Characterization of Triticale (× *Triticosecale* Wittmack) Accessions and Reciprocal Hybrids Possessing Wheat 1D Chromosome

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Summary

The aim of this study was to evaluate the genetic diversity of five triticale accessions and to perform reciprocal crosses involving wheat 1D chromosome in early generations. Morphological, cytological and seed protein analysis was applied in order to select the best individuals among initial triticale strains. Individual selection for spike traits was performed followed by chromosome examination and seed protein analysis. One hexaploid line (2n=42) and an octoploid strain (2n=56) with the D genome from *Triticum aestivum* L. cv. Mironovskaya 808, were the most distant among all triticale studied. They differed from each other on almost all traits analyzed, including storage proteins encoded at the loci of four genomes. Cytologically fixed plants of the two lines were involved in reciprocal crosses. Selected individuals in F_2 generation, containing 1D chromosome through the presence of the subunit pair 5+10, were selfed and measured for six traits. According to HMW glutenins and secalins, F_3 seeds were divided in eleven genotypes, among which G2 (2*-2r+6.5r-7+9-5+10-d2) and G5 (1-2r+6.5r-7+9/9r-5+10-d2) were the most frequently distributed, 14.3% and 11.4%, respectively. Some of them expressed structural changes marked by combining alleles in A- and B-genomes and *d1* and *d2* in *Gli-R2* loci. Our data suggested that selection in crosses between 6x and 8x triticales appeared as an effective approach for transferring 1D chromosome of a particular wheat variety into hexaploid triticale in association with diverse glutenin and secalin compositions.

Key words

triticale (6x and 8x), glutenins, secalins, wheat 1D chromosome, F_1-F_3 generations

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Introduction

Triticale (×*Triticosecale* Wittmack) is a man-made cereal crop that has been synthesized by crossing wheat (*Triticum aestivum* L.) with rye (*Secale cereale* L.). The first triticale was produced by Scottish scientist A. Stephen Wilson in 1875. Randhawa et al. (2015) summarized the various types of triticale that could be obtained with different chromosomal constitutions. Among them, hexaploid triticale (durum wheat × rye) has been the most successful due to its superior vigor, genetic stability and fertility. The octoploid forms (*T. aestivum* × *S. cereale*) suffered larger genetic instability and associated plant sterility (Mergoum et al., 2019).

The goal for breeding triticale is to produce varieties that combine the superior agronomic performance and the bread quality of wheat with the stress tolerance to biotic and abiotic factors affiliated with adaptability of rye. Hexaploid triticale is usually grown for feed grain and as a hay or a grazing crop. Moreover, triticale is a potential energy crop, and assumed source of biomass for bioethanol production. Most of the created triticale varieties are not suitable for human consumption due to their weak gluten strength, preharvest sprouting and shriveled grain (Bazhenov et al., 2015; Niedziela et al., 2016). Modern hexaploid cultivars of triticale are called secondary forms, because they have been created by crosses of only hexaploid lines (Randhawa et al., 2015) or spontaneously appeared in octoploid triticales (Zhou et al., 2012; Kalinka and Achrem, 2018). Increasing the genetic variability is the main component for effective breeding of agronomically valuable triticale lines. Numerous strains with various ploidy levels and gene combinations have been created between octoploid, hexaploid and tetraploid triticales in crosses to durum and bread wheat (Bernard and Bernard, 1987; Li et al., 2015; Kang et al., 2017; Kwiatek and Nawracała, 2018).

Baking quality of hexaploid triticale can be improved by introducing the D-genome chromosomes from common wheat (Sodkiewicz et al., 2011; Salmanowicz et al. 2013). Presto substitution lines 2D(2R), 3D(3R) and 6D(6R) showed a higher pre-harvest sprouting resistance than cv. Presto (Rybka, 2003). A hexaploid triticale 4D(4B) substitution line conferred stripe rust resistance (Kang et al., 2017). Many valuable traits of hexaploid triticale were associated with the presence of 2D(2R) chromosome (Lukaszewski and Gustafson, 1983; Rahmatov et al., 2016). In some cases, substituted triticales were better adaptive to the environment than the complete varieties (Xynias et al., 2008; Navarro-Contreras et al., 2014). Saranya and Reddy (2017) assessed 82 hexaploid triticale accessions to identify the replaced rye chromosomes and found 34 (41.5%) strains with one substituted chromosome pair and 24 (29.3%) had replacement of two pairs of rye chromosomes. Triticales with 2D(2R) and 4D(4R) substitutions had a higher and medium seed set, respectively.

