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Evaluation of grain yield and quality traits and identification of seed storage protein alleles in durum wheat genotypes

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Abstract

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The aim of modern selection is the creation of high-yielding varieties with improved grain quality. The investigation included collection of 8 durum wheat genotypes: Mirela, Heliks, Saya, MVPennedur, Marco Aurelio, Wintergold, D-8243, DF-009114002 and Agridur. Cultivars Heliks and Saya and line D-8243, were the highest yielding among all studied genotypes; averaged for three years, their yield varied from 530.8 kg/da (breeding line D-8243) to 644.1 kg/da (cultivar Heliks). According to the trait gluten strength, the highest yielding genotypes had the lowest SDS-sedimentation volume, which varied from 19.7 cm^3 in cultivar Heliks to 21 cm^3 in line D-8243, and respectively had the lowest SDS values. All other investigated genotypes were characterized by statistically significant higher values of the SDS-sedimentation volume, the variation being from 42.8 cm^3 in cultivar Wintergold to 73.5 cm^3 in line DF-009114002. Using the method of electrophoresis, the allele composition of the γ-gliadins, the high molecular (HMW-GS) and low molecular (LMW-GS) glutenins was studied. Six alleles of HMW were identified, which were coded for by loci *Glu-A1* and *Glu-B1*. In the LMW zone, eight alleles were expressed (loci *Glu-A3* and *Glu-B3*). The accessions having gliadin subunits *γ-42* (Heliks, Saya and D-8243) and *γ-45* (Mirela, MVPennedur, Marco Aurelio, Wintergold and DF-009114002) in locus *Gli-B1* were determined. Depending on the respective allele forms in loci *GliB1/GluB3*, the investigated genotypes were divided into two main types (LMW-1 and LMW-2), which determined the quality of the ground semolina. The quality scores and the genetic variability were calculated for the separate loci. Based on the obtained results, varieties Heliks, Saya by high-yield and Marco Aurelio, Mirela by high quality were selected as parental components for crosses.

Keywords: Triticum durum; grain yield; HMW-GS; LMW-GS; γ-gliadin; SDS-PAGE; A-PAGE

Introduction

Durum wheat is a traditional crop for Bulgaria, which has been grown in these lands since the time of the ancient Thracian tribes. It is an important cereal crop used primarily for human food due to its nutrition and technological properties (high content of proteins and carotinoids, hard endosperm with glassiness and strong gluten). These properties make it an irreplaceable component in the diet of people with deteriorated metabolism (diabetes type 2, macular degeneration and cataracts). Bulgaria is one of the regions with the most favorable conditions for growing of high-quality durum wheat in Europe, a large number of botanical varieties have been identified in this region (Ivanov, 1927; Gyurov, 1976). Therefore, its breeding improvement in Bulgaria has deep traditions, the beginning dating back 95 years ago at the Field crops institute in Chirpan (Dechev et al., 2011).

The suitability of durum wheat for processing into high-quality pasta products is due to a number of characteristics. The protein content and the gluten strength as factors of

culinary taste, and the color of the pasta products as a primary marketing characteristics are the most important quality priorities in the modern concept of technological quality of durum wheat (Taneva, 2019; Sharma et al., 2020).

The main purpose of the durum wheat breeding programs relates to the improvement of the gluten strength and increasing the concentration of yellow pigments in the grain in an attempt to meet the constantly increasing demands of industry towards the raw material quality and the expectations of the consumers (Roselló et al., 2018). The breeding improvement of grain quality is accompanied by a number of difficulties, such as: small differences in the variation of the genes controlling major qualitative parameters in the modern durum wheat varieties; difficult selection due to the fact that the expression of these parameters is largely dependent on the environmental conditions or on the interaction of the genotype with the environment and the presence of a negative correlation between yield and grain quality (Goutam et al., 2013; Kudryavtsev et al., 2014; Rapp et al., 2018; Taneva et al., 2019).

The selection of plants with suitable agronomy traits, including such related to the quality of the grain, is an important stage of the breeding process in the development of new varieties. In this relation, the breeders are relying on biochemical markers in the recent years, because their use in wheat is quite helpful for the identification of genotypes with good quality parameters of grain (Todorovska et al., 2007; Dechev et al., 2011; Taneva & Bozhanova, 2021).

The storage proteins are biochemical markers, which are characterized by a high level of polymorphism and stability. In the endosperm of durum wheat, the main storage protein subunits are the gliadins and glutenins, the composition and ratio of which determine the viscosity and elasticity of gluten (Chacón et al., 2020). The glutenins are multimeric aggregations consisting of multiple subunits connected by intermolecular disulfide bridges. They are divided into two main groups depending on the molecular weight of their subunits: HMW (high-molecular weight) and LMW (low-molecular weight) glutenin subunits (Todorov, 2006; Motalebi et al., 2007; Doneva, 2017). The high-molecular weight glutenins are coded for by a complex locus, *Glu-1*, on the long arm of the chromosomes from the first homologous group, while most of the low-molecular weight glutenins are coded for by a complex locus, *Glu-3*, which is closely connected with locus *Gli-1* (Porceddu et al., 1998; Gregová et al., 2012). The gliadins are coded for by complex loci Gli-1 (γ- and w-) and Gli-2 (α- and ß-) on the long arm of the chromosomes from first and sixth homologous groups, respectively. Two different profiles are known in durum wheat: LMW-1 and LMW-2 (Payne et al., 1984), which correspond to gliadin subunits *γ-42* and *γ-45.* Depending on the respective allele forms in loci *GliB1/GluB3*, *T. durum* are divided into two main types (LMW-1 and LMW-2), and they determine the quality of the durum wheat ground semolina.

