spaced at 25 cm.with one seed per hill on one side of the ridge. Data for yield and its components were recorded on individual plants chosen randomly from each polt.

Data analysis:

Data of the experiment were subjected to proper statistical analysis of variance according to Snadecor and Cochran (1967) for comparison between means, Duncan's multiple range test was used (Duncan 1955).

Genetic analysis:

The studied nine genotypes as well as the commercial cultivar were subjected to the following genetic analysis.

The Isozymes activity

The isozyme activities of 6-Phosphogluconate Dehydrogenase (6-PGH), Alcohol dehydrogenase (ADH) , Glutamate dehydrogenase (GDH) , Acid phosphatase (ACP), peroxidase (Prx..) and Esterase (Est.) were determined according to the methods of Torres and Diedenhofen (1976), Riesenberg and Soltis (1989), Dry (1985), Chance and Naehly (1955), Gillared and Dennind (1974) and Torre(1974), respectively.

Isozymes electrophoresis:

Six isozymes i.e., 6- Phosphogluconate Dehydrogenase (6-PGH) , Alcohol dehydrogenase (ADH),Glutamate dehydrogenase (GDH), Acid phosphatase (ACP), peroxidase (Prx..) and Esterase (Est.) were extracted from plant samples and polyacrylamide gel electrophoresis method was carried out according to the method described by Garkova *et al.*, (2000) . Technique for isozymes electrophoresis in 10genotypes of faba bean was summerized in the follwing Table (2).

Table (2): Technique for isozymes electrophoresis in ten genotypes of faba bean.

Isozymes	Electrode buffer.	Gel buffer	Staining
6-Phosphogluconate dehydrogenase (6-PGH)	65 mM L-histidine 15 mM citric acid	9 mM L-histidine	1
Alcohol dehydrogenase (ADH)	0.1M NaOH	15 mM Tris	2
Glutamate dehydrogenase (GDH)	65 mM L-histidine 15 mM citric acid	9 mM L-histidine	1
Acid phosphatase (ACP)	50mM Na acetate pH 5.0	50 m Na-α naphthyl acid phosphate	5
Peroxidase (Prx)	50 mL A.1M Na acetate , PH 4.7	3,3'5,5'-tetramethyl binzidine (TMBZ)	4
Esterase (Est.)	0.3 M boric acid pH 8.0	4 mM citric acid pH 7.8	3

Buffer systems are described by Soltis *et al.*, (1983).
** (1) Soltis *et al.*, (1983), (2) Torres (1974), (3) Torres and Diedenhofen (1976), (4) Graham *et al.*, (1964) and (5) Povercne *et al.*, (1988).

Gel analysis:

All gels resulted from six isozyme electrophoresis were scanned using Gel Doc-2001 Bio-Rad system. The densitometric scanning of the bands was performed on three direction characters. Each band was recognized by length, width and intensity. Accordingly, relative amount of each band quantity could be measured and scored.

RESULTS AND DISCUSSION

The analysis of variance for seed yield and its components for 10 faba bean genotypes are presented in Table (3).

Table (3): Mean squares for yield and its components of 10 faba bean genotypes at F_6 generation.

S.O.V.	d.f.	Characters			Seed weight g /plant
		No.of pods/plant	No.of seeds/plant	100seed/weight /g	
Rep.	2	18.88	5.707	0.709	20.112
Genotypes	9	69.07**	647.784**	94.415**	432.928**
Error	18	7.27	21.299	12.831	39.173

* and ** indicates significant at 0.05 and 0.01 levels of probability, respectively.

The results of the analysises variances analysis showed significant differences among genotypes which were detected for studied all traits in F_6 genertations. These results suggested that the comparison between genotypes should be made in order to determine the best performing genotypes under drought stress conditions. These results were completely coincide with those given by Omar (2003).

Data presented in Table (4) indicated the performance of 10 faba bean genotypes which were selected from F_6 generations as a best genotypes for yield and its components under rainfed conditions compared with the commercial cultivar Giza 461 (Omar, 2003).

Table (4): The mean performance of F_6 generation for yield and its components of 10 faba bean genotypes.

