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IDENTIFICATION OF SOME FABA BEAN (*VICIA FABA* L.) VARIETIES USING ELECTROPHORESIS PROTEIN AND ISOZYMES.

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ABSTRACT

The objectives of this study were to identify ten Faba bean (Vicia faba L.) varieties using electrophoresis protein and some isozymes. The results of electrophoresis protein revealed that total number of bands ranged from 8 in variety G.643 to 12 in varieties G.461 and Sa.2. Three common bands were found in all varieties, which have molecular weight of 84.742, 51.620 and 38.554 kda. Some varieties showed specific bands which could be used to distinguish between others. Each of Sa.1, M.1, Nu.1, G.843 and G.717 varieties had only unique band, with molecular weight of 223.041, 192.156, 141.056, 90.412 and 10.421 kda, respectively. Band for MW of 30.746 kda is found in in the tested varieties except G.643, G.843 and Sa.1, which could be considered as a negative unique marker. Bands of three isozymes systems (peroxidase Prx, glutamate oxaloacetate transaminase GOT and esterase Est.) were determined for identification of faba bean varieties based on polyacrylamide gel electrophoresis. Using the number of bands and its Rf values can be used to identify these varieties. The number of GOT bands indicated that the varieties G.717 and Nu.1 contained only four bands .To distinguish between these varieties upon RF values of these bands which have 0.125, 0.281, 0.346, 0.408 and 0.118, 0.232, 0.285, 0.355, respectively. Esterase bands of various varieties indicated that the G.3 Im variety have seven bands only which led to define this variety from the others . Also, Nu.1 variety contained five bands. Peroxides bands of varieties demonstrate that G.717 variety contained 5 bands which characterize it from the others. While, the other varieties contained from 2 to 3 bands which were differ in Rf values and can distinguish between them depending on the difference of their Rf values.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important leguminous pulses cultivated over wide areas of the whole world used for human consumption as well as for animal feeding. Faba bean considered an important component diet for people in developing countries. They consumed in the form of immature tender green pods, green mature seed or as dry seeds after slow boiling cooking. In Egypt, Faba bean seeds are among the major nutritional source of plant proteins. Protein content of seeds is basically determined by genetic factors as sources for different raw materials. Modern varieties of faba bean have been developed for higher content of proteins

which represent significant improvement over earlier varieties. Biochemical genetic fingerprinting can be considered as a good tool for identification and genetic evaluation of the conserved material. This fingerprinting would be achieved by protein banding pattern or isozymes polymorphism. Therefore, biochemical genetic fingerprinting would satisfy both adequacy and accuracy for the identification of the conserved material, Furthermore, electrophoresis polyacrylamide gel continues to play a major role in the experimental analysis of protein .Polyacrylamide gel electrophoresis (PAGE) is still the most widespread from of the technique, since it offers sufficient resolution for most situations coupled with ease of use and the ability to process many samples simultaneously for comparative purpose (Hames,1990).

The protein banding pattern was efficiently used to identify genotypes. Isozymes are enzymes that convert the same chemical substrate, but are not necessarily products of the same gene. Isozymes are products of orthologous genes, but differ in composition by one or more amino acids due to allelic differences. Isozyme electrophoresis has been successfully applied to many organisms from bacteria to numerous animal and plant species since 1960s (May, 1992).Polignatno and Sonnante (1991) detected the glutamate isomerase (PG 1) isoenzymatic band patterns of nine faba bean population from Ethiopia and Afghanistan using the starch gel electrophoresis method. The Ethiopian Populations exhibited a high polymorphism for the GOT (E.C.2.6 1.1) system, whereas the PGI system was monomorphic for all the studied population

The polymorphism observed for GOT (E.C.2.6. 1.1) may allow the identification of variants useful for breeding and for future evaluation of (V faba L.) collections. Mudzana *et al.* (1995) analyzed seed storage protein composition by electrophoresis. Bulked seed samples were used to characterize seed proteins. Tests confirmed that an integrated system using the three methods was able to distinguish a subset of 12 varieties. Naguib (2000) carried out investigation to evaluate the biochemical identification and electrophoretic methods testes of seven faba bean varieties (Giza 402, Giza Blanka, Giza 716, Line 40/93,Triple white and Bakestsni). This study showed that the some proteins with different molecular weight characterized every faba bean genotype under investigation.