The aim of this investigation was to evaluate durum wheat (*T. turgidum* L. var. *durum*) genotypes from different origin for their yield, grain quality, composition and ratio of the main storage protein subunits: gliadins and glutenins. Based on the obtained results, suitable parental components are expected to be selected for the development of high-yielding breeding lines (crosses) with strong gluten, which meet the modern criteria of technological properties.

Materials and methods

1. Materials

The investigation included collection of 8 durum wheat genotypes (*T. turgidum* L. var. *durum*, AABB, 2n = 2x = 28). Varieties and breeding lines of different origin was analyzed: varieties Mirela (Bulgaria), Heliks (Bulgaria), Saya (Bulgaria), MVPennedur (Hungary), Marco Aurelio (Italy), Wintergold (Germany) and lines D-8243 (Bulgaria) and DF-009114002 (Romania). Cultivar Agridur (France) with allelic composition *GluA1c* (null), *GluB1d* (6 + 8), *GluA3a* (6), *GluB3a* (2 + 4 + 15 + 19), *GluB2a* (12), *GliB1γ45* (LMW-2) was used as a standard in the electrophoretic investigations. Among the varieties and lines, those with high yield and high quality have been selected based on our previous research.

All genotypes were grown in field of Field Crops Institute – Chirpan in the competitive variety trials in three replications in three harvesting years 2018/2020 and were analyzed for yield and traits associated with grain quality. The size of each replication is 15 m^2. The genotypes were sown in the optimal periods (20–30.10) with 550 germinating seeds per meter square and the corresponding sowing rate for each genotype. Trials are harvested at full maturity. The predecessor was peas and the accepted technology for growing durum wheat in Bulgaria was used.

2. Methods

Content and quality of protein/wet gluten in grain

The protein content in the grain was determined by the method of Kjeldahl (N × 5.7) according to BSS EN ISO 20483:2006, and of the wet gluten – according to BSS EN ISO 21415-2:2008. The quality of the protein/gluten was evaluated for sedimentation value of the entire milled grain using sodium dodecyl sulfate (SDS) (ICC 151: 1990). The quality and quantity of the protein/gluten was determined at the Biochemistry Laboratory of FCI – Chirpan.

Extraction and electrophoresis (A-PAGE) of gliadins.

The extraction of gliadins was carried out with 70% ethyl alcohol, and the separation of the protein subunits itself was done through monomeric acid vertical gel electrophoresis (A-PAGE) according to Khan et al. (1983) with some modifications made at the Laboratory of Biochemistry of Cereal Crops at Dobrudzha Agricultural Institute – General Toshevo (DAI). The electrophoresis was performed on 8% separation gel, on 2 mm gel plate under 60 mA electric current, increased to 120 mA after running for 1 hour. The duration of the electrophoresis under these conditions was about 5 hours, at constant temperature of 10°C. After that, the gels were fixed and stained with 0.15% coomassie brilliant blue (CBB) R250, 20% ethanol and 12% trichloroacetic acid for 24 hours. This was followed by discoloration with distilled water.

Extraction and electrophoresis (SDS-PAGE) of glutenins

The extraction of the high-molecular weight glutenins was carried out by the method of Singh et al. (1991). To each sample of ground flour, 0.50 ml of 50% propanol were added to remove the albumins and globulins. This was followed by extraction of the glutenins, firstly by adding to the sample 0.1 ml of 50% (v/v) propanol, 0.08 M Tris – HCl, pH 8.0, containing 1% (w/v) of freshly added dithiothreitol (DTT). After 1-hour incubation at 65°C, 0.1 ml of 50% (v/v) propanol, containing 1.4% (v/v) of fresh 4–vinypyridine (VP) was added to each sample. Thus, the SH-groups in the samples were alkalized. This was followed by 1-hour incubation at 65°C and 10-minute centrifuge at 12000 g. 0.2 ml of each supernatant were transferred to a new Eppendorf tube, and 0.2 ml solution, containing 2% SDS, 0.08 M Tris – HCl (pH 8.0), 40% glycerol and 0.02% bromo phenol blue was added. The samples were mixed, incubated for 1 hour at 65 °C, centrifuged at 12000 g for 10 min, and were ready to use for SDS–PAGE analysis. By applying this extraction procedure, which was realized in four stages, maximum removal of the residual gliadins was done; these gliadins have the same molecular weight as the low-molecular glutenins and are an obstacle for their precise identification. Even clearer electrophoregram was obtained after additional alkalization of the protein molecules before treating them with SDS.

