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ORIGINAL ARTICLE

Genetic polymorphism of glutenin subunits with high molecular weight and their role in grain and dough qualities of spring bread wheat (*Triticum aestivum* L.) from Northern Kazakhstan

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Abstract

High-molecular weight glutenin (HMWG) subunits are an important component of the gluten protein complex in cereal grains and can influence the baking properties of wheat. The study of polymorphism of glutenin makes it possible to isolate preferred genotypes with higher grain quality. To determine the allelic state of the *Glu-1* loci controlling HMWG in 122 spring bread wheat varieties from Northern Kazakhstan, an SDS-based analysis was performed. The highest frequencies (in percentage) were detected in the alleles *Glu-A1b* (56.1), *Glu-A1c* (42.4), *Glu-B1c* (85.0), *Glu-D1a* (50.6) and *Glu-D1d* (46.9). A technological and baking evaluation of 33 varieties and 40 prospective selection lines on samples of bread wheat with known allelic compositions at the *Glu-1* loci was carried out. Associations between HMWG and several technological traits were established: component 2* (allele *Glu-A1b*) with gluten content and P/L ratio; 7 + 9 components (allele *Glu-B1c*) with valorimetric index; and components 2 + 12 (allele *Glu-D1a*) with the ratio *P/L*. In some cases, discrepancies between the predicted baking qualities based on HMWG subunits and the results of traditional technological and baking evaluations have been revealed.

Keywords Breeding · Genotype · Glutenin · *Glu*-locus · Polymorphism · Wheat

Introduction

The endosperm of bakery wheat contains about 70% starch and 10–15% proteins. These proteins consist of gliadin (about 40%), as well as high-molecular weight (about 10%) and low-molecular weight (30%) glutenins, which are key determinants in the baking quality of wheat (Branlard et al. 2003).

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The high-molecular weight glutenin (HMWG) subunits are controlled by the loci *Glu-A1*, *Glu-B1* and *Glu-D1,* localised in the long arms of chromosomes 1AL, 1BL and 1DL (Anjum et al. 2007). The *Glu-B1* and *Glu-D1* loci encode two types of HMWG: *x*- and *y*-types, with comparatively higher and lower molecular weights, respectively (Caballero et al. 2010), whereas the *Glu-A1* locus controls the synthesis of HMWG *x*-type only. The important role of HMWG in the baking properties of wheat has been shown in many published reports (Liang et al. 2010; Park et al. 2011; Izadi-Darbandi and Yazdi-Samadi 2012; Zaitseva et al. 2017). For example, the combination of HMWG components, *Dx5* and *Dy10*, at the *Glu-D1* locus was associated with superior baking characteristics, including SDS sedimentation and dough quality, compared to HMWG components *Dx2* and *Dy12* (Liang et al. 2010). The improved baking quality was related to HMWG component *Dx5,* with additional residues of cysteine involved in disulphide bridges compared to component *Dx2*. The second component, *Dy10,* contains a longer protein domain with several repeats and more hydrogen bonds, providing a more stable gluten compared to component *Dy12* (Wang et al. 2018). Wheat genotypes with *Glu-D1* allele subunits *Dx2* and *Dy12* were characterised by samples with poor baking qualities (Izadi-Darbandi and Yazdi-Samadi 2012; Goutam et al. 2015). Two new subunits, *1Dy12.6* and *1Dy12.7*, with similar molecular weight and electrical charge to *1Dy12*, were found in wheat germplasms using MALDI-TOF-MS. It was proposed that these two new subunits can improve gluten quality in grains due to their higher number of glutamine residues (Peng et al. 2015).

The locus *Glu-B1* encodes important subunits of *Bx7* HMWG components. Initially, only three alleles of the genes were described, including allele ***a*** for a single component *Bx7*; allele ***b*** for a pair of components, *Bx7* + *By8*; and allele ***c*** for another pair of components, *Bx7* + *By9*. Later, another subunit, *Bx7**, was found which differed from the *Bx7* component in the reference cv. Chinese Spring. The novel allele ***u*** was defined for a pair of components, *Bx7** + *By8* (Espí et al. 2012). Nevertheless, the effect of this *Bx7* component on dough elasticity and extensibility was demonstrated (Butow et al. 2003; Gao et al. 2018). The combination of *Bx20*/*By20* components of HMWG had a negative effect on dough quality (Pirozi et al. 2008; Sissons et al. 2005), and it was further suggested that the *By20* component had the greatest negative effect (Santagati et al. 2016).

Analyses based on DNA diagnostics are the favoured modern methods of plant genetics (Li et al. 2012; Paux et al. 2012; Shavrukov 2016). Nevertheless, the use of SDS-electrophoresis is still very relevant for the study of HMWG (Liu et al. 2010; Gao et al. 2010). The combination of urea/SDS-PAGE electrophoresis has yielded excellent results in the identification of new glutenin subunits (Liang et al. 2010; Xu et al. 2010; Niu et al. 2011; Zhang et al. 2012).

