INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
07-297/AWB/2008/10–101–104
http://www.fspublishers.org

Full Length Article

Study of Glutenin Subunits in Some Wheat Landraces from Northwest of Iran by SDS-PAGE Technique

N. CHAPARZADEH, O. SOFALIAN[1]†, A. JAVANMARD†, M.S. HEJAZI‡ AND L. ZARANDI†
Halophytes Biotechnology Center and †Department of Biology, Azerbayjan University of Tarbiat Moallem, Tabriz, Iran
‡Pharma-biotechnology Department, Faculty of pharmacy, Tabriz Medical University, Tabriz, Iran
[1]Correspondencing author's e-mail: sofalian@yahoo.com

ABSTRACT

Genetic variations of cultivars are very interesting in reducing genetic vulnerability and lead to stable control of production. This study was conducted to determine the variation of endosperm protein subunits of 42 wheat landraces from northwest of Iran. Results showed the existence of variation at HMW-Glutenins, LMW-Glutenins and ω-gliadins regions. Frequency of landraces with 2 + 12 subunits was more than landraces with 5 + 10. In addition, new protein subunits appeared at HMW-GS region, between 1 and 2 bands region (related to Glu-A1) and 2 and 3 bands region (related to Glu-D1). Tow-way analysis of protein content variance also showed the significant differences between experimental landraces.

Key Words: Genetic diversity; Glutenin; SDS-PAGE; Wheat

INTRODUCTION

Narrow genetic background has restricted improved varieties of main crop tolerance to biotic and abiotic stresses. Early farmers grew genetic blended cultivars (landraces) on a very large scale and thereby extended genotypes possess effective genetic make up against various plant diseases. Today it is realized that the use of genetically different varieties is an effective strategy in order to minimized genetic vulnerability (Smale *et al*., 2002). Depending on their geographical regions, landraces had specific genetic background that can be used in genetic research program (Allard, 1996). In addition, landraces are important genetic resources that improve gene pools of modern cultivars by introducing new alleles (Feldman & Sears, 1981; Nevo & Payne, 1987).

In order to evaluate genetic diversity the study of biochemical and molecular markers, less affected by environmental factors, is more important than morphological traits (Plaschke *et al*., 1990; Röder *et al*., 1998; Korzun *et al*., 1999). Seed storage proteins are the result of expression of genome and contain extensive genetic variation in wheat landraces. Thus, they are taken as good criteria for genetic diversity studies (Porceddue *et al*., 1998).

The most important part of these proteins was polymeric glutenin subunits and monomeric gliadins. Especially, glutenin components are important in quality determination as viscoelastic properties of bread wheat flour (Payne & Lawrence, 1983). HMW-GS are encoded by two type of genes called (x:y) that are located on three loci (Glu-A1, Glu-B1 & Glu-D1) placed on long arms of the group 1 chromosomes (Lawrence & Shepherd, 1981). Gene loci on chromosomes are controlled main LMW-GS (subunits B) (Payne *et al*., 1987). Payne *et al*. (1987) showed that allelic variation at Glu-D1 locus had greater effects than other loci on bread making quality. According to some studies, subunit combination 5 + 10 was good, whereas subunit combination 2 + 12 associated with poor bread making quality (Gupta *et al*., 1989, 1994). Therefore, local varieties are most important in breeding programs in order to improving crop quality, and biotic and abiotic stress resistance (Metakovsky & Branlard, 1998).

Northwest of Iran is amongst the main centers of origin and diversity of bread wheat (Harlan & Zohary, 1976). The present study was carried out to evaluate the biodiversity of wheat landraces in relation to storage protein subunits of this region.

