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Review

Allelic Variation of High-Molecular-Weight Glutenin Genes in *Triticum* Species and Triticale (× *Triticosecale* Wittmack)

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Abstract

High-molecular-weight glutenin subunits (HMW-GS) encoded by alleles at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci confer unique bread-making properties of common wheat (*Triticum aestivum* L.). The identification of HMW-GS is the prerequisite for pyramiding high-quality glutenin genes. The present review is designed to list all published HMW-GS alleles in *Triticum* species and triticale (A- and B genomes), focusing on methods for their identification. *T. monococcum* is characterized by 37 alleles at the *Glu-1* locus versus four alleles in *T. thaoudar* and 39 in *T. urartu*. In total, 80 alleles at *Glu-A1* of diploid *Triticum* species and about 42 alleles found in polyploid wheat landraces and varieties (4x and 6x), including triticale, were listed. Allelic variation at the *Glu-B1* locus is divided into 3 groups: *a* – *z*, *aa* – *az*, and *ba* – *ct*, comprising 121 alleles, of which 26 subunits have unspecified alleles. At least 51 allelic variants at locus *Glu-D1* of *Triticum* species were indicated, along with carriers of the species level. In addition, subunit-specific genetic loci have been tagged, facilitating molecular marker development of high-gluten wheat cultivars through marker-assisted breeding.

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Keywords
HMW-glutenins; allelic variation; *Triticum* species; triticale

1. Introduction

Three components, high-molecular-weight glutenins (HMW-GS), low-molecular-weight glutenins (LMW-GS), and gliadins, make up gluten, the major protein in the endosperm of wheat seeds [1]. The elasticity of the dough depends on the HMW-GS, while the low-molecular-weight glutenins mainly provide the elasticity and extensibility of the dough. Gliadins are factors affecting dough extensibility. Since the 1970s, the influence of these significant gluten components on the baking process of bread wheat has received much attention [2, 3].

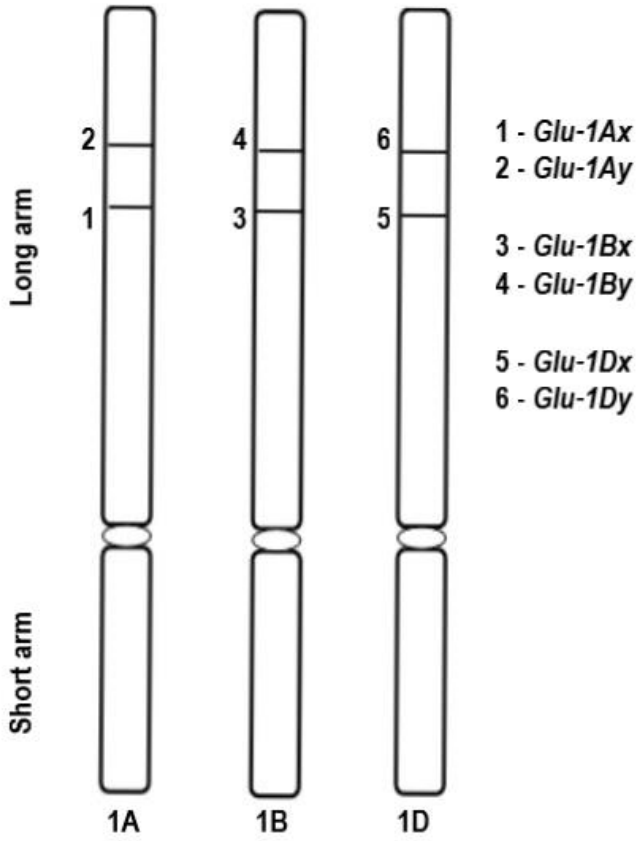

Figure 1 Schematic diagram of the gene loci of HMW-GS in wheat 1A, 1B and 1D chromosomes.

The *Glu-1* loci on the long arms of wheat group 1 (1A, 1B, 1D) chromosomes encode HMW-GS and are named *Glu-A1*, *Glu-B1*, and *Glu-D1* based on their chromosome names. Two genes are linked together, encoding two different types of HMW-GS, x- and y-type subunits at each locus (Figure 1). In sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), x-type subunits generally have a slower electrophoretic mobility and higher molecular weight than y-type subunits. Each subunit is assigned a unique number that is part of the numbers of all subunits in

SDS-PAGE. A single locus is indicated as the critical carrier from which the subunit is derived, and whether it is an x-type or y-type subunit. For clearer distinction, HMW-GS alleles can also be expressed as simple numbers, for example 5 + 10 (meaning Dx5 + Dy10) [4, 5].

Hexaploid wheat is typically characterized by three to five subunits, and this variation is due to both cultivar protein composition and presence of silent genes in the genotype. The *Glu-A1* locus encodes one subunit or none, *Glu-B1* two or one, and *Glu-D1* two subunits. In the 1980s, the *Glu-A1* locus was represented by three alleles, namely *a*, *b*, and *c*, versus eleven (*a* – *k*) at *Glu-B1* and six (*a* – *f*) at the *Glu-D1* locus [4, 6]. The last mentioned one has the strongest effect on wheat quality, followed by loci *Glu-B1* and *Glu-A1*. The variation in the protein composition is usually due to the high-molecular-weight glutenins, regardless of their small amount in the seeds, about 10%. Therefore, increasing the variation of HMW-GS alleles will potentially lead to more attention of researchers to wheat grain quality in crops breeding [7, 8]. This article aims to review all published HMW-glutenin alleles in *Triticum* species, including triticale A- and B genomes, focusing on methods for their identification and practical breeding aspects of recent molecular advances.

