YYÜ TAR BİL DERG
(YYU J AGR SCI)
2015, 25(2): 127-133

Geliş Tarihi (Received): 22.09.2014
Kabul Tarihi (Accepted): 13.01.2015

Araştırma Makalesi/*Research Article (Original Paper)*

Identification of Seed Storage Protein Polymorphism in Some Soybean (*Glycine max* Merril) Genotypes Using SDS-PAGE Technique

Omid SOFALİAN*, Pooran BANDARIAN, Ali ASGHARI, Mohammad SEDGHI, Behnam FIROOZİ

Agronomy and Plant Breeding Department, Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, Ardabil 179
*e-mail: sofalian@gmail.com; tel: +98(45)33512081

Abstract: Seed storage proteins are considered as biochemical markers, which are less affected by the environment and have high repeatability, and have become a powerful tool in studying genetic variation within and among genotypes in crop plants and also distinguishing cultivars of a particular crop species. In this study, 17 genotypes of soybean were studied using SDS-PAGE. The results showed that genetic diversity average for all detected loci including total proteins and low molecular weight (LMW-GS) was equal to 0.48 and for proteins with high molecular weight (HMW-GS) was 0.42. Additionally, Shannon average index were 0.4096, 0.41 and 0.60 respectively with regard to three storage proteins examined. While the cluster analysis produced from total proteins divided genotypes into four groups, proteins with low molecular weight (LMW-GS) and high molecular weight(HMW-GS) divided them into three groups. This polymorphism could be used in any biochemical marker based breeding programs to facilitate selection.

Key words: Cluster analysis, Genetic diversity, SDS-PAGE, Soybean

SDS-Page Tekniği Kullanılarak Bazı Soya (Gylicine max Merril) Genotiplerindeki Tohum Depo Protein Polimorfizminin Belirlenmesi

Özet: Tohum depo proteinleri, çevre tarafından daha az etkilenir ve yüksek tekrarlanabilirliğe sahiptir ve kültür bitkilerinde genotipleri arasındaki genetik varyasyonum belirlenmesinde ve aynı zamanda belirli ürün türlerinin çeşitlerinin ayırt edilmesinde güçlü bir araç haline gelen bir biyokimyasal belirteç olarak kabul edilmektedir. Bu çalışmada, 17 soya genotipi SDS-PAGE kullanılarak incelenmiştir. Sonuçlar, toplam protein ve düşük moleküler ağırlıklı (LMW-GS) lokuslar dahil olmak üzere tespit edilen tüm lokusların genetik çeşitlilik ortalamasının, 0.48 olduğunu ve yüksek molekül ağırlığına sahip proteinler (HMW-GS) için ise 0.42 olduğunu göstermiştir. Ayrıca, Shannon ortalama endeksi, bu üç depo protein grubu için sırasıyla 0.4096, 0.41 ve 0.60 olarak bulunmuştur. Toplam proteinler için oluşturulan kümeleme analizi genotiplerin dört gruba ayrırırken, genotipler düşük moleküler ağırlıklı (LMW-GS) ve yüksek moleküler ağırlıklı (HMW-GS) sahip proteinler bakımından üç gruba ayrılmıştır. Bu polimorfizm, herhangi bir biyokimyasal belirteç tabanlı ıslah programlarında seleksiyon amacı ile kullanılabilecektir.

