AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 5, pp. 840-851
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Wheat genetics and breeding

UDC 633.11:631.51:577.2

doi: 10.15389/agrobiology.2023.5.840eng
doi: 10.15389/agrobiology.2023.5.840rus

STUDY OF THE ALLELIC DIVERSITY OF THE *Glu-1* GENES IN THE DURUM WHEAT (*Triticum durum* Desf.) COLLECTION

V.A. KOROBKOVA[1] ✉, A.YU. KROUPINA[1], A.V. ARKHIPOV[1], A.S. YANOVSKY[2], A.D. VOROPAEVA[2], L.A. BESPALOVA[2], A.A. MUDROVA[2], L.A. NAZAROVA[1], M.M. MAGOMEDOV[3], P.Yu. KROUPIN[1], M.A. SAMARINA[1], D.S. ULYANOV[1], G.I. KARLOV[1], M.G. DIVASHUK[1]

[1]*All-Russian Research Institute of Agricultural Biotechnology*, 42, ul. Timiryazevskaya, Moscow, 127550 Russia, e-mail bowlingistka@gmail.com (✉ corresponding author), annshirley@yandex.ru, batler51@yandex.ru, lpukhova@yandex.ru, pavelkroupin1985@gmail.com, Samarina.homa@yandex.ru, uldas1508@gmail.com, karlovg@gmail.com, divashuk@gmail.com;
[2]*Lukyanenko National Grain Centre, Department of Breeding and Seed Production of Wheat and Triticale,* 1, Tsentral'naya Usad'ba KNIISH, Krasnodar, 350012 Russia, e-mail yanovskij81@list.ru, a.d.voropaeva@vk.com, bespalova_l_a@rambler.ru, mudrova.alya@mail.ru;
[3]*Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, Dagestan experimental station*, s. Vavilovo, Khazarskoe, Derbent District, Republic of Dagestan, 368600 Russia, e-mail magomed.magomedov.1122@mail.ru
ORCID:
Korobkova V.A. orcid.org/0009-0009-2330-3681
Kroupina A.Yu. orcid.org/0000-0003-3654-8310
Arkhipov A.V. orcid.org/0000-0003-1195-6086
Yanovsky A.S. orcid.org/0000-0003-3169-6066
Voropaeva A.D. orcid.org/0009-0008-3989-2188
Bespalova L.A. orcid.org/0000-0002-0245-7835
Mudrova A.A. orcid.org/0000-0001-7292-0867
Nazarova L.A. orcid.org/0000-0002-4056-8907
Magomedov M.M. orcid.org/0009-0006-0879-9226
Kroupin P.Yu. orcid.org/0000-0001-6858-3941
Samarina M.A. orcid.org/0000-0001-9102-4208
Ulyanov D.S. orcid.org/0000-0002-5880-5931
Karlov G.I. orcid.org/0000-0002-9016-103X
Divashuk M.G. orcid.org/0000-0001-6221-3659
The authors declare no conflict of interests
Acknowledgements:
Supported financially by the Russian Science Foundation, grant No. 21-16-00121 and State assignment FGUM-2022-0001
Final revision received June 13, 2023
Accepted August 22, 2023

Abstract

Durum wheat grain, which is used in the production of pasta and groats, must meet requirements for specific parameters of gluten quality. Its high quality depends on various factors, including the allelic state of the high-molecular-weight glutenin genes that encode subunits of wheat storage proteins (HMW-GS). This work presents, for the first time, the allelic structure of the *Glu-1* loci in a wide collection of facultative durum wheat varieties and breeding lines. The objective of this study was to identify the allelic state of the *Glu-A1* and *Glu-B1* genes which encode high-molecular-weight glutenins in this collection. Additionally, the study aimed to evaluate the impact of these genes on the gluten index. The material for the research consisted of a collection of 198 breeding lines and varieties of facultative durum wheat obtained from the Lukyanenko National Grain Center. The allelic state of HMW-GS was assessed using SDS-PAGE. The gluten index value was measured using the Perten Glutomatic® 2100 System (PerkinElmer, USA). To identify allelic variants of HMW-GS genes, KASP markers were also used. The Glu-Ax1/x2*_SNP marker was utilized to identify the alleles of *Glu-A1.* The BX7OE_866_SNP marker was employed to differentiate between the *Glu-B1b* (Bx7 + By8) and *Glu-B1al* ($Bx7^{OE}$ + By8) alleles, which cannot be distinguished through SDS-PAGE. The BX7OE_866_SNP marker is based on a single nucleotide polymorphism (SNP) in the promoter region: the C variant is associated with the *Glu-B1al* allele, which carries a duplicated copy of the Bx7 locus, while the G variant is associated with the absence of the Bx7 duplication. The allelic state of the *Glu-A1* and *Glu-B1* was identified by comparing the results obtained using the KASP and SDS-PAGE assays. According to the research results, three alleles were identified for *Glu-A1*, and eight alleles were identified for *Glu-B1.* The vast majority of the studied accessions contained the *Glu-A1c* allele (98 %), while the proportion of accessions with *Glu-A1a* and *Glu-A1b* accounted for 0.5 % and 1.5 %, respectively. For *Glu-B1*, there was a higher number of accessions with the *Glu-B1al* allele (60 %) compared to accessions carrying *Glu-B1d* (17 %) and *Glu-B1e* (12 %). The accessions containing subunits

Bx7 + By8 (*Glu-B1b*) accounted for 3 % of the collection. In addition, rare alleles *Glu-B1h* (1 %), *Glu-B1i* (1 %), as well as *Glu-B1z* (1 %) and *Glu-B1z** (5 %) were identified. The last two differed in a single nucleotide polymorphism in the promoter region of the gene that encodes the Bx7 subunit. There was a tendency towards the distribution and fixation of the *Glu-B1z** allele in durum wheat varieties. A positive effect of *Glu-B1d* on the gluten index and a negative effect of *Glu-B1e* on its value were also observed.

