

## Polymorphism of proteins in selected slovak winter wheat genotypes using SDS-PAGE

## Polymorfizmus bielkovín vo vybraných slovenských odrôdach pšenice letnej použitím SDS-PAGE

Dana MIHÁLIKOVÁ\*, Zdenka GÁLOVÁ, Lenka PETROVIČOVÁ and Milan CHŇAPEK

Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, \*correspondence: xmihalikovad@is.uniag.sk

### Abstract

Winter wheat is especially used for bread-making. The specific composition of the grain storage proteins and the representation of individual subunits determines the baking quality of wheat. The aim of this study was to analyze 15 slovak varieties of the winter wheat (*Triticum aestivum* L.) based on protein polymorphism and to predict their technological quality. SDS-PAGE method by ISTA was used to separate glutenin protein subunits. Glutenins were separated into HMW-GS (15.13%) and LMW-GS (65.89%) on the basis of molecular weight in SDS-PAGE. At the locus Glu-A1 was found allele Null (53% of genotypes) and allele 1 (47% of genotypes). The locus Glu-B1 was represented by the HMW-GS subunits 6+8 (33% of genotypes), 7+8 (27% of genotypes), 7+9 (40% of genotypes). At the locus Glu-D1 were detected two subunits, 2+12 (33% of genotypes) and 5+10 (67% of genotypes) which is correlated with good bread-making properties. The Glu – score was ranged from 4 (genotype Viglanka) to 10 (genotypes Viola, Vladarka). According to the representation of individual glutenin subunits in samples, the dendrogram of genetic similarity was constructed. By the prediction of quality the results showed that the best technological quality was significant in the varieties Viola and Vladarka which are suitable for use in food processing.

**Keywords:** prediction of technological quality, SDS-PAGE, storage proteins, winter wheat

## Abstrakt

Pšenica letná, forma ozimná sa využíva najmä na výrobu chleba. Špecifické zloženie zásobných bielkovín zrna a zastúpenie individuálnych podjednotiek určuje pekársku kvalitu pšenice. Cieľom tejto práce bolo analyzovať 15 slovenských odrôd pšenice letnej, formy ozimnej (*Triticum aestivum L.*) z hľadiska polymorfizmu bielkovín a predigovať ich technologickú kvalitu. Na separáciu glutenínových podjednotiek bola použitá metóda ISTA SDS-PAGE. Gluteníny boli rozdelené v SDS - PAGE na základe molekulovej hmotnosti na HMW-GS (15,13%) a LMW-GS (65,89%). Na lokuse Glu-A1 bola zistená prítomnosť alely 0 (53% genotypov) a alely 1 (47% genotypov). Lokus Glu-B1 bol reprezentovaný HMW-GS podjednotkami 6+8 (33% genotypov), 7+8 (27% genotypov), 7+9 (40% genotypov). Na lokuse Glu-D1 boli detegované dve podjednotky, a to 2+12 (33% genotypov) a 5+10 (67% genotypov), ktorá je spájaná s dobrými pekárskymi vlastnosťami. Hodnota Glu-skóre sa pohybovala od 4 (odroda Viglanka) do 10 (odrody Viola, Vladarka). Na základe zastúpenia jednotlivých glutenínových podjednotiek vo vzorkách bol zostrojený dendrogram genetickej príbuznosti. Predikciou kvality z výsledkov vyplýva, že najlepšiu technologickú kvalitu vykazovali odrôdy Viola a Vladarka, ktoré sú vhodné na využitie v potravinárskom priemysle.

**Kľúčové slová:** predikcia technologickej kvality, pšenica letná forma ozimná, SDS-PAGE, zásobné bielkoviny

## Introduction

One of the most important sources of nutrients and energy in human and animal beings are cereal grains. Cereal grains contain macro-nutrients, proteins, fats, carbohydrates and many essential nutrients such as amino acids, vitamins and fatty acids that are very important for human health. Especially, wheat and bread are the most common food in many countries (Chňapek et al., 2012, Tsao et al., 2012).

Next to rice, wheat is the second largest grown cereal crop in the world. Typically, wheat flour contains from 8 to 11% proteins. Based on their functional properties, wheat proteins can be divided into two different groups: non-gluten proteins (albumins and globulins) and wheat storage proteins called the gluten proteins (gliadins, glutenins). Of all the cultivated cereals, wheat flour is almost unique because it forms a dough. Gluten proteins are responsible for the dough forming capacity of wheat flour. Gluten permits the retention of gas bubbles during baking of a dough to give open textured and pleasant eating products (Manley et al., 2011). Amount of wet gluten is influenced by growing environment and it is correlated to the content of the grain proteins. Despite of this, influence of genotype is usually dominant for qualitative properties of gluten and the quantity of protein or gluten is not a measure for its quality (Šimić et al., 2006).

Wheat genome (A, B, D) and genetic analysis of wheat storage proteins discovered that genes encoding these proteins were present on some loci of wheat chromosomes. Gliadins are monomeric, alcohol soluble proteins, which have

molecular weight ranging from 30 kDa to 60 kDa. They are broadly divided into 4 categories ( $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\omega$ - gliadins). At the distal end of chromosomes 1AS, 1BS and 1DS are located 3 homologous loci Gli-A1, Gli-B1 and Gli-D1, which contain large number of genes encoding for many  $\gamma$ - and  $\omega$ -gliadins. At the short arm of the group 6 chromosomes (Gli-A2, Gli-B2, Gli-D2 chromosomes) there are genes responsible for the synthesis of  $\alpha$ -,  $\beta$ -, and some  $\gamma$ -gliadins. All gliadins contain intramolecular disulfide bond linkages, with exception of  $\omega$ -gliadins (Katyal et al., 2016; Shewry et al., 1986). Polymeric glutenin proteins, with very variable molecular masses, from approximately 300 kDa to more than 1 million kDa are composed of two groups of subunits. Low molecular weight glutenin subunits (LMW-GS) with molecular weight from 30 to 40 kDa are similar in structure and size to the  $\gamma$ -gliadins. LMW glutenin subunits localized on short arms of chromosomes 1A, 1B and 1D are encoded by genes at the Glu-A3, Glu-B3 and Glu-D3 loci. High molecular weight glutenin subunits (HMW-GS) range in molecular mass from 65 to 90 kDa are localized on the long arms of chromosomes 1A, 1B and 1D which are encoded by x and y type genes at the Glu-A1, Glu-B1 and Glu-D1 loci (Liu et al., 2009; Shewry, Tatham, 1990).

