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RESEARCH ARTICLE

Characterization of HMW glutenin subunits in European spring common wheat (*Triticum aestivum* L.)

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Abstract High molecular weight glutenin subunits plays an important role in conditioning the end-use quality of wheat products. The aim of the study was to determine the composition of HMW glutenin subunits, occurrence frequency of theses subunits and the potential of the end—use quality in spring wheat. The analysis included 81 European cultivars of spring wheat with a potential use in the food industry. Eight gene-specific markers were used to screen for HMW-GS. The analyzed genotypes showed twenty-five different allelic combinations at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. The results showed that the most common at the *Glu-A1* locus was Ax2* (58%), followed by Ax1 and Axnull (the same frequency of 21%). A high variation in allelic combinations was detected at the *Glu-B1* locus. The Bx7* subunit was present in 65% cultivars, Bx7 in 25%, Bx7 or Bx7* in 7.5% and Bx6 in 2.5%. The frequency of By9 was 59%, By8—21% and Bynull—20%. A higher frequency of Dx5 + Dy10 (80%) was observed at *Glu-D1* compared to Dx2 + Dy12 (16%) in the analyzed cultivars. A rare pattern of the Dx5 + Dy12 subunits was also detected (in 4% of cultivars tested). Ax2*, Bx7* + By9, Dx5 + Dy10 and Ax1, Bx7* + By9, Dx5 + Dy10, which determine good technological quality in wheat, were one of the most frequently detected allelic combinations at *Glu-1*. The conducted analysis showed that the tested cultivars were characterized by the potentially good and very good end-use quality of wheat products. These cultivars can be used effectively in the food industry.

Keywords European spring wheat (*Triticum aestivum* L.) · HMW-GS · Molecular markers · PCR

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Introduction

Gluten proteins play a major role in determining wheat technological properties. Two main fractions can be distinguished among them: glutenins and gliadins. Glutenins are divided into two groups: low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS). The quantity and composition of glutenins are important factors in determining wheat baking properties (D'Ovidio and Masci 2004; Figueroa et al. 2009; Payne et al. 1979).

HMW-GS are encoded by three complex loci: *Glu-A1*, *Glu-B1* and *Glu-D1* located near the centromeres on the long arms of group 1 chromosomes 1A, 1B and 1D. All loci have two closely linked genes that encode a higher molecular weight protein x-type and lower molecular weight protein y-type (Payne 1987; Shewry et al. 2003). A close relationship has been demonstrated between HMW-GS composition and wheat

baking quality (Dhaka and Khatkar 2015; Dobraszczyk and Morgenstern 2003). Typically, the *Glu-A1* locus encodes one or no subunits, *Glu-B1* two or one and the *Glu-D1* locus two subunits.

The presence of subunits encoded by the D genome followed by the B genome has a significant influence on good baking quality. Subunits encoded in the A genome have a lower influence on shaping these qualities (Obreht et al. 2007; Uthayakumaran et al. 2002).

The characterization of HMW-GS is an indispensable task in plant breeding aimed at the baking quality of wheat. HMW-GS variability can explain a significant part of the genetic variation affecting technological properties. Knowing the composition of HMW-GS, it is possible to make an approximate prediction of baking quality of wheat cultivars. The presence or absence of specific allelic variants of high molecular glutenins correlates with these features, and their variability depends mainly on the type of allelic variants present (Motawei 2008; Payne 1987; Song and Zheng 2007). However, it is often difficult to detect small differences between individual subunits using standard electrophoretic methods. The PCR method based on DNA markers provides an additional tool to overcome these constraints and additionally allows more accurate and faster characterization of glutenin subunit composition. A large number of PCR-based DNA molecular markers have been developed and applied on the basis of the available HMW-GS sequences. PCR-based markers are available to discriminate the important *Glu-1* alleles: Dx5, Dy10, Ax2*, Bx7, Bx7*, By8 and By9 (Abdel-Mawgood 2008; Kocourkova et al. 2008; Lafiandra et al. 1997; Ma et al. 2003; Xu et al. 2008).

In the present study, we used specific DNA markers to identify *Glu-1* alleles in 81 European cultivars of spring wheat. The aim of this study was to determine the frequency of occurrence of individual glutenin subunits at the *Glu-1* locus in the analyzed cultivars (Table 1).