Keywords: *Triticum durum*, durum wheat, high molecular weight glutenins, HMW-GS, *Glu-A1*, *Glu-B1*, KASP marker, SDS-PAGE, gluten index

Durum wheat (*Triticum durum* Desf.) is one of the main crops cultivated for food. Durum wheat grain is widely used for pasta and cereal production and in bakery [1]. Durum wheat grain for high-quality pasta production must have appropriate glassiness and contents of protein, gluten and carotenoids [2-4]. Consumers' interest in products with improved properties increases manufacturers' requirements for the quality of raw materials [5]. Particular attention is paid to gluten index, characterizing the strength of its intra- and intermolecular bonds. In breeding, the gluten index, along with classical methods of farinograms and mixograms or SDS sedimentation, is used to select the most promising lines of durum wheat [6, 7]. The gluten index is extremely important for modern technology of high-temperature drying widely used in the pasta industry in North America, Australia and Europe. This technology, which increases both the volume and efficiency of pasta production, is also used in Russia, but its wide distribution in our country and the CIS countries is hampered by the small number of durum wheat varieties with the required genetic basis that provides gluten of a certain quality [8, 9].

The quality of durum wheat gluten determined by its rheological properties, depends on the components of the gliadin and glutenin fractions of the protein, the ratio of gliadins and glutenins, allelic variants of gliadin and glutenin loci, and on the genotype-environment interaction [10-12]. Glutenins are storage proteins of wheat formed by interconnected polypeptide chains (subunits) [13, 14]. The glutenin fraction of the protein consists of high molecular weight (HMW) and low molecular weight (LMW) glutenins. The genes encoding high-molecular-weight glutenins are located in the *Glu-1* locus, while the genes for low-molecular-weight glutenins are located in *Glu-3* [15].

High- and low-molecular glutenins have different effects on the quality of durum wheat gluten and pasta. LMW glutenins have the greatest influence on the quality of grain and final products [10, 16, 17]. However, the influence of HMW glutenins is also important [11, 18, 19]. High molecular weight glutenins in durum wheat are encoded by the *Glu-A1* and *Glu-B1* loci, each for x- and y-subunits resulting from ancient duplication, while the y-subunit of *Glu-A1* is not expressed [13]. There is no clear opinion about the influence of the *Glu-A1* allelic state on the quality of durum wheat products due to the actual fixation of the *Glu-A1c* null allele in most world collections of modern varieties [19, 20]. *Glu-B1* alleles with a positive effect on the quality of durum wheat gluten and pasta include, for example, *Glu-B1b* (Bx7 + By8), *Glu-B1al* ($Bx7^{OE}$ + By8), *Glu-B1d* (Bx6 + By8), *Glu-B1h* (Bx14 + By15), *Glu-B1z* (Bx7 + By15), *Glu-B1e* (Bx20 + By20) is the allele with a negative effect [19, 21, 22].

The genes for high molecular weight glutenins are significantly diverse in allelic variants of protein subunits. In turn, these variants differ in the degree of influence on the quality of durum wheat grain. Therefore, it is of interest to assess the distribution of allelic variants of the HMW-GS genes and the combination of their alleles in different collections of durum wheat [16, 19, 23]. Despite the accuracy of lab-on-a-chip, liquid chromatography and mass spectrometry methods

for detecting subunits of grain storage proteins, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins remains the most accessible and widely used procedure [11, 21, 22].

The allelic state of *Glu* genes is detected by classical polymerase chain reaction (PCR) with separation of amplification products in an agarose or polyacrylamide gel, and competitive allele-specific PCR, or KASP assay [24]. KASP markers distinguish single nucleotide polymorphisms (SNPs) between alleles due to competition between two forward primers, the oligonucleotide sequences that are complementary to the target DNA region but differ at the 3′-end in target SNPs, and at the 5′-end have different "tails" with unique sequences. In addition to two alternative forward primers and one common reverse primer, the KASP kit includes two FRET (fluorescence resonance energy transfer) cassettes, each is a double-stranded oligonucleotide. One strand of the FRET cassette at the 3′-end is complementary to the unique "tail" of a particular forward primer, and at the 5′-end carries a covalently bound fluorophore (as a rule, one cassette carries FAM, the other HEX). The second strand of the FRET cassette complementary to the first strand carries a fluorophore quencher at the 3′-end.

In the first round of PCR, one of the alternative forward primers is annealed. In the competition between direct alternative primers, the primer corresponding to the SNP of the template DNA with which PCR is run has an advantage. In the second round of PCR, the reverse (common) primer is annealed. In the third round of PCR, the "tail" of the forward primer anneals the one of the two FRET cassettes that is complementary to the "tail" of the forward primer which had an advantage. As a result, the fluorophore and quencher are spatially isolated and a signal (FAM or HEX) is emitted. If a heterozygote is analyzed, two signals are detected simultaneously [24]. Due to the fact that the KASP assay is based on signal detection in real-time PCR, it does not involve the stage of fragment separation in a gel, which significantly speeds up the genotyping procedure.

