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GENETIC DIVERSITY AMONG FABA BEAN POPULATIONS BASED ON SEED STORAGE PROTEIN POLYMORPHISM

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ABSTRACT: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses were performed with total seed storage proteins extracted from twenty-one varieties and sixteen landraces of faba bean (*Vicia faba*) with the objective of assessing genetic diversity among these populations. Samples of faba bean landraces were collected from El-Sharkia governorate. Considerable variation in seed storage protein composition was found amongst the 37 faba bean genotypes examined and allelic variants were detected. A total of 30 distinguishable protein bands was revealed. These bands were present in some genotypes and absent in the other. The electrophoretic bands showed a wide variation in their molecular weight. Of these protein bands, 11 bands were found to be polymorphic. Differences have been noted in band intensity in the patterns of different genotypes. Most genotypes were readily identified by SDS-PAGE. Cluster analysis of the 37 faba bean populations showed three major groups and seven subgroups. Group I corresponded to the landraces and the local variety Rebaya 40, whereas group II corresponded to the German KT variety and group III belong to the rest of varieties. Genetic distances among faba bean varieties were mostly small with the exception of those between KT as well as Rebaya 40 and all other varieties. The 21 faba bean varieties were grouped into seven clusters. These cluster groupings were mainly due to differences in legumin and vicilin banding patterns, and showed correspondence with breeding and taxonomic

origin. The data showed that the two landraces LR 34 and LR 33 were the most divergent from all other landraces. Therefore, these two accessions may be important sources of genetic variation within this collection of faba bean germplasm. The varieties used in this study are more diverse than local landraces. The 16 faba bean landraces were grouped into eight clusters. Cluster analysis allowed for the detection of relationships among faba bean genotypes. These cluster groupings were mainly due to differences in legumin and vicilin banding patterns, and showed correspondence with breeding and taxonomic origin.

Keywords: Faba bean, diversity, SDS-PAGE, polymorphism, legumin and vicilin polypeptides and cluster analysis.

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important grain legume crop grown for its protein rich grains. It is an inexpensive source of protein in the diets of people in Egypt. Broadening of the genetic base and systematic exploitation of heterosis in faba bean requires reliable information on genetic diversity in the germplasm. Knowledge of genetic diversity within and among faba bean accessions would enable plant breeders to choose parental sources that will generate diverse populations for selection. Characterization of germplasm by means of protein fingerprinting techniques provides a tool for precise germplasm identification and a quantitative estimate of genetic diversity (Volodin *et al.*, 1984; Perrino *et al.*, 1987; Tucci *et al.*, 1987; Abdel-Tawab *et al.*, 1989; Polignano *et al.*, 1990 and 1991).

The major seed storage proteins of legumes are globulins (packaged into cotyledonary protein storage bodies), which are represented in most legumes by two different types of proteins; the legumin type polypeptides and the vicilin type polypeptides. Understanding the diversity for seed storage proteins in faba bean varieties and landraces will facilitate their use in genetic improvement of nutritional quality. The most commonly used biochemical markers, legumin and vicilin poly-peptides, have been used routinely for analysis of diversity in natural populations of faba beans. Biochemical and

molecular genetic markers can provide a relatively unbiased methods of quantifying genetic diversity in faba beans (Link *et al.*, 1995; Polignano *et al.*, 1998; Potokina *et al.*, 2000; Minelli and Cionini, 2002; Zeid *et al.*, 2003 and Santalla *et al.*, 2004).

The objective of this study was to investigate and characterize faba bean genetic diversity in a set of 21 varieties and 16 landraces obtained from throughout the El-Sharkia governorate on the basis of SDS-PAGE of seed storage proteins.

MATERIALS AND METHODS

Plant materials:

The plant material used in this study was a set of 37 faba bean genotypes. It consisted of 21 varieties. They were Pakistani, Nubaria 1, Triple White, Giza 2, Giza 40, Giza 3, Giza 461, Misr 1, Misr 2, Giza 843, Sakha 1, Sakha 2, Giza 717, Giza 716, Giza 429, Giza 643, English, Giza 674, Kleine Thuringer, KT, Roumi Acoador and Rebaya 40. These genotypes resulted from a wide range of crosses. All these genotypes with the exception of English and Kleine Thuringer (KT) were kindly supplied by Legume Res. Inst., ARC, Giza. Seeds of 16 faba bean landraces currently grown by farmers were obtained from throughout the El-Sharkia governorate. Seed sample of the studied faba bean landraces were kindly provided by Dr. A.R. Alkaddoussi, Professor of Plant Breeding, Faculty of Agriculture, Zagazig University. For purposes of this study, landraces were assigned numbers from 22 to 37.

Protein extraction and SDS-PAGE:

Ten seeds from each genotype were milled using a Micro-Mill. The total seed storage proteins were extracted from a bulk of ten seeds to obtain a combined average protein composition for each genotype rather than single seed analysis. Reduced protein extracts from bulk extractions were separated and classified into subunits by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to their molecular weight using a discontinuous buffer system according to a modified Laemmli (1970) procedure.

Data analysis:

To study clustering pattern among the studied faba bean

genotypes based on band differences in seed storage proteins, the bands generated from these variations were recorded for presence or absence. Genetic distance, calculated as an Euclidean metric distance, was computed between all pairs of populations. The Euclidean metric distance between two populations is equivalent to their total number of observed band differences. Genetic distance values between genotypes were used to produce a dendrogram of the relationships among faba bean populations by the unweighted pair-group method with arithmetic averages (UPGMA) as suggested by Sneath and Sokal (1973). The cluster analysis and dendrogram construction were performed with SPSS (1995).