In studies that incorporate wheat from different countries of the world, the obtained data reveal differing distributions of HMWG alleles in wheat samples grown in different climatic conditions (Table 1). This could indicate a possible relationship between baking characteristics and adaptability to climatic conditions.

Extensive information on the identification of wheat HMWG in Europe is available in the review by Tohver (2007) and a publicly available electronic database (Békés and Wrigley 2013). HMWG alleles and their effects on grain quality have been described for durum wheat (Nazco et al. 2014) and in wheat wild relatives (Obukhova 2014; Kozub et al. 2014; Li et al. 2015; Shiferaw and Kassahun 2017). The genetic diversity in spring and winter wheat from Kazakhstan has been reported earlier, but this information is far from complete (Bulatova 1985, Zaitseva et al. 2017). Glutenins and their influence on the baking characteristics of spring bread wheat in Northern Kazakhstan have not been addressed in any published work. Therefore, studies of glutenin polymorphism, as an important factor in dough quality, will help to identify the most valuable genotypes for further crosses. Ultimately, this knowledge can be used to obtain new promising wheat breeding lines with improved grain and baking qualities.

The aims of this study were, therefore, to: (1) identify and determine the allelic variability in the *Glu-1* locus in wheat from Kazakhstan; and (2) study the influence of HMWG alleles on baking quality in the varieties and prospective breeding lines of wheat via measurement of the technological characteristics as described in ISO or other standards.

Materials and methods

Environment and wheat germplasm material

The ecological conditions of Northern Kazakhstan can be characterised as strongly drought affected during most of the vegetative period. This study used accessions of 122 cultivars of bread wheat produced by various breeding institutions in Northern Kazakhstan over different years as well as 40 promising breeding lines selected by the Barayev Research and Production Centre of Grain Farming, in Northern Kazakhstan. Seeds of wheat were provided by the Bread Wheat Breeding laboratory.

Electrophoresis of proteins

Electrophoresis of glutenins was carried out in polyacrylamide gels with a concentrating and separating system (Laemmli 1970). To determine the degree of polymorphism, 100 seeds from each wheat accession were collected. The glutenin was extracted from each individual milled grain by the

Table 1 Variety of glutenin subunits in wheat of different origins

Countries	Glutenin-coding loci			References
	Glu-A1	*Glu-B1*	*Glu-D1*	
European countries				
Baltic countries	Null/1	7 + 9	5 + 10	Johansson et al. (2003)
Bulgaria	2*/null	7 + 9	5 + 10	Tsenov et al. (2009)
Czech Republic	1/null	7 + 9	5 + 10	Bradová and Šašek (2005)
France	Null	7 + 8/6 + 8	2 + 12/5 + 10	Branlard et al. (2003)
Portugal	2*/null	13 + 16	2 + 12	Nascimento et al. (1998)
Serbia	Null	7 + 9	5 + 10	Hristov et al. (2013)
Spain	2*	20	2 + 12	Ayala et al. (2016)
	1/2*	7 + 8	5 + 10	Sanchez-Garcia et al. (2015)
Ukraine	Null/2*	7 + 9/7/6 + 8	5 + 10/2 + 12	Zaika et al. (2014)
	1/2*	7 + 9	5 + 10	Kozub et al. (2014)
Asian countries				
China	1	13 + 16/17 + 18/6 + 16	5 + 10	Li et al. (2009)
	1/null	7 + 8/7 + 9	2 + 12	Novoselskaya-Dragovich et al. (2011)
India	2*/1	17 + 18/7 + 8	2 + 12	Bakshi and Bhagwat (2016)
Iran	2*	7 + 8/17 + 18	2 + 12	Bahraei et al. (2004)
	2*	6 + 8/7 + 8	2 + 12/3 + 12	Chaparzadeh et al. (2008)
	Null/2*	7 + 8/7 + 9	2 + 12/5 + 10	Shahnejat-Bushehri et al. (2006)
Japan	Null	7 + 8	2 + 12/2,2 + 12	Nakamura (2001)
Pakistan	2*	17 + 18	2 + 12/5 + 10	Yasmeen et al. (2015)
Russia	2*/1	7 + 9/7 + 8	5 + 10/2 + 12	Morgunov et al. (1990)
Tajikistan	2*	7 + 9/7 + 8	5 + 10	Mahkamov (2013)
Turkey	2*	7 + 8	5 + 10	Kilic et al. (2017)
North America				
Canada	1/2*	7 + 9/7 + 8	5 + 10/2 + 12	Lukow et al. (1989)
USA	1	7 + 9	2 + 12	Redaelli et al. (1997)
South America				
Argentina	1/2*	7 + 8*/7* + 9	5 + 10	Gianibelli et al. (2002)
Brazil	2*	7 + 9	5 + 10	Costa et al. (2013)
Africa				
Ethiopia	2*	7 + 9	5 + 10	Dessalegn et al. (2011)
Morocco	2*	17 + 18, 7*/7 + 9	5 + 10	Henkrar et al. (2017)
South Africa	1	7 + 8	5 + 10	de Villiers and Bosman (1996)

