

Diversity of seed storage proteins in common wheat (*Triticum aestivum* L.)

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Abstract

The objective of our study was to determine the composition of high-molecular weight-glutenin subunits (HMW-GS) in 120 cultivars of common wheat (*Triticum aestivum* L.). Fourteen alleles and 34 allelic compositions were detected using sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The most frequent HMW-GS alleles at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci were null (57.1%), 7 + 9 (43.3%) and 5 + 10 (61.9%), respectively. However, low-frequency HMW-GS alleles were also observed, such as 13 + 16, 20, 21, 7 and 18, encoded by the *Glu-B1* locus, and 4 + 12, encoded by the *Glu-D1* locus. The wheat-rye 1BL.1RS translocation was identified in 25 cultivars, using acid polyacrylamide gel electrophoresis. The Glu-score varied greatly, and some lines reached the maximum value of 10.

Keywords: high-molecular weight-glutenin subunits; quality; sodium dodecyl sulphate-polyacrylamide gel electrophoresis; *Triticum aestivum* L.; wheat

Introduction

Common wheat (*Triticum aestivum* L.) is the most widely grown wheat and it is one of the major food crops utilized all over the world. Identification and characterization of the genetic resources of wheat are highly desirable because the available information can be applied in further research and breeding. The improvement of wheat for use of the end-product has become an important part of wheat breeding programmes worldwide. It has been documented that quality is determined principally by the molecular structure of the seed storage proteins, glutenins and gliadins (Bushuk, 1998). Thus, not only protein quantity but also their quality is of great importance. High-molecular weight-glutenin subunits (HMW-GS) play a major role in this regard. The HMW-GS are encoded by genes at three *Glu-1* loci (*Glu-A1*, *Glu-B1* and *Glu-D1*), which are located on the

long arms of homologous group-one chromosomes. These loci encode an x-type subunit of higher molecular weight and a y-type subunit of lower molecular weight. It was demonstrated that good bread-making quality is highly associated with the presence of specific HMW-GS (Payne *et al.*, 1987). Since then, a number of novel GS have been identified and characterized. These novel alleles may be a potential genetic resource for the creation of wheat lines with improved quality (Gálová *et al.*, 2002; Dotlačil *et al.*, 2003; Ren *et al.*, 2008). Databases that contain information on wheat genotypes/cultivars and their HMW-GS compositions may offer breeders the prospect of further advancement by selecting wheat cultivars with beneficial HMW-GS. The objective of our study was to determine the composition of HMW-GS in 120 wheat cultivars (*T. aestivum* L.).

Materials and methods

A total of 120 hexaploid wheat cultivars (*T. aestivum* L.), provided by the Gene Bank of the Slovak Republic in

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Table 1. The frequencies of HMW-GS coded at the *Glu-1* loci

Locus	HMW-GS	Frequency (%)
<i>Glu-A1</i>	Null	57.1
	1	14.3
	2*	28.6
	7 + 9	43.3
<i>Glu-B1</i>	7 + 8	18.1
	6 + 8	18.1
	17 + 18	7.1
	7	9.4
	20	1.6
	13 + 16	0.8
	18	0.8
	21	0.8
	<i>Glu-D1</i>	5 + 10
2 + 12		37.3
4 + 12		0.8

Piešťany, were analyzed in this study. The wheat samples originated from 11 countries: Slovakia (SVK, 12), Czech Republic (CZE, 12), France (FRA, 12), Austria (AUT, 12), Italy (ITA, 12), Hungary (HUN, 12), former Yugoslavia (YUG, 12), Ukraine (UKR, 6), Russia (RUS, 6), Great Britain (GBR, 12) and the USA (12). The HMW-GS were extracted from randomly selected single seeds. Protein extraction, electrophoretic separation of glutenins and the detection procedures used were in accordance with the International Seed Testing Association (ISTA) standard procedure for use of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Wrigley, 1992). HMW-GS were identified by following the catalogue of HMW-GS alleles (Payne and Lawrence, 1983). In addition, the presence or absence of the secalin block (1BL.1RS translocation) in the genotypes was examined using acid polyacrylamide gel electrophoresis (A-PAGE), according to the standard ISTA reference method

(Draper, 1987). The bread-making quality of the grain was expressed as a score (Glu-score and Rye-score) derived from the presence or absence of specific high-molecular weight-glutenins or -gliadins.

Results and discussion

Fourteen alleles and 34 allelic compositions were detected in the set of 120 cultivars. The most frequent HMW-GS patterns were null, 7 + 9 and 5 + 10, which were present in 21 cultivars. Ten of the wheat accessions analyzed (8.3%) were observed to be heterogeneous in their glutenin profiles. Five cultivars were heterogeneous at one locus, two cultivars at two loci and another three varieties were heterogeneous at all three *Glu-1* loci.

