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Composition of gluten proteins of hexaploid triticale varieties from different origin

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Abstract

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A collection of 15 triticale varieties (*Triticosecale*) grown in Bulgaria, 17 varieties grown in Poland, 1 variety grown in France and 3 variety grown in Germany were analyzed using sodium dodecilsuphate polyacrylamide gel electrophoresis (SDS-PAGE) to describe allelic variability in the storage proteins encoded at the *Glu-1* (*Glu-A1*, *Glu-B1* and *Glu-R1*), *Glu-3* (*Glu-A3* and *Glu-B3*), *Glu-B2* and *Gli-R2* loci. Thirty-two alleles were identified. These alleles formed 40 allelic configurations including 37 specific for one cultivar each. Two new allelic forms for high-molecular weight (HMW) – secalins were found in *Glu-R1* loci and one new allelic form of 75K γ-secalins (named "*new"*) was found in *Gli-R2* loci. 83% of the triticale cultivars were homogenous, only 17% showed two or more diagrams respectively in HMW glutenin subunits, low-molecular weight (LMW) glutenin subunits and 75K γ-secalins. Thus, the total number of genotypes in the study increased to fifty-four. Twenty-nine varieties possessed allele *b* and seven varieties commonly grown in Bulgaria possessed allele '*a*' in *Glu-A1* loci. These alleles are associated with good bread-making qualities. According to the data on the allelic composition, the cultivars were classified depending on their origin; it was found out that all analyzed accessions from Poland, Germany and France, as well as those developed in Bulgaria, fell within the same group, namely the group of triticale varieties of winter type developed through European germ plasma.

Keywords: Triticosecale; storage proteins; SDS-PAGE; genetic variability; cultivars

Introduction

Evolutionally, triticale is a "young" crop (Salmanowicz et al., 2013; Sokol, 2014; Doneva et al., 2019; Daskalova et al., 2020). The first cultivars were introduced in production over 35 years ago. The areas sown with triticale worldwide are currently exceeding 3 million ha and are constantly increasing (Kandrokov et al., 2019; Mergoum et al., 2019). The on-going interest towards the crop is due to the high productivity potential and grain quality inherited from wheat, and the higher resistance to biotic and abiotic stress due to the rye genome (Randhawa, H. S, Bona L. & Graf, R., 2015). Synthetic amphiploids of the triticale type are increasingly more often being used in the contemporary breeding programs also as a genetic source for improvement of wheat through transfer of suitable genes inherited from rye (Hohmann, U., 1988; Sokol, 2014; Daskalova et al., 2020).

The amino-acid composition of triticale is characterized by a much higher content of glutamine acid, proline and lysine in comparison to common wheat. This allows using the crop as a source of animal food with improved biological value of protein. Furthermore, the green mass of triticale is more valuable than that of wheat and rye because it contains more digestible protein, and the flour from it is richer in carotenoids and mineral substances important for the nutrition

regime of the animals (Varughese et al., 1996; Sokol, 2014; Stoyanov, 2018; Doneva et al., 2019).

In the recent years, triticale was also found to possess high potential as an energy crop. In this relation, it is widely used as a source of biomass for production of bioethanol (Bazhenov et al., 2015; Niedziela et al., 2016).

The researches have shown that triticale has a genetic composition much similar to that of its parental components (wheat and rye) in the chromosomes of first and third homologous group, where the main loci coding for the storage proteins of this crop have been localized (Brzezinski, W. & Lukaszewski, A. J. (1998); Makarska, E., Ciołek, A. & Kociuba, W., 2008;).

In wheat and rye, the genes, coding for the high molecular weight glutenins and the high molecular weight secalins are localized in loci on the long arms of chromosomes 1A (*Glu-A1*) (Lawrence & Shepherd, 1980), 1B (*Glu-B1*) (Bietz et al., 1975), 1D (*Glu-D1*) (Orth & Bushuk, 1974), 1R (*Glu-R1* ore *Sec-3*) (Lawrence & Shepherd, 1981). In wheat, the gliadins are positioned on the short arms of chromosomes 1 and 6. They are coded for by genes localized in loci *Gli-1* (*Gli-A1*, *Gli-B1*, *Gli-D1*) and *Gli-2* (*Gli-A2*, *Gli-B2*, *Gli-D2*). In rye, one ω-secalin and two 40K γ-secalins are coded for by genes in locus *Gli-R1*(or *Sec-1*) (Shepherd, 1986), localized on the short arm of chromosome 1R, two ω-secalins are coded for by genes in locus *Gli-R3* (or *Sec-4*) (Carrillo et al., 1992), in chromosome 1RS, and the 75K γ-secalins are coded for by genes in locus *Gli-R2* (or Sec-2), localized in chromosome 2RS (Shewry et al., 1984). The low-molecular weight glutenins (LMW) both in wheat and rye are coded for by genes in locus *Glu-3*, which is closely related to locus *Gli-1* (Jackson et al., 1983).

