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The relationship between gluten proteins and loaf volume

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Abstract

The storage proteins influence technological quality value of grain wheat, dough quality traits, and loaf quality. The aim of this study is to estimate variability in the dry gluten content, loaf volume, and their relationships with encoding alleles of gliadin and glutenins. Wheat genotypes grown in two vegetation seasons (2015/16 and 2016/17) were studied. The technological quality traits analyzed in this study varied in wheat genotypes within and between vegetation seasons. In both vegetation seasons the highest dry gluten content was established in G-3621-1 (30.23% and 31.15%) and the highest value of loaf volume in G-3621-1 (530 ml and 540 ml). In both vegetation seasons the least dry gluten content was found in G-3606-6 (25.42% and 25.98%) and the least loaf volume in G-3606-6 (380 ml and 390 ml). The composition of gliadin and glutenin alleles in the wheat genotypes analyzed was different. The genotypes carrying Gli-B1b, Gli-D1b, Gli-D2b, and Glu-A1b, Glu-B1c, Glu-D1d had the highest gluten content, while the genotype that carried Gli-B1l and Glu-A1b, Glu-B1c, Glu-D1d had high bread volume. The results have shown relationships between gliadin and glutenin alleles and quality traits of grain, flour, and bread.

Key words: wheat, gliadin, glutenin, allele, bread, quality.

Introduction

The technological quality of wheat flour, dough, and bread is determined by the protein quantity and quality, as well as by the carbohydrate-amylase complex. The analysis of gluten protein quality and quantity during the breeding process, cultivation, and food processing by using standardized methods is necessary for estimating wheat quality (Hruškova et al., 2004; Zečević et al., 2021). The grain protein content of wheat is a genetic trait that varies under environmental factors and genotype-environment interaction. Among environmental factors, soil fertility, soil moisture, nitrogen nutrition, amount of precipitation, air temperature, etc. mainly influence the value of the grain protein content (Peršić et al., 2023). Nitrogen content and fertilizers have a positive influence on increasing the quality of grain protein (Annavarapu et al. 2021). Providing wheat nutrition at an optimal rate and time with nitrogen fertilizers in an available form used by the plant root contributes to the increase in grain protein content (Djokić et al., 1998; Yu et al., 2018) and bread-making quality parameters of wheat (Zečeveić et al., 2004). High temperatures have a negative influence on nitrogen translocation in building up protein in wheat grain. On average grain protein content varied in bread wheat genotypes in a ratio form by 10% to 15% (Shewry, 2007; Laidig et al., 2016). The protein content in grain usually varies even within the same wheat genotype depending on environmental conditions. Protein content is connected to gluten content which indicates the quality of wheat flour, dough, and bread (Knezevic et al. 2017a). Gluten proteins gliadin and glutenins amount to approximately 80%-85% in wheat grain proteins, while the remaining content includes non-gluten proteins, i.e., albumins and globulins ((Wrigley et al., 2006; Khan et al., 2013). The gliadin provides viscosity and the glutenin provides viscoelasticity properties to the dough. The absence of several allelic variants such as high molecular weight subunits of glutenin has an impact on the quality of bread (Payne et al.,1987; Dimitrijević et al., 1998; Wieser and Zimmermann, 2000). However, varieties are chosen for high glutenin content to increase the dough strength (Menkovska et al., 2002). Gliadins are controlled by genes located on the short arm of groups 1 and 6 of A, B, and D chromosomes (Sozinov and Poperelya, 1980), while the high molecular weight of glutenins encoded by genes located on the long arm of group 1 of A, B, and D chromosomes.

The aim of this work was to study the variability of technological quality properties of wheat genotypes grown on the basis of variation in (i) the dry gluten protein, (ii) loaf volume, and (iii) identification of the relationship between studied traits with encoding alleles for gliadin and glutenins.

Material and Methods

The 10 genetically divergent wheat genotypes (G-3626-1, G-3618-2, G-3606-4, G-3636-3, G-3627-1, G-3621-1, G-3606-5, G-3607-5, G-3606-6, and G-3632-2) were included for the analysis of dry gluten content, loaf volume and their relationship with encoding gliadin and glutenins gene alleles.