Three alleles were detected at the *Glu-A1* locus. The most abundant allele was *Glu-A1c*, encoding the null subunit (57.1%, Table 1). It was found mostly in cultivars bred in SVK, CZE, YUG and GBR (Table 2). In contrast, the alleles *Glu-A1b* (subunit 2*) and *Glu-A1a* (subunit 1) were present in only 14 and 29% of the genotypes, respectively. These alleles were observed mainly in Hungarian, Italian, Austrian and American wheat samples (Table 2). A high frequency of alleles encoding subunits 1 and 2* in wheat cultivars from the USA was observed also by Shan *et al.* (2007). These subunits (1, 2*) have a positive impact on the rheological properties of dough, so they are highly desirable in cultivars used for bread-making.

A total of nine HMW-GS were identified at the *Glu-B1* locus. Subunit 7 + 9 was the predominant one (43.3%, Table 1); it was abundant mostly in wheat bred in HUN, SVK, YUG, RUS and UKR. The subunit 6 + 8, which is associated with poor bread-making quality, was highly frequent in British and Austrian wheat samples (Table 2). Low-frequency HMW-GS, such as 13 + 16, 20, 21, 7 and

Table 2. The frequencies (%) of HMW-GS at the *Glu-1* loci according to the country of origin

Locus	HMW-GS	HUN	YUG	CZE	AUT	SVK	FRA	USA	UKR	RUS	ITA	GBR
<i>Glu-A1</i>	Null	33	75	75	42	100	62	43	50	62	23	67
	1	25	8	–	16	–	15	21	17	13	31	8
	2*	42	17	25	42	–	23	36	33	25	46	25
<i>Glu-B1</i>	7 + 9	67	69	33	42	67	18	40	57	57	29	8
	7 + 8	25	8	42	8	25	18	13	29	–	22	8
	6 + 8	–	15	17	50	8	9	27	–	–	–	59
	17 + 18	–	8	8	–	–	9	–	–	–	21	25
	7	–	–	–	–	–	46	20	–	29	14	–
	20	8	–	–	–	–	–	–	–	–	7	–
	13 + 16	–	–	–	–	–	–	–	–	14	–	–
	18	–	–	–	–	–	–	–	–	–	7	–
	21	–	–	–	–	–	–	–	14	–	–	–
<i>Glu-D1</i>	5 + 10	83	42	92	67	67	50	63	100	83	43	25
	2 + 12	17	58	8	33	33	50	31	–	17	57	75
	4 + 12	–	–	–	–	–	–	6	–	–	–	–

18, which are encoded by alleles at the *Glu-B1* locus were also detected (Table 1). An unpaired subunit 7 was present mainly in French cultivars and also in wheat samples originating from USA, RUS and ITA (Table 2). The rare subunit 21 was found in the cultivar Mironovskaja ulucsenaja (UKR). Subunit 18, which is expressed commonly coupled with subunit 17, was observed as an unpaired subunit in the Italian cultivar Glutinoso. Redaelli *et al.* (1997) observed expression of unpaired subunit 6 in some cultivars of *T. aestivum* L. They assumed that it was derived from the HMW-GS pair 6 + 8 (because it had the same mobility as subunit 6 of this pair), but that the expression of only subunit 6 was caused by a switch-off mutation in the coding sequence or in the regulatory region of the gene for subunit 8. Yuan *et al.* (2009) characterized two inactive γ -type genes for *Glu-B1* in *T. aestivum* ssp. *yunnanese* and ssp. *tibetanum*. They indicated that the silenced 1By genes were 1By9 and that the silencing was caused by deletions of nucleotide A, which resulted in frameshift mutations responsible for the presence of premature stop codons.

Most of the cultivars (61.9%, Table 1) were found to express the subunit pair 5 + 10, which is encoded by allele *Glu-D1d* at the *Glu-D1* locus. It has been shown that varieties that contain the pair 5 + 10 can form stronger dough than those that contain subunits 2 + 12 (Payne, 1987; Liang *et al.*, 2010). Thus, wheat carrying the pair 2 + 12 can be utilized in the production of cookies, crackers or pasta rather than in bread-making. In this study, subunit pair 2 + 12, encoded by allele *Glu-D1a*, was observed frequently in cultivars bred in ITA and GBR (Table 2). A high frequency of subunit pair 2 + 12 has been published for wheat originating in Asian countries, such as China and Japan (Nakamura, 2001; Li *et al.*, 2009), where wheat is used predominately in the production of noodles. Our analysis revealed also the presence of subunit 4 + 12, encoded by allele *c* at the *Glu-D1* locus, in one cultivar that originated in the USA.