Some of the first studies on the allele variation of storage proteins in triticale were carried out by Igrejas et al. (1999). He analyzed a collection of 14 Portuguese cultivars and established significant genetic variability in loci *Glu-1*, *Gli-1*, *Glu-3*, *Glu-B2* and *Gli-R2*, as well as differences in the intensity of some bands as compared to the electrophoretic spectrum of wheat. Later, over 130 European genotypes of the amphidiploid were investigated and new allele forms were identified in *Glu-1*, *Glu-3* and *Gli-1*, which coded high-molecular weight and low-molecular weight glutenin and secalin subunits. Amiour et al. (2002a) and Amiour et al. (2002b) characterized three subunits: '2r+9r', '2r+6.5r' and '6.5r', coded by alleles '*b*', '*d*' and '*e*', respectively, in locus *Glu-R1* and identified four alleles (‚*a*', ‚*b*', ‚*c*' and ‚*d*') in locus *Gli-R2* in the spectrum of secondary hexaplod triticale cultivars developed in Europe. Allelic variation in locus *Glu-R1* was also found by Bellil et al. (2010) in combination with the triplet 't1', coded by allele *Gli-R2c*, which was typical for 80.3% of the analyzed cultivars. Based on the data for the allelic frequencies in the glutenin and secalin loci of the triticale varieties developed in France and using the results of Amiour et al., (2002b) on cultivars developed in other countries, Bellil et al. (2010) applied cluster analysis and calculated the genetic distances between the separate groups of accessions depending on their origin.

The bread-making properties of the French triticale cultivars were analyzed (Bellil et al., 2010) and a comparison was made between the allelic frequencies in loci *Glu-A1* and *Glu-B1* to common and durum wheat cultivars from the world collection. The composition of the glutenin proteins and the qualitative parameters of winter triticale hybrids developed in Poland were studied (Makarska et al., 2008).

The results from the two investigations showed that the low gluten, the absence of D-genome and the presence of R-genome, as well as the high level of α-amylase influenced negatively the bread-making parameters of the synthetic amphidiploid regardless of the presence of alleles with high quality score in loci *Glu-A1* and *Glu-B1* (*Glu-A1a*, *Glu-A1b* and *Glu-B1b*).

The aim of this study was by using the polymorphism of the storage endosperm proteins in loci *Glu-1* (*Glu-A1, Glu-B1*, *Glu-R1*), *Gli-2* (*Gli-R2*) and *Glu-3* (*Glu-A3*, *Glu-B3*, *Glu-B2*) to identify, compare and classify by groups the different hexaploid triticale genotypes according to their origin.

Materials and Methods

A collection of seventeen hexaploid triticale varieties breeding of DANKO – Poland, three accessions received from IPK-Gatersleben, Germany, 1 variety from France and fifteen Bulgarian accessions, including eleven cultivars developed at Dobrudzha Agricultural Institute, 1 cultivar developed at the Institute of Plant and Genetic resources – Sadovo and one of Breeding House – Sadovo, which were included in the official varietal list of Republic of Bulgaria, as well as two new candidate-varieties, were analyzed (Table 1).

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

Table 1. Hexaploid triticale cultivars – origin

Cultivar	Origin
Doni 52	DAI – General Toshevo, Bulgaria
Dobrudzhanets	DAI – General Toshevo, Bulgaria
Blagovest	DAI – General Toshevo, Bulgaria
Borislav	DAI – General Toshevo, Bulgaria
Kolorit	DAI – General Toshevo, Bulgaria
Bumerang	DAI – General Toshevo, Bulgaria
Respekt	DAI – General Toshevo, Bulgaria
Akord	DAI – General Toshevo, Bulgaria
Atila	DAI – General Toshevo, Bulgaria
Lovchanets	DAI – General Toshevo, Bulgaria
Irnik	DAI – General Toshevo, Bulgaria
215/05-76	DAI – General Toshevo, Bulgaria
52/05-56	DAI – General Toshevo, Bulgaria
Rozhen	IPGR – Sadovo, Bulgaria
Musala	Breeding house – Sadovo, Bulgaria
Salto	DANKO, Poland
Twingo	DANKO, Poland
Fredro	DANKO, Poland
Remiko	DANKO, Poland
Subito	DANKO, Poland
Toledo	DANKO, Poland
Rotondo	DANKO, Poland
Palermo	DANKO, Poland
Silverado	DANKO, Poland
Kasyno	DANKO, Poland
Maestozo	DANKO, Poland
Avokado	DANKO, Poland
Alekto	DANKO, Poland
Trismart	DANKO, Poland
Algoso	DANKO, Poland
Berenico	DANKO, Poland
Trapero	DANKO, Poland
CS Tris	France
T-4-2	Germany
T-3-4	Germany
T-1-1	Germany

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 min, 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 35% separation gel by the method of Payne et al. (1980). By the method of Laemmli (1970), the electrophoresis occurred at constant current of 20 mA 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 & 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 & 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).

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 2, Figure 1, Figure 2, Figure 3).

