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## **GLUTENIN COMPOSITION OF WINTER WHEAT VARIETIES BRED IN DOBRUDZHA AGRICULTURAL INSTITUTE**

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### **Abstract**

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A total of 70 bread wheat varieties bred in Dobrudzha Agricultural Institute (DAI), Bulgaria were characterized for the composition of high-molecular-weight and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS, respectively) using SDS-PAGE. Twenty-five different HMW-GS patterns were observed. The most frequent combination was 2\*, 7+9, 5+10 (23.76%) and N, 7+9, 5+10 (14.85%). Twenty-eight different LMW-GS patterns were established as *Glu-A3c*, *Glu-B3b*, *Glu-D3c* and *Glu-A3e*, *Glu-B3b*, *Glu-D3c* being the most frequent. The average *Glu-score* of the investigated wheat varieties was comparatively high (8.0). One of the reasons for the high score was the gradually removing of subunit 2+12 at locus *Glu-D1*, which effect on bread making quality was negative. The potential of locus *Glu-D1* was still unused for improving end-use quality of wheat and for broadening the genetic diversity of germplasm. The established database describing the HMW and LMW-GS of DAI bread wheat varieties may be useful for breeders who want to use Bulgarian germplasm in their breeding programs.

*Key words:* HMW-GS, LMW-GS, bread wheat, DAI varieties

*Abbreviations:* DAI - Dobrudzha Agricultural Institute, HMW-GS – high-molecular-weight glutenin subunits; LMW-GS - low-molecular-weight glutenin subunits; SDS-PAGE - Sodium Dodecylsulphate PolyAcrylamide Gel Electrophoresis

### **Introduction**

Wheat storage proteins (gliadins and glutenins) are the main components of gluten, which is the main contributor to the rheological and bread-making properties of wheat flour. Glutenin proteins are divided into two groups: High and Low Molecular Weight Glutenin Subunits (HMW-GS and LMW-GS, respectively).

The genetics and biochemistry of HMW-GS in wheat is very well known now (Shewry et al., 1992).

The results have shown that three loci coding for HMW-GS are highly polymorphic in nature without the influence of environment (Lagudah et al., 1987). Considering these properties, allelic variation at the three loci coding for HMW-GS has been used either independently or in combination with other genetic markers to estimate the genetic variation in different wheat species and wheat cultivars from different countries (Nevo and Payne 1987; Buhraei et al., 2004; Bushehri et al., 2006; Sultana et al., 2007; Tohver,

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2007).

HMW subunits of wheat glutenin have become the most intensively studied group of wheat proteins despite of the fact that they account for only about 10% of the total proteins in the mature grain (Payne et al., 1980). The allelic variation in the polypeptide composition of HMW subunits was closely correlated with the bread making quality (Payne, 1987). These observations have since been confirmed and extended and are now routinely used in plant breeding programs (Shewry et al., 2001).

The LMW-GS represent about one-third of the total seed protein and nearly 60% of total glutenins (Bietz and Wall, 1973). But they have received less research attention than HMW-GS mainly because of difficulty in their identification. It has been reported a close linkage between the *Glu-3* loci encoding LMW-GS and the *Gli-1* loci for gliadins (Singh and Shepherd, 1988; Pogna et al., 1990). Because the gliadin composition can be screened more readily than specific LMW-GS, the gliadins are potentially useful as indicators of LMW-GS (Jackson et al., 1996). A number of studies have revealed that the allelic variation at the LMW-GS loci is associated with significant differences in dough quality in bread (Gupta et al., 1989, 1994) and durum wheat (Pogna et al., 1990; Ruiz and Carrillo, 1993).

The aim of this study was to discriminate the wheat varieties, bred in Dobrudzha Agricultural Institute, based on allelic variation and subunit composition of HMW and LMW glutenins.

## Material and Methods

Seventy winter wheat varieties (*Triticum aestivum* L.), bred in Dobroudja Agricultural Institute for the period 1967 till 2007, were included in the investigation. Some of the varieties were heterogeneous for high- and low-molecular weight glutenins and the *Glu-score* was given to the prevailed biotype, according to Ivanov et al. (2000) (Table 1).

Extraction of storage proteins was done on single seeds (10 to 40 for a variety) following sequential procedure (Singh et al., 1991). Electrophoresis of

reduced and alkylated proteins (HMW-GS and LMW-GS) was performed on SDS-PAGE (sodium dodecylsulphate polyacrylamide gels) according to Payne et al. (1980).