The wheat–rye 1BL.1RS translocation was identified in 25 cultivars by use of A-PAGE. No cultivars that originated in USA and FRA were found to carry this translocation. The presence of the 1BL.1RS translocation has a negative effect on the rheology of dough; it produces a sticky dough with a lack of overmixing, and low loaf volumes (Graybosch, 2001; Gobaa *et al.*, 2008; Zheng *et al.*, 2009).

The Glu-score and Rye-score varied greatly; some samples reached the maximum value of 10 (cultivars GK Kapos, GK David, Vlasta, Baltimor, Luzanovka, Maverick and Smuggler). These cultivars are supposed to have superior bread-making quality. The highest average value of Glu-score was achieved by Hungarian cultivars (8.1). In contrast, the HMW-GS composition of British wheat samples resulted in an average value of only 5.9.

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References

- Bushuk W (1998) Wheat breeding for end-product use. *Euphytica* 100: 137–145.
- Dotlačil L, Bradová J, Hermuth J and Stehno Z (2003) Diversity of HMW-Glu alleles in landraces and cultivars of winter wheat (*Triticum aestivum*), spelt (*Triticum spelta*) and emmer (*Triticum dicoccoides*). In: Pogna NE, Romano M, Pogna EA and Galterio G (eds) *Proceedings of the 10th International Wheat Genetics Symposium*. 1–6 September 2003, Paestum, Italy, pp. 475–477.
- Draper SR (1987) ISTA variety committee report of the working group for biochemical tests for cultivar identification 1983–1986. *Seed Science and Technology* 15: 431–434.
- Gálová Z, Michalík I, Knoblochová H and Gregová E (2002) Variation in HMW glutenin subunits of different species of wheat. *Rostlinna Výroba* 48: 15–19.
- Gobaa S, Brabant C, Kleijer G and Stamp P (2008) Effect of the 1BL.1RS translocation and of the *Glu-B3* variation on fifteen quality tests in a doubled haploid population of wheat (*Triticum aestivum* L.). *Journal of Cereal Science* 48: 598–603.
- Graybosch RA (2001) Uneasy unions: quality effects of rye chromatin transfers to wheat. *Journal of Cereal Science* 33: 3–16.
- Li Y, Huang Ch, Sui X, Fan Q, Li G and Chu X (2009) Genetic variation of wheat glutenin subunits between landraces and varieties and their contributions to wheat quality improvement in China. *Euphytica* 169: 159–168.
- Liang D, Tang J, Pena RJ, Singh R, He X, Shen X, Yao D, Xia X and He Z (2010) Characterization of CIMMYT bread wheats for high- and low-molecular weight glutenin subunits and other quality-related genes with SDS-PAGE, RP-HPLC and molecular markers. *Euphytica* 172: 235–250.
- Nakamura H (2001) Genetic diversity of high-molecular-weight glutenin subunit compositions in landraces of hexaploid wheat from Japan. *Euphytica* 120: 227–234.
- Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Physiology* 38: 141–153.
- Payne PI and Lawrence GJ (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 Research Communications* 11: 29–35.
- Payne PI, Nightigale MA, Krattinger AF and Holt LM (1987) The relationship between the HMW glutenin subunit composition and the breadmaking quality of British-grown wheat varieties. *Journal of the Science of Food and Agriculture* 40: 51–65.
- Redaelli R, Ng KW and Pogna NE (1997) Allelic variation at the storage protein loci of 55 US-grown white wheats. *Plant Breeding* 116: 429–436.
- Ren Y, Wang T, Xu Z-B, Yang Z-J and Ren Z-L (2008) Molecular characterization of a novel HMW-GS 1Dx5' associated with good bread making quality (*Triticum aestivum* L.) and the

- study of its unique inheritance. *Genetic Resources and Crop Evolution* 55: 585–592.
- Shan X, Clayshulte SR, Haley SD and Byrne PF (2007) Variation for glutenin and waxy alleles in the US hard winter wheat germplasm. *Journal of Cereal Science* 45: 199–208.
- Wrigley CW (1992) Identification of cereal varieties by gel electrophoresis of the grain proteins. In: Linskens HF and Jackson JF (eds) *Seed Analysis*. Heidelberg: Springer-Verlag, pp. 17–41.
- Yuan Z-W, Chen Q-J, Zhang L-Q, Yan Z-H, Zheng Y-L and Liu D-C (2009) Molecular characterization of two silenced γ -type genes for *Glu-B1* in *Triticum aestivum* ssp. *yunnanese* and ssp. *tibetanum*. *Journal of Integrative Plant Biology* 51: 93–99.
- Zheng S, Byrne PF, Bai G, Shan X, Reid SD, Haley SD and Seabourn BW (2009) Association analysis reveals effects of wheat glutenin alleles and rye translocations on dough-mixing properties. *Journal of Cereal Science* 50: 283–290.