Identification of Glutenin Markers in Cultivars of three Wheat Species

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Abstract: In a collection of 41 Slovak and European winter wheat cultivars (*Triticum aestivum* L.), 5 spelt wheat cultivars (*Triticum spelta* L.) and 3 durum wheat cultivars (*Triticum durum* DESF.) we investigated the qualitative composition of high-molecular-weight glutenin subunits (HMW-GS), the Gluten quality (GQ) score and the presence of the secale block 1B3. The highest frequency of the HMW-GS 0, 7 + 9 and 5 + 10 was found among the winter wheat cultivars. The highest GQ score of 10 was found in the cultivars SK-30 and FD-92017/1. A GQ score of 9 was found in the cultivars Astella, Ilona, Velta and MV-06-95. The HMW-GS 0 and 7 + 8, associated with a GQ of 4, were found in *T. durum*. The electrophoretic spectra of the spelt wheat cultivars were not homogeneous and represented 4 different lines.

Keywords: *Triticum aestivum* L.; *Triticum spelta* L.; *Triticum durum* DESF.; HMW glutenin subunits; secale block 1B3; electrophoresis; SDS-PAGE; A-PAGE

The storage proteins of the wheat grain consist mainly of two gluten fractions: glutenin and gliadin. Glutenins, especially their highly aggregative HMW fraction, is important because of its major effect on gluten structure stability and elasticity and thus the bread-making quality of wheat. The HMW-GS are controlled by the codominant alleles of the loci Glu-A1, Glu-B1 and Glu-D1 on the long arm of the homoeologous chromosomes 1A, 1B and 1D of hexaploid wheat. The relationship between presence or absence of specific HMW-GS and the breadmaking quality of wheat is known. The subunits 5 + 10 and 2 + 12, controlled from the locus Glu-D1, are the most important for the technological quality. The correlation between breadmaking quality and specific HMW-GS can be used for quality screening or prediction in wheat (Payne & Lawrence 1983; Payne *et al*. 1984, 1987; Šašek & Černý 1997).

Gliadins are coded by genes of the loci Gli-1 and Gli-2 located on the distal part of the short arm of chromosomes 1A, 1B, 1D, 6A, 6B and 6D. They are mostly monomeric proteins, that can be separated by electrophoresis into α-, β-, γ- and ω-gliadins. Gliadins represent an important part of the gluten complex and are mainly responsible for the viscosity parameters of dough. Some gliadin blocks have positive effects, while others have negative or no effects upon dough quality (Payne *et al*. 1984, 1987). The main goal of our work was to analyse and characterise the qualitative composition of HMW glutenin subunits and to predict the technological quality in the collection of the three wheat species, based on gliadin and glutenin markers.

MATERIALS AND METHODS

Plant material: 41 hexaploid winter wheat cultivars (*Triticum aestivum* L.) of European origin, including 20 from Slovakia, were obtained from the experimental station Želiezovce of the Central Institute for Control and Testing in Agriculture. 5 winter spelt wheat cultivars (*Triticum spelta* L.) and 3 durum wheat cultivars (*Triticum durum* DESF.) were obtained from the experimental base Dolná Malanta of the Department of Food

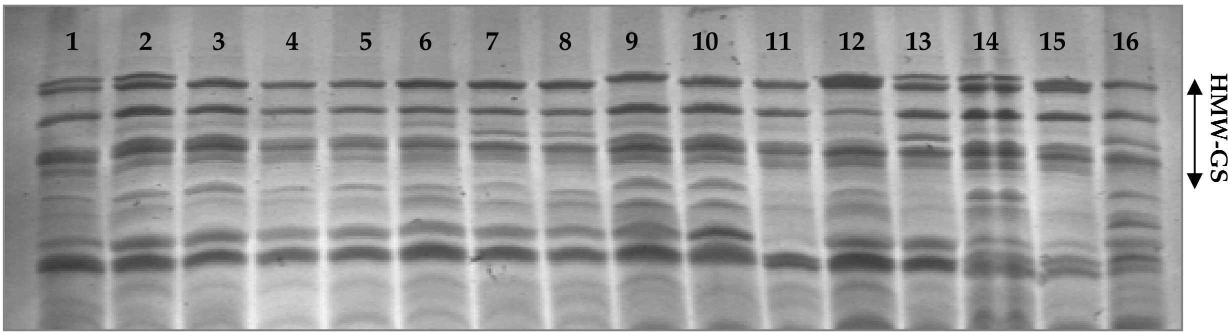
Supported by the Scientific Grant Agency of the Ministry of Education and the Academy of Science of the Slovak Republic – VEGA, Grant No. 1/7648/20.