High-molecular weight glutenin subunits (HMW-GS) were determined according the nomenclature of Payne and Lawrence (1983) and was given score by Payne et al. (1987).

Low-molecular weight glutenin subunits (LMW-GS) were determined by Gupta and Shepherd (1990) and Jackson et al. (1996).

Biotypes of varieties differing in glutenin composition were treated as separate cultivars when HMW-GS proportion and different HMW and LMW patterns were determined.

## Results and Discussion

For the 40-year winter wheat breeding period in Dobrudzha Agricultural Institute high-molecular-weight glutenin composition of cultivars was not an end in itself but a consequence of the efforts to breed cultivars with high productivity and quality. This explained to a certain extent comparatively low diversity in glutenin subunits. The other reason was using Bezostaya 1 as a parent in pedigree of the most varieties which was a prerequisite for their high to moderate quality traits.

In our investigation 25 different HMW patterns were determined. The most frequent was 2\*, 7+9, 5+10 (23.76%) followed by N, 7+9, 5+10 (14.85%). The other patterns shared among 0.99 to 6.93%. Ivanov et al. (2000) received similar results as they found 25.8% frequency for 2\*, 7+9, 5+10 and 11.9% for N, 7+9, 5+10 in 50 Bulgarian wheat varieties. The pattern 2\*, 7+9, 5+10 was also determined as most frequent in American hard red winter wheat (HRWW) varieties (Graybosch, 1992), in 1 267 CIMMYT bread wheats (Trethowan et al., 2001). Tohver (2007) observed as most frequent patterns 2\*, 7+9, 5+10 and 1, 7+9, 5+10 when studying Estonian winter wheat together with Nordic and Middle European wheats. Pattern N, 7+9, 5+10 was observed as most frequent in Austrian wheats (Groger

**Table 1**  
**Diversity of Glu-1 loci, score and pedigree of DAI wheat varieties**

Variety	Pedigree	Year of release	HMW-GS			Score
			<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>	
<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>
Er. 19-16	C 134 / San Pastore	1967	2*	7+9	2+12	7
Roussalka	C-13 / BAN 54	1971	N	7+9	2+12	5
(1)* Levent	Bezostaya 1 / Fiorello	1974	1	7+9	5+12	7
(2) Levent			2*	7+8	5+12	
(3) Levent			N	7+9	5+10	
(4) Levent			1	7+9	2+12	
(5) Levent			1	7+9	5+10	
(6) Levent			1	6+8	5+12	
(1)* Ludogorka	Bezostaya 1 / Etoal de Choasi	1974	N	7+9	5+10	7
(2) Ludogorka			2*	7+9	5+10	
(1)* Rubin	NS 175/2 / Bezostaya 1	1976	N	7+9	5+10	7
(2) Rubin			2*	7+9	5+10	
(3) Rubin			N	7+9	2+12	
Slavyanka	NS 313 / Bezostaya 1	1976	2*	7+9	5+10	9
Yubiley	Flenshman / Bezostaya 1	1976	2*	7+9	5+10	9
(1)* Kremena	894/Campadorro// Bezostaya 1	1977	N	7	5+10	6
(2) Kremena			N	7+9	5+10	
(3) Kremena			N	7	2+12	
(1)* Zlatoklas	894/ *2 Bezostaya 1// NS 171/2	1977	2*	6+8	5+10	7
(2) Zlatoklas			2*	6+8	2+12	
Kiten	Libellula/Bezostaya 1//NS 171/2 /3/19-45	1977	2*	7+9	2+12	7
Vega	25-66/301 / C-13 // NS 314	1977	N	7+9	2+12	5
Vratsa	Bezostaya 1 / Елия	1977	N	6+8	5+10	6
(1)* Trakya	Rumelia / Roussalka	1978	N	7+9	5+10	7
(2) Trakya			N	6+8	5+10	
(3) Trakya			N	6+8	2+12	
(4) Trakya			N	7+9	2+12	
Dobroudja 1	Libellula/ Bezostaya 1	1978	2*	7+9	5+10	9
Kaliakra 2	Ibo/La Prevision// Bez. 1/Skorosp. 35	1978	2*	7+9	2+12	7
(1)* Ogosta	№ 234 / C-13 // Bezostaya 1	1979	1	7+8	5+10	10
(2) Ogosta			2*	7+8	5+10	
Kubrat 1	Langedok / Er. 19-16 // Aurora	1979	2*	7+9	2+12	7
Toshevka	NS 314 / Bezostaya 1	1979	N	7+9	5+10	7
Charodeika	Roussalka / Rannaya 12 // Zlatna dolina	1980	1	7+9	2+12	7