Genotypes	Characters			
	No.of pods/plant	No.of seeds/plant	100seed/weight/g	Seed weight g /plant
1	29.23 d	93.37 d	78.07 bc	68.54 cd
2	27.63 d	87.23 d	72.23 cde	62.89 d
3	28.13 d	87.73 d	69.87 de	63.05 d
4	41.90 a	122.4 a	68.10 e	85.80 b
5	38.33 ab	111.3 b	84.93 a	98.15 a
6	29.07 d	90.33 d	70.77 de	62.19 d
7	34.83 bc	72.37 e	82.83 ab	82.02 b
8	32.27 cd	102.8 c	75.57 cd	79.72 bc
9	32.23 bc	108.4 bc	71.33 cde	82.26 b
10	29.17 d	87.50 d	75.73 cd	69.68 cd

L -values followed by same letter (s) are not different at <0.05 of Duncan's . Multiple Range test .

Genotypes no., 4 and 5 gave the highest values for number of seeds / plant and seed yield as compared with genotype no., 1 (commercial cultivar ,Giza-461) .Whereas, the genotypes number 5 and 7 gave the highest mean values for 100/seed weight. These results indicated that genotypes gave the highest values for yield and its components had the best ability to seed productivity under any conditions and it could be used in program for improving and selection for yield and its components under different stress conditions especially drought stress. This finding is totally aline with those obtained by Omar (2003). He found that genotype number 5 had the highest tolerance (DSI-c) for all studied traits under different water regeme.

On the other side EL-Hosary *et al.*, (2002) reported that genotypes identified as stress tolerant using stress susceptibility index should possess

tolerant mechanisms which may need to be incorporated into germplasm with higher yield potential for development of high yielding and stress tolerant cultivars.

Table (5):The activity levels of different isozymes for the ten genotypes

Isozymes / Genotypes	6-PGH Unit g^{-1}	ADH Unit g^{-1}	GDH Unit g^{-1}	ACP Unit g^{-1}	PRX Unit g^{-1}	EST Unit g^{-1}
1	190 ± 5	288 ± 5	195 ± 30	193 ± 5	1000 ± 5	156 ± 4
2	180 ± 4	298 ± 7	199 ± 12	190 ± 4	995 ± 4	145 ± 5
3	183 ± 5	290 ± 5	189 ± 20	180 ± 3	998 ± 7	180 ± 4
4	250 ± 4	415 ± 8	325 ± 12	210 ± 9	1500 ± 7	190 ± 1
5	230 ± 3	392 ± 7	310 ± 20	190 ± 8	1300 ± 5	181 ± 6
6	177 ± 4	280 ± 4	199 ± 30	181 ± 5	1000 ± 4	140 ± 5
7	210 ± 2	375 ± 4	285 ± 10	187 ± 6	1200 ± 6	172 ± 4
8	200 ± 2	310 ± 2	213 ± 12	180 ± 3	1100 ± 5	163 ± 4
9	195 ± 4	308 ± 3	233 ± 12	165 ± 4	1100 ± 5	164 ± 2
10	182 ± 4	304 ± 2	210 ± 14	155 ± 3	995 ± 3	158 ± 3

The isozymes activity

Isozyme activity levels were used as biochemical markers for the characterization of faba bean genotypes .Data tabulated in Table (5) showed the activity levels of six isozymes in the ten genotypes of faba bean. The results showed that the highest values of isozymes activity were associated with the genotypes no. 4 and 5 followed by the genotypes no.7, 8 and 9.

Isozymes electrophoresis:

Six isozymes i.e., 6- Phosphogluconate dehydrogenase (6-PGH) , Alcohol dehydrogenase (ADH),Glutamate dehydrogenase (GDH), Acid phosphatase (ACP), peroxidase (Prx..) and Esterase (Est.) were used to identify the biochemical markers for each of ten faba bean genotypes to distinguish its identity and properties.