Materials and methods

Plant material

The research material comprised 81 cultivars of spring common wheat from European breeders. The seeds of the tested cultivars were obtained from plant breeding companies from Poland (24), Czech Republic (8), Czech Republic/Denmark (4), Germany (12), Spain (4), Sweden (19), Norway (8), Sweden/Norway (1) and the United Kingdom (1) (Table 1).

DNA extraction

Genomic DNA was extracted from fresh leaf tissues using a modified CTAB method (Doyle and Doyle 1987). The quality and quantity of the isolated DNA was determined using a NanoDrop 2000 spectrophotometer. DNA samples were diluted to a working concentration of 20 ng/µl.

PCR amplification and electrophoretic analysis

PCR reaction was performed using primers for specific genes encoding HMW-GS listed in Table 2. Amplification was carried out in a Biometra T1 thermal cycler with a heated lid in a final volume of 20 µl. Reaction mixture composition and cycle temperatures were determined for each primer pair after optimizing reaction conditions (Table 3).

The PCR products were separated by electrophoresis in a 1.5% agarose gels containing 0.1% EtBr at 120 V for 2 h and visualized under a UV transilluminator and photographed using the PolyDoc System. GeneRuler^{TM} 100 bp DNA Ladder Plus was used to determine the molecular weights of the products.

Results

The present study used eight DNA markers to determine the composition of high molecular weight glutenin subunits in 81 European cultivars of spring wheat. The PCR results are presented in Table 3.

Identification of genes at the *Glu-A1* locus

PCR analysis of European wheat cultivars using marker P1 produced a 920-bp fragment in 17 (21%) cultivars, indicating the presence of the Axnull allele. This product was not amplified in the remaining 64 (79%) cultivars, suggesting the presence of another allele at the *Glu-A1* locus. Marker P2 showed the presence of one of the other two alleles: Ax1 or Ax2*. This marker generated a 1500-bp PCR product for the

Table 1 Composition of HMW-GS in European cultivars of spring common wheat determined by PCR

No.	Cultivar	Origin	Year of registration	HMW glutenin subunits			Alleles		
				Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A1*	*Glu-B1*	*Glu-D1*
1	Kamelia	Poland	2015	Null	7 + 8	5 + 10	c	b	d
2	Nimfa	Poland	2016	Null	7	5 + 10	c	a	d
3	Ostka Smolicka	Poland	2010	Null	7* + 9	5 + 10	c	c	d
4	Rusałka	Poland	2016	Null	7* + 9	2 + 12	c	c	a
5	Serenada	Poland	2015	2*	7	2 + 12	b	a	a
6	Koksa	Poland	2000	1	7 + 8	5 + 10	a	b	d
7	Korynta	Poland	2002	Null	7* + 9	2 + 12	c	c	a
8	Nawra	Poland	1999	Null	7 + 8	5 + 10	c	b	d
9	Harenda	Poland	2014	1	7* + 9	5 + 10	a	c	d
10	Izera	Poland	2012	Null	7* + 9	5 + 10	c	c	d
11	Parabola	Poland	2006	Null	7	5 + 10	c	a	d
12	Łagwa	Poland	2009	1	7* + 9	5 + 10	a	c	d
13	Żura	Poland	2002	1	7	2 + 12	a	a	a
14	Rospuda	Poland	2010	1	7* + 9	5 + 10	a	c	d
15	Zadra	Poland	2005	2*	7* + 9	2 + 12	b	c	a
16	Werbena	Poland	2009	1	7* + 9	5 + 10	a	c	d
17	Arabella	Poland	2011	1	7* + 9	5 + 10	a	c	d
18	Goplana	Poland	2015	Null	7/7*	5 + 10	c	a/aa	d
19	Kandela	Poland	2010	Null	7 + 8	2 + 12	c	b	a
20	Mandaryna	Poland	2014	1	7* + 9	2 + 12	a	c	a
21	Struna	Poland	2013	1	7	5 + 10	a	a	d
22	Brawura	Poland	2007	1	7* + 8	5 + 10	a	u	d
23	Katoda	Poland	2008	2*	7* + 9	5 + 10	b	c	d
24	Bombona	Poland	2005	2*	7 + 8	5 + 10	b	b	d
25	Alicia	Czech Republic	2016	1	7* + 9	5 + 10	a	c	d
26	Alondra	Czech Republic/ Denmark	2013	Null	7* + 9	5 + 10	c	c	d
27	Anabel	Czech Republic	2014	1	7* + 9	5 + 10	a	c	d
28	Astrid	Czech Republic/ Denmark	2012	1	6 + 8	5 + 10	a	d	d
29	Dafne	Czech Republic/ Denmark	2010	Null	7* + 9	5 + 10	c	c	d
30	Granny	Czech Republic	2006	1	7* + 9	2 + 12	a	c	a
31	Izzy	Czech Republic	2010	2*	7* + 9	5 + 10	b	c	d
32	Lotte	Czech Republic	2016	1	7* + 9	5 + 10	a	c	d
33	Registana	Czech Republic	2016	1	7* + 9	5 + 10	a	c	d
34	Septima	Czech Republic	2008	1	7* + 9	5 + 10	a	c	d
35	Tercie	Czech Republic/ Denmark	2008	1	7* + 9	5 + 10	a	c	d
36	Zuzana	Czech Republic	2003	1	7 + 8	5 + 10	a	b	d
37	KWS Akvilon	Germany	2013	1	7* + 9	5 + 10	a	c	d
38	KWS Buran	Germany	2012	Null	7* + 8	5 + 10	c	u	d
39	KWS Chamsin	Germany	2008	1	7* + 9	5 + 10	a	c	d