Therefore, electrophoretic analysis is considered an important tool for the identification of faba bean cultivars. Haider *et al.* (2001) carried out a study to determine the genetic diversity between faba bean and some related Vicia species. Thirteen taxa of wild germplasm representing six Vicia species (V. sativa, V. Villosa, V.monantha, V. narbonensis and V. cineria, in addition to V. faba) were used. Protein electrophoretic patterns indicated clear differences among different Vicia species as well as within the taxa of the same species. In all tested genotypes, protein patterns revealed polymorphism among the polypeptides with molecular weights lower than 45 kDa. Electrophoretic patterns indicated considerable variation among different Vicia species and also among different taxa of the same species. Data also showed that V. monantha and V.villosa had similar protein patterns, which are very close to V.cineria. V. faba showed the most distinct protein patterns differ from other wild species. Gadalla (2004) studied the SDS-PAGE for seed storage protein (water soluble) applied to study the genetic diversity of ten faba bean cultivars. The

maximum number of bands (27) appeared in Giza 643 and Giza 716 and the minimum number of bands (20) was detected in Giza 3 cultivars. Result revealed a total number of 32 bands with molecular weights ranging from about 11.00 to 91.00 k Da and the densitometry analysis of SDS-protein banding patterns were found to be useful in varietal identification of the studied faba bean cultivars.

MATERIALS AND METHODS

Varieties seed samples:

Seed samples of ten faba bean (Vicia faba L.) varieties were used in this study: {Sakha 1(Sa.1), Sakha 2(Sa.2), Nubaria 1(Nu.1), Masr 1(M.1), Giza 3 Im (G.3Im), Giza 461 (G.461), Giza 643(G.643), Giza 717(G.717), Giza 843(G.843) and Y. Elsedik (Y.S.) } . Samples were obtained from the Leguminous Crops Research Department (LCRD), Field Crops Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Methods of analysis:

A-The SDS-protein electrophoresis:

Protein extracts of seeds of various genotypes were identified by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970) as follows.

1-Preparation of samples:

Sample extract (400µl) equivalent to about 0.2 absorbance value per 10µl were added to 400µl sample buffer, thoroughly mixed and heated in a boiling water bath for 5 to10 min,then centrifuged at 1000 r.p.m. for 5 min.The supernatant was kept at 4 C for analysis. A known sample volume (20-30µl)was applied to each slot (0.5x1.6x.01cm).Air bubbles were avoided in order to obtain sharp separation

2-Qualitative and quantitative determination of protein bands:

The Rf values and approximate molecular weights used to determine the position of the protein bands. The gels were densitometrically scanned using color flatbed scanner (Espon GT 8000) connected with a computer and printer. The software was scan peack ll. The estimation of molecular weights of different protein bands was automatically done by the aid of a protein marker.

B-Isozymes electrophoresis:

Native polyacrylamide gel electrophoresis technique was used to identify the isozymes fingerprint of faba bean varieties such as esterase (EST), peroxidase (Prx) and glutamate oxaloacetate transaminase (GOT). Isozymes fractionation was performed on vertical slab (19.8cm x26.8cm x.02cm) using gel labconco electrophoresis apparatus according to Jonathan and wendel (1990)

RESULTS AND DISCUSSION

Protein electrophoresis [SDS-PAGE]:-

Electrophoresis assays have been widely used as a rapid and accurate testes to identify between the ten faba bean varieties. By the use of such appropriate and refined techniques, it is now possible to actually fingerprint each varieties to identity and its genetic potentialities.