Anahtar kelimeler: Kümeleme analizi, Genetik çeşitlilik, SDS-PAGE, Soya

Introduction

Diversity is the basis of all selection efforts, and the genotypic based selection requires genetic variability. It is clear that selection options are wider in a population with higher genetic diversity. The reduced genetic variance results in lower efficiency of the breeding program and leads to genetic vulnerability of crops against pests, diseases and environmental stresses. Researchers have focused on broadening the genetic sources of their breeding programto overcome these problems (Ciaffi et al. 1993). Soybean (*Glycine max*) is an annual diploid plant (2n = 40) and it belongs to legumes. Evaluation of food protein quality has particular importance for biological and economic reasons.In recent years, the use of plant protein sources in the daily diet has beenhighly recommended (Yang and Scrimshaw 1998). Plant protein sources are cheap and contain saturated fat that causes prevention of chronic diseases, especially cardiovascular disease, diabetes, etc. (Hahn and Schoberlein 1999). Biochemical properties of the seed

storage proteins become apparent with molecular weight in a range of 118 to 50-7586 Daltons (Akinamed et al. 2010). A number of researchers have focused on soy protein components with respect to their extraction, separation, classification and physicochemical properties. The classic method for separating soy protein into its components is the ultracentrifuge method, developed by Wolf and his colleagues (1956, 1968) who first classified soy proteins into 2S, 7S, 11S, and 15S components and later on estimated that 2S, 7S, 11S and 15S accounted for 22, 37, 31 and 11% of the total proteins of soybeans, respectively (Liu et al. 2007). In the soybean, globulins similar to Vicilin were isolated and they were named as Beta-Conglycinin. It was demonstrated that they found in six isomeric forms with molecular weights ranging from 150 to 175 kDa. Each isomer of Conglycinin has been diagnosed with one of three subunits, alpha, beta and gamma. Glycine (11s) and Beta-Conglycinin (7s) are the major proteins of soybean. The molecular weight of glycine is 350kDa, and it consists of at least six different subunits (Sathe et al. 1987). In the present study 17 soybean cultivars were studied using SDS-PAGE.

Materials and Methods

In this study, seeds of 17 different genotypes of *Glycine max* Merril were studied. Genotypes were obtained from Seed and Plant Institute of Karaj, Alborz province, Iran (Table 1).

Table 1. The names of plant materials used in this study

Code	Name	Code	Name
1	JK	10	033
2	Hamilton	11	Sahar
3	Interprize	12	M7
4	Lavina	13	B.P
5	Linford	14	Clean
6	Liana	15	Gorga
7	T.M.S	16	Calark
8	Stressland	17	DPX
9	Safyabad		

Protein extraction

Extraction of storage proteins was carried out by using Payne and Lawrence method (1983). In this study, SDS-PAGE method was used for protein electrophoresis (electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulfate). SDS-PAGE was performed with 10% separating gel and a 5% stacking gel according to the method previously described by Laemmli (1970). Data obtained from the decomposition of the proteins with low molecular weight (LMWs) and proteins with high molecular weight (HMWs), and soluble proteins (total proteins) for 17 genotypes of *Glycine max* Merril were analyzed using NTsys pc 2.2 and PopGen 32 softwares.

Results and discussion

The results showed that the mean genetic diversity for all loci was 0.48 and the mean Shannon index for proteins with low molecular weight was 0.41 and for soluble proteins was 0.40. In total, for 17 genotypes studied,there were 19 protein bands for proteins with low molecular weight, 18 protein bands for HMW proteins and 23 protein bands for soluble proteins in water and salt.For proteins with low molecular weight, the lowest number of bands observed was 2, and the highest number of bands observed was 16. While the highest number of bands in the case of soluble proteins in water and salt was 9, the lowest number of bands was 12 (Table 2). Hill and Breidenbach (1974), using the similar SDS-PAGE technique, found six bands in 7S and seven bands in 11S of soybean cultivar Portage, respectively. Fifteen polymorphic and four monomorphic(at gene loci of 5, 6, 7 and 15) gene loci were observed from the proteins with low molecular weight (Table 3), While the highest gene diversity was related to markers of 8, 18 and 19 with 48%, the lowest diversity was related to markers of 5, 6, 7 and 15with zero percent.In total, about 78.95% of gene locations were polymorphic. Although 17 gene locations for soluble proteins in water and salt were polymorphic,six were monomorphic (gene loci of 4, 5, 18, 21, 22 and 23) based on the results from the genetic diversity analysis (Table 2). While the highest gene diversity was related to markers of 7, 6, 10, 11, 19 and 20 with a percentage of 48, the lowest diversity was related to markers of

