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Research Article

Composition of High Molecular Weight Glutenin Subunits in Polish Common Wheat Cultivars (*Triticum aestivum* L.)

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The main goal of our study was to present research data on genes encoding high molecular weight glutenin subunits (HMW-GS) associated with high flour bread-making quality. This is the leading research objective in our institute in the area of wheat gluten in cultivars that have not been studied so far in that respect, but which can potentially be a valuable source of new information. Identification and characterization of high molecular weight glutenin subunits (HMW-GS) were performed using sequencing and SDS-PAGE and STS-PCR methods. Genes located in the vicinity of the *Glu-1* locus have been identified and characterized in 28 Polish cultivars of *Triticum aestivum*. The results were then analyzed using the following computer programs: Finch TV, BLAST, MEGA 4, Molecular Imager® Gel Doc™ XR, and Quantity One software (Bio-Rad). Three alleles *(a, b, c)* have been identified in the *Glu-A1* locus, 6 alleles *(a, b, c, d, e, k)* in the *Glu-B1* locus, and 2 alleles *(a, d)* in *Glu-D1* using the SDS-PAGE method. The amplification of specific HMW-GS sequences generated one product of 450 bp in *1Dx5* in 13 cultivars of old wheat and of 435 bp in *1Dx2* in 15 cultivars. The amplification products of primers for *1Dy10* and *1Dy12* genes were 422 bp and 552 bp in size, respectively.

Dedicated to Professor St. M. Rogalska who approved this research and who passed away on 17 April 2015.

1. Introduction

The main purposes of breeding bread wheat are good crop quality as well as bread-making quality of flour—parameters conditioned by genes encoding glutenin protein subunits. Inheritance and polymorphism of gluten proteins were studied already in the early 1980s. It was shown then that both inheritance and polymorphism are determined by polycistronic genetic loci located on the long arms of wheat chromosomes 1A, 1B, and 1D [1, 2]. The independent inheritance of subunits encoded by alleles located on different chromosomes and relatively easy identification of HMW-GS by means of electrophoresis have demonstrated that HMW-GS could serve as good markers in assisting in the selection of genotypes with better baking qualities. It was found that these proteins had a significant impact on technological quality of wheat and that the variability of their technological features was related to their physicochemical structure [3, 4]. Protein blocks that are direct products of individual (multiple) alleles are inherited in a simple manner and their variability does not depend on the environmental fluctuations. Genes encoding HMW-GS are characterized by the presence of a large region extending from the 5′ to the 3′ end of the repetitive nucleotide DNA sequences, measuring about 1200 bp 5′ of the start codon and 200–400 bp 3′ of the stop codon. Primers designed to track the presence of these alleles permit the amplification of 450 bp fragments, which are typical for *1Dx5* and *1Dy10* groups [5]. It has been repeatedly demonstrated that the difference between *1Dx2* and *1Dx5* genes lies in the cysteine codon located at the beginning of the repeated DNA domains. Cysteine is characteristic of the *1Dx5*-encoded subunit and probably plays an important role in the formation of physicochemical gluten properties. It has also been shown that *1Ax2* and *1Bx7* genes do not have additional cysteine codon,

and thus the gluten they produce has a modified matrix with a smaller number of disulphide bonds that enhance the elasticity and viscosity of the dough [6, 7].

In light of the aforementioned research, our study was aimed at the practical application and expanding the knowledge base. This is because growers still use glutenin markers to preselect genotypes with poor and good baking quality. However, the selection aimed at agricultural traits, such as resistance or yield potential, is conducted first; only then the introduced lines of wheat are grain-tested for their technological values and qualities.

The results obtained in this study help to better understand the population structure of old wheat cultivars in terms of the presence and type of glutenin genes. Furthermore, they will provide information on protein products of these genes and will allow identifying the frequency and nature of mutations occurring in certain regions of these genes as well as their impact on the quality of proteins they encode. These data were obtained by gene sequencing analysis, whereas protein products of these genes were analyzed using SDS-PAGE electrophoresis as well as technological quality analysis of grains and their gluten properties.

Therefore, the aim of this study was to investigate the presence of genes encoding high molecular weight glutenin subunits (HMW-GS) associated with high bread-making quality of flour. Moreover, we wanted to understand the sequence structure in those wheat cultivars that have not been studied so far in that respect but which potentially can be a valuable source of new information.