Biochemical fingerprinting was developed for identification of the ten faba bean varieties under study. Poly- morphism among different varieties for the biochemical genetic level was scored in the present investigation by the absence and presence of any given band in one or more varieties due to the differential expression of genes governing such protein.

Protein banding patterns of the studied faba bean varieties as revealed by SDS-PAGE for the total seed protein are shown in Tables (1 and 2) and Figure (1) .Regarding the presented data, the total numbers of bands in all of the studied faba bean varieties were 26 bands. There was clear variation in the number of the obtained bands. The obtained total number of bands between varieties was ranged from 8 in variety G.643 to 12 in G.461 and Sa.2 varieties. From results, G.3 Im, G.843 and Nu.1 varieties contained similar number of bands (9 bands). Meanwhile, G.717, Y.S. and Sa.1 varieties showed similar number of bands (10 bands).Whereas M.1 variety had the highest number of band (11).

The molecular weight (MW) ranged from 223.041 kda in Sa.1 variety to 6.211 kda in G.3 Im. variety. The molecular weight of the least protein band was 6.22 kda in G3.Im variety, whereas, the highest protein band was 223.041 kda in Sa.1 variety. Also there are three common bands were found in all varieties, with molecular weight of 84.742, 51.620 and 38.554 kda. Some varieties contained specific bands which could be used to identify them among others. For example, each Sa.1, M.1, Nu.1, G.843 and G.717 varieties had only unique band, which have molecular weight, 223.041, 192.156, 141.056, 90.412 and 10.421 kda, respectively (Table 1). Meanwhile, band of 42.674 kda MW was found in all varieties except for G.717, G.843 and Y.S. varieties. Similarly, 30.746 kda band was found in all varieties except for G.643, G.843 and Sa.1 .Which could be considered as negative unique marker (NUM) . whereas, band with of 162.211 kda MW was present only in G.843 and Sa.2 varieties. Also, band with 151.152 kda MW was present only in G.461 and Nu.1 varieties. Meanwhile, band with MW 68.411 kda is present only in G.643 and G.843 varieties. Similarly, band with molecular weight 21.320 kda was present only in G.461 and Sa.1 varieties. Whereas, bands with molecular weight 119.540 and 55.854 kda are present only in varieties M.1, G.717, Y.S., G.643 and G.461. Bands MW about 124.123 and 92.581 kda. are present in 50% of studied varieties (M.1, G.3 Im, G.717, Sa.1, Sa.2 and M.1, G.717, Y.S., Nu.1, Sa.2), respectively. These obtained results could be considered as positive unique marker (PUM). Hence, the different varieties were also varied in molecular weights of their protein fractions.

Along the same line Goodrich *et al.* (1985) reported that polyacrlamide gel electrophoresis in the presence of (SDS-PAGE) was used to analyze the seed proteins of faba bean cultivars. The (R_f) and the MW were used to determine protein bands. The MW of seed protein bands had a greet value in distinguishing between faba bean cultivars. Salama (1988) reported that using Disc-electrophoresis to compare between seed proteins of some faba bean cultivars, where he developed a distinctive proteins band patterns. Broad bean gave 10-12 bands, while dry bean. The use of SDS gradient gel electrophoresis on the protein of some faba bean cultivars showed different molecular weight of the separated proteins. Kamel and El-Mashad (1999); Mohamed (1999) and Gadala (2004) found that SDS-PAGE electrophoresis could be considered as a good tool for identification and characterization of faba bean genotypes. The

presence or absence of certain bands and the concentration of each band present in each protein can be used as a criterion of identification. In addition, the fingerprint of electrophoresis pattern would be the best tool for cultivars identification.

Table (1): Molecular weight (MW) of SDS-PAGE protein electrophoresis and the presence or absent of bands for each studied faba bean varieties.