KASP system markers effectively detect both single-nucleotide polymorphisms and insertions/deletions of a specific DNA region [25]. The basis of KASP markers can be either previously created classical PCR markers developed for polymorphisms between gene alleles, or single-nucleotide polymorphisms identified by of association genetics methods using high-throughput SNP genotyping [26]. KASP markers are widely used in plant breeding, including wheat, due to their high accuracy, efficiency and productivity, which allows large-scale screening of varieties, segregating populations and breeding lines [27]. KASP markers are used to analyze the allelic state of genes for economically valuable traits in wheat, including genes for storage proteins [28-30].

In previous studies of winter durum wheat varieties and breeding samples [23] and samples from the nursery for competitive variety testing of spring durum wheat [21] collected at the Lukyanenko National Grain Center (NGC), we analyzed the effects of allelic variants *Glu-A1* and *Glu-B1* on grain and pasta quality parameters.

This work is the first to report the structure of an extended collection of durum wheat varieties and spring-winter breeding lines for the combination of *Glu-1* gene allelic variants.

The purpose of the work was to identify allelles of the high molecular weight glutenin genes *Glu-A1* and *Glu-B1* using SDS-PAGE and KASP assay in a collection including varieties and promising breeding lines of durum wheat, and to assess their effect on the gluten index.

Materials and methods. A total of 198 breeding lines and spring-winter varieties of durum wheat of the Lukyanenko National Geain Center were tested.

The allelic state for HMW-GS was assessed by SDS-PAGE according to

previously described methods [23, 31]. The gluten index value was measured using the Perten Glutomatic® 2100 System (PerkinElmer, USA) according to the protocol [23, 32].

Genomic DNA was isolated from 4-day-old seedlings by CTAB method with some modifications using titer tubes for mass analysis [33].

To identify allelic variants of the *Glu-A1* gene, the Glu-Ax1/x2*_SNP marker was used, including the FAM primer 5′-AAGTGTAACTTCTCCGCAACG-3′ (it corresponds to the *Glu-A1c* null allele, Ax-null), HEX primer 5′-ACCTAAGTGTAACTTCTCGCAACA-3′ (it corresponds to the *Glu-A1a* allele encoding the Ax1 subunit or the *Glu-A1b* allele for the Ax2* subunit), general primer was 5′-CGAAGAAGCTTGGCCTGGATAGTAT-3′ (28).

Samples with the *Glu-B1b* allele (Bx7 + By8) carrying one copy of Bx7, and samples with the *Glu-B1al* allele ($Bx7^{OE}$ + By8) that overexpress the Bx7 due to duplication of a fragment of the locus [34], are indistinguishable when analyzed by SDS-PAGE. Thereof, the KASP marker BX7OE_866_SNP was used, including the HEX primer 5′-GTGGAATATTAGTGATGGCGTGAC-3′ corresponding to the presence of the *Glu-B1al* allele for $Bx7^{OE}$ + By8 subunits, the FAM primer 5′-GTGGAATATTAGTGATGGCGTGAG-3′corresponding to the absence of the *Glu-B1al* allele, and the general primer 5′-TTCTTCTCTCGTTGGCCTTATCGC-3′ [33]. The marker is based on a SNP in the promoter region, the C variant is associated with the *Glu-B1al* allele carrying a double copy of Bx7, G variant is associated with the absence of a double copy of Bx7 [28, 29].

PCR was performed in a 96-well PCR plate (BIO-RAD CFX96, Bio-Rad Laboratories, Inc., USA; Bio-Rad CFX Manager 3.1 software). The 10 μl PCR mixture contained 5 μl Master mix KBS-1050-102 with FAM, HEX, ROX dyes (LCG Biosearch Technologies, UK), 1.5 pM allele-specific primers, 3.75 pM general primer and 5 μl template DNA (50 ng per well). The touchdown protocol was as follows: 15 min at 94 °C for enzyme activation; 20 s at 94 °C, 60 s at 61-55 °C (temperature decreases by 0.6 °C with each cycle), 30 s at 94 °C (10 cycles); 20 s at 94 °C, 60 s at 55°C (35 cycles); signal reading 60 s at 37 °C.

The allelic status of the HMW-GS *Glu-A1* and *Glu-B1* genes was indicated based on KASP assay and SDS-PAGE analyis.

For statistically processing data on the gluten index expressed as a percentage, a logit transformation was performed [35]. All further calculations were performed with the transformed values. The significance of differences in the average gluten index was assessed using Tukey's test for multiple pairwise comparisons at a 95% confidence interval for one-way ANOVA models (https://www.statsmodels.org/dev/generated/statsmodels.sandbox.stats.multicomp.MultiComparison.html).

Results. KASP analysis and SDS-PAGE provided data on the structure of the collection of breeding lines and spring-winter varieties of durum wheat (Fig. 1, Appendix 1; the full data of the Appendix for 198 samples can be found at http://www.agrobiology.ru).

It was found that 194 samples from the collection (98%) had the Glu-A1c allele, which indicated the absence of the Ax subunit. Samples with the Glu-A1a (1 sample) and Glu-A1b (3 samples) alleles, carrying the Ax1 and Ax2* subunits, respectively, were also found.

