# Protein heterogeneity in European wheat landraces and obsolete cultivars

Edita Gregová<sup>1</sup>, Jiří Hermuth<sup>2</sup>, Ján Kraic<sup>1,\*</sup> & Ladislav Dotlačil<sup>2</sup>

<sup>1</sup>Research Institute of Plant Production, Laboratory of Cell & Molecular Biology, Bratislavská cesta 122, 92168 Piešťany, Slovak Republic (\*author for correspondence: Fax: 838 7726306; E-mail: lbmb@vurv.sk); <sup>2</sup>Research Institute of Crop Production, Gene Bank, Drnovská 507, 16106 Praha 6 - Ruzyně, Czech Republic

Received 29 July 1998; accepted in revised form 19 January 1999

Key words: glutenin, homogeneity, landrace, obsolete cultivars, storage proteins, Triticum aestivum L., wheat

## **Abstract**

Identity and present degree of genetic homogeneity and heterogeneity, respectively of 52 European wheat accessions, maintained in the collection of wheat genetic resources, have been characterized using analyses of glutenins by sodiumdodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Six of the analyzed wheat accessions were observed to be homogeneous, while 46 (88.5%) of them were heterogeneous in protein profiles. Heterogeneous accessions possessed 2 to 13 different protein lanes. Together, 17 high molecular weight glutenin subunit (HMW-GS) alleles have been found. The most frequent HMW-GS alleles at the *Glu-A1*, *Glu-B1*, and *Glu-D1* complex loci were 1, 7+9, and 2+12, respectively. However, also low frequented HMW-GS alleles or allelic combinations, such as 7+15, 13+16, 20, 6, 7, and 9 were observed. Furthermore, another new allele encoding HMW glutenin subunit with relative molecular weight 98.6 kDa has been found in one of the lines of the cultivar Eritrospermum 917. The Glu-score in the examined accessions varied in broad range, some of the lines reached the maximum value 10.

# Introduction

From the point of view of total number of accessions in the word's genebank collections, wheat ranks first. As in other crops, wheat landraces and primitive cultivars represent interesting biological material from the historical, geographical, and also genetical point of view. However, available data and characteristics of landraces and obsolete cultivars are mostly lacking and their history is usually unknown. Due to their morphological, physiological, agronomical, and technological parameters they are no more utilized by modern agriculture. In spite of this, landraces and obsolete cultivars belong to the most valuable genotypes. Some of them possess genes, not occurring in modern cultivars, although these genes can be valuable for increasing adaptability of the high productive genotypes, for the improvement of their quality as well as for the increase of their resistance to biotic and abiotic stresses. Landraces and obsolete cultivars are also important as a source of the genetic variation (Autrique et al., 1996). Especially if the genetic uniformity of wheats developed and grew in the last decades increased (Morgunov et al., 1990). Decreasing of genetic variation and extension of genetic erosion in different territories during the last decades has also been published on wheat (Hammer et al., 1996; Gregová et al., 1997). Therefore, collecting missions to find and save still various landraces in some regions are presently organized and rescued materials evaluated. Landraces and other old cultivars of wheat and other crops are often maintained in genebanks all over the world for several decades. History from their introduction up to present days seems to be very interesting. For example Cox & Worrall (1987) published an interesting history of wheat cultivar 'Kharkof' from its introduction from Russia to North America at the end of 19th century. They also detected and compared present protein heterogeneity of this wheat maintained at various places in the USA. Variation of seeds of oat,

barley and other crops after 110 years of sealed storage was studied by Steiner & Ruckenbauer (1995). Steiner et al. (1997) analyzed genetic identity and purity of oat seed samples found in the foundation stone of the Nürnberger Stadttheather of 1832.

The study of landraces attracts more attention, whereupon molecular markers have been set out to be intensively utilized for this purpose (Bretting & Widrlechner, 1995). Seed storage proteins are considered to be usable markers for the studies of wheat genetic resources (Wang et al., 1993; Redaelli et al., 1997). From them, glutenins are in addition associated with the technological quality of wheat flour (Kolster et al., 1991; Manley et al., 1992; Weegels et al., 1996) and the specific gliadin alleles are associated with plant earliness and cold resistance (Metakovsky & Branlard, 1998).

The main goals of present study were (1) to identify and characterize selected European wheat landraces and obsolete cultivars by glutenin profiles, (2) to detect the present degree of genetic homogeneity and heterogeneity respectively, in these profiles, (3) to screen them for the novel HMW-GS alleles, and (4) to select lines with a high value of Glu-score for further utilization.

## Material and methods

Fifty-two landraces and obsolete cultivars of winter wheat - Triticum aestivum L. (var. aestivum, ferrugineum, lutescens, albidum, milturum, velutinum, hostianum, aureum), originating from 14 European countries (Table 1) and maintained in the collection of wheat genetic resources in the Czech Genebank, Research Institute of Crop Production, Praha (Bareš et al., 1993, 1995), were analyzed. One hundred individual halved grains of each accession were used for this study. Glutenins were extracted, separated, and visualized according to standard SDS-PAGE technique for wheat (Wrigley et al. 1992; Kraic et al., 1995). The glutenin patterns were evaluated by densitometer (ImageMaster DTS, Pharmacia Biotech). Homogeneity and heterogeneity, respectively, in the protein composition were studied by comparison of the complete protein patterns revealed by SDS-PAGE. HMW-GS were identified following the descriptions of Payne & Lawrence (1983). The Glu-score, which predicts bread-making quality of wheat flour based on additive effect of HMW-GS, was calculated according to Payne (1987).

### Results

Evaluating the complete protein patterns analyzed by SDS-PAGE, there have been found only six of the examined accessions to be homogeneous. Remaining 46 (88.5%) accessions consist of at least two protein phenotypes (lines). The results of the study of all 52 wheat accessions are summarized in Table 1. Heterogeneous accessions mostly consisted of one major line and one or more minor lines. However, some of them have been observed to consist of two to three major lines in approximately equal rate and another minor lines. The lowest frequency of a minor line was 1%. Furthermore, there have been also revealed the heterozygous grains for HMW-GS composition in some of the accessions. As the most heterogeneous has been found Bulgarian genotype No. 11 (Sofia), which contained as much as 13 protein lines.

HMW-GS composition has been also determined. There were found 17 already known alleles or allelic pairs together at the locus Glu-I. Three the most frequent alleles — null, 1, and  $2^*$  — were detected at the Glu-AI locus, while alleles and allelic pairs 6+8, 7+8, 7+9, 13+16, 17+18, 7+15, 20, 6, 7, and 9 were found at the Glu-BI locus, and allelic pairs 2+12, 3+12, 4+12, and 5+10 at the Glu-DI locus. Most of the analyzed accessions possessed alleles and allelic pairs 1, 7+9, 2+12, evaluated as the most frequent HMW-GS pattern as well.

Possible use of landraces and obsolete cultivars for bread-making quality improvement can be to some extent indicated by Glu-score values. Some of the lines reached the maximum value 10. However, there was no possibility to calculate the final Glu-score value in some of the lines, because they possessed glutenin subunits, which have not been associated with bread-making quality.

In addition, a new HMW-GS, with the electrophoretic mobility between 8 and 9 subunits, was detected in one of the minor lines of Eritrospermum 917. Its relative molecular weight was calculated for 98.6 kDa.

## **Discussion**

The results demonstrate that the heterogeneity of the analyzed set of landraces and obsolete cultivars in protein patterns detected by SDS-PAGE is considerable. As much as 88.5% of landraces were observed to be heterogeneous. Probably some of landraces or cultivars arose by mixing of several genotypes during

Table 1. HMW-GS composition and genetic homogeneity of wheat landraces and obsolete cultivars

Name	Variety	Origin	Lines [%]	Glu- A1	Glu- B1	Glu- D1	Glu- score
Bílá od Dukovan	lutescens	CZE	97	0	7+9	2+12	5
			3	1	7+9	5+10	9
Ruská genealogická bělka	albidum	CZE	100	1	7+9	2+12	7
Slovenská 777	aestivum	SVK	77	1	7+9	3+12	7
			9	0	7+9	5+10	7
			8	1	7+9	5+10	9
			3	0	7+9	2+12	5
			1	0	6+8	2+12	4
			1	1	7+9	2+12	7
			1	1	7+8	2+12	8
Slovenská B	aestivum	SVK	97	1	7+9	3+12	7
			3	0	7+8	3+12	6
Trebišovská 76	aestivum	SVK	100	0	17+18	3+12	6
Vígăšská červenoklasá	ferrugineum	SVK	37	0	7+9	5+10	7
			36	1	7+9	2+12	7
			17	1	7+9	5+10	9
			4	0	6+8	2+12	4
			3	1	6+8	5+10	8
			2	0	7+9	2+12	5
			1				heter.
Vouska z Třemošnice	ferrugineum	CZE	52a	0	7+9	5+10	7
			27	0	7+9	2+12	5
			15b	0	7+9	5+10	7
			3c	0	7+9	5+10	7
			2d	0	7+9	5+10	7
			1				heter.
Židlochovická jubilejní osinatá	ferrugineum	CZE	97	0	7+9	2+12	5
			1	1	7+9	2+12	7
			1	2*	7+9	5+10	9
			1	0	7+8	2+12	6
Austro-Bankut Grannen	milturum	AUT	89	1	7+9	2+12	7
			3	0	7+9	2+12	5
			2	1	7+9	2+12	7
			2	0	7+8	2+12	6
			2				heter.
			1	0	6+8	5+10	6
			1	0	6+8	2+12	4
Bartweizen Linie a	aestivum	AUT	51	1	7+9	2+12	7
			26	0	7+8	2+12	6
			21	1	7+8	5+10	10
			2	1	7+9	5+10	9
Bergland	aestivum	AUT	96	0	7+8	5+10	8
			3	0	7+9	2+12	5
			1	0	7+9	5+10	7