4, 5, 18, 21, 22 and 23 with a percentage of zero. Of the total, approximately 73.91% of loci were polymorphic(Table 2).In the case of HMW proteins, the results showed that the mean genetic diversity for all genetic loci was equal to 0.42 and the mean Shannon index was equal to 0.60 (table 4). While the lowest number of bandswas observedin the populations 8 and 9, the highest number of bands was observed in the populations 10 and 17. Except for the locus 17, other 17 loci were polymorphic. The highest and lowest gene diversity wererelated to markers of 6 and 12 (0.49) and 17(0), respectively. Of the total, approximately 94.44% of loci were polymorphic. This results revealed that there is sufficient genetic variation among genotypes especially HMW-GS (with average of Nei's index equal to 42%) to benefit in marker assisted selection and other breeding programs. Odeigah and Osanyinpeju (1996) investigated the total seed protein, globulin and albumin fractions of 20 cowpea (*V. unguiculata*) accessions by SDS-polyacrylamide gel electrophoresis. While there was no correlation between seed colour and total seed protein banding pattern, the six insect-resistant cultivars were characterized by the presence of the 39 and 20 kD subunits. They suggested that the insect-resistant cultivars may be genetically related and the 39 and 20 kD subunits may be involved in the insect resistance mechanism.

Cluster analysis for proteins with low molecular weight was performed based on Jaccard's similarity coefficient and a UPGMA tree was constructed(Figure 1b). By examining the tree diagram at a distance of 0.65,threegroups occurred. Therefore, the first group consisted of JK cultivar exclusively. The second group was composed of five cultivars including Hamilton, Liana, T.M.S, Safyabadand Lavina. The third group included Sahar, M7, Interprize, 033, Streesland, B.P, Clean, Calark, Gorga, DPX, Linford genotypes.Based on this cluster it was concluded that the hybridization between JK and third group (especially 033) genotypes would be more useful as they showed greater genetic diversity. Dobhal (1995) showed significant variability between soybean accessions for yield components, allowing accessions to be grouped into 17 clusters. In their study no linear relationship between geographic and genetic distance was found.

The highest similarity was observed between the genotypes M7 and B.P; Clean and M7; Clean and B.P; Clean and Gorga; B.P and Gorga; M7 and Gorga; M7 and Calark; B.P and Calark; Clean and Calark, and between Calark and Gorga, with a similarity coefficient equal to 1 and the lowest similarity was observed between the genotypes JK and Lavina, and between the genotypes 033 and JK with a similarity coefficient equal to 0.37.The suitability of cluster analysis method was determined with considering significant cophenetic correlation (0.8).

Faisal Anwar Malik et al. (2009)assayed the genetic variation of seed protein by SDS-PAGE for ninety-two accessions of soybean (Glycine max). The germplasm represented five different origins/sources (Pakistan, USA, AVRDC, North Korea and Japan). Dendrogram constructed using Ward's method divided the accessions in two main groups consisting of four clusters. The results of cluster analysis indicated that genetic diversity between Pakistani and US or AVRDC accessions is much larger that the genetic diversity between Pakistani and North Korean or Japanese accessions. Although cluster analysis completely separated most of the Pakistani accessions from USA and AVRDC accessions, but could not distinguish between the accessions from Japan and North Korea. As the accessions from various sources differed considerably, it was difficult to establish any relationship between origin and clustering pattern.

