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# Classification of Bean Genotypes by Protein Profiles

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**ABSRACT**

Conservation of biodiversity is the main concept to provide sustainability. There are various methods to identify diversity of living organisms. SDS-PAGE is one the most reliable method to classification of plants. Dry bean is the most important pulse crop over the world besides Konya has the highest production in Turkey which is expected as the widest genetic variation among the genotypes. In the present study, a total of 22 bean genotypes (20 populations from Konya and 2 certificated varieties) were used to obtain the protein patterns by using SDS-PAGE methods. Diversity of the genotypes and their relatives were evaluated with dendrogram. Results implicated that protein profiles of the investigated beans provided a clear classification by view of selection criteria. Similarity dendrogram presented two main groups that showed the ranges nearly 20-75% and 50-90%, respectively. Furthermore, two of the local populations (PV 19 and PV 20) showed a subgroup with certified varieties (Akman-98 and Gina). A comparison of SDS-PAGE method showed that, the method could be used to find solutions for the taxonomic and evolutionary problems of the bean genotypes. Therefore, this method may be a useful tool for plant breeders to simplification of selection and classification on genotypes.

## 1. Introduction

Today, the food industry is focused on the production of plant protein isolates and especially legumes due to the increasing application of plant protein in food and non-food markets. The extension of plant protein isolates using in foods is increasing in parallel as functional ingredients to improve the texture, the nutritional quality of the product or for economical reasons. Legume proteins are used about 10 times less than meat, eggs and dairy products although the relatively low cost of protein. The functional properties of protein preparations depend, not only on factors which are related with the preparations themselves, such as composition of protein, procedure of preparation and the way how it has been produced, but are also responded from environmental factors. Probably, these might be the reasons for marginal differences in the emulsifying, foaming and gelling properties in numberless protein products (Makri et al. 2005; Kahraman 2017).

Konya is the biggest city as area in Turkey beside it has the most common bean area and production. Common beans are produced in almost all the province. So there are many variations among common bean genotypes as depending on climate changes (Ceyhan 2004). Therefore, the populations are valuable sources of well adapted germplasms to the pedoclimatic conditions of restricted geographical areas. Local ecotypes have taken place with thought of the farmers attribute to change the landraces. Konya City has 2.617.908 ha of agricultural land which is almost 10% of total in Turkey. Common bean has 19.184 ha production area and 72.869 tons of production in Konya. Most of the farmers (90%) are using populations (non-certificated lines) but, it is amazing that the farmers are still taking higher yields (380 kg da$^{-1}$) than the average value of Turkey.

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There are many researches which are focus on the taxonomy of common bean. They are based on morphology, karyology, palynology and molecular sequencing. Recently, old landraces have been gradually replaced by improved cultivars in response to market demand. So, there it is quite important to collecting, characterising and evaluating the local populations, before they get lost (Lepori and Baldi 1979; Kahraman et al. 2015).

Polyacrylamide Gel Electrophoresis (PAGE) is a biochemical method and is most widely used due to its validity and simplicity for describing genetic structure of plant collections. Seed protein patterns had been obtained successfully to figure out the taxonomic and evolutionary problems of several species (Ghafoor et al. 2000) by using Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis method (SDS). In Turkey, seed storage proteins had been used as genetic markers in analyses of genetic distances inside and among species, in terms of determining the taxonomic relationship (Açık et al. 2004; Babaoğlu et al. 2004; Tamkoc and Arslan 2011).

Aim of the present research was evaluating the utility of SDS-PAGE profiles as reliable tools for classification of bean genotypes which are widely produced in Konya. In general, taxonomists are supposed to develop the genotypes which are able to survive in different environmental conditions and producing more seed protein. Moreover, we wonder to see how much resolution would be provided for taxonomy of the bean genotypes and how much harmony would be displayed with previous molecular analyses as reported in previous studies relative to SDS PAGE method.

## 2. Materials and Methods

The seeds of the used bean genotypes that used as material in the present study were provided from the Selcuk University, Agricultural Faculty, Department of Field Crops. Selection of the used bean genotypes were made according to widely cultivation, yield stability, higher adaptation ability (Ceyhan et al. 2014). Table 1 shows the register number and agronomical characteristics of the used bean genotypes.

