International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2013 IJFAS Journal-2013-2-14/448-453
ISSN 2322-4134 ©2013 IJFAS

Analysis of genetic diversity in barley genotypes using storage proteins

Ali Reza Shekhghasemi[1*], Hossein Shahbazi[1], Ali Akbar Imani[1] and Marefat Ghasemi[2]

1. Department of Agronomy and Plant Breeding, Ardabil branch, Islamic Azad University, Ardabil, Iran
2. Agricultural Research Center of Ardabil Province

Corresponding author Email: Emo_worde@yahoo.com

ABSTRACT: Genetic variation is considered as a fundamental factor to plants breeding and makes it feasible to select plants with suitable traits. One of the methods to study genetic variation among plant species is using electrophoresis of seed storage proteins. In this research genetic diversity of 23 cultivars of barley genotypes with the use of Polyacrylamide gel electrophoresis SDS-PAGE was performed. Based on the revealing results, 21 bands (markers) in sheet of Polyacrylamide gel were apparent and the number of bands in each marker was different which indicates genetic diversity in genotypes under studies. According to tree diagram resulting from cluster analysis, these genotypes are in 3 groups. The amount of genetic diversity in genetic locus 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17and 18 respectively are equal to 0.49, 0.5, 0.09, 0.49, 0.38, 0.34, 0.34, 0.34, 0.28, 0.38, 0.38, 0.28, 0.5, 0.47, 0.38, 0.47 and 0.38 and its mean equals 0.37. According to the present results and high diversity in protein markers, these markers can be used to identify differences between varieties.

***Keywords*:** Electrophoresis, Seed storage proteins, Barley, Cluster analysis

INTRODUCTION

Hordeum contains 24 species and there are diploid and tetraploid. The oldest barley is produced from two rows varieties and wild species (H.spontaneum). It is notable that crop barley species are diploid, barleys that are cultivated in different parts of the world for producing seed are H.sativum species other species that are spread in different areas and under various circum stances are included in wild species are not cultivable (Yazdi Samadi and Abadmeyshani, 2004). Cropping barley is to produce its seed which is used in animal's and people's food and. Moreover it is applicable in industry to extract barley malt and in confectionaries. In our country it is mainly used to feed livestock and poultry and in a small quantity to produce nonalcoholic beer. Its use in people's diet is gradually decreasing and its use in livestock food and malt industry is increasing (Khodabandeh, 2003).

Among various factors, seed storage proteins are considered as influential factors in genetic diversity and difference in quality (Rodrigues-Quijano et al, 2001). One way to study genetic diversity among plant species is utilizing electrophoresis of seed storage proteins, because this method is cheaper and requires less effort (Metakovsky and Branlard, 1998). Storage proteins are polymorphic while extremely stable. Environmental factors affect the amount of storage protein although their presence in grown seeds have no or low impact. Therefore, electrophoresis pattern of grown seeds' proteins is a suitable criterion to identify different plant species and varieties. The fundamental reason for the use of electrophoresis pattern of seed storage proteins in classification is proteins to be direct products. Since then it is assumed that these patterns have to display a measure of genetic similarities and differences among plants that are being compared (Abadmeyshani and Shahnejat Boushehri, 1998). By means of analysis on seed storage proteins of Hordeum spontaneum using electrophoresis of globulin and albumin proteins, introduced SDS- PAGE method as a suitable way to identify varieties. Found a considerable variation among barley genotypes; in the mentioned study D- hordein proteins were remarkably similar to HMV glutenin proteins of wheat. Have investigated diversities of barley genotypes received from research institute of

seed and plant breeding and studied 64 genotypes by electrophoresis method and observed 51 polymorphic bands in proteins (B and D- hordein, C). Have suggested electrophoresis pattern of seed storage proteins as an appropriate technique for detection of barley varieties. Boulter et al (1966), analyzed application of protein band pattern in plant systematic. In this respect the evaluation of diversity among varieties and new genotypes of crops is one crucial step forward. Molecular methods such as electrophoresis of proteins due to high polymorphism are suitable method to evaluate diversity, similarity detection and genetic distance between genotypes and amount of genetic erosion among these varieties. The objective of this study is to estimate genetic diversity among 23 barley genotypes through storage proteins.