2. Allelic Variation at *Glu-A1* Locus

2.1 Diploid Triticum Species

Research on the HMW-GS in the A genome of diploid and hexaploid wheat species has begun in the second half of the last century [9]. The HMW subunits of *T. monococcum* ssp. *boeoticum* had a major x-subunit of slow mobility and several less prominent y-subunits of greater mobility, all of which fall within the mobility range of HMW subunits reported for bread wheat. In *T. monococcum* ssp. *monococcum* the range of the banding patterns for HMW subunits was similar to that of ssp. *boeoticum*. However, two accessions containing y-subunits were null for x-subunits. The HMW subunit banding patterns of *T. urartu* accessions were distinct from those of *T. monococcum*. All of them contained one major, and most contained one major y-subunit. The active genes for y-subunits, if transferred to bread wheat, may be useful in improving bread-making quality. 30 different alleles were described (*a* – *z*, *aa* – *ae*) at the *Glu-A1*^{m} locus (Table 1) [10]. The only *T. sinskajae* accession had the allele *o*, as did some specimens of cultivated einkorn wheat. Researchers found three allelic variants (alleles *a*, *b* and *c*) [11], and seven subunits, three for *Glu-A1x*, and four for *Glu-A1y* [12]. A wider variation for the Glu-A1x was detected [13-15]. The spectrum of A1y-subunits was extended by 1Ay8* and 1Ay12* [16] and 1Ay8.2 and 1Ay8.3 [17, 18]. At present, the A genome of *T. monococcum* (incl. ssp. *monococcum*, *atriaristatum*, *flavescens*, *halbohornemanii*, *hornemanii*, *laetissimum*, *macedonicum*, *pseudomacedonicum*, *nigricultum*, *pseudoflavescens*, *sofianum*, and *vulgare*) is characterized by 37 alleles with published 16 x-type and 8 y-type subunits.

1Ay-subunits are of great interest because they are always silent in bread wheat. Four y-type subunits in *T. monococcum* ssp. *aegilopoides* differed from those of *T. urartu* (Table 1) [19]. Seven y-type subunits in this species, named with Roman numbers, were additionally detected [15]. Wild diploid wheat exhibited active Ay-subunits, and consequently, they could be good sources for increasing the number of alleles encoding active subunits at the *Glu-A1* locus. This procedure was exploited with two lines of *T. boeoticum* ssp. *thaoudar* for introducing active Ay subunits into common wheat [20] to increase gluten strength. This was also observed in a cross between a *T. urartu* accession and durum wheat cv. Yavaros and 1Ax2* + 1Ay-null subunits transferred from *T.*

boeoticum through synthetic hexaploid wheat 8A-Tb into durum background [21, 22]. Due to the single accession of *T. sinskajae* involved in investigations, the published data are scarce. Summarizing, two *Glu-A1* allelic variants were described in *T. thaoudar* coding for subunits 39 – 42.

Table 1 HMW-GS variation at *Glu-A1* in diploid *Triticum* species.

Allele	Subunit	Carrier/species	Method*	Reference
-	Null	TM	1	[12]
-	1	TM	1	[12]
-	2*	TM, TB, Tsin	1	[12]
a – g	2.1a – 2.1g	TM	1, 2	[13, 14]
a – z	-	TM	1	[10, 11]
aa - ae	-	TM	1	[10, 11]
-	I - VI	TMA	1, 2	[15]
-	1Ay-null	TM	1	[12]
-	1Ay 1	TM, TB, Tsin	1	[12]
-	1Ay 2	TM	1	[12]
-	1Ay 3	TM	1	[12]
-	1Ay 8*	TM	1, 2	[16]
-	1Ay 8.2	TM	1, 2	[17]
-	1Ay 8.3	TM	1, 2	[18]
-	1Ay 12*	TM	1, 2	[16]
-	1Ay Ta-e1 – e3	TMA	1, 2	[19]
-	1Ay Ta-e	TMA	1, 2	[19]
-	I - VII	TMA	1, 2	[15]
o	-	Tsin	1	[10]
r	39 + 40	TT	1	[20]
s	41 + 42	TT	1	[20]
ac - as	I - XVII	TU	1	[21-23]
a - k	1Ax	TU	1, 3	[24]
-	1Ax-null	TU	1, 3	[24]
a - h	1Ay	TU	1, 3	[24]
-	1Ay-null	TU	1, 3	[24]
-	1Ay Tu-e1 – e2	TU	1, 2	[19]
-	1Ay Tu-s	TU	1, 2	[19]

TM, *T. monococcum*; TMA, *T. monococcum* ssp. *aegilopoides*; Tsin, *T. sinskajae*; TB, *T. boeoticum*; TT, *T. thaoudar*; TU, *T. urartu*

*1, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2, Polymerase chain reaction

3, Simple sequence repeats (microsatellites)

Different authors used different nomenclature of *Glu-A1* allelic variation in *T. urartu*. Seventeen alleles for the *Glu-A^{u}1* locus (*ac – as*) were published [23, 24]. A recent study reported different electrophoretic mobility among the 1Ax1 subunits of *T. urartu* compared to those of bread wheat

varieties Xiaoyan-54 and Cheyenne [25]. Four 1Ax subunits in 46 accessions showed slower electrophoretic mobility than the 1Ax2* present in Cheyenne. The electrophoretic mobility of the 1Ay subunits was faster than the 1Dy10 subunit of cultivated wheat with eleven 1Ax (*a* – *k*) and eight 1Ay (*a* – *h*) alleles, resulting in 18 HMW-GS genotypes, U1-U18 (Table 1). It was evident that the 1Ay genes from *T. urartu* were closer to those from *T. dicoccum* and *T. aestivum* than those from *T. monococcum* ssp. *aegilopoides* [19]. Finally, 39 HMW *Glu-1A* alleles in *T. urartu* were detected.

