Pak. J. Bot., 35(1): 61-68, 2003.

THE HIGH-MOLECULAR-WEIGHT GLUTENIN SUBUNIT COMPOSITION OF WHEAT (*TRITICUM AESTIVUM* L.) LANDRACES FROM PAKISTAN

RASHID ANWAR, SHAHID MASOOD, MIR AJAB KHAN* AND S.NASIM

Plant Genetic Resources Institute, NARC, Islamabad, Pakistan.

Abstract

The endosperm storage proteins of 127 wheat landraces collected from different parts of Pakistan were fractionated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) to determine their high molecular weight (HMW) glutenin subunit composition. A total of 13 different subunits were found in the present set of wheat germplasm. Of these alleles detected at all the *Glu-1* loci, three belong to *Glu-A1*, five each to *Glu B1* and *Glu D1* locus. Subunit null controlled by locus *Glu-A1*, 17+18 by *Glu-B1* and 2+12 by *Glu-D1*, predominantly. Different accessions possessed between three and five HMW glutenin subunit. The frequency of alleles in the entire set of germplasm ranged from 0.79 to 80.31% which indicate a higher degree of genetic diversity. The quality score calculated according to Payne (1986) ranged between 4 and 10 in the landraces examined. The information generated in this study could be utilized to improve the bread or chapati making quality.

Introduction

Glutenins and gliadins are two major groups of seed storage proteins in hexaploid wheat (*Triticum aestivum*, genome constitution AABBDD). Glutenins consist of long chain of polypeptides (subunits) linked by disulfide bonds. Reduction of these inter-chain bonds allows the separation of the glutenin subunits into high molecular weight (HMW) and low molecular weight (LMW). The high molecular weight glutenin subunits are coded by genes at three loci designated *Glu-A1*, *Glu-B1*, and *Glu-D1*. They are located on the long arm of chromosome 1A, 1B and 1D, respectively (Lawrence & Shepherd, 1981; Payne & Rhodes, 1982). Although HMW-GS accounts for around 10% of the wheat storage proteins, they play a key role in determining bread making quality (Payne *et al.*, 1984).

The genetics and biochemistry of high molecular weight (HMW) glutenin subunits in wheat is very well known (Shewry *et al.*, 1989, 1992). The results from various studies have shown that the three loci coding for HMW glutenin subunits are highly polymorphic in nature and their expression is strictly under the genetic control. Considering these properties, allelic variation at the three loci coding for HMW glutenin subunits has been used either independently or in combination with other genetic markers to estimate the genetic variation in different wheat species (Nevo & Payne, 1987; Felsenburg *et al.*, 1991; van-Hintum & Elings, 1991; Ciaffi *et al.*, 1993).

Wheat landraces and populations of wild relatives collected in various countries have been evaluated for their variation in seed storage proteins (Lafianadra *et al.*, 1993; Lagudah *et al.*, 1987; Nevo & Pyne, 1987). Significant correlation between bread making quality and HMW glutenin subunits have been observed in wheat from several countries. Results from all these studies agree that some HMW glutenin subunits, such as 5+10, are

*Quaid-i-Azam University, Islamabad, Pakistan.

more important than others for determining bread making quality. Some wheat breeding programs such as those at the former Plant Breeding Institute (PBI) and the International Maize and Wheat Improvement Center (CIMMYT) have already used HMW glutenin subunit composition as a selection criterion of selecting parents for improving bread making quality. A number of studies also looked at the HMW glutenin subunit composition of wheat from Australia (Lawrence, 1986); Canada (Lukow *et al.*, 1989); India (Bhagwat *et al*, 1988); Italy (Pogna *et al.*, 1989); the Soviet Union (Morgunov *et al.*, 1990); and Yugoslavia (Vapa *et al.*, 1988) to provide information on quality improvement

To date the HMW glutenin subunit composition of Pakistani wheat remains unexplored. The only exception is the report published by Tahir *et al.*, (1995). The present study was undertaken to generate information on HMW-Glutenin subunit composition in the local landraces of wheat from Pakistan. The information may also be of interest to plant breeders to develop varieties with good bread making quality.

Material and Methods

Plant material: A total of 127 accessions of local wheat germplasm were taken from the gene bank of Plant Genetic Resources Institute at National Agricultural Research Centre, Islamabad and subjected to SDS-PAGE analysis. Chinese spring, Norin 10, Hope and Gabo cultivars were used as standards.

