ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol.45(2): 735-748, (2007).

IDENTIFICATION OF COWPEAS, BEANS AND PEAS VARIETIES BY PROTEIN AND ISOZYME ELECTROPHORESIS ANALYSIS

BY

Bekhit, M.M.M.
Department of genetics, Faculty of Agriculture, Benha University, Egypt

ABSTRACT

Banding patterns of SDS-protein and isozymes (esterase, glutamate oxaloacetate transminase and peroxidase) were studied to identify two cowpea (*Vigna unguiculata*) cultivars, two common bean (*Phaseolus vulgaris*) cultivars and two pea (*Pisum sativum L.*) cultivars. The results revealed a wide range of variation in SDS protein banding patterns in cultivars of cowpea (δ-vignin, β-vignin and albumin). Low level of polymorphism was detected in common bean cultivars (one band in albumin) and in pea (one band in convicilin as well as one band in legumin). Meanwhile, the banding patterns of esterase revealed slightly higher polymorphism in cowpea cultivars.

INTRODUCTION

Analysis of variation in the electrophoretic patterns of seed proteins is a useful method for establishing relationships among plant accessions within a species (Gepts *et al.*, 1986). Many plant proteins exhibit extensive polymorphism in relation to size and charge and are encoded at several loci within the genome (Cooke 1986). Therefore, the study of polymorphic proteins by electrophoresis is one of the most convenient methods for describing variability in plant genetic resources and for cultivar identification (Ladizinsky and Hymowitz 1979). Moreover, because proteins are primary gene products they provide a valuable means of marking genetic systems and thus variation in protein composition can be a reflection of genotypic variation (Osanyinpeju and Odelgah 1998). Genetic variants revealing differences in composition of seed protein have been recorded in many species and genera of higher plants (Collada *et al.*, 1991; Ignacicimuthu and Arockiadass 1993 and Vandana and Dubey 1994).

Like other legume seeds, cowpea contains large amounts of salt soluble proteins, mainly vicilin 7S globulin and to lesser amount legumin-like 11S globulins. Both of proteins consist of several polypeptides which have been reported (Khan *et al.*, 1980; Murray *et al.*, 1983 and Fotso *et al.*, 1994). Freitas *et al.*, 2004 revealed that globulins constitute over 51 % of the total seed cowpea protein with albumins composing 45 %. The globulins fractionated into three main components; α-vignin, β-vignin and γ-vignin. α-vignin is the major nonglycosylated globulin. It consisted of 80 KDa subunit which upon reduction, produces two polypeptides (20 and 60 KDa). β-vignin is a major glycosylated

globulin, composed of two main polypeptides (55 and 60 KDa) with no disulfide bonds. γ-vignin a minor globulin is composed by one main type of subunit (22KDa), which upon reduction, is converted into a single heavier polypeptide chain (30 KDa) due to the presence of an internal disulfide bond.

In phaseolus (*Phaseolus vulgaris*), seed protein fractionation studies revealed the presence of three major soluble fractions: phaseolin (G_1), globulin-2 (G_2) and albumin (Hussein and Gamal El-Din, 1985). These fractions represent 36 to 46 %, 5 to 12 % and 12 to 16 % of the total seed protein, respectively. Isozyme banding patterns were used for detection of genetic diversity among cultivars (Weeden, 1984; Becerra-Velasquez and Gepts, 1994 and El-Fiky *et al.*, 2003).

Pea (*Pisum sativum L.*) is one of the most important legumes. There are thousands of commercial cultivars (Gantotti and Kartha 1986) and a huge number of accessions in germplasm collections, all of which are useful as genetic resources for breeding. Genetic characterization of these genotypes is desirable both for high efficiency of breeding strategies and for commercial seed production. In peas, seed storage proteins (Cooke 1983, Przybylska, 1986; Śuśka and Stejskal 1992), isoenzymes (Przybylaska, 1986; Swiecicki and Wolko, 1987; Śuśka 1993, Stejskal *et al.*, 1996) have been useful for cultivar/genotype identification. Both types of molecular markers may also be used for pea phylogenetic studies (Hoey *et al.*, 1996 and Samec *et al.*, 1998). The major storage proteins of *Pisum* have been identified (Matta *et al.*, 1981; Matta and Gatehouse, 1982; Gamal El-Din *et al.*, 1984; Hussein and Gamal El-Din, 1986; Cooke, 1983; Przybylska, 1986; Stejskal *et al.*, 1996 and Śuśka and Stejskal 1992). Most of authors classified the major storage proteins into convicilin, vicilin and legumin.