High molecular weight glutenin subunits are one of the key factors in the process of bread-making which affected gluten elasticity by promoting the formation of larger glutenin polymers. HMW-GS can influence dough characteristics and bread-making quality in positive and negative way. For example, Glu-B1i allele (17+18) and Glu-D1d allele (5+10) have a positive influence, whereas Glu-D1a allele (2+12) and Null have a negative effect. HMW-GS alleles influencing quality of the dough, have been given different quality scores. They are extensively used as markers in wheat breeding programs. Composition of HMW-GS alleles has been used for selecting preferable bread-making lines (He et al., 2005; Liu et al., 2009; Payne et al., 1987).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is one of the method for HMW-GS diagnosis which is based on electrophoretic mobility of proteins in polyacrylamide gels. SDS-PAGE has been considered to be relatively straight-forward. Major limitations of SDS-PAGE are low resolution and overestimation of molecular mass, but it is still the simplest and the cheapest technique suitable for fast and large-scale screening of HMW-GS for wheat genotypes in breeding programs (Chňapek et al., 2015; Liu et al., 2009).

The aim of the work presented here was to analyze 15 varieties of the winter wheat (*Triticum aestivum* L.) based on protein polymorphism, to identificate HMW-GS and LMW-GS and to predict technological quality of analyzed varieties by using SDS - PAGE.

## Materials and methods

### Plant material

For the analyses, seeds of 15 genotypes of hexaploid *Triticum aestivum* L. from Slovakia were obtained from Hordeum Ltd - Plant Breeding station in Sládkovičovo (Bertold, Silvanus, Viola, Filemon, Natanael, Rupert, Torysa) and Research and Breeding Station at Malý Šariš - The Research Institute of Plant Production, Piešťany (Klaudia, Stanislava, Ilona, Viglanka, Vladarka, MS 1744, Stelarka, PS Sunanka).

### **Protein extraction**

Proteins were extracted from individual grains according to standard method by ISTA (Wrigley, 1992). Seed storage proteins were isolated from whole, dry and mature grains. There were analyzed 5 individual grains from each genotype and each grain was measured and mechanically homogenized. 8 µl of extraction solution (4.25 ml stock solution, 0.75 ml 2-mercaptoethanol, 10 ml redistilled water) was added per 1 mg of grain after homogenization. Stock solution for protein extraction was composed of 12.5 ml 1 mol·dm<sup>-3</sup> TrisHCl (pH 6.8), 20 ml glycerol, 24.1 ml redistilled water, 4 g SDS, 20 mg Pyronin Y. Extraction of storage proteins was performed by shaking at 100°C for 30 min. After that, samples were centrifugated at 15 000 rpm for 10 minutes and transferred to a new tube.

### **Electrophoretic separation of proteins**

Detection of HMW-GS and LMW-GS was performed by the standard reference electrophoretic method by ISTA, using polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS-PAGE) as a separative medium (Wrigley, 1992). Molecular marker (M-3788) and 10 µl of each sample was loaded into gel. Separation of storage proteins was running 10 – 12 hours with constant temperature 15°C, current 10 mA, 500 V and 50 W in the vertical discontinual electrophoretic system Hoefer SE 600 DeLuxe by Thermo Fisher Scientific (USA).

### **Gel staining and image analysis**

Electrophoreograms were coloured in the mixture containing 190 ml 10% trichloroacetic acid and 10 ml 0.5% Comassie Brilliant Blue R250 in ethanol overnight. Electrophoretic profiles were visualized in photo device with black and white CCD camera with a filter and lenses. Doc-It LS Image analysis UVP software (USA) was used for analysis of electrophoretic gels and statistical interpretation of the electrophoreogram . A dendrogram based on Jaccard's coefficient and hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) was constructed. The Glu-score was calculated according to the catalogue of alleles for HMW-GS (Payne et al., 1987).

### **Results and discussion**

Wheat, one of the most important food crops in the world, is more widely cultivated and consumed in comparision with other major cereals. Because of its immense importance as food source for humans and animals, breeders efforts are centered to improve and study the agronomic traits of wheat, such as quality, nutrient use efficiency, yield and resistance to abiotic and biotic stresses. Wheat quality is influenced by multiple aspects of which involved combination of actions of multiple genes and being further influenced by environmental conditions (Ling et al., 2013). The environmental factors and the genetic background contribute to protein composition and polymerization behavior in mature wheat to similar extend (Johansson et al., 2013).

Protein content of cereal grains is primarily evaluated to indicate their technological and nutritional quality. Nutritional quality depends on high content of essential amino acids, while technological quality is characterized not only by the amount of proteins and their functional composition, but also by the proportion of individual fractions of storage proteins which related to gluten quality and its impact on the final product (Palenčárová, 2010; Petrovičová et al., 2013).

Gluten plays significant role in the processing properties of dough. Gliadin and glutenin are seed storage proteins of wheat gluten which are accumulated in starchy endosperm during grain filling. These storage proteins support subsequent seedling growth and in addition influence the quality of flour (Miura et al., 2013).