RESULTS AND DISCUSSION

Genetic polymorphism of seed storage proteins (legumins and vicilins):

Seed storage proteins of faba bean seeds (*Vicia faba* L.) were fractionated by SDS-PAGE to identify genetic polymorphism among studied faba bean varieties and landraces. The electrophoretic banding patterns of total storage proteins extracted from 37 faba bean genotypes representing the different botanical types (minor, equina and major) are presented in Figure (1) for varieties and in Figure (2) for local landraces. Considerable variation in total seed storage protein composition was found amongst the 37 faba bean genotypes examined and allelic variants with different molecular weights were detected. With presently used SDS-PAGE, a total of 30 distinguishable protein bands was revealed and these bands were not necessarily found in all examined faba bean genotypes. Usually 25 to 30 bands are produced from a single genotype, and the bands may differ in intensity as well as mobility and number, therefore, many different genotypic banding patterns were observed. In general, the diversity and similarities among the banding patterns of the studied faba bean populations did not reflect any association to a specific taxonomic group or to accession type (landraces vs. varieties). These findings are in agreement with those previously reported by Sasek and Prugar (1975); Abdalla and Gunzel (1979); Wolff (1980); Kaser and Steiner (1983), Gamal El-Din *et al.*, 1988 and Abdel-Tawab *et al.*, (1989).

These legumin and vicilin protein bands were resolved into four zones on the basis of their molecular weights (migrational distances). In addition, differences have been noted in band intensity for apparently equivalent protein bands in the patterns of different faba bean genotypes. The electrophoretic bands showed a wide variation in their molecular weight. Such variations were found to be higher in landraces than in varieties of faba bean. Common band patterns in all genotypes; monomorphic, were observed. While, 11 bands were not detected in some genotypes; polymorphic, (Figures 1 and 2). These results are in agreement with those of Barrat (1980), Stegemann *et al.*, (1980), Goodrich *et al.*, (1985), Polignano *et al*,. (1986 and 1987), Tucci *et al.*, (1987) and Fayed *et al.*, (1990) who detected variations in legumin and vicilin electrophoretic patterns among faba bean genotypes.

However, it is clear that for the faba bean genotypes examined here, the complex legumin and vicilin banding patterns seen on polyacrylamide gels can be explained in terms of allelic differences. Most genotypes were readily identified by SDS-PAGE. In this study the protein markers proved to be valuable in genotyping faba bean accessions. It is clear that this electrophoretic procedure proved to be useful in the identification of all the studied faba bean populations as it assigned an unique protein pattern for each genotype (Figures 1 and 2). The results of this study agree with those of many others in showing that SDS-PAGE of total seed storage proteins can be used to identify some or most faba bean genotypes (Volodin *et al.*, 1984; Perrino *et al.*, 1987 and Abdel-Tawab *et al.*, 1989). The data are useful for varietal identification and for breeders who seek to devise effective programs aimed at improving faba bean quality.

Genetic diversity of faba bean genotypes as determined by SDS-PAGE of seed storage proteins:

Analysis of genetic divergence in faba bean can provide some interesting information about differentiation, adaptability and interrelationships of genotypes. Only polymorphic bands were used in the construction of a binary matrix, reflecting the presence and absence of the legumin and vicilin protein

subunits in the different genotypes. The data obtained were transformed in genetic distances, which were used to group the varieties and landraces in a dendrogram by the UPGMA method. The linkage dendrogram provide visual idea about clustering and variability present in the populations. Cut off point at 10 dissimilarity points (Euclidean distances) was fixed as minimum dissimilarity. Based on the extent of relative dissimilarity among genotypes based on legumin and vicilin polypeptides, the 37 faba bean populations were grouped into three main clusters and seven subclusters (Figure 3). The obtained dendrogram discriminates all the faba bean populations examined. The dendrogram clearly demonstrated the ability of protein banding patterns to detect a large amount of genetic diversity in faba bean and to identify intergroup differences.

Three distinct groups were identified; that is, the landraces and the old local variety Rebaya 40 were segregated into one main group. The German KT variety belonging to the minor type remained separately as second group and the rest of faba bean varieties were grouped into third main group cluster. The clustering analysis further separated the varieties Roumi Acoador, Triple white and Sakha 2 in three small clusters within the larger varieties containing cluster. Cluster analysis also united the local variety Rebaya 40 and LR 33 and separated LR 34 into two discrete groups within the larger landraces-containing cluster. The clustering of faba bean genotypes using the storage protein data proved to be in accordance with their pedigree and taxonomic origin. Cluster analysis based on legumin and vicilin data clearly differentiate the genotypes according to their type (local landraces vs. varieties). Accordingly, the diversity analysis of both faba bean varieties and landraces was considered separately (Figure 3).

Genetic diversity of faba bean varieties as determined by SDS-PAGE of seed storage proteins:

The similarity coefficient values among the studied faba bean varieties based on band polymorphisms generated by SDS-PAGE of seed storage proteins are presented in Table (1). The similarity coefficients among faba bean varieties were found to have the range from 0.00 (distantly related) to 0.87 (most related). The

highest similarity value (0.87) was found between Nubaria 1 and Giza 3 and between Giza 461 and English, followed by a value of (0.86) between Sakha 1 and English. These data indicated that these faba bean populations were the most similar genotypes. The minimum similarity coefficient value (0.00) was found between the German variety KT (minor type) and the local Egyptian variety Rebaya 40 (equina type). This result indicated that these two distant populations showed significant genetic differentiation. It is also interesting to note that the similarity coefficient values between KT and all other genotypes were the lowest.