Another way to enlarge the genetic variability in triticale was the use of species from Triticinae in crosses to rye or triticale. For example, octoploid wheat/*Thinopyrum elongatum* (AABBDDEE) and *T. turgidum* (L.) (AABB)/*Thinopyrum elongatum* (EE) (Han, F. P, pc) allopolyploids (Hohmann, 1993; Yang et al., 2015) and numerous amphiploids between *Aegilops* species and diploid rye (Wojciechowska and Pudelska, 2002; Kwiatek and Nawracała, 2018) were produced. Some primary triticale lines via hybridization of synthetic wheat (SHW) with rye were selected (Hao et al., 2013).

The R genome of rye contributed the secalin loci, *Sec-1* (1RS), *Sec-2* (2RL), *Sec-3* (1RL), and *Sec-4* (1RS) into triticale (Bellil et al., 2010). Thus, the absence of the D genome resulted in the poor bread making quality of triticale. From secalins, only Sec-3 had some positive effects on dough properties (Jonnala et al., 2010). Therefore, the breeder' efforts were aimed at improving the quality of triticale grain by using D(R) substitutions and/or translocations. These studies indicated that the transfer of the D-genome chromosomes and/or chromosomal segments into hexaploid triticale was attractive for improving important traits (Feng et al., 2019).

The present study was conducted to (1) evaluate five triticale accessions, using morphological, cytological and storage protein analyses in order to determine their genetic diversity, and (2) make reciprocal crosses between the selected individuals to exploit the existing variability and introduce the wheat 1D chromosome into triticale for breeding of substituted hexaploid winter genotypes.

Materials and Methods

Plant Material, Experimental and Meteorological Conditions

Cultivated rye accession A9E0053 (N053) was derived from the Institute of Plant Genetic Resources - Sadovo, Bulgaria. Triticale N294 was obtained from the Genebank of IPK-Gatersleben, and three octoploid strains N31, N79 and N80, were supplied by Dr. Kussovska (2011) (Table 1). Amphiploid N77 was produced by us from crossing *T. aestivum* cv. Sadovska Ranozreika-4 with rye N053. Plants were grown first in the greenhouse (2015) and then in the field during 2016 and 2017 by sowing seeds from the best yielded plant per sample, in Varna (43°12' N, 27°54' E, 50 m). A set of 30 seeds from each selected individual was sown in the field, at a distance of 10 and 40 cm between plants and rows in a plot, respectively, in two replications. The soil of the experimental site was haplic chernozem. Forage peas preceded and no fertilizers were applied. Weeds were manually controlled and only insecticides were used during the growing seasons. The sowing dates were typical for Varna conditions, from 10th -20th October. Winter hardiness (WH) of each variant and a plant per pot were assessed according to the WH scale, 1=the most sensitive, 9=the hardiest (APHA, 2019).

Concerning the meteorological factors in the two years (2016-2017), the temperature in October was optimal for the seed germination in the whole period. The conditions were characterized with low precipitation level and open weather in winter seasons. The average monthly minimum temperatures in winter dropped below -7.0 °C in 2016, and -10.1 in 2017. Usual rains fell before the sowing in all seasons, but very week precipitation level occurred in December. Heavy rains that accelerated plant development came in October 2017. Concerning the three factors (average monthly temperature, average monthly minimum temperature and the rainfall), the meteorological conditions were satisfactorily good for plant germination and growth to harvesting during the period.

Table 1. Pedigree of the initial triticale accessions

Initial No.	Pedigree	Source	Ploidy level (2n)	Checking the best 2 plants[3] (2n)
N31	Mironovskaya 808 × BLSR[1]	Kussovska [26]	56	56
N77	Sadovska Ranozreika-4 × *S. cereale* N053[2]	TU-Varna	56	56
N79	1732 [(*vavilovii*)-Rusalka × Lozen 14]	Kussovska [26]	56	42
N80	1737 [(*dicoccoides*)-Rusalka × BLSR]	Kussovska [26]	56	42
N294	Trit. Mazkow × *Secale montanum*	IPK Gatersleben	42	42

Note: [1] Bulgarian Low Stem Rye, [2] Originated from the IPGR "K. Malkov"-Sadovo, Bulgaria, [3] Selfed seed fertility.

