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Genetic Variation Among Egyptian Cultivars of *Vicia faba* L.

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Abstract: Nine Egyptian cultivars of *Vicia faba* were analyzed using electrophoretic and quantitative data from seed albumins and globulins and 100 seeds weight to measure genetic variation among faba bean cultivars in Egypt. Wide genetic variation was indicated for all the traits studied. The elctrophoregrams showed identity profile for each cultivar supporting the validity of electrophoresis of seed protein components in cultivar identification and assessing genetic variation in *Vicia faba* and other out-breeding plants at the infra-specific level. Negative correlation was indicated between seed globulins and both seed albumins and seed weight. Principal component analysis and cluster analyses indicated higher role of seed albumins over seed globulins in genetic variation within *Vicia faba* in Egypt.

Key words: *Vicia faba*, genetic diversity, cultivar identification, seed proteins

INTRODUCTION

Seed storage proteins represent a major component of human protein intake, either directly or indirectly via their consumption by livestock. Whereas cereal grains contain less than 15% protein, legume seed generally contains more than 20% (Payne, 1983; Signor *et al*., 2005). On a world basis, legume seeds are the second most important protein source after cereals (Perrot, 1995; Signor *et al*., 2005).

Characterization of the genetic variation in the available germplasm is important for further improvement of crop yield and to impart resistance to biotic and abiotic stresses (Kour and Singh, 2004). Using the morphological data in cultivar identification, studying genetic diversity and phenetic classification of plants that was previously scored for many plants (Bult and Kiang, 1992; Zviniene and Pank, 1996) is very time consuming and may be unreliable (Ahmad *et al*., 1997) since some of these characteristics are strongly affected by the environment. These problems have now been overcome, in many crops, by the use of electrophoresis to discriminate cultivars on the basis of genotype-specific protein markers (Barratt, 1980; Stegemann, 1983; Hussain *et al*., 1986). In addition, evidences provided by the separation of seed protein components in polyacrylamide gels are mostly informative at the species and infra-specific levels (Nei *et al*., 1978; Ladizinsky and Hymowitz, 1979; Cooke, 1984; Badr, 1995). Seed proteins data have therefore been applied to study genetic diversity at infraspecific levels (Signor *et al*., 2005; Mustafa *et al*., 2006; Sammour *et al*., 2007) and also for cultivar identification (Cooke, 1984; Sammour, 1988, 1990b, 1992; Krochko and Bewley, 2000; Thanh *et al*., 2006). In addition to morphological and seed proteins electrophoretic profiles, molecular, isozymes and immunological data were utilized in studying genetic diversity, cultivar identification and assessment of taxonomic and genetic relationships in *Vicia* and many other plants at generic, specific and infra-specific levels (Torres *et al*., 1993; Conney *et al*., 1998; Croft *et al*., 1999; Shiran and Raina, 2001; Badr *et al*., 2002; Zeid, 2003; Sammour, 2005; Mustafa *et al*., 2005, 2006; Shiran *et al*., 2006; Sorkheh *et al*., 2007). Among the mentioned techniques, the seed protein electrophoresis is considered the cheapest and less time consuming.

Using the electrophoretic techniques to study the Egyptian crop cultivars will allow us to identify between cultivars and screen the purity of the ever expanding number of cultivars, to clarify taxonomic and evolutionary problems, to exploit the important traits of landraces and wild relatives and to evaluate the genetic variation among the collected accessions. All these information are important for crop improvement programs, certification authorities and also in genetic resource management (Forde and Gardiner, 1986; Gardiner and Forde, 1988; Sammour, 1990b; Kour and Singh, 2004).

Using electrophoresis of the total seed proteins showed some shortage in discrimination between *Vicia faba* cultivars (Gardiner and Forde, 1988; Thanh *et al*., 2006). In this study, the author tried to use electrophoretic data of both seed albumins and globulins in addition to some other traits to study the genetic variations among

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nine Egyptian cultivars of *Vicia faba*, to test the validity of the applied technique for faba bean cultivar identification and also as a basic requirement for further crop traits improvement programs.

MATERIALS AND METHODS

This study was conducted at Botany Department, Faculty of Science, Tanta University at 2005-2006. Seeds of *Vicia faba* L. cultivars were collected from the Agricultural research station, institute of legumes, Sakha, Kafr El-Sheikh, Egypt. The cultivars list is shown in Table 1.

