PROTEIN QUALITY OF BREAD WHEAT

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ABSTRACT

The storage proteins content and their composition have important role in determination of protein quality in bread wheat. The aim of this work is analysis of gluten content, loaf volume and their relationship with gliadin and high molecular weight glutenin subunits in bread wheat. In investigation included 10 wheat genotypes grown in two vegetation seasons (2015/16 and 2016/17) with different climatic conditions. In the first year, the genotype G-3634-2 had the lowest dry gluten content (21.20%) and loaf volume (380 *ml*), while genotype G-3622-1, had the highest dry gluten content (26.54%) and loaf volume (500 *ml*). In second year, the lowest dry gluten content (23.44%) and the lowest loaf volume was in wheat G-3601-4 (400 *ml*), while in genotype G-3622-1, found the highest dry gluten content (29.86%) and loaf volume (540 *ml*). Wheat genotypes which possess glutenin subunits 2* encoded by *Glu-A1b*, 7+9 encoded by *Glu-B1c*, and 5+10 encoded by *Glu-D1d*. For improving bread making quality are necessary select and wheat genotypes in terms of gluten protein composition (gliadin and glutenin's) and higher gluten content.

Key words: wheat, gluten protein, allele, quality.

INTRODUCTION

Wheat seed is the main source of protein in food products for man and animal nutrition. The bread wheat seed protein content varies in average between 12–14% (Shewry, 2007) In wheat seed endosperm are deposited proteins which differed according the molecular weight and solubility. The main fraction of endosperm storage proteins are gluten proteins and non-gluten proteins. Non gluten proteins are albumins (water-soluble) and globulins (salt-soluble), while gluten proteins are gliadins (alcohol-soluble) and glutenins (acid soluble and insoluble). Gluten proteins are very important for dough properties and bread making quality. Gliadins are globular structures, monomeric molecules (M_w 30-70kDa), linked by intra molecular disulfide bound and contribute to viscosity of dough, as well with quality of bread. Glutenin proteins are polymeric molecules which consists two types of subunits: high-molecular weight glutenin subunits (HMW GS 80-130kDa) and low molecular weight glutenin subunits (LMW GS 10-70 kDa) with intermolecular disulfide bounds. Glutenins are polypeptide molecules chain structure, linked by intermolecular disulfide bounds which contribute to elasticity of gluten

elasticity, dough extensibility. Gluten quality is influenced by genetic control and environmental factors.

Gliadins are controlled by genes located at the short arm of 1. group (*Gli*-1) and 6. (*Gli*-2) group of homologous chromosomes (Sozinov and Poperelya, 1980). The multiple allelism at each of these six *Gli*- loci has been identified in wheat varieties from Australia (Metakovsky et al. 1990), from Yugoslavia (Metakovsky et al. 1991; Vapa and Knežević, 1993; Knežević et al., 1993; 1994; 1997; 1998a; 1998b; Dimitrijević et al., 1998; 1999; Novoselskaya-Dragovich et al., 2005; Torbica et al., 2006), from Italy (Metakovsky et al., 1994) and from France (Metakovsky and Branlard 1998), from Spanish (Metakovsky et al. 2000), from Serbia (Knežević et al., 2006; 2007; 2017a) Russian (Novoselskaya-Dragovich, 2015) and from different world varieties of common wheat (Metakovsky et al., 2021).

The HMW-GS are controlled by gene alleles at the *Glu-A1, Glu-B1* and *Glu-D1* locus 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 (Payne and Lawrence 1983; Payne, 1987). The allele polymorphisms at each *Glu-1* loci and their connection with quality traits were identified in analysis wheat varieties from England (Payne, 1987), from France (Branlard et al. 1989), from Yugoslavia (Knežević et al., 1993), from Macedonia (Menkovska et al., 2002), from Algeria (Bellil et al., 2014), from Serbia (Knežević et al., 2017a; 2017b; 2018) from Europa (Hlozáková et al., 2015). The LMW-GS are encoded by genes *Glu-A3, Glu-B3,* and *Glu-D3* located on the short arms of chromosomes 1A, 1B, and 1D, respectively (Payne et al. 1987). The allele polymorphisms at these loci and allele association with dough quality in bread wheat (Jackson et al., 1983; 1996; Gupta et al., 1989; Gupta and Shepherd, 1990; He et al., 2005; Bellil et al., 2014; Goel et al., 2018) and pasta quality in durum wheat (Pogna et al. 1988) was established.