Cytological Analysis, Reciprocal Crosses and Selection of F_2 and F_3 Plants with a Wheat 1D Chromosome

For chromosome checking, root tips of the seedlings from two individuals with the highest fertility per each strain, were pretreated with a 1-bromonaphthalene solution and stained by the conventional Feulgen method. Cytologically fixed plants of N31 and N294 were involved in reciprocal crosses: N88 F_1[294-6 (2n=42)/31-20 (2n=56)] and N89 F_1[(31-20 (2n=56)/294-6 (2n=42)]. At least two F_1 plants per cross were checked for somatic chromosome number. Hybrids including F_2 plants were grown in pots outside the greenhouse. Three seeds per hybrid plant were used for grain protein analysis. Selected plants in F_2 generation, containing chromosome 1D (*Glu-D1d* locus, encoding the 5 + 10 subunits) were bagged for selfing and measured, plus winter hardiness assessment in the beginning of vegetation (March 1-10). From them, a sample of 10 seeds from the best F_2 plants with plump grains, totally 70 seeds in F_3 were involved in the SDS-PAGE procedure.

Agronomic Traits

All plants per plot were harvested and measured for seven characters: plant height, tiller number, grain number per selfed spike, heading date, spike length, spikelet number and grain weight per selfed spike. Five plants per plot were used to assess the distinctness of traits according to the protocol (CPVO, 2011) on the following characteristics: flag leaf (anthocianin coloration of auricles), time of ear emergence, flag leaf (glaucosity of sheath), flag leaf (glaucosity of blade-lower side), stem (density of hairiness of neck), awns above the tip of ear-length, and ear (density). Selected F_2 plants involving a wheat 1D chromosome were measured for plant height, heading date, spikelet number per spike, grain number per spike, way of hand thrashing (a three step assessment). Grain plumpness was estimated visually using a four-point scale (poor, satisfactory, good and excellent) (Bazhenov et al., 2015).

Seed Protein Analysis

HMW-GS and 75 kDa γ-secalins were extracted from 50 mg of triticale flour. To each sample, 0.5 ml 50% 1-propanol was added to remove the albumins, globulins, 40 kDa γ- and ω-secalins followed by centrifuging for 10 min at 12000 g. Then, the HMW-GS and 75 kDa γ-secalins were isolated from the sediment by an extraction with 0.08 M Tris-HCl buffer (pH 8.0) containing 50% (v/v) 1-propanol and 1% (w/v) DTT. After 1 hour of incubation at 65°C the reduced proteins were alkylated with 0.08 M Tris-HCl buffer (pH 8.0) containing 50% (v/v) 1-propanol and 1.4% (v/v) 4-vinylpyridine (VP) at 65°C for 30 min followed by centrifugation at 12000 g for 10 min. 0.2 ml of the obtained supernatant was aliquoted in a new tube and mix with 0.2 ml of solution containing 2% SDS, 0.08 M Tris-HCl (pH 8.0), 40% glycerol and 0.02% bromophenol blue carried over 0.2 ml of each sediment. Again incubation and centrifugation were processed under the same conditions as stated above.

Extracted glutenins were fractionated by electrophoresis on a vertical SDS-PAGE gel using the discontinuous Tris-HCl-glycine buffer system. A 14μl aliquot of a protein sample was loaded onto the upper 4.5% gel, and separation was performed on 12.0% polyacrylamide gel at constant electric power of 20 mA per gel at room temperature for 18-20 hours. The gels were stained overnight with 1% Coomassie Brilliant Blue R 250 mixed with acetic acid, methanol and water in a proportion 1:5:4. Secalin designations were performed (McIntosh et al., 2003) by Arabic figures and the letter 'r' regarding the protein mobility toward the subunits of standard triticale variety Rakita. For *Glu-R1* and *Gli-R2* loci, the nomenclature proposed was used (Amiour et al., 2002b).