Dry gluten was obtained after water rinsing the dough with 2% saline solution to remove the starch and water-soluble fractions. Wet gluten remains on the top of a sieve whose content is expressed as a percentage of flour.

$$\text{Wet gluten (\%)} = \frac{\text{gluten left on sieve } (g)}{\text{total gluten } (g)} \text{ x } 100$$

The wet gluten was dried and weighed on technical scales (ICC 106/2, 1992).

$$\text{Dry gluten (\%)} = \frac{\text{dry gluten } (g)}{\text{initial dough } (g)} \text{ x } 100$$

To evaluate loaf quality, the loaf volume (ml) was included according to the ICC-Standard No.131, 1992).

Alleles at three Gli-1 loci, three Gli-2 loci, and three Glu-1 loci were identified for these ten genotypes in the previous study (Knežević et al., 2022) and 24 different alleles encoding gliadins at six gliadin loci and eight different alleles at the three Glu-1 loci were found. Gliadin proteins were separated by using the acid PAG electrophoresis method developed by Novoselskaya et al. (1983), and, in order to determine gliadin blocks alleles at Gli-1 and Gli-2 loci, the method developed by Metakovsky (1991) was used. High molecular weight glutenins were separated by a sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) and to determine HMW-GS and identify Glu-1 alleles the Payne and Lawrence method (1983) was applied. Once gliadin and glutenin composition and their frequency were determined, the correlation between each allele and the value of technological quality traits (protein content, sedimentation volume, gluten content, and loaf volume) were analyzed.

Weather conditions in the vegetation period

The growth of wheat plants is highly influenced by environmental factors, primarily by the total amount of precipitation and its distribution during the vegetation season and average temperature, which affect the availability of absorption and utilization of nutrient fertilizers by plants. In the analysis of weather conditions (temperature and precipitation) during the two years of experiment and during the long-term period (2000-2010) differences were found between the two seasons (2015/16 and 2016/17). Also, the amount of precipitation and temperature values in both years of experiments were different in relation to the long-term period (2000-2010). According to the data, the temperature in February 2016 (8.8 °C) was significantly higher than in 2017 (5.2 °C) and in the long-term period (2.6 °C), while the temperature in March 2017 (10.8 °C) was significantly higher than in 2016 (7.8 °C) and in the long term period (52.9 °C). Other temperatures for the growing seasons were similar (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 °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 °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 °C	2000-2010	11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	8.50	76.50
Precipitation (mm)	2015/16	56.8	64.0	9.0	86.2	52.7	157.9	39.9	135.9	48.6	72.30	651.00
Precipitation (mm)	2016/17	84.1	77.6	9.4	22.0	35.0	57.0	82.0	100.0	56.0	41.10	523.10
Precipitation (mm)	2000-2010	61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.40	417.80

(*source: Republic Hydrometeorological Service of Serbia*)

December precipitation in both years was (9.0 mm in 2016) and (9.4 mm in 2017), which was significantly lower than in the long-term period (44.6 mm). However, precipitation in January and March 2016 (86.2 mm and 157.9 mm) was significantly higher than in 2017 (22.00 mm and 57.00 mm) and in the long-term period (30.00 mm and 33.2 mm). The total precipitation was different between the two vegetation seasons. Also, total precipitation in both vegetation seasons was different and higher than in the long-term period. The total precipitation in 2015/16 was 651 mm, 523 m in 2016/17 m, and for the long-term period, it was 417 mm. In 2015/16 precipitation was higher by 127.9 mm than in 2016/17 and by 233.2 mm higher than in the long-term period, while the total precipitation in 2016/17 was by 105.3 mm higher than in the long-term period. In the period from March to May 2015/2016, there was 333 mm of precipitation, while in the same period of 2016/17, there was 239 mm of precipitation. In both years, the wheat growing season was characterized by high rainfall in May (135.9 mm and 100 mm), which negatively affected plant health. During the grain-filling phase in the first year, the average temperature in April was higher and the average precipitation in May was higher and more favourable than in the second year of the experiment and in the ten year period (Table 1.).