Table 1. (continued)

Name	Variety	Origin	Lines [%]	Glu- A1	Glu- B1	Glu- D1	Glu- score
Tiroler Landweizen	aestivum	AUT	65 25	1	13+16	2+12	8 heter.
			7	2*	7+9	2+12	7
			3	0	6+8	2+12	4
Tschermaks Marchfelder	aestivum	AUT	97	0	7+9	2+12	5
			2	1	7+9	2+12	7
			1	1	6+8	4+12	5
No. 11 (Sofia)	aestivum	BGR	62	1	7+9	5+10	9
			18	0	7+9	5+10	7
			6	- 14			heter.
			2	2*	7+8	5+10	10
			2	0	20	2+12	3+?
			2	2*	7+9	5+10	9
			2	2*	6+8	2+12	6
			1	0	7+8	5+10	8
			1	0	6+8	5+10	6
			1	1	7	5+10	8
			1	1	7+9	2+12	7
			1	0	7+9	2+12	5
N. 201 (K )	4:	DCD	1	0	7+8	2+12	6
No. 301 (Kneza)	aestivum	BGR	46	1	13+16	2+12	8
			29	1	7+8	2+12	8
			6	2*	7+9	5+10	9
			6	1	7+9	5+10	9
			5	0	7+9	5+10	7
			3	1	7+9	2+12	7
			4	0	7+9	2+12	5
C	:14	CHE	1	0	7+8	2+12	6
Sarrayer 602H	milturum	CHE	97 2	0	7+8 6+8	2+12 2+12	6 4
			1	0	0+8 7+15	2+12	4 3+?
Troistorrents 15C	lutescens	CHE	98	1	7+13 7+8	2+12	8
Hoistoffents 13C	lutescens	CHE	2	1	7+8 7+15	2+12	o 5+?
Visperterminen 640E	ferrugineum	CHE	100	1	13+16	2+12	8
Glundon Ladden	velutinum	DNK	42	1	7+9	5+10	9
Giundon Ladden	veratinani	DIVIK	18	1	7+9	2+12	7
			17	0	7+9	2+12	5
			12	0	6+8	2+12	4
			6	Ü	0.0		heter.
			5	0	7+9	5+10	7
Automne rouge barbu	ferrugineum	FRA	100	1	7+9	5+10	9
Barbu de Maconnais	aestivum	FRA	99	0	6+8	2+12	4
			1				heter.
Barbu de Tronchet	ferrugineum	FRA	97	0	7+8	2+12	6
	C		2a	0	6+8	2+12	4
			1b	0	6+8	2+12	4
Blé des Vosgès	milturum	FRA	68	0	7+8	2+12	6
Ţ.			32	0	6+8	2+12	4
Victor	aureum	GBR	64	0	6+8	2+12	4
			36	0	20	2+12	3+?
Baltischer WW	milturum	GER	100	1	7+92+12	7	
Brauner Fuchs	ferrugineum	GER	100	1	7+9	5+10	9
Breisgauer glatter Landweizen	milturum	GER	58	1	7+8	5+10	10
			32	1	7+8	2+12	8
			9	1	7+9	2+12	7
			_				

Table 1. (continued)

Name	Variety	Origin	Lines [%]	Glu- A1	Glu- B1	Glu- D1	Glu- score
Dippes Dickkopf	aureum	GER	98	0	20	2+12	3+?
			1	0	7+8	2+12	6
			1	0	6+8	2+12	4
Hessische Landsorte	lutescens	GER	98	1	6+8	5+10	7
			1	0	6+8	5+10	6
			1				heter.
Rimpaus frueher Bastard	lutescens	GER	92a	0	7+8	2+12	6
			6b	0	7+8	2+12	6
			2				heter.
Zapfs Oberfraenkischer Land	milturum	GER	99	1	6+8	2+12	4
			1	1	7+8	5+10	10
Hatvan	aestivum	HUN	43	1	7+8	5+10	10
			13	1	7+9	2+12	7
			10	1	7+8	2+12	8
			10	0	7+8	5+10	8
			9	- 4			heter.
			5	2*	7+8	5+10	10
			5	1	7+9	5+10	9
			4	0	7+8	2+12	6
a 1 40			1	0	7+9	5+10	7
Szekacs 19	aestivum	HUN	66	0	17+18	2+12	6
			12	1	7+9	2+12	7
			12				heter.
			7	0	7+9	2+12	5
a			3	2*	7+9	2+12	7
Szekacs 17	aestivum	HUN	91	2*	7+9	2+12	7
Danista WW		TITINI	9	0	7+9	2+12	5
Banater WW	aestivum	HUN	95	1	7+9	5+10	9
			2 2	1	7+8	5+10	10
			1	2*	7+9	5+10	heter. 10
Mindeszentpusztai	hostianum	HUN	97	1	7+9 7+9		9
Windeszentpusztai	nostianum	HUN	3	1	7+9 7+9	5+10 2+12	7
Szekacs 1242	aestivum	HUN	72a	0	7+9 7+9	2+12	5
SZCRACS 12-2	acstrum	11011	19b	2*	7+9	5+10	9
			3c	2*	7+9	5+10	9
			3d	0	7+9	2+12	5
			1e	2*	7+9	5+10	5
			1	2*	7+9	4+12	6
			1	1	7+8	5+10	10
Hatvan 5612	aestivum	HUN	89	2*	7+9	5+10	9
	-		3	0	20	2+12	3+?
			3	-	-		heter.
			2	2*	7+8	5+10	10
			2	1	7+9	5+10	9
			1	1	7+8	5+10	10
Ks. Andrzej	ferrugineum	POL	55	1	6+8	5+10	7
v	J		33	1	7+9	5+10	9
			7	1	6+8	2+12	6
			4				heter.
			1	0	7+8	2+12	6
					-		

Table 1. (continued)

Name	Variety	Origin	Lines [%]	Glu- A1	Glu- B1	Glu- D1	Glu- score
Antoninska Wczesna	lutescens	POL	88	1	7+9	2+12	7
			10	1	6+8	5+10	8
			2				heter.
Stepowa	aestivum	POL	92a	0	17+18	2+126	
			3	0	7+9	2+12	5
			3	1	7	2+12	6
			1	2*	7+9	5+10	9
			1b	0	17+18	2+12	6
Zarja	lutescens	SUN	95	1	7+9	5+10	9
			4	0	7+8	5+10	8
			1	0	20	2+12	3+?
Dolis-Puri 35/4	aestivum	SUN	70a	1	7+9	5+10	9
			15	1	7+9	2+12	7
			11	0	7+9	2+12	5
			1b	1	7+9	2+12	7
			1c	1	7+9	2+12	7
			1d	1	7+9	5+10	9
			1	2*	7+9	5+10	9
Krymka mestnaja	aestivum	SUN	36	2*7+9	5+10	9	
			27	1	7+9	2+12	7
			23	0	7+9	2+12	5
			6				heter.
			4	0	7+9	5+10	7
			1	0	7+8	2+12	6
			1	1	20	2+12	4+?
			1	2*	6+8	5+10	8
			1	1	7+9	5+10	9
Kooperatorka	aestivum	SUN	56	0	6	2+12	3+?
			26	1	7+9	5+10	9
			4	1	9	2+12	5+?
			3	0	7+9	5+10	7
			3	1	6	2+12	5+?
			2	0	7+9	2+12	5
			2	1	7+9	2+12	7
			2	0	6.0	0.10	heter.
			1	0	6+8	2+12	4
F '. 017		CLINI	1	1	13+16	5+10	10
Eritrospermum 917	aestivum	SUN	83	1	9	2+12	5+?
			10	1	7+8	2+12	8
			4	0	9	2+12	3+?
			2	1	?	2+12	5+?
Lilradialro		CLINI	1	0	7+9	5+10	7
Ukrajinka	aestivum	SUN	93a	1 1	7+9	5+10 2+12	9 7
			3	1	7+9	2+12	7 hotor
				1	7.0	5   10	heter.
Gostianum 237	hostianum	SUN	1b 96	1 2*	7+9	5+10 5+10	9
Gostianum 257	nosuanum	SUN	96 2		7+8	5+10 2+12	10
			1	0	7+8	2+12	6 9
			1	1	7+9	5+10	
			1				heter.