2.2 Tetraploid and Hexaploid Triticum Species

Early studies initially identified several alleles at the *Glu-1* loci as 3, 11, and 6 for *Glu-1A*, *Glu-1B*, and *Glu-1D*, respectively [4]. The authors compared the subunits derived from the four nomenclature systems and recommended allele designations for the three loci. Since this catalog of alleles was published at *Glu-1* loci, more alleles have been identified [26-29]. One adopted nomenclature [30] for the alleles encountered in *T. dicoccum* was used for the new ones in *T. durum* [31]. The authors reported seven HMW subunits of *Glu-1A* alleles in the 520 durum wheat varieties. To date, 31 alleles at the *Glu-A1* locus plus 11 others encoding named subunits have been published (Table 2).

Table 2 HMW-GS variation at *Glu-A1* locus in *Triticum* species (4x and 6x) and triticale.

Allele	Subunit	Carrier	Method*	Reference
a	1	TA, TS, Tdic, HT	1, 4	[32-35]
b	2*	TA, HT	1, 4	[27, 35]
c	Null	TA, TS, HT	1, 4	[32, 34-36]
d - g	-	TA	1	[9, 27]
h	I	Tdic	1	[27, 30]
i	II	Tdic	1	[27, 30]
j	1', III	TD, Tdic	1	[27, 30]
k	26	TA	1	[27, 37]
l	-	TA	1	[27, 38]
m	-	TD	1	[27, 39]
n	1'', IV	TD	1	[27, 31]
o	2**, V	TD	1	[27, 31]
p	3*	TA	1	[27, 40, 41]
q	2***, VI	TD, TA	1	[27, 31]
r	39 + 40	TA	1	[27, 42, 43]
s	41 + 42	TA	1	[20, 27]
t	21*	TA	1, 2, 3	[44-47]
u	2^{*B}	TA	1, 5	[27, 48]
v	2.1*, VII	TC, TS, Tdic	2, 3, 4	[5, 49-51]
w	2', 2.1*	TS, TA, TT	1	[27, 52, 53]
x	2''	TA	1	[27]
y	2··	TA, TS, TD	1, 2, 4	[35, 54-56]
u-z	-	TA	1	[57]
-	1 + T1	TD, TA	3	[47]

-	1*	DIC	3	[5]
-	1‴	TA	1	[58]
-	1.1	TA	1,3,5,6	[59, 60]
-	1.2	TA	1	[61]
-	2.2 + 1Ay	DIC	1	[61]
-	21 + 21*	TA	3	[47]
-	1AxTd	DIC	5	[62]
-	1Ay21	TA	3	[47]
-	1Ay21*	TA	1, 2, 5	[45, 63]
-	1AyT1	TA	3	[47]
-	1AyTd-e, Td-s	Tdic	5	[19]

TA, *T. aestivum*; TS, *T. spelta*; TD, *T. durum*; Tdic, *T. dicoccum*; DIC, *T. dicoccoides*; TC, *T. compactum*; TT, *T. turgidum;* HT, Hexaploid triticale

*1, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2, Reversed-phase high-performance liquid chromatography

3, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

4, Allele-specific polymerase chain reaction markers

5, Polymerase chain reaction

6, Two-dimensional electrophoresis (IEF × SDS-PAGE)

Allelic variation at the *Glu-A1* locus affects grain end-use quality. A new high-molecular-weight glutenin subunit at the *Glu-A1* locus (designated 21*) has been published that is of better quality than the common allelic variants 2* and 1 [44]. Using the electrophoretic, reversed-phase high-performance liquid chromatographic (RP–HPLC) analyses and polymerase chain reaction (PCR), the authors revealed genes encoding the subunits 21* and 21*y [45]. When the two y-type subunits were transferred into the Australian bread wheat cultivar Livingston, one of them (1Ay21*) showed a higher quality potential than 1AyT1. According to the authors, both alleles increased grain protein content, dough properties, and bread loaf volume [47].

Some molecular markers, such as allele-specific polymerase chain reaction (AS-PCR) based on single nucleotide polymorphisms (SNPs), have proven to be a rapid and efficient screening tool for desirable HMW-GS. Various genes in the *Glu-A1* locus, such as 1Ax2*, 1Ax2.1*, 1Ax1, and 1Ax, can be selected by AS-PCR markers for efficient use in breeding. Three locus-specific sequence-tagged locus markers showed distinctive features for marking selected *Glu-A1* and *Glu-D1* alleles. For example, UMN19 marker was created to differentiate *Glu-A1b* (Ax2*) from *Glu-A1a* (Ax1) on *Glu-A1* locus [7, 64, 65]. Additionally, some primers have been described to precisely detect the 2* subunit and discriminate it from null and 1 glutenin subunit [65, 66]. Wheat lines with *Glu-A1a* or *Glu-A1b* showed higher dough strength than lines with null allele *Glu-A1c* [67]. The use of *Glu-A1* markers to screen wheat cultivars worldwide has shown that more than 50% of cultivars from different countries carry *Glu-A1b*. Several end-use seed quality markers are diagnostic as they have been established based on the sequences of the relevant genes [51, 68]. A new set of DNA markers helped to establish 11 haplotypes at the *Glu-A1* locus (H1 to H11) in three wheat species [69]. The major haplotypes in tetraploid wheat (*T. turgidum*) are H1, H8, and H9, containing both 1Ax and 1Ay subunits. The developed DNA markers are helpful in studying molecular variation and evolutionary mechanisms of orthologous *Glu-A1* regions in common wheat and related species.