Quantitative estimation of seed proteins: Albumin and Globulin proteins were extracted successively from 20 mg air-dried defatted seed meals in 1000 μL extraction solution (distilled water for albumins then 5% NaCl w/v for globulins) for 24 h at -4°C, according to Croy and Gatehouse (1985) with some modifications. The extracts were centrifuged for 10 min at 10000 g and quantitative estimation of total proteins was made according to Bradford (1976). Three replicas were made for each sample.

SDS-PAGE: For electrophoresis, extracts were mixed with 10% sucrose and used directly for electrophoresis or kept at -20°C until use. Dissociating polyacryamide gel electrophoresis (SDS-PAGE) was adopted after Laemmli (1970) with some modifications. Samples of 15 μL were applied onto main gels of 17% acrylamide concentration using bromophenol blue as a front dye. Electrophoresis was carried out using 25 mM tris/glycine buffer pH 8.3. The gel was stained overnight by slow shaking in 100 mL staining mixture (0.05% Coammassie Brillient Blue-R250 in 50% methanol, 7% glacial acetic acid and 43% distilled water) and de-stained by slow shaking in the stain solvent. The subunit molecular weight of the protein bands was determined according to Weber and Osberne (1969) through a standard curve established for each gel. Each band was considered as a character for which the presence or absence was coded by 1 or 0, respectively in a data matrix for statistical analysis. Density of the protein bands was not considered.

100 seeds-weight: Weight 100 seed in each sample and notes it; the difference in weight present among samples may indicate the genetic variation.

Data analysis: The genetic diversity among the populations of the studied cultivars (evaluated by Dice similarity index) and multivariate analysis (factor analyses and cluster analysis) were made using the software SPSS for windows package (Version 10) copyright (C) 1999, SPSS INC. A dendrogram was constructed through the complete linkage-joining rule. Nei's similarity index (Nei and Li, 1979) was calculated also among the studied cultivars where:

$$\text{Nei's similarity index} = \frac{\text{The No. of bands in common among all the cultivars}}{\text{Total No. of bands}}$$

The similarity matrix values were converted into percentages.

RESULTS

The results obtained in this study showed little variation in the number of bands of either globulins [13-16 bands] or albumins electrophoregrams [15-18 bands] (Fig. 1, Table 1). The studied cultivars showed higher Nei's similarity index for globulins (57.79%) than that for albumins electrophoregrams (42.857). The studied cultivars of *Vicia faba* exhibited wide genetic variation in relation to each of 100 seeds weight, albumin and globulin seed protein contents (Table 1). A negative correlation was exhibited between seed globulin content and both seed weight (-0.381) (significant) and seed albumin content (-0.602) (highly significant), while 100 seeds weight showed small positive non-significant correlation (0.121) with seed albumin content (Table 2).

The first two components account for 88.752% of the total variance of the studied. Separate percentages of variation attributed to the first two components are

Table 1: Hundred seeds weight, No. of bands in the electrophoregrams and seed contents of Albumin and globulin in faba bean cultivars

Cultivars	100 seeds wt.	No. of albumin bands	Albumin (mg g^{-1})	No. of globulin bands	Globulin (mg L^{-1})
Sakha 2	97.00	16	50.5±4.8	16	31.8±0.7
Giza843	75.90	16	58.3±3.5	16	26.5±1.5
Giza3	83.20	15	29.4±2.6	14	53.0±0.9
Sakha 1	59.24	18	36.1±4.4	14	46.6±1.3
Sakha3	94.94	15	30.8±4.0	13	42.4±2.9
Misr1	64.74	18	44.7±4.3	15	41.6±2.6
Giza461	86.94	15	36.9±3.8	15	25.1±3.2
Nobaria1	95.00	16	58.7±2.4	14	27.4±0.4
Giza716	84.00	17	39.5±4.2	16	26.3±2.6

Table 2: Correlation coefficients among seed weight, seed globulin and seed albumin contents of faba bean cultivars

Parameters		100 seeds wt.	Al Qn (mg g^{-1})	Gl Qn (mg g^{-1})
Correlation	100 seeds wt.	1.000		
	Al Qn (mg g^{-1})	0.121	1.000	
	Gl Qn (mg g^{-1})	-0.381	-0.602	1.000
Significance	100 seeds wt.			
	Al Qn (mg g^{-1})	0.286		
	Gl Qn (mg g^{-1})	0.033	0.0009	