Table 1. (continued)

Name	Variety	Origin	Lines [%]	Glu- A1	Glu- B1	Glu- D1	Glu- score
Ljutescens 1060/10	lutescens	SUN	69a	2*	7+8	5+10	10
			18b	2*	7+8	2+12	8
			6	1	7+8	2+12	8
			2c	2*	7+8	5+10	10
			2d	2*	7+8	2+12	8
			2	2*	7+9	5+10	9
			1				heter.
Ferrugineum 1234	ferrugineum	SUN	88	1	7+8	2+12	8
			4	1	6+8	5+10	8
			3	1	7+9	2+12	7
			3				heter.
			2	2*	7+9	5+10	9
Landvete fran Halland	lutescens	SWE	86	0	9	2+12	3+?
			9	1	6+8	2+12	6
			2	1	20	2+12	5+?
			2				heter.
			1	0	6+8	5+10	6
Limburgse Kleine Rode	aestivum	NLD	99	1	6+8	5+10	8
			1	1	7+8	2+12	8

a, b, c, d, e, f – lines identical in HMW-GS composition but different by other protein bands. heter. – grains heterogeneous in glutenin profile. ? - unknown effect of glutenin subunit on Glu-score CZE – Czech Republic; SVK – Slovakia; AUT – Austria; BGR – Bulgaria; CHE – Switzerland; DNK – Danmark; HUN – Hungary; POL – Poland; SUN – former Soviet Union; FRA – France; GBR – Great Britain; GER – Germany; SWE – Sweden; NLD – Netherlands.

maintenance. Such contamination caused by mechanical manipulations with the samples among cultivars and landraces is also considered; but on the other hand, it should not be so extensive to cause so high protein heterogeneity. Moreover, parents are not known and available in most accessions; therefore, concerning the heterogeneous accessions, it is impossible to find out which lines are natural and which are additions of another accession. In some cases, it would be possible to conclude, that all lines fundamentally differ from major lines, are additions and the cultivar could be homogenized. For accessions consisting of two or three major lines with the approximately same frequency and differing in most of protein patterns (Krymka mestnaja, Kooperatorka, Blé des Vosgès), it is not possible even to determine unambiguously, whether some of the lines are additions. Heterogeneous accessions could be derived from even more heterogeneous populations originally by natural and target selection. In this way such developed mixtures of cultivars could adapt to the new environmental conditions and show better stability of yield and resistance to pressure of pathogens.

Landraces mostly cover considerable range of species genetic variation. Comparing set of the Slovak landraces of wheat with a set of cultivars recently grown in Slovakia and Czech Republic, it has been found that all HMW-GS discovered in modern cultivars were also detected in landraces. However, it does not apply conversely. Modern Slovak and Czech cultivars do not contain all HMW-GS alleles and encoded subunits observed in Slovak landraces (Gregová et al., 1997). Similar conclusions based on comparison of the genetic diversity between landraces and modern cultivars of durum wheat with various ecogeographic origin were also formulated by Autrique et al. (1996). They discovered that landraces contained 99% of the RFLP fragments, observed in modern cultivars. High genetic variation in HMW-GS alleles has been observed by Ciaffi et al. (1993) in Jordanian and Turkish genotypes of wild emmer, Triticum turgidum ssp. dicoccoides. However, in some of geographic territories there have been found wheat landraces with the very low genetic variation. High variation in HMW-GS alleles was also detected among 52 accessions analyzed in this study. On the contrary very low genetic

variation in HMW-GS has been revealed among 60 landraces of hexaploid wheat originated from several territories in Afghanistan by Lagudah et al. (1987). For example, they detected only one allele encoding subunits 2+12 at the *Glu-D1* locus and none of the genotypes possessed HMW glutenin subunit 1.

In addition, using electrophoretic analyses of durum wheat landraces it is possible to detect new alleles encoding HMW-GS (van Hintum & Elings, 1991; Tahir et al., 1996). In the present study a new HMW-GS with electrophoretic mobility between 8 and 9 subunits in one of the lines of Eritrospermum 917 was detected. Since this line of Eritrospermum 917 contains 1 and 2+12 subunits, a new allele is likely located at the locus *Glu-B1*.

Our research showed that glutenins have proved to be a simple and effective tool for detection of genetic variation, genetic homogeneity or heterogeneity, respectively, for glutenins composition and for revealing of novel glutenin subunits within wheat landraces.

## Acknowledgements

This study of wheat landraces and their diversity was supported by grant no. 05-514-31 from the Ministry of Agriculture of Slovak Republic and by grant no. 521/97/0834 provided by Grant Agency of the Czech Republic. We also thank Miss Eleonóra Medová for precision technical assistance with the electrophoretic analyses.

## References

- Autrique, E., M.M. Nachit, P. Monneveux, S.D. Tanksley & M.E. Sorrells, 1996. Genetic diversity in durum wheat based on RFLPs, morphophysiological traits, and coefficient of parentage. Crop Sci. 36: 735–742.
- Bareš, I., L. Dotlačil, V. Holubec, Z. Kryštof, P. Mikoška, J. Rychtárik, Z. Stehno, M. Ševčík, V. Tisová, M. Vlasák & I. Faberová, 1993. Catalogue of genetic resources of cereals in Czech and Slovak collections, pp. 27–122, VÚRV, Praha, Czechoslovakia.
- Bareš, I., L. Dotlačil, Z. Stehno, I. Faberová & M. Vlasák, 1995. Original and registered cultivars of wheat in Czechoslovakia in the years 1918–1992, pp. 113–296, VÚRV, Praha, Czech Republic.
- Bretting, P.K. & M.P. Widrlechner, 1995. Genetic markers and plant genetic resources management. In: J. Janick (Ed.), Plant Breeding Review, pp. 11–86, John Wiley & Sons, Inc., New York,
- Ciaffi, M., D. Lafiandra, E. Porceddu & S. Benedettelli, 1993. Storage-protein variation in wild emmer wheat (*Triticum tur-gidum* ssp. dicoccoides) from Jordan and Turkey. I. Electro-

- phoretic characterization of genotypes. Theor. Appl. Genet. 86: 474–480.
- Cox, T.S. & W.D. Worrall, 1987. Electrophoretic variation among and within strains of 'Kharkof' wheat maintained at 11 locations. Euphytica 36: 815–822.
- Gregová, E., V. Tisová & J. Kraic, 1997. Genetic variability at the Glu-1 loci in old and modern wheats (*Triticum aestivum* L.) cultivated in Slovakia. Genet. Resour. Crop Evol. 44: 301–306.
- Hammer, K., K. Knüpffer, L. Xhuveli & P. Perrino, 1996. Estimating genetic erosion in landraces two case studies. Genet. Resour. Crop Evol. 43: 329–336.
- Kolster, P., F.A. van Eeuwijk & W.M.J. van Gelder, 1991. Additive and epistatic effects of allelic variation at the high molecular weight glutenin subunit loci in determining the bread-making quality of breeding lines of wheat. Euphytica 55: 277–285.
- Kraic, J., L'. Horváth, E. Gregová & I. Žák, 1995. The standard methods of electrophoretic separation of glutenins and gliadins of wheat by SDS-PAGE and PAGE. Rostl. Výr. 41: 219–223.
- Lagudah, E.S., R.G. Flood & G.M. Halloran, 1987. Variation in high molecular weight glutenin subunits in landraces of hexaploid wheat from Afghanistan. Euphytica 36: 3–9.
- Manley, M., P.G. Randall & A.E.J. McGill, 1992. The prediction of dough properties of South African wheat cultivars by SDS-PAGE analysis of HMW glutenin subunits. J. Cereal Sci. 15: 39–47.
- Metakovsky, E.V. & G. Branlard, 1998. Genetic diversity of French common wheat germplasm based on gliadin alleles. Theor. Appl. Genet. 96: 209–218.
- Morgunov, A.I., W.J. Rogers, E.J. Sayers & E.V. Metakovsky, 1990. The high-molecular-weight glutenin subunits composition of Soviet wheat varieties. Euphytica 51: 41–52.
- 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. & G.J. Lawrence,1983. Catalogue of 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. Commun. 11: 29–35.
- Radaelli, R.P.K., W. Ng & N.E. Pogna, 1997. Allelic variation at the storage protein loci of 55 US-grown white wheats. Plant Breed. 116: 429–436.
- Steiner, A.M. & P. Ruckenbauer, 1995. Germination of 110-year-old cereal and weed seeds, the Vienna sample of 1877. Verification of effective ultra-dry storage at ambient temperature. Seed Sci. Res. 5: 195–199.
- Steiner, A.M., P. Ruckenbauer & E. Goecke, 1997. Maintenance in genebanks, a case study: contaminations observed in the Nürnberg oats of 1831. Genet. Resour. Crop Evol. 44: 533–538.
- Tahir, M., T. Turchetta, R. Anwar & D. Lafiandra, 1996. Assessment of genetic variability in hexaploid wheat landraces of Pakistan based on polymorphism for HMW glutenin subunits. Genet. Resour. Crop Evol. 43: 211–220.
- van Hintum, T.J.L. & A. Elings, 1991. Assessment of glutenin and phenotypic diversity of Syrian durum wheat landraces in relation to their geographical origin. Euphytica 55: 209–215.
- Wang G., J.W. Snape, H. Hu & W.J. Rogers, 1993. The high-molecular-weight glutenin subunit compositions of Chinese bread wheat varieties and their relationship with bread-making quality. Euphytica 68: 205–212.
- Weegels, P.L., R.J. Hamer & J.D. Schofield, 1996. Functional properties of wheat glutenin. J. Cereal Sci. 23: 1–18.
- Wrigley, C.W., 1992. Identification of cereal varieties by gel electrophoresis of the grain proteins. In: H.-F. Linskens & J.F. Jackson (Eds.), Seed Analysis, pp. 17–41, Springer-Verlag, Berlin & Heidelberg, Germany.