Statistical Analysis

The data were statistically evaluated by analysis of variance to determine significant differences ($P < 0.05$) between the genotypes, using Develve statistical program (www.develve.net). The least significant difference (LSD) was calculated (Snedecor and Cochran, 1980).

Results

Characterization of Triticale Samples

To involve the triticale accessions in genetic studies, we first grew them in the field (2016-2017) and measured for some agronomical traits (Table 2). The most distinguishable form was N31 giving lower values on spikelet number (SN), grain number per selfed spike (GNSS) and grain weight per selfed spike (GWSS). Conversely, it increased plant height (PH), heading date (HD) and spike length (SL). So, this triricale was late heading and formed long and lax spikes with minimal seed fertility.

Table 2. Morphological performance of the triticale samples in two years

Triticale	PH (cm)	TN	GNSS	HD	SL (cm)	SN	GWSS (g)
N31	150 a	2.5 b	38 b	146 a	15.5 a	27 b	2.4 b
N79	120 b	2.5 b	80 a	140 b	13 b	34 a	3.6 a
N80	115 b	4.5 a	75 a	137 b	12.2 b	29 b	2.5 b
N294	114 b	3.4 ab	88 a	136 b	14 ab	36 a	4.0 a
$LSD_{5\%}$	10	1.5	18	4.5	2.3	4.0	1.4

Note: PH-Plant height, TN-Tiller number, GNSS-Grain number per selfed spike, HD-Heading date in days from January 1st, SL-Spike length, SN-Spikelet number, GWSS-Grain weight per selfed spike. Amphiploid N77 was excluded due to very large variation for PH and spike shape

For tiller number (TN) and SL it was equaled to N294, and similar to triticale N80 for SN and GWSS. Triticale N31 was remarkable about notes on some qualitative traits as anthocyanin coloration of auricles, time of ear emergence, glaucosity of sheath and leaf blade (lower side), length of awns above the tip of the ear, and ear density, as compared to N294 (Table 3). The former strain was also different from N79 and N80 on four qualitative traits of plant phenotype. Additionally, N294 was morphologically more similar to N80 in comparison to N79, irrespective of the differences between the two last accessions.

The two best individuals about seed fertility per strain were cytologically checked for mitotic chromosome number resulting in 2n=42 for triticale N79-7, N80-4 and N294-6. Octoploid level (2n=56) was observed for line N31-20 (Table 1). All inspected mitotic cells showed to have a constant chromosome number. F_1 plants displayed a mean of 48.2 somatic chromosomes (data not shown).

The results of SDS-PAGE are shown in table 4. The electrophoresis confirmed the cytological findings for the octoploid nature of N31-20 and hexaploid constitution of the other three lines. The triticale N77 expressed a pair of subunits 5+10, which is a characteristic for the presence of the *Glu-D1* locus, as N31-20 did. The latter octoploid triticale showed again differences in protein pattern of glutenins and secalins, which described it as a valuable genotype. It exhibited different proteins in G*lu-A1, Glu-B1, Glu-R1, Gli-R2, Glu-A3* and *Glu-B3* as compared to N294-6, and partly to N79 and N80. The main contrast appeared in *Glu-R1* and *Gli-R2*: 2r+6.5r and d2 for the octoploid line versus 2r+9r and d1 of hexaploid triticale N294. Also, different alleles were located at *Glu-A1* and *Glu-B1*, coding for fractions 1 and 7+9 (N31-20) and 2* and 6.8+20y (N294-6), respectively.

Seed Protein Analysis and Morphological Traits of F_2 Plants

After selfing the F_1 hybrids, 33 F_2 seeds were analyzed for the storage proteins. From them, 16 (48.5%) possessed the subunit pair 5+10, coded by alleles located in chromosome 1D from cv. Mironovskaya 808. Some of the hybrids died during the growth and 12 F_2 plants reached maturity (Table 5). Four of them (89-4/1, 89-7, 89-8/2 and 89-9) were nearly sterile producing only 1-4 seeds. The two plants from a N88 cross (294-6 × 31-20) yielded 84 grains in total, with very good seed phenotype and winter hardiness assessment. The analysis of secalins for *Glu-R1* and *Gli-R2* loci (Sec-3 and Sec-2) showed that plant 88-1 giving 25 seeds in 3 spikes had chromosomes from the two parents. Excessive in fertility were 2 individuals from the reciprocal cross (89-2 and 89-8) which generated four spikes each, with 293 grains in total, but differentiated on the level of hand threshing.