The aim of this work was (1) to use seed storage protein and isoenzyme markers for the identification of cowpea, phaseolus and commercial pea cultivars, (2) to evaluate potential genetic/taxonomic relationships among these cultivars.

MATERIALS AND METHODS

This investigation was carried out at biotechnology lab of the genetic department, Faculty of Agriculture, Benha University. Seeds representing 6 samples belonging to three species (*Vigna unguiculata, Phaseolus vulgaris and Pisum sativum L.*) were obtained from the institute of vegetable crops, Ministry of Agriculture, Giza, Egypt (Table 1). The molecular determination of seed storage proteins was achieved using one –dimensional SDS-polyacrylamide gel electrophesis.

Finely ground meals from freeze-dried cotyledons (5 mg of meal/ml of buffer) were extracted overnight with constant agitation at 4°C using 0.2 M Tris-HCL buffer pH 6.8 containing 2 % (w/v) sodium dodycyl sulfate and subsequently centrifuged at 9000 g for 5 min. A protion of the clear supernatant was subsequently taken for analysis.

Table (1): Description of the commercial varieties of cowpea (*Vigna unguiculata*), beans (*Phaseolus vulgaris*) and peas (*Pisum sativum L.*) used in this study.

Species	Cultivars	Description
Cowpea *Vigna unguiculata*	Dokky 331 Qaha 3	Commercial
Beans *Phaseolus vulgaris*	Giza 6 Coby	Commercial
Peas *Pisum sativum L.*	Snow wind Lincoln	Commercial

SDS-polyacrylamide-gel electrophoresis was performed according to the method of Laemmli (1970). SDS-denatured bovine serum albumin (66,000), oval albumin (45,000), glyceraldyhide-3 phosphate dehydrogenase (36.000), carbonic anhydrase (29.000), trypsinogen (24.000) and soybean trypsin inhibitor (20.000) were used for the calibration curve (El-Fiky *et al.*, 2002).

Isoenzyme assay: Three isoenzyme systems useful for cowpea, phaseolus and peas identification were used.

Esterase isozyme was performed on the gel using α-naphthyl acetate and fast blue RR (Gottlieb, 1974). One unit of esterase activity was the amount of enzyme, which liberates one µmol of nitrophenol per min.

Glutamate Oxaloacetate transminase (GOT) was analyzed according to Reitman and Frankel, 1957. One unit of GOT activity was the amount of enzyme which converts one µmol of α-ketoglutarate to L-glutamate per hour.

Peroxidase isozyme was detected on the gel using guiacol and hydrogen peroxide according to Show and Prasad (1970). One unit of peroxidase activity was the amount of enzyme, which causes one O.D. change at 470 nm per min. under assay conditions.

RESULTS AND DISCUSSION

Genetic diversity in cowpea storage protein:

The 7S or vicilin-like fraction (G-1), known as vignin was subsequently shown to be a heterogeneous globulin of about 170 KDa (Khan *et al.*, 1980 and Cerdeira *et al.*, 1985). Globulins are the major protein component in the seed storage legumes, especially *Vigna unguiculata*, as it reaches 72 % (Murray *et al.*, 1983). Therefore, globulin is responsible for the nutritional value of the seed. Freitas *et al.*, 2004 found that globulins (constitute 51 % of the total seed protein) and albumins (constitute 45 % of the total seed protein followed by glutelin (3 %) and prolamin (1 %).

The term vignin has been used to designate the 7S globulin from *Vigna unguiculata* seeds. The three major *Vigna* globulins are α-vignin, β-vignin and γ-vignin.

The two genotypes revealed different patterns of vignin polypeptides in SDS-PAGE. The total number of appeared bands was nine for vignin. These bands could be classified into three different groups: polypeptides of molecular weight 59.42, 45.19 and 19.91 KDa in the first group (α-vignin). The polypeptides of molecular weight 68.72, 55.29, 51.22, 31.07 and 24.31 KDa in the second group (β-vignin) and 21.76 KDa in the third group as γ-vignin. These groups revealed molecular weights close to those discussed by Freitas et al., 2004. Differences in the molecular weights of these polypeptide groups have been recorded. Therefore, the differences in polypeptides of these subunits between the present study and those previously reported could be attributed to the difference in genotypes or the difference in techniques used or both of them. Table (2) revealed that the α-vignin was represented by both varieties (Dokky 331 and Qaha 3). The second type (β–vignin) was demonstrated by both of varieties. The polypeptide 31.07 KDa was absent in Qaha 3 while the 24.31 KDa was absent in Dokky 331. The third type (γ-vignin) was represented in the two varieties. This suggests that these varieties may have different genetic mechanism controlling the biosynthetic pathway of the vignin fraction.