We identified 8 variants of alleles at the *Glu-B1* locus. The *Glu-B1b* allele, corresponding to the presence of Bx7 + By8 subunits, was detected in 6 samples. The combination of subunits Bx6 + By8, inherent to *Glu-B1d*, was identified in 34 samples, which is 17% of the studied collection. The *Glu-B1e* allele was carried by 24 samples with Bx20 + By20 subunits. Rare alleles were *Glu-B1h* (Bx14 + By15) and *Glu-B1i* (Bx17 + By18), each was found in only two samples, in 3552h59-18-

7 and COLOSSEO, and in 4814h20 and 3902h3-18-3, respectively. The largest part of the collection (118 samples, or 60%) was carriers of the *Glu-B1al* allele ($Bx7^{OE}$ + Bx8), which is characterized by overexpression of the Bx7 subunit due to duplication of a fragment of the locus [34].

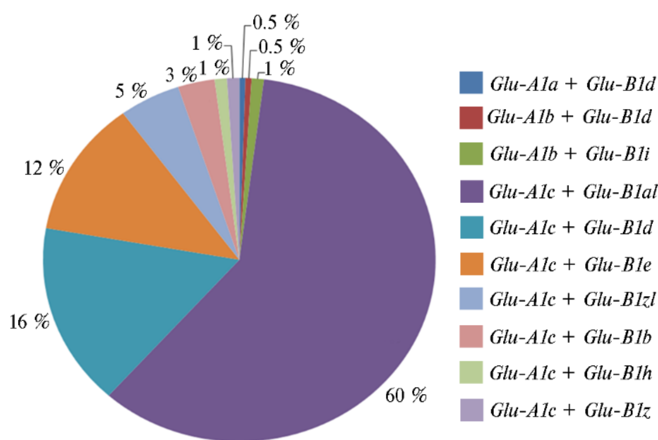

Fig. 1. Distribution of HMW-GS gene allele combinations in the collection of breeding lines and spring-winter varieties of durum wheat (*Triticum durum* Desf.) (Lukyanenko National Grain Center, 2021).

Appendix 1. Allelic variants of HMW-GS genes in durum wheat (*Triticum durum* Desf.) breeding lines and spring-winter varieties of a combined type (the collection of Lukyanenko National Grain Center, 2021)

No.	Accession	Allele *Glu-A1*	Allele *Glu-B1*
1	4801h142	*Glu-A1c*	*Glu-B1al*
2	4822h19	*Glu-A1c*	*Glu-B1al*
3	3902h3-18-3	*Glu-A1b*	*Glu-B1i*
4	4646h22	*Glu-A1c*	*Glu-B1al*
5	4583h47	*Glu-A1c*	*Glu-B1al*
6	4789h26	*Glu-A1c*	*Glu-B1al*
7	4290h4	*Glu-A1c*	*Glu-B1al*
8	4754h76	*Glu-A1c*	*Glu-B1al*
9	4507h14	*Glu-A1c*	*Glu-B1al*
10	4799h38	*Glu-A1c*	*Glu-B1b*
11	4387h8	*Glu-A1c*	*Glu-B1al*
12	4782h67	*Glu-A1c*	*Glu-B1b*
13	4503h23	*Glu-A1c*	*Glu-B1b*
14	4293h25	*Glu-A1c*	*Glu-B1b*
15	4810h54	*Glu-A1c*	*Glu-B1al*
16	4814h20	*Glu-A1b*	*Glu-B1i*
17	4291h83	*Glu-A1c*	*Glu-B1al*
18	4297h12	*Glu-A1c*	*Glu-B1al*
19	4316h90	*Glu-A1c*	*Glu-B1al*
20	4249h103	*Glu-A1c*	*Glu-B1d*

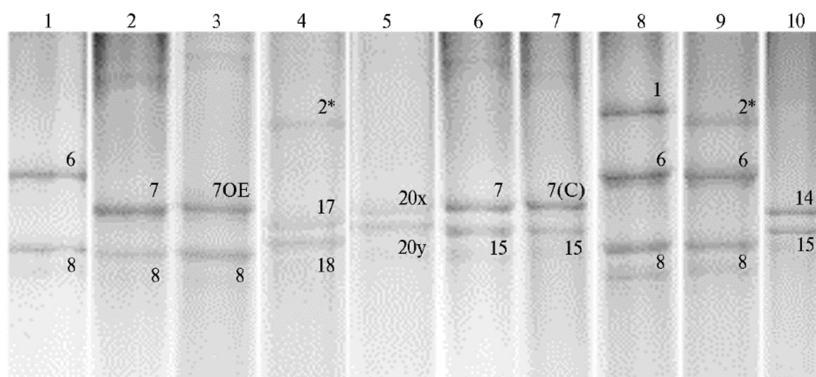

Fig. 2. Patterns of HMW-GS subunit combinations detected in the collection of breeding lines and spring-winter varieties of durum wheat (*Triticum durum* Desf.) by SDS-PAGE and confirmed by KASP analysis: 1 — 4598h48 (Bx6 + By8), 2 — ALLEMANNO (Bx7 + By8), 3 — 4583h47 ($Bx7^{OE}$ + By8), 4 — 4814h20 (Ax2*; Bx17 + By18), 5 — SAN MARCO (Bx20 + By20), 6 — 4593h36 (Bx7 + By15), 7 — 4808h8 (Bx7(C) + By15), 8 — 4626h42 (Ax1; Bx6 + By8), 9 — 4391h47 (Ax2*; Bx6 + By8), 10 — 3552h59-18-7 (Bx14 + By15) (Lukyanenko National Grain Center, 2021).