2. Materials and Methods

2.1. Plant Material. The present study examined the genes encoding HMW-GS in 28 old cultivars of wheat (*Triticum aestivum* L). All the material was obtained from the collection of the Institute of Plant Breeding and Acclimatization in Radzików (Poland).

2.2. Isolation of HMW Storage Proteins and Separation by SDS-PAGE. The HMW-GS composition of each cultivar was determined by SDS-PAGE using 11.5% (m/v) polyacrylamide gel. Identification of HMW glutenin alleles and subunits was performed according to the methods described by Payne and Lawrence [2] and McIntosh et al. [9].

2.3. Wheat DNA Purification. Genomic DNA was extracted from fresh 5-6-day-old etiolated coleoptiles from 28 cultivars of wheat. The isolation was performed using Wizard® Genomic DNA Kit (Promega).

2.4. PCR Analysis. The oligonucleotides used as primers were designed based on the data accessible in the literature (Table 1) and synthesized in the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences. PCR analyses were performed in three steps: (1) modification of single PCR analyses using genomic DNA from wheat cultivars with the known composition of glutenin subunits; (2) development of PCR analysis; and (3) application of the STS-PCR-based method for the identification of HMW glutenin alleles in Polish wheat cultivars. PCR reactions were performed in an MJ Mini™ PTC-200 thermal cycler (Bio-Rad) with a heated lid in the final volume of 25 μL.

The single PCR reaction mixture contained 1x buffer (Novazymes), 2 mM of $MgCl_2$, 2 mM of dNTP, 5 μM of each primer, 50 ng of genomic DNA, and 0.5 units of Allegro Taq DNA Polymerase (5 U/μL) (Novozymes). After the initial step at 95°C for 7 min, 35 cycles were performed, depending on the individual markers, at the following cycling temperatures:

1Dx2

94°C for 1′
55°C for 1′
72°C for 1′30″

1Dx5

94°C for 1′
52.5°C for 1′
72°C for 1′30″

1Dy10/12

94°C for 1′
58°C for 1′
72°C for 2′30″

After 35 cycles, the extension temperature was kept at 72°C for 10 min. Half of each PCR reaction product was analyzed on 1.5% (m/v) agarose gel run in 1x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2 EDTA, and pH 8.3) and exposed to UV light to visualize DNA fragments. Ethidium bromide was added to the gel to a final concentration of 0.1 μg cm^{-3}. The gel was analyzed and archived using the Molecular Imager Gel Doc XR software. Bands were scored and analyzed with the Quantity One software (Bio-Rad). The size of the products was determined by comparison with a DNA ladder (MassRuler, Fermentas). Software functions facilitated the accurate development of digital electrophoretic images and allowed creating a visual presentation in the form of dendrograms.

The presence or absence of bands was recorded as 1 and 0, respectively, in the form of the binary data matrix. The PIC (polymorphic information content) index for STS markers was calculated following the formula described by Ghislain et al. [8] for dominant marker system; that is, $PIC = 1 - p^2 - q^2$, where p is the band frequency and q is no band frequency.

2.5. Sequence Analysis of Selected HMW-GS Genes at Glu-D1 Loci. The selected HMW-GS *Glu-D1* genes, *1Dx5* and *1Dx2*, present in the studied wheat were sequenced in the laboratory of Macrogen Inc. (Seoul, South Korea). Fifty-six reactions were performed using primer pairs for the amplification of *1Dx5* and *1Dx2* genes both forward and reverse. A specific nucleotide sequence was obtained for each of the studied wheat cultivars. Bioinformatic analysis of the obtained *1Dx5*

Table 1: Sets of alleles: specific markers for the identification of wheat HMW glutenin genes used in the study.

Genes	Forward and reverse primer sequences (5′-3′)	Expected DNA fragment	References
1Dx2 or *1Dx5*	F: GCCTAGCAACCTTCACAATC R: GAAACCTGCTGCGGACAAG	413–430 bp or 450 bp	D'Ovidio and Anderson [5]
1Dy10 or *1Dy12*	F: GTTGGCCGGTCGGCTGCCATG R: TGGAGAAGTTGGATAGTACC	400 bp or 612 bp	Ahmad [6]

Table 2: Composition of the identified glutenin subunits of high molecular weight proteins in the studied wheat cultivars (SDS-PAGE).

