POLYMORPHISM OF GRAIN STORAGE PROTEINS IN TRITICALE LINES OF CIMMYT ORIGIN

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ABSTRACT

A collection of seventy Mexican Triticosecale samples originating from CIMMYT was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The studied lines are spring forms with high resistance to yellow rust and with high productive potential under the conditions of Bulgaria. Electrophoretic analysis of grain storage proteins encoded by loci Glu-1 (Glu-A1, Glu-B1 and Glu-R1), Glu-3 (Glu-A3 and Glu-B3), Glu-B2 and Gli-R2 showed the presence of eleven alleles encoding the high molecular weight (HMW) subunits (seven for glutenins and four for secalins), six for the low molecular weight (LMW) glutenin subunits and four for 75K γ - secalins. The formed allelic configurations were characterized by higher polymorphism at the Glu-A1 and Glu-B1 loci, where seven alleles were identified. The number of triticale lines possessing subunits ‚1'and ‚2*'at the Glu-A1 locus, coded by alleles ‚a'and ‚b' respectively, is the largest. These alleles are associated with good bread-making qualities of the flour. In the Glu-B1 locus, the fractional pair ‚7+18' (allele 'r') was found with the highest frequency, and in the locus Glu-R1 the fractional pair ‚6r+13r' (allele ‚c') was most often expressed. In the area of low molecular weight glutenins with higher allelic diversity is the Glu-B3 locus. Alleles 'a' and 'b', encoding 75K γ- secalins 'd1' and 'd2', were identified with the highest frequency in the Gli-R2 locus. The obtained results for the allelic composition of the storage proteins of the Mexican triticale lines will find application in the selection program of the Dobrudzha Agricultural Institute (DAI) for the creation of spring forms combining high productive potential, resistance to abiotic and biotic stress and quality.

Key words: SDS-PAGE, Grain storage proteins, Genetic diversity, Polymorphism

INTRODUCTION

Triticale (x *Triticosecale Wittmack*) is an artificially obtained amphiploid by hybridization between wheat (*Triticum turgidum* ssp. *durum* or *Triticum aestivum*) as a maternal component and rye (*Secale cereale*), which performs the function of pollinator. The resulting genotypes were hexaploid or octaploid depending on whether durum or bread wheat was used in the crosses with rye, respectively. The hybrids combine the good agrotechnical indicators of wheat with the high resistance to abiotic and biotic stress of rye (Salmanowicz et al., 2013; Sokol, 2014; Daskalova et al., 2021; Camerlengo and Kiszonas, 2023; Doneva et al., 2023; Sokol, 2014). The increased plasticity of triticale makes it suitable for cultivation under worse agro-meteorological conditions compared to wheat and other cereal crops (Kandrokov et al., 2019; Mergoum et al., 2019).

Triticale breeding, which began to develop very intensively in the second half of the 20th century, was aimed at creating varieties with higher yield, higher relative weight of the grain, reduced plant height and increased adaptability to changing climatic conditions. To date, the area sown with triticale