In samples with Bx7 + By15 subunits, polymorphism was observed for the

KASP marker. Two samples (4636h22 and 4593h36) have variant G, ten samples have variant C in the promoter region, and therefore we named the first variant *Glu-B1z* (Bx7 + By15), the second *Glu-B1z**, the subunits are conventionally designated Bx7(C) + By15 (Fig. 2). *Glu-A1a* and *Glu-A1b* in the studied durum wheat collection were found only in combination with *Glu-B1d* and *Glu-B1i*. *Glu-B1d* was also found in combination with *Glu-A1c*.

Figure 2 shows the patterns of protein subunits of high molecular weight glutenins, identified by SDS-PAGE com,ined with the KASP assay.

The effect of alleles of high molecular weight glutenins and their subunits on the gluten index is shown in the table. Due to the lack of samples with combinations of alleles *Glu-A1a* + *Glu-B1d* and *Glu-A1b* + *Glu-B1d*, statistical data are not submitted.

The gluten index in samples from the collection of breeding lines and spring-winter varieties of durum wheat (*Triticum durum* Desf.) depending on the alleles and subunits of HMW-GS (Lukyanenko National Grain Center, 2021)

Factor		Gluten index, %	The number of samples
By	By15	94.37^{ab}	14
	By18	99.11^{ab}	2
	By20	94.09^{b}	24
	By8	97.63^{a}	158
Glu-A1	*Glu-A1b*	98.91^{a}	3
	Glu-A1c	97.15^{a}	194
Glu-A1+Glu-B1	*Glu-A1b* + *Glu-B1i*	99.11^{ab}	2
	Glu-A1c + *Glu-B1al*	97.46^{ab}	118
	Glu-A1c + *Glu-B1b*	98.29^{ab}	6
	Glu-A1c + *Glu-B1d*	97.98^{a}	32
	Glu-A1c + *Glu-B1e*	94.09^{b}	24
	Glu-A1c + *Glu-B1h*	89.45^{ab}	2
	Glu-A1c + *Glu-B1z*	97.81^{ab}	2
	Glu-A1c + *Glu-B1z**	94.05^{ab}	10
Glu-B1	*Glu-B1al*	97.46^{ab}	118
	Glu-B1b	98.29^{ab}	6
	Glu-B1d	98.03^{a}	34
	Glu-B1e	94.09^{b}	24
	Glu-B1h	89.45^{ab}	2
	Glu-B1i	99.11^{ab}	2
	Glu-B1z	97.81^{ab}	2
	*Glu-B1z**	94.05^{ab}	10

N o t e. Statistically insignificant means based on Tukey's test are indicated by the same letters.

In 2021, samples with *Glu-B1i* had the maximum gluten index. Due to the low occurrence of this allele among the studied accessions, we could not identify its statistically significant differences from others, but it is of undoubted interest for further study. As for the contribution of each of the subunits to the increase in the gluten index, we can note a significant increase in this indicator due to the By8 subunit. A statistically significant decrease in the gluten index is associated with the By20 subunit, characteristic of *Glu-B1e*.

The *Glu-A1* locus in our studies was represented by three alleles with a predominance of *Glu-A1c* (98%). The predominance of this allele can be traced in many other durum wheat collections, regardless of the region of origin of the varieties [36-38]. Although the *Glu-A1c* allele is ubiquitous and is recorded even in varieties of modern breeding, among durum wheat samples there are genotypes with the *Glu-A1a* and *Glu-A1b* alleles [19, 23, 38]. In the collection we tested they accounted for 0.5 and 1.5%, respectively. Compared to modern varieties, landraces and old Mediterranean varieties are characterized by an increased frequency of the *Glu-A1a* allele occurrence (up to 38%) [37, 39]. The *Glu-A1a* and *Glu-A1b* alleles are of interest for further study because high gluten index scores were observed in samples in which they were combined with the *Glu-B1* locus alleles. For example, the highest gluten index was noted in the sample with the combination

Glu-A1b + *Glu-B1i*, however, due to the small sample, we were unable to identify statistically significant differences between this combination of alleles and other combinations (see Table).

The *Glu-B1* locus had 8 allelic variants. The *Glu-B1al* allele for subunits Bx7OE + By8 was dominant (60%). Due to the fact that SDS-PAGE does not distinguish $Bx7^{OE}$ + By8 (*Glu-B1al*) from Bx7 + By8 (*Glu-B1b*), we used the KASP marker which detects C/G polymorphism in the promoter region of the gene encoding subunit Bx7 [28]. In most other studies, KASP analysis was not performed and subunit composition was determined using SDS-PAGE. Combining data on *Glu-B1b* and *Glu-B1al*, we get a share of 63% (124 samples), close to the figures in European [37] and Australian [38] durum wheat collections. According to our data, samples with the *Glu-B1al* and *Glu-B1b* alleles had high gluten index (see Table).

The combination of subunits Bx6 + By8 (*Glu-B1d*) in soft wheat varieties leads to a deterioration in baking qualities, and in durum wheat varieties, on the contrary, is associated with high gluten quality indicators [37, 38, 40]. According to our data, samples carrying *Glu-B1d* were also characterized by high gluten index (see не представлены). This allele is moderately common in Mediterranean varieties [37], where it accounts for about 20% of accessions, similar to our study. A significant distribution of *Glu-B1d* is observed in US and Canadian cultivars (approximately 70%) [38, 41].

The *Glu-B1e* allele (Bx20 + By20) worsens gluten quality indicators [22, 40, 42]. In modern varieties, it is less common than in landraces and varieties of old selection [16, 37]. Although it reduces the gluten index value, this allele is present (15-20%) in modern durum wheat varieties [19, 21, 39]. This may be explained by the fact that the gluten index is not the only indicator that determines the quality of pasta. Since Bx20+By20 is still found in modern commercial varieties, such varieties are interesting for the search and study of other genetic systems that determine high quality pasta (LMW-GS, starch and protein content, gliadins, color index).