Technological and nutritional quality of different varieties of the winter wheat grains is influenced by differences in fractional composition of storage proteins (Gálová et al., 2012). Electrophoretic method SDS-PAGE is the most commonly used method for separation of these proteins. During SDS-PAGE, identification of phenotypic effect of individual alleles of each gene is performed by electrophoretic analysis of wheat grain glutenin proteins. After separation of storage protein by electrophoresis, results can be used for genetic analysis, which are applicable in breeding and genetic studies (Chňapek et al., 2014).

Storage proteins of analyzed wheat genotypes were separated by SDS-PAGE into three different groups, namely high molecular weight glutenin subunits (HMW-GS), low molecular weight glutenin subunits (LMW-GS) and the residual albumins and globulins according to their molecular weight (Table 1.).

Table 1. Molecular weight of protein subunits

Tabuľka 1. Molekulové hmotnosti bielkovinových podjednotiek

Fractions of storage proteins	Molecular weight of protein subunits (kDa)
HMW-GS <sup>1</sup>	136-92
LMW-GS <sup>2</sup>	85-30
alb+glo <sup>3</sup>	27-7

<sup>1</sup>HMW-GS – high molecular weight glutenin subunits, <sup>2</sup>LMW-GS – low molecular weight glutenin subunits, <sup>3</sup>alb+glo – albumins and globulins

<sup>1</sup>HMW-GS – vysokomolekulárne glutenínové podjednotky, <sup>2</sup>LMW-GS – nízkomolekulárne glutenínové podjednotky, <sup>3</sup>alb+glo – albumíny a globulíny

The results show that the molecular weight of the HMW-GS in the analyzed genotypes was in the range of 136 kDa to 92 kDa, LMW-GS was from 85 kDa to 30 kDa and residual albumins and globulins was from 27 kDa to 7 kDa. Comparable results of protein subunits in wheat detected also Socha (2011) who observed molecular weight of HMW-GS from 140 kDa to 80 kDa and LMW-GS from 80 kDa to 30 kDa.

Individual electrophoreograms of wheat genotypes were analyzed and there were detected differences in content of glutenin subunits. Percentage of HMW-GS, LMW-GS and residual albumins and globulins was determined by separation of storage proteins of analyzed wheat genotypes by SDS-PAGE (Table 2.).

Table 2. Percentage of HMW-GS, LMW-GS and residual cytoplasmatic proteins in analyzed genotypes of winter wheat

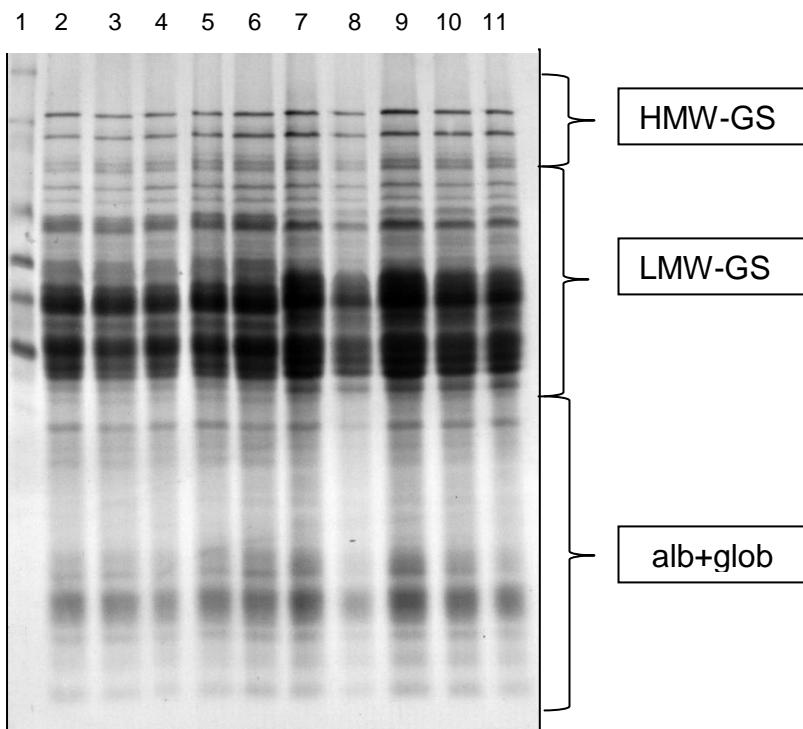
Tabuľka 2. Percentuálne zastúpenie HMW-GS, LMW-GS a zvyškových cytoplazmatických bielkovín analyzovaných odrôd pšenice letnej

Genotype	HMW-GS <sup>1</sup> (%)	LMW-GS <sup>2</sup> (%)	alb + glo <sup>3</sup> (%)
Bertold	15.03	69.94	15.03
Silvanus	15.20	62.19	22.55
Viola	14.50	68.12	17.37
Filemon	16.91	62.85	20.24
Natanael	14.49	64.75	20.75
Rupert	12.49	71.85	15.65
Torysa	12.17	73.72	14.08
Klaudia	18.74	54.68	26.56
Stanislava	18.38	54.33	27.29
Ilona	20.53	67.99	11.48
Viglanka	18.40	70.06	11.54
Vladarka	14.21	68.48	17.31
MS 1744	10.35	71.93	17.72
Stelarka	10.97	76.10	12.93
PS Sunanka	14.55	51.34	34.12
X <sup>4</sup>	15.13	65.89	18.97
σ <sup>5</sup> (%)	2.88	7.22	6.21
Min	10.35	51.34	11.48
Max	20.53	76.10	34.12
V <sup>6</sup> (%)	19.04	10.96	32.75