Table 3. Assessment of distinctness on some plant traits according to the CPVO protocol for triticale

CPVO N°	Characteristics	SM	Notes for triticale lines			
			N31	N79	N80	N294
5	Flag leaf: anthocianin coloration of auricles	47-51	5	7	5	3
6	Time of ear emergence	50-52	9	7	5	3
7	Flag leaf: glaucosity of sheath	55-65	1	3	9	7
8	Flag leaf: glaucosity of blade (lower side)	55-65	1	3	1	7
11	Stem: density of hairiness of neck	60-69	7	1	5	9
14	Awns above the tip of ear: length	80-92	5	9	9	1
18	Ear: density	80-92	1	3	2	3

Note: SM-Stage method (Zadoks scale), CPVO-Community plant variety office

Table 4. Storage protein analysis of triticale lines selected for high spike fertility and grain weight

Line	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-R1*	*Gli-R2*	*Glu-A3*	*Glu-B3*	*Glu-B2*
N31-20	1	7+9	5+10	2r+6.5r	d2	f	k	b
N77-2	1	7+9	5+10	6r+13r	d2	c	g	b
N79-7	2*	7+9	-	6.5r	t1	d	h	b
N80-4	2*	7+18	-	6.5r	t1	d	h	b
N294-6	2*	6.8+20y	-	2r+9r	d1	d	h	b

Table 5. Analysis of selected F_2 plants involving chromosome 1D from cv. Mironovskaya 808

Cross No	PH	HD	SNSS[1]	GNSS[1]	GP	ST	WH	*Glu-R1*	*Gli-R2*
88-1	122	17	35/30/29	10/11/4	a	3	8	2r+9r	d2
88-2	104	17	30/31	30/29	b	1	8	2r+6.5r	d2
89-2	127	14	23/20/21/19	32/16/19/14	a/d	1	9	-	d1
89-4/1	90	28	22/16/15	1	b	-	6	6.5r?	d2
89-4/2	100	12	30/23/28	8/10/10	d	1	8	-	d2
89-4	112	13	34/26	39	b	1	9	2r+6.5r	d2
89-5	100	17	23/22	13/18	b/d	1	7	6.5r?	d2
89-6	141	17	24/28/23	20/22/24	b/d	1	9	-	d2
89-7	100	20	27/25	2	b	-	7	2r+6.5r	d2
89-8	135	13	34/36/32/30	49/52/67/44	b/d	2/3	9	2r+6.5r	d1
89-8/2	128	25	35/26	1/3	b	-	6	2r+6.5r	d2
89-9	113	25	38/34	4/3	b/d	3	7	2r+6.5r	d1

Note: PH-Plant height in cm, HD-Heading date (in days from May 1st), SNSS-Spikelet number per selfed spike, GNSS-Grain number per selfed spike, GP-Grain plumpness (a-excellent, b-good, c-satisfactory, d-poor), ST-Scale of hand thrashing (1-normal, 2-mediate, 3-hard), WH-Winter hardiness assessment key, [1] Each cipher represents spikelet number or grains per single selfed spike, 6.5r?-difficulties in HMW subunit identification on SDS gel.

Both of them showed excellent winter surviving (the highest rating=9) and different combination of secalins. Plant 89-8 produced 212 seeds of 4 spikes and appeared as recombinant genotype (2r+6.5r and d1) involving secalins of parents (Table 5). In plants 89-2, 89-4/2 and 89-6 only sec-2 was expressed, possibly due to some additional chromosome restructuring events or a missing 1R chromosome. Furthermore, genes located on this chromosome might interact with genes from other chromosomes.

We introduced four notes to evaluate the grain plumpness (a-d, Table 5). Most of the plants yielded wrinkled seeds in variation between b (good) and d (poor), excepting N88-1. Some grains of the parental line N31-20 manifested excellent phenotype. Most of the spikes possessed normal hand threshing (rating=1) and were similar to the parent N294-6. From the best F_2 individuals, ten seeds per plant with excellent to good grain filling were chosen for the next SDS-PAGE procedure.