Production of the Slovak Agricultural University in Nitra. Marquis winter wheat and Chinese Spring spring wheat (*Triticum aestivum* L.) with well known HMW glutenin subunits composition were included as standards. All seeds were harvested in 2000.

Electrophoretic analysis. Glutenins and gliadins, were extracted from individually ground grains and fractionated by standard reference methods of polyacrylamide gel electrophoresis in acid medium (A-PAGE) (Draper 1987) and in presence of SDS (SDS-PAGE) (Wrigley 1992), as suggested by the International Seed Testing Association (ISTA). The gels were then stained by Coomassie Brilliant Blue R250 and washed with de-ionised water. The designation of HMW glutenin subunits followed the numbering system of Payne *et al*. (1987). The program GRAB-IT 259 was used to calculate quality scores. Overall quality scores for a given variety were obtained as the sum of the scores of individual HMW glutenin subunits. The rye-score was indicated by the presence or absence of the secale block 1B3.

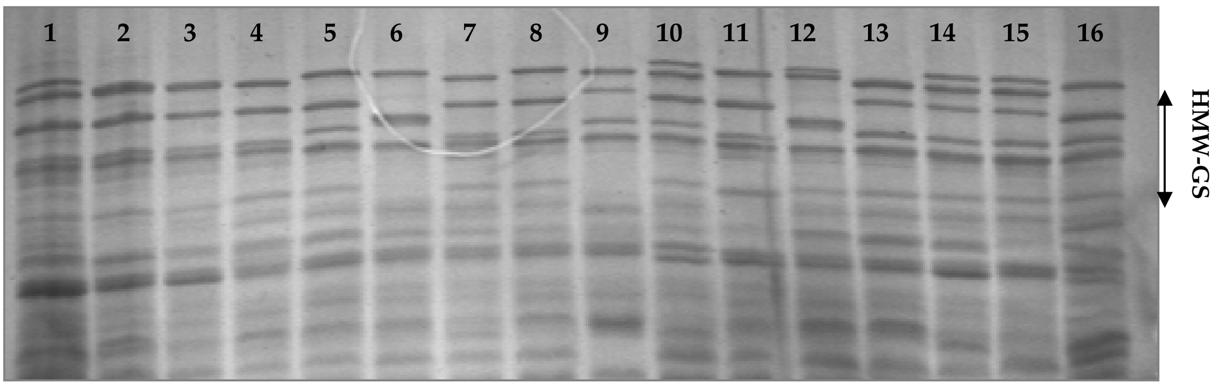
RESULTS AND DISCUSSION

Gliadin and glutenin proteins have a high genetic variability, expressed phenotypically with high heritability. The pattern of electrophoretic bands therefore does not depend on agro-ecological conditions (Gregová *et al*. 1995; Gálová *et al*. 2002) and can be used for the identification of cultivars and to predict some of their properties. Especially HMW-GS and gliadin blocks are useful genetic markers for technological quality of the wheat grain (Černý & Šašek 1996).



1 – Marquis, 2 – Velta, 3 – Arida, 4 – Istar, 5 – Armelis, 6 – SO-296, 7 – SO-355, 8 – SO-496, 9 – Vanda, 10 – Petrana, 11 – SK-22, 12 – SK-26, 13 – SK-30, 14 – MV-06-95, 15 – MV-230-96, 16 – Chinese Spring

Figure 1. Electrophoretic profile of HMW glutenin subunits in the grain of *Triticum aestivum* L.



