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STORAGE PROTEINS OF BULGARIAN VARIETIES AND ADVANCED LINES OF DURUM WHEAT

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Abstract

The research focused on 25 genotypes of durum wheat varieties and advanced lines created in the Field Crops Institute in Chirpan, Bulgaria. A-PAGE and SDS-PAGE methods were used to investigate the allelic composition of γ-gliadins, high molecular weight (HMW-GS), and low molecular weight (LMW-GS) glutenins. Two of the γ-gliadin fractions were identified in the analyzed genotypes. Gliadin fraction γ-45 in this investigation has a much lower frequency (24%) compared to gliadin fraction γ-42 (76%). According to the identified allelic combinations in the Gli-B1 and Glu-B3 loci of the studied genotypes, two main types were established (LMW-1 and LMW-2) that determine gluten strength. Protein subunit 2 (allele b) was identified in a significant part of the analyzed genotypes at the Glu-A1 locus, which is associated with higher gluten strength. In the Glu-B1 locus, genotypes with HMW-subunits 6+8, 7+8, 13+16, 14+15, 17+18 were established. Genotypes containing HMW-subunits 7+8 and 17+18 in combination with LMW-2 glutenin subunits have been identified, which are characterized by the best gluten properties. This information could be useful for identifying genes, for creating new durum wheat lines with improved gluten quality.*

Key words: *A-PAGE, SDS-PAGE, gliadins, glutenins, durum wheat.*

ITRODUCTION

An important characteristic related to durum wheat grain quality is gluten strength. The gluten network is mainly formed by the reserve proteins in the grain endosperm called prolamins. They are alcohol-soluble proteins and are divided according to their electrophoretic mobility into two groups: monomeric - gliadins (α-, β-, γ- and ω-) and polymeric - glutenins (high and low molecular weight glutenin subunits, HMW-GS and LMW-GS). The genes encoding most γ- and ω-gliadins and some β-gliadins are located in Gli-1 loci. The Gli-A2 locus on the short arm of chromosome 6A encodes α-gliadins, and Gli-B2 on chromosome 6B controls most of the β- and some γ-gliadins. Gliadins encoded by the Gli-B1 locus can be used as genetic markers for rapid quality assessment in early generations in breeding programs (Ruiz & Giraldo, 2021). HMW-GS are composed of x- and y-type subunits encoded by genes in Glu-A1 and Glu-B1 on the long arms of chromosomes 1A and 1B in durum wheat (Patil et al., 2015). LMW-GSs are classified into B, C, and D subunits according to their structure and functions. They are encoded on chromosome 1 at the Glu-A3 and Glu-B3 loci (1A and 1B) and at loci closely linked to Gli-1 and on chromosome 6 of the Gli-2 loci. The B-subunits are encoded by genes on chromosome group 1, while the C- and D-subunits are gliadin-like proteins and are encoded by genes on chromosome group 6 (De Santis et al., 2017). Allelic variation at the Glu-1 loci has a significant effect on wheat quality. Early studies reported 11 alleles (14 different subunits) at Glu-B1, 6 alleles (8 different subunits) at Glu-D1, and 1 or no alleles (2 different subunits) at Glu-A1 in about 300 common wheat cultivars (Payne & Lawrence, 1983; Yan et al., 2009). McIntosh et al. (2014) reported 11 allelic variants at Glu-A3 and Glu-B3 and 3 different allelic variants at the Glu-B2 loci of durum wheat. According to Aguiriano et al. (2006), HMW-GS had less effect compared to LMW-GS on durum wheat gluten quality. However, HMW-GS allelic status had a significant effect on gluten quality on average (Kroupina et al., 2023). According to the same authors, winter durum wheat is a younger and understudied crop compared to spring wheat and forms its grain under different environmental conditions. The effect of HMW-GS alleles on pasta quality may be different. A timely study of the allelic structure of HMW-GS genes and the effect of Glu-1 allelic status on the quality of durum wheat in local

