



Variations in protein of commercial legume seeds using SDS-PAGE

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Abstract

Six seeds belonging to legume species; *Vicia faba*, *Phaseolus vulgaris* and *Lens culinaris*; were analyzed using SDS-PAGE procedure. Extremely low polymorphism percentage (9.5%) was recorded in the protein pattern that scored a total number of 21 detectable bands with molecular weights ranging from 170 to 39 kDa. Only two bands with molecular weights 170 and 130 kDa disappeared in *Vicia faba* seeds purchased from Qalyubia. The UPGMA dendrogram confirmed the low genetic variability of the six samples that divided into two principle clusters with genetic distance of 0.25. The results led us to confirm that the quality of protein in the studied leguminous seeds was similar.

Key Words: Legumes, SDS-PAGE, Seeds, Dendrogram

1. Introduction: Legumes family (Fabaceae) are a very diverse group varying from perennial to annual, and include vines, trees, shrubs, herbs, and some aquatic species. It is considered as the second to grasses in agricultural importance and is the third largest family of higher plants (Young et al. 2003,199-204, and Omonhinmin et al., 2013,2157-2163). Their appearance ranges from small plants of the deserts or arctic/alpine areas to trees of the tropical rain forests. In the world, legumes are a vital vegetation type spread throughout the temperate and tropical regions (Rundel,1989,377-398). Legumes family includes some 670 genera (Polhill 1994), more than 21,000 species (Christou,1994,165-185). But, another phylogenetic analysis based on molecular studies determined 727 genera, 19,327 species (Lewis et al.,2005,130-162). They are known for

their characteristic seed-bearing pods and their nitrogen-fixing capabilities. Legumes are a vital part of many ecosystems

legumes are considered as a good source of vegetable protein because of their high protein content, ranging from 20% in pea (*Pisum sativum*) to 40% in lupine (*Lupinus albus*). Bean (*Vicia faba*) seeds is an annual plant in the legume family, exceeding a meter in length. It is a common food and is easy to digest. Its fruits can be kept frozen, and are eaten cooked, dried, or canned. The protein percentage in its seeds reaches about 30% and the carbohydrates percentage is 50-60%. *Phaseolus vulgaris* beans are a source of protein, carbohydrates and dietary fiber. Fiber may reduce how much cholesterol absorbed by the body. Enzymes in *Phaseolus* may also decrease how much starch the body

absorbs. Green beans strengthen the immunity of body, because they clearly contain anti-inflammatory and antioxidants substances, which help in identifying many immune diseases. Green beans are rich in vitamins such as vitamin C and zinc. Lentils (*Lens culinaris*) seeds are an important source of protein, vitamins, fiber and minerals. They are consumed split or whole and can be cooked in different ways, often used in stews, soups, dips and salads. They are considered as a healthy food choice contributing to feelings of fullness and potentially aiding digestion. They can improve soil fertility through nitrogen fixation (Cereletti,1979,165-185).

Because of their high content of protein, the legumes are considered to be a kindly and inexpensive alternative to animal proteins for human consumption mostly in the developing countries. Proteins are gene products that are often quite stable, and easy for handling (Iqbal et al.,2005,87-96). Furthermore, proteins can be utilized as biological markers and if properly analyzed and studied can lead to identification of candidates with valuable industrial, medicinal, or nutritional applications. Genomic or proteomic studies could supply these advantages in addition to being more dependable than field-based morphological, cytological or agronomic evidences.

Due to the storage protein is not sensitive to environmental variations, being the third-hand copy of DNA and reviewing the genetic make-up of the plant species (Singh et al.,2002,430–436), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was known as a widely used technique for resolving the total proteins and so very useful to study intra and inter specific relationships (Yousaf et al.,2008, 2293-2297). SDS-polyacrylamide gel electrophoresis is a dependable method of genetic description because electrophoretic profiles of the protein subunits are related to the genetic background of the proteins and can be used to validate the genetic make-up (Rehana et al., 2004,139-143).

Taking into account the importance of protein profiling, our study was conducted to characterize

and estimate variability in six legume seeds belonging to three species ; *Vicia faba*, *Phaseolus vulgaris* and *Lens culinaris*. We hope that the results of the research will give a good perception for the protein inside leguminous seeds, and thus the matter will be reflected in the economic and nutritional importance of these seeds.

2.Methods of Research and the tools used

Plant Materials

The seeds of legume species; *Vicia faba*, *Phaseolus vulgaris* and *Lens culinaris*; were purchased from markets from two governorates; Cairo and Qalyubia, Egypt as mentioned in Table 1.