6- Phosphogluconate dehydrogenase (6-PGH):

Banding patterns of 6-PGH isozymes for 10 faba bean genotypes are illustrated in Table (6) and Figure (1).The results indicated that the total number of bands were four bands, among them one band was monomorphic (6-PGH-1) in all genotypes and the remainder bands were polymorphic with percentage of 75% Table (11). Gene expression of this isozyme was the best in three genotypes no., 4, 5 and 7 which have maximum band numbers (four band). While the lowest gene expression was noticed in genotype no., 10 which has a minimum band number (one band).

Table (6): Electrophoretic patterns of 6-PGH isozymes of ten genotypes of faba bean.

Genotypes 6-PGH	1	2	3	4	5	6	7	8	9	10
1	+	+	+	+	+	+	+	+	+	+
2		+		+	+	+	+	+	+	
3	+		+	+	+		+	+	+	
4				+	+		+			
Total	2	2	2	4	4	2	4	3	3	1

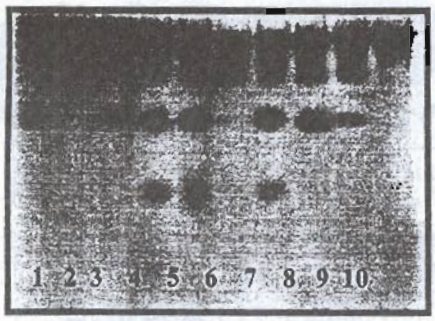

Figure (1) :(6-PGH)

Figure (2) :(ADH)

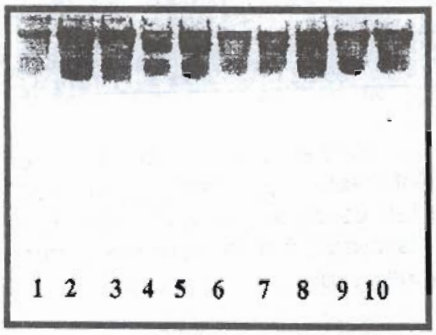

Figure (3) :(GDH)

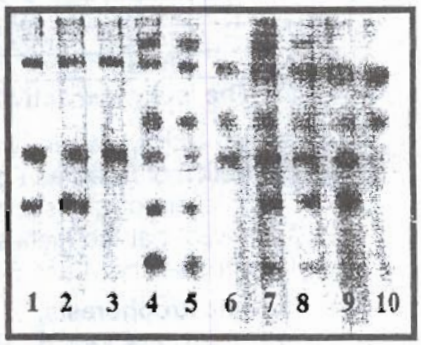

Figure (4): (ACP)

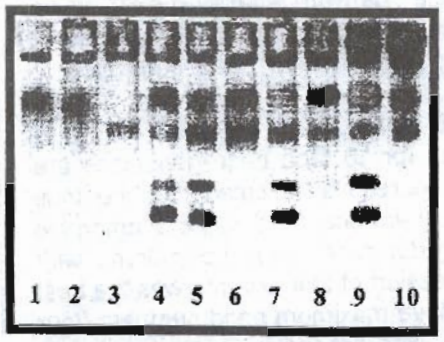

Figure (5): (Prx.)

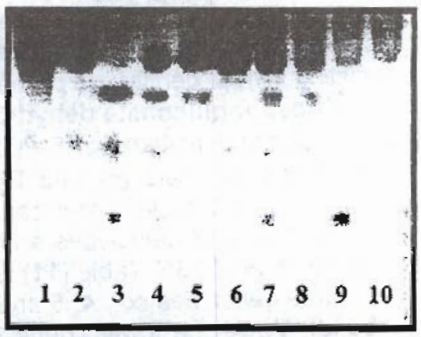

Figure (6): (Est.)

Figures (1-6): The electrophoresis patterns of six isozymes of ten faba bean genotypes .

1,2,3,4,5, 6,7,8,9 and 10 See Table (1)

Alcohol dehydrogenase (ADH):

Tables (7 and 12) and Figure (2) show the banding patterns of (ADH) isozyme for ten faba bean genotypes under study. The results revealed a high polymorphism with percentage of 80% in bands number between the studied genotypes.the differences in bands intensity were also noticed between and within all of the studied genotypes. Total of five bands were identified for the studied samples, which were present in some genotypes and absent in others, where band number 2 was detected in all genotypes as a common band and the other four bands were scored as polymorphic bands. The obtained results also revealed that band number 1 was present only in two genotypes no., 4 and 5 ,and could be distinguished among the other genotypes .Meanwhile band number 5 was present in four genotypes no., 4,5,7 and 8. On the other hand, the lowest gene expression was found in genotype no., 2 which has the minimum band number (one band).