The total number of bands of the albumin fraction electrophorized for the two varieties was nineteen. These bands ranged between 73.82 and 3.54 KDa. Table (2) and Figure (1) revealed that bands of molecular weight 48.90, 42.38, 38.94, 37.54, 34.21, 32.36, 27.20, 25.08, 13.19, 7.41, 6.54, 5.56, 4.45 and 3.54 KDa prevailed in both of the studied varieties. Variation in the appearance of five bands ranging from 73.82 to 3.93 could be easily detected in Table 2 and Figure 1.

It was found that there is no relationship between the variation in vignin and albumin fractions suggesting that these groups are under independent genetic control (Hussein and Gamal El-Din 1985).

Genetic diversity in bean storage protein:

The two varieties revealed the same patterns of phaseolin (G1-polypeptides) in SDS-PAGE. The total number of appeared bands was six for phaseolin. It is possible to classify these bands into three different categories: polypeptides that had the molecular weight of 50.02 and 49.07 KDa are found in one category (α-phaseolin). Polypeptides that had the molecular weight of 47.11 and 45.67 KDa are located in the second category (β-phaseolin). The third category is consisted of two polypeptides; 44.52 and 41.83 KDa as γ-phaseolin. These categories showed that these molecular weight nearly the same to those discussed by Hussein and Gamal El-Din (1985) and Brown et al., 1981. The differences in molecular weights between the present study and the previous studies may be attributed to varieties or techniques used or both of them.

Both of varieties; Giza 6 and Coby were identical in the three categories (Table 3 and Figure 1). This suggests that both of varieties may have the same genetic mechanism of the biosynthetic pathway of phaseolin fraction.

The total number of appeared bands for the albumin (G_2-fraction) was sixteen in a range of 36.52 to 3.07 KDa.

Table (2): Banding patterns and molecular weight (MW) of SDS proteins for two cultivars of cowpea (*Vigna unguiculata*).

Protein fraction	M. W. (KDa)	Cultivars	
		Dokky 331	Qaha 3
α-vignin	59.42	0	1
	45.19	1	1
	19.91	1	0
β-vignin	68.72	1	1
	55.29	1	1
	51.22	1	1
	31.07	1	0
	24.31	0	1
γ-vignin	21.76	1	1
Albumin	73.82	0	1
	48.90	1	1
	42.38	1	1
	38.94	1	1
	37.54	1	1
	34.21	1	1
	32.36	1	1
	28.81	1	0
	27.20	1	1
	25.08	1	1
	16.58	0	1
	13.19	1	1
	10.93	0	1
	07.41	1	1
	06.54	1	1
	05.56	1	1
	04.45	1	1
	03.93	0	1
	03.54	1	1

Table 3 shows that the band of molecular weight 12.62 KDa was found in Giza 6 and absent in Coby variety. Hussein and Gamal El-Din (1985) concluded that there is no relationship between the variation in phaseolin and albumin suggesting that these groups are under independent genetic control. The legumin fraction was represented by the polypeptides ranged from 55.41 to 74.57 KDa. Both of varieties are similar in legumin fraction.

Genetic diversity in pea storage protein:

As shown in Table (4) and Figure (1), both varieties Snow w nd and Lincoln revealed slight differences in convicilin banding pattern. The polypeptide with the molecular weight 63.76 KDa was absent in the snow wind variety and present in Lincoln variety. The presence of the polypeptide 70.94 KDa is an indicator of the presence of convicilin in both of varieties. This is in agreement with the previous studies (Hussein and Gamal El-Din, 1986). They stated that convicilin has distinctive amino acid composition, especially with respect to sulpher-containing amino acids, having one cysteine and one methionine residue per subunit.

Table (3): Banding patterns and molecular weight (MW) of SDS proteins for two cultivars of beans (*Phaseolus vulgaris*).

