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ALLELIC COMPOSITION OF HMW-GLUTENIN PROTEIN AND THEIR RELATIONSHIP WITH QUALITY OF WHEAT

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ABSTRACT

High molecular weight glutenin subunits (HMW-GS) proteins deposited in endosperm of wheat seed which have significant impact on bread quality. The HMW-GS encoded by genes located at the long arm of chromosomes 1A, 1B, 1D. The aim of this work was study allele polymorphysms at *Glu-A1*, *Glu-B1* and *Glu-D1* locus and loaf volume, grain protein content, sedimentation volume of eight wheat genotypes (G-3130, G-35183, G-3501, G3512, G-3574, G-3027, G-3075, G-3097) harvested in two years with various weather condition. For each genotypes, flour used for extraction of glutenin which separated by method of electrophoresis on SDS gel (11.8%). Electrophoregrams used for determining *Glu-1* alleles. Technological quality parameters analyzed by standard laboratory methods. The three alleles alleles (***a, b, c***) at the *Glu-A1*, three alleles (***b, c, d***) at the *Glu-B1* and 2 alleles (***a, d***) at the *Glu-D1* were identified. The highest protein sedimentation volume had wheat genotype G-3075 in the both years (54.0ml; 58.0ml) while the lowest sedimentation volume had G-3512 (34.0ml; 36.0ml). Grain protein content (GPC) was the highest in G-3075 in both years (14.20%; 15.40%) while the lowest GPC had G-3097 (11.60%) in first and G-3512 (12.60%) in the second year. Loaf volume was the highest in G-3075 in both year (520ml; 540ml) while the lowest was in G-3512 (400ml) in both years of experiment. The estimated quality traits varied depending on genotype and year. The better quality, in average, had the wheat genotypes which carried *Glu-D1d* allele.

Keywords: *Wheat, glutenin, Glu-1 allele, quality, polymorphism.*

INTRODUCTION

Wheat grain is important source of gluten proteins (gliadin and glutenin) which determine dough quality as well end use products (Shewry et al., 2003; Li et al. 2010). Glutenin proteins comprises two groups of subunits: high-molecular weight glutenin subunits (HMWGS) and low molecular weight glutenin subunits (LMWGS). The HMW-GS are controlled by gene alleles at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci on the long arm of chromosomes 1A, 1B and 1D, respectively. The each locus consisting of two tightly linked x-type and y-type alleles. The LMW-GS are encoded by *Glu-A3*, *Glu-B3*, and *Glu-D3* loci on the short arms of chromosomes 1A, 1B, and 1D, respectively (Payne et al. 1987). The high allele polymorphysms at each locus for storage proteins were identified ((Knežević et al., 1993; Novoselskaya-Dragovich, 2015). The composition of glutenin alleles, i.e, composition of encoded HMW-GS are in relationship with technological quality properties (Jondiko et al., 2012; 2003; Knezevic et al., 2016a), dough making quality and baking quality of wheat flour (Menkovska et al., 2002; Li et al. 2010). The previous studies showed that *Glu-D1* locus have significant influence to rheological and bread making quality. So, the HMW-GS subunit 1Ax1 and subunits pair 1Dx5+1Dy10 have the greatest relationships with flours with more suitable viscoelastic properties for bread making and that also result in bread with higher volume (Liang et al. 2010; Hernández et al. 2012; Blechl and Vensel, 2013). Also, other investigation showed that HMW-GSs such as 1Ax1, 1Ax2*, 1Bx7, and 1Bx17 + 1By18 have positive effects on dough characteristics, while 1AxNull, 1Bx20, 1Bx6 + 1By8, 1Dx2 +1Dy12 have negative effects on gluten quality and bread-making quality (Shewry et al., 2003). However, many factors are involved in variation of gluten proteins and that make dificulties for predicting bread making quality (Liu et al., 2016; Knezevic et al., 2017a; 2017b). The knowledge of diversity of wheat genotypes on the base of *Glu-1* allele composition is important, and can use in breeding program to create new new cultivars.

