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Research Article

Investigation of the Complex Influence of High- and Low-Molecular Glutenins and Crude Protein on the Quality of Bread Wheat (*T. aestivum L.*)

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Abstract

The relation between high- (HMW) and low-molecular (LMW) glutenins with the quality indicators - sedimentation value, valorimetric value and bread loaf of a collection of Bulgarian wheat varieties was studied.

A total of about 63% of the sedimentation value, 44% of the valorimetric value and 39% of the bread loaf was controlled by the two groups of glutenins and the crude protein.

The high-and low-molecular weight glutenins had an almost equal share in controlling the sedimentation value and the valorimetric value. The LMW glutenins had a significantly higher share in controlling bread loaf than that of HMW glutenins.

The participation of individual glutenin loci in quality control was not one-sided. It was mainly determined by genetic diversity and by the linking with the quality of the individual glutenin subunits.

The relative contribution of crude protein to the control of quality indicators during the various harvest years was highly variable, but its optimum quantity was a necessary condition for showing the positive effect of glutenin subunits on the quality of the wheat varieties.

Keywords: *Winter Wheat; HMW- and LMW-Glutenins; Crude Protein; Sedimentation Value; Valorimetric Value; Bread Loaf*

Introduction

The reserve proteins accumulated in the grain of wheat are the main components of gluten and are essential for determining the properties of dough and bread. They are subdivided into two groups - gliadins and glutenins and differ in their genetic condition and functional properties [37]. Gliadins mainly determine the stretch ability of wheat gluten [25]. The elasticity/strength of gluten is mainly determined by glutenins [7,8,21,23]. A characteristic feature of the genetic control of reserve proteins is the multiple allelism, which is the hereditary basis of their polymorphic structure and different alleles determine the expression of different protein subunits. Since each subunit is associated with a different quality of wheat gluten, there is a great variety in terms of baking qualities [1,4,15,17]. The specific features of the reserve proteins underlie their use as genetic markers to determine the hereditary quality potential of different wheat samples. The complex effect of prolamines and in particular high- and low-molecular gluten, on wheat quality is most pronounced in their ability to form together polymers of enormous molecular weight in the order of millions of daltons, on which the strength of wheat gluten depends [5,6,27]. The results of the studies of [18,32,35,36], show that the effect of allelic variation in gluten on quality is different depending on the

amount of protein in the wheat lines studied. These facts justify the need for study of the complex influence of high- and low-molecular glutenins and raw protein on wheat quality [9,10,14].

This article examines the results of a linkage study of some qualitative indicators - sedimentation value, valorimetric value and bread loaf with HMW and LMW alleles from the six Glu1 and Glu3 loci and the amount of crude protein.

Materials and Methods

Materials

34 varieties of common wheat (*T. aestivum* L., 2n = 28) breeding in Dobrudzha Agricultural Institute (Bulgaria) during the period 2009-2016, were studied: Todora, Bozhana, Aglika, Stoyana, Milena, Rada, Karat, Kristi, Antonovka, Karina, Korona, Kosara, Neda, Bolyarka, Lazarka, Demetra, Goritsa, Zhana, Kristalina, Laska, Fani, Merilin, Galateya, Sladuna, Kalina, Katarzhina, Enola, Kami, Kiara, Kristal, Dragana, Iveta, Pchelina, Tina.

Methods

Electrophoretic methods: Glutenins (HMW- and LMW-GS) were extracted according to [34]. The electrophoresis run on vertical apparatus in two ways: a) classical one-dimensional 12% polyacrylamide gel [20]; b) one-dimensional 10% polyacrylamide gel SDS-PAGE with addition of 4M urea [19]. Arrangement and numbering of HMW-GS in wheat was carried out according [30]. LMW-GS nomenclature in wheat [11] and combined method for LMW-GS and gliadin identification were adopted [16].