The main advantage of SDS-PAGE is that it allows for the simultaneous separation of high and low molecular weight glutenins. For a more precise identification of the allelic composition in the region of the low-molecular weight glutenins, where bands overlap, the electrophoresis was performed on a vertical apparatus in two variants: conventional monomeric polyacrylamide gel electrophoresis by the method of Laemmli (1970) on 10% separation gel, and monomeric polyacrylamide gel electrophoresis on 17% separation gel by the method of Payne et al. (1980). By the first method, the electrophoresis occurred under constant electric current of 20 mA on a plate at room temperature for 18–20 hours. The duration of the electrophoresis according to the second method was 3–4 hours at 60 mA. After running the electrophoresis, the plates were stained with 1% solution of Coomasie Brilliant Blue R-250, acetic acid, methanol and water at ratio (1:5:4) overnight. The discoloration was done with solution of acetic acid, methanol and distilled water (1:2:7) until the background was clean. A minimum of 50 grains were analyzed from each accession to determine the degree of its homogeneity.

Identification of the seed storage proteins.

The high-molecular weight (HMW) glutenin alleles (loci *Glu-A1* and *Glu-B1*) were identified according to the nomenclature of Payne & Lawrence (1983), and the low-molecular weight ones (LMW) (loci *Glu-A3*, *Glu-B3* and *Glu-B2*) – according to the nomenclature of Nieto-Taladriz et al. (1997), and γ-gladins (Gli-B1locus) were named according to Kudryavtsev et al. (1996).

Depending on the specific protein profile of the high-molecular glutenins, quality score was calculated for each genotype (Glu 1-score) (Payne et al., 1987).

The laboratory investigations on the seed storage proteins and their identification were carried out at the Laboratory of Biochemistry of Cereal Crops at DAI – General Toshevo.

3. Statistical analysis

The software package Statistica 13.0 (TIBCO, Software, 2018) was used for statistical processing of the data. Two-way analysis of variance (ANOVA) and multiple test for significance of the differences according to Duncan ($P < 0.05$) were applied (Duncan, 1955).

The genetic variation (H) in the separate loci was calculated through the index of Nei (1973), where *Pi* is the frequency of the alleles in the respective locus: $H = 1 - \sum Pi^2$.

Results

1. Evaluation of yield and grain quality

Prior to being analyzed for seed storage proteins, the selected durum wheat (*T. Turgidum* L. Var. *Durum*) genotypes from different origin were included in a three-year testing to determine grain yield and the quality and quantity of the protein/gluten in the grain. The results from the testing are presented in Table 1. Cultivars Heliks and Saya and line

D-8243, developed at FCI – Chirpan, were the highest yielding among all studied genotypes; averaged for three years, their yield varied from 530.8 kg/da (breeding line D-8243) to 644.1 kg/da (cultivar Heliks). All other breeding materials, Bulgarian and foreign, realized significantly lower yields, which varied from 367.5 kg/da in the Hungarian cultivar MVPennedur to 425.1 kg/da in the Romanian breeding material DF-009114002, and, according to the Duncan's multiple test for significance of the differences ($P < 0.05$), they fell within the same group.

According to the trait gluten strength, the highest yielding genotypes had the lowest SDS-sedimentation volume, which varied from 19.7 cm^3 in cultivar Heliks to 21 cm^3 in line D-8243, and respectively had the lowest gluten values. All other investigated genotypes were characterized by statistically significant higher values of the SDS-sedimentation volume, the variation being from 42.8 cm^3 in the German cultivar Wintergold to 73.5 cm^3 in the Romanian line DF-009114002. The results from the analysis of variance are presented in Table 2. The genotype had the highest effect on the variation of yield (61.7%), followed by the interaction between genotype and environment (24.5 %). The variation of the parameters content of raw protein (86.51 %) and content of wet gluten in grain (74.98 %) was influenced primarily by the conditions of the year. Considerably lower was the effect of the genotype and the genotype/environment interaction. Only the genotype affected significantly the SDS-sedimentation volume and it accounted for about 93% of the variation of this trait.

Allelic composition of storage endosperm proteins

By using the method of electrophoresis, the allelic composition of the high molecular weight (HMW) and the low molecular weight(LMW) glutenins and the γ-gliadins in the studied durum wheat genotypes (*T. Turgidum* L. Var. *Durum*, AABB, 2n = 2x = 28) was investigated.

The glutenin subunits identified in loci *Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3*, *Glu-B2* and the *γ*-gliadins in locus *Gli-B1* are presented in Table 3.

HMW Glutenin Subunits

In locus *Glu-A1* two alleles *'b'* and *'c'* were identified in the present research (Table 3, Fig. 1).