Results and Discussion

Dry gluten content

In this investigation, the dry gluten content varied and in both vegetation seasons the lowest was in the G-3606-6 genotype (24.42% and 25.98%) and the highest dry gluten content was in G-3621-1 (30.23% and 31.15%). The dry gluten content was different between the genotypes and between the two vegetation seasons analyzed. On average for all genotypes, the value of dry gluten content was approximately equal in both vegetation seasons (28.1% and 28.9%) (Table 2.).

The difference in the gluten content between the growing seasons was in the range from 0.39% (G-3627-1) to 1.928% (G3632-1). On average for all genotypes, the value of gluten content was 28.09% in the first vegetation season and 28.95% in the second which means that the gluten content was higher in the second vegetation season by 0.86% than in the first vegetation season (Table 2.).

In other studies, focusing at different wheat varieties, variations of wet gluten content have been found in the range from 31.4% to 45.1% (Šimić et al., 2006), 22.8% to 30.3% (Branković et al., 2018), and from 24% to 40.5% (Yang et al., 2014), which indicates that differences depend on genotypes and specific growing and environmental factors.

Table 2. Gluten content (%) in the wheat grain and Gli-1, Gli-2, and Glu-1 allele composition

	G-3626-2			G-3618-2			G-3606-4			G-3636-3			G-3627-1			G-3621-1			G-3606-5			G-3607-5			G-3606-6			G-3632-1			Average		
2015/16	28.35			27.66			27.33			26.85			27.94			30.23			30.10			28.81			25.42			28.23			28.09		
2016/17	29.64			27.92			28.92			27.56			28.33			31.15			30.76			29.26			25.98			30.02			28.95		
Average	28.99			27.79			28.12			27.20			28.14			30.69			30.43			29.04			25.70			29.12			28.52		
Chromosome/ Locus	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D
Gli-1	a	b	a	m	l	k	a	k	b/g	b	l	a	m/a	b	b/a	a	l	b	b	g	b	b	b	f	b	k	g	a	l	a	a	b	b
Gli-2	k	b	a	b	?	a	g/e	h	a	k	j	b	b	p	r	f	h	a	g	b	b	k/g	b	b	e	h	a/b	f	b	b	f	b	b
Glu-1	b	c	d	b	b	d	c	c	a	c	d	a	a	c	d	b	c	d	b	b	d	c	c	a	c	d	a	a	c	d	b	c	d

The amount of precipitation was satisfactory and values of temperature were high during the phase of grain filling. Analyzing the weather conditions for those two vegetation seasons, our investigation has found that all genotypes had a higher value of the gluten content in the second vegetation season, which indicates that those genotypes on average have shown a better response to weather conditions in the second growing season, as well as that there were more favourable values of precipitation and temperature for protein synthesis in the second vegetation season . Other studies showed that environmental factors have an influence on grain filling (Naeem et al., 2012; Knezevic et al., 2017b; Savill et al., 2018) and that high temperature at the end of grain-filling influences polymerisation of gluten proteins (Triboi et al., 2003), inhibits synthesis of starch (Hurkman et al., 2013).

Protein content in the wheat grain is a genetic trait that has greater variation under the impact of environmental factors (Hawkesford, 2017). However, the quality of protein is strongly genetically determined. The highest share in the grain protein content includes gluten proteins (80-85%), whose main components are gliadin and glutenins (Payne, 1987). The regime of nitrogen application (pre-anthesis and post-anthesis), temperature and timings, post-anthesis temperature, and watering regime have a strong influence on the accumulation of protein content (Godfrey et al., 2010; Knežević et al., 2016a; Zörb et al., 2018; Ma et al., 2019). The influence of environmental factors on gluten content was reported in a number of studies (Hurkman et al., 2013; Tóth et al., 2019; Horvat et al., 2021), which found that the amount of a gluten protein fraction is higher in wheat grown under high fertilization than without fertilization. Also, they found that high temperatures influenced the increase of gluten protein content per grain which is due to the inhibited synthesis of starch under a high temperature. Gluten quality is under genetic control with the interaction of environmental factors (Lookhart et al., 2001).