Protein extraction: A single grain from each spike was crushed into powder and 10 milligram wheat flour was taken in a microtube. One ml of extraction buffer (0.05M Tris + 0.2% SDS + 5M Urea, adjusted to pH 8.0 with HCl was added to each sample. After few minutes 10µl mercaptoethanol was added into each microtube and properly labelled for each accession. Material was mixed well with the help of Vortex mixer. The same process was repeated after 10 ~ 15 minutes and the extract were kept in refrigerator until electrophoresis was carried out.

Electrophoresis: The total seed protein was analyzed through slab type SDS-PAGE following the method of Laemmli (1970) using 11.5% polyacrylamide gel. A total volume of 10 µl protein extract solution was loaded into each well with the help of micro-syringe. Four wheat cultivars Chinese Spring, Norin 10, Gabo and Hope with known banding pattern were used as standards for the identification and comparison of generated bands. The electrophoresis was run at 100 V for first 30 minutes until a blue line of BPB solution passes through separation gel. Thereafter, the electrophoresis was run at 150 V till blue line passes through the bottom of gel plates. The gels were stained in a staining solution for 20 ~ 30 minutes over a shaker, shifted to another container having the destaining solution and kept until the protein bands become clear. After destaining, gels were dried using a Gel Drying Processor for about one hour at 60 ~70° C.

Scoring and data analysis: Allelic variation at *Glu-1* loci was recorded by numbering the bands at each subunit (*Glu-A1, Glu-B1 and Glu-D1*) on the basis of the catalogue by Payne & Lawrence (1983). Quality score was calculated according to Payne (1987) by adding together the score of individual subunit. The overall frequency of each subunit was worked out by computer software GSTAT.

Results and Discussion

The analysis of storage protein variation in wheat have proved to be a useful tool not only for the diversity studies but also to aid in optimising the variation in germplasm collections and in breeding cultivars with improved bread-making quality. The frequency of various alleles found in the entire set of germplasm at three *Glu-1* loci (*Glu-Al, Glu-B1* and *Glu-D1*) is given in Table 1. At the locus *Glu-A1* the subunit "null", which does not code for any protein was the most frequent and represented in 68.50% of the accessions. The remaining accessions were found to posses the subunit 2* (17.32%) and 1 (14.17%) at this locus. Generally, the null allele at *Glu-A1* locus is predominantly reported in wheat landraces (Lagudah *et al.*, 1987; Cross & Guo, 1993). Tahir *et al.*, (1996) also reported similar pattern in wheat landraces of Pakistan where more than 60% of the accessions possessed null allele followed by the allele 2* at this locus. While evaluating wheat for HMW glutenin subunit composition Tahir *et al.*, (1995) found that none of the cultivar possess null allele at the *Glu-A1* locus. It appears that wheat breeders in Pakistan have developed cultivars with superior quality by replacing the null allele with 2* which impart better quality to wheat flour.

Table 1. Allele frequencies of HMW glutenin subunits at *Glu-1* loci in 127accessions of bread wheat.

	Allele	No. of accessions	Proportion	Frequency
Glu-A1	1	18	0.1417	14.17
	2*	22	0.1732	17.32
	null	87	0.6850	68.50
Glu-B1	7	26	0.2047	20.47
	8	3	0.0236	2.36
	7+8	54	0.4252	42.52
	7+9	16	0.1260	12.60
	17+18	28	0.2205	22.05
Glu-D1	10	1	0.0079	0.79
	12	6	0.0472	4.72
	2+12	102	0.8031	80.31
	5+10	5	0.0394	3.94
	4+12	13	0.1024	10.24

The most frequent HMW glutenin subunits at *Glu-B1* locus were 7+8, which appeared in 54 (42.52%) accessions. It was followed by subunit 17+18 (22.05%) 7 (20.47%) and 7+9 (12.6%). It has been further noticed that in some of the accessions subunit 7 was found to be slightly faster as compared to Chinese Spring. The results are in agreement with Mir Ali *et al.*, (1999); Tahir *et al.*, (1996); Igrejas *et al.*, (1999); and Nakamura *et al.*, (1999) who reported the higher frequency of 7+8 allele than 17+18 at this locus. Payne & Lawrence (1993) and Mir Ali *et al.*, (1999) reported that 7+8 and 17+18 have more influence on quality than other Glu-B1 alleles.