The main objectives of the present study were to evaluate (i) the grain quality characteristics of wheat regarding to content of dry gluten, loaf volume (ii) composition of gliadin and glutenins copmonents (iii) identification alleles encoding gliadin and glutenins protens and (iv) relationship alelles with gluten and loaf volume.

MATERIAL AND METHODS

For this study, the 10 genetically divergent wheat genotypes (G-3632-1, G-3644-4, G-3619-3, G-3601-4, G-3626-2, G-3622-1, G-3617-1, G-3611-2, G-3634-2, G-3637-1) were used. The protein quality estimated on the base of composition of identified *Gli-1*, *Gli-2* and *Glu-1* alleles, encoding gluten proteins, gliadin and high molecular weight gluten subunits, content of dry gluten, loaf volume. Also, the association of *Gli*- alleles and *Glu-1* alleles with content of dry gluten and loaf volume were estimated.

The gliadins extracted from at least 30 single seeds, or more by 70% ethanol at room temperature for one hour. The extracts of gliadin loaded on prepared polyacrylamide gels 8.33%, on which separated gliadin by using method of acid PAG electrophoresis during 2.5 to 3 hours, in electric field under constant voltage from 550 V and in 5 mM aluminum lactate buffer pH=3.1 by method which were develop Novoselskaya et al. (1983). The separated gliadin subunits stained in 0.05% ethanol solution of Coomassie Brilliant Blue R250 in 250 ml 10% threechloroacetic acid (TCA) and obtained electrophoregrams were used for identification of gliadin alleles by using method Metakovsky (1991).

Glutenins were extracted from the residues after gliadin extraction. The residues treated by solution 120 mM (Tris-HCl - pH-6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) and boiled for 5 min and then centrifuged at 12000 rpm for 10 min. HMW glutenin subunits separated on 11.8% gel in electrical field of 20mA for 2h SDS-PAG electrophoresis method (Laemmli, 1970). After staining, by Coomassie Brilliant Blue dye, the electrophoregrams are used for determining HMW-GS and identification of *Glu-1* alleles (Payne and Lawrence, 1983).

Dry gluten obtained after water rinsing the dough with 2% saline solution, which is dried and weighed on a technical scale. The value in proportion quantity of dry gluten and the initial weight of the dough sample represents the percentage share of dry gluten. Loaf volume determined after baking by using standard laboratory methods.

Weather conditions in the vegetation period

The total amount of precipitation and average temperature per month and per year were different between experimental year during vegetative season (2015/16 and 2016/17) and differed in relation to the long-term period (2000-2010). In the first vegetation season, the average temperature was 9.9 ^{0}C and the total rainfall was 651.00 mm, which is significantly higher than in the second year as well than in ten-year period. In the second year the average temperature during the growing season was 8.7 ^{0}C and similar to ten-year period while the total rainfall 523.1 mm was significantly higher than in ten-year period. During the grain filling phase of plants in the second year in April the average temperature was higher and in May the average precipitation were higher and favorable than in first year of experiment and then in ten-year period (table 1).

Table 1. Average monthly temperatures and total monthly precipitation in Kraljevo

Parameter	Period	Oct	Nov	Dec	Jan	Feb	March	April	May	June	Xm	Total
Temperature ^{0}C	2015/16	11.6	7.3	3.3	-0.1	8.8	7.8	14.1	15.5	21.3	**9.96**	89.64
Temperature ^{0}C	2016/17	10.6	6.8	0.0	-4.7	5.2	10.8	11.1	16.8	22.1	**8.74**	78.66
Temperature ^{0}C	2000-2010	11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	**8.5**	76.5
Precipitation (mm)	2015/16	56.8	64.0	9.0	86.2	52.7	157.9	39.9	135.9	48.6	72.3	**651.0**
Precipitation (mm)	2016/17	84.1	77.6	9.4	22.0	35.0	57.0	82.0	100.0	56.0	41.1	**523.1**
Precipitation (mm)	2000-2010	61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.4	**417.8**

(*source: Republic Hydrometeorological service of Serbia)