Table 2. Analysis of variance (ANOVA) of trait yield and traits related to quality of protein/gluten of durum wheat genotypes during 2018/2020

Grain yield, kg/da				
Source	SS	MS	Significant	η^2%
Genotype	455130	65019	***	61.07
Year	102258	51129	***	13.72
GxY	182445	13032	***	24.48
Error	5470	228		
Protein content, %				
Source	SS	MS	Significant	η^2%
Genotype	2.340	0.334	***	2.83
Year	71.450	35.725	***	86.51
GxY	8.230	0.588	***	9.96
Error	0.575	0.024		
Wet gluten content, %				
Source	SS	MS	Significant	η^2%
Genotype	22.67	3.24	***	7.03
Year	232.72	116.36	***	74.98
GxY	54.47	3.89	***	17.55
Error	0.53	0.02		
SDS-sedimentation volume, cm^3				
Source	SS	MS	Significant	η^2%
Genotype	17035.25	2433.61	***	92.70
Year	0.87	0.44	n.s.	0.0047
GxY	1297.13	92.65	***	7.06
Error	42.00	1.75		

*** Significant at p< 0.001; ** Significant at p<0.01; * Significant at p<0.05; n.s. – not significant

Table 1. Mean values of the traits yield, protein content, wet gluten content and SDS-sedimentation volume during 2018/2020

Genotype	Grain yield, kg/da	Protein Content, %	Wet gluten content, %	SDS-sedimentation volume, cm^3
Heliks	644.08c	13.95a	27.82a	19.67a
Mirela	377.37a	14.62a	29.82a	47.17bc
Saya	578.83bc	14.42a	29.12a	20.67a
D-8243	530.78b	14.43a	29.25a	21.00a
MVPennedur	367.45a	14.50a	29.70a	52.33c
M. Aurelio	423.32a	14.22a	28.43a	59.83d
Wintergold	387.53a	14.52a	29.85a	42.83b
DF-009114002	425.12a	14.07a	28.62a	73.50e

Mean values (in each column), followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test (DMRT)

Table 3. 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-glutenins/ H*=0.42	*b* *c*	2* null	1 7	12.5 87.5
Glu-B1 /HMW-glutenins/ H=0.77	*b* *f* *h* *i*	7 + 8 13 + 16 14 + 15 17 + 18	4 1 2 1	50.0 12.5 25.0 12.5
Glu-A3 /LMW-glutenins/ H= 0.67	*a* *e* *f*	6 11 6 + 11 + 20	3 4 1	37.5 50.0 12.5
Glu-B3 /LMW-glutenins/ H= 0.80	*a* *b* *d* *e* *f*	2 + 4 + 15 + 19 8 + 9 + 13 + 16 2 + 4 + 15 + 17 + 19 2 + 4 + 15 + 16 + 18 2 + 4 + 15 + 17	1 3 1 2 1	12.5 37.5 12.5 25.0 12.5
Glu-B2 /LMW-glutenins/ H= 0.42	*a* *b*	12 null	1 7	12.5 87.5
Gli-B1 / γ-gliadins/ H= 0.25		γ-42/LMW-1 γ-45/LMW-2	3 5	37.5 62.5

*H – index of genetic variability (Nei, 1973; Hintum & Elings, 1991)

Allele '*c*' (null allele) was with much higher frequency – 87.5%, while allele '*a*' was typical for only one of the cultivars (Saya). The identified allelic composition in locus *Glu-A1* and the frequency of the subunits in it corresponded to a genetic variability which was a little below the average – H = 0.42.

Locus *Glu-B1* was characterized by high polymorphism. Alleles '*b*' and '*h*' were with the highest frequency, coded for by subunit pairs '7 + 8' и '14 + 15', respectively. Alleles '*f* ' and '*i*' were with relatively low frequency (12.5%). The genetic variability, calculated through H = 0.77, significantly exceeded the average.

LMW Glutenin Subunits

In this investigation, three alleles were expressed in locus *Glu-A3* – '*a*', '*e*' and '*f* ' (Table 3, Figure 1). Allele '*e*' was with the highest frequency (50%), followed by allele '*a*' (37.5%). With this allelic composition, the calculated genetic variability in locus (H = 0.67) was significantly above the average (H = 0.50).

Greater number of alleles were determined ('*a*', '*b*', '*d*', '*e*','*f* ') in locus *Glu-B3* in comparison to the other loci (Table 3, Figure 2), which was the reason for the high value of the genetic variability parameter – H = 0.80. Allele '*b*' was the most frequently expressed allele (37.5%), followed by allele '*e*' (25%).

Locus *Glu-B2* coded three allele variants, designated '*a*', '*b*' and '*c*'. In our investigation, allele '*b*', coding for subunit '*null'* was typical for seven of the analyzed genotypes (87.5%), while allele '*a*' (12) was with lower frequency – 12.5% (Table. 3, Figure 1). Allele '*c*' (12*) was not found. In this allele composition, the genetic variability in the locus was below the average (H = 0.42).