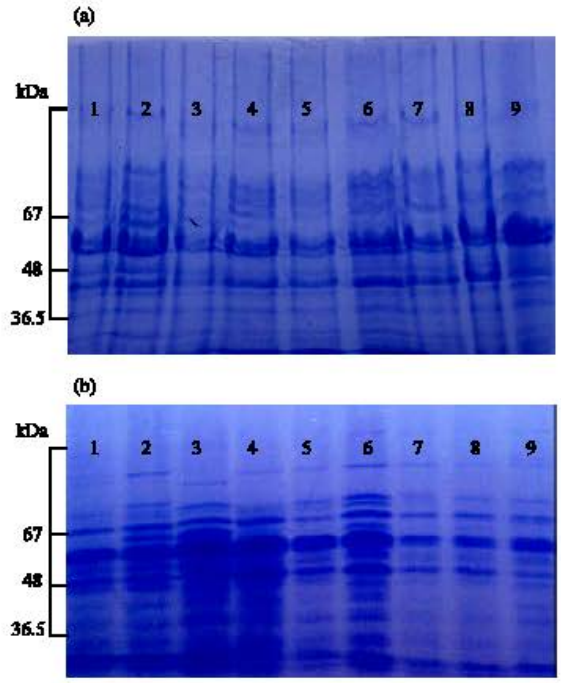

Fig. 1: Electrophoregrams of seed proteins (a) albumins and (b) globulins of *Vicia faba* cultivars: 1 (sakha2), 2 (Giza843), 3 (Giza3), 4 (sakha1), 5 (sakha3), 6 (misr1), 7 (Giza461), 8 (nobaria1), 9 (Giza716)

59.05 and 29.703%, with Eigen values of 1.771 and 0.891, respectively. Albumin content showed higher coefficient in the first component (PRIN1), while the 100 seeds wt. showed higher variation coefficient with the second component (PRIN2) (Table 3). The third component showed significant sharing of variation to associate with globulin content (not shown data). In this study, the first two component, were considered.

Cluster analysis of electrophoretic globulin data (Table 4) showed highest genetic similarity between misr 1 and giza 461 according to Dice measure. Farthest genetic distance was exhibited between giza 3 and each of sakha 2, giza 843 and giza 716 and between nobaria 1 and both sakha 2 and giza 843. However, highest genetic similarity index was exhibited between misr 1 and giza 461.

Based on electrophoretic data of albumin alone or aggregated with globulin data, nearest genetic distance was exhibited between sakha1 and misr 1, while farthest genetic distance was exhibited between giza3 and giza 716 according to Dice measure (Table 5, 6). Dendrograms based on cluster analysis according to Dice measure (Fig. 2-4) showed two major groups. In case of electrophoretic data of albumin alone or aggregated with globulin data, the smaller group included three cultivars (Fig. 2, 4), while in case of electrophoretic data of globulin alone, the smaller group included four cultivars. The studied cultivars were distributed into three groups at a genetic distance of 0.21, based on albumin electrophoretic data (Fig. 2) and at a genetic distance of 0.19, based on globulin electrophoretic data (Fig. 3). On aggregating

Table 3: Principal component analysis of *Vicia faba* cultivars, Eigen-values and percent of variation accounted by the first two components

Trait	PRIN1	PRIN2
100 seeds wt.	0.571	0.801
Albumin (mg g^{-1}) dry wt.	0.794	-0.494
Globulin (mg g^{-1}) dry wt.	-0.903	0.072
Eigen-value	1.771	0.891
Variation (%)	59.050	29.703
Variation cumulative (%)	59.050	88.752

Table 4: Genetic similarity index (Dice measure) among *Vicia faba* cultivars based on globulin electrophoretic data

Case	Sakha2	Giza843	Giza3	Sakha1	Sakha3	Misr1	Giza461	Nobaria1	Giza716
Sakha 2									
Giza843	0.531								
Giza3	**0.000**	**0.000**							
Sakha 1	0.500	0.500	0.464						
Sakha3	0.224	0.224	0.167	0.722					
Misr1	0.274	0.274	0.741	0.741	0.464				
Giza461	0.274	0.274	0.741	0.741	0.464	**1.000**			
Nobaria1	**0.000**	**0.000**	0.464	0.464	0.167	0.741	0.741		
Giza716	0.531	0.531	**0.000**	0.500	0.224	0.274	0.274	0.000	

Table 5: Genetic similarity index (Dice measure) among *Vicia faba* cultivars based on albumin electrophoretic data

Case	Sakha2	Giza843	Giza3	Sakha1	Sakha3	Misr1	Giza461	Nobaria1	Giza716
Sakha2									
Giza843	0.500								
Giza3	0.097	0.355							
Sakha1	0.529	0.294	0.394						
Sakha3	0.097	0.097	0.467	0.636					
Misr1	0.529	0.294	0.394	**1.000**	0.636				
Giza461	0.613	0.355	0.200	0.636	0.200	0.636			
Nobaria1	0.500	0.500	0.613	0.529	0.355	0.529	0.355		
Giza716	0.394	0.152	**0.000**	0.657	0.250	0.657	0.750	0.152	