AT the *Glu-D1* only three pairs of subunits 2+12, 4+12 and 5+10 were found in most of the accessions with the frequency of 80.13%, 10.24% and 3.94%, respectively. The subunits 10 and 12 were also found at this locus. The majority of the accessions were having subunit 2+12 which was recorded in 80% of the accessions. However, five accessions were found with 5+10 combination. Regarding the effect of *Glu-D1* allele on

Table 2. Allelic distribution in bread wheat accessions based on SDS-PAGE.

S. No.	Allelic combination	No.of Acc.	Quality score	Accessions
1	1, 17+18, 2+12	1	8	16430
2	1, 17+18, 5+10	2	10	16479, 16799
3	1, 7+8, 12	2	8	15730, 16206
4	1,7+8, 2+12	7	8	15940, 16094, 16204, 16338, 16341, 16346, 16885
5	1, 7+8, 4+12,	5	7	16456, 16460, 16468, 16477, 16883
6	1, 7+9, 12	1	7	16080
7	2*, 7, 2+12	1	7	15903
8	2*, 8, 4+12	1	6	16040
9	2*, 17+18, 2+12	2	8	16069, 16372
10	2*, 17+18, 4+12	2	7	16092, 16727
11	2*, 17+18, 5+10	2	10	15799, 16199
12	2*, 7+8, 12	1	8	15724
13	2*, 7+8, 2+12	8	8	15714, 15871, 15943, 16093, 16099, 16201, 16876, 17140
14	2*, 7+8, 4+12	2	7	16832, 16877
15	2*, 7+9, 2+12	2	7	16030, 16035
16	2*, 7+9, 4+12	1	6	16081
17	Null, 7, 2+12	25	5	15792, 16005, 16006, 16009, 16011, 16012, 16014, 16015, 16017, 16018, 16020, 16023, 16028, 16037, 16038, 16042, 16043, 16058, 16063, 16066, 16086, 16433, 16446, 16783, 16840,
18	Null, 8, 2+12	2	5	16772, 16880
19	Null, 17+18, 10	1	8	16073
20	Null 17+18, 2+12	17	6	16007, 16010, 16021, 16022, 16027, 16036, 16039, 16041, 16075, 16089, 16098, 16357, 16362, 16791, 16802, 16881, 16883
21	Null, 17+18, 5+10	1	8	16047
22	Null, 7+8, 12	2	6	15933, 17226
23	Null, 7+8, 2+12	26	6	15678, 15687, 15689, 15692, 15696, 15738, 15740, 15781, 15793, 16004, 16045, 16203, 16342, 16343, 16431, 16437, 16459, 16461, 16475, 16722, 16845, 16846, 16878, 17050, 17169, 17227
24	Null, 7+8, 4+12	1	5	15708
25	Null, 7+9, 2+12	11	5	15803, 16044, 16350, 16429, 16473, 16494, 16810, 16841, 16882, 16886, 17142
26	Null, 7+9, 4+12	1	4	16463