<sup>1</sup>HMW-GS (%) - high molecular weight glutenin subunits; <sup>2</sup> LMW-GS (%) – low molecular weight glutenin subunits; <sup>3</sup>alb + glo (%) – albumins and globulins; <sup>4</sup>x - average; <sup>5</sup>σ (%) - Standard deviation; <sup>6</sup>V (%) - coefficient of variation

<sup>1</sup>HMW-GS (%) – vysokomolekúrne glutenínové podjednotky; <sup>2</sup> LMW-GS (%) – nízkomolekúrne glutenínové podjednotky; <sup>3</sup>alb + glo (%) – albumíny a globulíny; <sup>4</sup>x – priemer; <sup>5</sup>σ (%) – smerodajná odchýlka; <sup>6</sup>V (%) – variačný koeficient

Electrophoretic spectrum of storage proteins of specific wheat genotype in SDS-PAGE represents Figure 1. High molecular weight glutenin subunits were separated in the upside and LMW-GS were separated in the middle part of the polyacrylamide gel.



HMW-GS – high molecular weight glutenin subunits, LMW-GS – low molecular weight glutenin subunits, alb+glob – albumins and globulins, 1 – high molecular marker M-3788, 2-6 – genotype MS 1588 (Stelarka), 7-11 – genotype PS 28/08 (PS Sunanka)

HMW-GS – vysokomolekulárne glutenínové podjednotky, LMW-GS – nízkomolekulárne glutenínové podjednotky, alb+glob – albumíny a globulíny, 1 – vysokomolekulárny marker M-3788, 2-6 – genotyp MS 1588 (Stelarka), 7-11 – genotyp PS 28/08 (PS Sunanka)

Figure 1. Electrophoretic spectrum of wheat storage proteins in SDS-PAGE  
Obrázok 1. Elektroforetické spektrum zásobných bielkovín pšenice v SDS-PAGE

Gluten is composed by gliadins and glutenins. Glutenins can be separated by SDS-PAGE into low molecular weight glutenin subunits and high molecular weight glutenin subunits. LMW-GS and HMW-GS are one of the essential quality determinants. They are responsible for dough extensibility and elasticity, and so they determine the processing qualities of a wide range of final wheat products (Rasheed et al., 2014).

The functional properties of gluten dependent especially on the quantity and the abundance of high molecular weight glutenin subunits. Content of HMW-GS was detected in the range of 10.35% to 20.53% with an average 15.13% and standard deviation 2.88% (Table 2.). The largest percent representation of HMW-GS was

proved in Ilona (20.53%) and the lowest part of HMW-GS was detected in MS 1744 (10.35%) and Stelarka (10.97%).

LMW-GS represent the highest content of proteins in analyzed samples with the average content 65.89% (Table 2.). Content of LMW-GS was detected in the range of 51.34% to 76.10%. Stelarka shows the highest content od LMW-GS (76.10%) and PS Sunanka (51.34%) and Stanislava (54.33%) the lowest percentage of LMW-GS.

In the collection of wheat (Table 2.) the content of albumins and globulins reached 11.48% to 34.12% with average content 18.97%. Results revealed that the highest representation of albumins and globulins had PS Sunanka (34.12%) and the lowest proportion was found in the Ilona (11.48%) and Viglanka (11.54%).

Gálová et al. (2011) used SDS-PAGE for detection of storage proteins and they detected similar percentage of HMW-GS (2.60% to 28.41%), LMW-GS (54.50% to 83.88%) and residual albumins and globulins (10.61% to 34.04%). Comparable results of glutenin subunits in wheat detected also Palenčárová and Gálová (2010). The average content of HMW-GS was 17.45% and LMW-GS 57.09%. Michalík et al. (2006) analyzed contents of HMW-GS and LMW-GS in wheat. Percentage of HMW-GS was 13.90% to 25.15%. Content of LMW-GS reached 38.16% to 57.05% and residual albumins and globulins had content 29.05% to 36.69%.

Table 3. Composition of glutenin subunits and Glu Score  
Tabuľka 3. Zastúpenie glutenínových podjednotiek a hodnota Glu-skóre

Genotype	Country of Origin	The year of registration	GLU-A1 <sup>1</sup>	GLU-B1 <sup>2</sup>	GLU-D1 <sup>3</sup>	Glu Score
Bertold	SR	2010	0	7+8	5+10	8
Silvanus	SR	2010	0	7+9	5+10	7
Viola	SR	2010	1	7+8	5+10	10
Filemon	SR	2011	0	7+9	2+12	5
Natanael	SR	2011	0	7+9	5+10	7
Rupert	SR	2012	0	7+9	5+10	7
Torysa	SR	1992	0	7+8	2+12	6
Klaudia	SR	2008	1	6+8	5+10	8
Stanislava	SR	2005	0	7+9	5+10	7
Ilona	SR	1989	1	6+8	2+12	6
Viglanka	SR	2010	0	6+8	2+12	4
Vladarka	SR	2013	1	7+8	5+10	10
MS 1744	SR	2014	1	7+9	2+12	7
Stelarka	SR	2013	1	6+8	5+10	8
PS Sunanka	SR	2013	1	6+8	5+10	8

<sup>1</sup>GLU-A1, <sup>2</sup>GLU-B1, <sup>3</sup>GLU-D1 – loci coded HMW-GS

<sup>1</sup>GLU-A1, <sup>2</sup>GLU-B1, <sup>3</sup>GLU-D1 – lokusy kódujúce HMW-GS

This work was focused on detection of genotypes and variability of electrophoretic spectra of individual HMW-GS (Table 3.) in relationship to technological quality of bread wheat. Quality of cereal seeds can be influenced in either positive or negative way by composition of HMW-GS. Technological quality is influenced by HMW-GS named 17+18, 7+9, 7+8 localized on Glu-B1 loci and 5+10 localized on Glu-D1 loci in positive way and presence of subunit Null and 2+12 localized on Glu-D1 leads to negative effect on technological quality of wheat grain (Chňapek et al., 2014).