MATERIALS AND METHODS

This study was conducted on a random sample of barley genotypes in the laboratory. SDS- RAGE electrophoresis of storage proteins of total data was applied for diversity analysis. After electrophoresis and dying gels, protein pattern was performed in one- zero scoring and by means of cluster analysis of simple matching coefficients through un weighted pair- group method using arithmetic average (UPGMA). Several processes in accordance with proposed method of the source (Valizadeh et al, 1994) were respectively carried out for electrophoresis test SDS- PAGE; protein extraction, preparation of polyacrylamide gels and necessary solution, preparation of dye solution, preparation of coloring solution, preparation of decoloring solution, preparation of electrode tampon and loading samples inside gel wells. After coloring and decoloring regarding the presence or absence of each band were respectively evaluated with zero- one and then recorded. In order to group studied varieties in terms of obtained data through protein banding pattern, due to frequent application in electrophoresis studies simple matching coefficients method was used to estimate the similarity between each couple of genotypes (Valizadeh et al, 1994) and diagram was drawn based on UPGMA. The following formula was also applied to estimate the amount of heterozygous among varieties. The amount of genetic diversity of Nei (1978) was estimated by $\zeta = 1 - \sum P_i^2$ formula in which Pi is the frequency of allele I in a genetic locus in the investigated community. The estimation of average genetic diversity (H) through means of ζ s (genetic diversity) in all genetic locus was carried out as below: in this formula N is the number of genotype, P_{ij}^2 is the frequency of allele I out of genetic locus of j and Ni is the number of genetic locus.

$$H = \frac{N}{N-1} * \frac{\sum_j (1 - \sum_i P_{ij}^2)}{N_i}$$

Table 1. Under study wheat genotypes

Number	Genotypes	Number	Genotypes
1	Saida/6/CitaS/4/Apm/Rl//Manker/3/Maswi/Bon/5/CopalS/7/Malouh	13	Sara/4/H.Spont.96-3/3/Roho//Alger/Ceres362-1-1
2	Saida/6/CitaS/4/Apm/Rl//Manker/3/Maswi/Bon/5/CopalS/7/Malouh	14	Mo.B1337/WI2291//Moroc9-75
3	Weeah11/WI2291/Bgs/3/ER/Apm//AC253	15	WI2269/Line251-11-2/3/Leb71/CBB37//Leb71/CBB29/4/Zanbakian
4	Moroc9-75/ArabiAswad/6/CI07117 9/DeirAlla106//Bda/3/Arar/5/11012-2/Impala//Birence/3/ArabiAbiad/4/5604/1025	16	Komplex/BCO47
5	Arar/Lignee527/3/Arar//CompCr29/C63	17	Clipper/Volla/3/Arr/Esp//Alger/Ceres362-1-1/4/Hml/5/Limon/Bichy2000
6	CM67/3/Apro//Sv.02109/Mari/4/Carbo	18	Matnan-01
7	Moroc9-75//H.spont.41-5/Tadmor	19	Salmas
8	SLB39-05/4/7028/2759/3/69-82//Ds/Apro	20	Beecher
9	SLB39-05/4/7028/2759/3/69-82//Ds/Apro	21	Lignee131
10	SLB39-05/4/7028/2759/3/69-82//Ds/Apro	22	makoei
11	SLB39-05/4/7028/2759/3/69-82//Ds/Apro	23	Sara/4/H.Spont.96-3/3/Roho//Alger/Ceres362-1-1
12	(Arar/H.spont.19-15//Hml/3/Babunj)*2		

RESULTS AND DISCUSSION

In this research, all of the storage proteins were extracted from seeds. The obtained gel from electrophoresis of seed total proteins was coded based on presence and absence of bands (protein pattern). The presence and absence of bands were illustrated respectively by number one and zero and eventually a matrix was created. According to the survey conducted by Rabie (1996) between two methods of electrophoresis A- PAGE and SDS-PAGE, SDS- PAGE yielded the best result. In order to identify molecular weight of protein bands, wheat seed

storage proteins which have recognized molecular weight were used (table 2). After that the following equation was determined between molecular weight logarithm and molecule vibrations on the basis of centimeters (figure 1).
5.2623 **+** distance (-0.0995 **=** LogMw)
R2 =0.92