addition of 250 µl of extraction buffer (Galili and Feldman 1983), followed by incubation for 2 h at room temperature in a 7-ml glass tube with constant stirring. For the extraction, 50 µl of alkylating solution (26% acrylamide) (Bulatova 1985) was added and placed in the bath with boiling water for 2 min. After cooling, 10 µl of the protein extract was separated in 10% polyacrylamide gel by SDS-electrophoresis in Tris–glycine buffer (pH 8.3). The 10% separating gel had the following composition: Tris buffer (pH 8.8) 2.5 ml; acrylamide (30%) 3.0 ml; H_2O 4.29 ml; SDS (10%) 100 µl; ammonium persulphate (10%) 100 µl; and TEMED 20 µl. The stacking gel consisted of: Tris buffer (pH 6.8) 1.0 ml; H_2O 3.145 ml; acrylamide (30%) 750 µl; SDS (10%) 50 µl; ammonium persulphate (10%) 50 µl; and TEMED 20 µl. The lower electrode buffer, for the '+' electrode, contained the following mixture: glycine 38 mmol, Tris 4.9 mmol per 1000 ml. The upper electrode buffer for the '−' electrode contained: glycine 191.8 mmol, Tris 24.7 mmol, and SDS 2.2 mmol per 1000 ml. For the electrophoresis, a vertical chamber (Hiyu Kalur, Estonia) was used, which makes it possible to obtain gel plates of 120 × 70 × 1 mm in size. Electrophoresis was performed at 200 V for 1.5 h. Fixation and staining were performed in 10% trichloroacetic acid with a 0.05% alcohol solution of Coomassie R-250 (Sigma-Aldrich,

Table 2 Scores of glutenin subunits based on their influence to baking characteristics

Score	Glutenin-coding loci, subunits (allele)		
	Glu-A1	*Glu-B1*	*Glu-D*
4	–	–	5 + 10 (***d***)
3	1 (***a***)	17 + 18 (***i***)	–
3	2* (***b***)	7 + 8 (***b***)	–
3	–	13 + 16 (***f***)	–
2	–	7 + 9 (***c***)	2 + 12 (***a***)
2	–	–	3 + 12 (***b***)
1	Null (***c***)	7 (***a***)	4 + 12 (***c***)
1	–	6 + 8 (***d***)	–
1	–	20 (***e***)	–

USA). Identification of glutenin subunits was carried out according to Payne and Lawrence's catalogue (1983). The Chinese Spring wheat variety was used as the standard for the preparation of glutenin genetic formulas. Each glutenin subunit or pair of subunits was assigned a quality score, from 1 to 3 units (loci *Glu-A1* and *Glu-B1*) and from 1 to 4 units (locus *Glu-D1*) (Table 2). A higher score corresponded to improved baking qualities (Lukow et al. 1989). The contributions of all HMWG subunits together to the quality of bread were assessed by the sum of all scores, with a maximum of 3 + 3 + 4 = 10 units.

Biochemical and technological assessment

Protein content was determined in whole grains using an Infrared analyser, InfraLum FT-10 (Russia) following published standards (AACCI 39-25.01 2011), and Bulk density was also estimated in accordance with existing standards (ISO 2003, 2019). The weights of 1000 grains were evaluated as a sum of two portions of 500 grains weighed separately. The gluten content and the gluten deformation index were determined as defined in: (Standard RK 2012).

Dough physical properties were studied on the Chopin Alveograph according to the following characteristics: dough deformation work (*W*) and dough tenacity/extensibility ratio (*P/L*) (ISO 2015). On the Brabender Farinograph, the following indicators were recorded: dough dilution degree (Farinograph units, *FU*); and the valorimetric index, *VI* (ISO 2013). The bread was baked from 100 g of flour and evaluated according to three main parameters: loaf volume, bread form stability and porosity (GOST 1988). As a result, the overall evaluation of baking bread qualities was made with maximum score of 5 units according to the method described earlier (The method 1988).

Statistical analysis

The calculation of intra-population genetic diversity ($\mu \pm S_{\mu}$) was carried out according to Zhivotovsky (1991). The genetic diversity (H) was calculated by the following formula (Nei 1973), where p_i is the frequency of studied alleles:

$$H = 1 - \sum p_i^2.$$

The associations between HMWG alleles and the technological parameters measured were based on contingency 2 × 2 tables with two inputs. The wheat accession and its identified glutenin alleles formed the first input, and the second input was derived from the group of wheat samples that shared the studied trait. The tests for independence or association of glutenin alleles were assessed by three criteria: χ^2 test, the association coefficient, Q, and the contingency (similarity) coefficient, V (Antamoshkin and Bakaeva 2011).