Nine of the 36 analyzed accessions were heterogeneous (25%). In the candidate-variety 52/05-56 and in cultivars Blagovest, Lovchanets, Fredro and CSTris, there was heterogeneity in the high-weight molecular glutenin subunits. Accession 215/05-76 was characterized by a heterogeneity spectrum both in the high-molecular weight glutenins and in 75K γ-secalins. In cultivars Irnik, Kasyno and Alekto, heterogeneity was registered in the high-molecular weight and the low-molecular weight glutenins, and in the 75K γ-secalins. The nine biotypes identified in cultivar Irnik are an evidence of its high degree of heterogeneity. It is explained by the insufficient purity of the cultivar, which could not be well determined only by the test performed prior to the registration of the variety. The reason is that this test is based on three criteria: distinctness, homogeneity and stability, determined primarily on the basis of morphological traits. Furthermore, although low (about 20%), allogamy in triticale is yet another reason for heterogeneity. Therefore, the use

Table 2. Composition of high-molecular weight glutenin subunits (HMW-GS), HMW-secalins, low-molecular weight glutenin subunits (LMW-GS) and 75K γ – secalins in cultivars and lines of hexaploid triticale

Cultivar	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
Doni 52	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
Dobrudzhanets	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
Blagovest-1	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
Blagovest-2	N/*c*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
Blagovest-3	1/*a*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
Borislav	N/*c*	7+9/*c*	6^{r}+13^{r}/*c*	*d'*	*b'*	*b*	d2/*b*
Kolorit	1/*a*	7+18/*r*	6.5^{r}/*e*	*d*	*i*	*b*	t1/*c*
Bumerang	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*k*	*b*	t1/*c*
Respekt	1/*a*	7+18/*r*	6^{r}+13^{r}/*c*	*d'*	*i*	*b*	d1/*a*
Akord	2*/*b*	6+8/*d*	6^{r}+13^{r}/*c*	*d'*	*d*	*b*	new/*ni*
Atila	1/*a*	7+9/*c*	6^{r}+13^{r}/*c*	*d'*	*i*	*b*	d1/*a*
Lovchanets-1	2*/*a*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
Lovchanets-2	1/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
Irnik-1	N/*c*	7+18/*r*	6^{r}+13^{r}/*c*	*d'*	*b*	*b*	new/*ni*
Irnik-2	1/*a*	7+18/*r*	6.5^{r}/*e*	*d*	*h*	*b*	t1/*c*
Irnik-3	N/*c*	7+18/*r*	6.5^{r}/*e*	*d'*	*b*	*b*	t1/*c*
Irnik-4	N/*c*	7+18/*r*	6^{r}+13^{r}/*c*	*d'*	*b*	*b*	d1/*a*
Irnik-5	1/*a*	7+18/*r*	6.5^{r}/*e*	*d'*	*b*	*b*	t1/*c*
Irnik-6	N/*c*	7+18 /*r*	6^{r}+13^{r}/*c*	*d'*	*h*	*b*	t1/*c*
Irnik-7	N/*c*	7+18 /*r*	6^{r}+13^{r}/*c*	*d'*	*h*	*b*	d1/*a*
Irnik-8	N/*c*	7+18 /*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	d1/*a*
Irnik-9	N/*c*	7+18 /*r*	6^{r}+13^{r}/*c*	*d*	*h*	*b*	t1/*c*
215/05-76/1	1/*a*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h'*	*b*	d1/*a*
215/05-76/2	N/*c*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*h'*	*b*	d1/*a*
215/05-76/3	N/*c*	6+8/*d*	6^{r}+13^{r}/*c*	*d*	*h'*	*b*	t1/*c*
52/05-56/1	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*b*	*b*	t1/*c*
52/05-56/2	1/*a*	7+8/*b*	6^{r}+13^{r}/*c*	*d*	*b*	*b*	t1/*c*
Rozhen/Sadovo	N/*c*	23+18/*ni*	6^{r}+13^{r}/*c*	*d*	*b'*	*b*	t1/*c*
Musala/Sadovo Seed house	N/*c*	23+18/*ni*	6^{r}+13^{r}/*c*	*d*	*b*	*b*	t1/*c*
Salto	2*/*b*	7+9/*c*	6^{r}+13^{r}/*c*	*d*	*k*	*b*	d2/*b*
Twingo	N/*c*	6+8/*d*	6.5*^{r}+13^{r}/*ni*	*e*	*k*	*b*	t1/*c*
Fredro/1	2*/*b*	7+8/*b*	6^{r}+13^{r}/*c*	*d*	*k*	*b*	t1/*c*
Fredro/2	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*k*	*b*	t1/*c*
Remiko	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d*	*k*	*b*	t1/*c*
Subito	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*e*	*k*	*b*	t1/*c*
Toledo	2*/*b*	7+18/*r*	6r+13r/*c*	*d*	*k*	*b*	t1/*c*
Rotondo	2*/*b*	7+18/*r*	6r+13r/*c*	*e*	*k*	*b*	t1/*c*
Palermo	2*/*b*	6.8+20y/*s*	2^{r}+9^{r}/*d*	*d*	*i*	*b*	t1/*c*
Silverado	2*/*b*	7+18/*r*	6^{r}+13^{r}/*c*	*d'*	*k*	*b*	d2/*b*
Kasyno/1	2*/*b*	7+18/*r*	5.8^{r}/*g*	*e*	*k*	*b*	d2/*b*
Kasyno/2	2*/*b*	7+18/*r*	5.8^{r}/*g*	*d*	*k*	*b*	d2/*b*
Maestozo	2*/*b*	7+18/*r*	5.8^{r}/*g*	*d*	*k*	*b*	d2/*b*
Avokado	2*/*b*	7+8/*b*	6^{r}+13^{r}/*c*	*e*	*b'*	*b*	t1/*c*