MW (Kda)	Varieties									
	M.1	G. 643	G. 3Im	G. 717	G. 461	G. 843	Y.S.	Nu.1	Sa.1	Sa. 2
223.041	-	-	-	-	-	-	-	-	+	-
192.156	+	-	-	-	-	-	-	-	-	-
182.541	-	-	-	+	+	-	+	-	+	+
162.211	-	-	-	-	-	+	-	-	-	+
151.152	-	-	-	-	+	-	-	+	-	-
141.056	-	-	-	-	-	-	-	+	-	-
132.211	-	+	-	-	-	-	-	-	-	-
124.123	+	-	+	+	-	-	-	-	+	+
119.540	+	-	-	+	-	-	+	-	-	-
101.006	-	-	+	-	+	+	-	-	-	-
92.851	+	-	-	+	-	-	+	+	-	+
90.412	-	-	-	-	-	+	-	-	-	-
84.742	+	+	+	+	+	+	+	+	+	+
76.623	+	-	-	-	+	-	-	-	+	+
68.411	-	+	-	-	-	+	-	-	-	-
55.845	-	+	-	-	+	-	+	-	-	-
51.62	+	+	+	+	+	+	+	+	+	+
42.674	+	+	+	-	+	-	-	+	+	+
38.554	+	+	+	+	+	+	+	+	+	+
30.746	+	-	+	+	+	-	+	+	-	+
26.777	-	-	-	-	-	+	+	-	-	-
21.320	-	-	-	-	+	-	-	-	+	-
18.058	+	+	+	+	-	-	+	-	-	+
10.421	-	-	-	+	-	-	-	-	-	-
7.846	-	-	-	-	+	+	-	-	+	+
6.211	-	-	+	-	-	-	-	-	-	-

(+) Band presence. (-) band absence.

Isozymes electrophoresis: -

The target of this investigation is to use three different isozymes systems (peroxides) Prx, esterase (Est) and glutamate oxaloacetae transamiase (GOT) for the identification of ten faba bean varieties under study based on polyacrylamide gel electrophoresis profiles.

1- Glutamate Oxaloacetate Tranaminase isozyme (GOT).

The glutamate oxaloacetate tranaminase (GOT) electrophoretic patterns of different faba bean varieties under investigation are presented in Table (3) and Fig.

(2). The number of GOT bands differed according to the faba bean varieties. The varieties, M.1, G.643, G.3 Im, G.717, G.461, G.843, Y.S, Nu.1, Sa.1 and Sa.2 varieties contained 3, 3, 2, 4, 3, 2, 2, 4, 3 and 2 bands, respectively. These results indicated that the various faba bean varieties had different GOT bands. According to the number of GOT bands it is possible to characterize faba bean varieties as follows; the faba bean varieties G.717 and Nu.1 contained only four bands to distinguish between these varieties depend on R_F values of such 4 bands which were 0.125, 0.281, 0.346, 0.408 for G.717 and of 0.118, 0.232, 0.285, 0.355 for Nu.1, respectively. The G.3 Im, G.843, Y.S. and Sa.2 faba bean varieties contained two bands. Their R_F values for each of the first and second bands were 0.283, 0.371, 0.092, 0.267, 0.248, 0.352 and 0.244, 0.360, respectively. Therefore, the differences in the R_F values of bands can be used successfully to identify these faba bean varieties.

Table (2): Ranges of molecular weight and its related number of bands for proteins of each of studied faba bean varieties

Varieties	Maximum MW (kda)	Minimum MW (kda)	Total bands
M.1	192.156	18.058	11
G.643	132.11	18.058	8
G.3 Im	124.123	6.211	9
G.717	182.541	10.421	10
G.461	182.451	7.846	12
G.843	162.211	8.46	9
Y.S.	182.541	18.058	10
Nu.1	151.152	6.211	9
Sa.1	223.041	7.864	10
Sa.2	182.541	7.864	12