Short communication

# Protein heterogeneity in European wheat landraces and obsolete cultivars: Additional information

# Edita Gregová\*, Jiří Hermuth¹, Ján Kraic and Ladislav Dotlačil¹

Research Institute of Plant Production, Department of Applied Biochemistry and Molecular Biology, Bratislavská cesta 122, 92168 Piešťany, Slovak Republic; <sup>1</sup>Research Institute of Crop Production, Gene Bank, Drnovská 507, 16106 Praha 6, Ruzyně, Czech Republic; \*Author for correspondence (e-mail: gregova@vurv.sk)

Received 18 July 2003; accepted in revised form 10 January 2004

Key words: Glutenins, Landraces, Obsolete cultivars, Storage proteins, Triticum aestivum L., Wheat

## Abstract

The objective of this study was to determine the composition of high molecular weight glutenin subunits of landraces and obsolete cultivars. Altogether glutenin profiles of 67 European wheats were analyzed by sodiumdodecylsulphate polyacrylamide gel electrophoresis. Nineteen of them were observed to be homogeneous, whereas 48 (71%) were heterogeneous in glutenin profiles. Heterogeneous accessions possessed from 2 to 9 different glutenin phenotypes. Seventeen high molecular weight (HMW)-glutenin subunits have been found, three belonged to Glu-1A, 11 to Glu-1B, and three to Glu-1D locus. The most frequented HMW-GS at the Glu-A1, Glu-B1, and Glu-D1 complex loci were 0, 7+9, and 2+12, respectively. However, allele low frequented in wheat such as 13+16, 20, 6, 7, 8, and 9 were observed also. Furthermore, other new alleles encoding HMW-GS at the locus Glu-1B with relative molecular weight 120 and 104 kDa have been found in one of the line of the Swedish cultivar Kotte. The Glu-1 quality score in the examined accessions varied broadly with some lines reaching the maximum value of 10.

# Introduction

Modern varieties are the product of formal, institutional, and scientific plant breeding, typically having a high degree of genetic uniformity, whereas landraces, which have been selected by farmers, tend to contain high levels of genetic diversity. They have high value for farmers (Brush and Meng 1998) and for breeders. Due to the limited genetic variation for economically important traits in modern cultivars, this germplasm has become a source of useful genes. They can be used as potential donor of genes encoding high protein content (Blum et al. 1987; Gregová et al. 1999). Seed storage proteins may also be considered as markers in wheat genetic resources

studies (Wang et al. 1993; Radaelli et al. 1997). Glutenins are major components of storage proteins in wheat and have a major role in determining of dough characteristics (Payne 1987; Manley et al. 1992; Weegels et al. 1996). Two major types of glutenins have been identified – high (HMW) and low (LMW) molecular weight glutenins. Genes coding for HMW-GS are located on *Glu-A1*, *Glu-B1*, and *Glu-D1* loci of chromosomes 1AL, 1BL, and 1DL, respectively.

The aim of this study was to continue in the HMW-GS analyses of European wheat landraces and obsolete cultivars, whose results have already been published (Gregová et al. 1999).

#### Material and methods

Sixty-seven landraces and obsolete cultivars of winter wheat – Triticum aestivum L. (var. aestivum, aureum, ferrugineum, lutescens, leucospermum, alborubrum, milturum, velutinum, hostianum, pyrothyx), originated from 15 European countries (Table 1), and maintained in the collection of wheat genetic resources at the Czech Gene Bank, Research Institute of Crop Production, Praha (Bareš et al. 1993), were analyzed. One hundred individual halved grains of each accession were used for analyses. Glutenins were extracted and electrophoresed according to standard SDS-PAGE procedure (Wrigley 1992), and patterns evaluated by densitometer (ImageMaster DTS, Pharmacia Biotech). Homogeneity/heterogeneity in protein composition were studied by comparison to complete protein patterns revealed by SDS-PAGE. High molecular weight glutenin subunits (HMW-GS) were identified according to Payne and Lawrence (1983). Glu-quality score was calculated according to Payne (1987).

# Results and discussion

Results showed high level of heterogeneity at all Glu-1 loci (Table 1). Altogether 17 different HMW-GS were detected. Nineteen, that is, 28%, of examined accessions showed homogeneous patterns, whereas remaining 48, that is, 72%, were heterogeneous, containing at least two different glutenin phenotypes. Some landraces consisted of 2-3 lines (Sipbachzeller zu 213004/G., Guttet 491 A, Unter Engadin 9, Landvete gran Uppsala, Zemka) in approximately equal rates plus some low frequency lines. The lowest frequency of line was 1%. In total, 16 already known alleles were detected in the 67 wheat accessions at the locus Glu-1. Polymorphism was present at each locus. The null allele has the highest frequency at the locus Glu-1A (49.3%). Allele 2\* was present in 5% of samples.

High polymorphism was observed at the locus *Glu-B1* where alleles 20, 6, 7, 8, and 9, and allelic pairs 6+8, 7+8, 7+9, 13+16, and 17+18 were detected, with pairs 7+8 and 7+9 being the most frequented. Combination 13+16 was

relatively rare being found in genotype Guttet 491 A, whereas combination 17+18 was present in cultivars from Hungary (Szekács, Fleischman 481) and from Poland (Ostka Czerwona Lopusta). Alelle 7 was identified in landraces from Poland (Wolynianka), former Soviet Union (Chulugo, Mos4, Zemka), Switzerland (Guttet 491 A), and France (Noe). Allele 8 was found in cultivar Banatka, allele 9 in cultivars Kotte (Sweden) and Rimpaus Dickkopf (Germany). allele 6 in landrace Saumur d'Automne from France. As for the locus Glu-D1 HMW-GS alleles 2+12 were detected in 69.8% and 5+10 in 26.7% of genotypes, respectively. Allelic pair 3+12 was present only in several cultivars with frequency less than 4%. Pattern 0, 7+8, 2+12 was present in the most of analysed accessions. As far as the Glu-score in concerned, some lines reached a value of 10. However it was not possible to calculate the final Glu-score value for some of the lines, because they possess HMW-GS, which have not been previously associated with bread-making quality. Some new HMW-GS alleles were detected by van Hintum and Ellings (1991), Tahir et al. (1996), Gregová et al. (1999) and others. Mutant 1Ax 2\* gene was found in Hungarian landrace Bánkúti 1202 and its positive influence on functional characteristics was already known (Juhász et al. 2001). A new HMW-GS pair with electrophoretic mobility between HMW-GS 7 and 8 was detected also in this study, in one of the lines of landrace Kotte. Since this line of Kotte contains 0 and 2+12 subunits, a new complex allele is likely located at the locus Glu-B1 and relative molecular weights of these subunits is 104 and 120 kDa, respectively. Use of genetically heterogeneous populations as gene sources and selection from landraces with good quality can play an important role by developing and improving new genotypes.

## Acknowledgements

The study was supported by grant No. 27-11 from the Ministry of Agriculture of Slovak Republic and by grant No. 521/03/1201 provided by Grant Agency of the Czech Republic.

Table 1. HMW-GS composition and genetic homogeneity of wheat landraces and obsolete cultivars.