Table 1
Agronomical characteristics of the used bean genotypes

| Genotypes | Agronomical characteristics |
|---|---|
| PV1 | 70-80 cm of plant height, straight developing, black color seed, horoz type. |
| PV2 | 50-60 cm of plant height, dwarf type, pods are flat and light green color, non-stringy, white color seed, dermason type. |
| PV3 | 50 cm of plant height, straight developing, leech, white color flower, pods are flat, dermason type and white color seed. |
| PV4 | Early maturing, leech, higher seed yield, pods are flat, mid-length, pods are green color and non-stringy, seeds are circular and white color. |
| PV5 | Semi wrapping, pods are light green and pink spotted and white color seed. |
| PV6 | 55-60 of plant height, straight developing, non-leech, white color flower, pods are flat, horoz type and white color seed. |
| PV7 | Dwarf, high seed yield, pods are smooth-green color and non-stringy, brown color seed. |
| PV8 | 50 of plant height, straight developing, non-stringy, white color flower, pods are flat and white color seed. |
| PV9 | Early maturing, semi wrapping, higher seed yield, pods are smooth, mid-length, non-stringy, and green color, seeds are circular and white color. |
| PV10 | Dwarf, high seed yield, pods are smooth, green color and non-stringy, brown color seed. |
| PV11 | 50-60 of plant height, straight developing, white color flower, pods are smooth, horoz type, white color seed. |
| PV12 | 60-70 of plant height, straight developing, leech, white color flower, pods are smooth, horoz type, white color seed. |
| PV13 | 50 of plant height, straight developing, leech, white color flower, pods are smooth, white color seed. |
| PV14 | Early maturing, semi wrapping, pods are light green and pink spotted, white color and circular seed. |
| PV15 | Early maturing, long pod, leech, dwarf, high seed yield, pods are smooth and green color, non-stringy, white color seed. |
| PV16 | 50 of plant height, straight developing, leech, white color flower, pods are smooth, white color seed. |
| PV17 | Dwarf, leech, white color flower, pods are smooth, dermason type, white color seed. |
| PV18 | Straight developing, leech, white color flower, pods are smooth, dermason type, white color seed. |
| PV19 | 40-50 of plant height, straight developing, white color flower, pods are smooth, dermason type, white color seed. |
| PV20 | Semi wrapping, leech, white color flower, pods are smooth, dermason type, white color seed. |
| PV21$^{certified}$ (Akman-98) | Certified variety: 60-70 of plant height, semi wrapping, leech, white color flower, pods are smooth, dermason type, white color seed. 23-26% protein ratio in seed, tolerant to virus and bacterial diseases. |
| PV22$^{certified}$ (Gina) | Certified variety: Developed from Romano type, early maturing, dwarf, pods are flat, green color, non-stringy, high yield, easy harvest. Advised for fresh consuming and canning, tolerant for bean mosaic virus. |

Isolation of seed total protein was made according to Saraswati et al. (1993) method. The total SDS-PAGE (Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis method) was made as report of the Laemli method (Laemli 1970; Maniatis et al. 1989). Overnight

fixing and staining of the gel proteins was carried out to Demiralp et al. (2000) method. Automatic scoring was made to seed storage protein profiles pattern of the bean genotypes. The computerize based program "Bio-Profiles Analysis" systems was used to achievement of a meaningful dendrogram to obtain the genetically distance.

## 3. Results and Discussion

Protein patterns of the 22 bean genotypes were obtained with SDS-PAGE methods in the study (Figure 1). Electrophoresis analysis of the seed proteins showed that all samples had a specific protein pattern. Clustering dendrogram showed basically 2 two main groups (Figure 2). The first main group was subdivided into 3 subgroups as well which had genetic distances between almost 20% and 75%. Upside cluster was included the genotypes 1, 2 and 3. Middle cluster was obtained the genotypes 5, 6 and 7 while the genotype 4 placed on the last subdivide group. Among these part members, the genotype 1 was placed in the first subgroup besides the genotypes 2 and 3 showed the second subgroup. As it seen on Figure 2, the genotype 4 showed the last component of the first main group. As a summarize of that group, the similarity was ranged from 35% to 75% while the closest genotypes were determined as 2 and 3.