Alekto/1	N/*c*	7+18/*r*	6^r+13^r/*c*	*e*	*i*	*b*	t1/*c*
Alekto/2	2*/*b*	7+8/*b*	6^r+13^r/*c*	*e*	*b'*	*b*	t1/*c*
Trismart	2*/*b*	7+18/*r*	2^r+9^r/*d*	*a*	*k*	*b*	t1/*c*
Algoso	N/*c*	6+8/*d*	5.8*^{r}+13^r/*ni*	*e*	*b'*	*b*	t1/*c*
Berenico	2*/*b*	7+8/*b*	6^r+13^r/*c*	*d*	*h*	*b*	t1/*c*
Trapero	2*/*b*	7+18/*r*	2^r+6^r/*ni*	*d*	*h*	*b*	t1/*c*
CS Tris/1	2*/*b*	7+18/*r*	6^r+13^r/*c*	*d*	*k*	*b*	t1/*c*
CS Tris/2	2*/*b*	7+8/*b*	2^r+9^r/*d*	*d*	*k*	*b*	t1/*c*
T-4-2	2*/*b*	7+18/*r*	2^r+9^r/*d*	*d*	*h*	*b*	d1/*a*
T-3-4	2*/*b*	7+18/*r*	2^r+9^r/*d*	*d*	*h*	*b*	d1/*a*
T-1-1	2*/*b*	7+18/*r*	2^r+9^r/*d*	*d*	*h*	*b*	d1/*a*

ni –unidentified allele

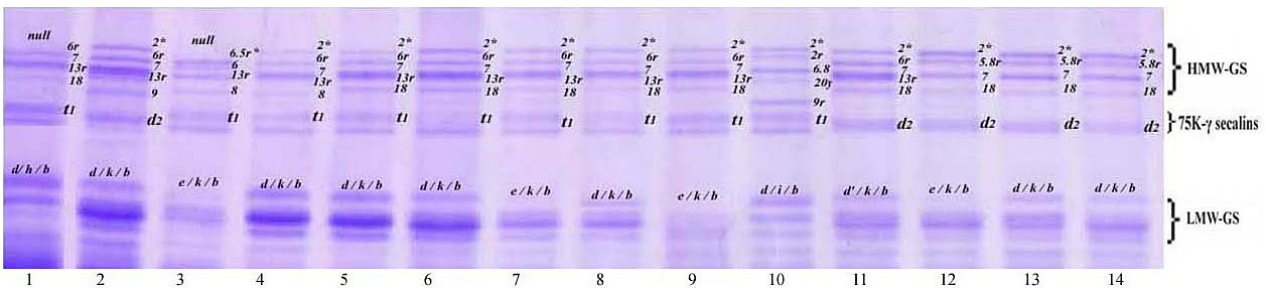

Fig. 1. Allelic composition of storage proteins (SDS-PAGE) – 1. Rakita (st), 2. Salto, 3. Twingo, 4. Fredro/1, 5. Fredro/2, 6. Remiko, 7. Subito, 8. Toledo, 9. Rotondo, 10. Palermo, 11. Silverado, 12. Kasyno/1, 13. Kasyno/2, 14. Maestozo

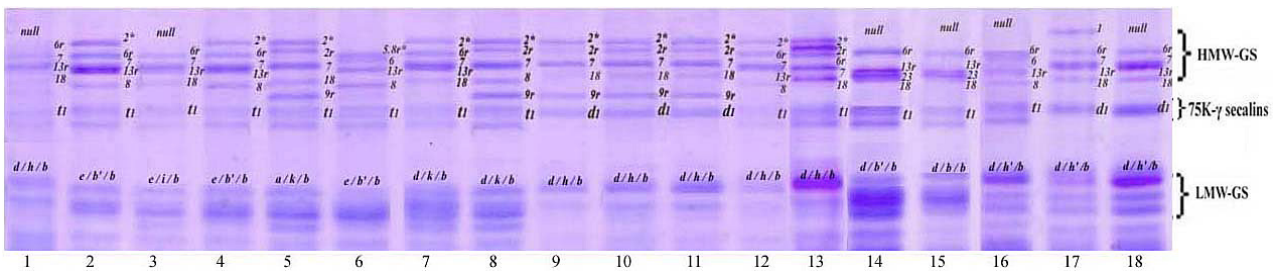

Fig. 2. Allelic composition of storage proteins (SDS-PAGE) – 1. Rakita (st), 2. Avokado, 3. Alekto/1, 4. Alekto/2, 5. Trismart, 6. Algoso, 7. CS Tris/1, 8. CS Tris/2, 9. T-4-2, 10.T-3-4, 11. T-1-1, 12. Berenico, 13. Trapero, 14. Rozhen, 15. Musala, 16. 215/05-76/1, 17. 215/05-76/2, 18. 215/05-76/3