The gluten content on average for all genotypes indicates good quality (Knezevic et al., 2016b). The main components of gluten proteins are gliadin and glutenins. Gliadins are a heterogeneous group of proteins that contain different types of polypeptide molecules (α-, β-, γ-, and ω-gliadins) with molecular mass between 16kDa to 50kDa. Glutenins contain two types of polypeptides, namely low molecular weight glutenin subunits (LMW GS) with molecular mass 20kDa to 50kDa and high molecular glutenin subunits (HMW GS) with molecular mass 50kDa to 200kDa which characterize intermolecular bonds (Bietz, 1997). Hydrated gliadin and glutenins interact through the formation of chemical bonds and begin to stick to each other, forming a very extensible, elastic structure that is responsible for the gas-holding ability of bread dough (Metakovsky et al. 1990). Gliadins are responsible for the viscosity of dough, and glutenins for the elasticity and strength of dough (Menkovska et al., 2002; Shewry, 2007). It means that by increasing viscosity gliadins reduce increasing elasticity of gluten complex, and those functional and rheological dough properties are influenced by the ratio of gliadin/ glutenin quantity as well as the ratio of high/ low glutenin polypeptide (Barak et al., 2013; 2015).

The HMW-GSs are encoded by three loci, Glu-A1, Glu-B1, and Glu-D1 on the long arm of chromosomes (Payne et al.,1987; Knežević et al., 1993), and LMW-GSs are encoded by genes located on the short arm of Glu-A3, Glu-B3, and Glu-D3. Gliadin is encoded by genes located on the short arm of group 1 and 6 of A, B, and D chromosomes (Sozinov and Poperelya, 1980), while high molecular weight glutenin is encoded by genes located on the long arm of 1A, 1B, and 1D chromosomes. The quality of wheat is based on the gluten proteins (gliadins and glutenins) which have a great influence on flour, dough properties, and quality of end-use products. The identification of alleles indicates their role and contribution to gluten and loaf quality properties.

This study analyzed the connection between the composition of alleles and the value of gluten content, and the association of each Gli-1, Gli-2, and Glu-1 alleles with the highest value of gluten content. Mainly, the genotypes that containing alleles with the highest frequency had on average the highest value of gluten content. The genotypes with the same allele at each Gli-1, Gli-2, and Glu-1 loci were grouped, and average values of gluten content were computed for each group. Average values of gluten content and associated alleles were estimated, i.e., which allele on each of three Gli-1, three Gli-2, and three Glu-1 loci was associated with the highest value of gluten content in both vegetation seasons.

Based on the presence of the same allele at the same locus, genotypes for each Gli-1, Gli-2 and Glu-1 locus were grouped and average values for gluten content were calculated. The group of genotypes with higher values of gluten content mainly possessed the most frequent allele at each locus mostly in both vegetation seasons, namely Gli-A1a (28.53%; 29.93%), Gli-B1b (28.36%; 29.07%), Gli-D1b (28.90%; 29.79%), Gli-A2f (29.23%; 30.58%), Gli-B2b (28.87%; 29.92%), Gli-D2b (28.49%; 29.40%), Glu-A1b (29.08%; 29.87%), Glu-B1c (28.48%; 29.55%), and Glu-D1d (28.75%; 29.64%) (Table 3.).