A novel x-type HMW-GS encoded by the *Glu-A1* locus was published as 2•• [54]. The authors describe its structure and function as highly favorable for grain quality, equal to subunit 2*. Subunit 2•• was later discovered in Spanish wheat cultivars [70]. For easy and fast identification of 2••, a PCR screening method was created. Alignment comparison of 2•• with 2* shows an 18 bp deletion present in the nucleotide sequence of 2*, but not in the 2•• sequence. This subunit scored higher qualitative values than protein 2*: high sedimentation volume, the low percentage difference between maximum peak height and 3 min post-peak height, and higher mixograph values. New subunits (1Ax2.2 and 1Ay) were recorded in the wild emmer accession D97. The 1Ax1.2 protein fraction was introduced into one of two newly obtained lines from the cross of the common wheat variety Chuannong 16 with the wild strain emmer [61]. Adding to the list of new genes is *Glu-A1x*, found in native wheat cultivars, encoding a subunit called 1‴. Recently, in *T. dicoccoides* acc. K5199, another subunit, 1AxTd, was identified using primers to reveal its sequence of nucleotides [58, 62].

3. Allelic Variation at *Glu-B1* Locus

3.1 HMW-GS Alleles a – z

In this group, 26 alleles were published for about 40 years. At first, 11 alleles (*a* – *k*) were revealed in *T. aestivum*, compared to subunit designations of various authors [4]. Another 9 alleles (I – IX) annotated by the proposed system in durum and common wheat cultivars were added, making their total number 20 [30, 31]. Later, alleles *m*, *n*, *o*, *q*, *x,* and *y* encoding I, II, III, V, VII, and VIII subunits were listed (Table 3). The established *o* and *s* alleles in bread wheat (6 + 22 and By18* subunits) were in conflict with the previously published *s* allele expressing 7 + 11 subunits [37, 42]. There is no information for two alleles (*t* and *v*) about their subunits. The *Glu-B1e* allele is responsible for Bx20 or 20x + 20y [27, 46].

The following four methods, SDS-PAGE, RP-HPLC, high-performance capillary electrophoresis (HPCE), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) were employed to analyze the HMW-GS compositions of 60 genotypes belonging to different 4x- and 6x-wheats [5].

Table 3 HMW-GS alleles *a* – *z* at *Glu-B1* locus in *Triticum* species and triticale.

Allele	Subunit	Carrier	Method*	Reference
a	7 + null	TA, HT	1	[27, 32, 33, 35]
b	7 + 8	TA, TS, HT	1	[2, 34]
c	7 + 9	TA, HT	1	[33, 46]
d	6 + 8	TD, TS, Tdic, HT	1	[34, 71-73]
e	20; 20x + 20y	TD, TA, TS, HT	1	[27, 33, 46, 74]
f	13 + 16	Tdic, TA, TS, HT	1	[2, 34, 36]
g	13 + 19	TA	1	[46, 75]
1g	14	TD, TA	1	[46, 76]
h	14 + 15	TA	1	[2, 74]
i	17 + 18	TA	1	[2, 33]
j	21; 21x + 21y	TA, HT	1	[27, 32, 77]
k	22	TA	1	[75, 78]

l	23 + 24	TA	1	[77, 79]
m	I	Tdic	1	[27, 30]
n	II	Tdic	1	[27, 49]
o	III; 6 + 22	Tdic	1	[27, 42]
p	23 + 18	Tdic, TA, HT	1	[30, 46]
q	V	Tdic	1	[27, 49]
r	By19	TD	1	[74, 77]
r (av)	7 + 18	HT	1	[32, 80]
s	11 + 7; y18*	TA	1	[27, 42]
t	-	TA	1	[27, 38]
u	7* + 8	TA	1	[35, 78]
v	-	TA	1	[27, 39]
w	6* + 8*	TA, TS	1	[39, 56]
x	VII	TT	1	[27, 81]
y	VIII	TD	1	[27, 81]
z	7 + 15	TD, TA	1	[33, 82]

TA, *T. aestivum*; TS, *T. spelta*; TD, *T. durum*; Tdic, *T. dicoccum*, TT, *T. turgidum*; HT, Hexaploid triticale

*1, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

A key objective was to compare and evaluate these methods regarding resolution, sensitivity, accuracy, and throughput. Comparative analysis demonstrated that each methodology has its advantages and disadvantages. The materials expressed a variety of 17 *Glu-B1* alleles, 9 of which belong to the group of *a* – *z*. Bx7 is one of the most critical subunits encoded by the *Glu-B1* locus and related to baking quality. Three variants can be distinguished among Bx7: Bx7, Bx7* and $Bx7^{OE}$. Novel DNA markers for accurate discrimination between the *Glu-B1* locus subunits Bx7 and Bx7* were described [56, 83]. The *Glu-B1* locus was more variable in durum wheat, with fifteen alleles represented, of which *Glu-B1b* (7 + 8), *-B1d* (6 + 8), and *-B1e* (20 + 20) were the most frequently occurring [71, 76].

3.2 HMW-GS Alleles aa – az

This group comprised 24 alleles, of which *ab*, *ax*, *ay* and *az* encoded XI, XV, XVI and XVII subunits found in tetraploid wheat, respectively [27, 84]. There are eight single bands in the list, that is Bx6, Bx7', Bx7*, Bx13, Bx18*, Bx37, By8, and By18, and one null allele, *Glu-B1ah* (Table 4). Two primer sets (P3 and P4) allowed for precise determination of the occurrence of individual x-type subunits. Bx7* is the most frequently identified subunit in wheat and occurred in 65% of the tested cultivars. Bx7 was identified in 25% of genotypes and Bx7 or Bx7* (not precisely identified) - 7.5%. A wide distribution of the Bx7* in wheat was recorded [76, 83, 85]. The Bx6 subunit is associated with poor baking quality and occurs with a relatively low frequency [86]. There were two variants of *Glu-B1b* (7 + 8), *Glu-B1u* (7* + 8) and *Glu-B1al* (7^{OE} + 8*), that could be differentiated by the presence of an 18 bp indel at a repetitive domain of hexapeptide motif [87]. The *Glu-B1al* lines with $Bx7^{OE}$ subunit well identified by SDS-electrophoresis, had higher dough strength than the lines with other *Glu-B1* alleles, and markers have been designed [88, 89]. A survey of diploid, tetraploid, and hexaploid

wheat using SDS-PAGE showed that the overexpressed phenotype of *Glu-B1a* (Bx7) and *Glu-B1al*, only occurred in *T. turgidum* and *T. aestivum* [67, 89, 90].