Name	Variety	Origin	Lines (%)	Glu-A1	Glu-B1	Glu-D1	Glu-score
Slovenská 2	aureum (Link) Mansf.	SVK	87	0	7+8	2+12	6
			10	1	7+8	2+12	8
			2	0	7+9	3+12	5
			1	1	7+9	5+10	9
Česká přesívka	milturum (Alef.) Mansf.	CZE	100	2*	7+9	5+10	9
Dobrovická 10	aestivum	CZE	100	0	20	2+12	4
Hanácka bělka	milturum (Alef.) Mansf.	CZE	100	1	7+9	2+12	7
Chlumecká 12	lutescens (Alef.) Mansf.	CZE	98	2*	7+9	5+10	9
	, ,		1	0	7+9	2+12	5
			1	1	7+9	2+12	7
Kostomlatská sametka	milturum (Alef.) Mansf.	CZE	84	0	7+9	2+12	5a
			5				heter.
			5	0	7+9	2+12	5b
			3	1	7+8	2+12	8
			1	0	7+9	2+12	5c
			1	1	7+9	2+12	7
			1	0	7+8	2+12	6
Pyšelka	pyrothrix (Alef.) Mansf.	CZE	100	0	20	2+12	4
Rokycanská sametka			86		7+8	2+12	
Rokycanska sametka	lutescens (Alef.) Mansf.	CZE		1			8
			12	1	7+9	2+12	7
			1	1	20	2+12	6
	(41.6) 3.6	A T 1777	1	0	7+9	2+12	5
Altsterier Linie a (Feldbach)	ferrugineum (Alef.) Mansf.	AUT	69	1	6+8	2+12	6
			23	1	7+8	2+12	8
			3	0	7+8	2+12	6
			3	1	7+8	2+12	8a
			1	1	7+8	2+12	8b
			1				heter.
Marienhofer Kolben	aestivum	AUT	78	1	7+8	2+12	8a
			10	1	7+8	2+12	8b
			6	1	7+8	5+10	10a
			2	1	7+8	5+10	10b
			2	1	7+8	2+12	8c
			2	0	7+8	2+12	6
Ritzelhofer Alt	lutescens (Alef.) Mansf.	AUT	89	0	7+9	2+12	5
	, ,		6	0	7+8	2+12	6
			2	0	6+8	2+12	4
			2				heter.
			1	1	7+9	2+12	7
Sipbachzeller zu 213004-G.	lutescens (Alef.) Mansf.	AUT	63	1	7+9	2+12	7
Siponement zu 21300 i G.	mieseens (Fileri) manon	.101	37	0	7+8	5+10	8
NO 159 (Kneza)	aestivum	BGR	77	1	7+9	2+12	7
100 135 (Ithioza)	acservent.	DOIL	9	0	7+9	5+10	7
			5	0	7+9	2+12	5
			5	1	7+9	5+10	9
			3	1	7+8	2+12	8
			1				
A 7C	f (A1-f) Mf	CHE		1	7+9	2+12	7
Ausserberrg 7C	ferrugineum (Alef.) Mansf.	CHE	100	1	6+8	2+12	6
Guttet 491 A	milturum (Alef.) Mansf.	CHE	45	1	7+8	2+12	8
			34	0	7+8	2+12	6
			14	1	13+16	2+12	8
			4	1	7	5+10	8
			3				heter.
Chermignon 906 D	ferrugineum (Alef.) Mansf.	CHE	100	0	20	2+12	4
Münstertaler	aestivum	CHE	96	1	7+8	2+12	8
			4	0	7+8	5+10	8

Table 1. (Continued)

Name	Variety	Origin	Lines (%)	Glu-A1	Glu-B1	Glu-D1	Glu-score
Sariese 840E	ferrugineum (Alef.) Mansf.	CHE	100	0	6+8	2+12	4
Unter Engadin 9	lutescens (Alef.) Mansf.	CHE	49	0	6+8	3+12	4
			43	1	6+8	3+12	6
			3	1	7+8	2+12	8
			4				heter.
			1	0	7+9	2+12	5
Verrosaz 1229 BA	lutescens (Alef.) Mansf.	CHE	89	0	20	2+12	4a
			9	1	20	5+10	6
			1	1	7+9	2+12	7
			1	0	20	2+12	4b
Kabel K. 162	lutescens (Alef.) Mansf.	DNK	66	1	6+8	2+12	6
	,		10	0	6+8	2+12	4
			10	1	20	2+12	6
			8	1	7+8	5+10	10
			2	1	7+8	2+12	8
			2	•	,		heter.
			1	0	7+8	2+12	6
			1	0	7+9	2+12	5
Lading Shaeghvede	aestivum	DNK	100	0	20	2+12	4
Lys Sterpressusisk	velutinum (Kob.) Mansf.	DNK	47	1	6+8	2+12	6
Lys Sterpressusisk	vetutinum (Koo.) Maiisi.	DINK	14	0	20	2+12	
							4
			14	0	6+8	2+12	4
			10	0	20	5+10	6
			6	2*	20	5+10	8
			3	1	6+8	5+10	8
			2	1	7+9	5+10	9
			2	0	6+8	5+10	6
			1	0	7+8	5+10	8
			1				heter.
Russisk Hvede Etarig	aestivum	DNK	97	0	7+9	2+12	5
			2	0	6+8	2+12	4
			1	1	7+9	2+12	7
Skaeg. Hvid. Glatavnet	aestivum	DNK	100	1	6+8	2+12	6
Warmland Lantvrete	velutinum (Kob.) Mansf.	DNK	84	1	6+8	2+12	6
			8	1	7+9	2+12	7
			6	0	20	2+12	4
			2	0	6+8	2+12	4
Bladette de PuyLaurens	lutescens (Alef.) Mansf.	FRA	99	0	7	2+12	4
			1	0	6+8	2+12	4
Blanc d' Arnay le Duc	lutescens (Alef.) Mansf.	FRA	100	1	20	2+12	6
Blanc de Lorraine	lutescens (Alef.) Mansf.	FRA	100	0	7+8	2+12	6
Ble Blanc a Duvet' Veloute	leucospermum (Koern.) Mansf.	FRA	54	1	7+9	2+12	7a
	• • • • •		18	1	7+9	2+12	7b
			13	0	6+8	2+12	4
			7	1	6+8	2+12	6
			6	2*	6+8	5+10	8
			2	0	7+9	5+10	7
Ble de Jura	lutescens (Alef.) Mansf.	FRA	100	1	7+8	5+10	10
Chiddam d' Automne a epi r.	milturum (Alef.) Mansf.	FRA	100	1	20	2+12	6
Noe	lutescens (Alef.) Mansf.	FRA	100	1	7	2+12	6
Saumur d' Automne	lutescens (Alef.) Mansf.	FRA	80	1	7+8	2+12	8
Samuel a Flatonnie	mesecis (Friend) Marion.	1 14/1	20	1	6	2+12	5+?
Val de Pres	ferrugineum (Alef.) Mansf.	FRA	44	0	20	2+12	4a
7 a1 dC 1 1C3	jerrugineum (MCI.) Malisi.	IIA	19	0	7+8	2+12	4a 6
			19	0	20	2+12	6 4b
			13	0	20	2+12	4c

Table 1. (Continued)

Table 1. (Continued)							
Name	Variety	Origin	Lines (%)	Glu-A1	Glu-B1	Glu-D1	Glu-score
			3	0	20	2+12	4d
			2	0	20	2+12	4e
			1	1	7+8	2+12	8
			1	2*	20	2+12	6
Standard White	lutescens (Alef.) Mansf.	GBR	100	0	20	2+12	4
Square Heads Master 13/4	lutescens (Alef.) Mansf.	GBR	100	0	20	2+12	4
Alter Deutscher unbegrannt	milturum (Alef.) Mansf.	GER	100	0	20	2+12	4
Bergers gelber Dickkopf	lutescens (Alef.) Mansf.	GER	98	0	6+8	2+12	4
			2	0	7+9	2+12	5
Hohenheimer Weidenstatter	milturum (Alef.) Mansf.	GER	95	0	7+8	5+10	8
			4	0	6+8	5+10	6
			1				heter.
Märkischer Landweizen	milturum (Alef.) Mansf.	GER	100	0	7+8	5+10	8
Rimpaus Dickkopf	aureum (Link) Mansf.	GER	60	0	20	2+12	4
			18	0	6+8	2+12	4
			6	1	20	2+12	6
			4	1	6+8	2+12	6
			3	2*	6+8	2+12	6
			1	2*	20	2+12	6
			1	0	9	2+12	3+?
			1	1	7+8	2+12	8
			6				heter.
Ritzelhofer	milturum (Alef.) Mansf.	GER	94	0	7+9	2+12	5
			4	0	7+8	2+12	6
			1	2*	7+8	2+12	8
			1	1	7+8	5+10	10
Samdomier	milturum (Alef.) Mansf.	GER	88	0	6+8	2+12	4
			5	1	7+8	2+12	8
			3	0	7+9	2+12	5
			1	0	6+8	5+10	6
			1	0	7+8	2+12	6
			1	1	7+8	2+12	8
			1	1	6+8	5+10	8
Stumpenweizen	milturum (Alef.) Mansf.	GER	100	0	7+8	2+12	6
Shireffs Dickkopf	lutescens (Alef.) Mansf.	GER	83	0	20	2+12	4
· ·			6	0	6+8	2+12	4
			4	1	20	2+12	6
			4	0	6+8	2+12	4
			2				heter.
			1	0	20	5+10	6
Eszterhazi mindenes	aestivum	HUN	62	0	7+9	3+12	5a
			32	0	7+9	3+12	5b
			2	1	7+8	5+10	10
Fleischman 481	aestivum	HUN	89	0	7+9	2+12	5
110000000000000000000000000000000000000		11011	11	0	17+18	2+12	6
Szekács	aestivum	HUN	93	2*	7+9	5+10	9
		11011	2	1	7+9	2+12	7a
			1	1	7+9	2+12	7b
			1	0	7+9	2+12	5
			1	0	17+18	5+10	8
			1	0	7+8	5+10	8
			1	0	7+8 7+9	5+10 5+10	7
Gelderse Ris	milturum (Alef.) Mansf.	NLD	97	1	7+9 7+8	2+12	8
OCIUCISC KIS	mitturum (Alet.) Mailst.	NLD				2+12	
Zeeuwse Witte	lutescens (Alef.) Mansf.	NLD	3 99	0	7+8 6+8		6
Lecuwse witte	iutescens (Alet.) Manst.	NLD	99	1	υτδ	2+12	6

Table 1. (Continued)