Analyzed genotypes of wheat were homogenous and single line and there were observed 9 electrophoretic profiles (Table 4.). The dominant composition of HMW-GS (26.7%) were 0, 7+9, 5+10 in genotypes Silvanus, Natanael, Rupert and Stanislava. The least frequent subunits composition (6.6%) were 0, 7+8, 5+10; 0, 7+8, 2+12; 0, 7+9, 2+12; 0, 6+8, 2+12; 1, 7+9, 2+12 and 1, 6+8, 2+12. The similar electrophoretic profiles of winter wheat were determined by authors Chňapek et al. (2004), Oslovičová et al. (2010), Gregová and Šliková (2011).

Table 4. Electrophoretic profiles of analyzed collection of winter wheat

Tabuľka 4. Elektroforetické profily analyzovaného súboru pšenice letnej

Electrophoretic profiles			Number of genotypes	Percentage of genotypes
0	7+8	5+10	1	6.6
0	7+8	2+12	1	6.6
0	7+9	5+10	4	26.7
0	7+9	2+12	1	6.6
0	6+8	2+12	1	6.6
1	7+8	5+10	2	13.3
1	7+9	2+12	1	6.6
1	6+8	5+10	3	20.0
1	6+8	2+12	1	6.6

Results show that on the Glu-A1 loci were determined two HMW-GS named Null and 1 (Figure 2.). The most frequent HMW-GS in collection of wheat was allele Null, which was identified in 53% of genotypes. Allele Null, which has a negative effect on technological quality of wheat grain was determined in genotypes Bertold, Silvanus, Filemon, Natanael, Rupert, Torysa, Stanislava and Viglanka.

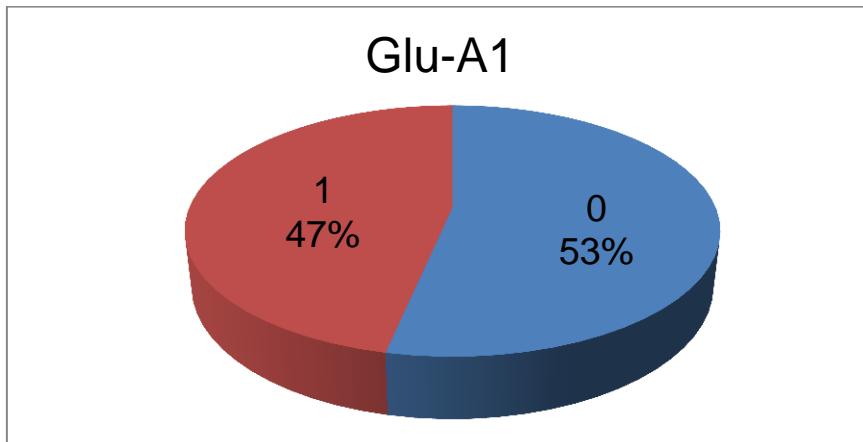


Figure 2. Percentage composition of alleles on Glu-A1 loci

Obrázok 2. Percentuálne zastúpenie alel na lokuse Glu-A1

On the Glu-B1 loci were detected presence of three HMW-GS named 6+8, 7+8 and 7+9 (Figure 3.). Allele 7+9 which has a positive effect on technological quality of wheat grain was the most frequent in collection of winter wheat (40% of genotypes). It was determined in genotypes Silvanus, Filemon, Natanael, Rupert, Stanislava a MS 1744. Allele 6+8 was detected in genotypes Klaudia, Ilona, Viglanka, Stelarka and Sunanka. In the 27% of genotypes was determined couple of subunits 7+8.

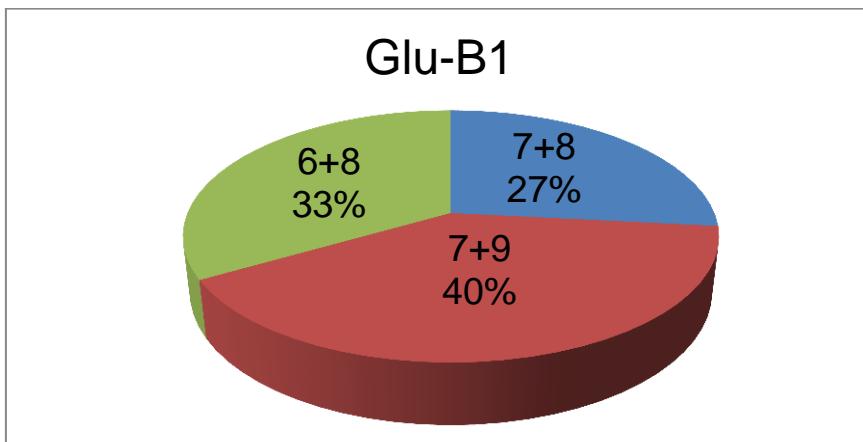


Figure 3. Percentage composition of alleles on Glu-B1 loci

Obrázok 3. Percentuálne zastúpenie alel na lokuse Glu-B1

Alleles localized on Glu-Di loci are the most important markers of technological quality of wheat grain flour (Chňapek et al., 2014). On the Glu-D1 loci were detected two HMW-GS alleles named 5+10 and 2+12 (Figure 4.).