Table 4 HMW-GS alleles *aa* - *az* at *Glu-B1* locus in *Triticum* species and triticale.

Allele	Subunit	Carrier	Method*	Reference
aa	X, 7*	TD, TA	1, 3	[86, 91, 92]
ab	XI	TD, TA	1	[27, 92, 93]
ac	16 + 6	TD	1	[31, 77]
ad	23 + 22	TD	1	[31, 77]
ae	18*	TA	1	[41, 77]
af	26 + 27	TA	1	[41, 77]
ag	28 + 29	TA	1	[27, 41]
ah	Null	TA	1	[77, 94]
ai	7'	TA	2	[77, 95]
aj	Null + 8	TA	1	[46, 74, 96]
ak	7* + 8*	TA	1	[42, 97]
al	7^{OE} + 8*; 7^{OE} + 8; 7 + 8*	TA, TD	1, 3, 5	[7, 35, 46, 76, 89, 98]
am	Null + 18	TA	1, 4	[35, 46, 68]
an	6 + null	TA, TD	1	[74, 82, 99]
ao	7 + 16	TA	1	[78, 99]
ap	30 + 31	TA	1	[77, 99]
aq	32 + 33	TA	1	[35, 100]
ar	34 + 35	TA	1	[77, 99]
as	13	TA	1	[77, 99]
at	13 + 18	TD, TA	1	[99, 101, 102]
au	37	TA	1	[77, 101]
aw	6.8 + 20y	HT	1	[32, 103]
ax	XV	Tdic	1	[49, 84]
ay	XVI	Tdic	1	[27, 84]
az	XVII	Tdic	1	[49, 84]

TA, *T. aestivum*; TD, *T. durum*; Tdic, *T. dicoccum*; HT, Hexaploid triticale

*1, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2, Two-dimensional electrophoresis (A-PAGE × SDS-PAGE)

3, Polymerase chain reaction

4, Allele-specific polymerase chain reaction markers

5, Multiplex PCR system

Three y-type subunits were identified at the *Glu-B1* locus. Primer sets were designed and used for 1By subunit determination [104]. A comparison of the results obtained for primers P5 and P6 indicated that the By9 subunit was present in 59% of cultivars studied against twice less content of By8 and By-null in them. The researchers drew attention to the discrimination of Bx7, Bx7* and $Bx7^{OE}$. The allele *Glu-B1i* encoding 17 + 18 subunits has been considered the most significant effect on gluten quality among 11 *Glu-B1* alleles in bread wheat [105]. Two SNP-based AS-PCR markers for

1By18 gene identification were developed and validated using many bread wheat varieties and RILs [68]. A set of 17 SNP markers representing the most frequent SDS-PAGE alleles at each locus was designed [46]. Four markers at the *Glu-B1* locus constituted a valuable toolbox for breeding wheat to improve end-use quality.

3.3 HMW-GS Alleles ba – ct

The section contains 46 alleles, of which 6 (*Glu-B1bl*, *-bw*, *-bz*, *-ck*, *-cn,* and *-cp*) encode single bands (Table 5). Data for *-bk/be*, *-bl/bf,* and *-bm/bg* alleles in the wheat gene catalog [27] and other studies [51, 52] are conflicting. Similar situations in *Glu-B1bh* encoded by 13 + 22 or 13 + 22* subunits [27, 52] and *Glu-B1cp* characterized by 20* or 14* subunits [50, 106]. The *Glu-B1al* gene ($Bx7^{OE}$ + By8*) improves dough strength over the more common allele *Glu-B1b* (7 + 8) and another promising allele *Glu-B1i* (17 + 18) [107]. Subunit $Bx7^{OE}$ is found only in high-gluten wheat materials.

Table 5 HMW-GS alleles *ba* - *ct* at *Glu-B1* locus in *Triticum* species and triticale.

Allele	Subunit	Carrier	Method*	Reference
ba	13* + 16	TS	1	[27, 108]
bb	6 + 18'	TS	1	[27, 108]
bc	6 + 17	TD	1	[27]
bd	20 + 8	TD	1	[102, 109]
be	-	DIC, TS	1	[52, 110]
bf	-	DIC, TS	1	[52, 110]
bg	-	DIC, TC, TS	1	[52, 110]
bh	13 + 22(22*)	TD, TS	1, 2	[27, 52, 102]
bi	13 + 22.1	DIC, TS	2	[5, 27, 52]
bj	14* + 15*	TS, TA	2	[27, 52, 111]
bk (be)	6.1 + 22.1	DIC,Tdic,TS	1	[5, 27, 52, 110]
bl (bf)	6.1 + null	DIC, TS	1	[5, 27, 52, 110]
bm (bg)	13* + 19*	TS	2, 3	[27, 51, 52]
bn	7 + 19	HT, TD	1, 4	[27, 76, 112]
bo	7 + 26	HT	1	[27, 112]
bp	7** + 8	TA	1	[27, 113]
bq	7 + 8**	TA	1	[27, 113]
br	7.1 + 7.2 + 8*	TA	4	[27, 114]
bs	7.3 + 7^{OE} + 8*	TA	4	[27, 114]
bt	17' + 18'	TT	1	[27, 115]
bu	17' + 18*	TT	1	[27, 115]
bv	13** + 8*	TT	1	[27, 115]
bw	8'	TT	1	[27, 115]
bx	7 + 17	TA	1	[27, 116]
by	7b* + 8	TA	3	[27, 98]
bz	7^{OE}	TA	3	[27, 98, 117]
ca	6 + 8b*	TA	3	[27, 98]
cb	7^{OE} + 8	TA	3	[27, 98]