RESULTS AND DISCUSSION

Gliadin alleles variability encoding gliadin proteins

The analysis of gliadin allele composition at *Gli-A1* and *Gli-A2* loci showed differences among the analyzed wheat genotypes. The 32 alleles at six *Gli-* loci were identified in studied ten wheat genotypes. The six alleles (*a. b. c. f. h. m*) at *Gli-A1*. five (***b. d. g. k. l***) at *Gli-B1*. three alleles (***a. b. k***) at *Gli-D1*. seven alleles (***a***. ***b. e. f. g. j. k***) at *Gli-A2*. six alleles (***a. b. e. o. p. v***) at *Gli-B2* and five alleles (***a. b. j. k. m***) at *Gli-D2* locus were identified (table 1). The gliadin allele polymorphisms of each ***Gli-1*** and ***Gli-2*** loci were established in numerous previous studies of wheat varieties (Knežević et al.. 1998a; 2006; 2007; Torbica et al.. 2006; Knežević et al.. 2017a. Metakovsky et al.. 2018; 2021; Utebayev et al.. 2019).

The heterogeneity of gliadin loci was found in three varieties. which represents 22.2% of heterogeneous genotypes from the total number of analyzed varieties. In those three genotypes the heterozygosity on four loci was identified. which represents 6.6% of heterozygosity 6.6% of heterozygous loci from the total number of analyzed *Gli–* loci in ten wheat genotypes. The two different alleles at two loci identified in the genotype G-3634-2 at the locus *Gli-D1* (***a*** and

b) and at *Gli-D2* (***m*** and ***b***). while two allele at one locus was identified in genotype G-3601-4. at the *Gli-A2* locus. (***b*** and ***j***) and in the genotype G-3622-1 at the locus *Gli-A1* (***b*** and ***c***) table 1.

The heterozygosity of *Gli*–loci indicates that wheat genotypes are not genetically homogenized for specified loci. which requires further selection in the aim to achieve genetic homozygosity of specified loci. The heterozygosity *Gli*–loci were found in numerous investigation (Knežević et al.. 2006; 2007; Knežević et al.. 2017a. Metakovsky et al.. 2018; 2021; Utebayev et al.. 2019).

Table1. Variation of *Gli-1. Gli-2 and Glu-1* alleles of dry gluten and loaf volume in wheat genotypes

Geno-type	*Gli- alleles*						*High molecular weight glutenin subunits*			*Glu-1 alleles*			Dry gluten *%*		Loaf volume (*ml*)	
	Gli-1 alleles			*Gli-2 alleles*												
	A1	*B1*	*D1*	*A2*	*B2*	*D2*	1AL	1BL	1DL	***A1***	***B1***	***D1***	2015/16	2016/17	2015/16	2016/17
G-3632-1	*a*	*l*	*a*	*f*	*b*	*b*	2*	7+9	5+10	***b***	***c***	***d***	24.80	28.16	480	500
G-3644-4	*f*	*b*	*b*	*e*	*o*	*j*	2*	7+9	2+12	***b***	***c***	***a***	26.42	28.32	480	510
G-3619-3	*h*	*d*	*b*	*b*	*a*	*k*	N	17+18	2+12	***c***	***i***	***a***	23.78	26.20	430	440
G-3601-4	*m*	*l*	*b*	*b+j*	*e*	*b*	N	6+8	2+12	***c***	***d***	***a***	22.92	23.44	400	400
G-3626-2	*b*	*b*	*a*	*k*	*b*	*a*	2*	7+9	5+10	***b***	***c***	***d***	24.12	28.38	480	520
G-3622-1	*b+c*	*b*	*b*	*a*	*v*	*b*	2*	7+9	5+10	***b***	***c***	***d***	26.54	29.86	500	540
G-3617-1	*b*	*g*	*a*	*e*	*b*	*m*	2*	7+9	5+10	*b*	*c*	*d*	24.86	27.96	460	480
G-3611-2	*f*	*l*	*k*	*b*	*p*	*b*	2*	7’+8	2+12	**b**	***u***	***a***	25.20	27.80	480	480
G-3634-2	*b*	*g*	*a+b*	*b*	*o*	*m+b*	N	6+8	2+12	***c***	***d***	***a***	21.20	24.14	380	410
G-3637-1	*m*	*k*	*k*	*g*	*b*	*j*	1	7+9	2+12	***a***	***c***	***a***	23.34	25.68	420	440

Glutenin alleles variability encoding high-molecular weight glutenin proteins

In ten wheat genotypes were identified nine alleles at three Glu-*1* loci. i.e. three of them (***a. b. c***) at the *Glu-A1*. four alleles (***c. d. i. u***) at the *Glu-B1* and two alleles (***a. d***) at the *Glu-D1* locus (table1).