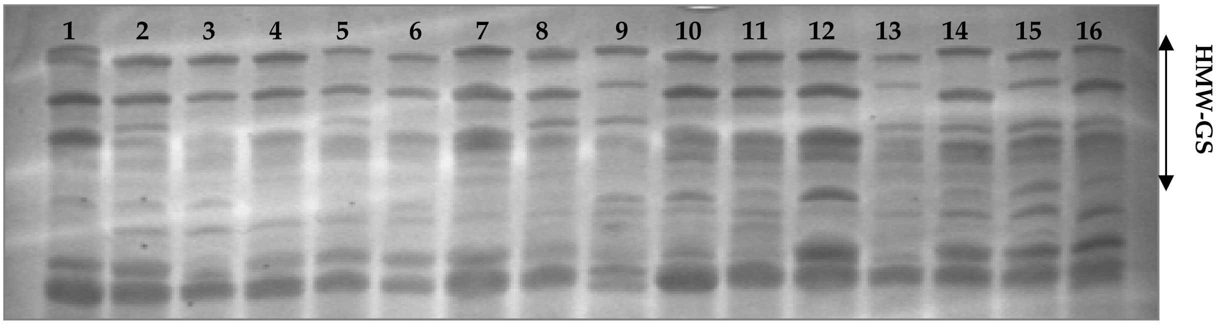
1 – Marquis, 2 – Astella, 3 – Ilona, 4 – Brea, 5 – Torysa, 6 – Charger, 7 – Ormil, 8 – Sideral, 9 – FD-90050, 10 – FD-92017/1, 11 – SG-U-7067, 12 – HE-6606, 13 – Vm-112, 14 – Ceb.-9703, 15 – LP-30729, 16 – Chinese Spring

Figure 2. Electrophoretic profile of HMW glutenin subunits in the grain of *Triticum aestivum* L.

Table 1. Composition of individual HMW glutenin subunits and secale block 1B3 in the collection of Slovak and European winter wheat (*Triticum aestivum* L.)

Cultivar	Country of origin	HMW glutenin subunits of loci			Glu-score	Rye-score
		Glu-A1	Glu-B1	Glu-D1		
Astella	Slovak Republic	2*	7 + 9	5 + 10	9	9
Arida	Slovak Republic	0	7 + 9	5 + 10	7	7
Ilona	Slovak Republic	2*	7 + 9	5 + 10	9	9
Istar	Slovak Republic	0	7 + 9	5 + 10	7	7
Armelis	Slovak Republic	0	7 + 9	5 + 10	7	7
SO-296	Slovak Republic	0	7 + 8	5 + 10	8	8
SO-355	Slovak Republic	0	7 + 8	5 + 10	8	8
SO-496	Slovak Republic	0	7 + 8	5 + 10	8	8
Vanda	Slovak Republic	0	7 + 9	2 + 12	5	5
Petrana	Slovak Republic	0	7 + 9	5 + 10	7	7
SK-22*	Slovak Republic	0	7 + 9	5 + 10	7	5
SK-26	Slovak Republic	2*	7 + 9	2 + 12	7	7
SK-30*	Slovak Republic	1	7 + 8	5 + 10	10	8
Torysa	Slovak Republic	0	7 + 8	2 + 12	6	6
Velta	Slovak Republic	1	7 + 9	5 + 10	9	9
RA-66	Slovak Republic	0	7 + 9	5 + 10	7	7
MS-1051-3	Slovak Republic	0	7 + 8	2 + 12	6	6
MS-944	Slovak Republic	0	6 + 8	5 + 10	6	6
PS-19/41	Slovak Republic	0	7 + 8	2 + 12	6	6
PS-41/98	Slovak Republic	0	7 + 9	2 + 12	5	5
HE-3625	Czech Republic	0	7 + 8	5 + 10	8	8
HE-6133	Czech Republic	0	7 + 9	5 + 10	7	7
HE-6606	Czech Republic	1	17 + 18	2 + 12	8	8
BR-881	Czech Republic	0	7 + 9	5 + 10	7	7
BR-816	Czech Republic	0	7 + 9	5 + 10	7	7
Brea	Czech Republic	0	7 + 9	5 + 10	7	7
BR-794	Czech Republic	0	7 + 9	5 + 10	7	7
SG-U-7067	Czech Republic	0	7 + 9	2 + 12	5	5
MV-06-95	Hungary	1	7 + 9	5 + 10	9	9
MV-230-96*	Hungary	2*	7 + 9	4 + 12	6	4
MV-04-96*	Hungary	0	7 + 8	2 + 12	6	4
CF-5172	France	0	6 + 8	2 + 12	4	4
Sideral	France	0	7 + 9	2 + 12	5	5
HYB-97-106	France	0	6 + 8	5 + 10	6	6
FD-90050	France	0	6 + 8	2 + 12	4	4
FD-92017/1	France	1	7 + 8	5 + 10	10	10
Vm-112	France	0	6 + 8	5 + 10	6	6
Ormil	France	0	7 + 9	5 + 10	7	7
Charger	Great Britain	0	17 + 18	2 + 12	6	6
Ceb.-9703	the Netherlands	1	6 + 8	5 + 10	8	8
LP-307292	Germany	1	6 + 8	5 + 10	8	8
Average					7.1	