Table 1 continued

No.	Cultivar	Origin	Year of registration	HMW glutenin subunits			Alleles		
				Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A1*	*Glu-B1*	*Glu-D1*
40	KWS Collada	Germany	2010	1	7	5 + 10	a	a	d
41	KWS Jetstream	Germany	2015	1	7* + 9	5 + 10	a	c	d
42	KWS Mistral	Germany	2015	1	7* + 9	5 + 10	a	c	d
43	KWS Scirocco	Germany	2008	Null	7* + 9	5 + 10	c	c	d
44	KWS Solanus	Germany	2015	1	7* + 9	5 + 10	a	a	d
45	Monsun	Germany	2004	1	7/7*	5 + 10	a	a/aa	d
46	Taifun	Germany	2003	Null	7* + 9	5 + 10	c	c	d
47	Trappe	Germany	2005	Null	7* + 9	5 + 10	c	c	d
48	Vanék	Germany	2004	1	7* + 9	5 + 10	a	c	d
49	Concil	Spain	2011	1	7/ 7* + 8	5 + 10	a	b/u	d
50	Escacena	Spain	2002	1	7* + 9	5 + 10	a	c	d
51	Jerezano	Spain	2004	1	7/ 7* + 8	5 + 10	a	b/u	d
52	Tejada	Spain	2009	1	7/ 7* + 8	5 + 10	a	b/u	d
53	Vinjett	Sweden	2001	2*	7* + 9	5 + 10	b	c	d
54	Diskett	Sweden	2009	2*	7* + 9	5 + 10	b	c	d
55	Sonett	Sweden	2010	2*	7/7*	5 + 10	b	a/aa	d
56	Boett	Sweden	2016	2*	7*	2 + 12	b	aa	a
57	Happy	Sweden	2015	2*	7* + 9	5 + 10	b	c	d
58	Berlock	Sweden	2014	2*	7* + 9	5 + 10	b	c	d
59	Miramis	Sweden	2016	2*	7* + 9	5 + 10	b	c	d
60	Countess	Sweden	2016	1	7	5 + 12	a	a	–
61	Nobless	Sweden	2014	1	7* + 9	2 + 12	a	c	a
62	Crickett	Sweden	2016	1	7* + 9	5 + 10	a	c	d
63	Rohan	Sweden	2016	1	7	2 + 12	a	a	a
64	Rouge	Sweden	2016	1	7* + 9	5 + 10	a	c	d
65	SW 01121	Sweden	2001	1	7* + 9	5 + 10	a	c	d
66	Zebra	Sweden	2002	2*	7* + 9	5 + 10	b	c	d
67	Jack	Sweden	2016	1	7* + 9	2 + 12	a	c	a
68	Bagett	Sweden	2012	2*	7* + 9	5 + 10	b	c	d
69	Dacke	Sweden	1991	2*	7	2 + 12	b	a	a
70	SW Kadrilj	Sweden	2005	1	7	5 + 10	a	a	d
71	Kungsjet	Sweden	2004	1	7	5 + 10	a	a	d
72	Bjarne	Sweden/Norway	2002	1	6 + 8	5 + 10	a	d	d
73	Berserk	Norway	2007	1	7 + 8	5 + 12	a	b	–
74	Demonstrant	Norway	2008	1	7* + 8	5 + 10	a	u	d
75	Krabat	Norway	2010	1	7* + 9	5 + 10	a	c	d
76	Laban	Norway	2011	1	7* + 9	5 + 10	a	c	d
77	Mirakel	Norway	2012	2*	7* + 9	5 + 10	b	c	d
78	Rabagast	Norway	2013	1	7* + 8	5 + 10	a	u	d
79	Seniorita	Norway	2014	2*	7 + 8	5 + 10	b	b	d