Technological methods: The grinding of the samples was carried out on a mill MLU-202 up to 70% flour. The sedimentation value of the wheat samples was determined by the method of [33], the rheological properties of the flour-the stability of the dough, the degree of softening and the valorimetric value and the bread loaf-according to the methods adopted in the technological laboratory of Dobrudzha Agricultural Institute, Bulgaria Crude protein content (%) was determined by standard Kjeldahl method (N x 7.5) [2].

Statistical analysis

Multiple correlation analysis [3] of STATISTICA package was performed for statistical data processing. The Glu-1 and Glu-3 alleles and the amount of crude protein were used as independent variables (X) and each of the above qualitative attributes was used as the dependent variable (Y). For this purpose, the high- and low-molecular-weight glutenin alleles were transformed into the numbers 1 and 0, which means, respectively, the presence and absence of an allele in the analyzed wheat sample. The indicator of genetic diversity - H at individual glutenin loci was determined by the method of [13]. As a result of the above statistical analysis, the following indicators have been identified: R is a multiple correlation coefficient expressing the degree of relationship between the independent variables (X) and the dependent variable (Y). R^2 - multiple determination coefficient, expressed as a percentage, indicates how much of the value of the dependent variable (Y) is determined by the corresponding independent variables (X). SE-standard error of estimation (prediction) reflecting the average difference between the true and predicted values of the dependent variable (Y).

Results

Allelic composition of HMW and LMW of the analyzed wheat varieties

The HMW allelic composition of the analyzed wheat samples is presented in table 1.

Locus	Alleles	Subunits	Number of varieties	Frequency, %
Glu-A1 H* = 0.60	*a* *b c*	1 2* Null	10 17 11	26.3 44.7 29.0
Glu-B1 H = 0.52	*b* *c*	7+8 7+9	15 23	39.5 60.5
Glu-D1 H = 0.27	*a* *b* *d*	2+12 3+12 5+10	5 1 32	13.2 2.6 84.2
Glu-1 H = 0.46				

Table 1: HMW glutenins composition in Glu-1 locus of wheat varieties during the period 2000-2016 (including two biotypes for the Laska variety, two for the Galateya variety and three for the Sladuna variety).
H-Genetic diversity index [13,26].

The Glu-A1 locus was characterized by a relatively high genetic diversity, H = 0.65. Its inherited potential is formed by three alleles - Glu-A1*a*, Glu-A1*b* and Glu-A1*c*. The '2 *' subunit (allel '*b*'), associated with high gluten quality, has the highest frequency (44.7%).

Subunit 'null' (allel '*c*') is next in frequency (29.0%). It is characterized by zero protein synthesis and results in low baking properties. Subunit '1' (allel '*a*') has the lowest frequency - 26.3%.

Although the allelic diversity at the Glu-B1 locus of *T. aestivum* is very large, only two alleles were identified in the present study (Table 1). The genetic diversity of the locus is at an average level - H = 0.52. This is a result of the high frequency of the allele '*c*' (60.5%) and the low frequency of the allele '*b*' (39.5%).

Three alleles encoding the '5 + 10', '2 + 12' and '3+12' fraction pairs were identified at the Glu-D1 locus. The '5 + 10' fractional pair has a high frequency, resulting in relatively low genetic diversity at the locus. The atypical fraction pair '3 + 12' is characteristic of only the two Fani biotypes. It is thought to be a mutant form of the '*x*-' and '*y*-' subunits of the main fraction pair '2 + 12' [19,24,35].

In general, the hereditary basis for the quality of the analyzed wheat samples, controlled by HMW, has an average level of genetic diversity - 0.46.

The LMW allelic composition of the analyzed wheat samples is presented in table 2.

Locus	Alleles	Number of varieties	Frequency, %
Glu-A3	*c*	29	72.5
H* = 0.45	*f*	1	2.5
	b	1	2.5
	e	6	15.0
	d	3	7.5
Glu-B3	*b*	24	60.0
H = 0.59	*f*	3	7.5
	g	2	5.0
	h	3	7.5
	j	8	20.0
Glu-D3	*c*	35	87.5
H = 0.22	*a*	5	12.5
Glu-3			
H = 0.42			

Table 2: LMW glutenins composition in Glu-3 locus of wheat varieties during the period 2000-2016 (including two biotypes for the Laska variety, two for the Galateya variety, two for the Tina variety and three for the Sladuna variety).