is increasing significantly, leading to a substantial increase in world production. The significant jump in the selection of triticale is due to its wide range of uses, from animal feed to various industrial applications. Although the technological and rheological properties of the dough obtained from triticale flour are of lower quality compared to those of wheat, in recent years it has been increasingly used in the modern food industry for the production of healthy foods. The reason for this is the high nutritional value of the flour. It is due to the increased protein content, the high proportion of soluble dietary fiber without starch, phenolic compounds with antioxidant activity, vitamins, etc. (Tohver et al., 2005; Jonnala et al., 2010b; Rakha et al., 2011; Dennett et al., 2013; Agil and Hosseinian, 2014; Pattison et al., 2014; Fraś et al., 2016; Langó et al., 2018b; Watanabe, 2019; Sirat et al., 2022). In addition, triticale flour is characterized by a higher level of the essential amino acid lysine, which increases the biological value of the protein, and this leads to a better balanced amino acid composition compared to wheat (Varugnese et al., 1996; Sokol, 2014; Doneva and Stoyanov, 2019; Doneva et al., 2023; Camerlengo and Kiszonas, 2023). The health benefits of consuming triticale foods have stimulated the selection of new varieties with the aim of making triticale more widely used in the human diet. Scientific research in recent years has shown that amphiploid has a high potential as an energy crop. In this regard, it is increasingly used as a source of biomass for the production of bioethanol (Bazhenov et al., 2015; Niedziela et al., 2016; Doneva and Stoyanov, 2019). Depending on the corresponding parental form of wheat, hybridization with rye produces triticale with different ploidy levels. Octaploid triticale genotypes (2n = 56 = AABBDDRR) result from the cross between bread wheat (2n = 42 = AABBDD) and rye (2n = 14 = RR). Crossing between tetraploid species of wheat (2n = 28 = AABB) and rye produces hexaploid amphiploids (2n = 42 = AABBRR). There are also tetraploid hybrids (2n = 14 = AARR), which are the result of the cross between hexaploid triticale and rye and subsequent self-fertilization. Breeding programs are aimed at obtaining hexaploid forms, which are more stable from a genetic point of view and realize high yields compared to octaploids, which in turn are characterized by better grain quality due to the presence of the D-genome (Lukaszewski, 2003, 2006; Daskalova et al., 2021; Camerlengo and Kiszonas, 2023) . Triticale hybrids obtained by crossing wheat and rye are classified as "primary triticale". Octaploid genotypes are obtained only as primary triticale. The classification also includes "secondary triticale", obtained in one of the following ways: by crossing primary lines of triticale, by crossing primary or secondary forms with wheat and rye, or by crossing primary with secondary triticale. Hexaploid triticale are either primary or secondary depending on how the amphiploid was obtained. When tetraploid wheat is crossed with diploid rye, primary hexaploid genotypes are obtained, and when octaploid forms are hybridized, hexaploid secondary amphiploids are obtained (Oettler, 2005; Bellil et al, 2010).

Ripe triticale grains are often wrinkled, similar to rye, and more elongated than durum and bread wheat. They consist of bran, germ and endosperm. The protein content of the grain of the hybrid varies (9-16%) depending on the genotype, the environment and the applied agrotechnical practices (Rakha et al., 2011; Sirat et al., 2022). Three protein fractions have been identified in the grain endosperm: albumins/globulins, gliadins and glutenins. Albumins and globulins are known to be enzymes and physiologically active proteins that do not form gluten. In contrast, the other two groups of proteins - glutenins and gliadins - are gluten-forming (Todorov, 2006) and are the main reserve proteins that are stored in the endosperm of the triticale grain. Glutenins are high molecular weight (HMW-GS) and low molecular weight (LMW-GS). High molecular weight glutenins are x- and y-type depending on the number of cysteine residues. HMW-GS are encoded by genes that are localized to loci in the long arms of 1A (Glu-A1) (Lawrence and Shepherd, 1980), 1B (Glu-B1) (Bietz et al., 1975) and 1D (Glu -D1) wheat chromosomes. LMW-GS are encoded by genes in the wheat Glu-3 locus (Glu-A3, Glu-B3, Glu-D3), which is closely related to Gli-1 (Jackson et al., 1983). Triticale also contains glutenin-like secalin subunits (HMW-SS and LMW-SS) encoded by genes on the long arm of rye chromosome 1R (Glu-R1 or Sec-3) (Lawrence and Shepherd, 1981). Gliadins are classified as α-, β-, γ-, and ω-. They are encoded by genes located in the Gli-1 (Gli-A1, Gli-B1, Gli-D1) and

Gli-2 (Gli-A2, Gli-B2, Gli-D2) loci located in the short arms corresponding to wheat chromosomes 1 and 6 (Metakovsky, 1991). Triticale also possesses one ω-secalin and two 40K γ-secalins encoded by genes at the Gli-R1(or Sec-1) locus (Shepherd, 1986), located on the short arm of chromosome 1R, two ω-secalins that are encoded from genes in the Gli-R3 (or Sec-4) locus (Carrillo et al., 1992), located on chromosome 1RS and 75K γ-secalins, which are encoded by genes in the Gli-R2 (or Sec-2) locus, located in chromosome 2RS of rye (Shewry et al., 1984).