Table 3. Alleles at Gli-1, Gli-2, and Glu-1 associated with the high value of gluten content (%) in wheat genotypes

Locus	Gli-A1	Gli-B1	Gli-D1	Gli-A2	Gli-B2	Gli-D2	Glu-A1	Glu-B1	Glu-D1
Alleles associated with high gluten content	**a**	**b**	**b**	**f**	**b**	**b**	**b**	**c**	**d**
Genotypes which carry the same, most frequent allele which on average had the highest gluten content	G-3626-2	G-3626-2	G-3606-4	G-3621-1	G-3626-2	G-3636-3	G-3626-2	G-3626-2	G-3626-2
	G-3606-4	G-3627-1	G-3627-1	G-3632-1	G-3606-5	G-3606-5	G-3618-2	G-3606-4	G-3618-2
	G-3621-1	G-3607-5	G-3621-1		G-3607-5	G-3607-5	G-3621-1	G-3627-1	G-3627-1
	G-3632-1	G-3626-2	G-3606-5		G-3632-1	G-3632-1	G-3606-5	G-3621-1	G-3621-1
								G-3607-5	G-3606-5
								G-3632-1	G-3632-1
Vegetation season	Average value of gluten content (%) in a group of genotypes which carry the most frequent allele								
2015/16	28.53	28.36	28.9	29.23	28.87	28.49	29.08	28.48	28.75
2016/17	29.93	29.07	29.79	30.58	29.92	29.40	29.87	29.55	29.64
Average	28.99	27.79	28.12	27.20	28.14	30.69	30.43	29.04	25.70

This combination of nine alleles was not found in the analyzed genotypes. However, out of this combination of nine alleles, six alleles were present in three genotypes (G-3621-1; G-3632-1; G-3626-2) , five alleles in one genotype (G-3606-5), in two genotypes (G-3627-1 and G-3607-5), three alleles in one genotype (G-3606-4), two allele in one genotype (G-3618-2) and one genotype (G-G-3636-3) with one allele. However, in G-3606-6 there were none of the nine alleles associated with the gluten content, and this genotype had the least gluten content in both vegetation seasons (25.42% and 25.98%).

The high and highest gluten content has been found in three genotypes that had six and in one genotype with five alleles associated with the gluten content. Therefore, alleles Gli-A1a, Gli-D1b, Gli-A2f, Glu-A1b, Glu-B1c, and Glu-D1d and the highest gluten content (30.23% and 31.15%)are present in G-3621-1; G-3632-1 includes Gli-A1a, Gli-A2f, Gli-B2b, Gli-D2b, Glu-B1c, and Glu-D1d and the high gluten content (28.23% and 30.02%); G-3626-2 includes Gli-A1a, Gli-B1b, Gli-B2b, Glu-A2b, Glu-B1c, and Glu-D1d and the high gluten content (28.23% and 30.02%). Also, the G-3606-5 genotype had high gluten content (30.10% and 30.76%), which had five (Gli-D1b, Gli-B2b, Gli-D2b, Glu-A1b, Glu-D1d) out of nine alleles associated with the gluten content. The differences between the genotypes that had the same number of alleles associated with a high value of gluten content are related to the differences in the composition of alleles and quality of encoded gluten proteins, as well as with additional effect alleles at Glu-3 loci which was not analyzed in this investigation. Based on the combination of nine alleles referred to, four alleles (Gli-B1b, Gli-D1b, Glu-B1c, Glu-D1d) had the G-3627-1 genotype, which had a high gluten content (27.94% and 28.33%), while G-3607-5 also had four alleles, but different composition (Gli-B1b, Gli-B2b, Gli-D2b, Glu-B1c), in which the gluten content was lower in two vegetation seasons (28.81% and 29.26%). The G-3636-3 genotype, which had a low gluten content (26.85% and 27.56%) had only one allele out of nine alleles (GliD2b) associated with the gluten content.

The genotypes that carried Gli-B1b, Glu-A1b, and Glu-D1d had the highest values of gluten content in both vegetation seasons, i.e., G-3621-1 (30.23% and 31.15%) and G-3606-5 (30.10% and 30.76%). In the G-3621 genotype Gli-A2f and Gli-B2h were found, which were low frequent and we can only assume that they had a positive contribution to the gluten content.

In their study Azadi and Bafrouei (2018) discussed that the most frequent alleles, Gli-A1b and Glu-A1a, as well as, Glu-D1d had a positive correlation with bread making quality and found that Glu-D1d was associated with higher grain yield than Glu-D1a. Also, the positive impact of HMW glutenin subunits Glu-B1 (7 + 8/7 + 9/17 +18) and GluD1 (5 + 10) encoded by Glu-B1c, Glu-B1b, Glu-B1i, and Glu-D1d on dough strength was established, presenting that those alleles significantly increased the dough strength (Lookhart et al. 2001; Sharma et al., 2020).