Results

The alleles of the *Glu-1* loci identified in 122 cultivars of wheat from Northern Kazakhstan are presented in the form of genetic formulas (Supplementary material 1). The results of the electrophoretic analysis revealed three, six and four alleles in loci *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. Polymorphic wheat samples represented 21.6% of all analysed accessions. Polymorphic spectra were found in wheat accessions with mixed grains carrying one or more glutenin-encoding loci.

Polymorphism in the *Glu-A1* locus was expressed as a combination of components: 2*/Null in 13 samples, and Null/1 in a single accession among the 122 studied, representing 10.65% and 0.82%, respectively. There were 62 and

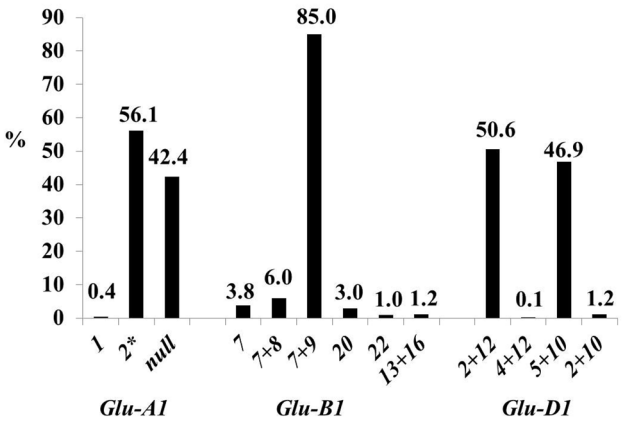

Fig. 1 Frequency of occurrence of protein components of glutenin-encoding loci in studied varieties of spring bread wheat from Northern Kazakhstan

46 samples (50.8% and 37.7%) determined as monomorphic in the components 2* and Null, respectively. Based on the statistical analysis, the frequencies of glutenin allele ***b*** (2*) and ***c*** (Null) were 56.1% and 43.4%, respectively (Fig. 1). The unique allele *Glu-A1a*, encoding component 1, was found in one of the biotypes in breeding line 35.86-94-166.

Polymorphism in locus *Glu-B1* was presented with six combinations of components: three samples were identified with genotypes 7 + 9/7 + 8 (alleles ***c*** + ***b***), two samples with 7/7 + 9 (alleles ***a*** + ***c***). In a few cases, the components of glutenins were found as following: 7 + 9/20 (alleles ***c*** + ***e***), 7 + 9/22/7 (alleles ***c*** + ***k*** + ***a***), 7 + 9/7 + 8/7 (alleles ***c*** + ***b*** + ***a***), and 7 + 9/13 + 16 (alleles ***c*** + ***f***). Allele ***c***, encoding pair components 7 + 9, was found in most cases with a maximum frequency of 84.9% (Fig. 1). The remaining alleles in the studied samples were much less frequent and their frequency did not exceed 6%.

Four alleles were identified in locus *Glu-D1*, ***a***, ***c***, ***d*** and ***e***, encoding the synthesis of HMWG components, 2 + 12, 4 + 12, 5 + 10 and 2 + 10, respectively. Polymorphism was detected in 21 cultivars with alleles ***d*** + ***a*** (components 5 + 10/2 + 12) and accounted for 17.2%. The remaining analysed varieties were monomorphic for two alleles, ***a*** (50.7%) coding for synthesis of the components pair 2 + 12, and ***d*** (47.0%) coding the components 5 + 10 (Fig. 1).

Genetic polymorphism has been previously identified in loci *Gli-A1*, *Gli-D1*and *Gli-A2* in biotypes of varieties Akmola 3 and Lutescens 94 (Utebayev et al. 2016). This fact justified the inclusion of two biotypes from Akmola 3 and five biotypes from Lutescens 94 in the list of studied accessions individually but not as a bulk. These biotypes are also found to differ in their glutenin spectra within each original variety. However, biotype 1 and 4 from Lutescens 94 were similar in glutenin formula, 2*, 7 + 9, 2 + 12, while all other three biotypes in the same cultivar appear to be polymorphic in all three *Glu-1* loci (Supplementary material 1).

In general, 32% of the 122 studied wheat accessions showed the most typical HMWG component formulas, 2*, 7 + 9, 2 + 12. Moderate frequencies (17.2% and 11.4%) were found in studied cultivars with the HMWG components Null, 7 + 9, 5 + 10, and 2*, 7 + 9, 5 + 10, respectively. The HMWG formula Null, 7 + 9, 2 + 12, was identified as the rarest and accounted for just 7.4% of all studied genotypes. The majority of studied spring wheat accessions (46.7%) had a grade of '7' for bread quality score. The maximum quality score recorded was '10', which was found in only two cultivars: Milturum 45 and Karabalykskaya 9; while a quality score of '9' was recorded for 12.3% of the studied wheat accessions.