*secale block 1B3

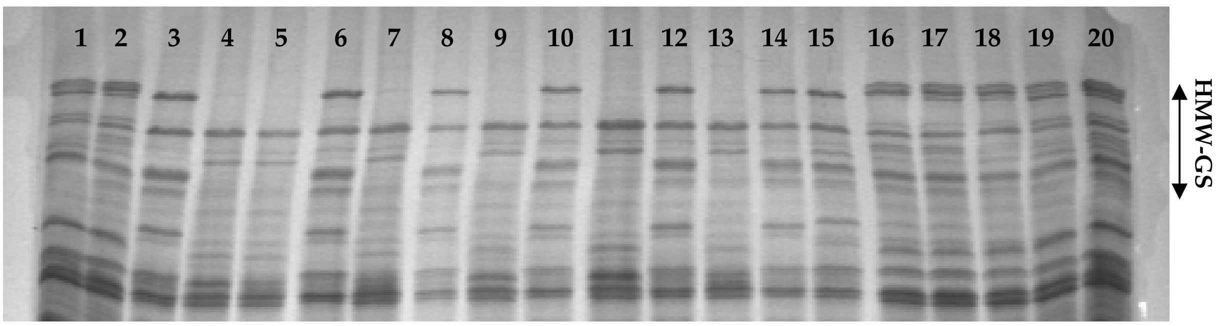


1 – Marquis, 2 – HE-3625, 3 – HE- 6133, 4 – Arida, 5 – MV-04-96, 6 – RA-66, 7 – PS-41/98, 8 – PS-19/41, 9 – CF-5172, 10 – BR-881, 11 – BR-816, 12 – BR-794, 13 – HYB-97-106, 14 – MS-1051-3, 15 – MS-944, 16 – Chinese Spring

Figure 3. Electrophoretic profile of HMW glutenin subunits in the grain of *Triticum aestivum* L.

Our analyses show (Table 1, Figures 1–3) that among the studied 41 winter wheat cultivars the most frequent banding paterns were subunits 0 (73%) from the locus Glu-A1, 7 + 9 (54%) from the locus Glu-B1 and 5 + 10 (66%) located on the locus Glu-D1. The subunits controlled by the locus Glu-D1 are the most important for technological quality. The subunits 5 + 10 have positive effects, while 2 + 12 have negative effects on quality. As summarised in Table 1, the pair of high quality subunits (5 + 10) was detected in 27 new breeding lines i.e. in 66% of the accessions. The highest GQ score of 10 was found in the lines SK-30 and FD-92017/1. The GQ score 9 was found in the cultivars Astella, Ilona, Velta and MV-06-95, i.e. in 10% of the accessions. The results show, that these high quality subunits are complemented with other high quality HMW glutenin subunits like 1, 2*, 7 + 8 and 7 + 9. Good technological quality can therefore be predicted for these accessions. The combinations of 5 + 10 with lower quality HMW-GS like 0 and 6 + 8 were detected in other accessions with a GQ-score from 5 to 8 (80%). In contrast, the lowest GQ-score of 4 was found in the accessions CF-5172 and FD-90050 with the subunits 0, 6 + 8 and 2 + 12. These accessions can be specified as unsuitable for bread making. The rare pair of subunits 17 + 18 with high positive effects on quality was found in the accessions Charger and HE-6606. The rare pair of subunits 4 + 12 with negative effects on quality was found in the accession MV-230-96. They are solitary like the subunits 20 and 3 + 12, which are less frequent in Slovak cultivars.