collections and especially the study of the germplasm would allow the timely identification of rare alleles and their atypical effects on gluten quality. Payne et al. (1987) reported a correlation between gluten strength as measured by SDS-sedimentation value and HMW-GS. The authors propose a numerical quality assessment scale (Glu score) that reflects the specific relationship between HMW-GS and gluten quality. LMW-GS are the best indicator of durum wheat gluten quality. The differences in the viscoelastic properties of gluten are due to the low molecular weight glutenins. Two types of LMW have been described in durum wheat - LMW-1 and LMW-2. LMW-1 binds to γ-42 gliadin and LMW-2 binds to γ-45 gliadin (Payne et al., 1984; Gergova et al., 2012). Prolamins are biochemical markers that are characterized by a high level of polymorphism and stability. They are encoded by multiple genes in complex loci, which is reflected in a high inter-varietal diversity of prolamins (Shewry et al., 2003). They are inherited co-dominantly, which is why electrophoretic profiles of proteins isolated from mature seeds are an excellent criterion for the characterization and identification of different plant populations and varieties (Taneva & Bozhanova, 2021). The ratio and composition of the gliadin and glutenin protein fractions in the grain endosperm determine the viscoelastic properties of gluten (Chacón et al., 2020). The gliadin fraction is responsible for the viscosity and extensibility of gluten, and the glutenin fraction for elasticity (Sissons et al., 2005a).

Information on allelic variation at the Glu-1 and Glu-3 loci is important in selecting suitable parents for crossing to create new durum wheat lines with improved gluten quality. Alleles present at these loci can have a large, combined effect on dough properties and suitability for specific end products. With a correct classification of glutenin alleles, it is possible to improve wheat quality by selecting alleles that exert beneficial effects and allelic combinations (Henkrar et al., 2017).

The present study aimed to identify the allelic composition of HMW, LMW glutenins, and gliadins of durum wheat cultivars and advanced lines developed at the Field Crops Institute in Chirpan.

MATERIALS AND METHODS

Materials

A collection of 25 genotypes of durum wheat (*T. turgidum* L. var. durum, AABB, 2n = 2x = 28) - varieties and advanced lines selected at the Institute of Field Crops, Chirpan - was analyzed. As a standard in the electrophoretic studies, the Agridur variety (France) with allelic composition Glu-A1c (null), Glu-B1d (6+8), Glu-A3a (6), Glu-B3a (2+4+15+19), Glu-B2a (12), GliB1γ-45 (LMW-2) was used. For the identification of the Glu-A1b (2*) allele, Bezostaya 1 variety (T. *aestivum* L., Russia) with allelic composition Glu-A1b (2*), Glu-B1c (7+9), Glu-D1d (5+10), Glu-A3a (6), Glu-B3a (2+4+15+19), Glu-B2a (12), GliB1γ-45 (LMW-2) was used as a standard.

Methods

The extraction of gliadins was performed with 70% ethyl alcohol, and the separation of the protein fractions itself was performed with one-dimensional acid vertical electrophoresis (A-PAGE) according to Khan et al. (1983) with certain modifications made in the Laboratory on Biochemistry of Grain and Wheat Crops of Agricultural Institute of Dobrudzha in General Toshevo. Electrophoresis was performed on an 8% separating gel, and the thickness of the gel plate was 2 mm at 60 mA DC, which after 1 hour was increased to 120 mA. The duration of electrophoresis under these conditions is about 5 hours at a constant temperature of 10°C. The gels were then fixed and stained with 0.15% Coomassie Brilliant Blue (CBB) R250, 20% ethanol, and 12% trichloroacetic acid for 24 h. This is followed by decolorization with distilled water.

Extraction and electrophoresis (SDS-PAGE) of glutenins

The extraction of high molecular weight glutenins was carried out according to the method of Singh et al. (1991). 0.50 ml of 50% propanol was added to each sample (ground to flour) to remove albumins and globulins. The glutenins were extracted by first adding 0.1 ml of 50% (v/v) propanol, 0.08 M Tris–HCl, pH 8.0, containing 1% (w/v) freshly added dithiothreitol (DTT) to the sample. After incubation for 1 h at 65°C, 0.1 ml of 50% (v/v)

propanol containing 1.4% (v/v) freshly added 4–vinylpyridine (VP) was added to each sample. In this way, alkylation of the SH-groups in the samples takes place. This was followed by incubation for 1 hour at 65°C and centrifugation for 10 minutes at 12,000 g. 0.2 ml of each supernatant was transferred to a new Eppendorf tube and 0.2 ml of a solution containing 2% SDS, 0.08 M Tris–HCl (pH 8.0), 40% glycerol and 0.02% bromophenol blue was added. Samples were vortexed, incubated for 1 h at 65°C, centrifuged at 12,000 g for 10 min, after which they could be used for SDS–PAGE analysis.