The index of genetic dissimilarity was used to calculate the pairwise distance matrix, which is presented in Table (2). The magnitude of genetic distances measured the extent of genetic diversity between the genotypes. Genetic distances were mostly small with the exception of those between KT as well as Rebaya 40 and all other varieties. Genetic distances ranged from 2.0 (most related) to 21.0 (distantly related). The highest magnitude of genetic distances among varieties was 21.0 and observed between the German variety KT and the local Egyptian variety Rebaya 40 followed by a distance of 17 between KT and Giza 2. The minimum genetic distance (2.0) was found between three pairs of faba bean varieties, i.e., Nubaria 1 and Giza 3; Giza 461 and English, Sakha 1 and English. Thus, the similarity between studied faba bean varieties could be ascribed to faba bean populations with a common origin and breeding history. The data presented in Table (2) indicated that the KT and Rebaya 40 were the most divergent genotype from all other genotypes. This suggested that there was enough genetic variability in legumin and vicilin polypeptides to set any faba bean genotype far apart from the others, especially KT and Rebaya 40.

The dendrogram produced from genetic distances between faba bean genotypes is shown in Figure (4). The linkage dendrogram provide visual idea about clustering and variability present in the populations. Based on the extent of relative dissimilarity among genotypes based on legumin and vicilin polypeptides, the 21 faba bean varieties were grouped into seven clusters. Cut off point at 7

dissimilarity points (Euclidean genetic distances) was fixed as minimum dissimilarity.

The grouping pattern and distribution of faba bean varieties into different clusters is given in Table (3). Cluster II was the largest having eight genotypes (Nubaria 1, Giza 40, Giza 3, Giza 461, Misr 2, Giza 843, Sakha 1 and English), while clusters I, IV and V had single genotype. The data suggested that the varieties KT (cluster I), Sakha 2 (cluster IV) and Rebaya 40 (cluster V) diverged most from the other genotypes. Cluster III consisted of two faba bean genotypes (Triple White and Roumi Acoador). Cluster VI contained five genotypes (Giza 717, Giza 716, Giza 429, Giza 643 and Giza 674). Cluster VII consisted of three genotypes (Pakistani, Giza 2 and Misr 1).

The average intra-cluster and inter-cluster genetic distances are presented in Table (4). The maxi-mum distance between clusters was between clusters I and II, followed by that between clusters I and VI, suggesting wide diversity between them. The minimum inter-cluster distance was observed between clusters V and VII, which was followed by clusters IV and V, indicating close relationship among the genotypes included. Generally, the magnitude of inter-cluster distances reflects the diversity exists among the studied faba bean genotypes. These results indicated wide genetic diversity between the studied faba bean varieties. The intra-cluster distance ranged from 0.00 (clusters I, IV and V) to 0.317 (cluster VII), indicating that the three varieties in cluster VII to be the most heterogeneous.

Cluster analysis using data on legumin and vicilin components, on the basis of Euclidean distances and measures of similarity (data on the absence / presence of bands in the electrophoretic pattern), allowed the detection of relationships among faba bean genotypes. These cluster groupings were mainly due to differences in legumin and vicilin banding patterns. Diversity analysis of the 21 faba bean varieties found high levels of genetic diversity among these varieties and sub-divided them into seven main groups with subdivision into sub-groups consistent with breeding history, origin and parentage of the varieties.

Genetic diversity of faba bean landraces as determined by SDS-PAGE of seed storage proteins:

The similarity coefficient values among the 16 studied faba bean landraces based on band polymorphisms generated by SDS-PAGE of seed storage proteins are presented in Table (5). The similarity coefficients among faba bean landraces were ranged from 0.20 (distantly related) to 0.87 (most related) with an average value of 0.514. The highest similarity value (0.87) was found between LR 25 and LR 28, followed by a value of (0.81) between LR 26 and LR 29. These data indicated that these faba bean populations were the most similar genotypes. The minimum similarity coefficient value (0.20) was found between LR 25 and LR 35. This result indicated that these two distant populations showed significant genetic differentiation. It is also of interesting to note that the similarity coefficient values between LR 34 as well as LR 33 and all other landraces were the lowest. Therefore, these two accessions may be important sources of genetic variation within this collection of faba bean germplasm.

Genetic distances between faba bean landraces are presented in Table (6). Genetic distances were mostly small with the exception of those between LR 33 as well as LR 34 and all other populations. Genetic distances ranged from 2.0 (most related) to 13.0 (distantly related). This showed that the range of genetic distances observed with landraces was lower than that observed between faba bean varieties. This demonstrates that the varieties used in this study are more diverse than local landraces and could be related to different origin of the genotypes, local and introduced varieties. In fact, utilization of genotypes with the same origin imply a low genetic diversity, since these genotypes may have exchanged genetic material through breeding programs. However, a high level of diversity may also be due to the level of polymorphism of the legumin and vicilin storage proteins. The highest magnitude of genetic distances among genotypes was between LR 28 and LR 34 followed by a distance of 12 between LR 33 and each of LR 28 and LR 29. The minimum genetic distance (2.0) was found between the two faba bean landraces LR 25

and LR 28, followed by a distance of 3.0 between three pairs of faba bean landraces LR 22 and LR 26; LR 26 and LR 29; LR 23 and LR 36. The data suggested that there was enough genetic variability in legumin and vicilin polypeptides to set any faba bean genotype far apart from the others, especially LR 33 and LR 34. The data presented in Table (6) indicated that the LR 33 and LR 34 were the most divergent landraces from all other genotypes. The similarity observed between the studied faba bean landraces could be ascribed to faba bean populations sharing a common ancestral population and geographical region.