Based on allele frequencies, the genetic variability (*H*) and intra-population diversity (μ) were estimated for the 122 studied wheat varieties (Table 3). At least one or two alleles were found with a high degree of frequency in each *Glu-1* locus. Three alleles (***a***, ***b*** and ***c***) were identified in *Glu-A1* locus, but only two alleles, ***b*** (0.561) and ***c*** (0.434), were found with maximum frequencies in the studied accessions. The study of *Glu-B1* revealed the presence of six alleles, from which allele ***c*** encoding the synthesis of the HMWG component pair, 7 + 9, occurred with maximal frequency (0.849). In our study, only four alleles out of seven described earlier were identified in the *Glu-D1* locus. The highest frequencies were found in alleles ***a*** and ***d*** of this locus with combinations of the HMWG components 2 + 12 (0.506) and 5 + 10 (0.469), respectively.

Table 3 Allele frequencies, intra-population diversity (μ) and genetic variability (*H*) of HMWG in 122 bread wheat cultivars studied by SDS-PAGE from Northern Kazakhstan

Glutenin-coding loci *Glu-1*	Allele	HMWG component	Frequency	$\mu \pm S_\mu$	*H*
Glu-A1	***a***	1	0.004	2.15 ± 0.122	0.497
	b	2*	0.561		
	c	Null	0.434		
Glu-B1	***a***	7	0.038	3.02 ± 0.271	0.273
	b	7 + 8	0.060		
	c	7 + 9	0.849		
	e	20	0.028		
	k	22	0.010		
	f	13 + 16	0.012		
Glu-D1	***a***	2 + 12	0.506	2.57 ± 0.173	0.523
	c	4 + 12	0.010		
	d	5 + 10	0.469		
	e	2 + 10	0.012		

The intra-population diversity (μ) reached its maximum value (3.02) in *Glu-B1,* with the presence of three very common alleles (***a***, ***b*** and ***c***). Loci *Glu-A1* and *Glu-D1* revealed a similar level of intra-population diversity, 2.15 and 2.57, respectively. However, the opposite results were found for genetic diversity (*H*), which was smallest in *Glu-B1*. It was directly related to a single allele ***c*** in *Glu-B1*, which was widely distributed among the studied wheat accessions. In contrast, several alleles with high frequencies were found in loci *Glu-A1* and *Glu-D1*.

To estimate the influence of HMWG components on technological traits, protein electrophoresis was carried out, and dough and bread baking qualities were evaluated in 40 advanced breeding lines and 33 selected varieties of spring wheat. These results are presented in Supplementary materials 2 and 3. The most appropriate threshold was established for each trait controlling grain quality (The method 1988), including: (1) protein, > 14.0%; (2) bulk density, > 750 g/l; (3) vitreousness, > 60%; (4) gluten content, > 28.0%; (5) GDI, gluten deformation index, within the range 45–80; (6) dough deformation energy, W, > 280 units

of the alveograph; (7) ratio *P/L*, within a range 0.8–1.5; (8) dilution of dough, > 60 units of the Farinograph; (8) valorimetric index, *VI*, > 70; (9) loaf volume, > 650 units; (10) bread form stability, within the range 0.41–0.60 units; (11) porosity, within the range 4.3–5.0 units; (12) bread baking qualities scores, within the range 4.3–5.0 units.

To test associations between allele frequency in loci *Glu-A1*, *Glu-B1* and *Glu-D1* with technological traits of grain and baking qualities, 73 wheat accessions (33 cultivars and 40 advanced breeding lines) were studied, with results presented in Supplementary materials 1, 2 and 3. In general, the most frequent alleles in each gliadin locus were *Glu-A1b*, *Glu-B1c*, *Glu-D1e* and *Glu-D1d* with frequencies of 0.70, 0.72, 0.54 and 0.45, respectively. It was revealed that the most commonly distributed alleles of gliadins were present in wheat accessions with both high and low grain and dough qualities. Regarding the χ^2 criterion, the only strong association was found between glutenin components and the three grain and dough characteristics, gluten content, *P/L* ratio and VI (Table 4).

Discussion

The results obtained in this study can be applied for the strategic selection of wheat genotypes with valuable baking qualities and combinations of glutenin alleles. Among the 122 studied bread wheat varieties in Kazakhstan, not all of them were widely used in grain production. However, this study represents an important overview of work how breeders' preferences in wheat grain and dough quality traits can affect the distribution of *Glu-1* alleles. The characteristics of each glutenin-encoding locus and corresponding alleles will be discussed separately.

Locus *Glu-A1*

All three known alleles, ***a***, ***b*** and ***c***, at this locus were identified in the studied wheat accessions. The allele ***a*** was unique to a single breeding line 35.86–94–166. Therefore, it can be argued that alleles ***b*** (56.1%) and ***c*** (43.4%) are the most common ones in wheat germplasms in Northern Kazakhstan. According to Bulatova (1985), wheats in the Southern Kazakhstan region also carried predominantly ***b*** and ***c*** alleles; however, the frequencies (95.8% and 4.2%, respectively) were quite different. These alleles ***b*** and ***c*** were also widespread in the neighbouring countries, such as Russia and Tajikistan (Morgunov et al. 1990; Mahkamov 2013).