H-Genetic diversity index [13,26].

Five alleles have been identified at the Glu-A3 locus. The major allele is '*c*', with a frequency of 68.3%, respectively. The other alleles have a low frequency.

A significantly higher value of the genetic diversity index was observed at the Glu-B3 locus, in which five alleles were also identified. The main allele is 'b' and the other four alleles have a low frequency.

Only two alleles were identified at the Glu-D3 locus - '*c*' and '*a*'.

The high frequency of the 'c' allele is the reason for the relatively low genetic diversity at this locus.

Generally, in the analyzed wheat varieties analyzed, LMW have a much lower genetic diversity than HMW.

Multiple correlation of HMW, LMW and crude protein with some qualitative features

HMW, LMW and crude protein/sedimentation

Table 3 presents the results regarding the complex effect of high- and low-molecular-weight glutenin subunits and crude protein on the sedimentation value of the analyzed wheat varieties.

Year	R	F-criterion	SE	R^2
2009	0.77	12.04***	5.21	0.60
2010	0.79	14.18***	6.15	0.62
2011	0.80	18.74***	10.33	0.65
2012	0.79	16.45***	16.45	0.63
2013	0.91	6.67***	6.38	0.82
2014	0.89	12.03***	4.15	0.79
2015	0.82	13.25***	7.08	0.67
2016	0.55	4.40***	8.94	0.31

Table 3: Multiple correlation coefficient reflecting the relationship of HMW, LMW and the content of crude protein with the sedimentation value of wheat varieties.

*** P < 0.001.

The values of the multiple correlation coefficient R (from 0.55 to 0.91) and the high degree of proof of criterion F (P < 0.001) are indicative of the presence of a well-expressed complex relationship of HMW, LMW and crude protein with sedimentation value. The multiple determination coefficients R^2 (%) indicate that 60 to 82% (ex-

cluding 2016) of variation in the sedimentation value of the analyzed wheat samples is due to the high- and low-molecular-weight glutenin alleles and the crude protein. On average over the period 2009-2016, HMW (Glu-1) controlled 21.99% of the variation in the sedimentation value of wheat samples. In all years, a significant share of this control is attributed to the Glu-A1 and Glu-B1 loci and less to the Glu-D1 locus (2.65%). LMW (Glu-3) accounted for 29.78% of the sedimentation. According to the effect on this trait the LMW loci are arranged in the following order: Glu-B3 > Glu-A3 > Glu-D3 during the analyzed period (Table 4).

During the study period HMW + LMW are responsible for 51.77% of the variation in sedimentation, and when added to the influence of the crude protein, this percentage increases to 63.75. The rest (36.25%) of the sedimentation control is due to other factors (Table 4).

Year	HMW				LMW				HMW+ LMW	Protein	Glu+ Protein	Others
	Glu A1	Glu B1	Glu D1	Glu 1	Glu A3	Glu B3	Glu D3	Glu3				
2009	7.62^{b}	5.63^{b}	4.86	18.11^{c}	7.47^{b}	13.71^{c}	1.67	22.85^{c}	40.95^{c}	18.85^{c}	59.80^{c}	40.20
2010	7.37^{c}	5.93^{c}	5.13^{a}	18.43^{c}	14.29^{c}	8.09^{a}	2.04	24.42^{c}	42.85^{c}	19.05^{c}	61.90^{c}	38.10
2011	13.1^{c}	7.03^{c}	3.53^{b}	23.65^{c}	7.27^{c}	9.66^{c}	9.75^{c}	26.68^{c}	50.33^{c}	14.47^{c}	64.80^{c}	35.20
2012	14.70^{c}	6.87^{c}	3.56^{b}	25.13^{c}	6.57^{c}	11.64^{c}	10.43^{c}	28.63^{c}	53.76^{c}	9.33^{c}	63.09^{c}	36.90
2013	16.64^{a}	15.05^{a}	2.51	34.2	8.30	23.58^{b}	2.33	34.21^{b}	68.41^{c}	13.70^{a}	82.11^{c}	17.89
2014	8.89^{a}	24.41^{c}	0.00	33.3^{c}	12.19^{a}	17.65^{c}	4.87^{a}	34.71^{c}	68.01^{c}	11.49^{a}	79.50^{c}	20.50
2015	5.08^{c}	2.82^{a}	1.60	9.50^{c}	14.56^{b}	38.46^{c}	3.29	56.31^{c}	65.81^{c}	1.69	67.50^{c}	32.50
2016	12.95^{b}	0.67	0.00	13.62^{b}	6.86^{b}	2.26	1.26	10.38^{a}	24.00^{b}	7.30^{b}	31.30^{c}	68.70
Average	10.79	8.55	2.65	21.99	9.69	15.63	4.46	29.40	51.77	11.99	63.75	36.25