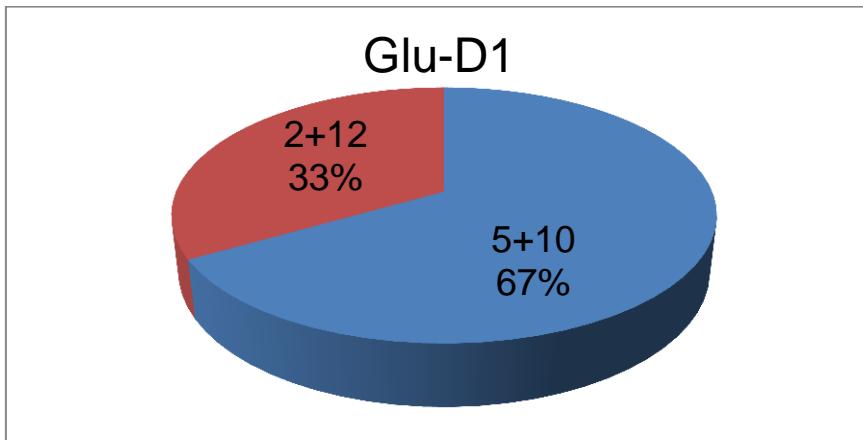


Figure 4. Percentage composition of alleles on Glu-D1 loci

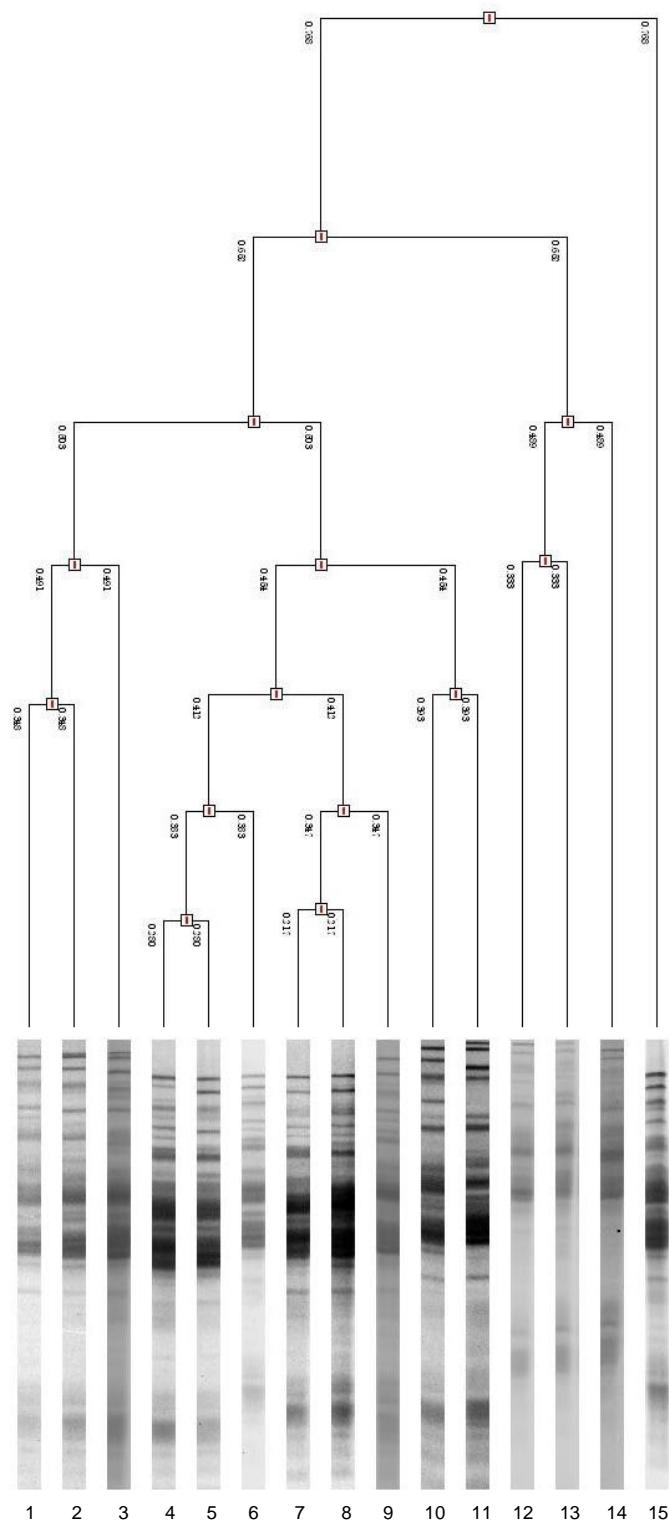
Obrázok 4. Percentuálne zastúpenie alel na lokuse Glu-D1

The most frequent presence of HMW-GS were 5+10 (67%) with positive effect on technological quality of wheat flour, which were detected in genotypes Bertold, Silvanus, Viola, Natanael, Rupert, Klaudia, Stanislava, Vladarka, Stelarka and Sunanka. Couple of subunits 2+12 have negative effect on technological quality of wheat grain. It was detected in 33% of analyzed genotypes which should be used for confectionery industry or such as food for cattle.

These results were consistent with research of Chňapek et al. (2004), Oslovičová et al. (2010), Gregová and Šliková (2011) and Ali et al. (2013). Tahir (2009) in research detected on Glu-A1 loci allele 1 (50%), on Glu-B1 loci were detected 3 alleles named 7 (50%), 7+9 (16.67%) and 20 (33.33%) and on Glu-D1 loci were detected couples of subunits 5+10 (33.33%) and 2+12 (66.67%).

Presence or absence of specific HMW-GS is the main indicator of the wheat technological quality. Glu-score of individual wheat genotypes contributes to bread making quality of wheat and can be calculated according to composition of individual alleles coded by A1, B1 and D1 loci of analysed bread wheat genotypes. Payne, et al. (1987) analysed collection of bread wheat genotypes and evaluated individual alleles by points depends on their contribution to bread making properties of selected genotype. The highest possible calculated Glu-score is 10. Good technological quality of wheat genotypes is characterized by Glu-score more than 7 (Chňapek et al., 2015).