The dendrogram produced from genetic distances between faba bean genotypes is shown in Figure (5). Based on the extent of relative dissimilarity among genotypes based on legumin and vicilin polypeptides, the 16 faba bean landraces were grouped into eight clusters. Cut off point at five dissimilarity points (Euclidean genetic distances) was fixed as minimum dissimilarity. The grouping pattern and distribution of faba bean genotypes into different clusters is given in Table (7). Clusters I, II and IV had single genotype. The data suggested that the landrace LR 33 (cluster I), LR 34 (cluster II) and LR 30 (cluster IV) diverged most from the other genotypes. Clusters III, VI and VIII consisted of three landraces, while clusters V and VII had two landraces (Table 7).

The average intra-cluster and inter-cluster genetic distances are presented in Table (8). The maxi-mum distance between clusters (0.590) was between clusters I and VII and between clusters II and VII, followed by a distance of 0.577 between clusters II and IV, suggesting wide diversity between them. The minimum inter-cluster distance (0.329) was observed between clusters III and V, which was followed by a distance of 0.358 between clusters III and VI, indicating close relationship among the genotypes included. Generally, the magnitude of inter-cluster distances reflects the diversity exists among the studied faba bean genotypes. These results indicated wide genetic diversity between the studied faba bean landraces. The intra-cluster distance ranged from 0.00 (clusters I, II and IV) to 0.224 (cluster III), indicating that the three landraces in cluster III to be the most heterogeneous.

Cluster analysis using data on legumin and vicilin components, on the basis of Euclidean distances and measures of similarity (data on the absence/presence of bands in the electrophoretic pattern), allowed the detection of relationships among faba bean genotypes. These cluster groupings were mainly due to differences in legumin and vicilin banding patterns, and showed correspondence with breeding and taxonomic origin. The existence of such a wide genetic diversity among faba bean genotypes based on seed storage protein polymorphisms were reported by Stegemann *et al.*, (1980); Abdel-Tawab *et al.*, (1989); Polignano *et al.* (1990 and 1991); Minelli and Cionini, (2002) and Santalla *et al.*, (2004).

The data indicated that legumin and vicilin cotyledonary proteins can provide a relatively unbiased methods of quantifying genetic diversity among faba bean genotypes. Several studies applied cluster analysis of faba bean genotypes based on legumin and vicilin components in order to identify the distribution of genetic diversity within the species (Link *et al.*, 1995 and Zeid *et al.*, 2001 and 2003). Analysis of legumin and vicilin proteins can also be a useful tool for evaluating genetic variability in faba bean germplasm collections for breeding purposes. The use of biochemical polymorphisms in cultivar identification is a well-known practice that is used in many countries (Barrat, 1980; Voldin *et al.*, 1984; Abdel-Tawab *et al.*, 1989; Polignano *et al.*, 1998, Potokina *et al.*, 2000 and Santalla *et al.*, 2004). They carried out cluster analysis, using data on isozyme components, based on Euclidean distances and measures of similarity (data on the absence/presence of bands in the electrophoretic pattern). This type of analysis in crop species is fundamental for designing optimal germplasm collection, management practices and for developing an index for parental selection.

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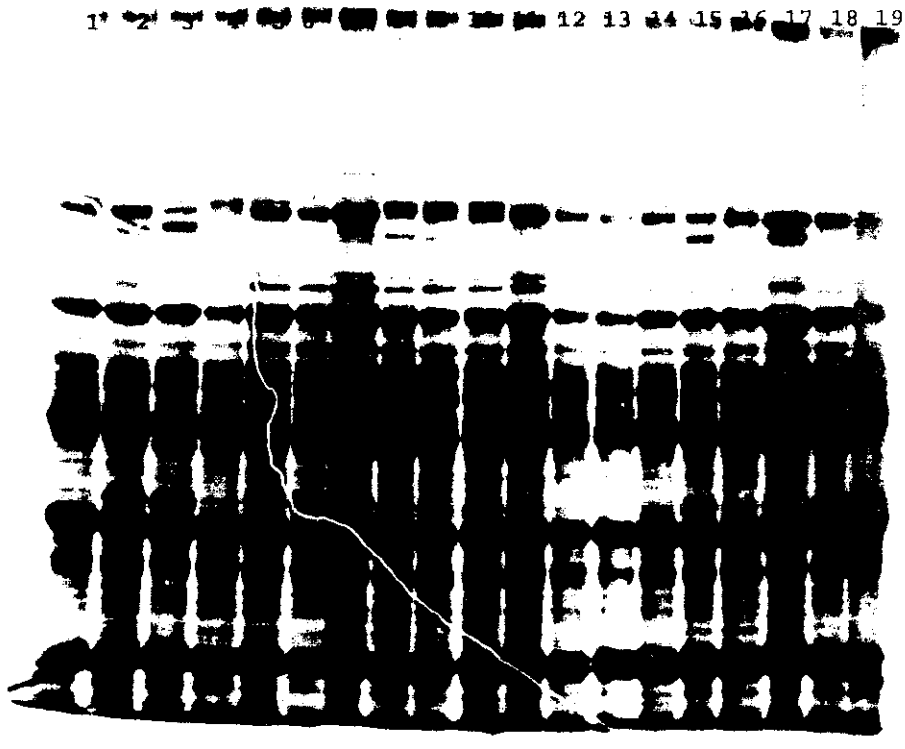

Fig. (1): A SDS polyacrylamide gel (SDS-PAGE) electrophoregram with legumin and vicilin protein polypeptides found in this set of faba bean varieties indicated. Lanes: 1, Pakistani; 2, Nubaria 1; 3, Triple White; 4, Giza 2; 5, Giza 40; 6, Giza 3; 7, Giza 461; 8, Misr 1; 9, Misr 2; 10, Giza 843; 11, Sakha 1; 12, Sakha 2; 13, Giza 717; 14, Giza 716; 15, Giza 429; 16, Giza 643; 17, English; 18, Giza 674 and 19, Kleine Thuringer (KT).