The aim of the present study was to identify the allelic composition of the reserve endosperm proteins in a collection of triticale lines of CIMMYT origin and, based on the obtained data, to calculate the allelic frequencies and genetic diversity in loci: Glu-1 (Glu-A1, Glu- B1, Glu-R1), Gli-2 (Gli-R2) and Glu-3 (Glu-A3, Glu-B3, Glu-B2). The obtained results will find application in the selection program of the Dobrudzha Agricultural Institute (DAI) for the creation of spring forms triticale combining high productive potential, resistance to abiotic and biotic stress and quality.

MATERIAL AND METHOD

- Material

Seventy Mexican spring-type triticale samples originating from CIMMYT were studied. The collection is distinguished by high resistance to yellow rust and promising productive potential under the climatic conditions of Bulgaria.

- Method

A minimum of 50 grains from each accession were analyzed to determine the degree of its homogeneity. The single grains were ground to fine flour, having preliminary removed their embryos. The extraction was carried out in several consecutive stages according to Singh et al. (1991). Initially, 0.1 ml 50% (v/v) propanol, 0.08 M Tris – HCl, pH 8.0, containing 1% (w/v) fresh dithiothreitol (DTT) were added to the sample. After 1-hour incubation at 65°C, 0.1 ml 50% (v/v) propanol, containing 1.4% (v/v) fresh 4–vinylpyridine (VP) were added to each sample. Thus, the SH-groups in the probes were alkalized. This was flowed by 1-hour incubation at 65°C and 10-minite 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% bromophenol blue were added to it. The samples were shaken, incubated for 1 hour at 65 °C, centrifuged at 12000 g for 10 minutes, and then were ready for SDS-PAGE analysis. The additionally alkalized protein molecules prior to their treatment with SDS allowed obtaining even clearer electrophoregram. For precise identification of the allelic composition, the electrophoresis was carried out on a vertical electrophoresis system 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 method of Laemmli (1970), the electrophoresis occurred at constant current of 20mA on a plate at room temperature for 18-20 hours. The duration of the electrophoresis by the method of Payne et al. (1980) was 3-4 hours at 60 mA. After running the electrophoresis, the gel plates were stained with 1 % solution of coomassie brilliant blue (CBB), R250, acetic acid, methanol and water at ratio (1:5:4) overnight. De-staining was done with solution containing acetic acid, methanol, distilled water (1:2:7) until clearing of the background.

The nomenclatures of Payne and Lawrence (1983) and Vallega and Waines (1978) were used for identification of HMW of triticale. The allelic composition of LMW was determined by using the bread wheat nomenclatures of Gupta and Shepherd (1990) and Jackson et al. (1996). The allelic forms in Glu-R1, Gli-R2 and in Glu-2 were identified according to the nomenclature suggested by Amiour et al. (2002a).

- Statistical analysis

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

RESULTS AND DISCUSSION

Genetic variation of the glutenin and secalin subunits was found in loci Glu-A1, Glu-B1, Glu-R1, Gli-R2, Glu-A3, Glu-B3 and Glu-B2, which were localized on the arms of chromosomes 1AL, 1BL, 1RL, 2RS, 1AS, 1BS (Table 1).

SDS-PAGE electrophoretic spectra showed that the analyzed samples were homogeneous. This result is an indicator of the presence of electrophoretic control already in the initial stages of the selection process. They are identified eleven alleles encoding the high molecular weight (HMW) subunits (seven for glutenins and four for secalins), six for the low molecular weight (LMW) glutenin subunits and four for 75K γ- secalins.