With the help of this extraction procedure, which is carried out in four stages, the maximum removal of the residual gliadins is achieved, which match in molecular weight with the low-molecular glutenins and prove to be an obstacle for their accurate identification. An even clearer electrophorogram is obtained after additional alkylation of the protein molecules before they are treated with SDS.

The main advantage of SDS-PAGE is that it enables the simultaneous separation of high and low molecular weight glutenins. To more accurately identify the allelic composition in the region of low molecular weight glutenins, where overlapping bands are observed, electrophoresis was performed on a vertical apparatus in two variants: classical one-dimensional polyacrylamide gel electrophoresis according to the method of Laemmli (1970) on a 10% separating gel and one-dimensional polyacrylamide gel electrophoresis on a 17% resolving gel by the method of Payne et al. (1980). In the first method, electrophoresis takes place at 20mA DC per plate at room temperature for 18-20 hours. The duration of electrophoresis according to the second method is 3-4 hours at 60 mA. After electrophoresis, the gel plates were stained with a 1% solution of Coomasie Brilliant Blue R-250, acetic acid, methanol and water (1:5:4) overnight. Destaining was performed with a solution of acetic acid, methanol, distilled water (1:2:7) until the background cleared (Doneva et. al, 2023). From each sample, 50 grains were analyzed to determine its degree of homogeneity.

Identification of storage endosperm proteins

The high molecular weight (HMW-GS) glutenin alleles (loci Glu-A1 and Glu-B1) were identified according to the nomenclature of Payne & Lawrence (1983), and the low molecular weight (LMW-GS) glutenin alleles (loci Glu-A3, Glu-B3 and Glu -B2) - according to the nomenclature of Nieto-Taladriz et al. (1997). Gliadin fractions at the Gli-B1 locus (γ-gliadins) were reported according to Kudryavtsev et al. (1996). Depending on the specific protein profile of high molecular weight glutenins, a quality score (Glu 1-score) was calculated for each genotype (Payne et al., 1987). Laboratory studies of reserve endosperm proteins and their identification were carried out in the Biochemistry Laboratory of the Institute of Field Crops, Chirpan.

RESULTS AND DISCUSSIONS

One of the most commonly used methods for the identification of prolamin alleles in durum wheat is SDS-PAGE. Compared to other methods, it is fast, accurate, and can be applied even on half a grain. The electrophoretic pattern can be used as a criterion for characterizing the genotype and establishing relationships between prolamin alleles and gluten quality. The relationship between reserve proteins and SDS-sedimentation value has been extensively studied. It has been found that a significant part of the variation in the properties of gluten is due to the glutenin composition. Different glutenin alleles in durum wheat cultivars are classified as positive, negative, and intermediate depending on whether they are associated with greater or lesser gluten strength (Giraldo et al., 2020). Table 1 presents the identified prolamin subunits in loci Glu-A1, Glu-B1, Glu-A3, Glu-B3, Glu-B2, and the γ-gliadins in locus Gli-B1 of the durum wheat genotypes included in the study.

The most common and most studied in durum wheat at the Glu-A1 locus are the alleles: a (subunit 1), b (2*), and c (null). According to most studies, the c (null) allele has a negative effect on gluten strength and occurs at the highest frequency in the landraces studied.

Table 1. Frequency of the high-molecular weight (HMW) and the low-molecular weight (LMW) glutenins subunits in loci Glu-A1, Glu-B1, Glu-A3, Glu-B3, Glu-B2 and the γ-gliadins in locus Gli-B1

Locus	Allele	Prolamin Subunits	Number of genotypes with corresponding allele	Frequency, %
Glu-A1 HMW-GS	b	2*	13	52
	c	null	12	48
Glu-B1 HMW-GS	b	7+8	3	12
	d	6+8	3	12
	f	13+16	3	12
	h	14+15	9	36
	i	17+18	7	28
Glu-A3 LMW-GS	a	6	2	8
	d	6+11	4	16
	e	11	16	64
	h	null	3	12
Glu-B3 LMW-GS	a	2+4+15+19	4	16
	b	8+9+13+16	19	76
	f	2+4+15+17	2	8
Glu-B2 LMW-GS	a	12	3	12
	b	null	22	88
Gli-B1 γ-gliadins		γ-42/LMW-1	19	76.0
		γ-45/LMW-2	6	24.0