Table (7) : Electrophoretic patterns of ADH isozymes of ten genotypes of faba bean.

Genotypes / ADH	1	2	3	4	5	6	7	8	9	10
1				+	+					
2	+	+	+	+	+	+	+	+	+	+
3	+		+	+	+	+	+	+	+	+
4	+			+	+		+	+	+	
5				+	+		+	+		
Total	3	1	2	5	5	3	4	4	3	2

Glutamate dehydrogenase (GDH):

Banding patterns of GDH for the faba bean genotypes are illustrated in Table (8) and Figue (3).This enzyme was identified by three bands among the studied genotypes. The second band was scored as a common band in all genotypes with differences in bands intensity. While, the remainder two band were distinguished for two faba bean genotypes no., 4 and 5 and could be considered as positive markers .

Table (8): Electrophoretic patterns of GDH isozymes of ten genotypes of faba bean.

Genotypes / GDH	1	2	3	4	5	6	7	8	9	10
1				+	+					
2	+	+	+	+	+	+	+	+	+	+
3				+	+					
Total	1	1	1	3	3	1	1	1	1	1

Acid phosphatase (ACP):

Acid phosphatase electrophoretic patterns of the ten faba bean genotypes are represented in Table (9) and Figure (4). This isozyme revealed high polymorphic level with percentage 83%, Table (12), where its pattern

gives six bands. Band number 2 scored as a common band in all genotypes. The results indicated that the highly expression in four genotypes no., 4 , 5 ,7 and 8 which they have six bands. While, the low gene expression with two

Table (9): Electrophoretic patterns of ACP isozyme of ten genotypes of faba bean.

Genotypes ACP	1	2	3	4	5	6	7	8	9	10
1				+	+		+	+		
2	+	+	+	+	+	+	+	+	+	+
3				+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	
5	+	+		+	+		+	+	+	
6				+	+		+	+		
Total	3	3	2	6	6	3	6	6	4	2

Peroxidase (Prx.) :

Banding patterns of Prx. isozymes for the ten faba bean genotypes are presented in Table (10) and Figure (5). A total of five bands were identified for the studied genotypes, which were present in some genotypes and absent in others. Band number 1 was scored as a common band in all genotypes .The remainder bands were polymorphic bands with percentage 80% Table (11) . The results indicated that bands number 4 and 5 were noticed in the genotypes no., 4, 5, 7 and 9 .It was obviously that band number 2 was in all genotypes except genotype no., 3 .Genotypes no., 1 and 2 expressed the same trend of gene expression and the same bands (two bands).

Table (10): Electrophoretic patterns of Prx. isozyme of ten genotypes of faba bean.

Genotypes Prx.	1	2	3	4	5	6	7	8	9	10
1	+	+	+	+	+	+	+	+	+	+
2	+	+		+	+	+	+	+	+	+
3			+	+	+	+	+	+	+	+
4				+	+		+		+	
5				+	+		+		+	
Total	2	2	2	5	5	3	5	3	5	3

Esterases (Est.):

Table (11) and figure (6) show the banding pattern of (Est.) isozymes for ten faba bean genotypes under study. The results indicated that a total number of four bands were identified for the studied genotypes, where, band number 3 was noticed in all ten genotypes as a common band and the three remainder bands were polymorphic with percentage 75%, Table (12). The result also revealed that the best gene expression was noticed with genotypes no. 4,5,7 and 9 which they have (four bands) as a maximum

bands . Meanwhile genotypes no., 1 and 2 performed a similar trend of the gene expression and the same of bands number were noticed (two bands) of these genotypes. On the other hand, band number 1 was present in all genotypes except genotype no., 3 and it could be considered as a negative marker to distinguish from the others.

Table (11): Electrophoretic patterns of EST isozyme of ten genotypes of faba bean.