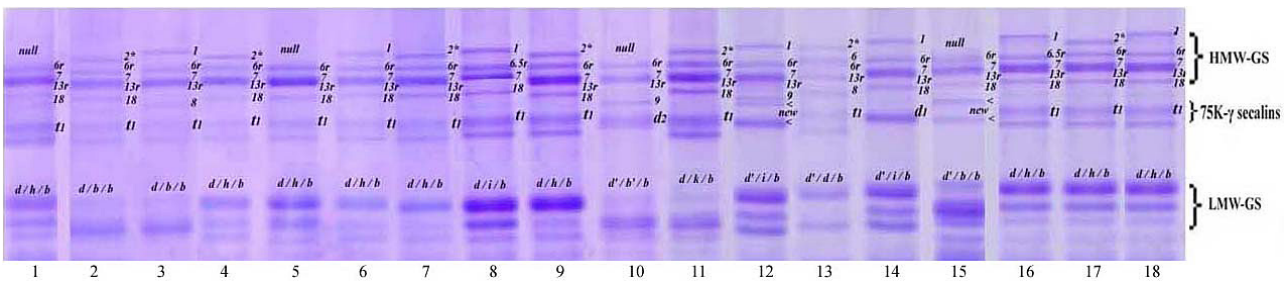

Fig. 3. Allelic composition of storage proteins (SDS-PAGE) – 1. Rakita (st), 2. 52/05-56/1, 3. 52/05-56/2, 4. Blagovest/1, 5. Blagovest/2, 6. Blagovest/3, 7. Doni 52, 8. Kolorit, 9. Dobrudzhanets, 10. Borislav, 11. Bumerang, 12. Atila, 13. Akord, 14. Respekt, 15. Irnik/1, 16. Irnik/3, 17. Lovchanets/1, 18. Lovchanets/2

of electrophoresis as a method for control of the purity of a given variety is required in the early stages of its breeding.

As a result of the SDS-PAGE electrophoretic analysis, a total of 32 alleles were identified, 16 of which in loci coding for the high-molecular weight (HMW) glutenin subunits, 12 in the loci coding for the low-molecular weight (LMW) glutenin subunits and 4 in locus *Gli-R2*, coding for 75K γ-secalins.

Two new allelic forms of HMW-secalins were identified in locus *Glu-R1*, coding for subunit pairs '6.5*r+13r' (cultivar Twingo) (Figure 1, line 3) and '5.8*r+13r' (cultivar Algoso) (Figure 2, line 6), respectively. The '*new*' allele in locus *Gli-R2,* coding for 75K γ-secalins was expressed in the electrophoretic spectra of cultivar Atila and in one of the biotypes of cultivar Irnik, respectively (Figure 3, lines 12 and 15). This allele was found for the first time in a research of Doneva et al. (2019).

Based on the genetic variation in the seven loci, 40 different allelic configurations formed, 37 of which were specific and typical only for one cultivar (Table 2). As a result from the heterogeneity determined in nine cultivars – Blagovest (3 biotypes), Lovchanets (2 biotypes), Irnik (2 biotypes), Fredro (2 biotypes), 215/05-76 (3 biotypes), 52/05-56 (2 biotypes), Kasyno (2 biotypes), Alekto (2 biotypes), CS Tris (2 biotypes), the total number of the genotypes analyzed in tis study reached 54 (Table 2, Table 3).

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 '*c*' was related to nil synthesis of protein, which determined low bread-making properties, while allele '*b*' and especially allele '*a*' were markers of good and high gluten quality in common winter wheat (Todorov, 2006). Allele '*b*' was with the highest frequency– 53.7%, followed by allele '*c*' (29.6%). The lowest frequency was that of allele '*a*' – 16.7%. The genetic variability in this locus was above the average – H = 0.60 (Table 3).