Payne and Lawrence (1983) and Payne *et al*. (1987) reduced the assigned GQ-score in presence of the secale-gliadin block 1B3 in the electrophoretic pattern. This block is known as a marker of low breadmaking quality but also of resistance to stem



1 – Frankenkorn, 2 – Bauländer Spelz, 3 – Arida, 4 – Vendur, 5 – Istrodur, 6 – Istar, 7 – Istrodur, 8 – Arida, 9 – Vendur, 10 – Istar, 11 – Vendur, 12 – Arida, 13 – Istrodur, 14 – Istar, 15 – SO-296, 16 – Rouquin, 17 – Schwabenkorn, 18 – Holstenkorn, 19 – Frankenkorn, 20 – Bauländer Spelz

Figure 4. Electrophoretic profile of HMW glutenin subunits in the grain of *Triticum spelta* L., *Triticum durum* DESF., *Triticum aestivum* L.

Table 2. Composition of HMW glutenin subunits in the grain of durum wheat (*Triticum durum* DESF.)

Cultivar	HMW glutenin subunits of loci			Glu-score
	Glu-A1	Glu-B1	Glu-D1	
Istrodur	0	7 + 8	–	4
Soldur	0	7 + 8	–	4
Vendur	0	7 + 8	–	4

rust. This indicates, that the secale-genes, present in it, are responsible for low gluten quality and include also the gene *Sr31* for stem rust resistance (ČERNÝ & ŠAŠEK 1996). In Table 1 can be seen, that this block is present in the two Slovak accessions SK-22 and SK-30 and in two Hungarian accessions, MV-230-96 and MV-04-96. The accessions SK-22, and SK-30 had a rye-score of 5 and 8, respectively, while both Hungarian accessions had a rye score of 4. Our results are similar to those of other authors who analysed Slovak and world collections of common wheats and the effects of particular HMW-GS on technological quality (GREGOVÁ *et al*. 1995; GÁLOVÁ *et al*. 1998, 2002). *Triticum durum* DESF. is grown in the Slovak Republic much less than *Triticum aestivum* L. Durum wheats are primarily used for pasta making, e.g. spaghetti, macaroni etc. Among the studied tetraploid wheats (Table 2, Figure 4) we detected the HMW-GS 0 and 7 + 8 with a GQ score of 4 in all three analysed cultivars. The D genome absence was expressed by the lack of HMW-GS, coded by the D genome. This was described in more detail by GREGOVÁ *et al*. (1995), KRAIC *et al*. (1997) and GÁLOVÁ *et al*. (1998).

Triticum spelta L. is an old crop known worldwide, experiencing some renaissance in the Slovak Republic because of its unique flavour, high vitamin content and a higher nutritious value than common wheat. The cultivation, final use and breeding methods of spelt wheat are increasingly studied (BOGNAR & KELLERMAN 1993; ABDEL-AAL *et al*. 1996; RANHOTRA *et al*. 1996). Our electrophoretic analyses of spelt wheat HMW-GS (Table 3, Figure 4) showed that these spelt wheat cultivars were not homogeneous and consisted of 3–4 different lines (A, B, C, D). The cultivar Bauländer Spelz showed four groups of electrophoretic profiles with different HMW-GS, with similar proportions of lines B (30%), C (35%) and D (30%). The most frequent line was C with the subunits 1, 13 + 16, 2 + 12 and a GQ score of 8. Within the cv. Frankenkorn, consisting also

Table 3. Composition of HMW glutenin subunits in the grain of spelt wheat (*Triticum spelta* L.)

Cultivar	Lines	Rel. frequency (%)	HMW glutenin subunits of loci			Glu-score
			Glu-A1	Glu-B1	Glu-D1	
Bauländer Spelz	A	5	1	7 + 8	2 + 12	8
	B	30	1	6 + 8	2 + 12	6
	C	35	1	13 + 16	2 + 12	8
	D	30	1	6 + 8	5 + 10	8
Frankenkorn	A	30	1	7 + 8	2 + 12	8
	B	10	1	6 + 8	2 + 12	6
	C	10	1	13 + 16	2 + 12	8
	D	50	1	6 + 8	5 + 10	8
Holstenkorn	A	65	1	7 + 8	2 + 12	8
	B	10	1	6 + 8	2 + 12	6
	D	25	1	6 + 8	5 + 10	8
Schwabenkorn	B	5	1	6 + 8	2 + 12	6
	C	65	1	13 + 16	2 + 12	8
	D	30	1	6 + 8	5 + 10	8
Rouquin	B	30	1	6 + 8	2 + 12	6
	C	50	1	13 + 16	2 + 12	8
	D	20	1	6 + 8	5 + 10	8