For high molecular weight proteins, cluster analysis showed that genotypes were separated into three groups including J.K, Linford, Sahar, Hamilton, Liana, T.M.S, B.P., Clean, Calark, O33 and D.P.X. in the first group, M7 and Gorga in the second group and finally Interprize, Lavina, Stressland and Safyabad in the third group (Figure 1c). AUPGMA tree was established based on Jaccard's similarity coefficient for the soluble proteins in water and salt (Figure 1a). By examining thetree diagramat the distance of0.76, fourgroups were determined. The first group consisted of T.M.S, Streesland, Safyabad, Liana, 033, Lavina, Interprize and JK genotypes. The second group contains Linford and Hamilton cultivars. The third group includes Sahar and M7 genotypes. Finally, the fourth group composed of B.P, Clean, Calark, Gorga and DPX genotypes. The similarity matrixfor the soluble proteins in water and salt was calculated based on the simple matching coefficient. While the highest similarity was observed between Liana and Safyabad genotypes with a similarity coefficient equal to 1, the lowest similarity was seen between Hamilton and Calark genotypes with a similarity coefficient equal to 0.30. The suitability of cluster analysis method was determined with considering cophenetic significant correlation (0.8). Genotypes with similar banding patterns are suggested to be studied for detailed agronomic and biochemical analyses, including 2D electrophoresis and DNA markers. Alipour et al. (2002) conducted an experiment

in order to study the genetic diversity in the electrophoretic patterns of seed proteins. Based on the relative mobility on the gel, 30 protein bands were observed, of which only 5 bands varied between the accessions. Cluster analysis based on qualitative evaluation of the patterns grouped the accessions into 8 clusters and classified the different bands into 3 groups. Ghafoor et al., (2003) reported similar results.