The identified rare alleles *Glu-B1h*, *Glu-B1z*, *Glu-B1z**, *Glu-B1i* are of interest for diversification of the gene pool of durum wheat to expand the range of technological qualities of food products from durum wheat. The small number of samples with the *Glu-B1i* (Bx17 + By18) and *Glu-B1h* (Bx14 + By15) alleles did not allow us to fully assess their effect on the gluten index, however, they were characterized by the highest and lowest average gluten index values, respectively (see Table). Other researchers have noted a reduced SDS sedimentation value when assessing gluten in samples with Bx14+By15 subunits compared to samples with Bx17+By18 [20].

Using KASP analysis, we identified two *Glu-B1z* variants (Bx7 + By15) with two SNPs in the promoter of the gene encoding the Bx7 subunit. Two samples had polymorphism G (the *Glu-B1z*), 10 samples had polymorphism C (the *Glu-B1z**). Since the promoter influences protein expression, it can be expected that the identified SNPs lead to phenotypic differences between varieties possessing the *Glu-B1z* and *Glu-B1z** alleles. This is indirectly confirmed by the differences in the average gluten index values between samples carrying the two indicated alleles (see Table). Due to the small sample size, the difference was not statistically significant, so the effect of this SNP requires further study. In addition, it is interesting that the number of accessions with the *Glu-B1z** allele (5%) prevailed over the number of accessions with the *Glu-B1b* allele (3%) widely used in durum wheat breeding, that is, the *Glu-B1z** allele is gradually spreading and becoming consolidated in modern varieties of durum wheat.

Thus, the analysis of 198 lines and varieties of durum wheat from the

collection of the Lukyanenko National Grain Centerwe revealed that the overwhelming majority of the studied samples contained the *Glu-A1c* allele (98%), and the samples with *Glu-A1a* and *Glu-A1b* accounted for 0.5 and 1.5%, respectively. At the *Glu-B1* locus, there was a superiority of the *Glu-B1al* samples (60%) over *Glu-B1d* (17%) and *Glu-B1e* (12%) samples. The samples with subunits Bx7 + By8 (*Glu-B1b*) accounted for 3% of the collection studied. In addition, we identified rare alleles *Glu-B1h* (1%), *Glu-B1i* (1%), *Glu-B1z* (1%) and *Glu-B1z** (5%). The last two alleles differ in single nucleotide polymorphism in the promoter region of the gene for the Bx7 subunit. There was a tendency for the *Glu-B1z** allele to become widespread and consolidated in durum wheat varieties. A positive effect of *Glu-B1d* on the gluten index and a negative effect of *Glu-B1e* were revealed. Previously we published data about promising winter durum wheat and spring durum wheat samples. The results for double-handling samples we described herein, together with previous data, expand understanding the influene of high molecular weight glutenins on the quality of grain, gluten and pasta. It may be useful in durum wheat breeding for improved varieties in demand.

REFERENCES

1. Mastrangelo A.M., Cattivelli L. What Makes Bread and Durum Wheat Different? *Trends in Plant Science*, 2021, 26(7): 677-684 (doi: 10.1016/j.tplants.2021.01.004).
2. Fu B.X., Wang K., Dupuis B., Taylor D., Nam S. Kernel vitreousness and protein content: relationship, interaction and synergistic effects on durum wheat quality. *Journal of Cereal Science*, 2018, 79: 210-217 (doi: 10.1016/j.jcs.2017.09.003).
3. Myasnikova M.G., Mal'chikov P.N., Shabolkina E.N., Sidorenko V.S., Tugareva F.V., Rozova M.A., Chakheeva T.V., Tsygankov V.I. *Zernobobovye i krupyanye kul'tury*, 2019, 4(32): 112-119 (doi: 10.24411/2309-348X-2019-11141) (in Russ.).
4. Kaplan Evlice A. The effect of durum wheat genotypes on cooking quality of pasta. *European Food Research and Technology*, 2022, 248(3): 815-824 (doi: 10.1007/s00217-021-03899-4).
5. Padalino L., Mastromatteo M., Lecce L., Spinelli S., Contт F., Del Nobile M.A. Effect of durum wheat cultivars on physicochemical and sensory properties of spaghetti. *Journal of the Science of Food and Agriculture*, 2014, 94(11): 2196-2204 (doi: 10.1002/jsfa.6537).
6. Clarke F.R., Clarke J.M., Ames N.A., Knox R.E., Ross R.J. Gluten index compared with SDS-sedimentation volume for early generation selection for gluten strength in durum wheat. *Canadian Journal of Plant Science*, 2010, 90(1): 1-11 (doi: 10.4141/CJPS09035).
7. Cecchini C., Bresciani A., Menesatti P., Pagani M.A., Marti A. Assessing the rheological properties of durum wheat semolina: a review. *Foods*, 2021, 10(12): 2947 (doi: 10.3390/foods10122947).
8. Shevchenko S.N., Mal'chikov P.N., Myasnikova M.G., Vinchentso N., Paskuale D.V., Dzhuliyani M. *Izvestiya Samarskogo nauchnogo tsentra Rossiyskoy akademii nauk*, 2018, 20(2-2), 220-230 (in Russ.).
9. Shevchenko S.N., Mal'chikov P.N., Myasnikova M.G., Natoli V., Chakheeva T.V. *Dostizheniya nauki i tekhniki APK*, 2019, 33(12): 38-42 (doi: 10.24411/0235-2451-2019-11207) (in Russ.).
10. Edwards N.M., Mulvaney S.J., Scanlon M.G., Dexter J.E. Role of gluten and its components in determining durum semolina dough viscoelastic properties. *Cereal Chemistry*, 2003, 80(6): 755-763 (doi: 10.1094/CCHEM.2003.80.6.755).
11. Sissons M.J., Soh H.N., Turner M.A. Role of gluten and its components in influencing durum wheat dough properties and spaghetti cooking quality. *Journal of the Science of Food and Agriculture*, 2007, 87(10): 1874-1885 (doi: 10.1002/jsfa.2915).
12. Fois S., Schlichting L., Marchylo B., Dexter J., Motzo R., Giunta F. Environmental conditions affect semolina quality in durum wheat (*Triticum turgidum* ssp. *durum* L.) cultivars with different gluten strength and gluten protein composition. *Journal of the Science of Food and Agriculture*, 2011, 91(14): 2664-2673 (doi: 10.1002/jsfa.4509).
13. Gianibelli M.C., Larroque O., MacRitchie F., Wrigley C.W. Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. *Cereal Chemistry*, 2001, 78(6): 635-646 (doi: 10.1094/CCHEM.2001.78.6.635).
14. Vorotyntseva M.V. *Trudy po prikladnoy botanike, genetike i selektsii*, 2021, 182(1): 168-185 (doi: 10.30901/2227-8834-2021-1-168-185) (in Russ.).
15. Pogna N.E., Autran J.C., Mellini F., Lafiandra D., Feillet P. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to gluten strength. *Journal of Cereal Science*, 1990, 11(1): 15-34 (doi: 10.1016/S0733-5210(09)80178-1).