The lowest frequency is the rare allele Glu-A1o (subunit V), which has a better effect on gluten strength than the a (1) and b (2*) alleles, but further studies are needed (Henkrar et al., 2017; Chacón et al., 2020; Ruiz & Giraldo, 2021). In contrast to the results reported in most of the studies carried out in this study, 52% of the analyzed Bulgarian genotypes are characterized by the presence of allele b (subunit 2*) in the Glu-A1 locus. The remaining 48% are characterized by the c allele at this locus and zero protein synthesis. According to Payne et al. (1987), subunit 2* has a high quality score of 3 and is associated with better gluten strength. The studied genotypes and their allelic configurations are presented in Table 2. Conflicting results have been reported regarding the effect of Glu-B1 alleles on gluten quality (Turchetta et al., 1995; Vazquez et al., 1996). Chacon et al. (2020) reported a large polymorphism at this locus. They found 7 alleles (a, b, e, f, an, aq and bd) from those previously cataloged by Mcintosh et al. (2014) alleles and reported some new allelic combinations. Chegdali et al. (2020) investigated the allelic composition of 95 durum wheat samples and found 12 new alleles, among them two new alleles in Glu-B1 (Glu-B1cp and Glu-B1co). According to several studies, alleles b (7+8) and d (6+8) have a positive effect and are associated with superior pasta quality parameters compared to the negative effect of allele e (20x+20y) (Babay et al., 2015; Magallanes-Lopez et al., 2017). Kroupin et al. (2023) found a negative effect of Glu-B1d on the traits gluten index and SDS-sedimentation value. Zhang et al. (2020) investigated the separate contribution of the Glu-B1x and Glu-B1y genes and concluded that, as in common wheat, the role of the x-type subunit is greater than that of the y-type subunit. They report that the Bx6 subunit has a stronger effect than the By8 subunit. In our study, 5 alleles were found - b (7+8), d (6+8), f (13+16), h (14+15), i (17+18). The highest frequency is allele h (36%), followed by allele i (28%). Relatively low frequencies (12%) were found for the remaining 3 alleles (Table 1). Oak et al. (2004) reported a positive effect on gluten strength of genotypes characterized by the h allele (14+15), and Kaur et al. (2015) for greater dough strength in the presence of subunits 14+15 and LMW-2 model. According to Branlard & Dardevet (1985), subunits 17+18 and 7+8 determine a positive effect on dough elasticity. Li et al. (2016) examined the Glu-B1 locus and sorted the effects of HMW-GS in the following order: 17+18 > 14+15 > 7+8 > 7+9. Al-Khayri et al. (2023) found a strong positive correlation of HMW-GS alleles – 7+8, 7+9, 13+16 and 17+18 with dough strength. According to Gregová et al. (2012), the combination of better alleles in Glu-B1 (subunits 17+18, 13+16 and 7+8) and Glu-3 (LMW-2 model) had a linear cumulative effect on dough strength.

In this study, four of the eight alleles described by Nieto-Taladriz et al were expressed. (1997) at the Glu-A3 locus. Allele e (subunit 11) is expressed with the highest frequency in the studied samples - 64%. Alleles d (16%), h (12%), a (8%) were expressed with lower frequencies. Different studies have reported different strength of effects of different alleles

at this locus. Negative effects for subunits 6 and 11 were reported by Aguiriano et al. (2009), and Chacon et al. (2020) for subunits 6+11. According to Nazco et al. (2014) the most common allele Glu-A3a (subunit 6) and the second most common allele Glu-A3d (subunits 6+11), Glu-B3a (2+4+15+19) and Glu-B2a (subunit 12) significantly increase the SDS-sedimentation value. A positive effect for subunits 6, 6+11, and null was also reported by Magallanes-Lopez et al. (2017). Roncallo et al. (2021) reported that the frequency with which the a (6) allele occurred did not change over the years, while the frequency of the c (6+10) and d (6+11) alleles increased.