Gliadin and glutenin have the largest share in the total protein content of the grain and contribute to the quality of dough and bread. Except for gliadin and high molecular weight glutenins, the important contribution to variation in protein content is attributed to low molecular weight glutenins encoded by Glu-D1 and Glu-A3 (Plessis et al. 2013).

Loaf volume

The analysis of bread quality showed that in both vegetation seasons the lowest value of loaf volume was in the G-3606-6 genotype (430 ml and 440 ml) and the highest loaf volume was in G-3621-1 (530 ml and 540 ml). The lowest value was different between the genotypes and between the two vegetation seasons analyzed. On average for all genotypes, the lowest value was approximately equal in both vegetation seasons (472 ml and 475 ml) (Table 4.).

The difference in loaf volume among the genotypes between the growing seasons was in the range from 10.0 ml (G-3618-1, G-3636-3, G-3627-1, G-3621-1, G-3606-6) to 20.0 ml (G-3606-4, 3606-5, G-3607-5). On average for all genotypes, the value of protein content was 472.0 ml in the first vegetation season and 475.0 ml in the second vegetation season whereas the loaf volume was higher in the second vegetation season by 3.0 ml than in the first vegetation season (Table 4.).

The genotypes that carried Gli-B1l, Gli-D1b, Glu-A1b, and Glu-D1b had the highest values of the loaf volume in both vegetation seasons, namely G-3621-1 (530.0 ml and 540.ml) and G-3606-5 (520.0 ml and 500.0 ml). In the G-3621 genotype Gli-A2f and Gli-B2h were present which were low frequent and we can only assume that they hade a positive contribution to the gluten content (Table 4).

Table 4. Variation in the loaf volume (ml) in wheat

	G-3626-2			G-3618-2			G-3606-4			G-3636-3			G-3627-1			G-3621-1			G-3606-5			G-3607-5			G-3606-6			G-3632-1			Average		
2015/16	460.0			480.0			420.0			430.0			500.0			530.0			520.0			460.0			430.0			490.0			472.0		
2016/17	460.0			490.0			440.0			420.0			490.0			540.0			500.0			480.0			440.0			490.0			475.0		
Average	460.0			485.0			430.0			425.0			495.0			535.0			510.0			470.0			435.0			490.0			473.5		
Chromosome/ Locus	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D
Gli-1	a	b	a	m	l	k	a	k	b/g	b	l	a	m/a	b	b/a	a	l	b	b	g	b	b	b	f	b	k	g	a	l	a	a	l	b
Gli-2	k	b	a	b	?	a	g/e	h	a	k	j	b	b	p	r	f	h	a	g	b	b	k/g	b	b	e	h	a/b	f	b	b	f	b	b
Glu-1	b	c	d	b	b	d	c	c	a	c	d	a	a	c	d	b	c	d	b	b	d	c	c	a	c	d	a	a	c	d	b	c	d

In this study the genotypes were grouped according to the same allele identified at the same locus for each three loci Gli-1, Gli-2, and Glu-1 and average values were computed for loaf volume. The group of genotypes with higher loaf volume mainly had the most frequent allele at each locus in both vegetation seasons, namely Gli-A1a (475.0 ml; 482.50 ml), Gli-B1l (482.5 ml; 485.00 ml), Gli-D1b (492.50 ml; 492.50 ml), Gli-A2f (510.00 ml; 515.00 ml), Gli-B2b (482.5 ml; 482.50 ml), Gli-D2b (475.0 ml; 472.50 ml), Glu-A1b (497.50 ml; 497.50 ml), Glu-B1c (476.70 ml; 483.30 ml), and Glu-D1d (496.7 ml; 495.00 ml) (Table 5.).