A relatively low association between alleles ***b*** and ***c***, and baking quality was previously reported (Lukow et al. 1989). However, in another report the ***b*** allele was indicated to be present in wheat germplasms with improved bread-making quality (Dias et al. 2017). In our current study, allele

Table 4 Association analysis of HMWG alleles with traits of grain and dough qualities in 33 wheat cultivars and 44 advanced breeding lines. *Q* is association coefficient, χ^2 is chi-square value; and *V* is contingency (similarity) coefficient in chi-square test

HMWG allele	Stat. index	Grain and dough quality characteristics									
		Protein content	Vitreousness	Gluten content	GDI	Deformation energy—W	P/L	Valorimetric index, VI	Bread form stability	Porosity	Bread-making quality scores
2*/***b**	*Q*	0.17	− 0.07	0.53	0.27	0.32	0.58	0.00	− 0.04	0.10	− 0.14
	χ^2	0.15	0.00	3.86*	0.38	0.89	5.31*	0.07	0.11	0.02	0.11
	V	0.05	0.00	0.23	0.07	0.11	0.27	0.03	0.04	0.02	0.04
7 + 9/***c***	*Q*	0.08	0.02	0.34	− 0.64	0.01	0.19	0.56	− 0.44	0.11	–
	χ^2	0.00	0.03	1.13	1.37	0.06	0.22	4.20*	0.18	0.03	0.52
	V	0.00	0.02	0.12	0.14	0.03	0.05	0.24	0.05	0.02	0.08
5 + 10/***d***	*Q*	− 0.16	0.04	− 0.39	0.34	− 0.02	− 0.63	0.32	0.04	− 0.06	− 0.12
	χ^2	0.19	0.00	2.19	0.63	0.03	7.12*	0.93	0.09	0.00	0.42
	V	0.05	0.00	0.17	0.09	0.02	0.31	0.11	0.04	0.00	0.08
2 + 12/***a***	*Q*	0.09	− 0.09	0.33	− 0.37	− 0.02	0.60	− 0.35	0.07	0.02	0.09
	χ^2	0.02	0.02	1.50	0.81	0.04	6.18*	1.23	0.05	0.03	0.42
	V	0.01	0.01	0.14	0.10	0.02	0.29	0.13	0.03	0.02	0.08

*$\chi^2_{St} = 3.84$ ($df = 1$)

b controls the synthesis of HMWG component 2*, and it was significantly associated with gluten content ($\chi^2 = 3.86$) and with gluten deformation index ($p = 0.95$, $df = 2$). Dough quality parameter *P/L* was balanced between elasticity and extensibility parameters, *P* and *L*, respectively. The most optimal interval *P/L* was determined as 0.8–1.5 for the environment of Northern Kazakhstan.

As was reported previously, allele *Glu-A1b* also correlated with the *P* parameter of dough quality while the influence of other glutenin alleles was found for parameter *L* (Branlard and Dardevet 1985). Based on our findings, we can hypothesise that the 2* HMWG component also affects *P/L* ratio. A chi-square test for association (0.58) and independence (0.27) showed a significant association between allele *Glu-A1b* and the *P/L* index (Table 4). The influence of climatic conditions on the expression of alleles *Glu-A1b* for drought tolerance has been discussed previously (Dobrotvorskaya and Martynov 2011). This was based on an earlier report that suggested strong breeding pressures on all studied varieties and advanced breeding lines from Northern Kazakhstan meant that they were already pre-selected for drought tolerance (Morgounov et al. 2001).

It must be stated that allele *Glu-A1c* was not always associated with low grain quality (Table 2). There are several accessions, among the 33 studied varieties and 40 advanced breeding lines with both allele ***c*** and high scores on baking characteristics. Therefore, allele ***c*** alone is insufficient to confer superior baking qualities, or there are other confounding factors potentially masking the effect of allele ***c*** on baking characteristics.

Locus Glu-B1

Among six identified alleles in the *Glu-B1* locus, maximum frequency (0.85) was found for the allele ***c***. At the same time, this allele had the highest frequency of occurrence in all four geographic regions of Northern Kazakhstan. It is important to note that this allele was reportedly found in Southern Kazakhstan wheats, however, at a lower frequency of 0.67 (Bulatova 1985). Allele *Glu-B1c* was quite common among high-quality wheat accessions in other countries, such as Russia (Dobrotvorskaya and Martynov 2011), Ukraine (Dobrotvorskaya and Martynov 2011; Zaika et al. 2014), China (Novoselskaya-Dragovich et al. 2011), several countries in Europe (Bradová and Šašek 2005) and America (Redaelli et al. 1997; Costa et al. 2013). As with allele *Glu-A1b*, a correlation between allele *Glu-B1c* in the studied wheat accessions and dry growth conditions was found in the present study. Additionally, it was reported that *Glu-A1b* was found in winter wheat growing in a wet- and frost-prone environment (Dobrotvorskaya and Martynov 2011). These facts may indicate that a very high degree of plasticity exists within wheat genotypes carrying allele *Glu-B1c*.