Cultivar	HMW glutenin subunits Locus Glu-1			Alleles Locus Glu-1			Bread-making quality scores 1–10°
	Glu-A1	Glu-B1	Glu-D1	Glu -A1	Glu-B1	Glu-D1	
Antonińska S.46	1	7 + 9	2 + 12	a	c	a	7
Balta	Null	20	2 + 12	c	e	a	4
Biała Kaszubska	1	7 + 9	2 + 12	a	c	a	7
Choryńska	Null	7 + 9	2 + 12	c	c	a	5
DańkowskaBiała	Null	7 + 9	5 + 10	c	c	d	7
Eka 132	1	6 + 8	2 + 12	a	d	a	6
Magnatka Rogalińska	1	6 + 8	2 + 12	a	d	a	5
Mudczanka Czerwona	2*	20	2 + 12	b	e	a	6
Murzynka Lipińskiego	1	20	5 + 10	a	e	d	8
Mydlniczanka	1	22	5 + 10	a	k	d	8
Niewylegająca	Null	7 + 9	5 + 10	c	c	d	7
Ostka Czerwona Łopuska	Null	7 + 9	5 + 10	c	c	d	7
Ostka Górczańska	Null	7	5 + 10	c	a	d	6
Ostka Kazimierska	1	7 + 8(9)	2 + 12	a	b/c	a	8/7
Ostka Mikulicka Selit	Null	7 + 8(9)	2 + 12	c	b/c	a	6/5
Ostka Nadwiślańska	Null	7	2 + 12	c	a	a	4
Poznańska	Null	7 + 8	2 + 12	c	b	a	6
Sielecka Genetyczna	1	7 + 9	5 + 10	a	c	d	9
Sobieszczyńska 44	1	7 + 9	5 + 10	a	c	d	9
Sobótka	1	20	5 + 10	a	e	d	8
Squarehead Grodkowicka	1	7 + 9	2 + 12	a	c	a	7
Srebrzysta	Null	6 + 8	5 + 10	c	d	d	6
Ślązaczka	Null	7 + 8	5 + 10	c	b	d	8
Tryumf Milulic	Null	7 + 8	5 + 10	c	b	d	8
Udyczanka Czerwona	1	22	5 + 10	a	k	d	8
Wysokolitewska Anonińska	1	7 + 9	2 + 12	a	c	a	7
Wysokolitewska Sztywnosłoma	Null	7 + 9	2 + 12	c	c	a	5
Żelazna	Null	20	2 + 12	c	e	a	4

and *1Dx2* gene sequences was performed using the following computer programs: FinchTV version 1.4.0, MEGA 4.0 [10], Protein Sequence Analysis Software, Shareware 1.2, and the GenBank database.

3. Results

3.1. SDS-PAGE Analysis of HMW Glutenin Subunits. The analysis of protein products of HMW-GS genes using SDS-PAGE electrophoresis allowed distinguishing protein blocks in the studied hexaploid wheat cultivars, as shown in Table 2. Electropherograms of HMW glutenin subunits indicated that the cultivars studied differed in respect to the composition of their HMW-GS. High molecular weight glutenin fractions were encoded by 11 alleles in the test cultivars. In the *Glu-A1* locus, 3 alleles (a, b, c) have been identified, in *Glu-B1* 6 alleles (a, b, c, d, e, k), and in *Glu-D1* 2 alleles (a, d) (Table 2). In 14 cultivars, the *Glu-A1* locus was determined as the *N* block *(Null)*, which is unfavourable because glutenins encoded by the genes in the so-called noncoding variant of the Null type lower the technological quality of wheat. However, subunit 2* was present in the *Glu-A1* locus of the

Table 3: The identified (SDS-PAGE) gluten subunits in the wheat varieties studied.