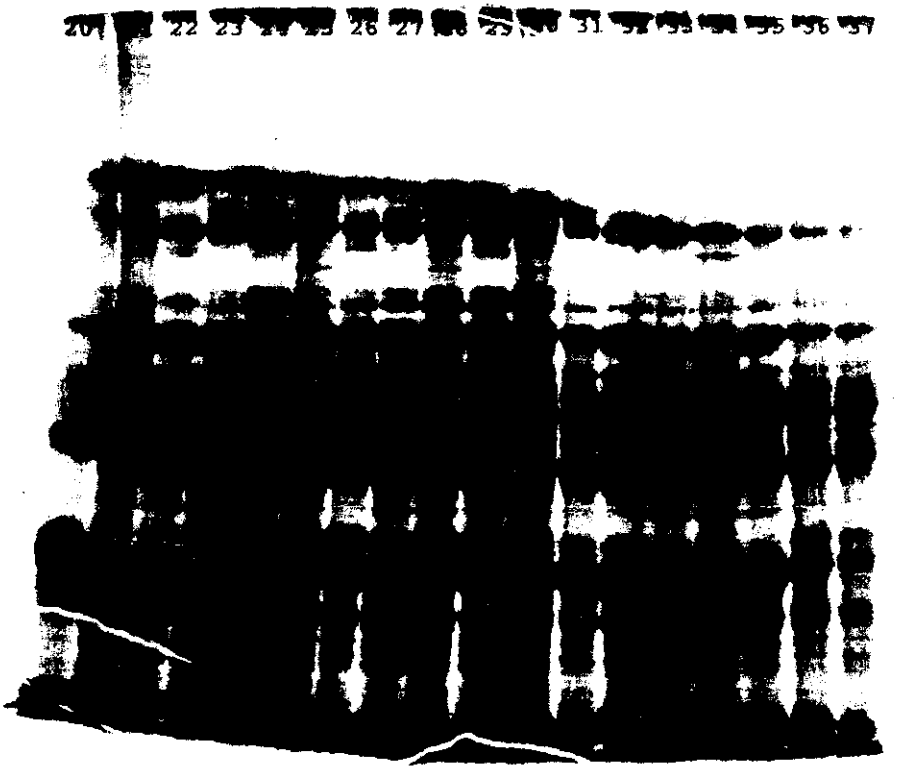

Fig. (2): A SDS polyacrylamide gel (SDS-PAGE) electrophoregram with legumin and vicilin protein polypeptides found in two varieties and sixteen faba bean landraces indicated. Lanes: 20, Roumi Acoador; 21, Rebaya 40; 22, LR 22; 23, LR 23; 24, LR 24; 25, LR 25; 26, LR 26; 27, LR 27; 28, LR 28; 29, LR 29; 30, LR 30; 31, LR 31; 32, LR 32; 33, LR 33; 34, LR 34; 35, LR 35; 36, LR 36 and 37, LR 37.

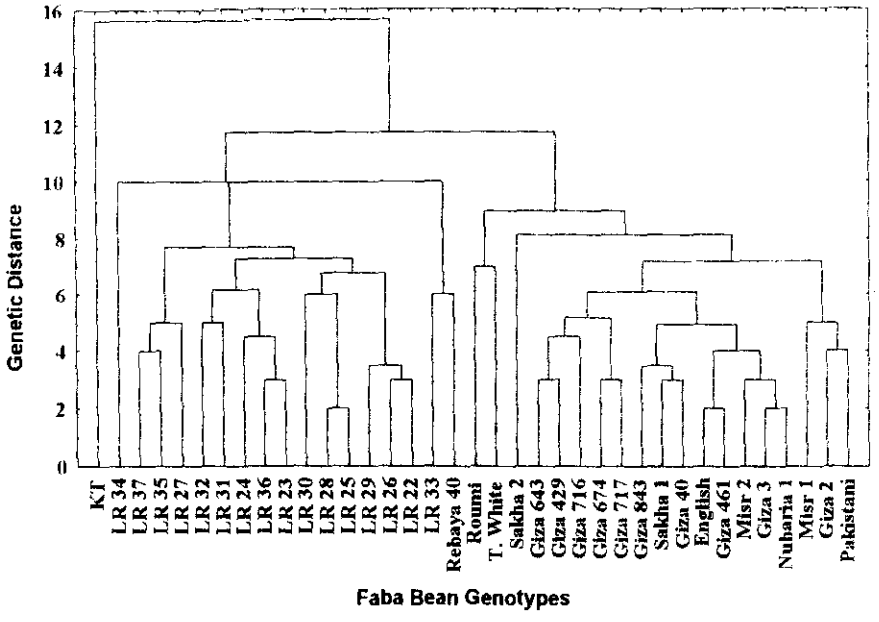

Fig. (3): Linkage dendrogram of studied faba bean varieties and landraces based on legumin and vicilin protein polypeptides revealed by SDS-PAGE of seed storage proteins.

Table (1): Similarity coefficients among the studied 21 faba bean varieties based on SDS-PAGE of legumin and vicilin storage protein polypeptides.

