

## Diversity of HMW-*Glu* Alleles and Evaluation of their Effects on some Characters in Winter Wheat Landraces and Old Cultivars

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**Abstract:** Earliness, morphological and agronomic characters and grain quality were studied in 123 European landraces and old cultivars of winter wheat in three-year field experiments. Simultaneously, HMW *Glu*-alleles were identified in these cultivars by means of SDS-PAGE. Within this set of cultivars 224 *Glu*-lines (with occurrence over 5% in the cultivar) were identified carrying 3 different allelic combinations at 1A, 10 combinations at 1B and 3 combinations at 1D chromosomes, respectively. Relatively rare alleles were 2\* at 1A and 3+12 at 1D as well as alleles 8, 6, 9, 7, 13+16 and 17+18 at 1B. Allele 20 at 1B was identified only in cultivars from DNK, CHE and EST. Allele 2\* at 1A locus was found mainly in cultivars from Eastern, South-Eastern and Central Europe. Allelic combination 17+18 at 1B was also characteristic of cultivars from Central Europe. However, the gluten patterns themselves were not a sufficient tool for geographic characterisation of cultivars. The composition of *Glu*-alleles influenced the earliness of cultivars (alleles 2\* at 1A, 17+ 18 and 6 at 1B and 3+12 at 1D). Spike length was positively affected by allele 1 at 1A and number of spikelets per spike by alleles 2+12 at 1D chromosome. Allele 2\* was also associated with lower grain weight per spike. Crude protein content was decreased in cultivars where GS at 1A locus was absent (0). The value of gluten index was considerably higher (59.2) in cultivars bearing allelic combination 5+10 at 1D. A number of alleles affected the values of SDS micro-sedimentation test.

**Keywords:** wheat; HMW glutenins; *Glu*-alleles; agronomic characters; grain quality

Modern cultivars of wheat as well as of many other crops are often quite uniform, with relatively narrow genetic constitution. Therefore the need of new sources of diversity for breeding is frequently discussed (DEVKOTA & SHAH 1998; MOGHADDAM *et al.* 1998). Landraces that were created through a combination of natural selection and selection by farmers (BELAY *et al.* 1995) have some valuable characters that can be utilised for improvement of newly bred cultivars and broadening of their diversity (TESEMMA *et al.* 1998; KELLER *et al.* 1991). Landraces and obsolete cultivars usually comprise much broader intra-specific genetic diversity than modern cultivars, therefore they are considered as a valuable part of gene pool (ZOU & YANG 1995; VOJDANI & MEYBODI

1993). The diversity of landraces is a result of different soil and climatic conditions in various regions and was also strongly influenced by local practices and specific demands for product quality and other characters (VAN HINTUM & ELINGS 1991). Tolerance to stresses (LI XINGPU *et al.* 1997) and yield stability are often mentioned as characteristic features of landraces (TESEMMA *et al.* 1998).

WANG and GUO (1992) found the similarity of gluten alleles in wheat in accordance with the geographical origin of cultivars. EHDAIE and WAINES (1989) reported significant genetic variability in spike productivity and tillering in wheat landraces. LI XINGPU *et al.* (1997) found wheat landraces resistant to stresses (frost, drought,

Research was carried out in the framework of the project “Diversity within and between Landraces and Old Cultivars of *Triticum aestivum*, *T. spelta* and *T. dicoccum* and its Utilization” as a part of Grant No. 521/00/1595 supported by Grant Agency of the Czech Republic.

salinity); OBARI (1990) confirmed earliness and good adaptability of landraces to high temperatures. Good grain quality characters were also reported in some wheat landraces and obsolete cultivars and much broader diversity of quality characters can be expected in them than in the presently grown cultivars. KELLER *et al.* (1991), WANG and GUO (1992), RODRIGUEZ-QUIJANO *et al.* (1994) and YANG and LIANG (1995) found a very high protein content in kernels of some landraces of common wheat. In the experiments carried out in RICP Prague selected landraces proved not only a high protein content but also convenient parameters of gluten quality (MICHALOVÁ & DOTLAČIL 1993; DOTLAČIL *et al.* 2000).