In locus Glu-A1, alleles '*c*', '*a*' and '*b*' were identified, which coded for the high-molecular weight glutenin subunits 'N', '2*' and '1', respectively. Allele '*a*' was with the highest frequency – 52.9 %, followed by allele '*b*' (28.6 %). Allele '*b*' and especially allele '*a*' were markers of good and high gluten quality in common winter wheat (Todorov, 2006). The lowest frequency was that of allele '*c*' – 16.7 %. Allele '*c*' was related to null synthesis of protein, which determined low bread-making properties. The genetic variability in this locus was above the average – H = 0.60 (Table 1).

Table. 1 Frequency of alleles and genetic variation in triticale lines of CIMMYT origin

Locus	Subunit/Allele	Number of biotypes	Frequency,%
Glu-A1	1 / *a*	37	52.9
H = 0.60	2* / *b*	20	28.6
	null / *c*	13	18.6
Glu-B1	7+18 / *r*	56	80.0
H = 0.34	7+9 / *c*	9	12.8
	7+8 / *b*	2	2.9
	23+18 / p	2	2.9
	6.8+20y / *s*	1	1.4
Glu-R1	6r+13r / *c*	61	87.1
H = 0.23	6.5r / *e*	6	8.6
	2r+6r / *ni*	2	2.9
	5.8r/g	1	1.4
Glu-A3	*d*	52	74.3
H = 0.42	*d'*	10	14.3
	e	6	8.6
	a	2	2.8
Glu-B3	*h*	38	54.3
H = 0.66	*b*	9	12.9
	k	8	11.4
	b'	6	8.6
	h'	5	7.1
	i	4	5.7
Glu-B2			
H = 0.00	*B*	70	100.0
Gli-R2	d1 / *a*	49	70.0
H = 0.47	d2 / *b*	11	15.7
	t1 / c	8	11.4
	null / *d*	2	2.9

ni - unidentified allele

A significant polymorphism of the storage endosperm proteins was found in locus Glu-B1, where five allelic forms were identified. A similar trend has been found in other studies (Amiur et al., 2002b; Bellil et al., 2010). Alleles '*b*' (2.9 %), '*p*' (2.9 %) and '*s*' (1.4 %), coding for subunit pairs '7+8', '23+18' and '6.8+20y' were comparatively rare. Allele '*c* ' was identified more frequently (12.8 %). The heritability potential of locus Glu-B1 was concentrated in allele '*r*', coding for subunit pair '7+18' to the highest degree (80.0 %). This pair of subunits was first identified in the electrophoretic spectra of Portuguese triticale cultivars (Igrejas, 1999) and occurs only in the Glu-B1 locus of this cereal (Amiur et al., 2002b). The genetic variability in locus Glu-B1 was a under the average – H = 0.34 (Table 1).

In locus Glu-R1 subunit pair '6r+13r' coded for by allele Glu-R1*c*, was dominant (87.1 %). Allele Glu-R1*e* (6.5r), identified in six lines, was with lower frequency (8.6 %), followed by unidentified allele encoding fractional pair 2r+6r, which was found in two accessions (2.9 %). The Glu-R1*g* allele occurs with the lowest frequency (1.4 %). In this allelic composition, the calculated genetic variability in locus Glu-R1 was below the average - 0.23 (Table 1). The low value of the indicator in locus Glu-B1 and locus Glu-R1 is due to the concentration of the hereditary potential of the loci mainly in one allel - '*r*' in Glu-R1 and '*c*' in Glu-B1, which have been found to occur with a very high frequency in many European triticale cultivars (Amiur et al., 2002a; Amiur et al., 2002b; Bellil et al., 2010; Doneva and Stoyanov, 2019).

75K γ-secalins coded for by locus Gli-R2 were represented by four allelic variants – '*a*', '*b*', '*c*' and '*d*'. The most frequent allele Gli-R2*a* (d1) was found in 49 genotypes (70 %), and alleles Gli-R2*b* (d2) and Gli-R2*c* (t1) were identified in 11 (15.7 %) and 8 (11.4 %) accessions, respectively. A 'null' allelic variant (Gli-R2*d*) of 75K γ-secalins was identified in the electrophoretic spectra of two lines. The genetic variability in locus Gli-R2 was 0.47 (Table 1).