Varieties	Pakistani (1)	Nubaria 1 (2)	T. White (3)	Giza 2 (4)	Giza 40 (5)	Giza 3 (6)	Giza 461 (7)	Misr 1 (8)	Misr 2 (9)	Giza 843 (10)	Sakha 1 (11)	Sakha 2 (12)	Giza 717 (13)	Giza 716 (14)	Giza 429 (15)	Giza 643 (16)	English (17)	Giza 674 (18)	KT (19)	Roumi (20)	Rehaya (21)
2	0.53																				
3	0.49	0.68																			
4	0.61	0.21	0.35																		
5	0.60	0.63	0.31	0.32																	
6	0.53	0.87	0.55	0.21	0.63																
7	0.51	0.67	0.48	0.31	0.58	0.67															
8	0.57	0.31	0.41	0.55	0.52	0.31	0.45														
9	0.58	0.80	0.48	0.24	0.69	0.80	0.72	0.50													
10	0.63	0.60	0.41	0.27	0.77	0.60	0.63	0.55	0.79												
11	0.73	0.67	0.48	0.42	0.82	0.67	0.72	0.50	0.73	0.79											
12	0.54	0.65	0.46	0.44	0.52	0.65	0.50	0.30	0.60	0.55	0.60										
13	0.46	0.60	0.41	0.46	0.50	0.60	0.34	0.40	0.65	0.43	0.51	0.55									
14	0.53	0.48	0.42	0.38	0.63	0.48	0.53	0.60	0.54	0.46	0.67	0.53	0.60								
15	0.58	0.54	0.48	0.42	0.69	0.40	0.43	0.65	0.46	0.51	0.73	0.47	0.51	0.67							
16	0.63	0.74	0.68	0.46	0.64	0.60	0.63	0.55	0.65	0.57	0.79	0.55	0.57	0.74	0.79						
17	0.73	0.80	0.61	0.42	0.69	0.80	0.87	0.50	0.73	0.65	0.86	0.60	0.51	0.67	0.59	0.79					
18	0.73	0.67	0.61	0.60	0.56	0.67	0.58	0.65	0.73	0.51	0.73	0.60	0.79	0.67	0.73	0.79	0.73				
19	0.27	0.35	0.27	0.17	0.32	0.22	0.22	0.31	0.31	0.13	0.31	0.29	0.26	0.48	0.44	0.53	0.31	0.44			
20	0.45	0.37	0.56	0.32	0.26	0.50	0.44	0.23	0.30	0.23	0.43	0.39	0.23	0.37	0.30	0.50	0.56	0.43	0.07		
21	0.45	0.39	0.32	0.19	0.37	0.39	0.39	0.20	0.35	0.43	0.48	0.22	0.30	0.14	0.35	0.30	0.48	0.35	0.00	0.49	

Table (2): Genetic distances, calculated as the total number of band differences, among the studied 21 faba bean varieties.

Varieties	Pakistani (1)	Nubaria 1 (2)	T. White (3)	Giza 2 (4)	Giza 40 (5)	Giza 3 (6)	Giza 461 (7)	Misr 1 (8)	Misr 2 (9)	Giza 843 (10)	Sakha 1 (11)	Sakha 2 (12)	Giza 717 (13)	Giza 716 (14)	Giza 429 (15)	Giza 643 (16)	English (17)	Giza 674 (18)	KT (19)	Roumi (20)	Rebaya (21)
2	7																				
3	8	5																			
4	4	11	10																		
5	7	6	11	11																	
6	7	2	7	11	6																
7	6	5	8	8	7	5															
8	5	10	9	5	8	10	7														
9	6	3	8	10	5	3	4	7													
10	5	6	9	9	4	6	5	6	3												
11	4	5	8	8	3	5	4	7	4	3											
12	9	6	9	11	8	6	9	12	7	8	7										
13	7	6	9	7	8	6	9	8	5	8	7	8									
14	7	8	9	9	6	8	7	6	7	8	5	8	6								
15	6	7	8	8	5	9	8	5	8	7	4	9	7	5							
16	5	4	5	7	6	6	5	6	5	6	3	8	6	4	3						
17	4	3	6	8	5	3	2	7	4	5	2	7	7	5	6	3					
18	4	5	6	6	7	5	6	5	4	7	4	7	3	5	4	3	4				
19	15	12	13	17	12	14	15	14	13	16	13	12	14	10	11	10	13	11			
20	9	10	7	11	12	8	9	12	11	12	9	10	12	10	11	8	7	9	16		
21	12	11	12	16	11	11	12	15	12	11	10	13	13	15	12	13	10	12	21	9	