Grain quality in wheat is determined mainly by the content and quality of storage proteins in kernels. These proteins are represented by monomeric gliadins and polymeric glutenins which comprise several types of subunits and are usually divided into HMW (High Molecular Weight) and LMW (Low Molecular Weight) glutenin subunits (GS); HMW-GS genes are localised at the *Glu-1* loci on long arms of 1A, 1B and 1D chromosomes (SHEWRY & TATHAM 1997). Their allelic variants are associated with differences in grain quality (PAYNE 1987; MANLEY *et al.* 1992; WEEGELS *et al.* 1996). They can also be linked with some other characters such as cold resistance (ŠAŠEK *et al.* 2000). Due to this specific nature HMW-GS genes can be utilised as markers of some important characters as well as for characterisation of cultivars and lines in wheat (ČERNÝ & ŠAŠEK 1996, 1998; ŠAŠEK *et al.* 1995).

The aim of this paper is to analyze diversity of HMW gluten subunits in evaluated landraces and old cultivars and to estimate possible effects of different *Glu*-alleles on important characters.

## MATERIAL AND METHODS

A set of 123 winter wheat landraces and obsolete cultivars originating predominantly from European countries was studied in three-year field experiments. Cultivars were sown on microplots of 1.5 m<sup>2</sup> in Prague-Ruzyně; seed from a previous growing season was used in the second and third year. Standard growing practices were used during vegetation, only application of growth regulators and use of fertilisers in spring were omitted. Earliness of heading and flowering (50% of spikes in the phase concerned), maturity for harvest (hard kernels in spikes) and grain filling period were recorded. Also plant height (stand in the field) was measured before maturity. Before harvest, 30 culms with spikes were cut from each plot and spike morphology, productivity and harvest index (HI) were determined. The rest of the plot was harvested and seed samples from each plot were analysed for crude protein content (method according to Kjeldahl, using Kjeltex Auto System II), SDS micro-sedimentation

test (modification by HÝŽA (1986) and gluten index (in accordance with ICC Standard No. 155, using Glutomatic 2 200). All evaluations and calculations were carried out each experimental year; the acquired data (mean values of characters for each cultivar in particular year) were used for statistical analyses.

Simultaneously with the field experiments, HMW-gluten patterns were characterised in 100 grains randomly taken from each harvested plot; individual grains were halved and analysed by a standard SDS-PAGE technique for wheat (WRIGLEY 1992; KRAIC *et al.* 1995). Glutenin patterns were evaluated by densitometry (Image Master DTS) and classification according to PAYNE and LAWRENCE (1983) was applied for identification of HMW *Glu*-alleles.

The HMW *Glu* patterns and results of the 3-year field trials were analysed by means of statistical software UNISTAT (ANOVA-hierarchical model: chromosomes/HMW subunits;  $\chi^2$ ). Only *Glu*-lines showing occurrence over 5% in cultivars (populations) were recorded as significant and included in evaluation (to avoid marginal effects of rare alleles as well as to eliminate possible admixtures in the case of *Glu*-lines with very low representation).

## RESULTS AND DISCUSSION

In the studied set of 123 cultivars two different gluten subunits (GS) were found on the 1A chromosome (or the active allele was absent), 10 different *Glu*-alleles and/or allelic combinations were identified on the 1B chromosome and 3 different allelic combinations on the 1D chromosome. GREGOVÁ *et al.* (1999) published complete HMW *Glu* patterns for 52 cultivars evaluated in this study and a paper providing the patterns for the rest of the cultivars is under preparation. Therefore, primary information on gluten subunits in individual cultivars is not a subject of this paper.

The occurrence of particular HMW *Glu*-alleles and their combinations is shown in Table 1. Among 224 identified *Glu*-lines (with occurrence over 5% in the cultivar/population) allele 1 was the most frequent one at 1A locus (48.7%), nevertheless, the absence of HMW-*Glu*-subunits (0) at 1A locus was also very common (44.6%). Allele 2\*, which was found in 6.7% of the examined cultivars, was relatively rare.

Similarly, only three different GS allelic combinations were identified at 1D locus, among them alleles 2+12 were the most common combination (63.4% lines) followed by 5+10 combination having about a half frequency of occurrence (33%). But such occurrence of allelic combination 5+10 at 1D locus in our materials is higher than that found by OVESNÁ *et al.* (2001), when only 3 cultivars out of 15 old and 7 new ones carried this combination. Allelic combination 3+12 was observed only in 5 cultivars

(3.6%) and can be considered as quite a rare one as it was also confirmed by ŠAŠEK *et al.* (1995) (in 4 accessions out of 393 cultivars).