Protein fraction	M. W. (KDa)	Cultivars	
		Giza 6	Coby
Legumin	74.57	1	1
	71.88	1	1
	66.74	1	1
	62.64	1	1
	55.41	1	1
α-phaseolin	50.02	1	1
	49.07	1	1
B-phaseolin	47.11	1	1
	45.67	1	1
γ-phaseolin	44.52	1	1
	41.83	1	1
G2 (Albumin)	36.52	1	1
	34.38	1	1
	32.47	1	1
	29.39	1	1
	26.80	1	1
	23.81	1	1
	22.27	1	1
	16.26	1	1
	12.84	1	1
	12.62	1	0
	9.72	1	1
	7.42	1	1
	6.32	1	1
	5.21	1	1
	3.92	1	1
	3.07	1	1

Both of varieties; Snow wind and Lincoln shows three vicilin bands (50.57, 48.62 and 34.06 KDa). These results are in agreement with the previous findings (Gatehouse *et al.*, 1981 and Hussein and Gamal El-Din, 1986).

Differences in legumin subunits were detected among both of varieties at the polypeptide 23.35 KDa (Table 4 and Figure 1). The differences in the legumin subunits between these two varieties suggests that legumin as an important gene product may be affected by a number of genes scattered on different chromosomes in addition to the major structural gene (s) located in chromosome vii (Hussein and Gamal El-Din, 1986). These genes may affect the biosynthetic pathway of legumin end product. Matta and Gatehouse (1982) stated that the major acidic subunits behaved as product of a single mendelian gene with at least five different possible alleles.

Table (4): Banding patterns and molecular weight (MW) of SDS proteins for two cultivars of pea (*Pisum sativum*).

Protein fraction	M. W. (KDa)	Cultivars	
		Snow wind	Lincoln
Convicilin	70.94	1	1
	67.39	1	1
	63.76	0	1
Vicilin	50.57	1	1
	48.62	1	1
	34.06	1	1
Legumin	61.57	1	1
	57.71	1	1
	56.25	1	1
	46.61	1	1
	43.75	1	1
	38.87	1	1
	35.51	1	1
	30.84	1	1
	26.80	1	1
	25.55	1	1
	23.35	1	0

Isozyme banding patterns in cowpea:

Table (5) and Figure (2) revealed the electrophoretic separation of esterase, GOT and peroxidase isozymes. The isozyme patterns of esterase show disappearance of two bands for the Dokky 331 cultivar with (RF of 0.496 and 0.585) and disappearance of two bands for the Qaha 3 with (RF of 0.479 and 0.577). Moreover, the banding patterns of GOT isozyme revealed unique bands for both of the two cultivars (RF of 0.414 and 0.531). Two bands for the Dokky 331 cultivar were disappeared with (RF of 0.772 and 0.979). In case of peroxidase banding patterns, it showed disappearance of three bands (RF of 0.546, 0.761 and 0.907, respectively) for the Dokky 331 cultivar.

Isozyme banding patterns in phaseolus:

Table (6) and Figure (3) show the banding of esterase, GOT and peroxidase isozymes. The banding patterns of esterase show unique band of both of cultivars; Giza 6 and Coby (RF of 0.530). On the other hand, the isozyme patterns of GOT isozyme revealed disappearance of two bands for the Giza 6 cultivar (RF of 0.579 and 0.689) and one band for the Coby cultivar (RF of 0.938). Concerning peroxidase banding patterns, it revealed disappearance of two bands for the Giza 6 (RF of 0.810 and 0.937) and three bands for Coby cultivar (RF of 0.756, 0.820 and 0.893, respectively).

Isozyme banding patterns in peas:

The banding patterns of esterase, GOT and peroxidase were found in Table (7) and Figure (4). The banding patterns of esterase revealed unique band for both of cultivars; Snow wind and Lincoln (Rf of 0.718). On the other hand,

the banding patterns of GOT isozyme show disappearance of different bands for Snow wind cultivar with (Rf of 0.172, 0.572 and 0.945, respectively) and two different bands for Lincoln with (Rf of 0.607 and 0.821). In case of peroxidase banding patterns, it revealed absence of three different bands for Snow wind cultivar with (Rf of 0.200, 0.449 and 0.741, respectively).

Depending on the obtained data for SDS protein patterns as well as esterase, GOT and peroxidase isozymes from two cultivars of cowpea, two cultivars of phaseolus and two cultivars of pea. It concluded that there is a variation between these cultivars. The banding patterns of protein reflected high levels of polymorphism in cowpea cultivars as compared to phaseolus and pea cultivars. Meanwhile, the banding patterns of esterase revealed slightly higher polymorphism in cowpea cultivars while GOT banding patterns show slightly higher polymorphism in pea cultivars than peroxidase. These data were in agreement with findings of Jaaska and Jaaska, 1988, Prestamo and Manzanol, 1993 and El-Fiky *et al.*, 2002.