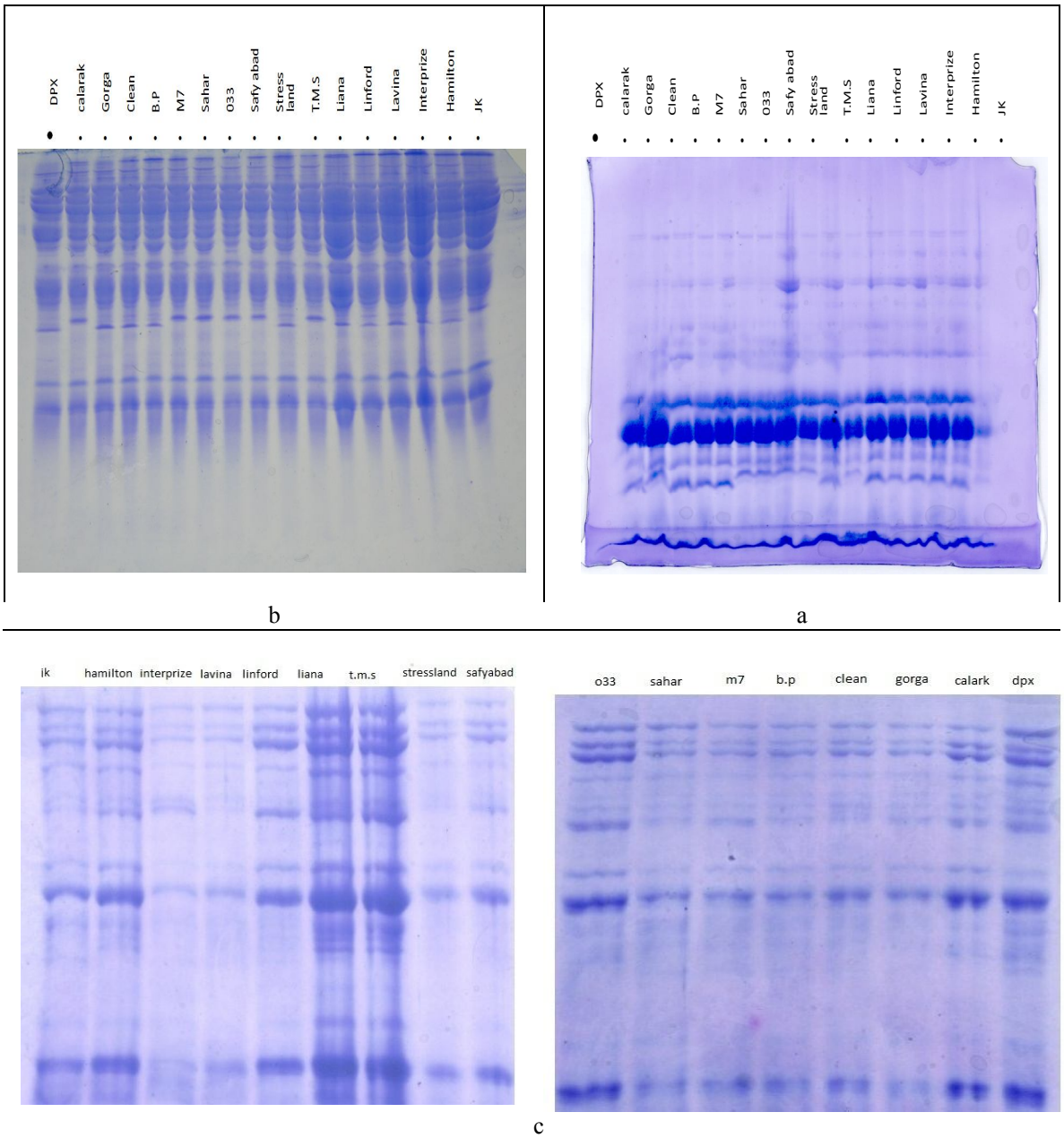

Figure 1. Electrophoretic pattern for a) soluble proteins b) low molecular weight proteins c) high molecular weight proteins

Table2. Gene diversity indices for water and salt soluble protein markers

Locus Number	Nei Index	Shanon Index	Locus Number	Nei Index	Shanon Index
1	0.29	0.46	13	0.41	0.60
2	0.29	0.46	14	0.41	0.60
3	0.29	0.46	15	0.45	0.64
4	0	0	16	0.29	0.46
5	0	0	17	0.20	0.36
6	0.48	0.67	18	0	0
7	0.48	0.67	19	0.48	0.67
8	0.20	0.36	20	0.48	0.67
9	0.20	0.36	21	0	0
10	0.48	0.67	22	0	0
11	0.48	0.67	23	0	0
12	0.35	0.54			

Table3. Gene diversity indices for LMW protein markers

Locus Number	Nei Index	Shanon Index	Locus Number	Nei Index	Shanon Index
1	0.45	0.64	11	0.11	0.22
2	0.45	0.64	12	0.11	0.22
3	0.41	0.64	13	0.29	0.46
4	0.45	0.64	14	0.41	0.6
5	0	0	15	0	0
6	0	0	16	0.29	0.46
7	0	0	17	0.29	0.46
8	0.48	0.67	18	0.48	0.67
9	0.41	0.6	19	0.48	0.67
10	0.11	0.22			

Table4. Gene diversity indcies for HMW protein markers

Shanon Index	Nei Index	Locus Number	Shanon Index	Nei Index	Locus Number
0.67	0.48	10	0.67	0.48	1
0.64	0.45	11	0.67	0.48	2
0.69	0.49	12	0.64	0.45	3
0.67	0.48	13	0.54	0.35	4
0.64	0.45	14	0.60	0.41	5
0.54	0.35	15	0.69	0.49	6
0.46	0.29	16	0.67	0.48	7
0	0	17	0.64	0.45	8
0.67	0.48	18	0.67	0.48	9