Inferior cluster of the second main group was gathered from the genotypes 8, 12, 9, 10 and 11 which the last two genotypes were determined as the closest with more than 90% similarity. Middle subgroup involved the genotypes 13, 15, 14, 17 besides 16 and 18. Similarity ratio in that group was between 70% to 80%. In the third subgroup, the genotypes 19, 22, 20 and 21 showed the similarity ratios between 50% to 70% values. A general evaluation of the second main group presented that the genotypes 8 and 12 provided the first subgroup while the genotype 20 and 21 was placed in the last subgroup alone. Additionally, the certified varieties Akman-98 and Gina showed similar protein profiles with other two population characterized bean genotypes (PV 19 and PV 20) while these ones showed a subgroup in the dendrogram.

Finally, the first main group included a total of seven bean genotypes while the second main group was consisted from 15 bean genotypes totally. The middle subgroup of the second main group was appeared as the crowded cluster. The mentioned protein profiles are assumed as a useful tool to present the similarity among the bean genotypes.

Methods which are using molecular markers as seed storage proteins or RAPD; provide a rapid way to discriminate between genotypes and have strong mutual correlation (Zivkovic et al. 2012). Identification of varieties is an important aspect in agricultural system. Identify and characterize on the basis of morphological characters in large number of varieties or landraces is difficult due to their non-stable and originate with regards of environmental and climatic conditions, and therefore phenotypic plasticity is an outcome of adaptation (Barbara et al. 1991; Ceyhan 2006a, 2006b; Ceyhan et al. 2012). A previous study was conducted to determine the genetic variation in seed composition which effected by the environmental factors. Mineral availability and sink strength were obtained as limiting for reserve accumulation. Genes and/or QTL controlling seed protein content and sulfur-amino acid levels were identified (Gallardo et al. 2008). Results were believed as a support to increase the nutritional value of legume seeds. It was recommended that genetic engineers supposed to effort about the proper structural requirements of the storage proteins to achievement stable accumulation in the vacuolar protein bodies by the way increasing of the amino acid composition of crop seeds (Sindhu et al. 1997). A previous study was also reported that diversity observed with proteins having intermediate or heavy molecular weight was more than that of light proteins. The results of that research could be used to study the genetic diversity (Marzooghian and Valizadeh 2011). Ferreira et al. (2003) revealed that their results were suggested that calcium and magnesium ions are also electrostatically involved in vivo in the macromolecular aggregation of legume seed storage proteins, ensuring their efficient packing inside the protein storage vacuoles. This mechanism was responsible for the typical insolubility of legume globulins in water. Golombec et al. (2007) were reported that changes in developmental processes like the duration of the seed filling period could also contribute to the higher protein content.

## 4. Conclusions

It can be stated that the various classes of plant storage protein (e.g. albumins, globulins, prolamins and glutelins) possess many useful attributes which permit them to be used for various food/non-food applications (Marcone 1999). Montoya et al. (2010) were revealed that breeding programs which are focused on highly-digestible phaseolin types could lead to the production of beans with higher protein quality. It was reported that the traditional varieties have been naturally selected by the many year practices for the local growing environment and contain important adaptive genes to survive in harsh climatic conditions. Therefore, detailed study is required prior to exploit the adaptive genes from these varieties. Also, the intensive and continuous focus on hybrid rice in the region can limit the number of crop varieties and traditional varieties can be lost forever. Hence, it is necessary to conserve old crop varieties and landraces which could be important sources of adaptive genes for future plant breeding programs (Jugran et al. 2010).

Konya region has a strong tradition of transhumance. This is more probable that the environment conditions changes depended to each zone. Consequently, using of the SDS-PAGE method provided a clear and useful classification of the bean genotypes. It is assumed that, SDS-

PAGE method could be used to determination of the taxonomic and evolutionary problems of the genotypes. The studied genotypes were indicated special protein profiles and patterns. As a result, the related bean genotypes placed meaningfully in the dendrogram.