The present study was carried out in the aim to determine the HMW-GS composition in 8 Serbian wheat genotypes, allele polymorphysms at the *Glu-1* loci and its relationship with protein sedimentation volume, protein content and loaf volume was determined.

MATERIAL AND METHODS

The eight genetically divergent wheat genotypes ((G-3130, G-35183, G-3501, G3512, G-3574, G-3027, G-3075, G-3097) were harvested in two years of experiment. During two years of experiment, those genotypes were grown on plots $5m^2$ in five replications. At least 30 single seed were used for extratcion of glutenin proteins. Wheat flour obtained by milling of grains on Bühler laboratory mill.

The flour used for extraction glutenin proteins. The 10 mg was weighed in 1.5ml microtube and for extraction was added 400µl protein extraction SDS buffer (120 mM Tris-HCl, pH=6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) and boiled for 5 min. The sample were centrifuged at 12000 rpm for 10 min. Protein resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-

PAGE) Laemmli, (1970) was performed with Bio-Rad equipment based on a previously described method He (2011) with 11.8% gel and electrophoresed at 20mA for 2h. Gels were stained by using Commassie Briliant blue dye resloved in 10% TCA and 250ml methanols. After staining, the obtained electrophoregrams are used for analysis and determining HMW-GS and identification of *Glu-1* alleles (Payne and Lawrence, 1983). Total protein content was determinated according to the Kjeldahl's method (N×5.7). Protein sedimentation volume analyzed by Zeleny method. Baking bread volume and the score was done by standard laboratory methods.

Climatic conditions in year of experiment during growing period

Experiment carried out on experimental field in two year which are characterized different regime of temperature and precipitation. Temperature values and precipitation amount measured and average values computed per months in both year of experimental investigation. Obtained values compared with average values of ten-year period (tab. 1). The average value of temperatures (8.3 ^{o}C) in the first year were similar to average of ten years' period (8.5 ^{o}C) and less than in second (11.0 ^{o}C) experimental year. The temperature and precipitation varied per months within years and were different between same period in two experimental years.

Table 1. Average values of monthly temperatures and precipitation during wheat growing period

Tem& Precpt	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
^{0}C	2005/06	11.5	5.6	3.3	-1.7	1.5	5.5	12.7	16.4	19.7	8.3	74.4
(mm)	2006/07	13.3	7.6	3.5	6.1	6.3	9.1	12.1	18.2	22.8	11.0	99.0
1990/2000		11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	8.5	76.7
	2005/06	49.0	54.8	47.1	27.9	38.1	116	86.3	29.6	84.8	59.3	533.7
	2006/07	16.7	13.7	51.9	45.3	32.1	62.9	3.6	118	25.3	41.1	369.9
1990/2000		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.4	417.8

In the first year 2005/06 of investigation the amount of precipitation was 533.7mm and significantly higher than in second 2006/07 (369.9mm) year and the ten-year period average (417.8mm). Amounts of precipitation in the first year are was more suitable for plant grooving than in second year and without big differences between minimum and maximal values per month, as in second year (in April – 3.6mm and in May 118mm). However, in second year, dry conditions are favorable for intensive grain filling and accumulation of protein. Environmental conditions during wheat grain development can affect wheat flour quality.

RESULTS AND DISCUSSION

The identified high-molecular wight glutenin subunits were present in different frequency of analyzed wheat genotypes. The eight different *Glu-1* alleles were determined, three at *Glu-A1*, three at *Glu-B1* and two at *Glu-D1* (tab. 2). At *Glu-A1* locus three x-type subunits 1, 2* and null controlled by alleles *Glu-A1a*, *Glu-A1b* and *Glu-A1c*, respectively were identified. The subunit 2* (ecoded by ***b*** allele) was determined the most frequently in four (50.0%) genotypes, followed by subunit null (encoded by ***c*** allele) in three (37.5%) and subunit 1 (encoded by ***d*** allele) in one (12.5%) wheat genotypes. The varying in frequency of glutenin allele in wheat cultivars were found in other research (Knežević et al., 1993; Yasmeen et al., 2015; Knezevic et al., 2016a).