In the analyzed triticale collection, eleven low-molecular weight glutenin subunits (LMW-GS) were identified. The alleles GluA3*d* (74.3 %) and GluA3*d*' (14.3 %) were found in the Glu-A1 locus with the highest frequency in the analyzed genotypes. GluA3*e* allele occurs with a lower frequency (8.6 %), while allele GluA3*a* was registered in the spectrum of only one accession. In this allelic composition the index of genetic variability was 0.42 (Table 1).

In locus Glu-B3 the main part of the heritability potential of the analyzed collection was concentrated in allele Glu-B3h (54.3 %). Next in frequency were alleles Glu-B3b (12.9 %), Glu-B3k (11.4%). Alleles Glu-B3b', Glu-B3h' and Glu-B3i were with the lowest frequency. They were identified in six, five and four genotypes, respectively. In this locus the genetic variability was comparatively high – H = 0.66 (Table 1).

Locus Glu-B2 was characterized by extremely low polymorphism represented by one allelic variant - Glu-B2*b*. This determined the nil value of the parameter genetic variability (Table 1).

The identified alleles of the reserve endosperm proteins in loci Glu-1 (Glu-A1, Glu-B1), Glu-3 (Glu-A3, Glu-B3), Glu-B2 and Gli-R2 form 24 allelic configurations, which are presented in Table. 2.

The allelic composition of glutenins and gliadins is a determining factor for triticale quality. Glutenins encoded by chromosomes 1A and 1B have been shown to influence gluten quality characteristics much more than glutenins encoded by chromosome 1R. The alleles *'a'* (subunit 1) and *'b'* (subunit 2*) at the Glu-A1 locus found with high frequency in the present study have a high quality score and increase gluten quality (Todorov, 2006). The high molecular weight glutenin alleles encoded by the Glu-B1 locus also have a very strong effect. In our study, the allele *'r'*, encoding the fractional pair '7+18', was found with the highest frequency, followed by the allele *'c'*, encoding the pair of subunits '7+9'. The *'b'* (7+8) allele is less common. This allele, as well as the *'d'* allele (6+8), has been shown to have the strongest effect on gluten quality in triticale (Salmanowisz et al., 2013).

Tohver et al. (2005) found that the high molecular weight glutenin subunit 2* (chromosome 1A) and the fractional pairs 7+26 and 7+19 (chromosome 1B) represented the best combination of alleles in the amphiploid with a positive effect on gluten quality. Several authors prove that glutenins encoded by the Glu-R1 locus, inherited from rye, negatively affect the quality parameters, and the different combinations between HMW-GS/SS and LMW-GS/SS can significantly change the baking qualities of the different genotypes (Makarska et al., 2008; Belill et al., 2010; Camerlengo & Kiszonas , 2023). In triticale grain, the proportion of ω-gliadins is greater compared to α-, β- and ɣ-gliadins (Pruska-Kędzior et al., 2017). α- and β-gliadins are not involved in the formation of gluten (Salmanowicz and Novak, 2009) and, together with low molecular weight glutenins, have a weaker effect on the viscoelastic properties of triticale dough. The most significant influence on the formation of strong gluten in triticale is the absence of the D-genome. It has been proven that through introgression of high- and low-molecular glutenin alleles encoded by the 1D-chromosome, the quality of triticale lines and varieties is significantly increased (Lukaszewski, 2006; Martinek et al., 2008; Jonnala et al., 2010; Daskalova et al., 2021).