MATERIALS AND METHODS

Seeds of 42 wheat landraces of Northwest of Iran obtained from national gene bank of Iran and seed storage proteins were extracted according to procedure described by Payne and Lawrence (1983). Based on Lawrence and Shepherd (1981) method with slight modification, extracted protein fractionated by one-dimensional (sodium dodecyl sulfate polyacrylamid gel electrophoresis) SDS-PAGE (10%). Identification of the subunits were performed based on catalogue described by Payne *et al*. (1981). Subsequently cluster analysis was made with NTSYS software after gel scoring (James Rohlf, 2000). In addition, the total extracted

To cite this paper: Chaparzadeh, N., O. Sofalian, A. Javanmard, M.s. Hejazi and L. Zarandi, 2008. Study of glutenin subunits in some wheat landraces from northwest of Iran by SDS-PAGE technique. *Int. J. Agri. Biol.*, 10: 101–104

protein content of seed was calculated spectrophotometerically using lowry (Lowry *et al*., 1951) method in quintuplicate.

RESULTS AND DISCUSSION

Identification of the HMW glutenin subunits confirmed the probable polymorphism of these subunits in the experimental landraces. A majority of the subunits encoded by Glu-D_1 locus were 2 + 12, 3 + 12 and 4 + 12, while 5 + 10 subunit was in the lower frequency (Fig. 2b). In addition, in some of the experimental landraces there was new subunit in the region between 2 and 4 subunits that encoded by Glu-D_1 locus (Fig. 2b). None of our samples involved one subunit encoded by Glu-A1 locus, which proposed that this subunit after breeding efforts of hybridization and selection procedures might be added to genetic background of local varieties but the null and 2 * subunits were predominant (Fig. 2a). Results showed the 6 + 8 and 7 + 8 subunits encoded by Glu-D1 locus were more predominant than others (Fig 2c). In agreement with these results, Valizadeh *et al*. (2001) mentioned the lower frequency of 5 + 10 and lack of 1 subunits in some other landraces from Iran.

Singh *et al*. (2007) divided 32 Indian wheat landraces to two groups based on HMW-Gs subunits. One group (with 23 accessions) indicated genetic homology and another group (with nine accessions) was genetically heterogeneous. They identified new subunits at both groups. Also the highest frequency of subunits at Glu-1 loci were related to subunits of 2, 17 + 18 and 2 + 12 at Glu-A1, Glu-B1 and Glu-D1 loci, respectively. Gregova *et al*. (1999, 2006) studied two groups of European wheat landraces and obsolete cultivars. In the first study, subunits 1, 7 + 9 and 2 + 12 encoded by Glu-A1, Glu-B1 and Glu-D1 loci were predominant respectively. The second study showed majority of cases were null allele at Glu-A1 locus, 7 + 8 allele at Glu-B1 locus and 2 + 12 allele at Glu-D1 locus. In Romanian wheat landraces, null allele at Glu-A1 locus, 7 + 9 allele at Glu-B1 locus and 5 + 10 allele at Glu-D1 locus, had the highest frequency (Popa *et al*., 2003).

The study of allelic variation at the loci encoding HMW-Gs subunits in Japanese and Chinese hexaploid wheat demonstrated predominance in null allele at Glu-A1 locus, 7 + 8 allele at Glu-B1 and 2 + 12 allele at Glu-D1 locus, which agreed the studies from Japan (Nakamura, 2000). Tahir *et al*. (1996) reported that the highest frequency were found for the null allele at Glu-A1 locus, 17 + 18 allele at Glu-B1 locus and 2 + 12, at Glu-D1 locus in the hexaploid wheat landraces of Pakistan. In agreement of these studies, our results also showed the lower frequency of valuable bread making subunits of landraces. This fact showed that there must be further efforts in order to reach high bread making quality flour in relation to landraces all over the world. This showed that composition of subunits in any region had adaptive value with respect to eco-geographic condition (Nevo & Payne, 1987).