The best technological quality on the basis of Glu-score calculation (Table 3.) showed genotypes Viola and Vladarka (Glu-score 10). Genotypes Bertold, Klaudia, Stelarka and Sunanka with Glu-score 8 have very good technological quality. Genotype Viglanka reached the worst Glu-score 4 and it is not recommended for bread-making.



1 – Viglanka, 2 – Ilona, 3 – Viola, 4 – MS 1744, 5 – Vladarka, 6 – Bertold, 7 – Stelarka, 8 – PS Sunanka, 9 – Filemon, 10 – Stanislava, 11 – Klaudia, 12 – Natanael, 13 – Rupert, 14 – Torysa, 15 - Silvanus

Figure 5. Dendrogram of the analyzed wheat genotypes

Obrázok 5. Dendrogram analyzovaných odrôd pšenice

A dendrogram (Figure 5.) was constructed based on polymorphism of HMW-GS using the UPGMA algorithm, giving an overview of their genetic similarity and relationships. Analyzed collection of wheat genotypes was divided into two major clusters with dissimilarity 0.768. One of them was divided into two sub-clusters with dissimilarity 0.660. Genotype Silvanus was isolated to the free-standing cluster with dissimilarity 0.768 because it was the most genetically different from another analyzed wheat genotypes.

## Conclusions

Storage protein profiles in 15 genotypes of winter wheat (*Triticum aestivum* L.) was evaluated by SDS-PAGE. Utilization of SDS-PAGE analysis of winter wheat proteins is useful tool for differentiation, identification and characterization of some technological important properties. The results showed that LMW-GS represent the highest content of proteins in analyzed samples with the average content 65.89%, content of residual albumins and globulins was 18.97% and HMW-GS 15.13%. Wheat genotypes were homogenous, single line and the dominant composition of HMW-GS were 0, 7+9, 5+10. Viola and Vladarka had the best technological quality and were suitable for bread-making.

## Acknowledgements

This work was funded by European Community under project ITMS 26220220180: Building Research Centre "AgroBioTech" (50%) and KEGA project No 021SPU-4/2015 (50%).

## References

- Ali, A., Arshad, M., Mastrangelo, A.M., De Vita, P., Gul-Kazi, A., Mujeeb-Kazi, A. (2013) Comparative assessment of glutenin composition and its relationship with grain quality traits in bread wheat and synthetic derivatives. *Pakistan Journal of Botany*, 45 (special issue), 289-296.
- Chňapek, M., Gálová, Z., Gregáňová, Ž. (2004) Protein Markers of the Technological Quality of Wheat Grain In: Collective of authors eds. (2004) *Aktuálne problémy riešené v agrokomplexe*. Nitra: Slovak University of Agriculture. 235-241.
- Chňapek, M., Palenčárová, E., Gálová, Z., Balážová, Ž., Tomka, M. (2012) Proteomics analysis of wheat and barley grain. *Journal of Microbiology, Biotechnology and Food Sciences*, 1 (February Special issue), 622-631.
- Chňapek, M., Tomka, M., Peroutková, R., Gálová, Z. (2014) Polymorphism of HMW-GS in Collection of Wheat Genotypes. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, 8 (7), 652-657.

- Chňapek, M., Peroutková, R., Vivodík, M., Gálová, Z. (2015) Identification of Technologically Important Genes and their Products in the Collection of Bread Wheat Genotypes. *Journal of Microbiology, Biotechnology and Food Sciences*, 4 (special issue 2), 26-29. DOI: [10.15414/jmbfs.2015.4.special2.26-29](https://doi.org/10.15414/jmbfs.2015.4.special2.26-29)
- Gálová, Z., Kečkešová, M., Kopálová, Z., Chňapek, M., Poláčková, A. (2011) Quality of Cereals, pseudocereals and legume from the point of view gluten free diet. *Potravinárstvo*, 5 (special issue), 268 – 273.
- Gálová, Z., Palenčárová, E., Chňapek, M., Balážová, Ž. (2012) Využitie obilnín, pseudoobilnín a strukovín v bezlepkovej diéte. Nitra: Slovak University of Agriculture.
- Gregová, E., Šliková, S. (2011) Characterization of genotypes of grain wheat collection by storage proteins. In: Nové poznatky z genetiky a šľachtenia poľnohospodárskych rastlín. Zborník z 18. vedeckej konferencie. Piešťany: VÚRV. 22-24.
- He, Z.H., Liu, L., Xia, X.C., Liu, J.J., Pena, R.J. (2005) Composition of HMW and LMW glutenin subunits and their effects on dough properties, pan bread, and noodle quality of Chinese bread wheats. *Cereal Chemistry*, 82, 345-350. DOI: [10.1094/CC-82-0345](https://doi.org/10.1094/CC-82-0345)
- Johansson, E., Malik, A.H., Hussain, A., Rasheed, F., Newson, W.R., Plivelic, T., Hedenqvist, M., Gällstedt, M., Kuktaite, R. (2013) Gluten protein structures: Variation in wheat grain and for various applications. In: He, Z., Wang, D. eds., Proceeding: 11th International Gluten Workshop. Beijing, China, August 12-15, 2012, Mexico : International Maize and Wheat Improvement Center.
- Katyal, M., Virdi, A.S., Kaur, A., Singh, N., Kaur, S., Ahlawat, A.K., Singh, A.M. (2016) Diversity in quality traits amongst Indian wheat varieties I: Flour and protein characteristics. *Food Chemistry*, 194, 337-344. DOI: [10.1016/j.foodchem.2015.07.125](https://doi.org/10.1016/j.foodchem.2015.07.125)
- Ling, H. Q., Zhang, A., Wang, D., Liu, D., Wang, J.Y., Sun, H., Fan, H.J., Li, Z.S., Zhao, Y., Wang, D.W., Zhang, K.P., Yang, Y.S., Wang, J.J., Dong, L. (2013) Wheat A genome sequencing and its application for quality modification. In: He, Z., Wang, D. eds., Proceeding: 11th International Gluten Workshop. Beijing, China, August 12-15, 2012, Mexico : International Maize and Wheat Improvement Center.
- Liu, L., Wang, A., Appels, R., Ma, J., Xia, X., Lan, P., He, Z., Bekes, F., Yan, Y., Ma, W. (2009) A MALDI-TOF based analysis of high molecular weight glutenin subunits for wheat breeding. *Journal of Cereal Science*, 50 (2), 295-301. DOI: [10.1016/j.jcs.2009.05.006](https://doi.org/10.1016/j.jcs.2009.05.006)
- Manley, D., Pareyt, B., Delcour, J.A. (2011) Wheat flour and vital wheat gluten as biscuit ingredients. In D. Manley, ed. (2011) *Manley's Technology of Biscuits, Crackers and Cookies*. Oxford: Woodhead Publishing. 109-133.