Note: 7,8 and 12 were scored 2 when they were individually present

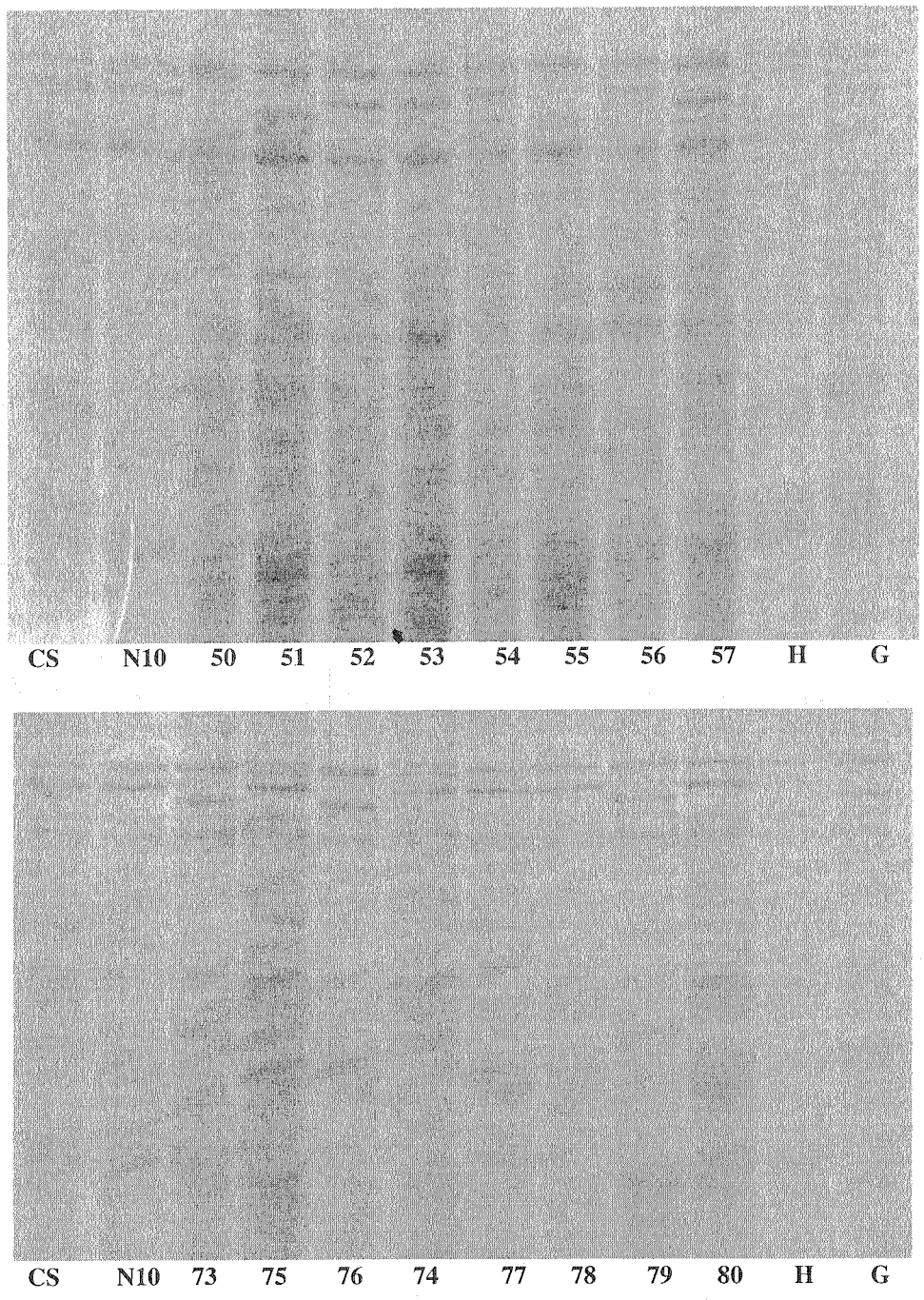

Fig. 1. Allelic variation of the HMW glutenin subunits of the wheat landraces of Pakistan and Chinese Spring (CS), Norin 10, (N10), Hope (H) and Gabo (G).

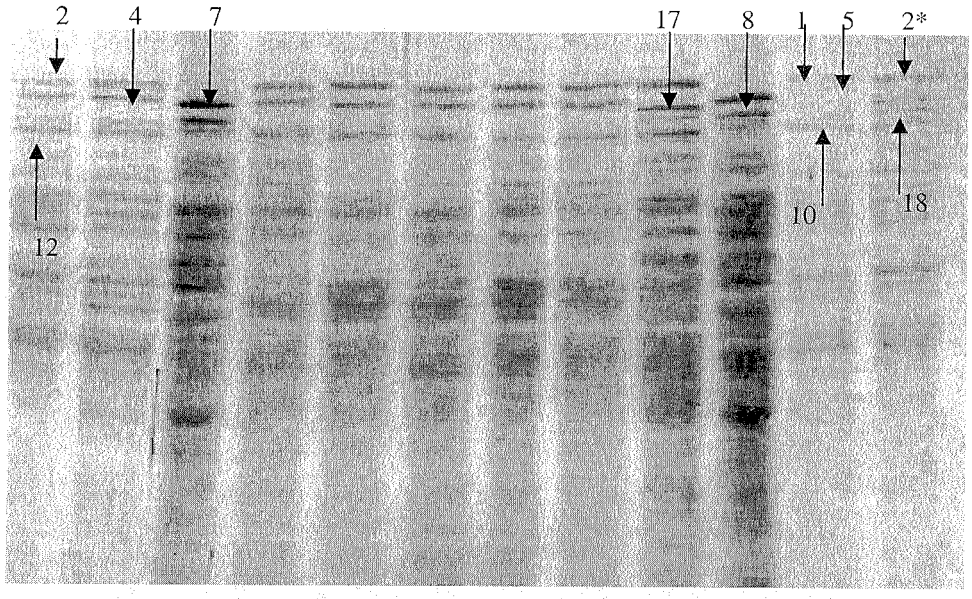

Fig. 2. SDS-PAGE separation of high molecular weight glutenin subunits of *Triticum aestivum* accessions from Pakistan.