Composition of HMW glutenin subunits	Number of varieties	Frequencies [%]
1/7 + 9/2 + 12	4	14.3
N/20/2 + 12	2	7.1
N/7 + 9/2 + 12	2	7.1
N/7 + 9/5 + 10	3	10.7
1/6 + 8/2 + 12	2	7.1
1/7 + 9/5 + 10	2	7.1
1/22/5 + 10	2	7.1
N/7 + 8/5 + 10	2	7.1
1/20/5 + 10	2	7.1
N/7/5 + 10	1	3.6
1/7 + 8(9)/2 + 12	1	3.6
N/7 + 8(9)/2 + 12	1	3.6
N/7/2 + 12	1	3.6
N/7 + 8/2 + 12	1	3.6
N/6 + 8/5 + 10	1	3.6
2*/20/2 + 12	1	3.6
Total number of cultivars	28	
Total percentage		100

cultivar Mudczanka Czerwona, while subunit *1* was found in 13 remaining cultivars. The *Glu-D1* locus was responsible for the biosynthesis of subunits *2 + 12* and *5 + 10,* while the *Glu-B1* locus encoded subunits *6 + 8, 7 + 8, 7 + 8(9), 7 + 9, 7, 20,* and *22*. In the *Glu-B1* locus in Ostka Kazimierska and Ostka Mikulic Selit cultivars, an additional band appeared characteristic of the *7 + 8(9)* HMW-GS. It is, therefore, possible that these cultivars are characterized by a system of subunit *7 + 8* or *7 + 9* in the *Glu-B1* locus (Table 2). Each subunit observed in the electropherogram of the studied cultivars occurred at a different frequency rate. The *1/7 + 9/2 + 12* arrangement had the highest percentage (14.3%) and was followed by *N/ 7 + 9/5 + 10* (10.7%) (Table 3), while HMW-GS *N/7/5 + 10, 1/7 + 8(9)/2 + 12, N/7 + 8(9)/2 + 12, N/7/2 + 12, N/7 + 8/2 + 12, N/6 + 8/5 + 10,* and 2*/*20/2 + 12* occurred at the lowest frequency of 3.6%. The mean value of 7.1% was observed for the following HMW-GS arrangements: *1/6 + 8/2 + 12, 1/7 + 9/5 + 10, 1/22/5 + 10, N/7 + 8/5 + 10, 1/20/5 + 10, N/20/2 + 12,* and *N/7 + 9/2 + 12* (Table 3).

We also calculated the *Glu-1* quality score for the analyzed cultivars based on the numeric scale developed by Payne [11] (Table 2). Balta, Ostka Nadwiślańska, and Żelazna cultivars were found to have the lowest glutenin score (4 points), whereas the *Glu-1* quality index in the remaining cultivars matched those of Sielecka Genetyczna and Sobieszyńska 44 cultivars (9 points), which are cultivars of very high quality (Table 2).

3.2. STS-PCR Analysis of HMW Glutenin Genes. As regards the *1Dx5* and *1Dx2* genes, one amplification product was identified for each of them: 450 bp for *1Dx5* and 435 bp for *1Dx2*. Relatively high PIC values (0.50) were observed in the studied genes (Table 4). Primer amplification products for *1Dy10* and *1Dy12* genes were 422 bp and 552 bp in size, respectively. The cultivars with *1Dx5* and *1Dx2* alleles associated with *1Dy10* and *1Dy12* formed *Dx5 + Dy10* and *Dx2 + Dy12* systems. The ability to distinguish wheat cultivar genotypes, as measured by the PIC index, was the same for *1Dy10* and *1Dy12* markers as for STS *1Dx2* and *1Dx5*.

Table 4: Characteristics of STS markers polymorphism.

Genes HMW-GS	PIC[a]	PCR product size [bp]
1Dx5	0,50	450
1Dx2	0,50	435
1Dy12	0,50	552
1Dy10	0,50	422

[a]PIC: polymorphic information content, Ghislain et al. [8].

3.3. Sequencing Analysis of 1Dx5 and 1Dx2 Genes. As a result of *1Dx5* and *1Dx2* sequencing, 56 specific nucleotide sequences of suitable length were acquired for 28 studied cultivars. Comparing the structure of *1Dx5* and *1Dx2* genes in different cultivars using the *ClustalW* software, 117 parsimoniously informative sites (parsim-info sites) were found for *1Dx5* and 204 for *1Dx2* as well as 395 polymorphic sites for *1Dx5* and 535 for *1Dx2* (Table 5). Using the extra "Analysis" option, which is available in the *ProtSA* software, information was obtained on the percentage composition of purine and pyrimidine bases as well as the total weight of nucleotides present in the examined sequences. The average percentage of AT/GC pairs in cultivars was 58.80/41.20 for the *1Dx2* gene and 32.25/61.30 for the *1Dx5* gene (Table 5).