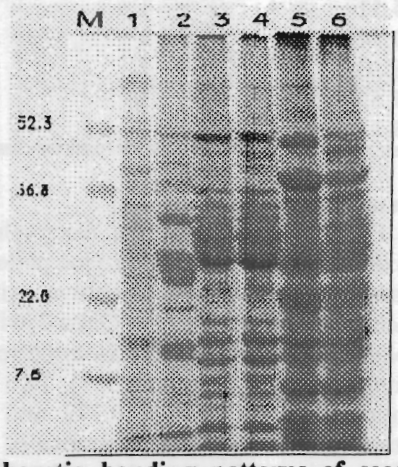

Figure (1): Electrophoretic banding patterns of seed storage protein of *Vigna, Phaseolus and Pisum* cultivars. Lanes 1-6: represent Dokky 331, Qaha 3, Giza 6, Coby, Snow wind and Lincoln, respectively.

Figure (2): Electrophoretic banding patterns of esterase isozyme of *Vigna, Phaseolus and Pisum* cultivars. Lanes 1-6: represent Dokky 331, Qaha 3, Giza 6, Coby, Snow wind and Lincoln, respectively.

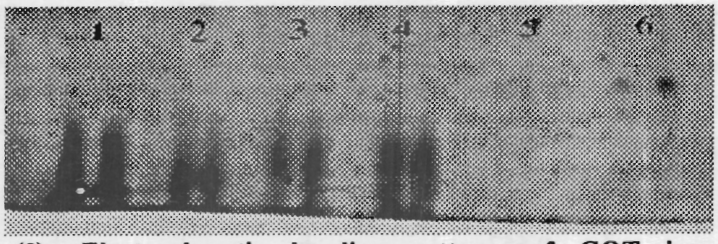

Figure (3): Electrophoretic banding patterns of GOT isozyme of *Vigna,Phaseolus and Pisum* cultivars. Lanes 1-6: represent Dokky 331, Qaha 3, Giza 6, Coby, Snow wind and Lincoln, respectively.

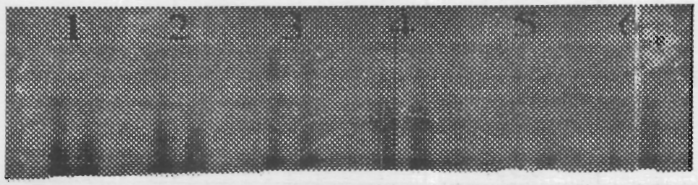

Figure (4): Electrophoretic banding patterns of peroxidase isozyme of Vigna, Phaseolus and Pisum cultivars. Lanes 1-6: represent Dokky 331, Qaha 3, Giza 6, Coby, Snow wind and Lincoln, respectively.

Table (5): Banding patterns and relative mobilities of three isozymes for two cultivars of cowpea (*Vigna unguiculata*).

Isozyme	RF	Cultivars	
		Dokky 331	Qaha 3
Esterase (Est)	0.479	+	-
	0.496	-	+
	0.577	+	-
	0.585	-	+
GOT	0.414	+	+
	0.531	+	+
	0.772	-	+
	0.862	+	+
	0.979	-	+
Peroxidase (Prx)	0.546	-	+
	0.629	+	+
	0.761	-	+
	0.839	+	+
	0.898	+	+
	0.907	-	+

Table (6): Banding patterns and relative mobilities of three isozymes for two cultivars of beans (*Phaseolus vulgaris*).

Isozyme	RF	Cultivars	
		Giza 6	Coby
Esterase (Est)	0.530	+	+
GOT	0.276	+	+
	0.303	+	+
	0.557	+	+
	0.579	-	+
	0.689	-	+
	0.938	+	-
Peroxidase (Prx)	0.668	+	+
	0.712	+	+
	0.756	+	-
	0.810	-	+
	0.820	+	-
	0.893	+	-
	0.937	-	+

Table (7): Banding patterns and relative mobilities of three isozymes for two cultivars of pea (*Pisum sativum*).