Table I. Comparison of protein content means in wheat landraces

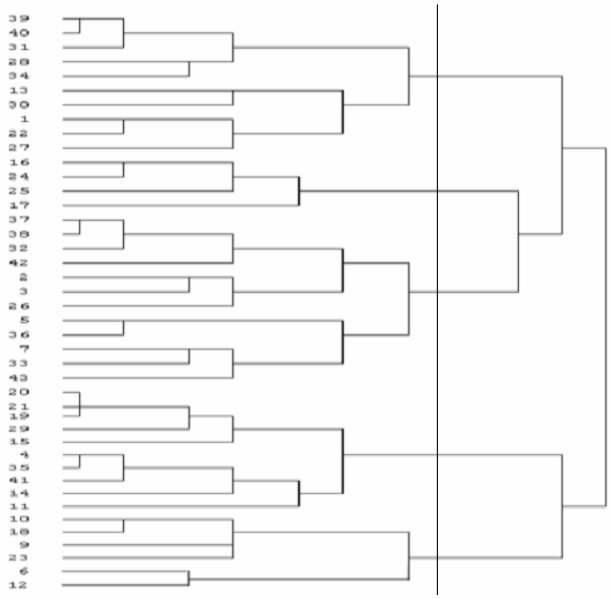
Landrace	Protein content	Landrace	Protein content (mg g^{-1} FW)	Landrace	Protein content (mg g^{-1} FW)
1	65	15	56	29	63.16
2	60.16	16	66.65	30	54.00
3	59.87	17	65.00	31	63.16
4	62.16	18	64.85	32	63.86
5	60.66	19	63.96	33	66.00
6	55.00	20	61.16	34	81.00
7	63.86	21	62.16	35	72.53
8	59.17	22	54.00	36	60.76
9	65.00	23	57.27	37	61.16
10	61.16	24	69.00	38	63.06
11	50.00	25	64.45	39	60.57
12	68.44	26	62.16	40	43.31
13	69.74	27	63.76	41	45.10
14	55.00	28	58.36	42	51.44
					$LSD_{0.05}$ = 8.7

Table II. Two way analysis of variance based on protein content in the wheat landraces

Source of Variation	df	Means of squares
Replication	4	535 **
Landrace	41	180 **
Experimental error	164	54.3

** , means the significant difference in p≤ 0.01

Fig. 1. Cluster analysis of HWM-Glutenins in wheat landraces



Assessment of the protein content of experimental materials performed using by Lowry method (Lowry *et al*., 1951). This method is sensitive to low concentration of protein. Price (1996) suggested concentration of 0.005 – 0.1 mg of protein per ml. Two- way variance analysis based on protein content showed significant results and there was

Fig. 2. Frequency of different alleles encoded by: (a) Glu-A_1, (b) Glu-D_1, (c) Glu-B_1 in the experimental wheat landraces

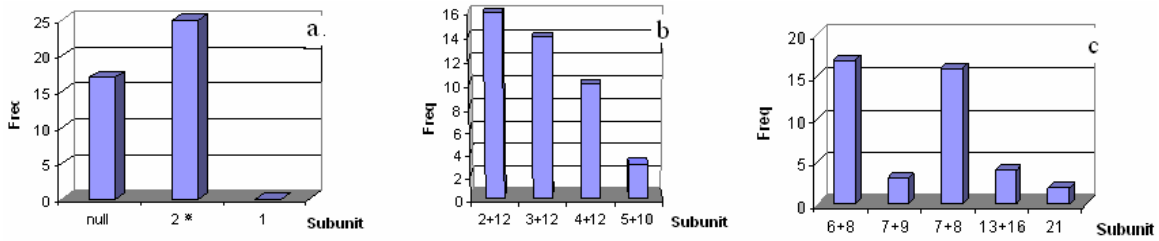
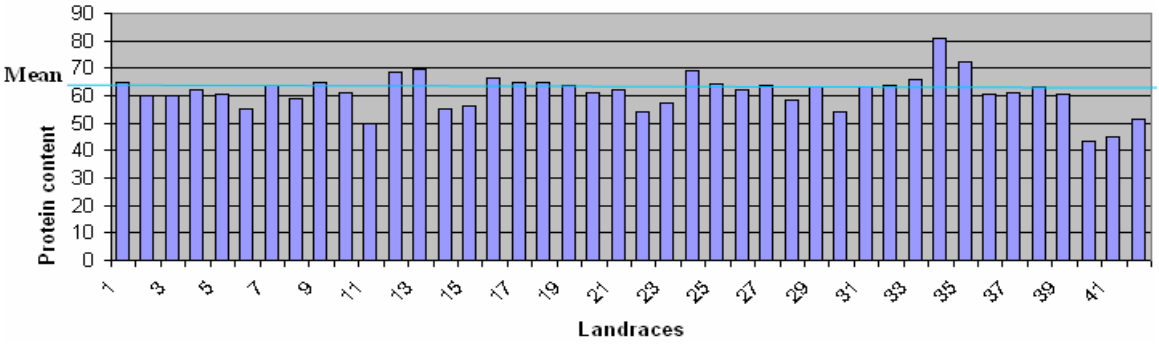