In locus *Glu-B1*, alleles '*c*' (5.5%), '*d*' (7.4%), '*ni*' (3.7%) and '*s*' (1.9%), coding for subunit pairs '7+9', '6+8', '23+18' and '6.8+20y' were comparatively rare. Higher was the frequency of allele *GluB1b,* coding for subunit pair 7+8 (13%). The heritability potential of locus *Glu-B1* was concentrated in allele '*r*', coding for subunit pair '7+18' to the highest degree (70.4%). Therefore, the genetic variability was a little under the average – H = 0.48 (Table 2). In locus *Glu-R1* subunit pair '6r+13r' coded for by allele *Glu-R1c*, was dominant (70.3%). Allele *Glu-R1d* (2r+9r), identified in six cultivars, was with lower frequency (11.1%), followed by allele *Glu-R1e* (6.5r), which was found in four accessions (7.4%). Allele *Glu-R1g* (5.8r) was registered only in three biotypes. Subunit pair '2r+6r' was identified only in the spectrum of cultivar Trapero. Worth mentioning are the two new subunit pairs identified in the *Glu-R1* locus of cultivars Twingo and Algoso. They consisted of two subunits – x and y type. The electrophoretic mobility of the y-type subunits coincided with that of subunit 13r, while the x-type subunit in the electrophoretic spectrum of cultivar Twingo was expressed at the level of y-type subunit '6.5r', and in the spectrum of cultivar Algoso it had the same electrophoretic mobility as y-type subunit '5.8r'. Probably this is an instance of x-type subunits with lower molecular weight unidentified up to now, which, together with the y-type subunit '13r' formed new allelic forms designated in this study as '6.5*r+13r' and '5.8*r+13r' (Fig. 1, Fig. 2). In this allelic composition, the calculated genetic variability in locus *Glu-R1* was below the average – 0.48 (Table 3).

Table 3. Frequency of alleles and genetic variation in triticale varieties

Locus	Subunit/Allele	Number of biotypes	Frequency, %
Glu-A1	*c*	16	29.6
H = 0.60	*a*	9	16.7
	b	29	53.7
Glu-B1	*r*	38	70.4
H = 0.48	*c*	3	5.5
	d	4	7.4
	b	6	11.1
	s	1	1.9
	23+18/*ni*	2	3.7
Glu-R1	*c*	38	70.3
H = 0.48	*e*	4	7.4
	d	6	11.1
	g	3	5.5
	$5.8^{*r}+13^{r}$/*ni*	1	1.9
	$6.5^{r}+13^{r}$/*ni*	1	1.9
	$2^{r}+6^{r}$/*ni*	1	1.9
Glu-A3	*d*	34	63.0
H = 0.54	*d'*	11	20.4
	e	8	14.8
	a	1	1.8
Glu-B3	*h*	17	31.5
H = 0.76	*b*	7	13.0
	i	5	9.2
	k	16	29.6
	d	1	1.8
	b'	5	9.3
	h'	3	5.6
Glu-B2			
H = 0.00	*b*	54	100.0
Gli-R2	*c*	37	68.5
H = 0.49	*a*	9	16.7
	b	6	11.1
	new/*ni*	2	3.7

ni – unidentified allele

75K γ-secalins coded for by locus *Gli-R2* were represented by four allelic variants –'*a'*, '*b'*, '*c'* and '*new'*. The most frequent allele *Gli-R2c* (t1) was found in 37 genotypes, and alleles *Gli-R2b* (d2) and *Gli-R2a* (d1) were identified in 9 and 6 accessions, respectively. A new allelic variant of 75K γ-secalins was identified in the electrophoretic spectra of two cultivars – Akord and Irnik. This allele, designated as '*new*', consisted of two subunits, indicated by arrows in Figure 1. They differed by molecular weight and electrophoretic mobility in comparison to the bands, forming the known subunit pairs 'd_1', 'd_2' and the triplet 't_1'. The genetic variability in locus *Gli-R2* was 0.49 (Table 3).

In the analyzed triticale collection, nine low-molecular weight glutenin subunits were identified. For this purpose, the nomenclature of common wheat introduced by Gupta and Shepherd (1990) and Jackson et al. (1996) were used.

GluA3d (63%) and *GluA3d'* (20.4%) occurred with the highest frequency in the investigated biotypes. A comparatively small part of the heritability potential in locus *GluA3* was formed by allele *GluA3e* (14.8%), while allele *GluA3a* was registered in the spectrum of only one accession. In this allelic composition, the index of genetic variability was 0.60 (Table 3).

In locus *Glu-B3* the main part of the heritability potential of the analyzed collection was concentrated in alleles *Glu-B3h* (31.5%) and *Glu-B3k* (29.6%). Next in frequency were alleles *Glu-B3b* (13%), *Glu-B3b'* (9.3%) and *Glu-B3i* (9.3%). Alleles *Glu-B3h'* and *Glu-B3d* were with the lowest frequency – they were identified in three lines (215/05-76/1, 215/05-76/2, 215/05-76/3) and one genotype (Akord), respectively. In this locus the genetic variability was comparatively high – H = 0.79 (Table 3).

Locus *Glu-B2* was characterized by extremely low polymorphism represented by one allelic variant – *Glu-B2b.* This determined the nil value of the parameter genetic variability (Table 3).