Isozyme	RF	Cultivars	
		Snow wind	Lincoln
Esterase (Est)	0.716	+	+
GOT	0.172	-	+
	0.303	+	+
	0.572	-	+
	0.607	+	-
	0.821	+	-
	0.945	-	+
Peroxidase (Prx)	0.200	-	+
	0.302	+	+
	0.449	-	+
	0.741	+	-
	0.766	-	+

REFERENCES

Becerra-Velasquez, U.L. and Gepts, P. (1994): RFLP diversity of common bean (*Phaseolus vulgaris*) in its centers of origin. Genome, 37: 256-263.

Brown, J.W.S.; McFerson, J.R.; Bliss, F.A. and Hall, T.C. (1982): Genetic divergence among commercial classes of *Phaseolus vulgaris* in relation to phaseolin pattern. Hort. Science, 17: 752-754.

Cerdeira, A.L.; Cole, A.W. and Luthe, D.S. (1985): The seed proteins of cowpea (*Vigna unguiculata L. Walp.*) J. Exp. Bot., 31: 1599-1611.

Cooke, R. J. (1983): The characteristics of *Pisum sativum L.* (field pea) cultivars by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. J. Nat. Inst. Agr. Bot., 16: 213-220.

Cooke, R. J. (1986): Gel electrophoresis: a role in agriculture. Electrophoresis., 7: 203-217.

Collada, C.; Caballero, R.G.; Casado, R.; Salced, G. and Aragoncillo, C. (1991): Subunit structure of legumin-like globulins of Fagus sylvatica seeds. J. Exp. Bot., 42: 1305-1310.

El-Fiky, Z.A.; Hussein, M.H.; Mohamed, E.M. an Hussein, H.A. (2002): Biochemical and molecular genetic studies using SDS-protein, isozymes and RAPD-PCR in some common bean (*Phaseolus vulgaris L.*) cultivars. Arab. J. Biotech., 5(2): 249-262.

Fotso, M.; Azanza, J.L.; Pasquet, R. and Raymond, J. (1994): Molecular heterogencity of cowpea (*Vigna unguiculata Fabaceae*) seed storage proteins. Pl. Syst. Evol., 191: 39-56.

Freitas, R.L.; Teixeira, A.R. and Ferreira, R.B. (2004): Characterization of the proteins from *Vigna unguiculata* seeds. J. Agric. Food chem.., 52 (6):1682-1687.

Gamal El-Din; Hussein, E.H.A.; Müller, H.P. and Stegemann, H. (1984): Phylogenetic studies on Pisum. II. Electrophoretic identification of Taxa. Egypt. J. Genet. Cytol. 13: 143-157.

Gantotti, B. V. and Kartha, K. K. (1986): Pea. In: Evans, D. A.; Sharp, W. R.; Amnirato, P. V. (ed): Handbook of plant cell culture, Techniques and applications, Macmillan, New York. 4:370-418.

Gatehouse, J.A.; Croy, R.R.D.; Morton, H.; Tyler, M. and Boulter, D. (1981): Characterization and subunit structures of the vicilin storage proteins of pea (*Pisum sativum L.*) Eur. J. Biochem. 118: 627-633.

Gepts, P.; Osborn, T. C.; Roshk, K. and Bliss, F. A. (1986): Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*). Econ. Bot. 40: 451-468.

Gottlieb, L.D. (1974): Genetic confirmation of the origin of Clarkia lingulata. Evolution, 28: 244-250.

Hoey, B.K.; Crowe, K.R.; Jones, V.M. and Polans, N.O. (1996): A phylogenetic analysis of *Pisum* based on morphological characters and allozyme and RAPD markers. Theor. Genet., 92: 92-100.

Hussein, E.H.A. and Gamal El-Din, A.Y. (1985): Genetic variability in protein patterns of Phaseolus spp. Egypt. J. Genet. Cytol. 14(1): 143-152.

Hussein, E.H.A. and Gamal El-Din, A.Y. (1986): Heterogeneity of *Pisum* storage proteins due to different chromosomal rearrangements. Egypt. J. Genet. Cytol. 15: 75-82.

Igancimuthu, S. and Arockiadass, A. (1993): Induced protein and isozyme variation in *Vigna radiata* var. Ps 16. Madras Agric. J. 80: 252-254.

Jaaska, V. and Jaaska, V. (1988): Isozyme variation in the genera *Phaseolus* and *Vigna* (Fabaceae) in relation to their systematic asparatate aminotransferase and superoxide dismutase. Plant systematic and Evolution, 159: 145-159.

Khan, M.R.I.; Gatehouse, J.A. and Boulter, D. (1980): The seed proteins of cowpea (*Vigna unguiculata L. Walp.*). J. Exper. Bot., 31: 1599-1611.