The less common allele ***b*** (6.0%) controls the first component pairs 7 + 8. Advantages and disadvantages of allele *Glu-B1b* compared to allele *Glu-B1c* have been explored experimentally. Thus, the replacement of allele ***b*** (components 7 + 8) by allele ***c*** (components 7 + 9) improved bread baking quality (Mansur et al. 1990), and this is in direct contrast to earlier reports of *Glu-B1b* in wheat accessions with good quality characteristics of bread (Branlard and Dardevet 1985). Contradictions in these results can probably be explained by the influence of additional low-molecular weight glutenins, and perhaps also by gliadin.

According to early studies of wheat in Northern Kazakhstan, only two alleles, ***b*** and ***c***, in locus *Glu-B1* were identified, where allele ***b*** had 13.5% of their distribution (Morgunov et al. 1990). However, our results show that despite most spring wheat carrying allele ***b***, there was a wider allelic diversity identified in Northern Kazakhstan due to an introgression of genotypes from other climatic zones in the breeding process. Of special interest is allele ***f*** (1.2% distribution frequency) which controls the synthesis of glutenin subunits 13 + 16. This relatively rare allele was found in only two wheat accessions: Karabalykskaya 9 and in one biotype of the advanced breeding line 35.86–94–166. Very high grain and dough quality assessment scores of wheats with allele *Glu-B1f* have attracted the attention of breeders who have to utilise this glutenin allele in further breeding programmes for grain quality.

The association between allele *Glu-B1c* with the valorimetric index, *VI*, was found based on the dough quality evaluation from grains of varieties of spring bread wheat and advanced breeding lines. The *VI* shows the elastic properties of the dough and its stability to mixing, where a higher *VI* value generally indicates better quality for bread baking. The association of *Glu-B1c* with *VI* was significantly high (Table 4). The association (*Q*) and contingency (*V*) coefficients reached 0.56 and 0.24, respectively, and a chi-square test yielded a score of $\chi^2 = 4.2$ ($p = 0.95$, and $df = 2$). The null-hypothesis supposes that glutenin components 7* + 9 are encoded by allele *Glu-B1c* (Gianibelli et al. 2002). The glutenin components *Bx20/By20* controlled by the allele ***e***, may receive negative attention from wheat breeders. On the dough quality scale (Lukow et al. 1989), the allele *Glu-B1e* (subunits *Bx20/By20*) showed the lowest score results and strong negative effects (Sissons et al. 2005; Pirozi et al. 2008). Nevertheless, recent published reports indicated that the switching 'off' of the gene encoding the synthesis of glutenin subunits *Bx20/By20* led to a decrease of *VI*, reduced content of crude protein and gluten, poor dough stability and bread porosity (Liu et al. 2016). Therefore, it is necessary to study and analyse more carefully those wheat samples carrying allele *Glu-B1e* in their genotype, and to conduct additional studies of this allele.

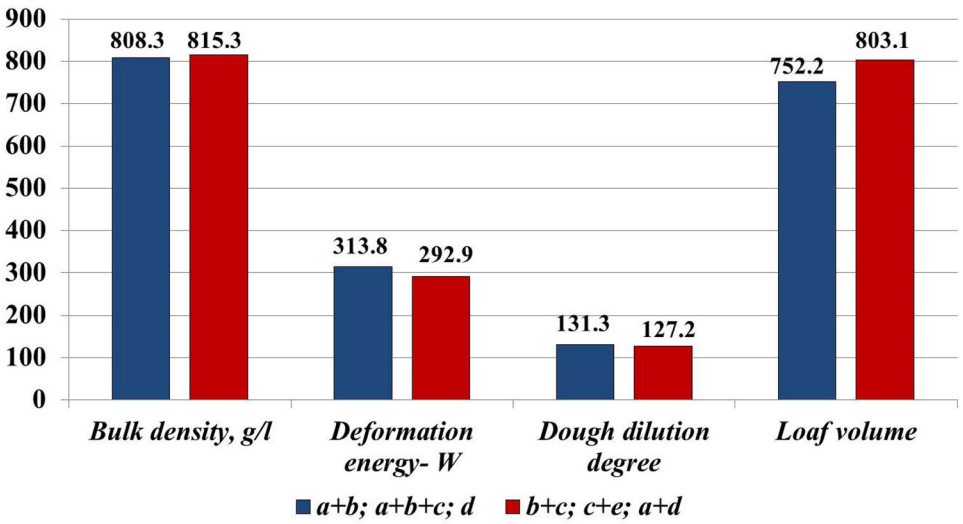

Fig. 2 Comparison of four dough and bread-making traits in two groups of wheat arranged based on biochemical analysis of components and real scores of the best wheat accessions