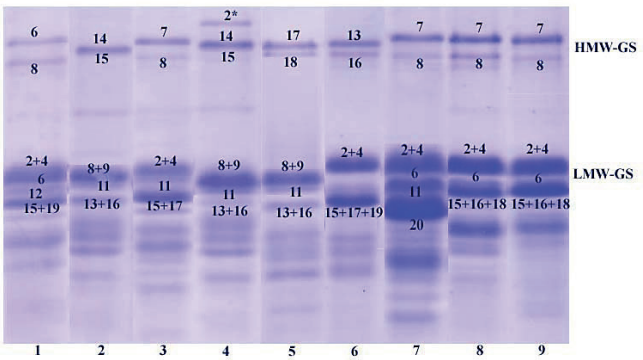

Fig. 1. SDS-PAGE (10% separating gel) of HMW and LMW glutenins 1. Agridur (standard); 2. Heliks; 3. Mirela; 4. Saya; 5. D-8243; 6. MVPennedur; 7. Marco Aurelio; 8. Wintergold; 9. DF-009114002

Gliadins

In the studied varieties and lines of durum wheat the gliadin subunit *γ-45* occurred with much higher frequency (62.5%) in comparison to gliadin subunit *γ-42* (37.5%) (Table 3, Figure 2). The low value of the genetic variability index in locus *Gli-B1* – H=0.25 was due to the high concentration of heritability potential in the *γ*-gliadin subunit *45*, which is favorable for gluten quality. Depending on the allele forms in loci *Gli-B1/Glu-B3*, the durum wheats are usually divided into two main types – LMW-1, related to *γ-42* and LMW-2, related to *γ-45*. The obtained results showed that cultivars with LMW-2 type of the low-molecular weight glutenins were predominant in our study, which was due to the higher frequency of gliadin subunit *γ-45* (Table 3, Figure 2). The cultivars of this type were of foreign origin, and cultivar Mirela was developed at DAI – General Toshevo. The breeding materials of type LMW-1, related to *γ-42* and with weaker gluten were developed at FCI – Chirpan.

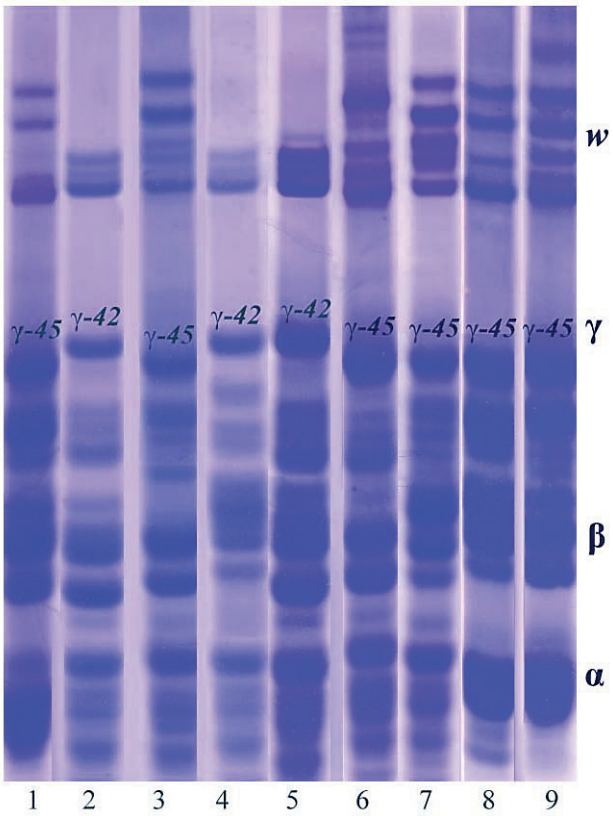

Fig. 2. A-PAGE of gliadins: 1. Agridur (standard); 2. Heliks; 3. Mirela; 4. Saya; 5. D-8243; 6. MVPennedur; 7. Marco Aurelio; 8. Wintergold; 9. DF-009114002

The alleles identified in loci *Glu-A1, Glu-B1, Glu-A3, Glu-B3, Glu-B2* and *Gli-B1* formed seven allele configurations (table 4). With the exception of one of the configurations, which was determined in two genotypes, all other configurations were present in only one durum wheat accession.

Based on the results from the electrophoretic analysis and the data on the grain yield and the quality and quantity of the protein/gluten in the grain, four durum wheat cultivars were selected – Heliks, Marco Aurelio, Mirela and Saya to be included in a breeding program for developing of high-yielding varieties with increased gluten strength. Cultivars Heliks and Saya were selected for their high yield potential and high quality score. Cultivars Marco Aurelio and Mirela contain gliadin subunit γ45 and respectively have high gluten, although being with lower yields.

Discussion

Enhancing yield and grain quality are important aims of the breeding programs on durum wheat, but the existing negative correlation between them makes their simultaneous improvement difficult (Rapp et al., 2018).