SDS-PAGE technique

SDS-PAGE was performed in 14 % acrylamide slab gels depending on the system of (Laemmli,1970,680-685). The extraction of protein was conducted by mixing some seeds of each species with an equal weight of clean, pure, sterile fine sand. The seeds were then ground to fine powder using a mortar and pestle and homogenized with 1.5 M Tris-HCl buffer, pH 8.8 in clean Eppendorf tube and left in refrigerator overnight (Badr,1995,183-191). Then 20 μ of each sample supernatant, after mixing with bromophenol blue stain, was loaded in the gel. After experiment finished, the gel was stained in coomassie brilliant blue-R250 solution, then destained and photographed to be analyzed.

Data Analysis

Differences of intensity among bands of the different samples were not considered. The clear defined bands are used to estimate levels of polymorphism by dividing the polymorphic ones by the total number of gained bands. Then the presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to construct dendrogram among the six samples using computer program SPSS-11.

3. Results of Research

The SDS-protein profile of the six seeds of legume species; *Vicia faba*, *Phaseolus vulgaris* and *Lens culinaris* is appeared in Fig. (1). Table (2) revealed a total number of 21 detectable bands with molecular weights ranging from 170 to 39 kDa. Extremely low polymorphism percentage (9.5%) was recorded in the protein profile as measured in Table (3). Nearly no intra and inter specific variations were observed in protein profile. Two bands with molecular weights 170 and 130 kDa disappeared in *Vicia faba* seeds purchased from Qalyubia.

The UPGMA dendrogram (Fig.2) confirmed the low genetic variability of the six samples. It divided them into two principle clusters with genetic distance of 0.25. Samples; *Vicia faba* (from Cairo), *Phaseolus vulgaris* (from Cairo and Qalyubia) and *Lens culinaris* (from Cairo and Qalyubia) were grouped in one cluster, whereas, the other cluster included *Vicia faba* (from Qalyubia). According to results of the SDS-PAGE, the overall blueprint of seed storage proteins show very low degree of heterogeneity may be attributed to family homogeneity or purity in protein content.

Table (1): . Names and purchasing places of the seeds of the legume species.

No.	Species	Governorate
1	<i>Vicia faba</i>	Qalyubia
2	<i>Vicia faba</i>	Cairo
3	<i>Phaseolus vulgaris</i>	Qalyubia
4	<i>Phaseolus vulgaris</i>	Cairo
5	<i>Lens culinaris</i>	Qalyubia
6	<i>Lens culinaris</i>	Cairo

Table 2. Molecular weights of the seed protein bands and their presence (+) or absence (-) in the legume seeds.

Mw (kDa)	1	2	3	4	5	6
170	-	+	+	+	+	+
163	+	+	+	+	+	+
160	+	+	+	+	+	+
150	+	+	+	+	+	+
130	-	+	+	+	+	+
115	+	+	+	+	+	+
100	+	+	+	+	+	+
97	+	+	+	+	+	+
94	+	+	+	+	+	+
80	+	+	+	+	+	+
75	+	+	+	+	+	+
72	+	+	+	+	+	+
67	+	+	+	+	+	+
63	+	+	+	+	+	+
60	+	+	+	+	+	+
56	+	+	+	+	+	+
53	+	+	+	+	+	+
51	+	+	+	+	+	+
49	+	+	+	+	+	+
42	+	+	+	+	+	+
39	+	+	+	+	+	+

Table 3. Number and types of the SDS-PAGE bands as well as the polymorphism percentage generated in the six legume seeds.

System	Monomorphic band	Polymorphic band		Total bands	Polymorphism (%)
		Unique	Shared		
SDS-	19	0	2	21	9.5