The polymorphisms of glutenin alleles established in previous numerous investigations of bread wheat (Menkovska et al.. 2002; Tohver. 2007; Bellil et al.. 2014; Knežević et al.. 2017a; 2017b; 2018; Hlozáková et al.. 2015).

Frequency of identified alleles at *Gli-1. Gli-2* and *Glu-1* loci

The frequency of identified gliadin alleles was different and varied between 10% and 40%. The highest frequency expressed alleles ***b*** (40.0%) at the ***Gli-A1*** locus. while the lowest had allele ***a*** (10%) and (***a*** and ***h***). At the ***Gli-B1*** locus the highest frequency (30.0%). had two alleles ***l*** and ***b***. and the lowest had alleles ***k.*** (10%). At the ***Gli-D1*** locus the most frequent was two allele ***a*** and ***b*** (40.0%). and the lowest frequency had alleles ***k*** (10%). At the ***Gli-A2*** locus the most frequent was allele ***b*** (40.0%). while the four alleles ***a. f. g. k.*** had the lowest frequency (10.0%). At the ***Gli-B2*** locus the most frequent was allele ***b*** (40.0%). while the lowest and

equal frequency (10%) had four alleles (***a. e. p. v)***. At the ***Gli-D2*** locus the most frequent was allele ***b*** (40.0%) and the lowest frequency had allele ***a*** and ***k (***10%). table 2.

The frequency of glutenin alleles varied at all three loci. in ratio from 10% to 60%. At the ***Glu-A1*** locus the highest and equal frequency found for alleles ***b*** (60.0%). while the lowest had alleles ***a*** (10%). At the ***Glu-B1*** locus the most frequent was allele ***c*** (60.0%). while the lowest frequency had allele ***i*** and ***u*** (10.0%). At the ***Glu-D1*** locus the highest frequency had allele ***d*** (60.0%). while the lowest frequency had alleles ***d*** (40%) table 2.

The highest frequency of alleles could be results of their association with some desirable quality traits (grain hardness. flour and dough quality traits. lipid and starch quality properties) and breeders directed selection of plants genotypes with that traits (Javornik et al.. 1991; Metakovsky et al.. 1991; Menkovska et al.. 1995;1997; 2000; Dimirijevic et al.. 1998; He et al.. 2005; Knežević et al.. 2016; 2017a; 2017b; 2018). Also. some protein encoding alleles can be located close to genes which control some desirable traits of adaptability as well as frost resistance and resistance to diseases (Lfiandra et al.. 1987; Knežević et al.. 1998b; Dimitrijević et al.. 1999). The reason for existing high frequency of alleles should be small diversity of wheat genotypes which use as parents in hybridization in breeding program. Differences in allele frequencies. analyzed similar way. in other studies which reported variability in allele composition and frequency (Knežević et al.. 1998a; 2017b; Metakovsky et al.. 1994; 1998; 2000; 2021; Lookhart et al.. 2001; This et al.. 2001; Bellil et al.. 2014; Hlozáková et al.. 2015; Novoselskaya Dragovich. 2015; Goel et al.. 2018; Utebayev et al. 2019).

Table 2. Frequency of alleles at *Gli-1. Gli-2* and *Glu-1* loci

Gliadin alleles												Glutenin alleles					
Gli-A1		*Gli-B1*		*Gli-D1*		*Gli-A2*		*Gli-B2*		*Gli-D-2*		*Glu-A1*		*Glu-B1*		*Glu-D1*	
Allele	%	Allele	%	Allele	%	Allele	%	Allele	%	Allele	%	Allele	%	Allele	%	Allele	%
a	10	*b*	30	*a*	40	*a*	10	*a*	10	*a*	10	*a*	10	***c***	60	*a*	60
b	40	*d*	10	*b*	40	*b*	40	*b*	40	*b*	40	*b*	60	***d***	20	*d*	40
c	-	*g*	20	*k*	20	*e*	20	*e*	10	*j*	20	*c*	30	***i***	10		
f	20	*k*	10			*f*	10	*o*	20	*k*	10			***u***	10		
h	10	*l*	30			*g*	10	*p*	10	*m*	20						
m	20					*j*	-	*v*	10								
						k	10										

Content of dray gluten

In analysed wheat genotypes. content of dry gluten was different in two year of experiment. In the first vegetation season 2015/16 the dry gluten content varied between 21.20% (G-3634-2) and 26.54% (G-3622-1). while in second vegetation season 2016/17 varied from 23.44% (G-3601-4) to 29.86% (G-3622-1) table 1.