cc	7^{OE} + 8a*	TA	3	[27, 98]
cd	7^{OE} + 8b*	TA	3	[27, 98]
ce	7 + 8a*	TA	3	[27, 98]
cf	20* + 33*	TD	1	[100, 118]
cg	13 + 16*	TD	1	[100, 118]
ch	7 + 22	TA, TD	1	[82, 100]
ci	7 + 22*	TD	1	[100, 118]
cj	13* + 15*	TD	1	[100, 118]
ck	Null + 15	TA, TC	1	[60, 119]
cl	14 + 8	TM	1	[118, 119]
cm	6 + 8*	TM, TD	1, 4	[76, 119]
cn	17 + null	Tsph	1	[27, 119]
co	20 + 22*	TD	1	[106, 118]
cp	20*, 14*	TD, TA	1	[50, 106]
cq	7 + 33	TA	1	[50]
cr	14 + 22*	TD	1	[82]
cs	6* + 15*	TD	1	[82]
ct	6 + 20y	TD	1	[82]

TA, *T. aestivum*; TD, *T. durum*; TS, *T. spelta*; Tdic, *T. dicoccum*; DIC, *T. dicoccoides*; TT, *T. turgidum*; TC, *T. compactum*; TM, *T. macha*; Tsph, *T. sphaerococcum*; HT, Hexaploid triticale

*1, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2, Two-dimensional electrophoresis (A-PAGE × SDS-PAGE)

3, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

4, Polymerase chain reaction

SDS-PAGE has difficulty distinguishing HMW glutenins with similar electrophoretic mobilities, such as By8 and By8* [104]. Multiplex PCR can simultaneously detect different target genes in one PCR reaction, improving efficiency and reducing costs compared to single PCR markers. Positive results were obtained when multiplex PCR detected the presence of Ax2*, $Bx7^{OE,}$ and Dx5 [87, 120] on an agarose gel-based codominant attachment region marker for the $Bx7^{OE}$ subunit homozygosity test at the *Glu-B1* locus [7]. Researchers used a combined method (MALDI-TOF–MS and genotyping markers) to identify lines containing $1Bx7^{OE}$ from an experiment with 217 wheat genotypes [117]. This approach is of practical importance when the goal is to select plants with the *Glu-B1al* allele for creating lines with increased dough strength in the wheat breeding programs.

Different authors marked differently the subunit expression of *Glu-B1al*: 7 + 8*, 7^{OE} + 8, and 7^{OE} + 8* [46, 98, 121]. The allele encoding the overexpression of the 7^{OE} subunit was previously found to have a large positive influence on bread-making quality [97, 122]. MALDI-TOF-MS is a powerful technique to rapidly identify the diversity of HMW-GS alleles at the *Glu-B1* locus. Its high resolution also led to the identification of a novel HMW-GS 7b* + 8 in the Japanese germplasm Eshimashinriki [98], as well as the discrimination of protein subunits 7^{OE}, 8a* and 8b* associated with superior seed quality, which was not possible by the use of SDS-PAGE method.

Glu-B1al is not the only allele in *Glu-B1* with a duplication of the x-type glutenin gene. *Glu-B1al*, *Glu-B1br*, and *Glu-B1bs* provide overexpression of subunits of the same size and electrophoretic mobility as Bx7, but *Glu-B1br* appear to differ from the others in its effect on glutenin

polymerization. The similarity in the DNA sequences of the three alleles, as well as the identical duplication linkages in the three alleles to the same places found in diploid, tetraploid, and hexaploid *Triticum* species [88], indicate that these alleles originated from a common ancestor. In wheat, the Argentinian wheat cultivar Klein Universal II has been shown to be a *Glu-B1al* donor in several common wheat cultivars [87]. The VQ0437 genotype probably inherited *Glu-B1bs* from the CD87 variety [114].

The *Glu-B1ch* (7 + 22) allele was reported in one Mediterranean strain and three Iranian landraces [100]. Recent authors have also linked *Glu-B1ch* to low gluten quality. Conversely, some haplotypes carrying 7 + 15 or 7 + 22 banding patterns gave excellent glutenin profiles with elevated SDSS (sodium dodecyl sulfate micro sedimentation assay) values. Their use in selection should be more careful in durum wheat breeding programs [82]. The Italian Polesine (14 + 22*) has a unique protein pattern associated with low gluten quality but with a high grain protein content value. Subunit 22* has been published as a rare protein variant [100].

Another unique banding pattern (6* + 15*) was obtained in the local variety Haurani. A previous study described this wheat variety's 6 + 16 pairs [123]. The authors found that subunit 6* had a higher mobility than subunit 6 in the SDS-PAGE. Various quality parameters were investigated over 2 years to establish that the 6* + 15* subunits could be a helpful breeding resource for grain quality. A new combination of subunits at the *Glu-B1* locus (6 + 20y) was published, found only in the Italian cultivar Capeiti 8. However, previous studies reported different patterns for this cultivar, such as 20x + 20y and 7 + 8 [93]. The 6 + 20y pair was not evaluated phenotypically, which also applies to the 14 + 22*, 6 + 20y, and 6* + 15* glutenins, tentatively designated as *-B1cr*, *-B1ct,* and *-B1cs* alleles, respectively [27].

Interesting subunits were identified in the European spelt wheat, such as 1By19*. The allele structure was analyzed by cloning and sequencing. SNP-based molecular markers have been developed using different F_2 populations derived from crosses between spelt and bread wheat cultivars and recombinant inbred lines [51].