the wheat quality, the largest effect of this locus was reported as compared to *Glu-A1* and *Glu-B1*, with subunits 5+10 having a significantly higher effect than that of 2+12 (Lafiandra *et al.,* 1993). In total, 13 *Glu 1* alleles were identified, three at *Glu-A1* and five each at *Glu-B1* and *Glu D1*.

The accessions belonging to different regions of Pakistan were partitioned into 26 different HMW glutenin subunit combinations (Table 2). Of these subunit null, 7+8 and 2+12, was the most frequent, as it was found in 26 accessions. The other frequent subunit composition was null, 7 and 2+12, which was recorded in 25 accessions. Several other subunits with different unique compositions were also found in this group of accessions. The gel depicting the allelic variation and separation of HMW-GS is presented in Fig.1 and 2, respectively.

The overall HMW glutenin subunit quality score calculated according to Payne & Lawrence (1983) ranged from 4 to 10 (Table 1). The lowest score of 4 was observed in less than 1% landraces studied. About 78 % had a score of 5, 6 or 7. The other group of 21% accessions were having a quality score of 8 and above indicating the usefulness of these lines for quality improvement. According to Payne (1987) the minimum score value for a variety is normally three, and the maximum is ten. A large proportion of wheat in Pakistan is used for Chapati-flour, which needs medium to strong gluten strength, whereas, wheat in Pakistan is also used for bread making which requires strong gluten. The accessions PAK 15799, PAK 16199, Pak 16479 and PAK 16799 shows a high *Glu-1* quality score of 10 and were best among others. The information generated from this study could be utilized to devise efficient breeding programs aimed at improving bread or chapati making quality. Further studies are needed to estimate the correlation between glutenin alleles obtained and certain other components of bread making quality.

References

Bhagwat, S.G. and C. R. Bhatia. 1988. *Variation in high molecular weight glutenin subunits of Indian wheat varieties and their Glu-1 quality score. Proc. 7th Int. Wheat Genetics Symposium*, p. 933-935. Cambridge, England.

Ciaffi, M., D. Lafiandra, E. Porceddu and S. Benedettelli. 1993. Storage protein variation in wild emmer wheat (*Triticum turgidum* spp. *dicoccoides*) from Jordan and Turkey. I. Electrophoretic characterization of genotypes. *Theor. Appl. Genet.*, 86: 474-480.

Cross, R.J. and B. Guo. 1993. Glutenin variation in a diverse pre-1935 world wheat germplasm collection. In: *Biodiversity and wheat improvement* (Ed.): A.B. Damania, John Wiley and Sons Publishers, U.K. pp 287-298.

Felsenburg, T., A.A. Levy, G. Galili and M. Feldman. 1991. Polymorphism of highly molecular weight glutenins in wild tetraploid wheat: spatial and temporal variation in a native site. *Israel J. Botany*, 40: 451-479.

Igrejas, G., H. Guedes-Pinto, V. Carnide and G. Branlard. 1999. The high and low molecular weight glutenin subunits and ω-gliadin composition of bread and durum wheats commonly grown in Portugal. *Plant Breeding*, 118: 297-302.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

Lafiandra, D., D. Ovidio, E. Porceddu, B. Margiotta and G. Colaprico. 1993. New data supporting high molecular weight glutenin subunit 5 as the determinant of quality differences among the pair 5+10 Vs 2+12. *J. Cereal Science*, 18: 197-205.

Lagudah, E.S., R.G. Flood and G.M. Halloran. 1987. Variation in high molecular weight glutenin subunits in landraces of hexaploid wheat from Afghanistan. *Euphytica*, 36: 3-9.

Lawrence, G. J. 1986. The high molecular weight glutenin subunit composition of Australian wheat cultivars. *Aust. J. Agric. Res.*, 37: 125-133.