Fig. 3. Difference between protein content (mg g^{-1} fresh weight) of wheat landraces ($LSD_{0.05}$ = 8.7)



significant differences between means assessments of protein contents (Fig. 3 & Table I). This confirmed the existence of genetic difference and existence of genetic variance between landraces (Table II). This genetic variance could be use in any selection programs in order to develop seed with high quality and quantity. It observed not only in the case of subunits but also in the case of protein content there were genetic variations in the studding landraces.

Additionally, in order to better study the observed variation, cluster analysis of the scored HMW-Glutenin subunits was performed (Payne & Lawrence, 1983). Based on simple matching coefficient and furthest neighbors linkage method (Fig. 1) for grouping the landraces we found five cluster in the seed bulk of landraces. In order to better identify the subunits, SDS-PAGE performed for single seed too. According to the cluster analysis, there were similarities between and within clusters.

CONCLUSION

Variation in glutenin structural subunits can be exploited by wheat breeders for the introduction of new varieties with improved bread-making quality. This study confirmed that landraces of Northwest of Iran have valuable biodiversity in order to use in any bread-making breeding and to increase quality (protein type with respect to subunits) and quantity (protein content). The existence of some new subunits was also documented, which may cause and preferred by hidden natural selection to local environment. In order to stop genetic erosion, it is necessary to preserve the common wheat germplasms. This evaluation can be used to predict bread making quality and introduction of new alleles.

Acknowledgment. We thank the National Plant Genetic Resource of Iran for providing seeds.

REFERENCES

Allard, R.W., 1996. *Principles of Plant Breeding,* 2nd edition, p: 254. John Wiley and Sons, New York

Feldman, M. and E.R. Sears, 1981. The wild gene resources of wheat. *Sci. America*, 44: 102–12

Gregova, E., J. Hermuth, J. Kraic and L. Dotlacil, 1999. Protein heterogeneity in European wheat landraces and obsolete cultivars. *Genetic Resour. Crop Evol.*, 46: 521–8

Gregova, E., J. Hermuth, J. Kraic and L. Dotlacil, 2006. Protein heterogeneity in European wheat landraces and obsolete cultivars. Additional information II. *Genetic Resour. Crop Evol.*, 53: 867–71

Gupta, R.B. and F. MacRitch, 1994. Allelic variation at glutenin subunit and gliadin loci, Glu-1, Glu-3 and Gli-1 of common wheat. II. Biochemical basis of the allelic effects on dough properties. *J. Cer. Sci.*, 19: 19–29

Gupta, R.B., N.K. Singh and K.W. Shepherd, 1989. The cumulltive effect of allelic variation in LMW and HMW glutenin subunits on physical dough properties in progeny of two bread wheat. *Theor. Appl.Genet.*, 77: 57–64