The data obtained on the frequencies of the alleles coded for by loci *Glu-A1*, *Glu-B1*, *Glu-R1*, *Gli-R2*, *Glu-A3*, *Glu-B3* and *Glu-B2* on the Bulgarian triticale cultivars and the cultivars with origin from other European countries (Poland, Germany and France) allow doing certain grouping of the separate accessions according to their origin (Table 4).

The allelic variation determined in locus *Glu-A1* showed that the genotypes could be divided into two main groups. Group '1' was characterized by allele *GluA1b*, coding for subunit '2*', which was typical for the analyzed cultivars from France, Germany and Poland (85 – 100%). The frequency of this allele in the Bulgarian cultivars was lower – 24.2%. Group '2' included the other two allelic forms – *Glu-A1c* and *Glu-A1a.* Allele '*c*', which is related to nil synthesis of protein, was identified in 44.8% of the Bulgarian genotypes and in 15% of the Polish ones. It was not present in the cultivars with origin from France and Germany. Allele *Glu-A1a*, coding for subunit 1, was typical only for the cultivars from Bulgaria (31%).

The allelic variation in locus *Glu-B1* showed that the cultivars could be divided into three groups. Group '1' was characterized by allele *Glu-B1r* (7+18), which occurred with high frequency in all cultivars regardless of their origin. It was most typical for the German (100%) and Bulgarian (76%) cultivars, and in the varieties with origin from Poland and France its frequency was 60% and 50%, respectively. In group '2' the dominant allele was '*c*', coding for subunit pair '7+9'. It was most typical for the French triticale cultivars (50%). The alleles, which fell within group '3' were '*IV'* (23+18),'s' (6.8+20y), '*d*' (6+8) and '*b*' (7+8). They showed certain specificity depending on the origin of the accessions. Thus, for example, alleles'd' and '*b*' were identified both in Polish and Bulgarian cultivars, but were not typical for varieties with origin from France and Germany. Allele '*IV'* was registered only in Bulgarian breeding materials (6.9%), while's' was identified in a single accession from Poland.

In locus *Glu-R1*, the obtained results on the allelic frequencies showed that group '1' was characterized by allele *Glu-R1c*, coding for fraction pair 6r+13r. It was registered mainly in Bulgarian (86.2%), Polish (60%) and French (50%) cultivars. In group '2', dominant was allele'd', which was identified in all cultivars with origin from Germany, in 50% of the French cultivars and in 10% of the Polish accessions. All other allelic forms fell within group '3', the dominant alleles being '*g'*, determined in 15% of the Polish cultivars, and allele '*e*', identified in some accessions from Bulgaria (13.8%).

Based on the allelic variation in locus *Glu-A3*, two main groups were outlined. Group '1' encompassed a large number of cultivars from all four countries, which possessed allele *Glu-A3d*. 34.5% of the Bulgarian and 5% of the Polish accessions were referred to group '2', where the main allele was *Glu-A3d'*. The other two alleles, *Glu-A3e* and *Glu-A3a*, were typical only for cultivars of Polish breeding.

The allelic variation in locus *Glu-B3* divided the genotypes into two main groups.

In group '1', alleles *Glu-B3h* and *Glu-B3κ* were dominant. Allele '*h*' was typical for the cultivars of Bulgarian breeding (41.4%). It was also identified in all three cultivars from Germany and in 10% of the Polish accessions. Allele '*k*' was registered with high frequency (65%) in the Polish genotypes and in cultivars Bumerang (Bulgaria) and CS Tris (France). In group '2', dominant were alleles *Glu-B3i* and *Glu-B3b'*, which were identified only in accessions with or-

Table 4. Allelic frequencies per locus per country where triticale cultivars originated

Locus	Origin and number of cultivars				
Allele/subunit	Total 54	BG 29	POL 20	FR 2	GE 3
Glu– A1					
c (null)	29.6	44.8	15.0	–	–
a (1)	16.7	31.0	–	–	–
b (2*)	53.7	24.2	85.0	100.0	100.0
Glu–B1					
r (7+18)	70.4	76.0	60.0	50.0	100.0
c (7+9)	5.5	10.3	5.0	50.0	–
d (6+8)	7.4	3.4	5.0	–	–
b (7+8)	11.1	3.4	25.0	–	–
s (6.8+20y)	1.9	–	5.0	–	–
IV (23+18)	3.7	6.9	–	–	–
Glu–R1					
c(6^r+13^r)	70.3	86.2	60.0	50.0	–
e (6.5^r)	7.4	13.8	–	–	–
d (2^r+9^r)	11.1	–	10.0	50.0	100.0
g (5.8^r)	5.5	–	15.0	–	–
ni (5.8^{*r}+13^r)	1.9	–	5.0	–	–
ni (6.5^{*r}+13^r)	1.9	–	5.0	–	–
ni (2^r+6^r)	1.9	–	5.0	–	–
Glu–A3					
d	63.0	65.5	50.0	100.0	100.0
d’	20.4	34.5	5.0	–	–
e	14.8	–	40.0	–	–
a	1.8	–	5.0	–	–
Glu–B3					
h	31.5	41.4	10.0	–	100.0
b	13.0	24.1	–	–	–
i	9.2	10.3	10.0	–	–
k	29.6	3.5	65.0	100.0	–
d	1.8	3.5	–	–	–
b’	9.3	6.9	15.0	–	–
h’	5.6	10.3	–	–	–
Glu–B2					
b	100.0	100.0	100.0	100.0	100.0
Gli–R2					
c (t1)	68.5	69.0	75.0	100.0	–
a (d1)	17.6	20.7	–	–	100.0
b (d2)	11.1	3.4	25.0	–	–
ni (new)	3.7	6.9	–	–	–