Seed Protein Analysis of F_3 Seeds

A total of 70 grains were investigated. The chromosome 1D with the *Glu-D1d* allele was not observed in 28 plants (39.9%). Forty-two plants contained the locus encoding the 5 + 10 subunits, and 35 from them set up eight representative genotypes (G), numbered from 1 to 8 in table 6 and Fig. 1. Genotypes 2 (2*-2r+6.5r-7+9-5+10-d2) and G5 (1-2r+6.5r-7+9/9r-5+10-d2) were the most frequently distributed among the investigated plants, 14.3% and 11.4%, respectively (Table 6). Among all, the least common was G8 (1-6.8+20y/7+9-5+10-d1) with frequency of 1.4%. Seven other plants that were not included in Fig. 1, assembled three additional genotypes (numbered 9-11) as follows: G9 (1–2r+6.5r-7+9-5+10-d2), G10 (1–6.5r?-20y/7+9-5+10-d1), and G11 (2*-7+9-5+10). The last protein pattern was represented by only one plant. Seed setting and grain plumpness will be the main criteria for effective selection of proper genotypes to breed the substituted triticale lines.

Table 6. SDS-PAGE patterns of seed proteins of selected F_3 plants bearing chromosome 1D from cv. Mironovskaya 808

Nos in Fig. 1	G	Parent/F_3 plant	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-R1*	*Gli-R2*	Frequency, %
1		Rakita (st)	Null	7 + 18	-	6r + 13r	t1	-
2		294-6	2*	6.8 + 20y	-	2r + 9r	d1	-
3		31-20	1	7 + 9	5 + 10	2r + 6.5r	d2	-
4	1	88-1/3-4	2*	7 + 9	5 + 10	2r+9r+6.5r?	d2	5.7
5	2	88-2/3-10	2*	7+9	5 + 10	2r + 6.5r	d2	14.3
6	3	89-2/3-3	1	6.8+20y/7+9	5 + 10	-	d2	8.6
7	4	89-4/1-1	1	7 + 9	5 + 10	-	d2	2.9
8	5	89-4/3-3	1	7 + 9/9r	5 + 10	2r + 6.5r	d2	11.4
9		89-5/2-3	2*	7 + 9	-	2r + 6.5r	d2	-
10	6	89-8/2-1	1	7 + 9	5 + 10	2r + 9r	d1	2.9
11	7	89-8/2-2	1	6.8+20y/7+ 9	5 + 10	2r + 6.5r	d1	2.9
12	8	89-8/2-3	1	6.8+20y/7+9	5 + 10	-	d1	1.4

Note: G-Representative genotype, 6.5r?-difficulties in HMW subunit identification on SDS gel