Table 1 continued

No.	Cultivar	Origin	Year of registration	HMW glutenin subunits			Alleles		
				Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A1*	*Glu-B1*	*Glu-D1*
80	Willy	Norway	2016	1	7* + 9	5 + 12	a	c	–
81	Tybalt	Great Britain	2003	Null	7	5 + 10	c	a	d

Table 2 PCR primer sequence for the amplification of specific HMW-SG genes

Locus	Gene/allele	Primer name	Forward and reverse primer sequence (5′-3′)	Product size (bp)	References
Glu-A1	*Axnull*	P1	F: ACGTTCCCCTACAGGTACTA R: TATCACTGGCTAGCCGACAA	920	Lafiandra et al. (1997)
	*Ax1 or Ax2**	P2	F: CCATCGAAATGGCTAAGCGG R: GTCCAGAAGTTGGGAAGTGC	1500 or 1400	Lafiandra et al. (1997)
Glu-B1	*Bx6, Bx7, Bx7**	P3	F: CAAGGGCAACCAGGGTAC R: AGAGTTCTATCACTGCCTGGT	3 groups: 1 band of 850–920 4–5 bands of 420–640 2 bands180–280	Butow et al. (2004)
	Bx6	P4	F: CGCAACAGCCAGGACAATT R: AGAGTTCTATCACTGCCTGGT	680 and 870	Ma et al. (2003)
	*Bx7, Bx7**			~630 and 766	
	Bx17			669	
	By9	P5	F: TTCTCTGCATCAGTCAGGA R: AGAGAAGCTGTGTAATGCC	662	Lei et al. (2006)
	By16	P6	F: GCAGTACCCAGCTTCTCAA R: CCTTGTCTTGTTTGTTGCC	1 band of 290–400	Lei et al. (2006)
	Bynull or By20			2 bands of 290–400 3 bands of 290–400	
	By8,By8, By9*				
Glu-D1	*Dx5*	P7	F: CGTCCCTATAAAAGCCTAGC R: AGTATGAAACCTGCTGCGGAC	478	Ma et al. (2003)
	Dy10 or Dy12	P8	F: GTTGGCCGGTCGGCTGCCATG R: TGGAGAAGTTGGATAGTACC	576 or 612	Abdel-Mawgood (2008)

Ax1 allele in 47 (58%) cultivars and 1400-bp PCR product for the Ax2*allele in 17 (21%) cultivars.

Identification of genes at the *Glu-B1* locus

Four DNA markers were used for the detection of alleles at the *Glu-B1* locus (two pairs for "x" and "y" types).

Primer set P3 amplified 3 groups (I–III) of PCR products of different sizes. The Bx7 and Bx7* subunits produced 1 band of 860 bp in group I, 4 bands of 420–600 bp in group II and 2 bands of 180–280 bp in group III. The PCR products for the Bx7* subunit had smaller fragments in groups II and III than the amplification products for the Bx7 subunit. The Bx6 subunit generated 1 band of 920 bp in group I, 5 bands