The amount of precipitation was satisfactory and values of temperature were high during the phase of seed filling in second vegetation season what was more favorable for protein synthesis n second than in first year of investigation. The analysed wheat genotypes on average. showed a better response to weather conditions in the second growing season. This is in agreement with results reported in earlier investigation. which established that high temperature at the end of grain-filling influence on polymerisation of gluten proteins (Triboi et al.. 2003). inhibited synthesis of starch (Hurkman et al.. 2013) and that environmental factor influence on efficiency of grain filling (Naeem et al.. 2012; Torbica et al.. 2008; Knežević et al.. 2017a).

Loaf volume

The study of loaf volume showed differences among wheat genotypes within each vegetative season as well between first and second vegetative season. In average the higher value of loaf volume expressed wheat in second vegetative season (2016/17) than in first vegetative season. In the first vegetation season 2015/16 the loaf volume was the lowest 380 *ml* in G-3634-2 and the highest 500 *ml* in G-3622-1 wheat genotypes. In second vegetation season 2016/17 the lowest value of loaf volume was found in G-3601-4 (400 *ml*) and the highest loaf volume was in wheat G-3622-1(540 *ml*) table 1.

Gliadins are positive associated with dough elasticity and high molecular weight-glutenin subunits with strength of dough (Payne. 1987). The matrix of gliadin and glutenins determine gas retention during dough fermentation as well as during bread baking**.** what influence to dough development time. ad loaf volume. In this study established that genotypes (G-3622-1) which possess 2*. 7+9. 5+10. encoded by alleles ***Glu-A1b***. ***Glu-B1c***. ***Glu-D1d***. had the highest dry gluten content and the highest loaf volume in both vegetation season of experiment.

The relationship between *Glu-1* alleles of the HMWG subunits and the bread-making quality was determined in earlier studies which showed positive relationships 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 (Payne. 1987; Lafiandra. et al.. 1987; Gupta et al.. 1989; Metakovsky et al.. 1990; Torbica et al.. 2007; Amjid et al.. 2013; Vaiciulyte-Funk et al.. 2015; Knežević et al.. 2017a; 2018). However. the lowest dry gluten content and the lowest loaf volume had genotype G-3634-2 in the first vegetation season and G-3601-4 in second vegetation season and in both of them identified glutenin subunits 6+8 encoded ***Glu-B1c*** and subunits 2+12 encoded ***Glu-D1d***. The previous investigation showed that these subunits associated with poor dough and bread quality (Menkovska et al.. 2002; Shewry et al.. 2003; Wrigley et al.. 2006; Torbica et al.. 2007; Knežević et al.. 2017b).

CONCLUSIONS

This study showed variability of gliadin and glutenin allele composition and different combination of alleles in analyzed wheat genotypes. At the six *Gli*–loci and were identified 32 gliadin alleles. and at the three Glu-1 loci were identified nine alleles encoding HMW glutenin subunits. Each wheat genotypes characterized specific composition of gliadin and glutenin alleles. The frequency of gliadin alleles varied between 10% and 40%. while frequency for high molecular weight glutenin varied between 10% and 60%. The highest frequency for gliadins *Gli-A1b. Gli-B1a+l. Gli-D1a+b. Gli-A2b. Gli-B2b. Gli-D2b.* and glutenin proteins *Glu-A1b. Glu-B1c. Glu-D1a.* were established in studied 10 wheat genotypes. The studied wheat genotypes showed differences according to dry gluten content and loaf volume within one year of experiment as well as differences between two year of experiment for gluten content and loaf volume. what indicate influence of environment on quality traits. The highest dry gluten content and loaf of volume had G-3622-1whet genotype in both year of experiment. while the lowest values had G-3634-2 in the first vegetation season and G-3601-4 in second vegetation season. Genotypes which carried glutenin subunits 2* encoded by *Glu-A1b.* 7+9 encoded by *Glu-B1c* and subunits 5+10 encoded by *Glu-D1d* in average had high value of dry gluten content and loaf volume.

Acknowledgement

This work supported financially by the Ministry of Education. Science and Technological development of Republic Serbia. Belgrade. in program of Project TR31092 and program 451-03-68/22-14/200189

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