This combination of nine alleles was not found in the genotypes analyzed in this study. However, out of this combination of nine alleles, G-3621-1 had seven alleles (Gli-A1a, Gli-B1l, Gli-D1b, Gli-A2f, Glu-A1b, Glu-B1c, and Glu-D1d), which had the highest value of loaf volume in two vegetation seasons (530.0 ml and 540.0 ml). The G-3632-1 genotype also had the same number of alleles, i.e., seven, but their composition was different (Gli-A1a, Gli-B1l, Gli-A2f, Gli-B2b, Gli-D2b, Glu-B1c, and Glu-D1d), in which the loaf volume was 490.0 ml and 490.0 ml. Those two genotypes, G-3621-1 and G-3632-1, had the same, five alleles and two different alleles at four loci, namely Gli-D1(b and a) Gli-B2 (h and b), Gli-D2 (a and b), and Glu-A1(b and a).) Out of the combination of nine alleles referred to, five alleles (Gli-D1b, Gli-B2b, Gli-D2b, Glu-A1b, and Glu-D1d) had the G-3606-5 genotype, which had a very high value of the loaf volume (520.0 ml and 500.0 ml), while G-3626-2 also had five alleles, but with different composition (Gli-A1a, Gli-B2b, Gli-D2b, Glu-A1b, and Glu-D1d), in which the loaf volume was lower in two vegetation seasons (460.0 ml and 460.0 ml). The G-3636-3 genotype, which had the lowest loaf volume (430.0 ml and 420.0 ml) had only two alleles out of nine alleles (Gli-B1l, GliD2b), which can be associated with a high value of the loaf volume (Table 5.).

Table 5. Alleles at *Gli-1*, *Gli-2* and *Glu-1* associated with the high value of loaf volume (*ml*) in wheat genotypes

Locus	Gli-A1	Gli-B1	Gli-D1	Gli-A2	Gli-B2	Gli-D2	Glu-A1	Glu-B1	Glu-D1
Alleles associated, with high loaf volume	a	l	b	f	b	b	b	c	d
Genotypes which carry the same, most frequent allele which on average had the highest loaf volume	G-3626-2	G-3618-2	G-3606-4	G-3621-1	G-3626-2	G-3636-3	G-3626-2	G-3626-2	G-3626-2
	G-3606-4	G-3636-3	G-3627-1	G-3632-1	G-3606-5	G-3606-5	G-3618-2	G-3606-4	G-3618-2
	G-3621-1	G-3621-1	G-3621-1		G-3607-5	G-3607-5	G-3621-1	G-3627-1	G-3627-1
	G-3632-1	G-3632-1	G-3606-5		G-3632-1	G-3632-1	G-3606-5	G-3621-1	G-3621-1
								G-3607-5	G-3606-5
								G-3632-1	G-3632-1
Vegetation season	Average value of loaf volume (ml) in a group of genotypes which carry the most frequent allele								
2015/16	475.0	482.5	492.5	510.0	482.5	475.0	497.5	476.7	496.7
2016/17	482.5	485.0	492.5	510.0	482.5	472.5	497.5	483.3	495.0
Average	460.0	485.0	430.0	425.0	495.0	535.0	510.0	470.0	435.0

The differences between the genotypes that had the same number of alleles associated with a high value of the loaf volume are due to the differences in the composition of alleles and quality of encoded gluten proteins (their amino acid composition and amount), as well as additional effect alleles at Glu-3 loci, enzymes, etc.

The storage proteins, gliadin and glutenin, have the largest share in determining the quality of dough and bread. The relationship between Glu-1 alleles of the HMWG subunits and the bread-making quality was determined (Payne, 1987; Lafiandra, et al., 1987; Metakovsky et al., 1990; Gupta et al., 1989; Amjid et al., 2013; Goutam et al., 2015; Knežević et al., 2017b; 2018; Kumar et al., 2018; Rai et al., 2019). The study of 96 double haploid lines (Ahn et al., 2014) showed that the volume of a bread loaf was mainly influenced by the allelic variations on Glu-D1, and also found that, among 14 allelic compositions, lines carrying all of Glu-D1d, Glu-A3d, Glu-B3d, and Pinb-D1b alleles had higher volume of bread loaves than the other combination of alleles.