Table 3 PCR components and amplification profile

Primer name	PCR components	Amplification cycle
P1	1 × PCR Buffer (Fermentas),250 μM of each dNTP, 2 mM $MgCl_2$, 5 pM of each primer, 60 ng of template DNA and 0.5 U Taq DNA Polymerase (Fermentas)	1 × 94 °C 5′; 38 × (94 °C 1′; 64 °C 45″; 72 °C 1′30″); 1 × 72 °C 10′
P2	1 × PCR Buffer (Fermentas), 250 μM of each dNTP, 1.6 mM $MgCl_2$, 5 pM of each primer, 60 ng of template DNA and 0.5 U Taq DNA Polymerase (Fermentas)	1 × 94 °C 5′; 35 × (94 °C 1′; 60 °C 45″; 72 °C 1′30″); 1 × 72 °C 10′
P3	1 × PCR Buffer (Fermentas), 250 μM of each dNTP, 1.8 mM $MgCl_2$, 5 pM of each primer, 60 ng of template DNA and 0.5 U Taq DNA Polymerase (Fermentas)	1 × 95 °C 5′; 35 × (94 °C 45″; 58 °C 45″; 72 °C 1′); 1 × 72 °C 10′
P4	1 × PCR Buffer (Fermentas), 200 μM of each dNTP, 1.6 mM $MgCl_2$,5 pM of each primer, 60 ng of template DNA and 0.5 U Taq DNA Polymerase (Fermentas)	1 × 95 °C 5′; 35 × (94 °C 30″; 58 °C 30″; 72 °C 2′); 1 × 72 °C 10′
P5	1 × PCR Buffer (Fermentas), 150 μM of each dNTP, 1.6 mM $MgCl_2$, 5 pM of each primer, 60 ng of template DNA and 0.5 U Taq DNA Polymerase (Fermentas)	1 × 95 °C 2′; 38 × (94 °C 30″; 59 °C 30″; 72 °C 1′30″); 1 × 72 °C 10′
P6	1 × PCR Buffer (Fermentas), 250 μM of each dNTP, 1.6 mM $MgCl_2$, 5 pM of each primer, 60 ng of template DNA and 0.5 U Taq DNA Polymerase (Fermentas)	1 × 95 °C 5′; 38 × (94 °C 30″; 65 °C 30″; 72 °C 1′30″); 1 × 72 °C 10′
P7	1 × PCR Buffer (Fermentas), 250 μM of each dNTP, 1.8 mM $MgCl_2$, 5 pM of each primer, 60 ng of template DNA and 0.5 U Taq DNA Polymerase (Fermentas)	1 × 95 °C 5′; 35 × (94 °C 45′; 58 °C 30″; 72 °C 1′); 1 × 72 °C 10′
P8	1 × PCR Buffer (Fermentas), 250 μM of each dNTP, 1.8 mM $MgCl_2$, 5 pM of each primer, 60 ng of template DNA and 0.5 U Taq DNA Polymerase (Fermentas)	1 × 94 °C 5′; 40 × (94 °C 1′; 63 °C 1′; 72 °C 1′); 1 × 72 °C 10′

of 420–640 bp in group II and 2 bands of 180–280 bp in group III. Marker P4 produced 2 bands of ~ 630 bp and 766 bp for cultivars containing Bx7 and Bx7* subunits and 2 bands of 680 bp and 870 bp for the Bx6 allele. Similarly to primer set P3, the Bx7*subunit had smaller fragments in comparison to the Bx7 subunit. PCR analysis of 81 European wheat cultivars using markers P3 and P4 showed the presence of the Bx7 gene in 20 (25%) cultivars, Bx7* in 53 (65%) cultivars, Bx7 or Bx7* in 6 (7.5%) cultivars and Bx6 only in 2 (2.5%) cultivars.

Marker P5 produced a 662-bp fragment, which turned out to be specific for the By9 gene. The remaining products suggested the presence of other By genes. The By9 allele has been identified in 48 genotypes (58%). Primer set P6 confirmed the presence of the By9 allele in 48 cultivars. This marker generated 2 bands of 290–350 bp for this allele. Additionally, primer pair P6 allowed the identification of the By8 allele in 17 genotypes (21%) and the Bynullallele in 16 cultivars (20%). The By8 subunit produced 3 bands of 290-400 bp and Bynull generated 1 PCR product of 290 bp.

Identification of genes at the *Glu-D1* locus

Two primer sets identified the allelic composition at the *Glu-D1* locus. Primers P7 was specific for the Dx5 allele and produced a 478-bp fragment. Marker P8 amplified a 576-bp PCR product for the Dy10 allele and 612-bp fragment for the Dy12 allele. The Dx5 + Dy10 composition of HMW glutenin subunits was the most frequent (80%, 65 cultivars). The Dx2 + Dy12 alleles were detected at the *Glu-D1* locus in 13 cultivars (16%). A rare 5 + 12 allele was found in the remaining 3 cultivars (4%).