Ladizinsky, G. and Hymowitz, T. (1979): Seed protein electrophoresis in taxonomic and evolutionary studies. Theor. Appl. Genet., 54: 145-151.

Laemmli, U.K. (1970): Proteins during the assembly of head of bacteriophage T., Nature 227: 680-685.

Matta, N.K. and Gatehouse, J.A. (1982): Inheritance and mapping of storage protein genes in *Pisum sativum L.*, Heredity, 48 (3): 383-392.

Matta, N.K.; Gatehouse, J.A. and Boulter, D. (1981): Molecular and subunit heterogeneity of legumin of *Pisum sativum L.* (Garden pea). A multi-dimensional gel electrophoretic study. J. Exper. Bot., 32: 1295-1307.

Murray, D.R.; Mackenzie, K.F.; Vairinhos, F.; Peoples, M.B. and Atkins, C.A. (1983): Electrophoresis studies of the seed proteins of cowpea (*Vigna unguiculata (L.) Walp.*). Z. Pflanzenphysiol. 109: 363-370.

Osanyinpeju, A.O. and Odelgah, P.G. (1998): Variation in seed proteins from mutagen-treated cultivars and selected lines of *Vigna unguiculata (L.) Walp.* Plant breeding., 117: 361-365.

Prestamo, G. and Manzano, P. (1993): Peroxidase of selected fruits and vegetables and the possible use of ascorbic acid as an antioxidant. Hort. Science, 28:48-50.

Przybylska, J. (1986): Identification and classification of the *Pisum* genetic resources with the use of electrophoretic protein analysis. Seed Sci. Technol., 14: 529-543.

Samec, P.; Pośvec, Z.; Stejskal, J.; Naśinĕc, V. and Griga, M. (1998): Cultivar identification and relationships in *Pisum sativum L.* based on RAPD and iso enzymes. Biologia plantarum. 41(1): 39-48.

Show, C.R. and Prasad, R. (1970): Starch gel electrophoresis of enzymes: A compilation of recipes. Biochem. Genet., 4: 297-320.

Stejskal, J.;Pośvec, Z. and Griga, M. (1996): Utilization of iso enzymes and protein spectra for identification of pea (*Pisum sativum L.*) cultivars. Biologia, 51:99.

Šuśka, M. (1993): Iso enzymes from pea (*Pisum*) leaves and their use in cultivar identification. Genet. Šlecht (Praha). 29: 27-33.

Šuśka, M. and Stejskal, J. (1992): The electrophoretic identification of pea (*Pisum sativum L.*) cultivars by seed protein analysis. Rost. Vŷroba (Praha). 38:203-208.

Swiecicki, W.K. and Wolko, B. (1987): Application of electrophoretic methods of isozymes separation to genetical characterization of pea (*Pisum sativum L.*S.Lat.) cultivars. Genet. Pol. 28: 89-99.

Reitman, S. and Frankel, S. (1957): Determination of glutamic oxaloacetic and glutamic pyruvic transferase. Am. J. Clin. Path., 28: 26.

Vandana, A.T. and Dubey, D.K. (1994): Frequency and spectrum of mutations induced by ethyl methane sulfonate (EMS) and diethyl sulfonate (DES) in lentil var. K-85. LENS news l. 21:16-18.

Weeden, N.F. (1984): Distinguishing among white seeded bean cultivars by means of allozyme genotypes. Euphytica, 33: 199-208.

تعريف الأصناف فى اللوبيا، الفاصوليا والبسلة بإستخدام التفريد الكهربى للبروتين والمشابهات الانزيمية.