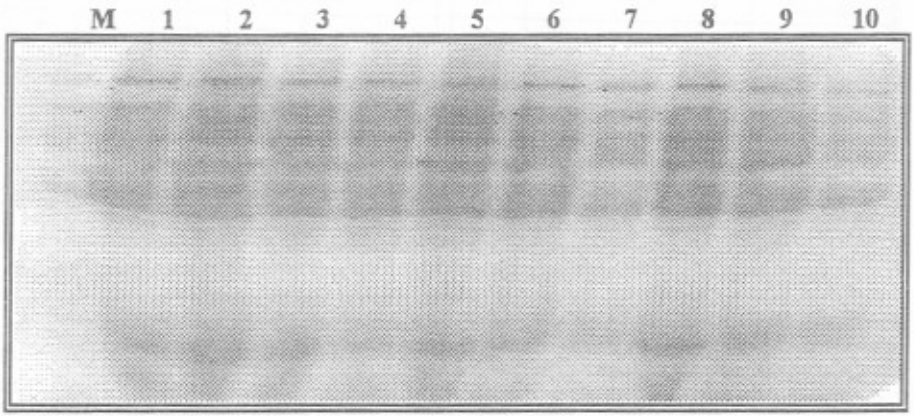

Figure (1): SDS-PAGE of total protein patterns of the ten faba bean varieties.

Results in Table (3) showed that each of the varieties M.1, G.643, G.461 and Sa.1 contained three bands. The R_F values for the first, second and third bands were differed (0.210, 0.291, 0.356; 0.288, 0.371,0.452; 0.232, 0.282, 0.346; and 0.275, 0.358, 391, respectively).The glutamate oxaloacetate tranaminase (GOT)

produced similar number of bands but the location and R_F values of the bands were different. Hence, these values can be adequately used for identification among these varieties.

Table (3): **The Rf of Glotamate oxaloacetate Transaminase isozyme (GOT) bands for ten faba bean varieties.**

Varieties	GOT-1	GOT-2	GOT-3	GOT-4
M. 1	0.210	0.291	0.356	-----
G.64	0.288	0.371	0.452	----
G.3 Im	0.283	0.371	----	-----
G.717	0.125	0.281	0.346	0.408
G. 461	0.232	0.282	0.346	-----
G.843	0.092	0.267	----	-----
Y.S.	0.248	0.352	----	----
Nu.1	0.115	0.232	0.285	0.355
Sa.1	0.275	0.358	.0391	----
Sa.2	0.244	0.360	----	----

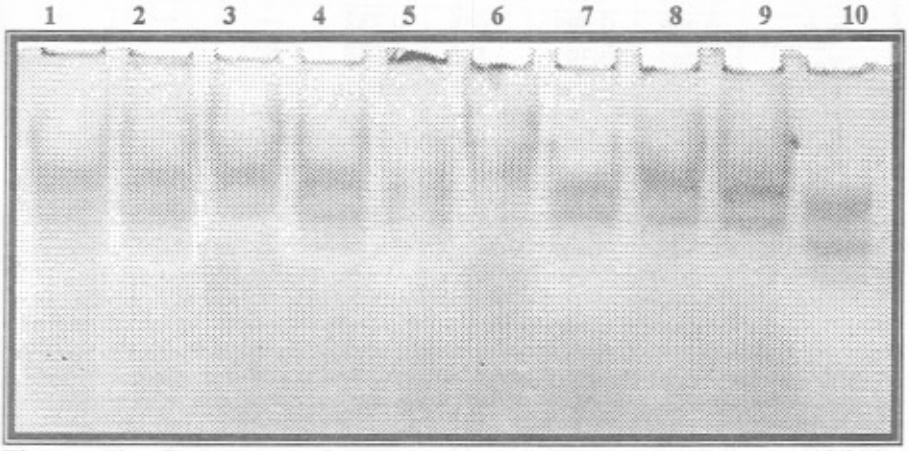

Figure (2): Zymogram of glutamate oxaloacetate tranaminase (GOT) isozyme for the ten faba bean varieties under study.

2- Esterase (Est) isozyme.