Table 1 (continued)

1	2	3	4	5	6	7
(1)*Kardam	Er. 19-16-14/ NS 171/2 // Aurora	1981	1	7+9	5+10	9
(2) Kardam			2*	7+9	5+10	
(1)*Skitia	Sonalika 64/Nainari 60//Zlatna dolina	1981	1	7+9	2+12	7
(2) Skitia			N	6+9	5+10	
(3) Skitia			N	6+8	5+10	
(4) Skitia			N	7+9	5+10	
Pliska	Wisconsin 5517-5-5-1p3 / Roussalka	1981	N	7+9	5+10	7
(1)* Kaloyan	Snem / Aurora // Roussalka	1982	1	7	2+12	6
(2) Kaloyan			2*	7+9	2+12	
(1)* Trayana	Ogosta / 50-17 // Aurora	1982	N	6+8	5+10	6
(2) Trayana			N	7+9	2+12	
Rekviem	NS 171/2/Ск. 35// Русалка/Зл. Долина	1982	1	7+9	2+12	7
(1)* Krapets	323 / 3165-7 // 997-2 / 5864-4	1983	2*	7+9	5+10	9
(2) Krapets			2*	6+8	5+10	
(3) Krapets			N	6+8	5+10	
(1)* Yantar	Aurora / Era	1983	1	7+8	5+10	10
(2) Yantar			1	7+9	5+10	
(3) Yantar			2*	7+8	5+10	
(4) Yantar			2*	7+9	5+10	
Zagore	Wisconsin 5517-5- 5-1p3 / Roussalka	1983	1	6+8	2+12	6
Prostor	Roussalka / Rannaya 12 // Nadadores 63	1986	2*	7+8	2+12	8
Yasen	Kiten / Kardam	1988	2*	7+9	5+10	9
Pryaspa	Aurora / Era	1988	1	7+8	5+10	10
Slavyanka 196	NS 313 / Bezostaya 1	1988	2*	7+9	5+10	9
Dobrotitsa	2558-128 / 4199 // 5460-1039	1992	1	7+9	5+10	9
Shabla	2350-60 / 5460-845 // 3387-121	1993	N	7+9	5+10	7
(1)* Svilena	863-A-31 / Super Zlatna // 76-24-5	1994	1	7+9	5+10	9
(2) Svilena			1	7+9	2+12	
Milena	203-238 / Vedrina // 593-51	1996	2*	7+9	5+10	9
Perla 2	Olesen dwarf / Odesskaya 16 // Promin	1996	2*	7+9	5+10	9
Todora	11-57 / Trayana	1996	1	7+9	2+12	7
Zora	GP 2558-128/5517-A- 5-5-1p3/Roussalka	1996	2*	7+9	5+10	9

Table 1 (continued)

1	2	3	4	5	6	7
Kristal	F 9-75/ 3837-5-5 // 7523-11	1996	N	7+9	5+10	7
Preslav	Armada/3/2350-60/	1996	2*	7+9	5+10	9
Laska	F 2498-W1-2 / Obrii	1997	N	7+8	5+10	8
Albena	selection from F 1959 W1-2	1997	N	7+8	5+10	8
Dobroudjanka	Pliska/2* Albidum 114	1997	2*	7+9	5+10	9
Aglika	GP 2558-128 / Pliska	1997	2*	7+9	5+10	9
Elitsa	Yznaya zarya / Pliska	1997	2*	7+9	5+10	9
Enola	Kiten/4360-5//Trakya /3/ Hersonskaya 552	1997	1	7+9	5+10	9
Galateya	504-18/Libellula// Bezostaya 1/3/ Pliska	1999	N	7+9	5+10	7
Ideal	M 51/112 (Bez1) / M 524 (Sadovo 1)	1999	N	7+9	5+10	7
Karat	Bezostaya. 1/Zl dolina// Sava/3/371-2023	1999	N	7+9	2+12	5
(1)** Mizia	Vratsa / Shipka	2000	N	6+8	5+10	6
(2) Mizia			N	7+9	5+10	7
(1)** Progress	Shabla / 1027-1	2000	2*	7+9	5+10	9
(2) Progress			2*	7+9	2+12	7
(1)** Ludogorie	So 290/576 /4888-682 //	2000	2*	7+9	2+12	7
(2) Ludogorie	Pliska /3/Madara		N	7+9	5+10	7
Iveta	Obrii / Mironovskaya 61	2000	N	7+8	5+10	8
Slaveya	GP 2558-128 / *2 Pliska	2000	2*	7+9	5+10	9
Liliya	Pryaspa / Mironovskaya 27	2000	2*	7+9	5+10	9
Ktisti	Miryana / Pryspa // Enola	2003	2*	7+9	5+10	9
Zlatitsa	Er. 10071 / Pryaspa	2003	1	7	5+10	8
Zlatina	Obrii / 129-171-1	2003	2*	7+8	5+10	10
(1) *Antonovka	Fuss / *2 Pryspa	2004	1	7+8	5+10	10
(2) Antonovka			2*	7+8	5+10	10
Karina	Antilema 1-8 / 2* Pliska	2004	N	7+9	5+10	7
Bolyarka	Pryaspa / F 2076 W2-11	2004	1	7+8	5+10	10
Neda	GP 2558-128//Sadovo 1/Roason	2004	1	7+8	5+10	10
Demetra	Er. 1613/86 / Эп. 103//	2004	2*	7+9	5+10	9
Anna	Lepoklasa x Pryaspa	2005	2*	13+16	5+10	10
Antitsa	627/86-9-14 x F 302- K4 -221	2006	2*	7+9	5+10	9
Korona	Pryaspa/Olvia // Manital/Flamura 80	2006	N	7+8	2+12	6
Lazarka	Una / Flamura 85	2007	2*	7+8	5+10	10
Dona	Milena x Lut.1210KG-10977-20	2007	2*	7+9	5+10	9
Merilin	Lut. 598kG2852-21/5 IWWSN-T-118-12	2007	2*	7+8	5+10	10