Table 4: Proportion of HMW, LMW and crude protein in the formation of the sediment value of wheat varieties - 2009-2016.

$^{a, b, c}$ = Proof of the correlations at P < 0.05; 0.01; 0.001 respectively.

HMW, LMW and crude protein/valorimetric value

The multiple correlation coefficients expressing the complex relationship of HMW, LMW and crude protein with the valorimetric value of wheat samples have very good statistical evidence (Table 5).

Year	R	F-criterion	SE	R^2
2009	0.62	5.73***	6.56	0.39
2010	0.65	7.84***	4.64	0.42
2011	0.75	13.06***	9.94	0.56
2012	0.65	6.40***	13.68	0.43
2013	0.86	8.37***	6.45	0.75
2014	0.62	3.10**	8.38	0.39
2015	0.64	5.98***	10.17	0.41
2016	0.52	4.79***	8.28	0.27

Table 5: Multiple correlation coefficient reflecting the relationship of HMW, LMW and the content of crude protein with valorimetric value of wheat varieties.

** P < 0.01, *** P < 0.001.

Their values range from average in 2016 to high in 2013. This also reflects on the multiple determination coefficients - R^2. Apparently, the years have a certain effect on the interconnections between the biochemical and technological parameters studied.

It was found that on average for the period 2009 - 2016, HMW and LMW control a total of 35.57% of the variation in the valorimetric value of wheat samples. With the inclusion of crude protein this percentage increased to 45.15. HMW account for 16.48% of the value of the valorimetric index. In the individual years, there is no unidirectionality in the ordering of the three Glu-1 loci, but the Glu-A1 and Glu-B1 loci have a significant influence on the indicator over the study period and to a lesser extent Glu-D1 locus (Table 6).

HMW control 19.09% of the valorimetric value. Glu-A3 and Glu-B3 loci account for a significant proportion of this control. The influence of Glu-D3 is weak and insignificant in some years. The control of the other factors is significant - 54.85% (Table 6).

Year	HMW				LMW				HMW+ LMW	Protein	Glu+ Protein	Others
	Glu A1	Glu B1	Glu D1	Glu 1	Glu A3	Glu B3	Glu D3	Glu 3				
2009	6.32[b]	4.42[b]	4.01	14.75[c]	12.62[c]	3.48	1.91	18.01[c]	32.76[c]	5.84[a]	38.60[c]	61.40
2010	0.51	2.38[a]	5.79[b]	8.68[b]	4.39[a]	6.31[a]	0.48	11.18[b]	19.86[c]	22.04[c]	41.90[c]	58.10
2011	11.12[c]	6.12[c]	5.11[c]	22.35[c]	8.68[c]	5.28[c]	7.68[c]	21.64[c]	43.99[c]	12.21[c]	56.20[c]	43.80
2012	12.10[c]	3.28[b]	1.10	16.48[c]	4.26[b]	7.12[c]	6.63[c]	18.01[c]	34.49[c]	8.11[b]	42.60[c]	57.40
2013	2.56	33.14[c]	1.77	37.47[b]	19.45[b]	12.58	2.34	34.37[b]	71.84[c]	2.66	74.50[c]	25.50
2014	0.98	3.76	1.05	5.79	12.18	8.57	0.00	20.75	26.54	12.46[b]	39.00[b]	61.00
2015	6.28[b]	6.91[b]	0.92	14.11[c]	6.15[a]	12.2[b]	0.00	18.35[b]	32.46[c]	8.54[b]	41.00[c]	59.00
2016	3.95[b]	5.36[b]	2.86	12.17[c]	3.15	6.51[b]	0.74	10.40[b]	22.57[c]	4.83[a]	27.40[c]	72.60
Average	5.48	8.17	2.83	16.48	8.86	7.76	2.47	19.09	35.56	9.59	45.15	54.85