Data in Table (4) and Fig. (3) protein bands of esterase (Est) for ten faba bean varieties under study. The numbers of obtained bands were 6, 4, 7, 6, 4, 6, 4, 5, 6 and 6 for M.1, G.643, G.3 Im, G.717, G.461, G.843, Y.S, Nu.1, Sa.1 and Sa.2, respectively. In order to distinguish between such faba bean varieties, the following results and information could be considered:

a- The G.3 Im variety contained seven bands. which were 0.271, 0.455, 0.542, 0.569, 0.650, 0.711 and 0.835. This is unique feature of these studied variety.

b- The Nu.1 variety contained five bands. The R_F values of these bands were 0.242, 0.395, 0.585, 0.738 and 0.843.

c- Each of the M.1, G.717, G.843, Sa.1 and Sa.2 faba bean varieties contained six bands. The R_F values for the second band of EST for these varieties were 0.465, 0.432, 0.391, 0.475 and 0.344, respectively. Therefore, identifying among these varieties depending on the obtained R_F values.

d- The same trend can be followed to distinguish between faba bean varieties which contained four esterase bands (G.643, G461and Y.S.). Science the R_F values for the third band of EST were of Rf 0.721, 0.744 and 0.655. Hence, EST produced a similar number of bands, but the R_F values were different. Therefore, these results can easily be used to distinguish between these faba bean varieties.

Table (4): The Rf of esterase isozyme (EST) bands for the ten faba bean varieties under studies.

Varieties	EST-1	EST-2	EST-3	EST-4	EST-5	EST-6	EST-7
M.-1	0.354	0.465	0.596	0.650	0.725	0.743	----
G.643	0.442	0.585	0.721	0.841	----	----	----
G.Im	0.271	0.455	0.542	0.596	0.650	0.711	0.835
G.717	0.370	0.432	0.521	0.585	0.712	0.743	----
G.461	0.265	0.595	0.744	0.820	---	---	---
G.843	0.321	0.391	0.642	0.722	0.775	0.835	---
Y.S.	0.290	0.598	0.655	0.732	---	---	---
Nu.1	0.242	0.395	0.585	0.738	0.843	---	---
Sa.1	0.290	0.475	0.538	0.601	0.702	0.843	---
Sa.2	0.252	0.344	0.453	0.596	0.711	0.843	---

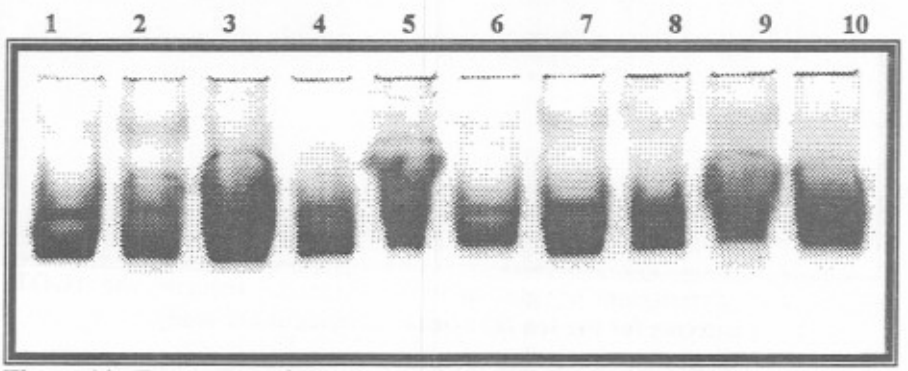

Figure (3): Zymogram of esterase (Est) isozyme for the ten faba bean studied varieties 5-3- Peroxides (Prx) isozyme.