Harlan, J.R. and D. Zohary, 1966. Distribution of wild wheats and barley. *Sci.*, 153: 1074–80

James Rohlf, F., 2000. *NTSYSpc Numerical Taxonomy and Multivariate Analysis System User Guide,* p: 38. New York University

Korzun,V., M.S. Roder, K. Wendehake, A. Pasqualon, C. Lotti, M.W. Ganal and A. Blanco, 1999. Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. *Theor. Appl. Genet.*, 98: 1202–7

Lawrence, G.L. and K.W. Shepherd, 1981. Inheritance of glutenin protein subunits of wheat. *Theor.Appl.Genet.*, 60: 333–7

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin–Phenol reagents. *J. Biol. Chem.*, 193: 265–75

Metakovsky, E.V. and G. Branlard, 1998. Genetic diversity of French common wheat germplasm based on gliadin alleles. *Biomed. Life Sci.*, 96: 209–18

Nakamura, H., 2000. Allelic variation at high molecular weight glutenin subunit loci, Glu-A1, Glu-B1 and Glu-D1, in Japanese and Chinese hexaploid wheat. *Euphytica*, 112: 187–93

Nevo, E. and P.I. Payne, 1987. Wheat storage protein diversity of HWM glutenin subunits in wild emmer from Isreal. 1. Geographical patterns and ecogeographical predicability. *Theor. Appl. Genet.*, 74: 827–36

Payne, P.I. and G.J. Lawrence, 1983. Catalogue of alleles for the complex gene loci, Glu-A_1, Glu-B_1 and Glu-D_1which code for high-molecular weight subunits of glutenin in hexaploid wheat. *Cer. Res.Commun.*, 11: 29–35

Payne, P.I., M.A. Nightingale, A.F. Krattiger and L.M. Holt, 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Food Agric.*, 40: 51–65

Plaschke, J., M.V. Ganal and M.S. Roder, 1995. Detection of genetic diversity in closely-related bread wheat using microsatellite markers. *Theor. Appl. Genet.*, 91: 1001–7

Popa, M., E. Gregova and J. Kraic, 2003. Romanian wheat (*Triticum aestivum* L.) landraces characterized by seed storage proteins. *Biodiversity FAO.*, 135: 53–8

Porceddue, E., D. Lafiandra and D. Kasarda, 1998. Variation in endosperm protein composition and technological quality properties in durum wheat. *Euphytica*, 100: 197–205

Price, N.C., 1996. *Proteins, Labfax*, Oxford Academic Press

Röder, M., V. Korzun, K. Vendehake, J. Plaschke, M.H. Tixier, P. Leroy and M. Ganal, 1998. A Microsatellite map of wheat. *Genetics*, 149: 2007–23

Singh, A.M., J.J. Deveshwar, A.K. Ahlawat and B.B. Singh, 2007. Identification of novel variants of high molecular weight glutenin subunits in India bread wheat landraces. *Cer. Res. Commun*., 35: 99–108

Smale, M., I. Ortiz-Monsterio, M. Warburton, B. Skovmand, M. Reynolds, J. Crossa, R. Singh and R. Trethowan, 2002. Dimensions of diversity in modern spring bread wheat in developing countries from 1965. *Crop Sci.*, 42: 1766–79

Tahir, M., T. Turchelta, R. Anwar and D. Lafiandra, 1996. Assesment of genetic variability in hexaploid wheat landraces of Pakistan based on polymorphism for high molecular weight glutenin subunits. *Genetic Resour. Crop Evol.*, 43: 211–20

Valizadeh, M., H. Shahbazi and O. Sofalian, 2001. *Comparative Analysis of Iranian Northwest Landraces by SDS-PAGE and ACID-PAGE*. Abstracts of the XVIth EUCARPIA Congress. Edinburgh, Scotland

(Received 04 September 2007; Accepted 05 October 2007)