\*prevailed biotype (Ivanov et al., 2000)

\*\*undetermined prevailed biotype

**Table 2**  
**Diversity of Glu-3 loci for LMW-GS in the DAI wheat varieties**

Variety	Number	LMW-GS		
		<i>Glu-A3</i>	<i>Glu-B3</i>	<i>Glu-D3</i>
Lazarka	1	b	b	c
Levent (5), Slavyanka, Yubiley, Zlatoklas, Kiten, Vratsa, Trakya (1), Kaliakra 2, Toshevka, Prostor, Yasen (1), Perla 2, Zora, Preslav, Aglika, Elitsa, Progres, Iveta, Slaveya, Kristi (1), Merilin	21	c	b	c
Er. 19-16, Roussalka, Levent (3), Vega, Trakya (2), Kaloyan (2), Trayana	7	c	h	c
Kubrat 1, Kardam (2), Skitia (1), Slavyanka 196, Ideal	5	c	f	c
Levent (6), Skitia (2), Ludogorie (2), Zlatina, Demetra,	5	c	b	a
Charodeika, Pliska, Dobrotitsa (1), Galateya, Karina	5	c	g	a
Kaloyan (1), Shabla (1), Enola, Karat, Ludogorie (1)	5	c	j	c
Levent (1), Rubin (2), Dobroudja 1	3	c	i	c
Levent (2), Kremena	2	c	i	a
Rekviem, Shabla (2)	2	c	j	a
Dobrotitsa (2), Dobroudjanka	2	c	g	c
Zagore	1	c	h	a
Todora	1	c	f	a
Levent (4), Ludogorka, Mizia	3	d	b	c
Rubin (3)	1	d	i	c
Laska (1)	1	d	h	c
Ogosta (1), Yantar, Pryaspa, Kristi (2), Antonovka, Bolyarka, Neda, Anna, Korona	9	e	b	c
Krapets (3), Svilena	2	e	j	c
Rubin (1)	1	e	i	c
Ogosta (2)	1	e	h	c
Zlatitsa	1	e	d	c
Kristal	1	e	j	a
Krapets (1)	1	e	b	a
Kardam (1), Krapets (2)	2	f	b	c
Yasen (2), Albena, Liliya, Antitsa	4	f	j	c
Milena	1	f	h	c
Laska (2)	1	f	g	c
Dona	1	f	f	c

et al., 1997), in Slovak varieties (Gregova et al., 2007), in Serbian wheats (Dencic and Kobiljski, 2008).

Ivanov et al. (2000) observed 26 heterogeneous for HMW-GS varieties. In seed production process some of these varieties have been made uniform and

homogeneous according to the recommendation of Todorov et al. (2002). Such varieties were Prostor, Roussalka, Yasen, Kiten, Slavyanka196, Toshevka and Vratsa. According to Pogna et al. (1989) 10.8% of Italian wheat varieties were heterogeneous for their high-molecular composition. For Yugoslavian wheats this percent was 15 (Vapa, 1989). Heterogeneity gave the possibility to better bread making qualities by choosing biotype with the best combination of glutenins (Lawrence et al., 1987).