Discussion

The determination of HMW-GS composition in wheat cultivar collections from different countries has been

conducted in many studies (Atanasova et al. 2009; Brönneke et al. 2000; Henkrar et al. 2017; Jin et al. 2011; Ma et al. 2003; Moczulski and Salmanowicz 2003). The adequate composition of these proteins plays a particularly important role in conditioning the technological properties of wheat. For this reason, the determination and characterization of HMW-GS are extremely useful for assessing baking quality of wheat. Diagnostic PCR analysis with primers specific not only for individual genes, but also for nearly identical alleles of a given gene represents a valuable tool for identifying genotypes with desirable end-use quality at an early stage of breeding selection.

Three alleles, *a*, *b* and *c* (subunits 1, 2* and null) at the *Glu-A1* locus were identified in the studied collection of European spring wheat cultivars. Ax1 (58%) had the highest frequency and was followed by Ax2* and Axnull (the same frequency of 21%). The Ax1 subunit predominated in cultivars from central Europe (Poland, Czech Republic and Denmark − 40%), but to a large extent it was also identified in cultivars from northern (34%) and western (26%) Europe. The Ax2* subunit was identified in cultivars from northern (71%) and central (29%) Europe only, while Axnull was detected in central (71%) and western (29%) Europe only. A similar result was obtained by Ghazy et al. (2012), who analyzed 29 cultivars of spring wheat from Saudi Arabia. They identified the Ax1 allele in 79% of screened genotypes and Ax2* occurred in 21% of tested genotypes. However, many studies reported that the Ax2* allele was the most frequent subunit at the *Glu-A1* locus. Jin et al. (2011) characterized HMW-GS composition for 718 cultivars and advanced lines from 20 countries. They showed the highest frequency of Ax2*—43.3%. High frequencies of Ax2* subunit were present in cultivars from Canada, Romania, Russia and USA. Jin et al. showed low presence of Ax2 * from Chile, China and Germany. In the presented work, a similar result was found no cultivar Ax2* among genotypes from Germany. The analysis carried out by Henkrar et al. (2017) was similar to the study of Jin et al. (2011). These authors identified Ax2* in 60% of Moroccan bread wheat cultivars, while Ax1 in 25% and Axnull in 15%. The Ax1 and Ax2* subunits at the *Glu-A1* locus have a significantly positive impact on the processing quality of wheat products. Their presence increases dough strength, which determines better baking quality and better quality parameter values than in wheat with the null allele (Anjum et al. 2007; Brönnekei et al. 2000; He et al. 2005; Li et al. 2010; Liang et al. 2010; Liu et al. 2005; Luo et al. 2001; Meng and Cai 2008).

Glu-1 genes show high variation. The most diverse is the *Glu-B1* locus. Bx7 is one of the most important subunits encoded by the *Glu-B1* locus and related to baking quality. Three variants can be distinguished among Bx7: Bx7, Bx7* and $Bx7^{OE}$. Differences between these variants are inconsiderable. The results of research carried out using primer sets P3 and P4 complemented each other and allowed for precise determination of the occurrence of individual "x"-type subunits. Bx7* is the most frequently identified subunit in wheat. In the present study, the Bx7* subunit occurred in 65% of the tested cultivars. Bx7 was identified in 25% of genotypes and Bx7 or Bx7* (not precisely identified)—7.5%. A high level of Bx7* was recorded by Espí et al. (2012), Henkrar et al. (2017) and Janni et al. (2017). The Bx6 subunit is associated with poor baking quality and occurs with the relatively lowest frequency. In our study, this subunit was identified only in 2.5% of cultivars, and in the work carried out by Janni et al. (2017) only in 5% of genotypes.

In the present study, three "y"-type subunits were identified at the *Glu-B1* locus. Primer sets designed by Lei et al. (2006) were used for By subunit determination. Comparison of the results obtained for primers P5 and P6 indicated that the By9 subunit was present in 59% of cultivars, By8 in 21% and Bynull in 20% of cultivars.