According to Chacon et al. (2020), locus Glu-B3 is characterized by a very large polymorphism. They reported epistatic interactions of Glu-B3 alleles over some Glu-A3 and Glu-1 alleles, implying that the effect of these two loci is highly dependent on Glu-B3 alleles (Ruiz & Giraldo, 2021). Three Glu-B3 alleles were expressed in this study – a (2+4+15+19), b (8+9+13+16), and f (2+4+15+17). The highest frequency in the analyzed Bulgarian durum wheat genotypes was found for allele Glu-B3b (76%). Studies by several authors have associated this allele with poor gluten quality (Sissons et al., 2005b; Magallanes-Lopez et al., 2017). Babay et al. (2015) reported that of all prolamins, those with LMW-GS composition a a a (for Glu-A3, Glu-B3, and Glu-B2 loci, respectively) when bound to Glu-A1c and Glu-B1d, yielded the most - good quality semolina. In Mediterranean durum kinds of wheat, allele a (2+4+15+19) occurs with the highest frequency and is associated with excellent gluten quality. Only four of the analyzed Bulgarian genotypes express this allele – Elbrus, Predel, D-8091, and D-232 (Table 2). The Glu-B3f (2+4+15+17) allele was characterized by a moderate but positive effect on gluten quality (Magallanes-Lopez et al., 2017). In the studied sample, this allele occurs with a very low frequency - 8%. Two of the tested samples expressed the favorable gluten quality allele f – D-146 and D-148.

One of the best indicators for assessing gluten strength in early generations is the gliadins encoded by the Gli-B1 locus, as they are more easily and rapidly visualized in A-PAGE gels. Interrelationships were found between some gliadins in Glu-B1 and some LMW-GS in Glu-B3. According to several authors (Ruiz et al., 2018; Ruiz & Giraldo, 2021), gliadin γ-45 is associated with subunits 2 and 4, gliadin γ-44 is associated with subunit 3, and γ-42 with 7 or 8. In the studied durum wheat genotypes, two of the nine γ-gliadin fractions encoded by the Gli-B1 locus were found. In the genotypes analyzed, the gliadin fraction γ-42 (76%), associated with the LMW-1 pattern and poor gluten quality, occurs with a higher frequency. Gliadin fraction γ-45 and LMW-2 pattern occurs with a significantly lower frequency (24%) in the analyzed durum wheat varieties and lines. The favorable gluten quality gliadin fraction γ-45 was found for six of the analyzed genotypes – Elbrus, Predel, D-8091, D146, D-148, and D-232.

The identified alleles at the Glu-A and Glu-B loci form 16 allelic configurations (Table 3). With the highest frequency (28%) in this study is the allelic combination: b h e b b. Despite the expression of the gluten quality-beneficial subunits: 2* and 14+15, which ensure strong gluten, the presence of the Glu-B3b allele has a negative impact. The effect of the allelic combination b i e b b (8% frequency) is similar. A high-quality score was calculated for a number of the studied genotypes - 5 and 6. These are the genotypes: Belosvava, Vazhod, Zvezditsa, Deni, Trakiets, Kehlibar, Reyadur, Saya, Deyche, Chirpanche. Despite the expression of the favorable HMW-GS of these genotypes, the presence of the LMW-1 pattern suppresses their positive effect. They can be used as donors of good HMW alleles to increase gluten strength. With a high-quality score of 6 is line D-148, which is characterized by the expression of gluten quality-beneficial HMW subunits 2* and 14+15 in combination with the LMW-2 pattern (allele f). Favorable gluten quality allelic combinations - LMW-2 pattern, but lower quality scores were found for Elbrus cultivar and line D-8091. The best allelic combination according to Babay et al. (2015), Glu-A3a, Glu-B3a, and Glu-B2a was found in this study for two of the analyzed genotypes - the Predel cultivar, which is a standard for quality and yield in Bulgaria, and the D-232 line.

Table 2. Fractional composition of the storage proteins of durum wheat varieties and lines