Much higher diversity was recorded at 1B locus, where 10 different alleles and/or their combinations could be identified, among them alleles 7+9 (present in 39.7% of characterised *Glu*-lines), 7+8 (22.3% of *Glu*-lines), 6+8 (16.1% of *Glu*-lines) and allele 20 (present in 12.1% *Glu*-lines) can be considered as common and broadly distributed. On the contrary, alleles 8, 6, 9, 7 and allelic combinations 13+16 and 17+18 were scarce (they were found only in 0.4–3.1% of all characterised *Glu*-lines). This finding is again in agreement with the results published by ŠAŠEK *et al.* (1995).

HMW glutenin subunits can be employed for characterisation of cultivars with respect to the country or region of their origin (VAN HINTUM & ELINGS 1991). Such attempt was also made in this study in 224 *Glu*-lines that were identified (with occurrence over 5%) in 123 landraces and obsolete cultivars from European countries (only two cultivars originate from New Zealand); the results are summarised in Table 2.

Geographic distribution of HMW *Glu*-alleles implies that most alleles with frequent occurrence (such as alleles 1 at 1A; 7+9, 7+8 and 6+8 at 1B; 2+12 and 5+10 at 1D) also have a broad geographic distribution. These alleles are not usually specific to a country or region and can be found in a majority of geographic groups of cultivars. On the contrary, the absence of some alleles could be considered as specific to several countries (e.g. absence of *Glu*-alleles 7+8 at 1B in cultivars from Denmark, Estonia, Great Britain, Georgia or absence of *Glu*-alleles 6+8 at the same locus in cultivars from Russia, Ukraine, Hungary, Georgia, Estonia and Bulgaria). The absence of *Glu*-alleles 7+9 at 1B was characteristic of cultivars coming from

the Netherlands, Great Britain and Switzerland. Quite a specific geographic origin was found in relatively frequent allele 20, which was identified in cultivars originating from Denmark, Switzerland, France, Great Britain, Germany, Sweden and former Czechoslovakia. In the case of 1D chromosome the absence of the most frequent allelic combination 2+12 was observed only in cultivars from 2 countries with low representation of accessions (1; 3), therefore this GS pattern can probably be considered as commonly distributed. A similar situation was recorded in gluten subunits 5+10 (these GS were absent only in cultivars from Great Britain and New Zealand).

As concerns rare GS, allele 2\* at 1A locus was found in cultivars from Hungary (5), Ukraine (3), Czech Republic (2) and in single cultivars from Austria, Bulgaria, Denmark, France and Russia. It seems that the occurrence of this allele is typical on cultivars from Eastern, South-Eastern and Central Europe. Similarly, GS 3+12 at 1D chromosome were recorded only in cultivars close to Central Europe (Czech Republic – 3, Hungary – 2, Switzerland – 2). Nevertheless, this allelic combination was also found in one cultivar from Georgia. Among the relatively rare allelic combinations at 1B, GS 17+18 were found in 5 cultivars from Poland, Hungary and former Czechoslovakia and this combination seems to be characteristic of Central Europe. Gluten subunits 13+16 at 1B were found in cultivars from Switzerland, Austria and Bulgaria. Allele 7 was identified in cultivars from Ukraine (2), France (2) and in single cultivars from Georgia, Poland and Russia. Allele 9 at 1B was identified exclusively in 3 cultivars from Sweden and Ukraine and alleles 6 and 8 in single cultivars from Russia, France and Hungary.

Even though the presence of several HMW *Glu*-alleles (especially on 1A and 1B chromosomes) can be considered as specific to some regions of Europe, the GS

Table 1. The frequency of HMW *Glu*-alleles identified in the set of 123 landraces and old winter wheat cultivars

Chromosome 1A					Chromosome 1B					Chromosome 1D				
Allele	$n_1$	(%)	$n_2$	(%)	Allele	$n_1$	(%)	$n_2$	(%)	Allele	$n_1$	(%)	$n_2$	(%)
0	57	47.2	100	44.6	7+9	45	36.6	88	39.7	2+12	79	64.2	142	63.4
1	58	46.3	109	48.7	7+8	28	22.7	51	22.3	5+10	39	31.7	74	33.0
2*	8	6.5	15	6.7	6+8	21	17.1	36	16.1	3+12	5	4.1	8	3.6
					20	16	13	27	12.1					
					17+18	3	2.4	5	2.2					
					13+16	3	2.4	4	1.8					
					7	3	2.4	7	3.1					
					9	2	1.6	3	1.3					
					8	1	0.8	1	0.4					
					6	1	0.8	2	0.9					