- Michalík, I., Gálová, Z., Urminska, D., Knoblochová, H. (2006) Bielkovinový komplex zrna obilnín a pseudoobilnín In: Collective of authors eds. (2006) Výživná a technologická kvalita rastlinných produktov a ich potravinárske využitie. Nitra: Slovak University of Agriculture. 68-101.
- Miura, M., Kawaura, K., Nakamura, M., Kouyama, H., Ogihara, Y. (2013) Profiling gliadins from common wheat using two-dimensional gel electrophoresis. In: He, Z., Wang, D. eds., Proceeding: 11th International Gluten Workshop. Beijing, China, August 12-15, 2012, Mexico : International Maize and Wheat Improvement Center.
- Oslovičová, V., Gálová, Z., Chňapek, M., Balážová, Ž. (2010) Identification of Triticum aestivum L., Triticum spelta L. and Triticum durum DESF. genotypes on the HMW-GS base. Plant Soil and Environment, 56 (2), 82-86.
- Palenčárová, E. (2010) Detekcia a charakteristika celiakálne aktívnych bielkovín v niektorých cereáliách, pseudocereáliách a strukovine. Nitra: Slovak University of Agriculture.
- Palenčárová, E., Gálová, Z. (2010) Detection of Celiac Active Proteins by Electrophoretic and Imunochemical Methods. Potravinárstvo, 4 (special issue), 485-490.
- Payne, P.I., Nightingale, M.A., Krattiger, A.F., Holt, L.M. (1987) The relationship between HMW glutenin subunit composition and the bread-making quality of British- grown wheat varieties. Journal of the Science of Food and Agriculture, 40, 51-65. DOI: [10.1002/jsfa.2740400108](https://doi.org/10.1002/jsfa.2740400108)
- Petrovičová, L., Gálová, Z., Chňapek, M., Kečkešová, M. (2013) Protein Polymorphism in Genetic Resources of Rye using SDS-PAGE. Journal of Microbiology, Biotechnology and Food Sciences, 2 (Special issue 1), 1531-1539.
- Rasheed, A., Xia, X., Yan, Y., Appels, R., Mahmood, T., He, Z. (2014) Wheat seed storage proteins: Advances in molecular genetics, diversity and breeding applications. Journal of Cereal Science, 60 (1), 11-24. DOI: [10.1016/j.jcs.2014.01.020](https://doi.org/10.1016/j.jcs.2014.01.020)
- Shewry, P.R., Tatham, A.S., Forde, J., Kreis, M., Miflin, B.J. (1986) The classification and nomenclature of wheat gluten proteins: A reassessment. Journal of Cereal Science, 4, 97-106. DOI: [10.1016/S0733-5210\(86\)80012-1](https://doi.org/10.1016/S0733-5210(86)80012-1)
- Shewry, P.R., Tatham, A.S. (1990) The prolamin storage proteins of cereal seeds: structure and evolution. Biochemical Journal, 267, 1-12. DOI: [10.1042/bj2670001](https://doi.org/10.1042/bj2670001)
- Šimić, G., Horvat, D., Jurković, Z., Drezner, G., Novoselović, D., Dvojković, K. (2006) The Genotype Effect on the Ratio of Wet Gluten Content to Total Wheat Grain Protein. Journal of Central European Agriculture, 7 (1), 13-18.
- Socha, P. (2011) Optimalizácia stanovenia prítomnosti špeciálnych skupín celiakálne aktívnych bielkovín. Nitra: Slovak University of Agriculture.

Tahir, N.A. (2009) Evaluation of Hexaploid Wheat Varieties for Making Bread by High Molecular Weight (HMW) and Low Molecular Weight (LMW) Analysis. Jordan Journal of Biological Sciences, 2 (2), 55-62.

Tsao, R., Yu, L.L., Shahidi, F. (2012) Cereals and pulses – an overview. In: L. L. Yu, R. Tsao, F. Shahidi, eds. (2012) Cereals and Pulses. Nutraceutical properties and health benefits. Oxford: Wiley-Blackwell. 1-6. DOI: [10.1002/9781118229415.ch1](https://doi.org/10.1002/9781118229415.ch1)

Wrigley, C.W. (1992) Identification of cereal varieties by gel electrophoresis of the grain proteins. Seed Analysis, 17-41. DOI: [10.1007/978-3-662-01639-8\\_2](https://doi.org/10.1007/978-3-662-01639-8_2)