In the studied collection of DAI wheat varieties in *Glu-A1* locus three alleles were determined *a*, *b*, *c* (subunits 1, 2\* and N). The biggest share was for 2\*, followed by N and 1 (Figure 1). The most diverse was *Glu-B1* locus, where 6 alleles were found. Allele *c* (subunits 7+9) had the biggest part 64.4%, the next was allele *b* (subunit 7+8) - 17.8% and allele *d* (subunit 6+8) - 11.9%. In *Glu-D1* locus 3 alleles were observed - *d* (subunit 5+10) with 71.3% share, allele *a* (subunit 2+12) with 25.7% share and rare allele 5+12 with 2.97%.

Distribution and frequency of glutenin alleles was dependent on traditions of using wheat in different countries, on breeding aims of different research programs, and on used parental material as well. That's why when breeding was directed towards increasing bread-making qualities and traditions were to use wheat mainly for bread, there was concentration of "strong" alleles, which positively influenced these traits.

Ukrainian and Russian wheat were good illustration for that. The frequency of 2\* in *Glu-A1* locus was more than 70% in these varieties. Subunits 7+9 in *Glu-B1* locus had 85.3 and 80.6% share, subunits 2+12 in *Glu-D1* locus – 52.9 and 52.1%, subunit 5+10 in the same locus had 47.1 and 39.5% share in Ukrainian and Russian varieties, respectively (Rabinovich et al., 1998). In Australian wheat varieties the frequency of subunits 1 and 2\* in *Glu-A1* locus was 47.1 and 45.4%, respectively. In *Glu-B1* the subunits with the highest frequency were 17+18 (39.3%) and 7+8 (36.4%). In *Glu-D1* subunit 2+12 was more often met than subunit 5+10 – 67.8% to 30.5% (Ma et al., 2003). Similar distribution of "strong" subunits was observed in other breeding programs too, mainly in Nordic and Middle European countries (Tohver, 2007). The distribution of glutenin subunits was not the same in wheat varieties used mainly for noodles. Such varieties were these from Asian countries – Japan, China (Nakamura et al. 1999; Nakamura, 2000).

Studying the wheat storage proteins was proofed to be very useful tool not only in observing diversity and variation in different germplasm collections but in breeding varieties with improved bread-making qualities. It is possible to assess these qualities using *Glu-score* of HMW-GS nevertheless that they were complex and strongly influenced by environments. Bulgarian varieties had comparatively high average *Glu-score* (8.0) and it ranged from 5 to 10. It determined the

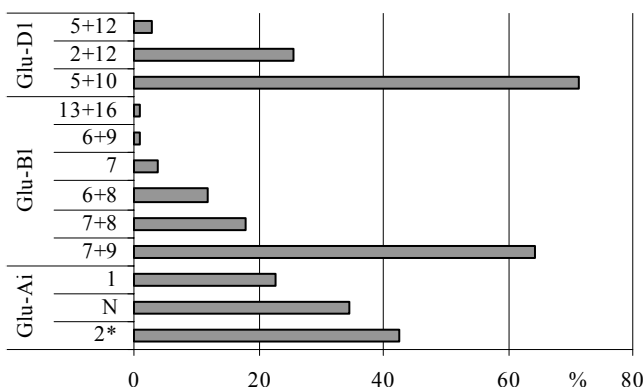


Fig. 1. Distribution of HMW-GS in the DAI winter cultivars, %

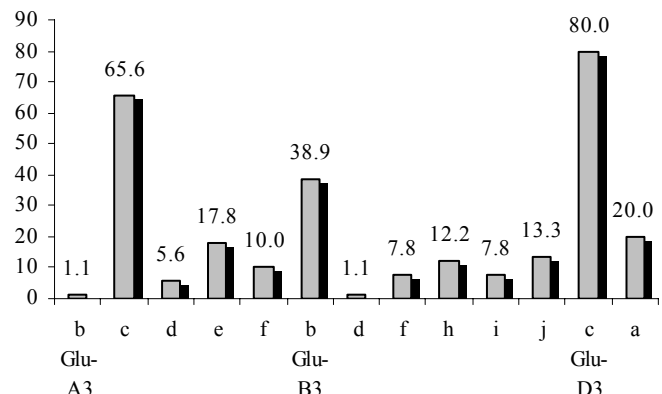


Fig. 2. Distribution of LMW-GS in the DAI winter varieties, % into every locus

good quality of most of the varieties. It was observing increase of average score by decades – in the seventies it was 7.2, in the eighties – 7.9, in the nineties – 8.1 and during the new millennium – 8.0. One of the reasons the *Glu-score* of Bulgarian wheats to increase was the gradual falling away of subunit 2+12 in *Glu-D1* locus, which had negative influence on bread-making qualities.