1Dx2 and *1Dx5* nucleotide sequences were analyzed in silico using the *BLAST2* software and compared to other

Table 5: Comparison of the nucleotide composition of DNA regions in *1Dx5* and *1Dx2* genes of the analyzed wheat cultivars.

The analyzed sequences	*1Dx5* gene	*1Dx2* gene
Total aligned sequential sites	838	1575
Conserved sites- C%	400	217
Variable sites-V%	395	535
Parsim-info sites-Pi%	117	204
Singleton sites- S%	265	239
The average percentage of AT pairs	32.25	58.80
The average percentage of GC pairs	61.30	41.20

nucleotide sequences found in the GeneBank database. The resulting orthologous sequences were aligned using the *Alignment-BLAST* function. Thus, the similarity of *1Dx2* and *1Dx5* genes of *Triticum aestivum* L. to other related species, such as *Aegilops tauschii, Triticum turgidum,* and *Psathyrostachys huashanica* was demonstrated.

4. Discussion

The data available in the literature demonstrate that glutenins are a group of storage proteins that play a key role in the quality parameters of wheat. It is commonly accepted that rheological properties of the gluten complex are related to the presence or absence of specific subunits of these proteins [12–19]. According to Zientarski and Waga [20], using this group of storage proteins as markers, may provide a more objective view of the relationship between their physicochemical structure and the variability of the genes encoding them. In this study, the composition of HMW-GS in the studied group of 28-old wheat cultivars was determined using the SDS-PAGE method. Eleven different protein subunits, which are a combination of three alleles, have been identified, *1 (Glu-A1a), 2* (Glu-A1b),* and *null (Glu-A1c)* in genome A; 6 alleles, *7 (Glu-B1a), 7 + 8 (Glu-B1b), 7 + 9 (Glu-B1c), 6 + 8 (Glu-B1b), 20 (Glu-B1e),* and *22 (Glu-B1k)* in genome B; and 2 alleles, *2 + 12 (Glu-D1a)* and *5 + 10 (Glu-D1d)* in genome D (Table 2). It has been demonstrated that the encoded fraction in chromosome 1A occurs significantly more frequently in the forms containing the *null (Glu-A1c)* variant (50%) than in those containing *2* (Glu-A1b)* (3.57%) and slightly more frequently than in the forms containing the *1 (Glu-A1a)* variant (46.43%). According to Lafiandra et al. [21], the noncoding *null* variant was probably formed due to clogging of structural genes within the *Glu-A1* locus. In Polish cultivars and breeding strains of winter wheat, *Glu-A1-null* outnumbers allelic variants of chromosome 1A [16, 22]. In terms of quality traits, the presence of the null variant is disadvantageous, as it adversely affects technological properties of gluten, whereas subunits *1* and *2** may, in fact, improve them [16]. So far, it has not been determined what is causing the quantitative dominance of the null variant over the coding variants *1* and *2**. It is believed that genes affecting HMW-GS are coupled with other genes important for various utility traits, such as those responsible for fertility, while the selection preferring high values of yield structure eliminates subunits *1* and *2** already at the early breeding stages [16]. In Poland, a detailed analysis of the subject was conducted by many authors. They found that *1 (Glu-A1a)*, *7 + 9 (Glu-B1c),* and *5 + 10 (Glu-D1d)* alleles were associated with high values of wheat flour baking properties. In turn, allelic variants of *null (Glu-A1c), 6 + 8 (Glu-B1d),* and *2 + 12 (Glu-D1a)* were associated with low values of wheat grain technological traits. Furthermore, it was observed that *1 (Glu-A1a)* and *5 + 10 (Glu-D1d)* were found in cultivars characterized by low grain yield, while *null (Glu-A1c)* and *2 + 12 (Glu-D1a)* were associated with the highest average yield values. This indicates that alleles encoding HMW-GS that favourably affect technological quality are also associated with low parameters of yield structure, whereas alleles adversely affecting this trait are associated with high fertility.