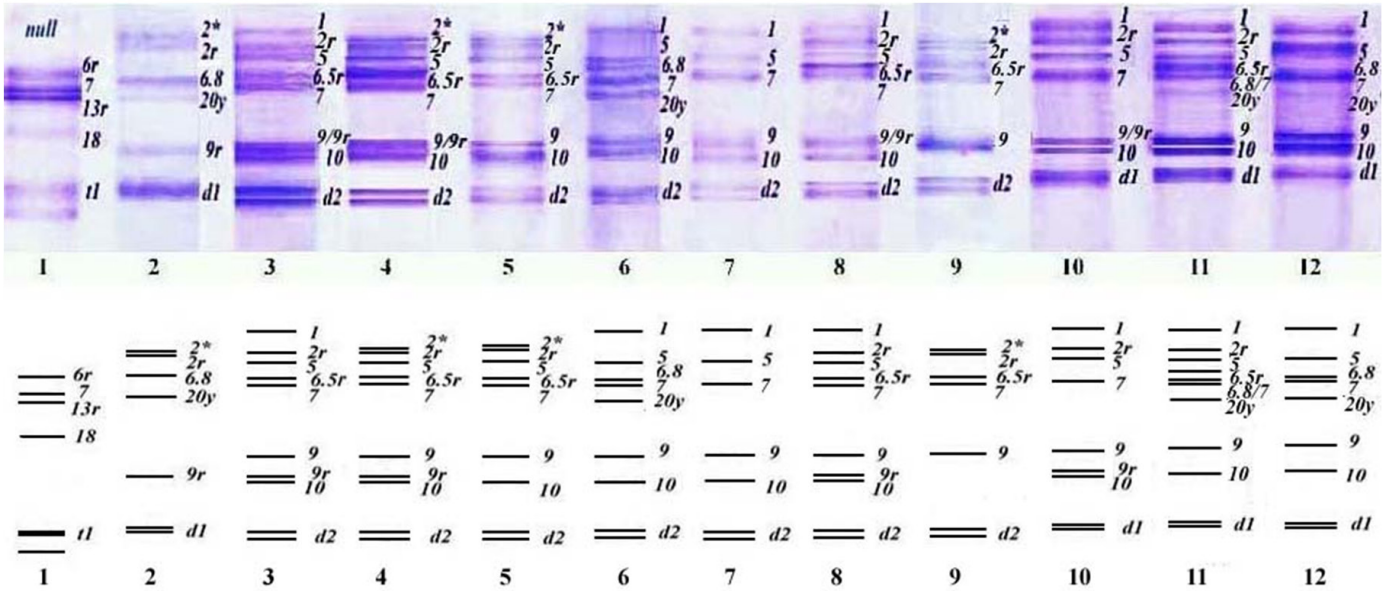

Figure 1. SDS electrophoregram of storage proteins of selected F_3 individuals from the reciprocal crosses N88 (294-6/31-20) and N89 (31-20/294-6), attended by a diagram with drawn bands: 1:Rakita (st); 2:N294-6 parent; 3:N31-20 parent; 4:88-1/3-4; 5:88-2/3-10; 6:89-2/3-3; 7:89-4/1-1; 8:89-4/3-3; 9:89-5/2-3; 10:89-8/2-1; 11:89-8/2-2; 12:89-8/2-3

Discussion

The analysis of storage protein alleles is well known as a powerful tool for genotyping plant genetic resources. In 68 rye inbred lines, 9 HMW-SS compositions were described (Salmanowicz et al., 2014). Amiour et al. (2002b) characterized three subunits (2r+9r, 2r+6.5r and 6.5r) at *Glu-R1* belonging to three different allelic forms *b, d* and *e*. Later, these alleles were found in hexaploid triticale in combination with t1 (Bellil et al., 2010). 75K gamma-secalins, encoded at *Gli-R2*, corresponded to the triplet bands and d1 and d2 equated to two double bands. The majority of cultivars (80.3%) possessed the triplet t1 (*Gli-R2a*). Five alleles (*a, b, c, d* and *e*) at *Gli-R2* were found in secondary hexaploid triticale cultivars grown in Europe (Amiour et al., 2002a). Among them, allele *c* was widely distributed in triticale. Triticale accessions in this study revealed wide genetic variation

regarding the seed proteins including secalins. We studied five triticales, two of which (N31-20 and 77-2) were octoploids with known origin of the wheat D-genome, cvs. Mironovskaya 808 and Sadovska ranozreika-4, respectively. Kussovska (2011) carried out agronomic traits on 12 new triticale lines, isolated in the process of cytological stabilization of several triticale lines, selected from population 1737-1. The author described newly synthesized alloplasmic octoploid triticale lines. For its diverse expression of morphological traits, line N31-20 was involved in crosses with triticale line 294-6 to produce the reciprocal F_1 hybrids. The chromosome number in the hybrid plants of the octoploid × hexaploid cross was generally lower than the expected 49 (Lafferty and Lelley, 2001). Our cytological data confirmed this finding (data not shown). Amphiploid N77-2 expressed 6r+13r in *Glu-R1*, coded by *c* allele, which is highly distributed in DAI-General Toshevo's triticale varieties (Doneva and Stoyanov, 2019).