igin from Bulgaria and Poland. The other two alleles – *Glu-B3b* и *Glu-B3h*' were specific for our own breeding.

In locus *Glu-B2*, all cultivars, regardless of their origin, possessed allele *Glu-B2b*.

The allelic variation in locus *Gli-R2* divided the cultivars into two main groups. In group '1', predominant was allele *Gli-R2c*, which was identified in 75% of the Polish, 69% of the Bulgarian genotypes and in cultivars with origin from France. Next in frequency in this group was allele *Gli-R2a*, which was typical of the analyzed cultivars from Germany and for 20.7 % of the Bulgarian accessions. In group '2', the dominant allele was *Gli-R2b*, which was registered in 25% of the Polish cultivars and in 3.4% of the Bulgarian ones. Allele '*new*' was identified only in two Bulgarian cultivars – Akord and Irnik.

This research showed that some of the alleles identified in the investigated loci occurred only in individual cultivars and lines from a certain country; thus, for example, alleles *GluA1a*, *Glu-B1IV*, *Glu-R1e* and *Gli-R2 new* were typical of the Bulgarian breeding; allele '*g*' and subunit pairs '2r+6r', '5.8*r+13r' and '6.5*r+13r' from *Glu-R1* occurred only in the French amphiploids. Nevertheless, the investigation carried out revealed that all analyzed accessions from Bulgaria, Poland, Germany and France had the same allelic composition in loci *Glu-A1*, *Glu-B1*, *Glu-A3* и *Glu-B2* regardless of their origin.

The identified alleles *Glu-A1b*, *Glu-B1r*, *Glu-A3d* and *Glu-B2b* occurred with high frequency in the genotypes from all four breeding centers. These results largely support the classifications of Bellil et al. (2010) and Amiour et al. (2002b) based on the comparison of triticale cultivars from different origin. The authors cited above divided the amphiploids into two large group according to the country they were developed in.

Group '1' included accessions from Great Britain, Poland, Germany, Sweden and France, which are mainly of winter type and were developed from European embryo plasma. The results from our electrophoretic analysis showed that genotypes from Bulgaria, too, could be added to this group. Bellil et al. (2010) and Amiour et al. (2002b) referred the accessions to group '2', dividing them into two subgroups: cultivars from Spain, which were bred on the basis of tetraploid wheat from Iberia (Sanchez-Monge, 1996), and spring type cultivars developed from germ plasma from CIMMYT, which were two types – with genome from rye or with *2D/2R* translocation.

The obtained results on the variation of the investigated cultivars and accessions according to their electrophoretic spectrum explicitly demonstrated the presence of significant genetic variability. However, this variability is strict and specifically grouped according to the country of origin. This indicates that the triticale breeding programs have a comparatively narrow genetic basis, which is a major problem for the crop, as a whole. Nevertheless, the detailed information this study provided allowed selecting those genotypes, which have perspective potential depending on the specific breeding goals. This would further allow combining the alleles, which are valuable for the practice, thus facilitating the occurrence of new combinations significant for the improvement work on triticale.

Conclusions

The storage endosperm proteins are characterized by high polymorphism regardless of the climatic conditions and the ontogenetic stage of the plant development. This allows using them for determining of the genetic variation, and identifying and differentiating the variability of the genotypes.

The electrophoretic method, based on the storage endosperm proteins, is comparatively cheap and fast. The identification of the electrophoregrams provides information on a number of genetic, biochemical and technological properties of the storage endosperm proteins, which is related to the identity of the cultivars, similar to a "fingerprint".

In this study, the reproduction of the electrophoresis and the good separation of the storage endosperm proteins on polyacrylamide gel was utilized to identify and compare different genotypes of hexaploid triticale. According to the data on the allelic composition, the cultivars were classified depending on their origin; it was found out that all analyzed accessions from Poland, Germany and France, as well as those developed in Bulgaria, fell within the same group, namely the group of triticale varieties of winter type developed through European germ plasma.

In parallel, the fractionation and visualization of the storage proteins allowed identifying two new allelic forms, which coded for subunit pairs '5.8*r+13r' and '6.5*r+13r' in locus *Glu-R1*, for which information from similar researches was not found. Evidence was provided for the expression of an allele identified in our previous research and designated as *'new'* in locus *Gli-R2* in the spectra of cultivars Atila and Irnik.

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