Name	Variety	Origin	Lines (%)	Glu-A1	Glu-B1	Glu-D1	Glu-score
			1				heter.
Dreadnought	lutescens (Alef.) Mansf.	NZL	59	0	6+8	2+12	4a
			41	0	6+8	2+12	4b
Ostka Czerwona Lopusta	ferrugineum (Alef.) Mansf.	POL	80	1	7+8	2+12	8
			11	0	17+18	2+12	6
			3	1	7+8	2+12	8
			3	1	17+18	2+12	8
			2	1	7+8	2+12	8
			1	1	6+8	5+10	8
Wolynianka	aureum (Link) Mansf.	POL	87	1	7+9	5+10	9
			5	1	7+9	2+12	7
			5	1	7	5+10	8
			3	0	6+8	2+12	4
Banatka	ferrugineum (Alef.) Mansf.	SUN	97	0	8	5+10	5+?
			2	0	6+8	2+12	4
			1	1	7+8	2+12	8
Chulugo	lutescens (Alef.) Mansf.	SUN	83	1	7	3+12	6
			6	1	7+9	5+10	9
			4	1	7+9	2+12	7
			2	0	7	2+12	4
			2	0	7+9	2+12	5
			2	1	7	2+12	6
			1	0	7+8	3+12	6
Ljutescens 116	lutescens (Alef.) Mansf.	SUN	98	1	7+8	5+10	10
			1	0	7+9	5+10	7
			1				heter.
Ljutescens 329	lutescens (Alef.) Mansf.	SUN	81	1	7+8	5+10	10a
			9	1	7+8	5+10	10b
			6	1	7+9	5+10	9
			4	1	6+8	5+10	8
Mos 4	alborubrum (Koern.) Mansf.	SUN	94	1	7+9	2+12	7
			5	1	7	5+10	8
			1	1	7+9	5+10	7
Novokrymka 204	aestivum	SUN	95	1	7+9	5+10	7a
			5	1	7+9	5+10	7b
Zemka	aestivum	SUN	34	1	7+9	5+10	9
			23	1	7+9	2+12	7
			21	1	7	2+12	6
			7	1	7	5+10	8
			4	1	7+8	5+10	10
			4	0	7+9	2+12	5
			3	2*	7+9	5+10	9
			2	0	7+8	5+10	8
			2	0	7+9	5+10	7
Extra square head	aestivum	SWE	83	0	6+8	2+12	4a
•			15	0	6+8	2+12	4b
			1	0	20	2+12	4
			1	0	7+8	2+12	6
Kotte	lutescens (Alef.) Mansf.	SWE	42	0	6+8	2+12	4
	` '		40	0	uk+uk	2+12	3+?
			7	0	9	2+12	3+?
			3	0	7+9	2+12	5
			3	1	6+8	2+12	6
			3	1	7+9	5+10	9
			1	2*	7+9	5+10	9
			-	_			-

Table 1. (Continued)

Name	Variety	Origin	Lines (%)	Glu-A1	Glu-B1	Glu-D1	Glu-score
Landvete fran Uppsala	lutescens (Alef.) Mansf.	SWE	44	0	7+9	2+12	5
**			35	0	7+8	2+12	6
			11	0	20	2+12	4
			9	1	20	2+12	6
			1	0	7+8	5+10	8
Renodlade square head	lutescens (Alef.) Mansf.	SWE	93	0	7+8	5+10	8
			2	1	6+8	2+12	6
			2	0	6+8	2+12	4
			1	1	7+8	5+10	10
			1	1	20	5+10	8
			1				heter.

a, b, c, d, e, f – lines identical in HMW-GS composition but different by other protein bands; uk – unknown HMW-GS; heter. – grains heterogeneous in glutenin profile; ? – not known effect of glutenin subunit on Glu-score; CZE – Czech Republic; SVK – Slovakia; AUT – Austria; BGR – Bulgaria; CHE – Switzerland; DNK – Denmark; HUN – Hungary; POL – Poland; SUN – former Soviet Union; FRA – France; GBR – Great Britain; GER – Germany; SWE – Sweden; NLD – Netherlands.

#### References

- Bareš I., Dotlačil L., Holubec V., Kryštof Z., Mikoška P., Rychtárik J., Stehno Z., Ševčík M., Tisová V., Vlasák M. and Faberová I. 1993. Catalogue of Genetic Resources of Cereals in Czech and Slovak Collections. VÚRV, Praha, Czechoslovakia, pp. 27–122.
- Blum A., Sinmena B., Golan G. and Mayer J. 1987. The grain quality of landraces of wheat as compared to modern cultivars. Plant Breed. 99: 226–233.
- Brush S.B. and Meng E. 1998. Farmers valuation and conversation of crop genetic resources. Genet. Resour. Crop Evol. 45: 139–150.
- Gregová E., Hermuth J., Kraic J. and Dotlačil L. 1999. Protein heterogeneity in European wheat landraces and obsolete cultivars. Genet. Resour. Crop Evol. 46: 521–528.
- Juhász A., Tamás L., Karsai I., Vida G., Láng L. and Bedö Z. 2001. Identification, cloning and characterization of a HMWglutenin gene from an old Hungarian wheat variety Bánkúti 1201. Euphytica 119: 75–79.
- Manley M., Randall P.G. and McGill A.E.J. 1992. The prediction of dough properties of South African wheat cultivars by SDS-PAGE analysis of HMW glutenin subunits. J. Cereal Sci. 15: 39–47.
- 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 Lawrence G.J. 1983. Catalogue of 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. Commun. 11: 29–35.
- Radaelli R.P.K., Ng W. and Pogna N.E. 1997. Allelic variation at the storage protein loci of 55 US-grown white wheats. Plant Breed. 116: 429–436.
- Tahir M., Turchetta T., Anwar R. and Lafiandra D. 1996.
  Assessment of genetic variability in hexaploid wheat landraces of Pakistan based on polymorphism for HMW glutenin subunits. Genet. Resour. Crop Evol. 43: 211–220.
- van Hintum T.J.L. and Ellings A. 1991. Assessment of glutenin and phenotypic diversity of Syrian durum wheat landraces in relation to their geographical origin. Euphytica 55: 209–215.
- Wang G., Snape J.W., Hu H. and Rogers W.J. 1993. The high-molecular-weight glutenin subunit compositions of Chinese bread wheat varieties and their relationship with bread-making quality. Euphytica 68: 205–212.
- Weegels P.L., Hamer R.J. and Schofield J.D. 1996. Functional properties of wheat glutenin. J. Cereal Sci. 23: 1–18.
- Wrigley C.W. 1992. Identification of cereal varieties by gel electrophoresis of the grain proteins. In: Linskens H.-F. and Jackson J.F. (eds), Seed Analysis, Springer-Verlag, Berlin and Heidelberg, Germany, pp. 17–41.

Short Communication

# Protein heterogeneity in European wheat landraces and obsolete cultivars: Additional information II

Edita Gregová<sup>1,\*</sup>, Jiří Hermuth<sup>2</sup>, Ján Kraic<sup>1</sup> and Ladislav Dotlačil<sup>2</sup>

<sup>1</sup>Department of Applied Biochemistry and Molecular Biology, Research Institute of Plant Production, Institute of Applied Genetics and Breeding, Bratislavská cesta 122, 92168 Piešťany, Slovak Republic; <sup>2</sup>Research Institute of Crop Production, Gene Bank, Drnovská 507, 16106 Praha 6, Ruzyně, Czech Republic; \*Author for correspondence (e-mail: gregova@vurv.sk; phone: +421-33-7722311; fax: +421-33-7726306)

Received 28 July 2005; accepted in revised form 27 January 2006

Key words: Glutenins, Landraces, Obsolete cultivars, Storage proteins, Triticum aestivum, Triticum spelta, Wheat

#### **Abstract**

The endosperm storage protein of 46 European wheat (*Triticum aestivum* L.) landraces and obsolete cultivars have been fractionated by SDS-PAGE to determine the composition of high molecular weight glutenin subunits (HMW-GS) composition. It has been discovered that about 46% of the wheats were heterogeneous, comprising 2–11 different glutenin profiles. Eighteen of them were observed to be homogeneous. A total of 13 HMW-GS alleles, including 3 at the *Glu-A1*, 8 at the *Glu-B1*, and 3 at the *Glu-D1* loci were revealed. HMW-GS null controlled by locus *Glu-A1*, subunits 7 + 8 by *Glu-B1*, and 2 + 12 by *Glu-D1* predominated. However low frequented alleles such as 17 + 18, 20, 6, and 7 were observed. Furthermore, other new alleles encoding HMW-GS at the locus *Glu-B1* have been found in one of France cultivar (Saumur d'Automne). The glutenin-based quality score ranged from 4 to 10.