Eagles et al. [23] found the greatest HMW-GS polymorphism in the *Glu-B1* locus, in which over 30 alleles could be distinguished. The relationship between the presence of specific alleles in HMW-GS is extensively described in the literature [24–28]. In general, the data found in the literature suggest that an allele of one subunit has a higher negative impact on the dough energy than alleles of two subunits [21, 25, 27]. An example of this may be the effect of the *Glu-B1a* allele encoding subunit *7*. However, according to Pilch [26], this allele had the more detrimental effect on gluten than *Glu-B1b* alleles of two subunits *7 + 8* or the *Glu-B1c* allele in subunits *7 + 9*. The variability of HMW-GS subunits within the Glu-B1 locus observed in this study indicated that the following blocks favourably affected gluten quality in the studied wheat cultivars: *7 + 8 (Glu-B1b)* and *7 + 9 (Glu-B1c)*, while *7 (Glu-B1a), 6 + 8 (Glu-B1d), 20 (Glu-B1e),* and *22 (Glu-Bk)* were found to exert a negative effect on this trait.

When analyzing the *Glu-1D* locus in this study, it was noted that the presence of the *2 + 12* block was associated with poor baking quality, while the presence of the *5 + 10* block was associated with high flour baking quality. These subunits occurred with a similar frequency of 53.57% and 46.43% for *2 + 12 (Glu-D1a)* and *5 + 10 (Glu-D1d)*, respectively, in the pool of 28 wheat cultivars.

Because of the simple Mendelian inheritance of the genes encoding glutenin protein subunits, the identification of homozygous systems in electrophoretic images obtained from most cultivars, lines, or breeding strains is not difficult. In some cases, however, the electrophoretic image of bands is similar in different genotypes. In such cases, a heterozygous image can be studied, for example, *null, 1,* or *7 + 8(9),* in one form [21, 27]. Similar relationships of wheat cultivar heterogeneities in respect to the composition of HMW-GS in *Glu-B1* loci were also observed by Gregová et al. [27] and Fang et al. [29]. In the present study, such relationships of heterozygous band patterns of *7 + 8(9)* were detected in two cultivars: Ostka Kazimierska and Ostka Mikulic Selit within genome B (Table 2). In the remaining cultivars analyzed in the study, homozygous band patterns were identified which corresponded to beneficial or unfavourable glutenin subunits with a high or low number of quality points on a scale from 1 to 10° proposed by Payne [11].

The lowest glutenin quality score was observed in Balta, Żelazna, and Ostka Nadwiślańska (4 quality points). The first two of the aforementioned cultivars had the set of HMW-GS blocks, *N/20/2 + 12*, and the latter, *N/7/2 + 12*. Only two cultivars; that is, Sielecka Genetyczna and Sobieszyńska 44 reached the highest glutenin quality score (9 points); they contained the beneficial glutenin block *1/7 + 9/510* (Table 2). These results demonstrate that polymorphism of HMW-GS may, in some combinations of subunits, impair the technological quality of a given wheat form. This can be explained by the hypothesis described above [3, 23, 30–32]; namely, that one allele encoding one subunit present in a system of glutenin protein blocks has a stronger adverse effect on the technological value of flour than one allele of two subunits. These results suggest that HMW-GS polymorphisms observed in the common wheat cultivars, as determined by SDS-PAGE, are related to the technological quality variability assessed using the *Glu-1* index on a ten-point scoring scale proposed by Payne [11].

STS-PCR reactions generated products ranging from 422 bp to 552 bp in the *Glu-D1* region. In 15 cultivars, PCR products of 450 bp *(1Dx5)* and 422 bp *(1Dy10)* were amplified in *Glu-D1*. The following products were observed in the remaining cultivars: 435 bp *(1Dx2)* and 552 bp *(1Dy12)*. A similar gene amplification was obtained by Ahmad [6], De Bustos et al. [33], Zúñiga et al. [34], and Zamani et al. [35]. In addition, it was found that the resulting amplification products were characterized by a high PIC index of 0.50 (Table 4). The relatively high PIC index in the analyzed STS-HMW-GS markers proved their usefulness for the genetic variability analysis within *Glu–D1* loci of HMW-GS genes.