$n_1$  = 123 cultivars (dominant *Glu*-lines in cultivars) and  $n_2$  = 224 *Glu*-lines with occurrence over 5% in cultivars

Table 2. Geographic distribution of HMW-*Glu* alleles in identified *Glu*-lines (224) derived from 123 winter wheat landraces and old cultivars

Country of origin	Total number of <i>Glu</i> -lines		A1		B1										D1		
	0	1	1	2*	7+8	6+8	7+9	17+18	13+16	7	8	9	6	20	2+12	3+12	5+10
AUT	16	5	10	1	8	1	6	0	1	0	0	0	0	0	12	0	4
BGR	11	4	6	1	1	0	9	0	1	0	0	0	0	0	4	0	7
CHE	13	6	7	0	5	3	0	0	2	0	0	0	0	3	10	2	1
CSK	28	15	11	2	3	1	21	1	0	0	0	0	0	2	14	3	11
CZE	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
DNK	16	9	6	1	0	5	6	0	0	0	0	0	0	5	12	0	4
EST	3	0	3	0	0	0	3	0	0	0	0	0	0	0	0	0	3
FRA	21	10	10	1	6	5	3	0	0	2	0	0	1	4	18	0	3
GBR	6	5	1	0	0	1	0	0	0	0	0	0	0	5	6	0	0
GEO	5	1	4	0	0	0	4	0	0	1	0	0	0	0	2	1	2
GER	23	14	9	0	8	6	4	0	0	0	0	0	0	5	18	0	5
HUN	22	10	7	5	4	0	15	2	0	0	1	0	0	0	10	2	10
NLD	3	0	3	0	1	2	0	0	0	0	0	0	0	0	2	0	1
NZL	2	2	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0
POL	13	4	9	0	2	4	4	2	0	1	0	0	0	0	8	0	5
RUS	6	1	4	1	1	0	3	0	0	1	0	0	1	0	2	0	4
SWE	15	10	5	0	3	6	1	0	0	0	0	2	0	3	13	0	2
UKR	20	3	14	3	8	0	9	0	0	2	0	1	0	0	9	0	11
Total	224	100	109	15	51	36	88	5	4	7	1	3	2	27	142	8	74



patterns could hardly be used as the only and exclusive tool for characterisation of European landraces and obsolete cultivars since there are often single cultivars bearing unaccustomed gluten subunits. This fact could illustrate an intensive exchange of local cultivars throughout Europe even in early decades of the last century. On the contrary, the absence of some broadly distributed alleles could indicate breeding based only on local resources.

Characterisation of cultivars using the composition of *Glu*-alleles in principal *Glu*-lines (in multi-lines or populations) was applied in the subsequent step of evaluation. The number of analysed *Glu*-lines was reduced to 123 in this way. However, as it is shown in Table 1, this reduction did not lead to the elimination of rare alleles in the analysed set and relative frequencies of all GS-alleles in both sets ( $n_1 = 123$ ;  $n_2 = 224$ ) were also in good conformity. This conformity was tested by  $\chi^2$  test and proved as significant at the level  $P = 0.87$  for chromosome 1A,  $P = 0.99$  for 1B and  $P = 0.93$  for 1D. Somewhat lower values of significance for 1A and 1D are caused mainly by a low number of recorded allelic combinations. Because of the high conformity of both sets and considering the occurrence of additional marginal *Glu*-lines as random, the cultivars could be further characterised by the *Glu*-patterns of principal lines. This approach allowed a simple estimation of effects of GS-alleles on some evaluated characters.

HMW *Glu*-alleles can be used as markers of important characters (WANG *et al.* 1993; ČERNÝ & ŠAŠEK 1996). Therefore, we also tested relevant effects of HMW *Glu*-alleles on other evaluated characters; the results are summarised in Table 3.

A significant effect on some characters was found in *Glu*-alleles on all chromosomes 1 (1A, 1B, 1D). At 1A locus, the composition of *Glu*-alleles has significant effects on earliness of heading and maturity when the presence of allele 2\* resulted in earlier development (by 2–3 days). Earliness of heading, flowering and maturity was also affected by the composition of alleles at 1B (presence of GS 17+ 18 and probably also allele 6 were associated with earliness) and 1D (where the presence of GS 3+12 has positive effects on earliness). The only significant interaction was 1A × 1B × 1D for heading time.