3.4 HMW-GS Unspecified Alleles

This group includes 27 subunit patterns involving 12 single bands. Six of them are y-type subunits, By8*, By8.1, By8.1*, By16*, By20, and ByTd (Table 6).

Table 6 HMW-GS subunits at *Glu-B1* locus with unspecified alleles.

Allele	Subunit	Carrier	Method*	Reference
-	1.1 + 15*	DIC	3	[5, 111]
-	6 + 15	TD	1	[2, 124]
-	6 + (8)	TD	1	[40]
-	6 + 8**	TA	4	[42]
-	6*	TA	1	[125]
-	6* + 8	TA	1	[5]
-	6* + 16	TD	1	[102]
-	6* + 22*	DIC	3	[5]
-	6** + 8	TA	1, 4	[126]
-	6.1 + 22	TA	1	[2, 46]

-	6.1* + 8.1*	TA	1	[96]
-	6.5	TA	1, 3	[127]
-	7 + 11	TA	1	[74]
-	7* + 9	TA	1, 4	[35, 56]
-	7.1*	TA	1	[128]
-	14	TA	1	[42]
-	14 + 19	TA, TD	1, 4	[46, 76]
-	14 + 20	TD	4	[76]
-	14.1	TA	4	[42]
-	23 + 8	TA	1	[46]
-	By8*	TA	4	[42]
-	By8.1	TA	4	[60]
-	By8.1*	TA	1	[128]
-	By16*	DIC	1, 2, 3	[105]
-	By20	TA	1	[61]
-	ByTd	DIC	4	[62]
-	hs*	TS	1	[34]

DIC, *T. dicoccoides*; TA, *T. aestivum*; TD, *T. durum*; TS, *T. spelta*

*1, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2, Capillary electrophoresis

3, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

4, Polymerase chain reaction

The germplasms used generally expressed good match between the MALDI-TOF-MS determined molecular masses and these deduced from the coding sequences [5]. The authors compared lines with different subunits at *Glu-1* loci, demonstrating that MALDI-TOF-MS is suitable for screening HMW glutenins and identifying new genotypes. In Syrian durum genotypes, *Glu-B1b* and *Glu-B1d* were the most frequent alleles, and the 6 + 15 subunit was found at high frequencies [124]. A comparison showed the effects of several Glu-B1 subunits, including 6.1 + 22, on dough strength (W) and tenacity (P) wheat flour. Three other subunits, 1Bx14, 14 + 19 and 23 + 8 occurred less frequently, between 0.2 and 0.5 percent. Two subunits, 6 + 8** and By8* were very scarce, found in Asian wheat genotypes, while 1Bx14.1 was designated as a new subunit [42].

The Chinese wheat landraces contained novel alleles (1Bx7.1* and 1By8.1*), indicating that this group was also a potential resource for improving wheat quality [78, 128]. A novel high-molecular-weight glutenin subunit encoded by the *Glu-1B* locus was identified in the French genotype Bagou, named 1Bx6.5. This subunit differed in SDS-PAGE from the well-known 1Bx6 and 1Bx7 subunits, encoded at this locus [127]. Several other subunits with unspecified alleles are shown in Table 6.

4. Allelic Variation at *Glu-D1* Locus

At least 51 alleles were identified at the *Glu-D1* locus of hexaploid *Triticum* species (Table 7). Carriers of alleles involved *T. aestivum* (exclusively wheat landraces, historical and modern varieties), *T. macha*, *T. compactum*, *T. spelta*, *T. sphaerococcum,* and *T. petropavlovskyi*. *Aegilops tauschii* (DD, 2n = 14) genes representing 42 alleles (*t*, *x*, *y*, *z*, *aa – ak*, *aq – az*, *ba – bn*, and *br – bt*) were listed here without subunits following the accepted order [27]. There were several single

subunits: 1Dx2, 2.2*, 5, 36, 38 and 1Dy subunits, 10, 10.1, 11, 12, 12*, 12.1, 12.1* and 12.2. Allele *i* encoded null at *Glu-D1-1* as found in Nap Hal variety expressing both high protein and high lysine contents [27, 129], and in cultivar Lontoi from Finland [46]. The first six alleles (*a* – *f*) were identified earlier [4]. Some conflicts were found in *Glu-D1am* allele (subunits 2 + 12'or 2 + 12*) and *Glu-D1br* (2.8 + 12 or 5*t + 10.1t).

Table 7 HMW-GS allelic variation at *Glu-D1* locus.

Allele	Subunit	Carrier	Method*	Reference
a	2 + 12	TA, TM, TC, Tsph	1, 2, 3	[96, 119, 130]
b	3 + 12	TA, TC, TM	1	[119, 131]
c	4 + 12	TA	1, 3, 4, 5, 6	[7, 46, 130]
d	5 + 10	TA, TC, TS	1, 2, 3, 5, 6, 7	[7, 130, 132, 133]
e	2 + 10	TA	1	[74, 78]
f	2.2 + 12	TA	1, 3, 7	[46, 130, 133]
g	5 + 9	TA	1	[27, 37]
h	5 + 12	TA	1	[35, 78]
i	Null	TA	1	[46, 78]
j	2 + 12*	TS, TA	1	[96, 113]
k	2 + null	TA	1	[113, 134]
l	Null + 12	TS, TC, TA	2, 3	[50, 130]
m	1Dy10	TA	1	[74, 135]
n	2.1 + 10	TA	1	[33, 135]
o	2.1 + 13	TM	4	[27, 136]
p	36	TA	1	[27, 137]
q	2 + 11	TA	1	[27, 78]
r	2.3 + 12	TS	1	[27, 98]
s	38	TA	1	[27, 101]
t	43 + 44	AT	-	[27]
u	2 + 10'	TA	1	[35]
v	2.1 + 10.1	TA	4	[50, 96]
w	5* + 10	TA	1	[27, 121]
x	2 + T2	AT	-	[27]
y	3 + T2	AT	-	[27]
z	3 + 10	AT	-	[27]
aa - ak		AT	-	[27]
ah	1.5 + 10	TA	1	[5]
al	2.2*	TA	1	[27, 138]
am	2 + 12'(12*)	TS, TA	1	[49, 116, 134]
an	2 + 12*	TA, TS	1	[27, 113, 139]
ao	2.4 + 12	TS	1	[27, 108]
ap	2.5 + 12	TS	1	[108, 139]
aq - az		AT	-	[27]
ba - bn		AT	-	[27]