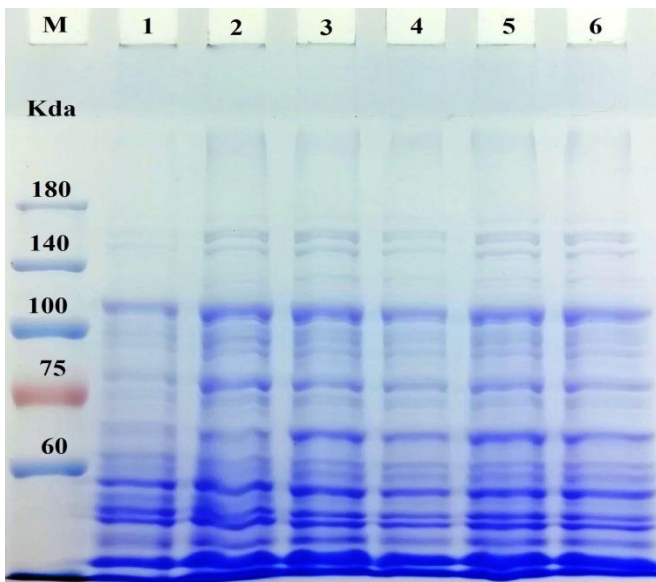


Figure 1. Seed protein profile of the six legume seeds using SDS-PAGE. M: Marker

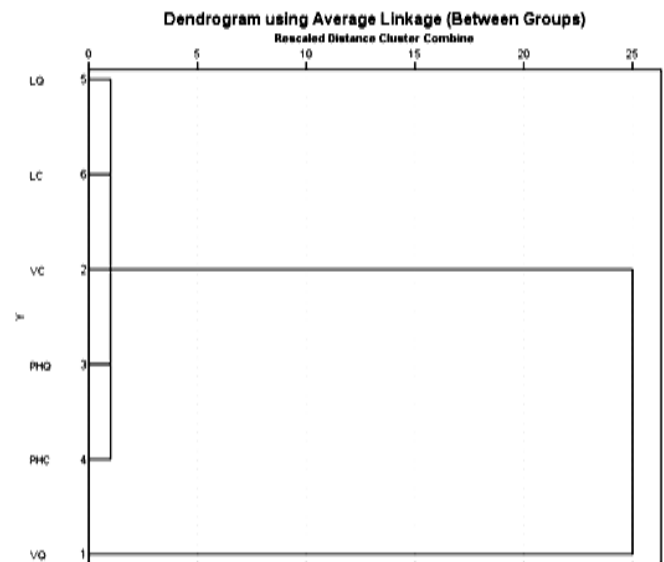


Figure 2. The dendrogram showing genetic relationship among of the legume species.

4. Interpretation of Results

Different electrophoretic methods based on storage protein profiles have been utilized for the characterization and identification of cultivars and crops. Some authors proposed that seed protein patterns may be useful for taxonomic relationships within some species (Duran et al., 2005, 1320-1328).

The SDS-protein profile of the six seeds of legume species; *Vicia faba*, *Phaseolus vulgaris* and *Lens culinaris* is appeared in Fig. (1). Table (2) revealed a total number of 21 detectable bands with molecular weights ranging from 170 to 39 kDa. Extremely low polymorphism percentage (9.5%) was recorded in the protein profile as measured in Table (3). Similar findings were detected by Zubaida et al., (2006, 99-106) and Aniel et al. (2010, 23-27) scored 15 protein bands with molecular weights ranging from 80.8 to 22.4 kDa in seeds of 10 cultivars of *C. annuum* L.

Nearly no intra and inter specific variations were observed in protein profile. Two bands with molecular weights 170 and 130 kDa disappeared in *Vicia faba* seeds purchased from Qalyubia. The UPGMA dendrogram (Fig.2) confirmed the low genetic variability of the six samples.

It divided them into two principle clusters with genetic distance of 0.25. Berber and Yaşar (2011, 1085-1090) characterized 28 bean cultivars from Turkey by the numerical analysis of seed protein patterns obtained by SDS-PAGE technique. Their study indicated that the numerical analysis offered no final answer to the identity of bean genotypes because that it was not sufficient as a typing tool for the differentiation of bean genotypes cultivated in Turkey.

According to results of the SDS-PAGE, the overall blueprint of seed storage proteins show very low degree of heterogeneity may be attributed to family homogeneity or purity in protein content. Odeigah et al. (1999, 127-131) and Fufa et al. (2005, 133-146) detected a similar visualization.

Seed protein patterns of 47 accessions of eleven species of legumes were studied, by extracting the total proteins from ten single seeds and performing SDS-PAGE. All eleven species were clearly recognizable from their protein banding patterns, but only *Phaseolus vulgaris* expressed high intraspecific variations. Variation in other species was very limited (Valizadeh, 2001, 287-292). Ghafoor et al. (2003, 613-624) and Nisar et al. (2007, 1575-1581) detected low intraspecific diversity in *Cicer arietinum* depending on SDS-PAGE for seed protein patterns and concluded that the storage seed protein is a very conservative trait and that the seed-protein patterns are greatly species-specific.

5. Conclusion

In conclusion, nearly no intra and inter specific variations were detected in The seeds of legume species; *Vicia faba*, *Phaseolus vulgaris* and *Lens culinaris*, therefore application of other techniques such as native-PAGE is recommended for more characterization and discrimination among these seeds. However, our results lead us to confirm that the quality of protein in the studied leguminous seeds is similar, which indicates that the use of any of them in the field of nutrition will be similar in terms of benefiting from the protein component.

Conflict of Interests

The authors have not declared any conflict of interests.

Acknowledgement

First of all we thank God for the most mercy for enabling us to present this project in the best form that we wanted to be, we would like to thank our supervisor of this project, Dr. Shawkat Mahmoud Ahmed for his valuable help and advice to come out with this project.

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