Results in Table (5) and Fig (4) represent the esterase (EST) electrophoretic patterns for different faba bean varieties under study. The number of (EST) bands were 3, 4,4,5,3,4,3, 3, 4 and 4 for the respective M.1, G.643, G.3 Im, G.717, G.461, G.843, Y.S, Nu.1, Sa.1 and Sa.2 faba bean varieties. These results showed that the various faba bean varieties had different EST bands. From these results, the only faba bean variety can be identified is G.717 since it contained five bands of R_F 0.045, 0.082, 0.121, 0.191 and 0.253. Hence, the number of band and the R_F values can be used to identify this variety.

Each of M.1, Y.S. and Nu.1 varieties had three bands. Their R_F values for first, second and third bands were 0.085, 0.153, 0.191; 0.070, 0.160, 0.201; and 0.070, 0.151, .0191, respectively. Therefore, it is not possible to distinguish between these faba bean varieties because their almost similar R_F . Dealing with faba bean varieties which comprised four bands, one can look at the R_F values of these bands are more identical for identification . In order to distinguish between G.643, G.3 Im, G.843, Sa.1 and Sa.2 varieties, the R_F values for fourth bands for each of the varieties were different which were 0.254, 0.246, 0.263, 0.196 and 0.212, respectively).Hence, these varieties had the same number of bands but the different Rf values.

Table (5): Rf of peroxidase isozyme (Prx) bands for the ten faba bean varieties under study.

Varieties	Prx 1	Prx 2	Prx 3	Prx 4	Prx 5
M.1	0.085	0.153	0.191	-	
G.643	0.045	0.116	0.181	0.254	---
G.3 Im	0.034	0.131	0.196	0.246	---
G.717	0.045	0.082	0.121	0.191	0.253
G.461	0.034	0.114	0.264	----	----
G.843	0.038	0.082	0.181	0.263	----
Y.S.	0.070	0.160	0.201	----	----
Nu.1	0.070	0.151	0.191	----	----
Sa.1	0.038	0.062	0.141	0.196	----
Sa.2	0.036	0.121	0.181	0.212	----

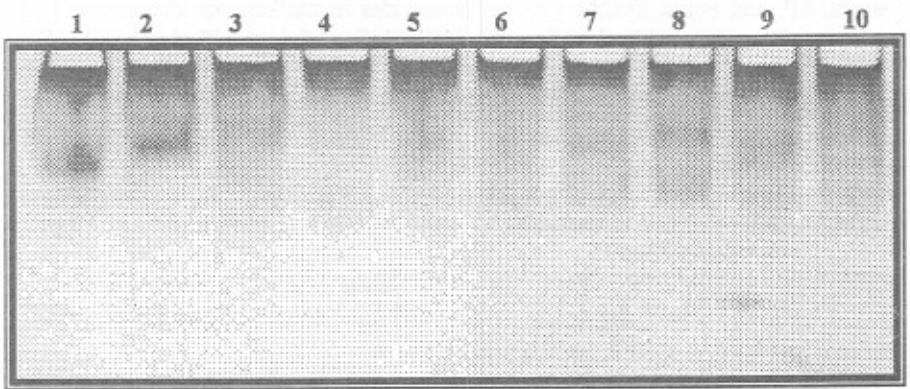

Figure (4): Zymogram of peroxidase (Prx) isozyme for the ten faba bean varieties under studied.

Results presented in this study for Isozymes electrophoresis of varieties under investigation are along the same line for many authors which studied different isozymes for varieties identification.(Amet 1986; Bonetti *et al*., 1995; Choer *et al*., 1999 and Eeswara and Peiris 2001). Also, De Mora *et al*., (1983) used polyacrylamide electrophoresis to GOT isoenzyme variation in differentiating between cultivars of *Vicia faba* L. Meanwhile, Salama (1988) reported that esterase pattern for seed protein was found to be helpful to differentiate between different varieties of cowpea and broad bean. Moreover, Polignano and Sonnante (1991)

observed a total of six bands for GOT isoenzymatic system in nine faba bean populations from Ethiopia and Afghanistan. The GOT patterns polymorphism was of clear differences in band patterns between populations. Also, Choer *et al.* (1999) used horizontal polyacrylamide gel electrophoresis to analyse esterase, leucine amino peptidase, aspartateaminotransferase and 6-phosphogulconate dehydrogenase iso enzymes from seeds of beans (Phaseolus vulgaris L.). The Isoenzymes are valuable markers for cultivar identification and varietal purity tests in seeds. Meanwhile, Tarek (2005) illustrated that the isoenzymes were used to assess the genetic diversity of melon .