As for the morphological characters, only effects on spike length (positive effect of allele 1 at 1A) and number of spikelets per spike (lower number of spikelets in cultivars carrying allele 2\* at 1A locus, positive effect of GS 2+12 on 1D chromosome) were observed.

Spike productivity characters were affected only by alleles at 1A locus while allele 2\* was associated with lower grain weight per spike. Lower harvest index was found in cultivars carrying allele 1 at 1A locus, and on the contrary, allelic combination 5+10 at 1D slightly increased the value of harvest index.

As it is commonly known, HMW-GS are closely associated with grain quality characters (WEEGELS *et al.* 1996;

ČERNÝ & ŠAŠEK 1996). Such association was also confirmed in this study. Crude protein content in grains was significantly affected only by the composition of alleles at 1A locus while the absence of GS (0) resulted in lower crude protein content whereas the effects of alleles 1 and 2 were equal. The value of gluten index (GI) was strongly affected by the composition of alleles on 1D chromosome; allelic combination 5+10 showed considerably higher GI (59.2) in cultivars carrying these subunits whereas the combination 3+12 caused a low value of GI (46.0). Some effects were observed on the value of SDS micro-sedimentation test when allele 2\* at 1A and allelic combination 5+10 at 1D increased the value of SDS test; positive effects of the same alleles on baking quality and their utilisation in a breeding program were recently reported by DE BUSTOS *et al.* (2001). As concerns the alleles located at 1B, a lower SDS value was recorded in cultivars carrying allele(s) 8, 20 and 6+8, on the contrary, positive effects could be expected in cultivars carrying alleles 7+9 and especially 13+16. The SDS micro-sedimentation test was also significantly affected by 1A × 1D interaction when allele 2\* at 1A and 5+10 at 1D resulted in a higher SDS-value (7.46 ml) than the other combinations of GS.

**Abbreviations:** HMW = high molecular weight gluten subunits; GS = gluten subunits; GI = gluten index; HI = harvest index.

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Received for publication November 25, 2002

Accepted after corrections December 5, 2002

## Abstrakt

DOTLAČIL L., GREGOVÁ E., HERMUTH J., STEHNO Z., KRAIC J. (2002): **Diversita HMW- Glu alel a hodnocení jejich účinků na některé znaky u krajových a starých odrůd ozimé pšenice.** *Czech J. Genet. Plant Breed.*, **38**: 109–116.

V tříletých polních pokusech byly studovány agronomické znaky a znaky kvality zrna u 123 krajových a starých evropských odrůd pšenice ozimé. Současně byly s využitím SDS-PAGE identifikovány HMW *Glu*-alely. V rámci studovaného souboru odrůd bylo identifikováno 224 *Glu*-linií (s výskytem vyšším než 5 % v odrůdě), které vykazovaly 3 různé alelické

kombinace na 1A, 10 kombinací na 1B a 3 kombinace na 1D chromosomech. Poměrně vzácně se vyskytovaly alely 2\* na 1A a 3+12 na 1D, jakož i alely 8, 6, 9, 7, 13+16 a 17+18 na 1B. Alela 20 na 1B byla zjištěna pouze u odrůd z DNK, CHE a EST. Alela 2\* v lokusu 1A byla nalezena především u odrůd z východní, jihovýchodní a střední Evropy. Také kombinace alel 17+18 na 1B byla charakteristická pro odrůdy ze střední Evropy. Samotné charakteristiky gluteninů však nebyly dostačující pro geografickou charakterizaci původu odrůd. Kombinace gluteninových alel měla vliv na ranost odrůd (alely 2\* na 1A, 17+18 a 6 na 1B a 3+12 na 1D). Délka klasu byla pozitivně ovlivněna alelou 1 na 1A a počet klásků na klas alelami 2+12 na 1D chromosomu. Výskyt alely 2\* byl rovněž spjat s nižší hmotností zrna na klas. Obsah hrubého proteinu byl nižší u odrůd s chybějící *Glu*-alelou v 1A lokusu (0). Hodnota gluten indexu byla značně vyšší (59,2) u odrůd s alelickou kombinací 5+10 na 1D. Řada alel měla vliv na hodnoty SDS mikrosedimentačního testu.

**Klíčová slova:** pšenice; HMW gluteniny; *Glu*-alely; agronomické znaky; kvalita zrna

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