The results from the three-year testing to determine grain yield and the quality and quantity of the protein/gluten in the grain of the selected durum wheat (*T. Turgidum* L. Var. *Durum*) genotypes from different origin show that they differed significantly by grain yield and by gluten strength, but did not considerably differ by protein content and wet gluten. Cultivars Heliks and Saya and line D-8243, developed at FCI – Chirpan, were the highest yielding among all studied genotypes. All other breeding materials, Bulgarian and foreign, realized significantly lower yields. According to the trait gluten strength, the highest yielding genotypes had the lowest SDS-sedimentation volume – cultivar Heliks and line D-8243 and respectively had the lowest gluten values. All other investigated genotypes were characterized by statistically significant higher values of the SDS-sedimentation volume. Was established preponderance of non-additive effects in inheritance of grain yield, protein and wet gluten content? It is recommended that an effective selection would start in later segregating generations (Taneva et al., 2019; Dragov, 2020; Dragov, 2021). On the other hand, additive genetic effects preponderance in inheritance of SDS-sedimentation volume, and selection can begin in early segregating generations (Taneva et al., 2019). This can be very important and can torque creating of high quality durum wheat variety.

Based on the analysis of variance on the yield variation and the traits related to the quantity of protein/gluten, the genotype, the year of growing and their interaction had statistically significant effect. The genotype had the highest effect on the variation of yield. The variation of the parameters content of raw protein and content of wet gluten in grain was influenced primarily by the conditions of the year. Only the genotype affected significantly the SDS-sedimentation volume. Other authors also report that protein content and wet gluten content are affected much greater extent by environmental conditions, while the SDS-sedimentation volume of the genotype (Nachit et al., 1995; Kilic & Yagbasanlar 2003).

The composition and ratio of the main storage protein fractions, gliadins and glutenins, determine the viscosity and elasticity of gluten in durum wheat. By using the method of electrophoresis, the allelic composition of the seed storage proteins in the studied durum wheat genotypes (*T. Turgidum* L. Var. *Durum*, AABB, 2n = 2x = 28) was investigated.

In locus *Glu-A1*, the expression of five alleles – '*a*' (1), '*b*' (2*), '*c*' (null), '*o*' (V) and 'new' has been reported (McIntosh et al., 2014). Two of them – *,b'* and *'c'* were identified in the present research. They were first discovered by Payne & Lawrence (1983). Allele '*c*' (null allele) was with much higher frequency, while allele '*a*' was typical for only one of

Table 4. Configurations of storage proteins of durum wheat cultivars

Cultivar	*Glu-A1*	*Glu-B1*	*Glu 1-score*	*Glu-*A3	*Glu-*B3	Glu-B2	Gli-B1	Frequency, %
Heliks	c null	*h* 14 + 15	4[a], 3[b]	*e* 11	*b* 8 + 9 + 13 + 16	*b* null	γ *-42* LMW-1	12.5
Mirela	*c* null	*b* 7 + 8	4	e 11	*f* 2 + 4 + 15 + 17	*b* null	γ *-45* LMW-2	12.5
Saya	*b* 2*	*h* 14 + 15	6[a], 5[b]	e 11	*b* 8 + 9 + 13 + 16	*b* null	γ *-42* LMW-1	12.5
D-8243	*c* null	*i* 17 + 18	4	e 11	*b* 8 + 9 + 13 + 16	*b* null	γ*-42* LMW-1	12.5
MVPen-nedur	*c* null	*f* 13 + 16	4	a 6	*d* 2 + 4 + 15 + 17 + 19	*b* null	γ*-45* LMW-2	12.5
Marco Aurelio	*c* null	*b* 7 + 8	4	*f* 6 + 11 + 20	*a* 2 + 4 + 15 + 19	*a* 12	γ *-45* LMW-2	12.5
Winter-gold	*c* null	*b* 7 + 8	4	a 6	*e* 2 + 4 + 15 + 16 + 18	*b* null	γ *-45* LMW-2	25.0
DF-009114002	*c* null	*b* 7 + 8	4	a 6	*e* 2 + 4 + 15 + 16 + 18	*b* null	γ *-45* LMW-2	25.0

[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.

the cultivars. Similar results have been presented in other researches as well. Thus, for example, Aguiriano et al. (2008) reported high frequencies of *Glu-A1* alleles '*a*' and '*c*' (33% and 38%, respectively) in a collection of 52 Spanish durum wheat accessions. A number of other researches confirmed this tendency and found out that in many cases the frequency of allele '*c*' exceeded 50% (Raciti et al., 2003; Nazco et al., 2014; Babay et al., 2015; Doneva, 2017; Chacón et al., 2020). Locus *Glu-B1* was characterized by high polymorphism. Based on previous researches, seven alleles were identified – '*a*' (7), '*b*' (7 + 8), '*e*' (20x + 20y), '*f*' (13 + 16), '*an*' (6), '*aq*' (32 + 33), '*bd*' (20x + 8), which were included in the catalog (McIntosh et al. 2014), but new combinations of subunits are also being identified (Chacón et al., 2020). In our study, we identified four alleles – *b*, *f*, *h*, *i*. Considerable allelic variability in locus *Glu-B1* has been confirmed by other authors, as well, when analyzing genetic plasma from Spain (Chacón et al., 2020), Portugal (Igrejas et al., 1999) and Turkey (Güleç et al., 2019).