Variety Line	Gli-B1	Glu-A1		Glu-B1		Quality score	Glu-A3	Glu-B3	Glu-B2	LMW type
		subunits	allele	subunits	allele		subunits allele	subunits allele	subunits allele	
Beloslava	γ-42	2*	b	17+18	i	6	e 11	b 8+9+13+16	b null	LMW-1
Vazhod	γ-42	2*	b	17+18	i	6	h null	b 8+9+13+16	b null	LMW-1
Progres	γ-42	null	c	13+16	f	4	h null	b 8+9+13+16	b null	LMW-1
Viktoriya	γ-42	null	c	13+16	f	4	e 11	b 8+9+13+16	b null	LMW-1
Zvezditsa	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Deyana	γ-42	null	c	13+16	f	4	e 11	b 8+9+13+16	b null	LMW-1
Elbrus	γ-45	null	c	6+8	d	2	d 6+11	a 2+4+15+19	b null	LMW-2
Deni	γ-42	2*	b	17+18	i	6	e 11	b 8+9+13+16	b null	LMW-1
Trakiets	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Kehlibar	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Reyadur	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Tserera	γ-42	2*	b	6+8	d	4	e 11	b 8+9+13+16	b null	LMW-1
Predel	γ-45	null	c	17+18	i	4	a 6	a 2+4+15+19	a 12	LMW-2
Raylidur	γ-42	2*	b	6+8	d	4	h null	b 8+9+13+16	b null	LMW-1
Saya	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Heliks	γ-42	null	c	14+15	h	3[a] ,4[b]	e 11	b 8+9+13+16	b null	LMW-1
Viomi	γ-42	null	c	17+18	i	4	e 11	b 8+9+13+16	b null	LMW-1
Deyche	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Dechko	γ-42	null	c	17+18	i	4	e 11	b 8+9+13+16	b null	LMW-1
Ilira	γ-42	null	c	7+8	b	4	e 11	b 8+9+13+16	b null	LMW-1
Chirpanche	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
D-8091	γ-45	null	c	17+18	i	4	d 6+11	a 2+4+15+19	a 12	LMW-2
D-146	γ-45	null	c	7+8	b	4	d 6+11	f 2+4+15+17	b null	LMW-2
D-148	γ-45	2*	b	14+15	h	5[a], 6[b]	d 6+11	f 2+4+15+17	b null	LMW-2
D-232	γ-45	null	c	7+8	b	4	a 6	a 2+4+15+19	a 12	LMW-2

[a]According to Branland & Dardevet (1985), Glu 1-score of '14 + 15' in locus *Glu-B1* is 3.
[b]According to Bahraei et al. (2004), Glu 1-score of '14 + 15' in locus *Glu-B1* is 2.

Table 3. Configurations of storage proteins of the investigated durum wheat genotypes

Glu-A1	Glu-B1	Glu-A3	Glu-B3	Glu-B2	Gli-B1	Genotipes, number	Frequency, %
b 2*	h 14+15	e 11	b 8+9+13+16	b null	LMW-1	7	28
b 2*	i 17+18	e 11	b 8+9+13+16	b null	LMW-1	2	8
c null	f 13+16	e 11	b 8+9+13+16	b null	LMW-1	2	8
c null	i 17+18	e 11	b 8+9+13+16	b null	LMW-1	2	8
b 2*	i 17+18	h null	b 8+9+13+16	b null	LMW-1	1	4
c null	f 13+16	h null	b 8+9+13+16	b null	LMW-1	1	4
c null	d 6+8	d 6+11	a 2+4+15+19	b null	LMW-2	1	4
b 2*	d 6+8	e 11	b 8+9+13+16	b null	LMW-1	1	4
c null	i 17+18	a 6	a 2+4+15+19	a 12	LMW-2	1	4
b 2*	d 6+8	h null	b 8+9+13+16	b null	LMW-1	1	4
c null	h 14+15	e 11	b 8+9+13+16	b null	LMW-1	1	4
c null	b 7+8	e 11	b 8+9+13+16	b null	LMW-1	1	4
c null	i 17+18	d 6+11	a 2+4+15+19	a 12	LMW-2	1	4
c null	b 7+8	d 6+11	f 2+4+15+17	b null	LMW-2	1	4
b 2*	h 14+15	d 6+11	f 2+4+15+17	b null	LMW-2	1	4
c null	b 7+8	a 6	a 2+4+15+19	a 12	LMW-2	1	4

CONCLUSIONS

The allelic composition of γ-gliadins, HMW and LMW glutenins of the 25 studied durum wheat genotypes was analyzed. Seven alleles encoded by Glu-A1 and Glu-B1 loci and nine alleles encoded by Glu-A3, Glu-B2 and Glu-B3 loci were identified. Two of the nine possible γ-gliadin fractions were detected, with only six of the examined samples expressing the γ-45 gliadin fraction associated with better semolina quality. Subunits characterized by a positive effect on gluten strength were identified, as well as genotypes with a combination of alleles associated with higher quality. Subunits: 2*; 14+15 and 17+18, are with high frequency in the studied genotypes and its positive effect is reported by many authors. The four analyzed advanced lines are characterized by very good allelic combinations and LMW-2 pattern. They are an indicator of the progress of the breeding work in terms of quality. Local genotypes possessing alleles with a positive effect on gluten strength can be used as parents in breeding programs to improve the quality of durum wheat.

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