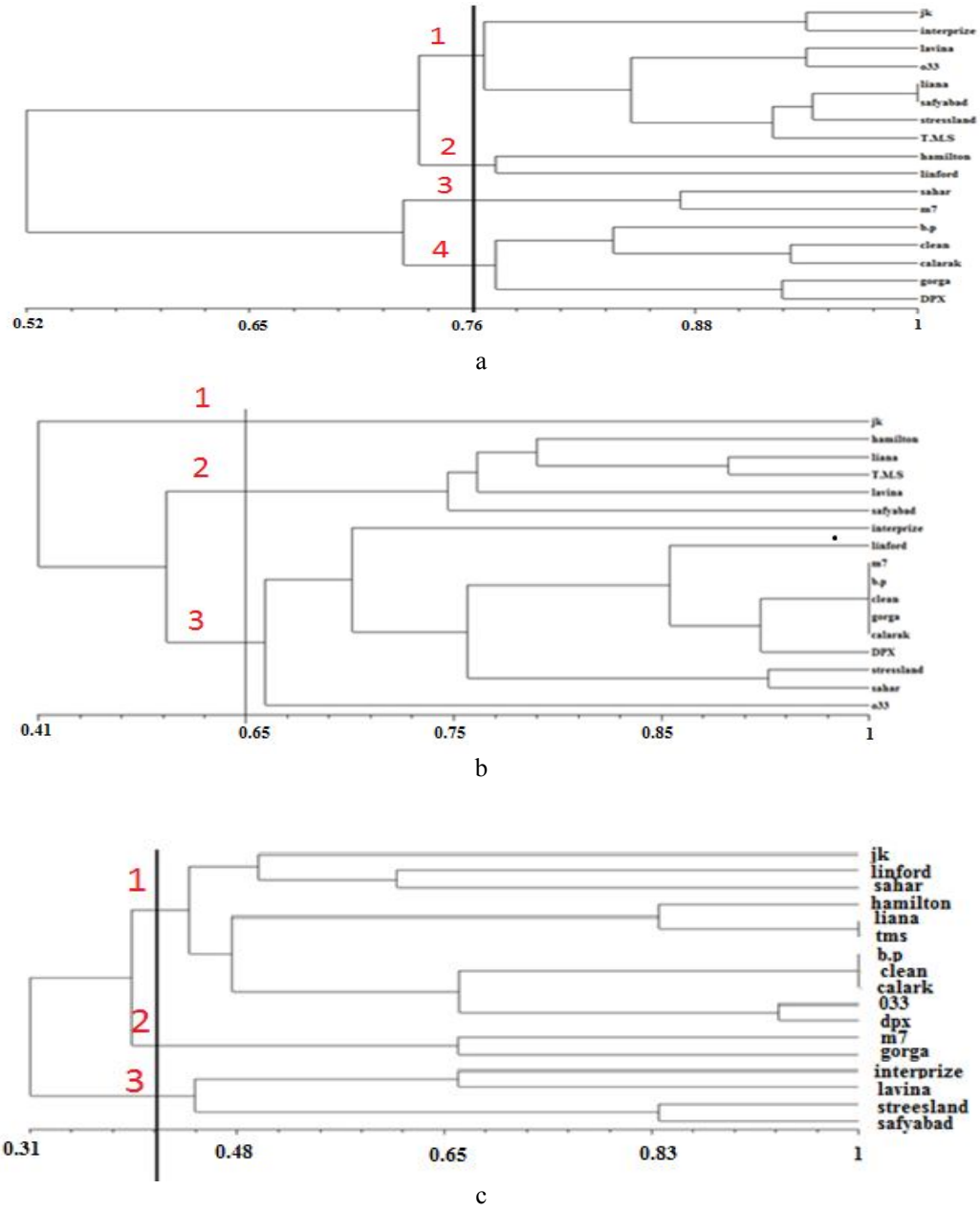

Figure 1. Dendrograms derived from cluster analysis using complete linkage method for in water and salt soluble proteins (a) and proteins with low (b) and high (c) molecular weight

Conclusion

Seed storage protein electrophoresis is a reliable method and it can be used to examine genetic differences and relationships among plant varieties. Seed storage proteins have been widely used in genetic studies because they are stable, uniform and reproducible (Payne and Lawrence 1983). Characteristics of seed storage protein electrophoresis have made it a powerful tool for investigation of communication (Naghavi et al. 2010), discrimination of genotypes and differentiation of plant species. In other words, various methods of electrophoresis, especially seed storage protein electrophoresis by SDS-PAGE in recent years has been very useful in the study of intra- and inter-species variation in plants. The results of this study revealed that there is sufficient genetic variation among soybean genotypes around storage proteins specially HMW-GS (with average of Nei's index equal to 42%). It also could be suggested that other

complementary biochemical analysis such as 2D electrophoresis carried out on genotypes with the same electrophoretic pattern in order to recognition of probable differences among them.