The electrophoretic profiles of the glutenin subunits coded for by locus *Glu-A3* were characterized by eight alleles – '*a*' (6), '*b*' (5), '*c*' (6 + 10), '*d*' (6 + 11), '*e*' (11), '*f*' (6 + 11 + 20), '*g*' (6 + 10 + 20), '*h*' (null) (Nieto-Taladriz et al., 1997). In this investigation, three of them were expressed – '*a*', '*e*' and '*f* '. A number of researchers reported that allele '*a*', related to good quality, was typical for many durum wheat cultivars with origin from Portugal, Algeria, Spain and other countries (Nieto-Taladriz et al., 1997; Igrejas et al., 1999; Cherdouh et al., 2005; Aguiriano et al., 2008). The heritability potential of quality in locus *Glu-B3* has a wide genetic basis due to the expression of nine alleles, which were catalogued – '*a*' (2 + 4 + 15 + 19, '*b*' (8 + 9 + 13 + 16), '*c*' (2 + 4 + 14 + 15 + 19), '*d*' (2 + 4 + 15 + 17 + 19), '*e*' (2 + 4 + 15 + 16 + 18), '*f*' (2 + 4 + 15 + 17), '*g*' (2 + 4 + 15 + 16), '*h*' (1 + 3 + 14 + 18), '*i*' (7 + 8 + 14 + 18) (Nieto-Taladriz et al., 1997), and to new combinations of subunits identified in later study (Ruiz et al., 2018). In this research, a greater number of alleles were determined ('*a*', '*b*', '*d*', '*e*','*f* ') in this locus in comparison to the other loci. Allele '*b*' was the most frequently expressed allele, followed by allele '*e*'. Other studies on populations of Mediterranean durum wheats identified allele '*a*' as having the highest frequency. Alleles '*h*', '*i* '(Aguiriano et al., 2008) and '*c*' (Moragues et al., 2006) were also identified with high frequencies. Our results and the results of other researchers confirmed that this locus was with the highest polymorphism among all prolamine loci (Cherdouh et al., 2005; Aguiriano et al., 2008; Ribeiro et al., 2011). Locus *Glu-B2* coded three allele variants, designated '*a*', '*b*' and '*c*'. In our investigation, allele '*b*' was typical for seven of the analyzed genotypes, while allele '*a*' was with lower frequency. Allele '*c*' was not found. Some authors reported relatively even distribution of the two alleles '*a*' and '*b*' among the accessions of the analyzed populations (Moragues et al., 2006; Chacón et al., 2020). In other researches, one of the two alleles was

predominant (Cherdouh et al., 2005; Nazco et al., 2014). The data on allele '*c*' showed it was extremely rare, being present only in some Spanish landraces (Chacón et al., 2020).

In the studied varieties and lines of durum wheat, two of the nine γ-gliadin subunits, coded for by locus *Gli-B1* were identified – *γ-40*, *γ-41*, *γ-42*, *γ-43*, *γ-44*, *γ-45*, *γ-46*, *γ-47* and '*null*'. The gliadin subunit *γ-45* occurred with much higher frequency in comparison to gliadin subunit *γ-42*. Identical tendency has been observed in other researches as well (Kudryavtsev et al., 1996; Aguiriano et al., 2008; Babay et al., 2015). A certain correlation between *γ*-subunits *45* and *42* and gluten strength has been determined in durum wheat. Subunit *45* is related to strong gluten, and *42* – to weak gluten (Todorov, 2006). It was found out, however, that all durum wheat varieties containing *γ*-gliadin subunit *45*, differed from those containing subunit *γ-42* by their low-molecular weight glutenin composition, as well. There is a genetic relationship between the gliadins and low-molecular weight glutenin subunits (LMW). A number of authors concluded that the γ-gliadin subunits were only genetic markers of quality, and low molecular weight subunits are associated with the quality variation in the durum wheat progenies (Payne et al., 1987; Singh & Shepherd, 1988; Boggini & Pogna, 1989; Pogna et al., 1990; Todorov, 2006; Doneva, 2017). In this relation, depending on the allele forms in loci *Gli-B1/Glu-B3*, the durum wheats are usually divided into two main types – LMW-1, related to *γ-42* and LMW-2, related to *γ-45* (Payne et al., 1984; Gregová et al., 2012). The obtained results showed that cultivars with LMW-2 type of the low-molecular weight glutenins were predominant in our study, which was due to the higher frequency of gliadin subunit *γ-45*. The cultivars of this type were of foreign origin, and cultivar Mirela was developed at DAI – General Toshevo. The breeding materials of type LMW-1, related to *γ-42* and with weaker gluten were developed at FCI – Chirpan, a fact demonstrating the necessity of further more purposeful breeding work to improve the gluten strength in the new varieties.

When comparing the results from the SDS-sedimentation volumes (Table 1) and the presence of gliadin subunits γ-45 and γ-42 (Figure 2), the correlation between the higher sedimentation volume and the presence of gliadin subunit γ-45, reported by other authors too (Liu et al., 1996) becomes evident.