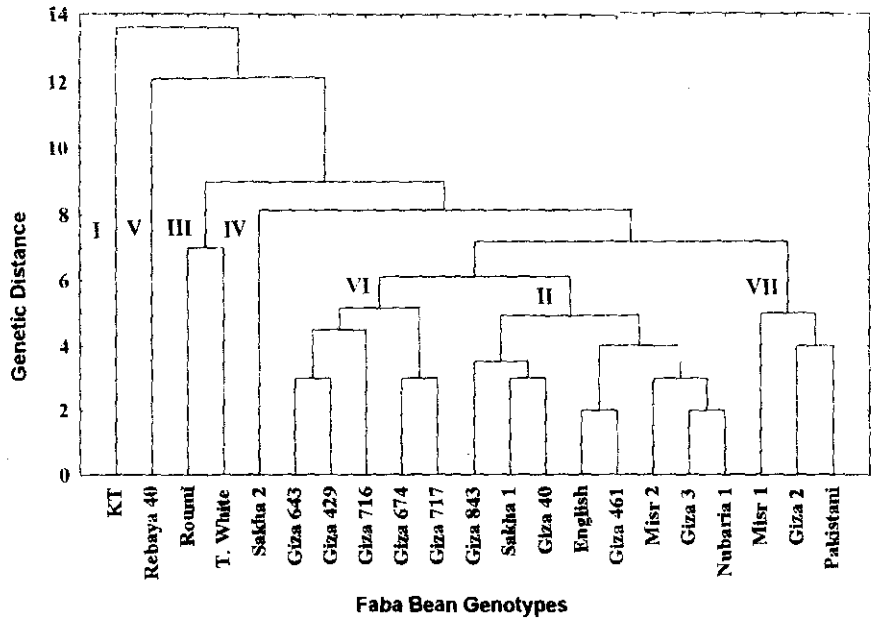

Fig. (4): Linkage dendrogram of the studied 21 faba bean varieties based on legumin and vicilin protein polypeptides revealed by SDS-PAGE of seed storage proteins.

Table (3): Grouping pattern of the studied 21 faba bean varieties.

Cluster	No. of genotypes	Faba bean varieties falling in cluster
I	1	KT
II	8	Nubaria 1, Giza 40, Giza 3, Giza 461, Misr 2, Giza 843, Sakha 1 and English.
III	2	Triple White and Roumi Equador
IV	1	Sakha 2
V	1	Rebaya 40
VI	5	Giza 717, Giza 716, Giza 429, Giza 643 and Giza 674
VII	3	Pakistani, Giza 2 and Misr 1

Table (4): Intra-cluster (in bold) and inter-cluster genetic distances among seven clusters of the studied 21 faba bean varieties.

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	**0.000**						
Cluster II	0.798	**0.284**					
Cluster III	0.628	0.603	**0.230**				
Cluster IV	0.581	0.554	0.406	**0.000**			
Cluster V	0.604	0.532	0.449	0.272	**0.000**		
Cluster VI	0.697	0.522	0.461	0.485	0.503	**0.304**	
Cluster VII	0.559	0.579	0.411	0.348	0.265	0.487	**0.317**

Table (5): Similarity coefficients among the studied fifteen faba bean landraces based on SDS-PAGE of legumin and vicilin storage protein polypeptides.

Landraces	LR 22	LR 23	LR 24	LR 25	LR 26	LR 27	LR 28	LR 29	LR 30	LR 31	LR 32	LR 33	LR 34	LR 35	LR 36
LR 23	0.40														
LR 24	0.54	0.74													
LR 25	0.46	0.54	0.54												
LR 26	0.80	0.61	0.74	0.54											
LR 27	0.58	0.39	0.39	0.43	0.53										
LR 28	0.43	0.67	0.67	0.87	0.67	0.39									
LR 29	0.75	0.55	0.55	0.61	0.81	0.62	0.62								
LR 30	0.52	0.70	0.45	0.65	0.58	0.53	0.67	0.63							
LR 31	0.46	0.67	0.54	0.46	0.54	0.43	0.58	0.61	0.65						
LR 32	0.30	0.63	0.63	0.43	0.50	0.30	0.44	0.56	0.45	0.69					
LR 33	0.26	0.58	0.32	0.26	0.32	0.40	0.26	0.26	0.51	0.52	0.45				
LR 34	0.34	0.40	0.40	0.34	0.40	0.36	0.22	0.58	0.33	0.47	0.64	0.45			
LR 35	0.35	0.60	0.45	0.20	0.45	0.61	0.29	0.41	0.48	0.50	0.37	0.62	0.30		
LR 36	0.32	0.80	0.67	0.46	0.54	0.43	0.58	0.48	0.52	0.59	0.56	0.52	0.47	0.50	
LR 37	0.50	0.60	0.60	0.35	0.60	0.61	0.45	0.56	0.48	0.65	0.66	0.62	0.44	0.67	0.65

Table (6): Genetic distances, calculated as the total number of band differences, as revealed by SDS-PAGE of legumin and vicilin protein polypeptides, among the studied 16 faba bean landraces.

Landraces	LR 22	LR 23	LR 24	LR 25	LR 26	LR 27	LR 28	LR 29	LR 30	LR 31	LR 32	LR 33	LR 34	LR 35	LR 36
LR 23	9.0														
LR 24	7.0	4.0													
LR 25	8.0	7.0	7.0												
LR 26	3.0	6.0	4.0	7.0											
LR 27	6.0	9.0	9.0	8.0	7.0										
LR 28	8.0	5.0	5.0	2.0	5.0	8.0									
LR 29	4.0	7.0	7.0	6.0	3.0	6.0	6.0								
LR 30	8.0	5.0	9.0	6.0	7.0	8.0	6.0	6.0							
LR 31	8.0	5.0	7.0	8.0	7.0	8.0	6.0	6.0	6.0						
LR 32	11.0	6.0	6.0	9.0	8.0	11.0	9.0	7.0	9.0	5.0					
LR 33	12.0	7.0	11.0	12	11.0	10.0	12.0	12.0	8.0	8.0	9.0				
LR 34	11.0	10.0	10.0	11.0	10.0	11.0	13.0	7.0	11.0	9.0	6.0	9.0			
LR 35	9.0	6.0	8.0	11.0	8.0	5.0	9.0	9.0	9.0	7.0	10.0	7.0	12.0		
LR 36	10.0	3.0	5.0	8.0	7.0	8.0	6.0	8.0	8.0	6.0	7.0	8.0	9.0	7.0	
LR 37	7.0	6.0	6.0	9.0	6.0	5.0	7.0	7.0	9.0	5.0	6.0	7.0	10.0	4.0	5.0