References

Akinamed, SA, Oben, TT, Soetan, KO (2010). Biochemical characterization soybean (*Glycine max*) TGX1485-1D seed proteins. pp. 851-856.

Alipour H, Rezai A, Meibodi SAM, Taheri M (2002) Evaluation of genetic variation in soybean lines using seed protein electrophoresis. J. Sci. Technol. Agric. Nat. Resour. 5(4): 85-96.

Ciaffi M, Lanfiandra D, Porceddu E, Benedettelli S (1993). Storage protein variation in wild emmer (*Triticum turgidum* ssp. dicoccoides) from Jordan and Turkey. 2. Patterns of allele distribution. Theor. Appl. Genet. 86: 518-5250.

Dobhal VK (1995) Genetic divergence in soybean. Legume Res. 18(1): 29-34.

Faisal Anwar Malik M, Qureshi SA, Rashid Khan M, Ashraf M, Javed A (2009).Evaluation of genetic diversity in soybean (*Glycine max*) lines using seed protein electrophoresis. Aust. J. Crop Sci. 3(2):107-112.

Ghafoor A, Gulbaaz FN, Afzal M, Ashraf M, Arshad M (2003). Inter-relationship between SDS-PAGE markers and agronomic traits in Chickpea (*Cicer arietinum*). Pak. J. Bot. 35(4): 613-624.

Hahn H, Schoberlein W (1999). Characterizationand identification of Festulolium hybrids byelectrophoresis of seed proteins. Seed Science and Technology, 27: 525-542.

Hill JE, Breidenbach RW (1974). Proteins of soybean seeds. I. Isolation and characterization of the major components. Plant Physiol. 53:742–746.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4.Nature, 227: 680-685.

Liu S, Zhou R, Tian S, Gai J (2007). A Study on Subunit Groups of Soybean Protein Extracts under SDS-PAGE.J.Am. Oil Chem. Soc. 84:793–801.

Naghavi A, Sofalian O, Asghari A, Sedghi M (2010). Relation between freezing tolerance and seed storage proteins in winter bread wheat (*Triticum aestivum* L.). Tur. J. F. Crops, 15: 154-158.

Odeigah PGC, Osanyinpeju AO (1996). Seed protein electrophoretic characterization of cowpea (*Vignaun guiculata*) germplasm from IITA gene bank.Genetic Resources and Crop Evolution. 43: 485-491.

Payne PI, Lawrence J (1983).Catalogue of alleles for the complex gene loci Glu-B1 and Glu-D1 which code for high molecular weight subunits of glutenin inhexaploid wheat. Cereal Research Communications, 11: 29-35.

Sathe SKGG, Lilley A, Mason C, Weaver CM (1987).High-resolution sodium dodecyl sulfate polyacrylamide gel electrophoresis of soybean (*Glycine max* L.)seed proteins. Cereal Chem.64:380-384.

Wolf WJ, Briggs DR (1956).Ultracentrifugal investigation of the effect of neutral salts on the extraction of soybean proteins. Arch.Biochem.Biophys. 63:40–49.

Wolf WJ, Tamura T (1968). Heat denaturation of soybean 11S protein. Cereal Chem. 46:331–344.

Yang V, Scrimshaw N (1998). Soybean protein in human nutrition. J. Amer. Oil. Chem. Soc. 56:110-120.