Another study showed that a combination of Glu-B1 (7 + 8)/(7 + 9) and Glu-D1(5 + 10), Glu-B1c, Glu-B1b, and Glu-D1d had positive relationships with the gluten quality, loaf volume, quality score, and that those alleles even neutralized a negative effect of secalins on end-use quality in the genotypes which carry chromosomal translocation 1B/1R (Sharma et al., 2020; Singh and Gupta, 2021). However, except for the positive gliadin and high molecular weight glutenin, the LMWGS alleles Glu-A3, Glu-B3 along with HMWGS Glu-D1 had great contributions to dough properties and improved end-use quality (Sharma et al., 2020).

Conclusion

Base on the results, differences in wheat genotypes were established for the gluten content and loaf volume, within each vegetation season and between vegetation seasons. The highest dry gluten content and loaf of volume has been found in the G-3621-1 genotype in both vegetation seasons (in the first 30.23% and 530 ml; in the second, 31.15% and 540 ml), while the G-3606-6 genotype had the lowest values in both vegetation seasons (in the first 25.42% and 430 ml; in the second, 25.98% and 440 ml).

The genotypes which carried the allele b at Gli-B1, allele b. at Glu-A1 (encoded 2* subunit), allele b. or c. at Glu-B1 (encoded 7+8 or 7+9 subunit), and d. at Glu-D1 (encoded 5+10 subunit) had the highest values of the gluten content and loaf volume (G-3621-1, G-3606-5).Those alleles are associated with a high value of the gluten content and loaf volume.

The genotypes that carried the allele b at Gli-B1, allele a at Glu-A1 (None subunit), allele d. at Glu-B1 (encoded 6+8 subunit), and a. at Glu-D1 (encoded 2+12 subunit) had the lowest values of the gluten content and loaf volume (G-3606-6). Those alleles are associated with a lower value of the gluten content and loaf volume.

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Веза између протеина глутена и волумена хлеба

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Резервни протеини ендосперма семена утичу на вредност особина технолошког квалитета семена пшенице, особине квалитета теста и квалитет хлеба. Циљ овог рада је био оцена варијабилности садржаја сувог глутена и запремине хлеба, као и њихове повезаности са кодирајућим алелима глијадина и глутенина. У изучавања је укључено 10 генетички дивергентних генотипова пшенице (G-3626-1, G-3618-2, G-3606-4, G-3636-3, G-3627-1, G-3621-1, G-3606-5, G -3607-5, G-3606-6, G-3632-2). Код ових генотипова је анализиран, садржај сувог глутена и запремине хлеба у две вегетационе сезоне (2015/16 и 2016/17). У истраживањима је установљено варирање вредности особина технолошког квалитета код анализираних генотипова пшенице у обе вегетационе сезоне, као и варирање вредности особина између вегетационих сезона код истог генотипа. У обе вегетационе сезоне утврђен је највећи садржај сувог глутена код генотипа G-3621-1 (30,23% и 31,15%) и највећа вредност запремине векне код генотипа G-3621-1 (530 ml и 540 ml). У обе вегетације је најмањи садржај сувог глутена био код генотипа G-3606-6 (25,42% и 25,98%), а најмања запремина векне у G-3606-6 (380 ml и 390 ml). Састав алела глијадина и глутенина код анализираних генотипова пшенице је био различит. Генотипови код којих су били идентификовани алели: Gli-B1b, Gli-D1b, Gli-D2b i Glu-A1b, Glu-B1c, Glu-D1d су имали највећи садржај глутена, а генотипови који је носиоци алела Gli -B1l и Glu-A1b, Glu-B1c, Glu-D1d су имали веће вредности запремине хлеба. Добијени резултати су показали да постоји повезаност између алела глијадина и глутенина са испољавањем вредности особина квалитета семена, брашна и хлеба.

Кључне ријечи: пшеница, глијадини, глутенини, алели, хлеб, квалитет.

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