Table 6: Genetic similarity index (Dice measure) among *Vicia faba* cultivars based on albumin and globulin electrophoretic data

Case	Sakha2	Giza843	Giza3	Sakha1	Sakha3	Misr1	Giza461	Nobaria1	Giza716
Sakha 2									
Giza843	0.560								
Giza3	0.074	0.258							
Sakha 1	0.560	0.385	0.442						
Sakha3	0.151	0.151	0.397	0.712					
Misr1	0.482	0.309	0.543	**1.000**	0.626				
Giza461	0.543	0.362	0.420	0.724	0.312	0.819			
Nobaria1	0.362	0.362	0.611	0.543	0.312	0.641	0.525		
Giza716	0.482	0.309	**0.000**	0.655	0.258	0.576	0.641	0.106	

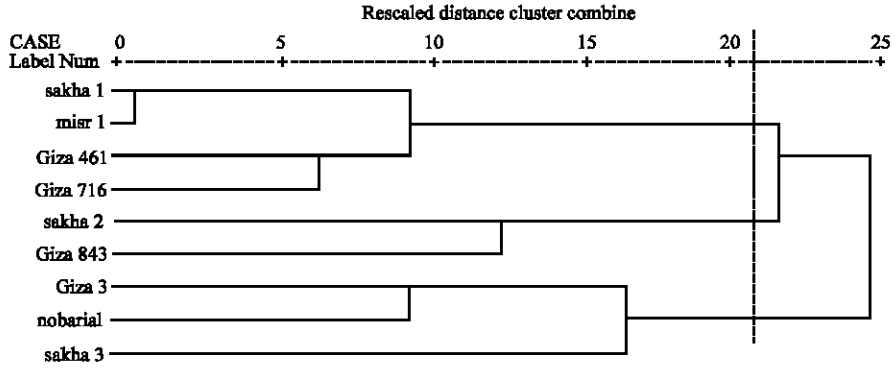

Fig. 2: Dendrograms based on electrophoretic data of seed albumins according to Dice

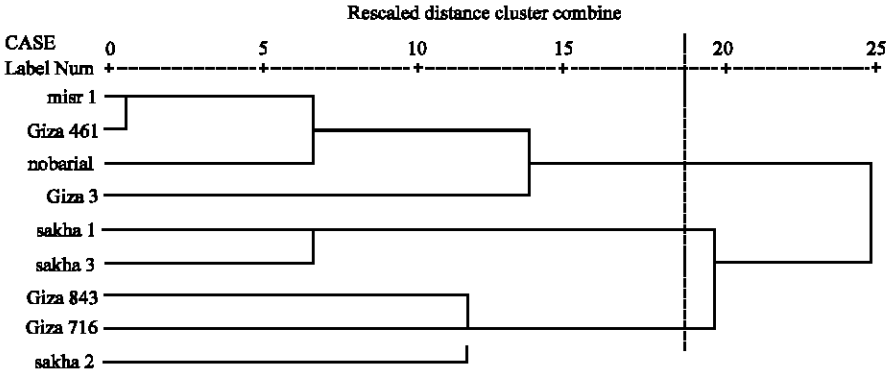

Fig. 3: Dendrograms based on electrophoretic data of seed globulins according to Dice

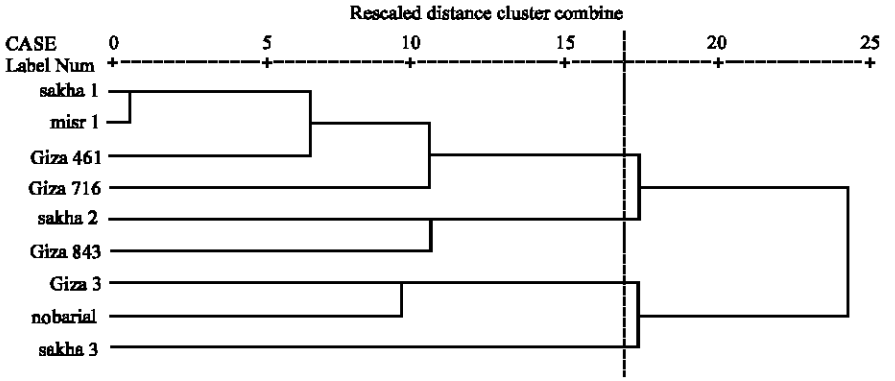

Fig. 4: Dendrograms based on electrophoretic data of seed albumins and globulins according to Dice

electrophoretic data of albumins and globulins, an arbitrary genetic distance of 0.17 was used to divide the studied cultivars into four groups (Fig. 4).