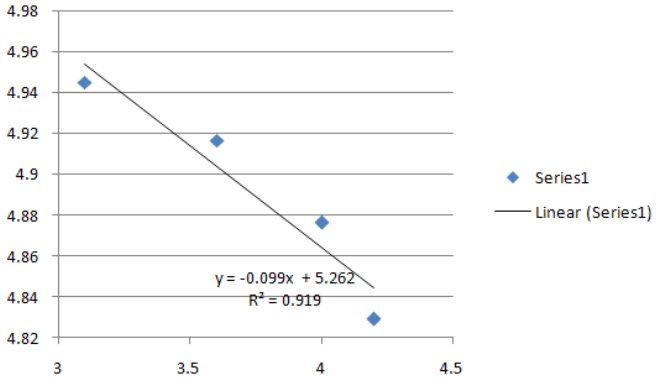

Figure 1. Linear diagram and regression line equation

Table 2. Protein bands molecular waight out of wheat seed storage proteins

sub unit	(KD) molecular wight	CM
5	88114	3.1
7	82529	3.55
8	75239	3.95
10	67476	4.3

The diagram of protein bands is illustrated in figure 2. The number of band presence by genotype seperation is given in table 3 and molecular weight of its bands is given in table 4. In general 14 bands were examined in studied genotypes and their molecular weight was whithin the range of 21.72 to 82.04 KD. The maximum band number (14) was observed in genotypes 2 and 21 and the minimum band number (9) was observed in genotype 3, 13 and 17.

Table 3. The number of band presence resulted from electrophoresis of proteins by genotype separation

Genotype number	band number	Genotype number	band number
1	13	13	9
2	14	14	13
3	9	15	10
4	10	16	11
5	11	17	9
6	11	18	12
7	11	19	11
8	11	20	10
9	12	21	14
10	11	22	10
11	12	23	13
12	11		

Table 4. Observed band resulted from electrophoresis of proteins and relative vibration and their molecular weight

band number	presence number	MV molecular weight (KD)	band number	presence number	MV molecular weight (KD)
1	10	82.04	10	17	41.26
2	12	80.19	11	17	39.41
3	22	73.16	12	17	36.80
4	10	71.51	13	10	32.07
5	16	55.58	14	15	30.63
6	6	53.09	15	23	27.32
7	19	47.34	16	16	25.50
8	17	46.27	17	10	23.81
9	5	44.20	18	16	21.72