bo	5' + 12	TA	1	[27, 107]
bp	2.1' + 12	TS	1	[27, 52]
bq	2.6 + 12	TA	1	[27, 96, 140]
br	2.8 + 12	TA	1	[134]
br	5*t + 10.1t	AT	-	[27]
bs - bt		AT	-	[27]
bu	2' + 12	TA	1	[27, 116]
bv	2'' + 10	TA	1	[27, 116]
bw	2'' + 12	TA	1	[27, 116]
bx	2^{+} + 12	TA	1	[50]
by	2^{+} + 12^{+}	TA	1	[50]
-	1.5* + 10	TA	1, 6	[141]
-	2 + 12.2*	TA	1, 6	[60, 141]
-	2 + 12.3	TA	1, 6	[74, 142]
-	2 + 12.6	TA	1, 3, 6	[60, 143]
-	2 + 12.7	TA	1, 3, 6	[60, 143]
-	3* + T2	TA	1	[58]
-	5	TA	1	[74]
-	5** + 10	TA	1	[96]
-	1Dy10.1	Tpetr		[144]
-	1Dy11	TA	1	[74]
-	1Dy12*	TA	1	[74]
-	1Dy12.1	TA	1	[74]
-	1Dy12.1*	TA	1	[74]
-	1Dy12.2	TA	1	[74]

TA, *T. aestivum* (includes wheat landraces, historical and modern wheat varieties); TS, *T. spelta*; TC, *T. compactum*; TM, *T. macha*; Tpetr, *T. petropavlovskyi*; Tsph, *T. sphaerococcum*; AT, *Aegilops tauschii*

*1, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

2, Reversed phase-high performance liquid chromatography (RP-HPLC)

3, Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS)

4, Two-dimensional (LEF × SDS-PAGE) analysis

5, Multiplex PCR

6, Allele-specific polymerase chain reaction markers (AS-PCR)

7, Lab-on-a-chip capillary electrophoresis (Lab-on-a-Chip)

In the past decades, various techniques have been developed to characterize the allelic variation of HMW-GS. These include SDS-PAGE, two-dimensional electrophoresis (2-DE), PCR, high-performance capillary electrophoresis (HPCE), RP-HPLC, MALDI-TOF-MS, and lab-on-a-chip capillary electrophoresis [5, 130, 145]. MALDI-TOF-MS was characterized as an accurate and high-throughput method for determining the HMW-GS composition of wheat varieties. Until now, only five active *Glu-1Dy* genes have been cloned and characterized from bread wheat. The allelic variation at the *Glu-1* locus in 485 Chinese wheat landraces using MALDI-TOF-MS method characterized and

identified several novel Glu-1D subunits [60, 143]. Researchers successfully separated 1Dx subunits 2, 2.2, 5, and 1Dy10, 12 in HMW-GS profiles of 38 Korean wheat cultivars [130]. The identified HMW-GS of each variety precisely agreed with the results previously obtained using RP-HPLC combined with SDS-PAGE [146].

To facilitate early selection of HMW-GS alleles in breeding programs, DNA-based molecular markers were developed by KASPar assays with a high degree of concordance between HMW-GS identified by SDS-PAGE and molecular markers, despite several discrepancies between the two methods [46]—the Mk-D1-2-1 marker at *Glu-D1-2* distinguished subunits 10 and 12 as in SDS-PAGE identification. Further, a new agarose gel-based multiplex PCR system for detecting three high-quality HMW-GS of Ax2*, Bx7^{OE,} and Dx5 was established. These multiplex markers will effectively develop strong-gluten wheat cultivars via marker-assisted breeding [7, 147].

Wheat lines missing one subunit at Glu-D1, either x- or y-subunit, attract great interest for the potential grain quality [148]. The effect of subunit Dy10 on wheat dough properties and end-use quality was studied by generating and analyzing a deletion mutant with the Dy-null allele. The Dy-null allele might be exploited by wheat breeding programs [149].

5. Conclusion

The analysis and characterization of HMW-GS banding patterns in wild *Triticum* specimens and landraces of wheat and their comparison with cultivated wheat is a successful strategy to introduce exotic genes into elite germplasms to improve wheat grain quality. Production of lines with different x- and y-subunits through the pyramiding of subunit-specific genetic loci may be facilitated by molecular markers for the specific glutenin fraction variants. Breeders can now efficiently select the desirable subunit in the early generations of wheat quality crosses using AS-PCR markers. In practice, it is preferable to have more information about subunits for each gene related to quality in individual forms and the subsequent evaluation of these proteins for best quality parameters. This will increase the selection efficiency and facilitate the creation of elite wheat and triticale lines with high glutenin subunit contents for quality improvement in breeding programs.

Author Contributions

The authors contributed to the study conception and design. Alleles at *Glu-A1* and *Glu-B1* loci were performed by P. Spetsov, and alleles at *Glu-D1* – by N. Daskalova. The first draft of the manuscript was written by PS and both authors commented on the final version of the manuscript.

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