In three pair of eight wheat genotype the same composition of glutenin subunits were found. Among eight wheat genotypes the five different Glu-1 allele composition was identified. The glutenin subunits 2*, 7+9, 5+10 were found in G-3130 and G-3127 wheat genotype, 2*, 7+8, 5+10 in G-35183 and G-3075, while N, 7+9, 2+12 were identified in G-3501 G-3097. Wheat genotypes G-3712 and G-3574 had differnt combination of HMW glutenin subunits (tab. 2).

Protein content in seed of wheat genotypes is important quality parameter which used for estimation of selected genotype of wheat in breeding program (Knežević et al., 2016b). In this study of eight wheat genotypes identified different value of seed protein content. In the first year of investigation protein content in seed varied from 11.6% (G-3097) to 14.20% (G-3570), while in the second year, content of protein in seed was the lowest in G-3512 (12.60%) and the highest in G-3075 (15.40%) tab. 2. Mainly, in all wheat genotypes, protein content value was the higher in second year of experiment than in first zear of experiment, what indicate the more favorable condition in stage of grain filling. In second average temperature in temperature in May was 18.24^{0}C and June 22.8^{0}C, while in the first year was lower in May-16.4^{0}C and June-19.7^{0}C. Also, amount of precipitation was higher in second year (May-118mm and June-25.3mm) than in the first year (May-29.6mm and June-84.8mm). Protein content is geneticaly controlled, but affect of environmental factors (temperature, precipitation) have great on expression this trait (Godfrey et al., 2010; Knezevic et al., 2017b). Amount of gluten protein fraction will be higher when using fertilizer than without using. The high temperature influence to increasing content of gluten protein, what is the results of inhibition of starch synthesis (Hurkman et al., 2013).

Table 2. Glutenin allele encoding HMW GS composition and technological quality of winter wheat genotypes

Geno-type	*High molecular weight glutenin subunits*			*Glu-1 alleles*			*Grain protein content %*		*Protein sedimentation volume (ml)*		*Loaf volume (ml)*		*Quality score*
	1AL	1BL	1DL	***A1***	***B1***	***D1***	2005/06	2006/07	2005/06	2006/07	2005/06	2006/07	
G-3130	2*	7+9	5+10	***b***	***c***	***d***	13.60	14.60	42.0	48.0	480	480	9
G-35183	2*	7+8	5+10	***b***	***b***	***d***	13.40	15.00	52.0	54.0	500	500	10
G-3501	N	7+9	2+12	***c***	***c***	***a***	12.20	13.00	38.0	46.0	420	460	5
G-3512	N	6+8	2+12	***c***	***d***	***a***	11.80	12.60	34.0	36.0	400	400	4
G-3574	1	7+9	5+10	***a***	***c***	***d***	12.80	14.00	42.0	44.0	460	500	9
G-3027	2*	7+9	5+10	***b***	***c***	***d***	13.60	14.80	44.0	50.0	460	480	9
G-3075	2*	7+8	5+10	***b***	***b***	***d***	14.20	15.40	54.0	58.0	520	540	10
G-3097	N	7+9	2+12	***c***	***c***	***a***	11.60	13.00	40.0	46.0	440	450	5

Genotype with high protein content mainly have high protein sedimentation volume and loaf volume, in this study. Genotypes G-35183 and G-3075 withcomposition of glutenin subunits 2*, 7+8, 5+10 had the highest protein content, the highest protein sedimentation volume and the highest loaf volume in both year of experiment (tab. 2). The investigation of Brazilian wheat cultivars showed that protein content varied from 10.04 to 15.10%, while correlation between the protein content and quality parameters of the grain and flour in wheat genotypes did not find (Costa et al., 2017). However, another investigation showed that HMW-GS have connection with functional properties of wheat dough (Dvořáček et al., 2013). So, positive association of glutenin component 5+10 encoded by ***d*** allele at *Glu-D1* and component 2* encoded by ***d*** allele at *Glu-A1* with dough quality, bread volume were found (Vázquez et al., 2012; Vaiciulyte-Funk et al., 2015).