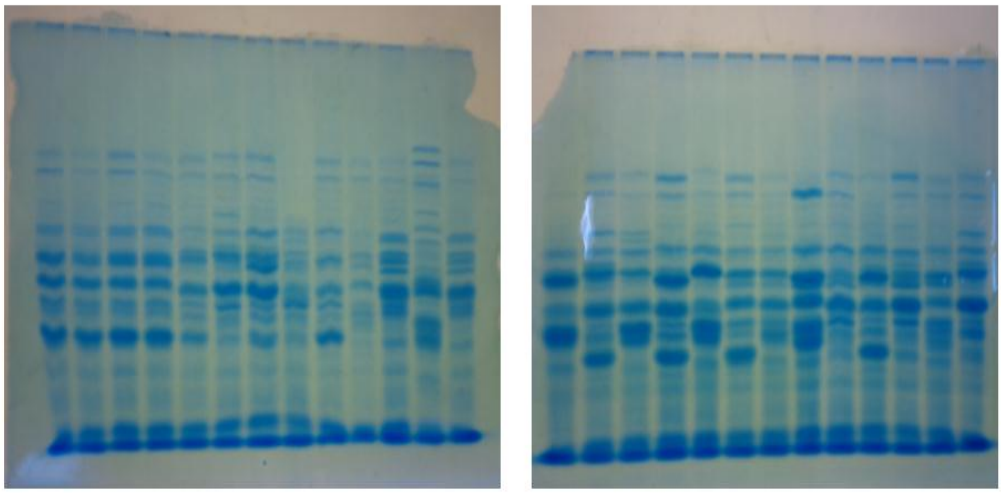

Figure 2. Band pattern of protein sub units

In seed storage proteins analysis based on SDS- PAGE method, total of 21 band patterns were identified. According to the obtained results, 23 studeid genotypes were divided into 3 groups (figure 3). Hence the first group consisting of 12 genotype had the most members genotypes number 1, 3, 4, 8, 9, 10, 11, 14, 16, 18, 22 and 23 respectively. This group contained two sub- groups: the first sub- group was consisted of 2 genotypes of 1 and 23 and the second sub group was consisted of 10 genotypes of 3, 4, 8, 9, 10, 11, 14, 16, 18 and 22 and genotypes 8 with 10 and 9 with 11 had identical electrophoretic pattern. The second group contained 7 genotypes and the second group had two sub- groups. The first sub group consisted of 4 genotypes was 2, 21, 19 and 20 and the second sub- group consisted of 3 genotypes were 5, 6 and 7. The third group with 5 genotypes of 12, 13, 15 and 17 were genotypes that were in the third group. This group was divided into 2 sub- groups: the first sub- group was consisted of genotypes number 12, 13 and 15 and the second sub- group was consisted of genotype number 17. Similarity coefficient between genotypes in protein bands was estimated by jaccard method:

A: Band numbers existed in each two types.
B: Band numbers limited to the first type.
C: Band numbers limited to the second type.

It is notable that these coefficients within the range of 0.24 and 1 are varied and the more is the similarity coefficient between two genotypes, the more similar two genotypes are in terms of protein bands and biochemistry. According to table 5 the minimum similarity coefficient was between genotypes (1 with 3, 5, 7, 18 and 22), (2 with 17), (3 with 12, 20 and 21) (4 with 12, 13 and 15), (6 with 11 and 16), (8 with 12 and 13), (9 with 12, 13 and 15), (10 with 12, 13 and 15), (11 with 12, 13 and 15), (12 with 16, 18 and 22), (13 with 22), (14 with 15), (15 with 16,and 22), (17 with 20 and 21) and genotype 22 with 23 which implies the radical difference of these genotypes with respect to seed total proteins. To obtain maximum heterozygous in producing hybrid, genotypes that have the most difference in electrophoretic pattern of protein bands are converged. The amount of genetic diversity in genetic locus of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17 and 18 are equal to 0.49, 0.5, 0.09, 0.49, 0.38, 0.34, 0.34, 0.34, 0.28, 0.38, 0.38, 0.28, 0.5, 0.47, 0.38, 0.47 and 0.38 respectively and its mean was 0.37 (table 6) as it can be observed genetic diversity in genetic locus of band number 3 was less in comparison with other genetic locus. While bands 2 and 13 had the highest genetic diversity, they can be taken into account in genetic identification and their grouping as well. Based on genetic diversity mean it can be concluded that genetic diversity in total proteins in SDS was to some extent low and in order to group and identify genotypes it can be efficient enough by merely itself. Germplasm of the products with Pakistani origin in terms of seed storage proteins diversity in chickpeas (Ghafoor and Arshad, 2008), wheat (Sultana et al, 2007), lentil (Sultana et al, 2006), black gram (Ghafoor and Arshad, 2005), peanut (Javaid and et al, 2004) and black eyed peas (Iqbal and et al, 2003) was under investigation.