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توصيف بعض أصناف الفول البلدى بإستخدام التفريد الكهربى للبروتين والمشابهات الإنزيميه

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أجري هذا البحث على عشرة أصناف من الفول البلدي وهي سخا١- ،وسخا-٢، نوباريه-١، مصر-١، جيزه-٣ محسن، جيزه-٤٦١، جيزه-٦٤٣، جيزه-٧١٧، جيزه-٨٤٣، يوسف الصديق.والهدف هو و توصيف هذه الأصناف عن طريق التفريد الكهربى لبروتينات البذوربإستخدام طريقة SDS-PAGE للتعرف على مكوناتها فى مناطق الاوزان الجزيئيه المختلفه. كما تم دراسة ثلاثة من المشابهات الإنزيمية وهى (جلوتامات اكسالواستيات ترانس أمينيز ــ الاستيريز ــ البيروكسيديز) عن طريق التفريد الكهربي لمكوناتها. وكانت أهم النتائج المتحصل عليها كالاتى:أوضح التفريد الكهربى لبروتينات البذور أن أن إجمالي عدد حزم البروتين يتراوح بين ٨ حزم في الصنف جيزة ٦٤٣ إلى ١٢ حزمة في كلاً من جيزة ٤٦١ ، سخا ٢ . كما وجدت ستة حزم مشتركة في جميع الأصناف ذات وزن جزيئي ٨٤,٧٤٧، ٥١,٦٢٠، ٣٨,٥٥٤ كيلودالتون. ومن النتائج أمكن الحصول على دلائل موجبة فى صورة حزمة مميزة لكل صنف من الأصناف الآتية: جيزة-٣ محسن، جيزة ٧١٧، جيزة ٨٤٣، نوبارية-١، مصر-١، سخا-١ عند الحزم ذات الأوزان الجزيئية

(٦,٢١١-١٠,٤٢١-٩٠,٤١٢-١٤١,٠٥٦-١٩٢,١٥٦-٢٢٣,٠٤١ كيلودالتون) على الترتيب ومن النتائج أيضا أمكن الحصول على دلائل سالبة فى صورة حزمة واحدة ذات الوزن الجزيئى (٣٠,٧٤٦ كيلو دالتون) مميزة للأصناف جيزة-٦٤٣، جيزة-٨٤٣، سخا-١.أما على مستوى المشابهات الإنزيمية فقد دلت النتائج أنه باستخدام عدد المكونات البروتينية وقيم الـ R_F لبعض المشابهات تحت الدراسة يمكن توصيف وتمييز هذه الأصناف. حيث تبين النتائج الخاصة بالجلوتامات أكسالواسيتات ترانس أمينيز أن الصنفان جيزة- ٧١٧ ونوبارية ـ١ يحتويان على أربعة مكونات بروتينية مختلفة عن باقي الأصناف ومختلفة في قيمة الـR_F. ومن نتائج الاستيريز فقد دلت أن الأصناف أعطت اختلاف في عدد المكونات (٤ـ٧ مكونات) حيث احتوى الصنف جيزة -٣ محسن وحده على ٧ مكونات بروتينية.تميزه عن باقي الأصناف.أما بالنسبة لنتائج البيروكسيديزفقد تبين وجود خمسة مكونات بروتينية للصنف جيزه-٧١٧ وهي مميزة له عن باقي الأصناف. بينما احتوت باقي الأصناف على عدد من ٣ ـ ٤ مكونات بروتينية مختلفة عن بعضها في قيم الـR_F.