of four lines, the line D with the subunits 1, 6 + 8, 5 + 10 and Glu-score 8 prevailed. Three groups of electrophoretic patterns were found within all remaining spelt cultivars. In the cv. Holstenkorn dominated line A (65%) with the subunits 1, 7 + 8, 2 + 12 and a GQ-score of 8. In the cvs. Rouquin and Schwabenkorn again line C prevailed with 50% and 65%, respectively. Based on the GQ-score of 8 these spelt wheat cultivars can be classified as wheats with good technological quality. Rodriguez-Quijano *et al*. (1990) surveyed the HMW-GS composition in 118 Spanish spelt wheat cultivars. They also have found not homogeneous HMW-GS patterns in some spelt cultivars. They identified a then new pair of subunits 13 + 16 in 12% of the accessions. We have found these subunits in the spelt cultivars Bauländer Spelz, Rouquin and Schwabenkorn. They found also a new allele 2.3 of the locus Glu-D1. Our results show, that our spelt wheat accessions have similar HMW-GS patterns as Slovak common wheats and that they are suitable for cultivation under conditions of the Slovak Republic. Our results have showen, that glutenin and gliadin markers are suitable for a fast identification and classification of cultivars and for the detection of lines and mutants. Genes coding glutenin and gliadin provide a simple system for the description of the genetic structure of wheat cultivars or populations, that can be homogeneous, consisting of several lines or polymorphic in the patterns of gliadins and glutenins. This is of particular importance for breeding. The ISTA-recommended methods of SDS-PAGE and A-PAGE are fast and reliable tools for the detection of HMW glutenin subunits and gliadins, that can be used for the characterisation of wheat cultivars and of some of their properties such as technological quality.

Acknowledgement: Authors thank the Central Institute for Control and Testing in Agriculture at Bratislava and the Department of Food Production at the Slovak Agricultural University in Nitra for supplying the plant material.

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Received for publication January 14, 2003
Accepted after corrections February 25, 2003

Abstrakt

Starovičová M., Gálová Z., Knoblochová H. (2003): **Identifikácia gluteínových markerov v rôznych odrôdách troch druhov pšenice**. Czech J. Genet. Plant Breed., **39**: 51–57.

Kolekcia 41 slovenských a európskych odrôd a novošľachtencov pšenice letnej formy ozimnej (*Triticum aestivum* L.), 5 odrôd špaldovej pšenice (*Triticum spelta* L.) a 3 odrôd tvrdej pšenice (*Triticum durum* DESF.) bola analyzovaná a charakterizovaná na základe kvalitatívneho zastúpenia vysokomolekulárnych gluteínových podjednotiek (HMW-GS) a detekcie sekalínového bloku 1B3. V genotypoch 41 slovenských a európskych pšeníc (*Triticum aestivum* L.) sa najčastejšie vyskytovali HMW gluteínové podjednotky 0 (73 %), 7 + 9 (54 %) a 5 + 10 (66 %). Najvyššie Glu-skóre (10) dosiahli dva genotypy (SK-30, FD-92017/1 a najvyššie Glu-skóre (9) štyri genotypy (Astella, Ilona, Velta a MV-06-95). HMW gluteínové podjednotky 0, 7 + 8 s Glu-hodnotením 4 boli stanovené v tvrdej pšenici (*Triticum durum* DESF.). Elektroforetické spektrá analyzovaných špaldových pšeníc sa vyznačovali nehomogénnosťou a vyskytovali sa pri nich štyri rôzne línie.

Kľúčové slová: *Triticum aestivum* L.; *Triticum spelta* L.; *Triticum durum* DESF.; HMW gluteínové podjednotky; sekalínový blok 1B3; elektroforéza; SDS-PAGE; A-PAGE

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