Two regions of HMW-GS were sequenced in the analyzed wheat cultivars, namely, *1Dx5* and *1Dx2*. These were selected because their dominant role in influencing wheat quality traits is well documented.

Research conducted by Anjum et al. [36] demonstrated that there were small sequence differences in *1Dx2* and *1Dx5* of *Triticum aestivum* L. Similar results were obtained in this work. The analysis of the structure of these genes revealed that the AT/GC ratio was different for *1Dx5* and amounted to 32.25/61.30 and 58.80/41.20 for *1Dx2* (Table 5). The high structure variability within the *1Dx2* gene was demonstrated when *1Dx2* and *1Dx5* sequences were aligned in the ClustalW software, as many polymorphic sites were found between them, that is, 395 *(1Dx5)* and 535 *(1Dx2)* (Table 5). Homology of the discussed nucleotide sequences was high and ranged from 93% to 78% for *Glu-Dx2-D1a* and from 100% to 99% for *Glu-Dx5-D1d*. Such a high degree of similarity indicates that these sequences must be homologous, that is, inherited from a common ancestor. This situation is not unusual since these genes have a common origin and similar biological functions.

Different allelic variants of the *Glu-1* gene were similar to one another both in the direct comparison of the obtained *1Dx2* and *1Dx5* sequences and when compared to the GeneBank sequences. It is understandable because all these sequences encode some HMW-GS variants. Despite the enormous homology of these sequences, polymorphic sites, mainly single nucleotide polymorphisms (SNP), do occur in them. In the case of the HMW-GS-Glu-1Dx2 gene, predominantly SNP mutations and small indel mutations were found relative to the GeneBank sequences. A small difference (only one transition) was observed between the sequence encoding *1Dx5* of *Triticum aestivum* and database sequences. When *1Dx5* was compared to the selected GeneBank sequences, only a few substitutions were detected. Lu et al. [37] also noted a high degree of homology of the sequence encoding HMW-GS in genome D. These authors studied sequences derived from *Aegilops tauschii* and *Triticum aestivum* and found a high degree of homology between them, but they also discovered single SNP mutations. These small differences in the nucleotide sequence can cause huge changes in the quality of wheat.

5. Conclusion

In this work, HMW-GS glutenin subunits, considered beneficial for gluten quality, have been identified based on electrophoretic SDS-PAGE analysis. In addition, subunits that negatively affect the technological value of common wheat grains have been identified. The presented data showed that the Glu-B1 locus was most variable. The polymorphism detected at the DNA level for the D genome using STS markers was consistent with the data obtained by SDS-PAGE at the level of high molecular weight glutenin subunits, HMW-GS. Two cultivars of common wheat have been distinguished based on Glu-1 technological quality indicators: Sielecka Genetyczna (1/7 + 9/5 + 10) and Sobieszyńska 44 (1/7 + 9/5 + 10), with very good baking quality. The comparative characteristics of *1Dx2* and *1Dx5* gene sequences demonstrated that there were differences in the occurrence of point mutations, transversions, transitions, and insertion and deletion. The genotypic variation in the contribution of glutenin to bread-making quality is due to variation in the number of specific HMW subunits. In wheat, the bread-making character of dough is strongly influenced by genetic background, determining protein concentration and composition. Presence or absence of specific allelic variants of HMW-GS is correlated with bread-making quality. HMW-GS *5 + 10* at the *Glu-D1* locus is related to better bread-making quality as compared to HMW-GS *2 + 12*. The reason for the better quality of wheat cultivars having HMW subunit pair *5 + 10* is related to the extra cysteine group on *1Dx5* compared to *1Dx2*, which enables the formation of additional disulphide link and increased polymer size. Glutenins y-type subunits possess more cysteine residues than x-type subunits. Therefore, they are capable of more inter- and intramolecular disulfide bonds formation, which mediates the aggregation of HMW-GS with the involvement of LMW-GS and results in an improved dough quality. In conclusion, it should be noted that valuable HMW-GS glutenin subunits are constantly being sought. In addition, the effect of other proteins in the gluten composition contributes to the improvement of wheat quality, which is why in further studies I will concentrate on testing of low molecular weight gluten protein subunits, LMW-GS, and gliadins, especially ω and γ, which are the basis of gluten physicochemical properties.