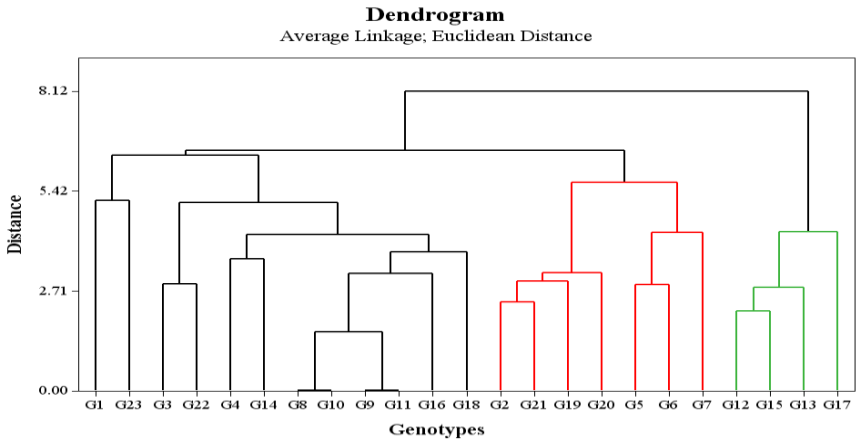

Figure 3. Dendogram obtained from cluster analysis by UPGMA method in barley genotypes based on band electrophoretic pattern

Table 5. Jaccard similarity coefficient f 23 barley genotype's protein bands

G23	G22	G21	G20	G19	G18	G17	G16	G15	G14	G13	G12	G11	G10	G9	G8	G7	G6	G5	G4	G3	G2	G1	
																						1	G1
																					1	0.61	G2
																				1	0.5	0.33	G3
																			1	0.61	0.67	0.61	G4
																		1	0.61	0.56	0.72	0.33	G5
																	1	0.89	0.61	0.56	0.61	0.44	G6
																1	0.67	0.78	0.61	0.56	0.5	0.33	G7
															1	0.56	0.44	0.56	0.72	0.67	0.72	0.56	G8
														1	0.94	0.50	0.39	0.50	0.78	0.61	0.78	0.61	G9
													1	0.94	1	0.56	0.44	0.56	0.72	0.67	0.72	0.56	G10
												1	0.94	1	0.94	0.50	0.39	0.50	0.78	0.61	0.78	0.61	G11
											1	0.28	0.33	0.28	0.33	0.67	0.78	0.67	0.39	0.33	0.5	0.56	G12
										1	0.78	0.28	0.22	0.28	0.22	0.44	0.67	0.56	0.39	0.44	0.5	0.44	G13
									1	0.44	0.44	0.83	0.78	0.83	0.78	0.67	0.56	0.67	0.83	0.56	0.83	0.56	G14
								1	0.39	0.83	0.83	0.22	0.28	0.22	0.28	0.61	0.72	0.61	0.33	0.50	0.44	0.39	G15
							1	0.28	0.78	0.44	0.33	0.83	0.78	0.83	0.78	0.44	0.33	0.44	0.61	0.67	0.72	0.56	G16
						1	0.56	0.72	0.56	0.78	0.67	0.39	0.44	0.39	0.44	0.56	0.67	0.56	0.50	0.67	0.39	0.44	G17
					1	0.50	0.72	0.44	0.72	0.50	0.39	0.78	0.72	0.78	0.72	0.50	0.50	0.61	0.56	0.72	0.78	0.39	G18
				1	0.72	0.56	0.56	0.61	0.78	0.56	0.56	0.61	0.67	0.61	0.67	0.56	0.67	0.78	0.61	0.56	0.83	0.44	G19
			1	0.83	0.56	0.39	0.50	0.56	0.72	0.50	0.61	0.56	0.61	0.56	0.61	0.61	0.61	0.72	0.56	0.39	0.78	0.5	G20
		1	0.78	0.83	0.67	0.39	0.61	0.56	0.83	0.50	0.61	0.67	0.61	0.67	0.61	0.61	0.61	0.72	0.67	0.39	0.89	0.61	G21
	1	0.44	0.44	0.61	0.78	0.50	0.61	0.33	0.61	0.28	0.28	0.78	0.83	0.78	0.83	0.61	0.50	0.61	0.67	0.83	0.56	0.39	G22
1	0.39	0.72	0.61	0.56	0.5	0.56	0.67	0.61	0.78	0.56	0.56	0.61	0.56	0.61	0.56	0.67	0.44	0.44	0.61	0.44	0.61	0.56	G23