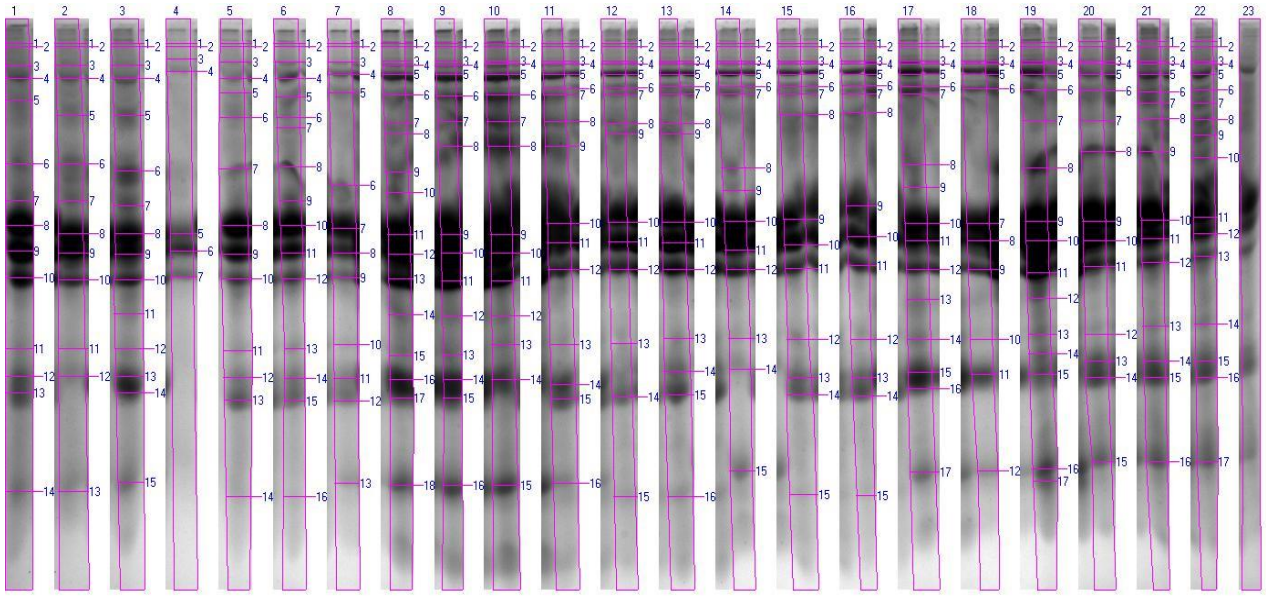

Figure 1

Protein patterns of the used bean genotypes in SDS-PAGE methods

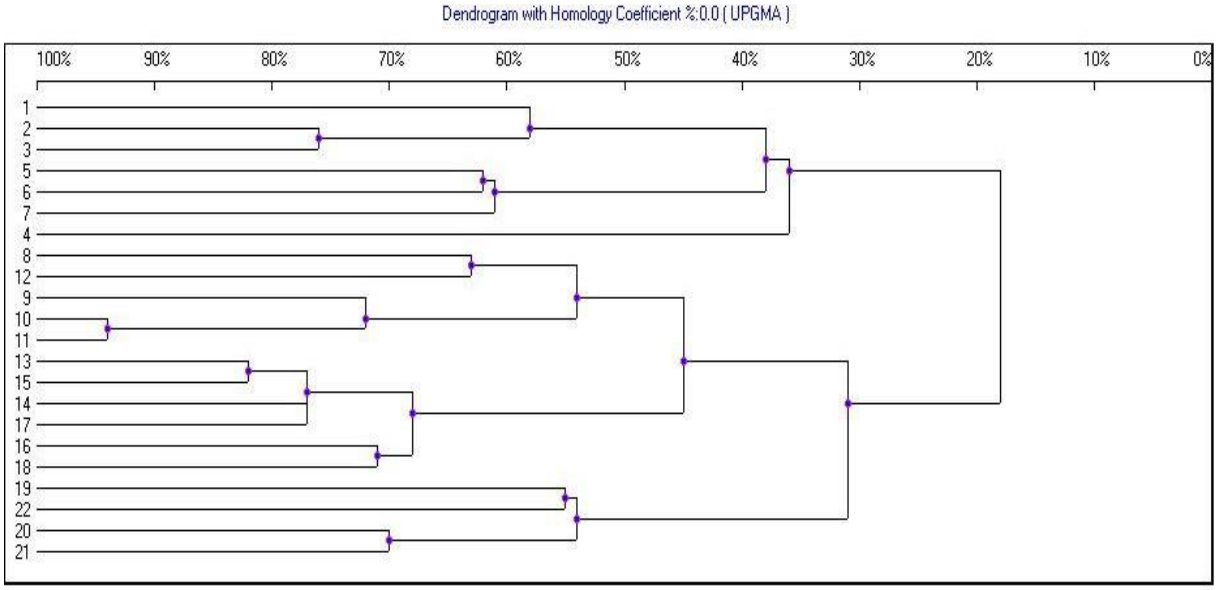


Figure 2

Protein patterns dendrogram of the 22 genotypes

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