Abbreviations

HMW:	High molecular weight
HMW-GS:	High molecular weight glutenin subunit
LMW-GS:	Low molecular weight glutenin subunit
STS-PCR:	Sequence-tagged site-polymerase chain reaction
PIC:	Polymorphic information content
SDS-PAGE:	Sodium dodecyl sulphate polyacrylamide gel, electrophoresis
Glu-1:	Loci encoding gluten protein
HMW-GS Glu-A1, Glu-B1, Glu-D1:	Complex gene loci encoding high molecular weight (HMW) subunits
SNP:	Single nucleotide polymorphisms.

Disclosure

This article does not contain any studies with human participants or animals performed by the author.

Conflicts of Interest

The author declares that there are no conflicts of interest.

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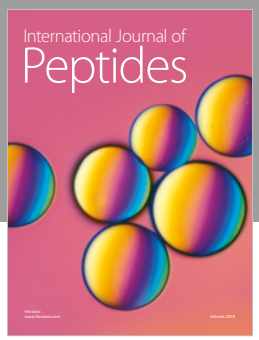

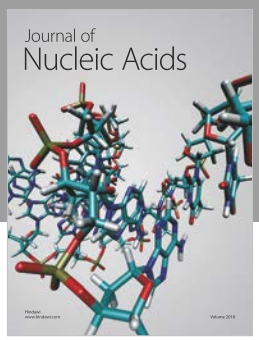

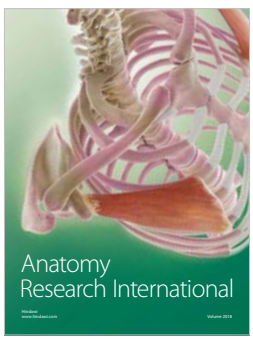

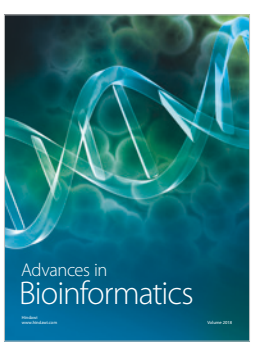

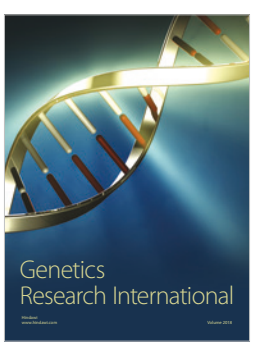

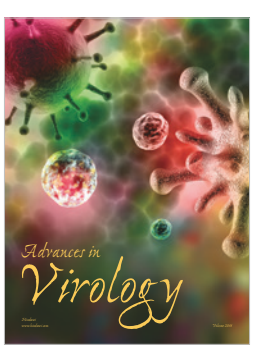

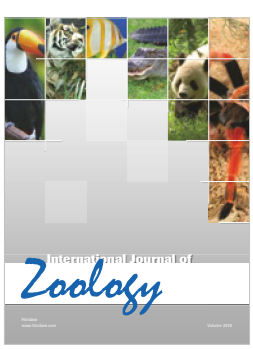

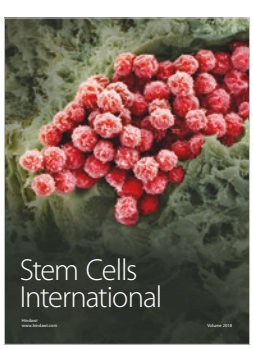

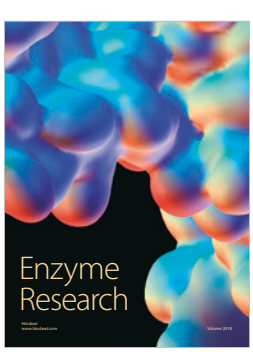

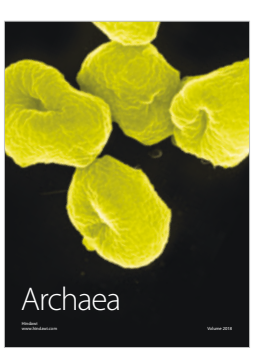


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