According to Morgunov et al. (1993) the increasing of *Glu-score* was connected with decreasing the diversity of glutenin alleles. Similar were the conclusions of Tahir et al. (1995), Sultana et al. (2007). It was obvious the weak usage of potential that was hidden in *Glu-B1* locus. The broadening of genetic base and improving of bread-making qualities of cultivars will be achieved when include subunits 17+18, 13+16, 14+15 from *Glu-B1* locus, which positively influenced these qualities (Bahraei et al., 2004; Deng et al., 2005; Liu et al., 2007).

Twenty eight different LMW-GS combinations were observed in the studied winter bread wheat collection (Table 2). The most widely met was *Glu-B3c*, *Glu-B3b*, *Glu-D3c* pattern (23.3%), followed by *Glu-A3a*, *Glu-B3b*, *Glu-D3c* - 10.0%. The other combinations took from 1.11 to 5.56%. The biggest diversity was found in *Glu-B3* locus where 7 alleles were met. In one quarter of combination the allele *Glu-B3b* was found. The alleles *Glu-B3j* and *Glu-B3h* covered 17.9%. The other alleles that were observed in *Glu-B3* locus were *Glu-B3i* (14.3%), *Glu-B3g* (10.7%), *Glu-B3f* (10.7%) and the rarest was found *Glu-B3d* (3.57%). In locus *Glu-A3* were found 5 alleles - *Glu-A3c* (46.4%), *Glu-A3e* (25.0%), *Glu-A3f* (17.9%), *Glu-A3d* (10.7%) and *Glu-A3b* (3.57%). In locus *Glu-D3* the most frequent was allele *Glu-D3c* (71.4%) and allele *Glu-D3a* had 28.6%.

Inside locus *Glu-A3* the biggest part was for subunit *c* - 65.6 %, followed by *e* - 17.8%, while the other were fewer than 10% (Figure 2). In locus *Glu-B3* allele *b* had the biggest share - 39%, followed by almost equal portions of alleles *h* and *j* (12-13 %). In locus *Glu-D3* allele *Glu-D3c* took 4/5 of all cases. Such distribution of low-weight-molecular subunits was mainly due to large-scale usage of cultivar

Bezostaya 1 directly as a parent in eighties and indirectly during last 10 years of XX century. As a result was the highest percent (57.9 %) of *Glu-3* pattern which was the same as in this variety - *c b c*.

Jackson et al. (1996) also observed high diversity in locus *Glu-B3* followed by *Glu-A3* and *Glu-D3* in collection of 49 European wheats. Similar findings had Igrejas et al. (1999) when studying 63 Portuguese bread wheats; Meng et al. (2007) in collection of 41 spring wheats. When observing 55 American wheat varieties with white kernel Redaelli et al. (1997) founded the biggest variation in *Glu-A3* locus. Zhang et al. (2008) determined as most frequent allele *Glu-A3d* (45.7%), followed by *Glu-A3a* (24.3%) and *Glu-A3c* (21.4%) when examined 70 Chinese varieties and lines.

## Conclusion

In the studied collection of Bulgarian winter bread wheat varieties, bred in Dobrudzha Agricultural Institute, the widespread high molecular weight patterns were 2\*, 7+9, 5+10 and N, 7+9, 5+10 and for low molecular patterns these were *Glu-A3c*, *Glu-B3b*, *Glu-D3c* and *Glu-A3e*, *Glu-B3b*, *Glu-D3c*. HMW-G subunits 2\*, 7+9 and 5+10 had the biggest share. The most frequent LMW glutenin alleles were *Glu-A3c*, *Glu-B3b* and *Glu-D3c*. Bulgarian varieties were determined as high qualitative according to the high molecular *Glu-score*. Still unused remain the potential of locus *Glu-B1* for improving genetic diversity of high-molecular glutenins and for improving bread-making qualities of varieties. The attempt to card-index the Bulgarian wheat varieties for their high- and low-molecular glutenins will be useful for breeders who want to use Bulgarian wheat varieties in their breeding programs.

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