The baking value of hexaploid triticale can be improved by introgression of wheat chromosome 1D. This trait is most strongly affected by high molecular weight glutenin subunits, which are encoded by locus *Glu-D1*. The wheat D genome, carrying valuable genes for breeding, has been involved in crosses with triticale to obtain D/R, or D/A and D/B substitutions (Budak et al., 2004; Kozub et al., 2007). A few studies involved primary octoploid triticale to investigate certain aspects of genomic transformations in the early generations (Kalinka and Achrem, 2018), or to obtain secondary hexaploid triticale by crossing triticale (2n=42) or common wheat with an octoploid triticale (Tang et al., 2014; Kang et al., 2016). We used octoploid triticale N31-20 in crosses with line N294-6 (2n=42) as both showed contrasting plant traits, including different proteins encoded at *Glu-B1, Glu-D1, Glu R1* and *Gli-R2* loci, as compared to other triticales. Researchers used the substituted triticale lines (2n=42) in crosses with the octoploid triticale to obtain F_1 hybrids and further screened the first backcross plants by SDS-PAGE to verify the presence of chromosome 1D and to determine the Glu-D1 allele. Reduction to the hexaploid level occurred quickly (Lafferty and Lelley, 2001).

Among the utilized breeding procedures for triticale improvement, introducing D-genome chromosomes into 6x triticale was quite recently exploited. Triticale D-genome chromosome introgression lines have been derived from wheat-rye-*Psathyrostachys huashanica* trigeneric hybrids (PHW-SA, 2n=8x=56, AABBDDNsNs crossed with triticale) (Kang et al., 2016), or by creating *Aegilops tauschii* Coss.(DD, 2n=14) × *S. cereale* (RR, 2n=14) amphiploid as a bridge in crosses to wheat (Kwiatek and Nawracała, 2018). Direct crossing between hexaploid and octoploid triticales (Tang et al., 2014) is an effective way of genome rearrangements in F_2-F_5 generations followed by self-fertilization of selected individuals. Using the D-genome of *T. aestivum* cv. Panda, various single substitutions in hexaploid winter triticale with improved resistance to wheat leaf rust, preharvest sprouting and low alpha-amylase activity in grain were obtained (Sodkiewicz et al., 2011).

Differences between the R and D genomes of hexaploid triticale and bread wheat were responsible for differences in agronomic and quality traits of the two cereals (Budak et al., 2004). The D(R) substitutions reduced the amount of rye germplasm present in triticale. The 2D(2R) substitution was among the most widespread substitutions in triticale, but reduced grain productivity regardless of promoting early heading and anthesis (Bazhenov et al., 2015). The authors investigated early generations of winter hexaploid triticale crosses to examine the effect of 2D(2R) substitution on grain yield, plant height and seed dormancy. In the first F_2 generation 31 plants did not carry the substitution, while the rest of 39 individuals contained the chromosome 2D in homozygous and heterozygous state. Nearly the same result was obtained in the second F_2 population: 52.9% of the studied plants was the carrier of chromosome 2D. We examined seeds by SDS-PAGE in segregating generation to find 16 (48.5%) in F_2 and 42 (59.9%) in F_3 as bearers of wheat 1D chromosome. The difference between our results and those mentioned above, although it was not a large one, might be due to ploidy level of the parents used: 6x × 8x and vice versa here, and 6x × 6x in Bazhenov et al. (2015).

To enhance the triticale genetic variation, more diverse types of substitutions are urgently needed. Many biotic and abiotic stress tolerance genes are also located on the wheat D-chromosomes. Substitutions 1D(1A) and 3D(3A) of triticale have introduced adult plant resistance to wheat leaf rust and preharvest sprouting (Sodkiewicz et al., 2011). Enhanced fungal resistance in substitutions has also been reported (Kang et al., 2016, 2017). Many of the end-use quality characteristics of Presto triticale have been improved by introducing wheat 1D and 6D chromosomes (Budak et al., 2004).

Conclusion

The results showed that the five triticale accessions exhibited a great genetic diversity on various traits studied, including glutenins and secalins. Crosses between hexaploid and octoploid strains could be of a special interest for breeding through introgression of wheat 1D chromosome into triticale associated with additional genome restructuring events. Hybrid plants were produced and investigated by selfing individuals with high seed set and well to excellently grain filling. According to the high molecular weight glutenins and secalins, F_3 seeds carriers of chromosome 1D of cultivar Mironovskaya 808 were grouped in 11 genotypes. Two of them (2*-2r+6.5r-7+9-5+10-d2) and (1-2r+6.5r-7+9/9r-5+10-d2) were predominant with 14.3% and 11.4%, respectively. The triticale D-genome substitution hybrid plants described in this study might be new initial germplasm for hexaploid triticale breeding due to various combinations occurred between loci at the A, B, D and R genomes.

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