The alleles identified in loci *Glu-A1, Glu-B1, Glu-A3, Glu-B3, Glu-B2* and *Gli-B1* formed seven allele configurations. Four genotypes (Mirela, Aurelio, Wintergold and DF-009114002) possessed subunit pair '7 + 8' (allele '*b*') in locus *Glu-B1*. Although in common winter wheat allele '*b*' has a high quality score (3) and affects positively the bread making properties of flour, the researches on durum wheat demonstrate that this subunit pair has a negative effect on the quality of the semolina, especially in the cases when combined with subunit *'1'* in locus *Glu-A1*. (Oak et al., 2004; Fan et al., 2009). In our study, '7 + 8' was in combination with allele '*c*' in locus *Glu-A1*, which was characterized by zero synthesis of protein and which determined poor gluten quality. According to a number of authors, in spite of the positive correlation between the high-molecular weight glutenins and quality, they alone were not sufficient to explain the variation in the values of the individual quality parameters. It is necessary to include the other storage proteins, too, as genetic markers (Hamer et al., 1992; Todorov, 2006). As already mentioned above, LMW-1 and LMW-2-glutenins and their respective gliadin subunits *γ42* and *γ45* have a considerable effect on the gluten quality of durum wheat. In cultivars Mirela, Marco Aurelio, Wintergold and line DF-009114002, the high-molecular weight subunits '*null'* and '7 + 8' were in combination with gliadin subunit *γ45*, which significantly increased gluten strength. Our results from the investigations on the quality of gluten determined according to its colloid properties in a solution of SDS-lactic acid (Table 1) confirmed that these varieties possessed strong gluten and their SDS-sedimentation volume (cm^3) was with high values.

Line D-8243 contained subunit pair '17 + 18' (allele '*i*') in locus *Glu-B1*, which was with high quality score (Table 2), but on its own, this allele was not efficient for the quality of pasta products (Butow et al., 2003; Güleç et al., 2019). In this particular case, the combination of allele *GluA1c* with gliadin subunit *γ-42* had a further negative effect on the quality parameters. Cultivars Saya and Heliks were characterized by subunit pair '14 + 15' (allele '*h*'), which guaranteed high gluten quality (Brites & Carrillo, 2003), but the presence of LMW-1 glutenin type (*γ-42*) had a negative effect. Our assumption is that cultivar Saya has higher quality parameters due to the high quality score of subunit '2*' (allele '*b*') in locus *Glu-A1*. The results from the investigations on the gluten strength confirmed that line D-8243 and cultivars Saya and Heliks were characterized by poor gluten based on the SDS-sedimentation volume, but line D-8243 and cultivar Saya were with higher content of protein and gluten.

The expectations for cultivar MVPennedur were for strong gluten due to the combination between subunit pair '13 + 16' (allele '*f*'), which had high quality score and gliadin subunit *γ-45*. These expectations were confirmed by our results, showing that this cultivar possessed high SDS-sedimentation volume and higher content of protein and gluten.

Conclusions

Evaluation was made on the durum wheat, *T. Turgidum* L. Var. *Durum*, (AABB, 2n = 2x = 28), breeding materials from different origin for their yield, quantity and quality of

protein/gluten, and the composition and ratio of the main storage protein subunits, the gliadins and glutenins determining the viscosity and elasticity properties of the gluten in durum wheat, was determined.

It was found out that the highest yielding cultivars Heliks and Saya and line D-8243, developed at FCI – Chirpan, were characterized with the weakest gluten.

By using the method of electrophoresis, the allelic composition of γ-gliadins, high-molecular weight (HMW) and low-molecular weight (LMW) glutenins was studied. Six alleles of HMW, coded for by loci *Glu-A1* and *Glu-B1* and eight alleles of LMW, coded for by loci *Glu-A3* and *Glu-B3* were identified.

The genotypes possessing gliadin subunits *γ-42* (Heliks, Saya and D-8243) and *γ-45* (Mirela, MVPennedur, Marco Aurelio, Wintergold and DF-009114002) in locus *Gli-B1*, markers for poor and high gluten quality, respectively, were determined. According to their respective allelic forms in loci *GliB1/GluB3*, the studied accessions were divided into two main types (LMW-1 and LMW-2), on which the quality of the ground semolina from durum wheat depended.

The quality scores and the genetic variability in the separate loci were calculated. Cultivar Saya was with the highest Glu-1 score (6^a, 5^b), followed by cultivar Heliks (4^a, 3^b), and loci *Glu-B3* (H=0.80) and *Glu-B1* (H=0.77) were with the highest polymorphism.

The correlation between the higher sedimentation volume and the presence of gliadin subunit γ-45 in the investigated breeding materials was confirmed.

Based on the obtained results, cultivars Heliks (highest yield and high quality score), Marco Aurelio (*γ-45*), Mirela (*γ-45*) and Saya (high yield and high quality score) were selected as parental components.

This study showed that by using the results from the electrophoretic method, purposeful breeding could be carried out through selection of parental forms with suitable qualitative properties. Thus, the use of the seed storage proteins as bio chemical markers in conventional breeding accelerates the process and widens the possibility to obtain new high-yielding cultivars with enhanced quality, which meet the modern standards.

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