Table 2 Alleic configuration of storage proteins of triticale lines of CIMMYT origin

№	HMW			LMW			75K γ –sec
	Glu-A1 subunit/ allele	*Glu-B1* subunit/ allele	*Glu-R1* subunit/ allele	*Glu-A3* allele	*Glu-B3* allele	*Glu-B2* allele	*Gli-R2* subunit/ allele
1	2*/*b*	7+18/*r*	5.8^{r}/*g*	*a*	*h*	*b*	d1/*a*
2	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
3	2*/*b*	7+18/*r*	2^{r}+6^{r}/*c*	*d*	*b*	*b*	d1/*a*
4	Null/*c*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	d1/*c*
5	Null/*c*	7+9/*c*	6^{r}+13^{r}/*c*	*a*	*h*	*b*	d1/*c*
6	1/*a*	23+18/*p*	6.5^{r}/*e*	*e*	*i*	*b*	d2/*b*
7	2*/*b*	7+18/*r*	6r+13r/*c*	*d*	*i*	*b*	d1/*a*
8	1/*a*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*k*	*b*	d1/*a*
9	1/*a*	7+18/*r*	6.5r/*e*	*d'*	*i*	*b*	d1/*a*
10	Null/*c*	7+18/*r*	6^{r}+13^{r}/*c*	*d'*	*b*	*b*	t1/*c*
11	1/*a*	7+18/*r*	6^{r}+13^{r}/*c*	*e*	*i*	*b*	t1/*c*
12	N/*c*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h'*	*b*	t1/*c*
13	Null/*c*	7+8/*b*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
14	1/*a*	7+9/*c*	6.5r/*e*	*d'*	*b*	*b*	d1/*a*
15	1/*a*	7+18/*r*	6.5r/*e*	*d*	*h'*	*b*	t1/*c*
16	1/*a*	7+18/*r*	6.5^{r}/*e*	*d'*	*b*	*b*	d2/*b*
17	2*/*b*	7+9/*c*	6^{r}+13^{r}/*c*	*d'*	*k*	*b*	d2/*b*
18	1/*a*	7+9/*c*	6r+13r/*c*	*d'*	*b*	*b*	d2/*b*
19	1/*a*	7+9 /*c*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	d1/*a*
20	Null/*c*	7+18 /*r*	6^{r}+13^{r}/*c*	*e*	*k*	*b*	t1/*c*
21	1/*a*	7+18 /*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	d2/*b*
22	Null/*c*	7+18 /*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	d2/*b*
23	2*/*b*	7+8/*b*	6^{r}+13^{r}/*c*	*d*	*h'*	*b*	Null/*d*
24	Null/*c*	7+9/*c*	2^{r}+6.5^{r}/*b*	*e*	*k*	*b*	Null/*d*

The identified allelic combinations in the Mexican triticale lines, which are not found in the Bulgarian varieties selected in Dobrudzha Agricultural Institute – General Toshevo (Doneva and Stoyanov, 2019), combined with the high resistance to yellow rust and the promising productive potential under

the climatic conditions of Bulgaria, show that these samples can find application as new sources of genes in our selection program with the aim of improving the quality and expanding the possibilities of application of the cereals.

CONCLUSIONS

Based on the study, the established alleles at loci Glu-A1, Glu-B1, Glu-R1, Glu-A3, Glu-B3, Glu-B2, Gli-R2 form twenty-four configurations. The frequencies of Glu-A1*a* and Glu-A1*b* alleles, whith quality indicators, are relatively high. In the Glu-B1 locus, the proportion of Glu-B1*c* and Glu-B1*b* alleles that increase baking qualities is relatively low. A fractional pair 7+18, which is encoded by the Glu-B1*r* allele, was found in 56 % of the studied lines. Its influence on the final quality indicators has not yet been clarified. The identified alleles at loci Glu-R1 and Gli-R2 were inherited from rye. They have a negative effect on quality, but increase resistance to abiotic and biotic stress. The highest values of the index of genetic variation (H) were calculated at loci Glu-A1 and Glu-B3. At the Glu-B2 locus, the value of this indicator is zero.

The results obtained in the present study show that the triticale lines from CIMMYT have a high potential for quality and sustainability and are included in the triticale breeding program of the Dobrudzha Agricultural Institute.

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