Table 6. Type, number and amount of genetic diversity in genetic locus

Number genotyp / bands	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	genetic diversity	Mean genetic diversity
1	1	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	1	1	1	0	0	0.49	
2	0	0	1	1	0	0	1	1	1	1	1	0	0	1	0	1	1	0	0	0	0	1	1	0.5	
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0.09	
4	0	1	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	1	0	0	1	0	1	0.49	
5	1	1	0	0	1	0	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	0.38	
6	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	0.34	
7	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	1	1	1	1	1	1	0.34	
8	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0.34	
9	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0.28	0.37
10	1	1	1	0	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0.38	
11	1	1	0	1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	0	0.38	
12	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	0	0.5	
13	1	1	1	0	0	0	0	1	1	1	1	0	0	0	1	1	0	1	0	0	0	1	0	0.47	
14	1	1	0	1	1	1	1	0	0	0	0	1	1	1	0	0	1	0	1	1	1	0	1	0.38	
15	1	1	1	1	0	0	0	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	0.47	
16	0	0	1	0	1	1	1	0	0	0	0	1	1	0	0	0	1	1	0	0	0	1	0	0.38	
17	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	0	1	1	0	1	0.47	
18	1	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	1	1	1	0	0	0.38	

REFERENCES

Abd Mishanei S and A. Shah Nejat Boushehri, 1998. Breeding Supplementary Volume 2. Tehran University Press.

Ashraf, S and Y. Hiroshi. 2006. Future of Rice-Fish Culture, Desert Aguaculture and Feed Development in Africa: The Case of Egypt as the Lieding Country in Africa. Jpan International Research Center for Agricultural Science. 40 (4): 351-360.

Boulter, D., Thurman, A and Turner, B.L 1966. The use of cise electrophoresis of plant proteins in systematic. Taxon. 15: 135-142.

Ghafoor, A. and Z. Ahmad. 2005. Diversity of agronomic traits and total seed protein in black gram [Vigna mungo (L.) Hepper]. Acta Biologica Cracoviensia, Series Botanica, Poland, 47(2): 69-75.

Ghafoor, A. and M. Arshad. 2008. Seed protein profiling of Pisum sativum (L) germplasm using Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for investigation of biodiversity. Pak. J. Bot., 40(6): 2315-2321.

Iqbal, M.S., A. Ghafoor, A.S. Qureshi and Z. Ahmad. 2003. Genetic dissimilarities in cowpea (Vigna unguiculata (L.) Walp.) for protein peptides and their significance for quantitative traits loci. Pak. J. Bot., 35(3): 377-386.

Javaid, A., A. Ghafoor and R. Anwar. 2004. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pak. J. Bot., 36(1): 25-29.

Khodabandeh, N. 2003. Cereals. Tehran University Press. P. 506.

Metakovsky, E.V and G. Branlard. 1998. Genetic diversity of French common wheat germplasm hased on gliadin alleles. Theor. Appl. Genel, 96: 209-218.

Nei, M. 1978. Estimation of the average heterozygosis and genetics distance from a small number of individuals. Genetics, 89: 583-590.

Rabeie, b. 1996. Protein variation through electrophoresis, SDS-PAGH 16 rice cultivars and their association with quantitative traits in field trials. Master's thesis. Faculty of Agriculture Tabriz.

Rodrigues-Quijano, M., M.T. Nieto-Taladriz., M. Gomez and J.M. Carrillo. 2001. Quality influence of some HMW gluteini subunits X and Y type coded by Glu-D1 locus pruc. 6th Int 1 Wheat Con. 5-9 June, Budapest, Hungary, 26 pages.

Sultana, T., A. Ghafoor and M. Ashraf. 2006. Geographic patterns of diversity of cultivated lentil germplasm collected from Pakistan, as assessed by seed protein assays. Acta Biologica Cracoviensia, Series Botanica, Poland, 48(1): 77-84.

Sultana, T., A. Ghafoor and M. Ashraf. 2007. Genetic variability in bread wheat (Triticum aestivum L.) of Pakistan based on polymorphism for high molecular weight glutenin subunits. Genetic Resources and Crop Evolution, 54: 1159-1165.

Valizadeh, M, K. K. Kang, & T. Kameya.1994, Genetic resemblance among nine medicago species. Journal of Agriculture Science and Technology (JAST), in press.