DISCUSSION

This study was conducted to study the genetic variations among the Egyptian cultivars of *Vicia faba* as a basic requirement for further crop traits improvement programs and to test the validity of the applied technique for faba bean cultivar identification. In the present investigation, high genetic variation was observed for the amount of albumin and globulin proteins of the seed meal and 100-seed weight. Also, in spite of the narrow variation in the number of bands in both albumins and globulins electrophoregrams, the Nei's similarity coefficient among all the studied cultivars was small (57.79% for globulins and 42.857 for albumins), indicating a wide genetic variation in both seed albumins and globulins of the studied faba bean cultivars. This genetic variation may be indicated in the polymorphism exhibited in the minor bands and in the major bands intensities, which gave each cultivar its specific electrophoregram for either albumins or globulins. These identity electrophoregrams of the studied cultivars can be used as passport data for their genetic identity and can be used as a good tool for testing core collection concepts and organizing genetic diversity in *Vicia faba*. These results support the validity of seed protein electrophoresis as a powerful tool for cultivar identification, clarifying taxonomic and evolutionary problems and studying genetic diversity (Ladizinsky and Hymowitz, 1979; Cooke, 1984; Sammour, 1988, 1990a,b; 1992; Krochko and Bewley, 2000; Signor *et al*., 2005; Mustafa *et al*., 2006; Thanh *et al*., 2006; Sammour *et al*., 2007). Also, the wide genetic variation observed for seed albumins and globulins and seed weight, which is a genetic trait (Rees, 1997; Sammour *et al*., 2007), indicated that improvement through simple selection for these traits is possible. However, broadening the genetic base from diverse sources is recommended to include most of the genetic determinants of these traits (Laghetti *et al*., 1998; Ghafoor *et al*., 2001; Sammour *et al*., 2007).

The association between globulins and both seed weight and albumins in the seed of the studied cultivars exhibited negative values which was significant in case of seed weight and highly significant in case of seed albumins. This suggests some kind of association in the genetic control of these traits, which may be supported by the high percentage of variance (88.752% of the total variance) accounted for by the first two principal components in multivariate analysis. These results may be in contradiction with other previous views (Polignano *et al*., 1979; Dixit *et al*., 1995; Granati *et al*., 2003). This contradiction is probably because the author used the seed protein components, albumins and globulins, rather than the total seed proteins. Thanh *et al*. (2006) concluded that using protein components (Albumins, globulins, prolamins and glutelins) in cultivar identification is recommended to expose the minor genetic variation between the cultivars of a specific crop. This was applied previously for *Lathyrus* sp. (Przybylska *et al*., 1999) and *Phaseolus lunatus* L. (Vargas *et al*., 2000). The conclusion of Thanh *et al*. (2006) may be supported by the present principal component analysis, where the PRIN1 accounted for 59.05% of the total variance with the highest load for seed albumins (0.794), which indicates crucial role of seed albumin variation in genetic variation and discriminating the faba bean cultivars. Further research is needed to determine exactly the genetic control of this correlation. These facts call for more large collections to be analyzed and for more extensive research work to ascertain the contents of the protein components in the seeds and their relationships with other seed traits.

The superior role of seed albumins over seed globulins in cultivar discrimination and genetic variation in *Vicia faba* at the infra-specific level is indicated in the results of cluster analysis of electrophoretic data that showed similarity in genetic relationships among the studied cultivars and the topology of the dendrograms in case of seed albumin alone and aggregated with seed globulin data. This could be obviously noted in grouping the same three cultivars, Giza3, Nobaria1 and Sakha3 in one group in the dendrograms based on albumin alone or aggregated with globulin.

Nadal *et al*. (2007) reported that Natural out-crossing is the major cause of loss of varietal purity in faba bean (*Vicia faba* L.) affecting both the regeneration-multiplication of germplasm collections and the production of certified or basic seed by plant breeders. Faba bean is a partially cross-pollinated species with a rate of out-crossing ranging from 4 to 84% (Bond and Poulsen, 1983). The results obtained here supports the validity of seed proteins electrophoresis for cultivar identification, studying genetic diversity and taxonomic relationships of *Vicia faba* and also other out-breeding plants at the intra-specific level.

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