High protein concentrations often lead to high Zeleny sedimentation values. In the analyzed wheat wheat genotypes, differences were detectable on the base sedimentation values were obtained. The Zeleny sedimentation values varied in both year of experiment. The lowest value of protein sedimentation had G-3512 (34.0ml in the first year and 36.0ml in the second year) while the highest protein sedimentatio volume had G-3075 (54.0ml in the firs year and 58.0ml in the second year) tab. 2. In average for all wheat genotypes, protein sedimentation values were higher in second year of experiment. In wheat genotypes which have composition of HMW GS 2*, 7+8, 5+10 (G-3075 and G-35183) sedimentation volume was significant higher than in genotypes which had glutenin components null at 1AL, 6+8 at 1BL and 2+12 at 1DL chromosome (G-3512). Mainly, the higher sedimentation volume had wheat genotypes with 2* than null at 1AL chromosome. Also, sedimentation volume in genotypes which posses 5+10 subunits was higher than in genotypes with 2+12 subunits at the 1Dl chromosome (tab. 2). Similar resluts reported for Yugoslav wheat cultivars ((Knežević et al., 1993).

The genotype G-3075 had the highest loaf volume in the first year (520*ml*) as well in the second year (540*ml*). The lowest loaf volume had G-3512 in the first year

(400*ml*) and in second year (400*ml*). This genotypes have glutenin subunits 6+8 encoded by ***d*** allele at *Glu-B1* and subunits 2+12 encoded by ***a*** allele at *Glu-D1* (tab. 2). The previous studies showed that these subunits associated with poor dough and bread quality (Bakshi and Bhagwat, 2016).

All the HMW subunit combinations resulted in bread with good and similar appearance. The highest loaf volumes were obtained for wheat genotypes that contained subunit 2*, 7+8 and 5+10 subunits encoded by *Glu-A1c*, *Glu-B1b* and *Glu-D1d* alleles. On the base of revealed values for each HMW glutenin subunits contribution to quality (Payne, 1987) we estimate *Glu-1* quality score which was the highest (QS=10) in genotypes G-3075 and G-35183 with *Glu-A1* allele composition ***b, b, d,*** which encode 2*, 7+8 and 5+10 subunits, while the lowest (QS=4) had genotype G-3512 which is glutenin alele composition ***c,d,a***, encoding N, 6+8 and 2+12 (tab.2). The allelic variation at HMW-GS and LMW-GS and environmental conditions are important factors that influence the wheat flour quality parameters (Branlard et al., 2003; (Knežević et al., 2017b). Alleles (***a, b***) encoded at the *Glu-A1* were associated with a higher loaf volume compared to the Null subunit (***c***), and allele *Glu-D1d* encoding 5+10 associated with good baking quality (Peña et al. 2005; Vázquez et al. 2012). However *Glu-B1c* encoding 7+9 subunits are associated with low baking quality, whereas ***i*** and ***b*** allele encoding 17+18 and 7+8 subunits are associated with good baking characteristics (Menkovska et al., 2002; Liang et al. (2010).

CONCLUSION

This investigation showed allele polymorphisms at all six *Glu-1* loci and were identified 8 glutenin alleles. In this study identified five different glutenin allele formula. Among the eight genotypes for three pair of genotypes identified the same composition ofglutenin subunits encoded by same Glu-1 allele. According to values of grain protein content, sedimentation volume and loaf volume, for studied wheat genotypes were established differences between genotypes in both years which characterized diferent climatic condition. The highest protein content (14.20%; 15.40%), Zeleny sedimentation volume (54.0ml; 58.0ml) and loaf of volume (520ml; 540ml) had G-3075 wheat genotype, while G-3512 had the lowest protein content (12.60% in second year) Zeleny sedimentation volume (34.0ml; 36.0ml) and loaf volume (400ml in both year). Genotypes which carried glutenin subunits 2* encoded by *Glu-A1b,* 7+9 encoded by *Glu-B1c* and subunits 5+10 encoded by *Glu-D1d* had the highest Zeleny sedimentation volume of proteins, protein content in seed and loaf volume. The results may be used as guidelines for the breeding purposes to create wheat cultivars with better bread making quality.

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