Janni et al. (2017) analyzed nineteen bread wheat and twelve durum wheat cultivars. Among bread wheat cultivars, the By8 and By9 genes were identified in 16%. Other "y"-type alleles were identified in the remaining cultivars. These results showed a different frequency of subunits compared to the current study. The results of research conducted by Henkrar et al. (2017) and Jin et al. (2011) were also different. The analyses carried out by Henkrar et al. (2017) showed the presence of By9 in 35% of cultivars and By8 in 25%. However, Jin et al. (2011) identified the By8 allele in 27.4% of all analyzed cultivars and By9 in 36.8%. Among spring wheat, they found the By8 allele in 31.1% and the By9 allele in 22.8%.

In this study Bx7* + By9 was the most commonly identified allelic combination. Bx7* + By9 was detected in 22 cultivars from central Europe, 16 cultivars from northern Europe and 10 cultivars from

western Europe. Espí et al. (2012) were identified the Bx7* in 69% of tested cultivars. They draw attention to the discrimination of Bx7, Bx7* and $Bx7^{OE}$. Espí et al. (2012) indicates several scientific papers in which insufficient attention was paid to this problem and too little detailed analysis was carried out. Many authors (Branlard et al. 2003; Morgounov et al. 1993; Ribeiro et al. 2011) have not identified the allelic composition 7* + 8 or 7* + 9 among several thousand varieties studied. In the light of current results, it is likely that many of them have not been correctly identified, e.g. in the study Moczulski and Salmanowicz (2003). Moczulski and Salmanowicz (2003) analysed 76 Polish cultivars and they detected the high variability within the *Glu-B1* locus (Bx6 + By8—24.8%, Bx7 + By8—13.6%; Bx7—11.8%, Bx7 + By9—43.2%, Bx17 + By18—6.6%), but they not identified the allelic composition 7* + 8 or 7* + 9. Therefore, these results can vary greatly despite the same origin of cultivars. All differences may be due to the high variability within the *Glu-B1* locus, country of origin or not correct identifying of subunits.

Three allelic combinations, 5 + 10, 2 + 12 and 5 + 12, occurred in chromosome 1D. Many studies demonstrated that the 5 + 10 subunit was dominant in relation to the 2 + 10 subunit. A high frequency (98%) of 5 + 10 subunits was demonstrated by Ali et al. (2013) (in Pakistani spring wheat), 71% by Atanasova et al. (2009) (in cultivars from Bulgaria), 73% by Dias et al. (2017) (in Brazilian wheat cultivars), 85% by Henkrar et al. (2017) (in Moroccan bread wheat) and 62% by Jin et al. (2011) (in common wheat cultivars from 20 countries around the world). In our study, the analyses based on specific DNA markers for the *Glu-D1* locus confirmed higher frequency of Dx5 + Dy10 (80%) in the tested genotypes. This allelic combination was occur very frequently in all tested genotypes from northern, cental and western Europe. The 5 + 10 allelic combination is associated with good baking quality, whereas the 2 + 10 subunit combination conditions poor baking quality. For that reason, wheat cultivars with Dx5 + Dy10 are more desirable in wheat breeding (Abdel-Mawgood 2008; Barak et al. 2013). The Dx2 + Dy10 allelic combination was carried by 16% of cultivars. In addition, the configuration of bands indicated the presence of rare 5 + 12 subunits at the *Glu-D1* locus (4%). D'Ovidio et al. (1994) has already mentioned the occurrence of 5 + 12 subunits in wheat cultivars. The presence of this allelic combination was confirmed by Atanasova et al. (2009), who identified the 5 + 12 allele in 2.97% of wheat cultivars. On the basis of this study and tests carried out by many authors of publications can confirm a significant advantage of 5 + 10 regardless of the country of origin.

Among the wheat cultivars analyzed, 25 allelic patterns at the *Glu-1* locus were identified. One of the most preferred sets of allelic combinations at Glu-1 is Glu-1A2*, Glu-1B7* + 9, Glu-1D 5 + 10. In this study, 10 wheat cultivars showed the presence of this combination. However, Ax1, Bx7 * + By9, Dx5 + Dy10 was the most common combination, as it was present in 23 cultivars. These two sets of alleles determine the increase in the extensibility as well as elasticity, viscosity and consistency of the dough, which in turn affects good baking quality. The remaining allelic patterns were present in single cultivars. The vast majority of the analyzed cultivars were characterized by allelic systems determining good or very good baking quality, indicating the suitability of these cultivars in the food industry.

Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent The authors agreed to the publication of the manuscript. All authors read and approved the final manuscript.

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