Locus Glu-D1

Locus *Glu-D1* was represented by four alleles identified as ***a***, ***c***, ***d*** and ***e***, two of which ***a*** and ***d*** showed maximal frequencies in the studied wheat accessions at 0.51 and 0.47, respectively. Two other alleles were very rare in the current study. In contrast, in Southern Kazakhstan, the allelic diversity of locus *Glu-D1* was reportedly much larger (Bulatova 1985). Analyses of wheat glutenins from different climatic zones revealed the widespread distribution of alleles ***a*** and ***d***, controlling HMWG components 2 + 12 and 5 + 10, respectively (Nakamura 2001; Gianibelli et al. 2002; Ayala et al. 2016). The low allelic diversity of glutenin in wheat varieties from Northern Kazakhstan may be the result of strong selection pressure by breeders in the dry environment. The allele ***a*** was reported to be associated with drought tolerance, while the allele ***d*** was mostly distributed in wet environments (Dobrotvorskaya and Martynov 2011). Thus, many papers indicated that allele ***d*** was present in wheats with high baking quality (Luo et al. 2001, Wang et al. 2018), which is possibly related to a dual role of allele ***d*** in both grain quality and in adaptive traits. A rare allele *Glu-D1c* controlling gliadin components 4 + 12 was found in two varieties Dostyk and Pamyati Movchana. Further, only a single biotype in the cultivar Pamyati Movchana carried allele ***c***, which could indicate heterogeneity within this variety. In this context, it would be very helpful to identify and select stable breeding lines of cultivar Pamyati Movchana based on polymorphic HMWG spectra, with further analysis of each biotype and breeding line separately. This presents the opportunity to study the nature of various *Glu-D* alleles for grain and dough based on the background of cultivar Pamyati Movchana. Additionally, conflicting results were found in literature and in our study regarding allele *Glu-D1c* (controlling components 4 + 12), where an association with the lowest scores on grain and dough qualities was previously reported (Lukow et al. 1989), but no negative impact on dough and bread-making qualities in varieties Dostyk was identified in our current study. Both alleles ***a*** and ***d*** of *Glu-D1* were associated with rheological characteristics of dough for *P/L* (Table 4). The association was confirmed by the statistical criteria: $|Q| \geq 0.5$ and $|V| \geq 0.3$ (Antamoshkin and Bakaeva 2011).

Comparison of two groups of wheats

To determine the potential efficiency of the predicted evaluation of glutenin components for various traits of dough and baking qualities, two groups of 18 varieties and breeding lines in each were arranged from the studied set of wheat accessions, based on either HMWG component analysis or grain and dough technological traits. The wheat accessions in the first group were selected based on HMWG components, 2*/1, 7 + 9/7 + 8/7, and 5 + 10, with alleles ***a*** + ***b***, ***a*** + ***b*** + ***c***, and ***d***, respectively, for three *Glu* genes, *-A1*, *-B1*, and *-D1*. This group had the highest score (8–10 out of a maximum of 10) of glutenin subunits with their influence to baking characteristics. It was predicted that wheat in the first group must have a very high score for grain and dough qualities. The second group combined the best wheat accessions with confirmed highest bread-making scores (from 4.6 to 4.8 out of maximum 5). In our study, wheat germplasms in the second group carried glutenin components 2*/Null, 7 + 9, and 5 + 10/2 + 12 with alleles ***b*** + ***c***, ***c*** + ***e***, and ***a*** + ***d***, respectively, for three *Glu* genes, *-A1*, *-B1*, and *-D1*. Accordingly, the scores on glutenin subunits with their influence to baking characteristics were between 5 and 7 out of 10. The comparative analysis of the two groups of selected wheat accessions revealed that two traits (dough deformation energy and dilution degree) were slightly higher in group 1,

while two other traits (bulk density and loaf volume) were higher in group 2 (Fig. 2). All differences were statistically insignificant, regarding Student's *t* test. The remaining traits for grain and dough qualities showed a similar level of differences (Supplementary material 3).

The prediction of dough and bread baking qualities based on glutenin spectra analyses showed no significant differences following statistical tests; therefore, the method employed in our study may prove to be very valuable. Nevertheless, the data we obtained contain some mismatches in the scores, indicating for other possible factors. There are several reports describing the relevant factors in assessing the quality of wheat for baking bread. These factors are, in their order of importance: the presence of HMWG (47–60%); the polymorphism of low-molecular weight glutenin, gliadin, protein content and α-amylase activity (31%); and a confirmed translocation on chromosomes 1BL/1RS (7%) (Wrigley et al. 2009).

The examination of the genetic control of HMWG and identification of new and rare glutenin subunits revealed during this study of wheat accessions is an extremely important step for the improvement of grain, dough and bread-making qualities. Wheat breeding programmes can benefit greatly from data on genetic polymorphism in glutenin subunits which reveal widespread, rare and unique alleles of *Glu* loci.

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Author contribution statement MU supervised the research and wrote the initial versions of the manuscript. KK and BS generated laboratory data with bread making and statistical analysis. SD and NB assisted in manuscript drafting and revising. YS coordinated and revised the final version of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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