# Introduction

The genes controlling synthesis of glutenins are located in hexaploid wheat (*Triticum aestivum* L.) at chromosomes 1A, 1B, and 1D, genes controlling synthesis of high-molecular-weight glutenin subunits (HMW-GS) are located at the loci *Glu-A1*, *Glu-B1* and, *Glu-D1*. Glutenins are composed from HMW-GS and low-molecular-weight subunits (LMW-GS). Analysis of large numbers of wheat cultivars by SDS-PAGE has been proved that all loci display large allelic variations. Payne and Lawrence (1983) demonstrated that there are 3 alleles at the loci *Glu-A1* in hexaploid wheat – allele a controlling synthesis of subunit 1, allele b for synthesis of subunit 2\*, and allele c controlling

so called null allele. At the locus Glu-B1 11 different alleles have been discovered. Alleles a, e, j, and k control synthesis of glutenin subunits (7, 20, 21, and 22), alleles b, c, d, f, h, and i for synthesis of subunits pairs (7 + 8, 7 + 9, 6 + 8, 13 + 16, 13 + 19, 14 + 15, and 17 + 18). Existence of 6 alleles at the locus Glu-D1 was revealed: alleles a, b, c, d, e, and f have control occurrence of each of the glutenin subunits pairs (2 + 12, 3 + 12, 4 + 12, 5 + 10, 2 + 10, and 2.2 + 12). Specific HMW-glutenin allelic variants were associated with bread-making quality (Payne 1987).

During the last few years an increasing interest of wheat breeders for genetically adapted and diverse raw material can be detected, mainly influenced by need for quality traits, specific adaptability, and yield stability of wheat. Wheat landraces are varieties that were improved by farmers over many generations without the use of modern breeding techniques. Genetic erosion is a process linked with modern agriculture and implies that the normal addition and disappearance of genetic variability in a population is altered so that net change in diversity is negative (Gregová et al. 1997). Hammer et al. (1996) compared collection missions in Albania and Italy during different time periods and concluded that genetic erosion had occurred at similar rates in both places. Using electrophoretic analyses of wheat glutenins it is sometimes possible to detect new HMW-GS alleles also in landraces (van Hintum and Ellings 1991; Tahir et al. 1996; Gregová et al. 1999; Juhász et al. 2001; Gregová et al. 2004). The aim of this study was to continue in the analysis of HMW-GS pattern in European wheat landraces and obsolete cultivars started several years ago (Gregová et al. 1999; Gregová et al. 2004).

The objective of this work was to detect and interpret genetic background for bread-making quality based on variations of HMW-GS in *Triticum aestivum* L. landraces and obsolete cultivars originated from the Europe.

# Material and methods

Forty-six landraces and obsolete cultivars of winter wheat - Triticum aestivum L. (vars. aestivum, aureum (Link) Mansf., alborubrum (Koern.) Mansf., ferrugineum (Alef.) Mansf., graecum (Koern.) Mansf., lutescens (Alef.) Mansf., milturum (Alef.) Mansf., T. spelta L. var. viridarduini) Jacubz. et Puchalski, originated from 9 European countries (Table 1), maintained in the collection of wheat genetic resources of the Czech Gene Bank, RICP, Praha-Ruzyně, were analyzed. Each halved seed was tested for uniformity of the glutenin banding patterns. Glutenins extracted from 100 individual grains of each accession were electrophoresed. Bread wheat genotypes with different and known HMW-GS combinations were used as markers. According to the procedure of Wrigley et al. (1992), HMW-GS were separated by polyacrylamide gel electrophoresis in presence of sodium-dodecyl-sulfate (SDS-PAGE). Using the bread wheat with different HMW-GS combinations as references, HMW-GS were classified according to Payne and Lawrence (1983). Glutenin patterns were evaluated by densitometer (Image Master DTS, Pharmacia Biotech). Glu-quality score was calculated according to Payne et al. (1987).

### Results and discussion

Fourteen different Glu-1 encoded allelic variants were identified among the 46 genotypes of wheat landraces, resulting from combination of 3 alleles of Glu-A1, 8 of Glu-B1 and 3 of Glu-D1 loci (Table 1). Also one novel allelic variant at the Glu-B1 locus was identified. Twenty-one, i.e. 46%, of examined wheat accessions were homogeneous in glutenin patterns, remaining 25 (i.e. 54%) were found as heterogeneous, containing at least two glutenin phenotypes. All three allelic variants were detected at the Glu-A1 the most frequent allele was Glu-A1c (null allele) in 55 lines and Glu-A1a (HMW-GS 1) which have found in 36 lines. The HMW-GS 2\* of allele *Glu-A1c* appeared only in 5 lines (Hatzfeld A, Berchtesgadener Vogel, Rouge de Bordeaux, Bled du Lot, and Allies). Generally, the Glu-A1c (null allele) is predominantly reported in wheat landraces (Lagudah et al. 1987; Tahir et al. 1996).

High polymorphism was observed at the locus Glu-B1 where alleles 20, 6, 7, and allelic pairs 6 + 8, 7 + 8, 7 + 9, and 17 + 18 were observed. For the Glu-B1 locus, the allele Glu-B1b (HMW-GS 7 + 8) was the most frequent (29%) among the evaluated lines of wheat landraces and presence of this allele is associated with a good bread-making quality. By contrast, this allele is very rare and appears with low frequency in bread wheat. The allele Glu-B1d (subunits 6 + 8) has possessed 24 lines (25%). On the contrary allele Glu-B1c (subunits 7 + 9), Glu-B1e (subunit 20), and Glu-1Ba (subunit 7) were identified with occurrence over 10%. The allele Glu-B1an (subunit 6) was rare and was found only in two accessions (Odesskaya 16 and Saumur d'Automne). This subunit was previously detected in two landraces of bread wheat by Rodriguez-Quijano et al. (1990) and Gregová et al. (1999). Relatively rare allele Glu-B1i (subunits 17 + 18), associated with good bread-making quality, was found in two accessions. A new allele at the Glu-B1 was found in one line of France landrace Saumur d'Automne.

Table 1. HMW-GS composition and genetic homogeneity of wheat landraces and obsolete cultivars.

Name	Variety	Origin	Lines [%]	Glu-A1	Glu-B1	Glu-D1	Glu-score
Svalöfs Sonnen	lutescens	SWE	93	0	6 + 8	2 + 12	5
			5	0	7 + 8	2 + 12	6
			2	1	6 + 8	2 + 12	6
Sigyn II	lutescens	NOR	94	1	7 + 9	2 + 12	7
			3	1	7 + 8	2 + 12	8
			3	1	7 + 9	2 + 12	7a
Kruisings Angel Tarwe	graecum	NLD	97	0	20	2 + 12	5
			3	0	7 + 8	2 + 12	6
Tucks W.	ferrugineum	GBR	100	1	7 + 9	5 + 10	9
Little Tich	lutescens	GBR	100	0	7 + 9	2 + 12	5
Rieti 11	aestivum	ITA	100	0	20	5 + 10	6
Aquileja	milturum, aureum	ITA	100	1	7	2 + 12	6
Odesskaya 16	aestivum	SUN	61	1	7 + 8	5 + 10	10
			18	1	7 + 9	5 + 10	9
			13	0	7 + 8	5 + 10	8
			7	0	6 + 8	5 + 10	6
			1	0	6	5 + 10	5 + ?
Rinner	aestivum	AUT	98	0	7 + 9	5 + 10	7
			2	1	7 + 9	5 + 10	9
Admonter Früh	lutescens	AUT	100	1	7 + 9	5 + 10	9
Wagrein (Salzburg) (Nr.252004)	aestivum	AUT	72	0	6 + 8	2 + 12	4
,			28	1	17 + 18	2 + 12	8
Harrachsweizen	aestivum	AUT	100	0	7 + 9	5 + 10	7
Strubes General von Stocken	lutescens	GER	100	1	7 + 8	2 + 12	8
Ackermanns Bayernkönig	milturum	GER	94	0	7 + 8	2 + 12	6
, ,			6	0	6 + 8	2 + 12	5
Salzmünder Wetzelweizen	aestivum	GER	100	0	7 + 8	2 + 12	6
Sperlings Sinslebener N. Z.	aureum	GER	95	1	6 + 8	2 + 12	6
			5	0	6 + 8	2 + 12	4
Hohenwettersbacher – Braun	milturum	GER	100	0	7 + 8	2 + 12	7
Werthers Ettersbergerw.	lutescens	GER	100	0	6 + 8	2 + 12	4
Von Stieglers Sieges	milturum	GER	94	1	6 + 8	2 + 12	6
6 6			5	1	7 + 8	5 + 10	10
			1	1	7 + 9	5 + 10	9
Rimpaus Barun	milturum	GER	100	0	6 + 8	2 + 12	4
Hatzfeldt A	milturum	GER	94	0	7 + 8	5 + 10	8
			3	2*	7 + 8	5 + 10	10
			1	0	7 + 8	2 + 12	6
			2				heter.
Hohenwettersbacher-b. Dick.	aestivum	GER	100	0	6 + 8	2 + 12	4
Strubes Dickkopf	lutescens	GER	65	1	20	2 + 12	6
•			35	0	20	2 + 12	4
Hatzfeldt E	lutescens	GER	41	0	7 + 8	2 + 12	6a
			28	0	7 + 8	2 + 12	6b
			17	0	7 + 8	2 + 12	6c
			14	0	7 + 8	5 + 10	8
Ebersbacher Weiss	aureum	GER	60	0	6 + 8	2 + 12	4
			40	0	20	2 + 12	4
Berchtesgadener Vogel	ferrugineum	GER	99	0	17 + 18	2 + 12	6
			1	2*	7 + 8	2 + 12	8
Von Reininghaus Alpen	lutescens	GER	73	0	7 + 8	2 + 12	6
- •			27	0	6 + 8	2 + 12	4
Blaukorn	viridarduini	GER	98	0	6 + 8	2 + 12	4
			2				heter.
	milturum	GER	69	0	7 + 8	2 + 12	6a
Toster Rot	muumm	OLIC	0,7	•	,		
Toster Rot	mutaram	GER	22	0	7 + 8	2 + 12	6b

Table 1. Continued.