16. Subira J., Peca R.J., Álvaro F., Ammar K., Ramdani, A., Royo C. Breeding progress in the pasta-making quality of durum wheat cultivars released in Italy and Spain during the 20th Century. *Crop and Pasture Science*, 2014, 65(1): 16-26 (doi: 10.1071/CP13238).
17. Ruiz M., Bernal G., Giraldo P. An update of low molecular weight glutenin subunits in durum wheat relevant to breeding for quality. *Journal of Cereal Science*, 2018, 83: 236-244 (doi: 10.1016/j.jcs.2018.09.005).
18. Raciti C.N., Doust M.A., Lombardo G.M., Boggini G., Pecetti L. Characterization of durum wheat Mediterranean germplasm for high and low molecular weight glutenin subunits in relation with quality. *European Journal of Agronomy*, 2003, 19(3): 373-382 (doi: 10.1016/S1161-0301(02)00091-6).
19. Roncallo P.F., Guzmбn C., Larsen A.O., Achilli A.L., Dreisigacker S., Molfese E., Astiz V., Echenique V. Allelic variation at glutenin loci (*Glu-1*, *Glu-2* and *Glu-3*) in a worldwide durum wheat collection and its effect on quality attributes. *Foods*, 2021, 10(11): 2845 (doi: 10.3390/foods10112845).
20. Al-Khayri J.M., Alshegaihi R.M., Mahgoub E.I., Mansour E., Atallah O.O., Sattar M.N., Al-Mssallem M.Q., Alessa F.M., Aldaej M.I., Hassanin A.A. Association of high and low molecular weight glutenin subunits with gluten strength in tetraploid durum wheat (*Triticum turgidum* spp. *durum* L.). *Plants*, 2023, 12(6): 1416 (doi: 10.3390/plants12061416).
21. Kroupin P.Y., Bespalova L.A., Kroupina A.Y., Yanovsky A.S., Korobkova V.A., Ulyanov D.S., Karlov G.I., Divashuk M.G. Association of high-molecular-weight glutenin subunits with grain and pasta quality in spring durum wheat (*Triticum turgidum* spp. *durum* L.). *Agronomy*, 2023, 13(6): 1510 (doi: 10.3390/agronomy13061510).
22. Giraldo P., Ruiz M., Ibba M.I., Morris C.F., Labuschagne M.T., Igrejas G. Durum wheat storage protein composition and the role of LMW-GS in quality. In: *Wheat quality for improving processing and human health*. G. Igrejas, T.M. Ikeda, C. Guzmán (eds.) Springer International Publishing, Cham, Switzerland, 2020: 73-108 (doi: 10.1007/978-3-030-34163-3_5).
23. Kroupina A.Y., Yanovsky A.S., Korobkova V.A., Bespalova L.A., Arkhipov A.V., Bukreeva G.I., Voropaeva A.D., Kroupin P.Y., Litvinov D.Y., Mudrova A.A., Ulyanov D.S., Karlov G.I., Divashuk M.G. Allelic variation of *Glu-A1* and *Glu-B1* genes in winter durum wheat and its effect on quality parameters. *Foods*, 2023, 12(7), 1436 (doi: 10.3390/foods12071436).
24. He C., Holme J., Anthony J. SNP genotyping: the KASP assay. In: *Crop breeding: methods and protocols*. D. Fleury, R. Whitford (eds.). Humana New York, NY, 2014: 75-86 (doi: 10.1007/978-1-4939-0446-4_7).
25. Nikitina E.A., Arkhipov A.V., Min'kova Ya.V., Yanovskiy A.S., Korobkova V.A., Samarina M.A., Chernook A.G., Krupin P.Yu., Karlov G.I., Divashuk M.G. *Izvestiya Timiryazevskoy sel'skokhozyaystvennoy akademii*, 2022, 1(6): 79-93 (doi: 10.26897/0021-342X-2022-6-79-93) (in Russ.).
26. Semagn K., Babu R., Hearne S., Olsen M. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. *Molecular Breeding*, 2014, 33: 1-14 (doi: 10.1007/s11032-013-9917-x).
27. Allen A.M., Barker G.L.A., Wilkinson P., Burridge A., Winfield M., Coghill J., Uauy C., Griffiths S., Jack P., Berry S., Werner P., Melichar J.P.E., McDougall J., Gwilliam R., Robinson P., Edwards K.J. Discovery and development of exome-based, co-dominant single nucleotide polymorphism markers in hexaploid wheat (*Triticum aestivum* L.). *Plant Biotechnology Journal*, 2012, 11(3): 279-295 (doi: 10.1111/pbi.12009).
28. Rasheed A., Wen W., Gao F., Zhai S., Jin H., Liu J., Guo Q., Zhang Y., Dreisigacker S., Xia X., He Z. Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. *Theoretical and Applied Genetics*, 2016, 129: 1843-1860 (doi: 10.1007/s00122-016-2743-x).
29. Ravel C., Faye A., Ben-Sadoun S., Ranoux M., Dardevet M., Dupuits C., Exbrayat F., Poncet C., Sourdille P., Branlard G. SNP markers for early identification of high molecular weight glutenin subunits (HMW-GSs) in bread wheat. *Theoretical and Applied Genetics*, 2020, 133: 751-770 (doi: 10.1007/s00122-019-03505-y).
30. Korobkova V.A., Bespalova L.A., Yanovskiy A.S., Voropaeva A.D., Arkhipov A.V., Shirnin S.Yu., Chernook A.G., Nikitina E.A., Ul'yanov D.S., Mudrova A.A., Bukreeva G.I., Karlov G.I., Divashuk M.G. *Kormoproizvodstvo*, 2023, 4: 25-31 (doi: 10.25685/KRM.2023.4.2023.004) (in Russ.).
31. Singh N., Shepherd K., Cornish G. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *Journal of Cereal Science*, 1991, 14(3): 203-208 (doi: 10.1016/S0733-5210(09)80039-8).
32. *Perten Glutomatic-PERGM. PerkinElmer*. Available: https://www.matt.nz/Products/Grain/Grain-Applications/Flour-Milling/Glutomatic/. Accessed: 19.05.2023.
33. Murray M.G., Thompson W.F. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 1980, 8(19): 4321-4326 (doi: 10.1093/nar/8.19.4321).
34. Ragupathy R., Naeem H. A., Reimer E., Lukow O.M., Sapirstein H.D., Cloutier S. Evolutionary origin of the segmental duplication encompassing the wheat *GLU-B1* locus encoding the overexpressed Bx7 ($Bx7^{OE}$) high molecular weight glutenin subunit. *Theoretical and Applied Genetics*, 2008, 116: 283-296 (doi: 10.1007/s00122-007-0666-2).