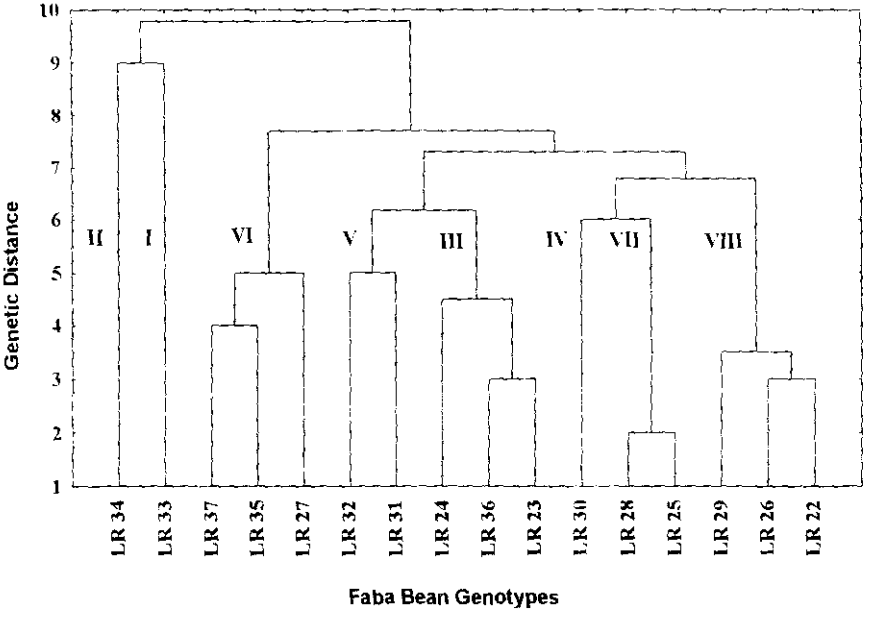

Fig. (5): Linkage dendrogram of the studied 16 faba bean landraces based on legumin and vicilin protein polypeptides revealed by SDS-PAGE of seed storage proteins.

Table (7): Grouping pattern of the studied faba bean landraces.

Cluster	No. of genotypes	Faba bean landraces falling in cluster
I	1	LR 33
II	1	LR 34
III	3	LR 23, LR 24 and LR 36
IV	1	LR 30
V	2	LR 31 and LR 32
VI	3	LR 27, LR 35 and LR 37
VII	2	LR 25 and LR 28
VIII	3	LR 22, LR 26 and LR 29

Table (8): Intra-cluster (in bold) and inter-cluster genetic distances among eight clusters of the studied 16 faba bean landraces.

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	**0.000**							
Cluster II	0.522	**0.000**						
Cluster III	0.471	0.503	**0.224**					
Cluster IV	0.492	0.577	0.426	**0.000**				
Cluster V	0.469	0.435	0.329	0.435	**0.194**			
Cluster VI	0.442	0.535	0.358	0.464	0.390	**0.232**		
Cluster VII	0.590	0.590	0.369	0.408	0.435	0.448	**0.123**	
Cluster VIII	0.566	0.499	0.381	0.422	0.407	0.372	0.391	**0.192**

التباعد الوراثى بين عشائر الفول البلدى على اساس التعدد المظهرى للبروتينات المختزنة فى البذور

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اجريت تحليلات التفريد الكهربائي في وجود الصوديوم دودوسيل سلفات (SDS-PAGE) على البروتينات المختزنة فى البذور و المستخلصة من ٢١ صنف من اصناف الفول البلدى وكذلك ١٦ من السلالات المحلية المنزرعة فى محافظة الشرقية بهدف تعيين التعدد المظهرى الوراثي للبروتينات المختزنة وقد استخدم التعدد المظهرى الوراثي للبروتينات المختزنة فى تقدير التباعد الوراثى بين التراكيب الوراثية المستخدمة فى الدراسة. وقد تم الكشف عن عدة تباينات من البروتينات المخترنة في هذه المجموعة من الفول البلدى وأعطى التفريد الكهربي ٣٠ حزمة من البروتينات وهى موجودة في بعض التراكيب الوراثية وتغيب من البعض الأخر مع وجود اختلافات في الوزن الجزيئى. وقد وجد ان ١١ حزمة كانت polymorphic وقد وجدت إختلافات فى كثافة الحزم فى طرز التراكيب الوراثية المختلفة وقد تم التعرف وتمييز معظم الأصناف بواسطة SDS-PAGE. وقد استخدمت مصفوفة وجود أو غياب حزم البروتينات في حساب المسافات الوراثية. وقد قسم تحليل المجموعات cluster analysis على اساس المسافات الوراثية المحسوبة التراكيب الوراثية الى ثلاثة مجاميع رئيسية clusters وتحت سبعة مجاميع. وقد اشتملت المجموعة الأولى على كل السلالات المحلية بالأضافة الى الصنف المحلى القديم رباية ٤٠، وقد اشتملت المجموعة الثانية على الصنف الألمانى KT بمفرده. وقد احتوت المجموعة الثالثة على بقية اصناف الفول البلدى المدروسة. وكانت المسافات الوراثية المحسوبة بين الأصناف صغيرة باستثناء تلك المحسوبة بين كل من KT و الصنف المحلى القديم رباية ٤٠ وبقية الأصناف الأخرى. وقد قسمت اصناف الفول البلدى المدروسة الى سبعة مجاميع clusters وكان هذا التقسيم متوافق مع المنشأ والوضع التقسيمى. وتوضح البيانات بأن كلا من السلالتين LR 34 و LR 33 كانتا الأكثر تباعداً من بقية السلالات. وقد قسمت سلالات الفول البلدى المدروسة الى ثمانية مجاميع clusters.