Name	Variety	Origin	Lines [%]	Glu- $A1$	Glu-B1	Glu-D1	Glu-score
			4	0	7 + 8	2 + 12	6c
Shireffs Dickkopf	lutescens	GER	100	0	20	2 + 12	4
Krapphausener dichter Epp	lutescens	GER	100	1	20	2 + 12	6
Gros Bleu	lutescens	FRA	100	0	6 + 8	2 + 12	4
Dattel	alborumbrum	FRA	100	1	20	2 + 12	6
Bon Fermier	lutescens	FRA	100	0	7 + 8	2 + 12	6
Roux des Ardennes	aestivum	FRA	95	1	7 + 9	2 + 12	7
			1	1	7 + 8	5 + 10	10
			4				heter.
Rouge de Bordeaux	milturum	FRA	100	2*	7 + 9	5 + 10	10
Rouge d'Altkirch	milturum	FRA	100	1	6 + 8	5 + 10	8
Ble de la Vallouise	lutescens	FRA	100	1	7 + 8	5 + 10	10
Ble de Crepi	lutescens	FRA	100	0	7 + 9	2 + 12	5
Ble du Lot	lutescens	FRA	97	2*	7	5 + 10	8
Die da Eot	intescens	1 10 1	3	1	20	2 + 12	6
Blanc de Flandre	lutescens	FRA	68	1	20	$\frac{2}{2} + \frac{12}{12}$	6
Biane de Fiandre	inicscens	1 10/1	31	0	20	$\frac{2}{2} + \frac{12}{12}$	4
			1	0	6 + 8	$\frac{2}{2} + \frac{12}{12}$	4
Vilmorin 23	lutescens	FRA	51	0	7	4 + 12	3a
Viiiioiiii 23	intescens	TIM	22	0	7	$\frac{4}{2} + \frac{12}{12}$	4a
			19	0	7	4 + 12	3b
			8	0	7	$\frac{4+12}{2+12}$	4a
Allies		FRA	6 46	1	7	$\frac{2 + 12}{2 + 12}$	4a 6
Ames	aureum	ГKA		0	7	5 + 10	
			21 7	1	6 + 8	5 + 10 5 + 10	6 8
			5				
				0	6 + 8	2 + 12	4
			4	1	6 + 8	2 + 12	6
			2	1	7 + 8	2 + 12	8
			2	0	6 + 8	5 + 10	6
			2	0	7	2 + 12	4
			2	2*	6 + 8	5 + 10	8
			1	0	7 + 8	2 + 12	6
			1	0	7 + 9	2 + 12	5
			7				heter.
Ble de Champagne	aestivum	FRA	94	1	7	2 + 12	6
			6	0	6 + 8	5 + 10	6
Japhet	lutescens	FRA	47	0	7 + 9	2 + 12	5
			32	1	7	2 + 12	7
			14	1	7	5 + 10	8
			2	1	6 + 8	2 + 12	6
			5				heter.
Saumur d' Automne	lutescens	FRA	72	1	7 + 8	2 + 12	8
			20	1	6	2 + 12	5 + ?
			8	1	7* + 8	2 + 12	5 + ?

 $a,\,b,\,c$  – Lines identical in HMW-GS composition but different by other protein bands.

Evaluated lines present a high level of homogeneity at the Glu-D1 locus, in fact 70% of them showed the Glu-D1a allele (subunits 2 + 12), although these subunits are associated with poor quality of bread wheat. Another allele associated

with good quality found with a relatively high frequency was Glu-Dld (subunits 5 + 10) found in 28% of lines. Two lines of landrace Vilmorin 23 had the Glu-Dc allele (subunits 4 + 12).

heter. - Grains heterogeneous in glutenin profile.

<sup>? -</sup> Not known effect of glutenin subunit on Glu-score.

 $AUT-Austria, GBR-Great\ Britain, FRA-France, GER-Germany, SWE-Sweden, NLD-Netherlands\ ,\ NOR-Norway, ITA-Italy,\ SUN-former\ Soviet\ Union.$ 

Altogether 30 combinations at the Glu-A1, Glu-B1, and Glu-D1 loci were detected. There is a clear dominance of combinations 0, 7 + 8,2 + 12 and 0, 6 + 8, 2 + 12. Possible use of landraces and obsolete cultivars for bread-making quality improvement can be in some extent indicated by Glu-score values. Some of the lines reached the maximum value 10 (Roux des Ardennes, Rouge de Bordeaux, Ble de Vallouise, Von Stieglers Sieges, Hatzfeldt A, and Odeskaya 16). However, there was no possibility to calculate the final Glu-score value in some of the lines, one combination present the novel alleles and two combinations present Glu-Blan (subunit 6), because they possess HMW-GS, which have not been associated with bread-making quality. Rare HMW-GS pair 7\* + 8 containing subunit 7\*, with electrophoretic mobility of protein band faster than subunit 7, at the locus Glu-B1 was identified. Two types of subunit 7 were previously identified based on slight differences in electrophoretic mobility (Marchylo et al. 1992). A second type, with slightly faster mobility than subunit 7, was named 7\* in genotypes Norstar. The same combination of subunit 7\* + 8, designated Glu-Blu was found only in the genotypes Owens and Fiorello (Ng et al. 1989; Pogna et al. 1989). Biochemical and molecular characterization accomplished in future study will provide detailed information about the subunits 7\* + 8 where we will compare subunit 7\* from genotypes Norstar, Fiorello, and Owens with subunit 7\* identified in landrace Saumur de Automne.

# Acknowledgements

This work was supported by project No. 2003 SP27/0280D01/0280D01 supplied by the Ministry of Agriculture of the Slovak Republic, project No. APVT-27-014504 from the Agency for Support of Science and Technology, and project No. 521/03/1201 provided by Grant Agency of the Czech Republic.

# References

- Gregová E., Hermuth J., Kraic J. and Dotlačil L. 1999. Protein heterogeneity in European wheat landraces and obsolete cultivars. Genet. Resour. Crop Evol. 46: 521–528.
- Gregová E., Tisová V. and Kraic J. 1997. Genetic variability at the Glu-1 loci in old and modern wheats (*Triticum aestivum* L.) cultivated in Slovakia. Genet. Resour. Crop Evol. 44: 301–306.
- Gregová E., Hermuth J., Kraic J. and Dotlačil L. 2004. Protein heterogeneity in European wheat landraces and obsolete cultivars: Additional information Genet. Resour. Crop Evol. 51: 569–575.
- Hammer K., Knüpffer K., Xhuveli L. and Perrino P. 1996. Estimating genetic erosion in landraces – two case studies. Genet. Resour. Crop Evol. 43: 329–336.
- Juhász A., Tamás L., Karsai I., Vida G., Láng L. and Bedö 2001. Identification, cloning and characterization of a HMWglutenin gene from an old Hungarian wheat variety, Bánkúti 1201. Euphytica 119: 75–79.
- Lagudah E.S., Flood R.G. and Halloran G.M. 1987. Variation in high molecular weight glutenin subunits in landraces on hexaploid wheat from Afghanistan. Euphytica 36: 3–9.
- Marchylo B.A., Lukow O.M. and Kruger J.E. 1992. Quantitative variation in high molecular weight glutenin subunit 7 in some Canadian wheats. J. Cereal Sci. 15: 29–37.
- Ng P.K.W., Pogna N.E., Mellini F. and Bushuk W. 1989. *Glu-1* allele compositions of the wheat cultivars registered in Canada. J. Genet. Breed. 43: 53–59.
- 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 Lawrence G.J. 1983. Catalogue of 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. Commun. 11: 29–35.
- Pogna N.E., Mellini F., Beretta A. and Dalbelin Deruffo A. 1989. The high-molecular-weight glutenin subunits of common wheat cultivars grown in Italy. J. Genet. Breed. 43: 17–24.
- Rodriguez-Qiujano M., Vázquez J.F. and Carrillo J.M. 1990. Variation of high molecular weight glutenin subunits in Spanish landraces of *Triticum aestivum* ssp. *vulgare* and ssp. *spelta*. J. Genet. Breed. 44: 121–126.
- Tahir M., Turchetta T., Anwar R. and Lafiandra D. 1996. Assessment of genetic variability in hexaploid wheat land-races of Pakistan based on polymorphism for HMW glutenin subunits. Genet. Resour. Crop Evol. 43: 211–220.
- Van Hintum T.J.L. and Ellings A. 1991. Assessment of glutenin and phenotypic diversity of Syrian durum wheat landraces in relation to their geographical origin. Euphytica 55: 209–215.
- Wrigley C.W. 1992. Identification of Cereal Varieties by Gel Electrophoresis of the Grain Proteins. Springer, Heidelberg, pp. 17–41.