Table 6: Proportion of HMW, LMW and crude protein in the formation of the valorimetric value of wheat varieties - 2009-2016.

[a, b, c] = Proof of the correlations at P < 0.05; 0.01; 0.001 respectively.

HMW, LMW and crude protein/bread loaf

The multiple correlation coefficients (Table 7) expressing the complex relationship of HMW, LMW and crude protein with the bread loaf have very good statistical evidence (R = 0.46 - 0.85, F = 2.99 ** - 7.24 ***). Over the years, there is a well-defined complex relationship (R) between the glutenins and the crude protein on the one hand and bread loaf on the other hand which ranged from R = 0.46 in 2015 to R = 0.85 in 2013.

Year	R	F-criterion	SE	R2
2009	0.66	6.24***	53.91	0.43
2010	0.62	5.39***	34.08	0.38
2011	0.64	7.06***	46.10	0.41
2012	0.57	5.91***	46.15	0.32
2013	0.85	7.24***	44.27	0.72
2014	0.62	3.06**	47.57	0.39
2015	0.46	2.99**	52.83	0.21
2016	0.52	5.69***	49.33	0.27

Table 7: Multiple correlation coefficient reflecting the relationship of HMW, LMW and the content of crude protein with bread volume of wheat samples.

** P < 0.01, *** P < 0.001.

However, in some years this correlation is manifested to a much lower extent due to the strong influence of other factors on this indicator (60.7%) (Table 8).

For the study period, LMW, HMW, and crude protein controlled 39.30% of the variation in bread loaf. In 2013, when control of the other factors was lowered, HMW + LMW + crude protein (71.7%) had a significant impact on the bread loaf (Table 8). Unlike sedimentation and valorimetric value, the proportion of LMW is significantly higher - 22.26%, compared to 9.91% for HMW. This finding is one-way for all years 2009 - 2016.

Discussion

In the present study, it was found that HMW and LMW had almost the same share in controlling the main indicators related to gluten strength - sedimentation value and valorimetric value. Similar equivalence of the two glutenin groups in the sedimentation control and other qualitative indicators are also indicated by [22,28]. The association of these proteins with quality indicators is determined mainly by their molecular weight and by the ability of their subunits to participate in the polymerization process, which plays a major role in the formation of gluten with good physical properties [12,38]. HMW have an advantage in this regard [29].

The higher ability of high molecular weight subunits to form polymers is due to their structure.

On the other hand, the amount of storage proteins is of great importance for their effect on gluten strength. As mentioned, LMW have roughly the same proportion with HMW in controlling the basic qualitative indicators (sedimentation and valorimetric