35. Warton D.I., Hui F.K. The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, 2011, 92(1): 3-10 (doi: 10.1890/10-0340.1).
36. Gregová E., Medvecká E., Jómová K., Šliková S. Characterization of durum wheat (*Triticum durum* Desf.) quality from gliadin and glutenin protein composition. *Journal of Microbiology, Biotechnology and Food Sciences*, 2021: 610-615.
37. Nazco R., Peca R.J., Ammar K., Villegas D., Crossa J., Moragues M., Royo C. Variability in glutenin subunit composition of Mediterranean durum wheat germplasm and its relationship with gluten strength. *The Journal of Agricultural Science*, 2014, 152(3): 379-393 (doi: 10.1017/S0021859613000117).
38. Sissons M.J., Ames N.P., Hare R.A., Clarke J.M. Relationship between glutenin subunit composition and gluten strength measurements in durum wheat. *Journal of the Science of Food and Agriculture*, 2005, 85(14): 2445-2452 (doi: 10.1002/jsfa.2272).
39. Moragues M., Zarco-Hernбndez J., Moralejo M.A., Royo C. Genetic diversity of glutenin protein subunits composition in durum wheat landraces [*Triticum turgidum* ssp. *turgidum* Convar. *durum* (Desf.) MacKey] from the Mediterranean Basin. *Genetic Resources and Crop Evolution*, 2006, 53: 993-1002 (doi: 10.1007/s10722-004-7367-3).
40. Ammar K., Kronstad W.E., Morris C.F. Breadmaking quality of selected durum wheat genotypes and its relationship with high molecular weight glutenin subunits allelic variation and gluten protein polymeric composition. *Cereal Chemistry,* 2000, 77(2): 230-236 (doi: 10.1094/CCHEM.2000.77.2.230).
41. Chegdali Y., Ouabbou H., Essamadi A., Cervantes F., Ibba M.I., Guzmán C. Assessment of the glutenin subunits diversity in a durum wheat (*T. turgidum* ssp. *durum*) collection from Morocco. *Agronomy*, 2020, 10(7): 957 (doi: 10.3390/agronomy10070957).
42. Brites C., Carrillo J.M. Influence of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits controlled by *Glu-1* and *Glu-3* loci on durum wheat quality. *Cereal Chemistry*, 2001, 78(1): 59-63 (doi: 10.1094/CCHEM.2001.78.1.59).