مخلوف محمد محمود بخيت
قسم الوراثة، كلية الزراعة، جامعة بنها،مصر

تم تحديد البصمات الوراثية لصنفين من أصناف اللوبيا (دقى ٣٣١ وقهـا ٣)، صنفين من أصناف الفاصوليا (جيزة ٦ وكوبى) وصنفين من أصناف البسلة (سـنوويند ولينكولن) والتى تزرع فى مصر. استخلصت البروتينات الكلية وكذلك نشاط مشابهات ثلاثة إنزيمات (الاستريز، الجلوتاميت اكسالواسيتيت والبيروكسيديز). وقد أسـتخدمت طريقة التفريد الكهربى بإستخدام الـ PAGE لتحديد البصـمات الوراثيـة لكـل مـن البروتين ومشابهات الانزيمات. فى حالة اللوبيا، أظهرت النتائج أن عدد الحزم الكليـة لنوع البروتين فيجنين كانت تسعة مقسمة الى ثلاثة أنواع مختلفة. بالنسبة للنـوع الفـا فيجنين، اختفت الحزمة ذات الوزن الجزيئى ٥٩،٤٢ كيلودالتون من الصنف دقى ٣٣١ بينما اختفت الحزمة ذات الوزن الجزيئى ١٩،٩١ كيلودالتون من الصنف قها ٣. فـى حالة النوع الثانى، أختفت الحزمة ذات الوزن الجزيئى ٣١،٠٧ كيلودالتون فى الصنف قها ٣ بينما أختفت الحزمة ذات الوزن الجزيئى ٢٤،٣١ كيلودالتون من الصنف دقـى ٣٣١. بالنسبة للإلبيومين، كان عدد الحزم الكلية ١٩ حزمة. أختفت الحزم ذات الوزن الجزيئى ٧٣،٨٢،١٦،٥٨ ، ١٠،٩٣ ، ٣،٩٣ على التوالى فى الصنف دقى ٣٣١ بينمـا أختفت الحزمة ذات الوزن الجزيئى ٢٨،٨١ كيلودالتون فى الصنف قهـا ٣. بالنسـبة للفاصوليا، كان عدد الحزم الكلى لبروتين الفاصولين ستة حزم. لم تلاحظ اى اختلافات بين الصنفين جزة ٦ وكوبى بالنسبة لهذا النوع من البروتين. بالنسبة للإلبيومين، كـان الاختلاف الوحيد فى الحزمة ذات الوزن الجزيئى ١٢،٦٢ كيلودالتون حيث انهـا لـم تظهر فى الصنف كوبى. بالنسبة للنوع الثالث وهواللجيومين، تراوح الوزن الجزيئـى للحزم من ٥٥،٤١ الى ٧٤،٥٧ كيلودالتون. فى حالة البسلة، وجد ان هناك اختلافا فـى حزمة واحدة (٦٣،٧٦ كيلودالتون) حيث كانت غائبة فى الصنف سـنوويند. بالنسـبة لبروتين الفيسلين، لم توجد اى اختلافات بين الصنفين. فى حالة الالبيومين، كان هنـاك اختلافا فى حزمة واحدة (٢٣،٣٥ كيلودالتون) حيث انها كانت غائبـة فـى الصـنف لينكولن.

بينت النتائج عند دراسة نشاط مشابهات الانزيمات فى اللوبيا اختلافات بـين الصنفين دقى ٣٣١ وقها ٣ حيث أظهر التفريد الكهربى للإستريز غياب حزمتان فـى الصنف دقى ٣٢١ وغياب حزمتان بالنسبة للصنف قها ٣. كما بين التفريـد الكهربـى للـGOT غياب حزمتان بالنسبة للصنف دقى ٣٣١. أما فى حالـة التفريـد الكهربـى للمشابه الانزيمى البيروكسيديز، كانت هناك ثلاثة حزم غائبة فى الصنف دقـى ٣٣١.

بالنسبة لنشاط مشابهات الانزيمات فى الفاصـوليا، بـين التفريـد الكهربـى للأستريز أن هناك حزمة واحدة متفردة فى كل من الصنفين جيزة ٦ وكوبى. من ناحية اخرى، أظهر نشاط المشابه الانزيمى GOT غياب حـزمتين فـى الصـنف جيـزة ٦ وحزمة واحدة بالنسبة للصنف كوبى. بالنسبة للمشابه الانزيمى بيروكسيديز، أظهـرت النتائج غياب حزمتين فى الصنف جيزة ٦ وثلاثـة حـزم بالنسـبة للصـنف كـوبى.

بالنسبة لنشاط مشابهات الانزيمات فى البسلة، أظهر التفريد الكهربى للأستريز أن هناك حزمة واحدة متفردة فى كل من الصنفين سنوويند ولينكولن. من ناحية اخرى، أظهر نشاط المشابه الانزيمى GOT غياب ثلاثة حزم مختلفة فى الصنف سنوويند وحزمتان مختلفتان بالنسبة للصنف لينكولن. بالنسبة للمشابه الانزيمى بيروكسيديز، أظهرت النتائج غياب ثلاثة حزم مختلفة فى الصنف سنوويند وحزمة بالنسبة للصنف لينكولن.