Year	HMW				LMW				HMW+ LMW	Protein	Glu+ Protein	Others
	Glu A1	Glu B1	Glu D1	Glu 1	Glu A3	Glu B3	Glu D3	Glu 3				
2009	2.32	1.21	1.72	5.25	12.06^{c}	10.53^{a}	3.66^{a}	26.25^{c}	31.50	12.00^{c}	43.50^{c}	56.50
2010	2.08	0.84	7.08^{a}	10.00^{a}	10.37^{c}	5.66^{a}	0.00	16.03^{b}	26.03^{c}	12.17^{c}	38.20^{c}	61.80
2011	4.86^{b}	2.05^{a}	2.76^{a}	9.67^{b}	4.67^{b}	9.33^{c}	5.05^{c}	19.05^{c}	28.72^{c}	12.18^{c}	40.90^{c}	59.10
2012	7.24	0.69	1.03	8.96	6.98	7.82	3.36	18.16	27.12	5.48^{b}	32.60	67.40
2013	20.16^{a}	9.24	0.00	29.4^{a}	4.21	35.49^{b}	0.00	39.70^{b}	69.10^{c}	2.60	71.70^{c}	28.30
2014	2.09	6.97^{a}	0.00	9.06	13.08^{a}	1.66	3.31	18.05	27.11^{a}	11.59^{a}	38.70^{c}	61.30
2015	0.40	1.24	0.81	2.45	4.86	10.88	1.62	17.36^{a}	19.81^{a}	1.52	21.33^{b}	78.70
2016	0.00	0.00	3.99	3.99	4.33^{a}	19.18^{c}	0.00	23.51^{c}	27.50^{c}	0.00	27.50^{c}	72.50
Average	4.89	2.17	2.78	9.91	7.57	12.57	2.13	22.26	32.11	7.19	39.30	60.70

Table 8: Proportion of HMW, LMW and crude protein in the formation of the bread loaf of wheat varieties - 2009-2016.

$^{a, b, c}$ = Proof of the correlations at $P < 0.05$; 0.01; 0.001 respectively.

value), despite the less favorable structure of their subunits for macropolymer formation. They compensate for this deficiency by about three times the amount with which they participate in the glutenin complex compared to HMW [12]. The results show that the involvement of the individual glutenin loci in the control of quality indicators is mainly determined by their genetic diversity. An example of this is the arrangement of HMW loci according to their effect on quality - Glu-A1 > Glu-B1 > Glu-D1. The locus with the highest genetic diversity, Glu-A1 (H = 60) has the largest share in the control of the quality indicators. The locus with the lowest genetic diversity - Glu-D1 (H = 0.27) had the least influence on the quality indicators. The genetic diversity, however, cannot be taken as an absolute indicator of the involvement of the relevant glutenin locus in quality control. It is known that a significant proportion of glutenin alleles are equivalent or similar in their effect on quality indicators, such as HMW alleles that determine the expression of subunits 1 and 2 * of the Glu A1 locus, at 7 and 6 + 8, at 7 + 8 and 17 + 18 from the Glu B1 locus and others [31].

Along with the glutenins, the amount of crude protein has a significant impact on the quality of the wheat varieties studied. Its relative involvement in quality control has been very variable over the years. A similar variation in the relative proportion by which the crude protein influences the different qualitative parameters is indicated by other authors [22,36]. In addition [18] state that the effect of varying HMW alleles depends on the amount of crude protein in the flour. The authors cited above found a difference in the bread loaf of wheat groups containing the opposite of their effect on the quality of the 5 + 10 and 2 + 12 fractional pairs at a protein content of 9.2 to 14%. The 5 + 10 fraction does not have a positive effect on quality beyond the specified limits.

The results of this study confirm the need for a comprehensive approach that, in addition to HMW glutenins, also includes the use of LMW glutenins as genetic markers, both in the evaluation of the source breeding material and in the breeding process. The optimal amount of crude protein is a necessary condition for the positive effect of glutenin subunits on the quality of wheat samples.

Conclusion

The results of the present study show that, by means of multiple correlations, a well-expressed complex association of glutenins and crude protein was established with some basic qualitative indicators.

HMW, LMW and crude protein control the following in total:

- About 64% of the sedimentation value, of which 52% from glutenins and 12% from crude protein;
- About 45% of the valorimetric value, of which 36% from glutenins and 9% from crude protein;
- About 39% of the bread loaf, of which 32% from glutenins and 7% from crude protein. HMW and LMW have been found to have almost the same share in controlling the main indicators related to gluten strength - sedimentation value and valorimetric value.

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