Lawrence, G.J. and K.W. Shepherd. 1980. Variation in glutenin protein subunits of wheat. *Aust. J. Biol. Sci.*, 33: 221-233.

Lawrence, G.J. and K.W. Shepherd. 1981. Chromosomal location of genes controlling seed proteins in species related to wheat. *Theor. Appl. Genet*, 59: 25-31.

Lukow, O.M., P.I. Payne and R. Tkachuk. 1989. The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. *J. Sci. Food Agric.*, 46: 451-460.

Margiotta, B., G. Colaprico, R.D. Ovidio and D. Lafiandra. 1993. Characterization of high M_r subunits of Glutenin by Combined Chromatographic (RP-HPLC) and Electrophoretic separations and Restriction Fragment Length Polymorphism (RFLP) Analyses of their Encoding Genes. *Journal of Cereal Sci.*, 17: 221-236.

MirAli, N., M.I.E. Arabi and B. Al-Safadi. 1999. High molecular weight glutenin subunits composition of Syrian grown bread wheat and its relationships with gluten strength. *J. Genet. & Breed.*, 53: 237-245.

Morgunov, A.I., W.J. Rogers, E.J. Sayers and E.V. Metakovsky. 1990. The high-molecular-weight glutenin subunit composition of Soviet wheat varieties. *Euphytica*, 51: 41-52.

Nakamura, H., A. Inazu and H. Hirano. 1999. Allelic variation in high-molecular-weight glutenin subunit Loci of *Glu-1* in Japanese common wheats. *Euphytica*, 106: 131-138.

Nevo, E. and P.I. Payne. 1987. Wheat storage proteins: diversity of HMW glutenin subunits in wild Emmer from Israel. *Theor. Appl. Genet.*, 74: 827-836.

Payne, P.I. 1986. Varietal improvement in the bread making quality of wheat: Contributions from biochemistery and genetics and future prospects from molecular biology. BCPC MONO *Biotechnology and Crop Improvement and Protection*, 34: 69-81.

Payne, P.I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread making quality. *Ann. Rev. Plant Physiol.*, 38: 141-153.

Payne, P.I. and A.O. Rhodes. 1982. Cereal storage proteins structure and role in agriculture and food technology. *Pl. Physiol.*, 14: 346-369.

Payne, P.I. and G.J. Lawrence. 1983. Catalogue of the alleles for the complex gene loci, *Glu-A1*, *Glu-B1*, *Glu-D1* which code for high-molecular weight subunits of glutenin in hexaploid wheat. *Cereal Res. Com.*, 11: 29-35.

Payne, P.I., L.M. Holt, E.A. Jacksen and C.N. Law. 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. *Phil. Trans. R. Soc. Lond.*, B304, 359-71.

Pogna, N.E., F. Mellini, A. Beretta and A. Dal Belin Peruffo. 1989. The high-molecular-glutenin subunits of common wheat cultivars grown in Italy. *J. Genet. and Breed.*, 43:17-24.

Shewry, P.R., N.G. Halford and A.S. Tatham.1992. High molecular weight subunits of wheat glutenin. *J. Cereal Sci.*, 105-120.

Shewry, P.R., N.G. Halford and A.S. Tatham. 1989. The high molecular weight glutenin subunits of wheat, barley and rye: genetics, molecular biology, chemistry and role in wheat gluten structure and function. *Oxford Survey of Molecular and Cell Biology*, 6: 163-219.

Tahir, M., S.A. Hussain., T. Tuchchetta and D. Lafiandra. 1995. The HMW glutenin subunit composition of bread-wheat varieties bred in Pakistan. *Plant Breeding*, 114:1-3.

Tahir, M., T. Turchetta, R. Anwar and D. Lafiandra. 1996. Assessment of genetic variability in hexaploid wheat landraces of Pakistan based on polymorphism for HMW glutenin subunits. Genet. *Resources and Crop Evolution*, 43: 211-220.

van-Hintum, T.J.L. and A. Elings. 1991. Assessment of glutenin and phenotypic diversity of Syrian durum wheat landraces in relation to their geographical origin. *Euphytica*, 55: 209-215

Vapa. L. and M. Savic. 1988. The HMW glutenin subunit composition of wheat varieties bread in Yugoslavia. *Proc. 7th Int Wheat Genetic Symposium*, p. 1019-1023. Cambridge, England.

(Received for publication 16 September 2001)