Genotypes EST	1	2	3	4	5	6	7	8	9	10
1	+	+		+	+	+	+	+	+	+
2			+	+	+		+	+	+	
3	+	+	+	+	+	+	+	+	+	+
4			+	+	+		+		+	
Total	2	2	3	4	4	2	4	3	4	3

Table (12) : Number and types of bands as well as the percentage of the total polymorphism generated by six isozymes 6-PGH, ADH, GDH, EST, PEX and ACP in the ten genotypes of faba bean

Isozymes	Monomorphic Bands	Polymorphic		Total bands	Polymorphic %
		Unique	non-unique		
6-PGH	1	3	-	4	75 %
ADH	1	4	-	5	80 %
GDH	1	2	-	3	66 %
ACP	1	5	-	6	83 %
PEX	1	4	-	5	80 %
EST	1	3	-	4	75 %

Generally, from the foregoing presentation, the results of the isozyme electrophoretic banding patterns indicated that genotypes no.4 and 5 gave the highest gene expression for the isozymes accompained with yield and its components as well as the isozymes associated with drought resistance .

These genotypes were distinguished from the other genotypes and also the commercial cultivar Giza-461 through two positive markers with isozyme GDH and one positive marker with isozyme ADH . Furthermore, the results of electrophoretic banding pattern indicated a differences in gene expression either for the commercial cultivar or the studied genotypes, where genotype no.3 was distinct from the other genotypes by two negative markers which associated with isozymes,Prx. and Est.

Meanwhile genotypes no.,4,5,7 and 9 gave the highest gene expressions with Est. and Prx. Isozymes, whereas, genotypes no,.4,5,7 and 8 gave the highest gene expressions with ACP isozyme.Commenting on the overall results , it could be concluded that genotypers no., 4 and 5 were the best genotypes of faba bean breeding program where under drought condition gave the maximal productivity.

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التباين الوراثي لبعض التراكيب الوراثية من الفول البلدي المتحملة للجفاف باستخدام مشابهات الإنزيمات

فريدة محمد السعيد – سيد عبد السلام عمر
قسم الأصول الوراثية – مركز بحوث الصحراء – المطرية – القاهرة

تمت دراسة تسعة تراكيب وراثية من الفول البلدي تم انتخابها علي أساس مدي درجة تحملها للجفاف من الجيل الخامس ومقارنتها بالصنف التجاري (جيزة -٤٦١) وقد تم زراعتها في الصوبة السلكية بمركز بحوث الصحراء في موسم ٢٠٠٢/٢٠٠٣ لتقييم المحصول و مكوناته و دراسة التباين الوراثي لهذه التراكيب الوراثية باستخدام التفريد الكهربي لستة من مشابهات الإنزيمات المختلفة و المرتبطة بالمحصول والمقاومة للجفاف.

قد أوضحت النتائج وجود اختلافات معنوية بين التراكيب الوراثية المختلفة لصفات المحصول تحت الدراسة.و أظهرت التراكيب الوراثية رقم ٤ ، ٥ ، ٧ اعلي قيم لصفات المحصول تحت ظروف التجربة في الجيل السادس مقارنة بالصنف جيزة ٤٦١ و باقي التراكيب الوراثية الأخرى. كما أعطت هذه التراكيب أيضا بالإضافة إلى التراكيب رقم ٨ ، ٩ اعلي قيم لنشاط المشابهات الإنزيمية. أوضحت نتائج التفريد الكهربي أن التراكيب الوراثية رقم ٤ ، ٥ تعطى اعلي قيم للتعبير الجيني للإنزيمات المرتبطة بالجفاف و أيضا المرتبطة بالمحصول و مكوناته.

و لذلك تعتبر التراكيب الوراثية رقم ٤ ، ٥ من افضل التراكيب الوراثية بناءا علي سلوكها الوراثي في تحملها للجفاف واعطائها أعلى إنتاجية والتي يمكن الاعتماد عليها في برامج التربية لمقاومة الجفاف واستخدامها كآباء و كسلالات جديدة تلائم الزراعة تحت الظروف الجافية.