ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 105, No. 2 (2018), p. 113–122
DOI 10.13080/z-a.2018.105.015

Transfer of novel storage proteins from a synthetic hexaploid line into bread wheat

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Abstract

Pure line selection for the presence of Glu-D1-4t+10.1t in high molecular weight glutenin subunits was applied in a cross between a synthetic hexaploid wheat (SHW) 530-1 (*Triticum dicoccum* / *Aegilops tauschii* acc. 19088) (2n = 42, $BBA^uA^uD^tD^t$) and two common wheat (*T. aestivum* L.) cultivars 'Albena' and 'Slaveya' (2n = 42, BBA^uA^uDD). Grain number and seed weight per plant of selected $BC_1F_{5\text{-}7}$ individuals accompanied this high molecular weight subunit pair to create 9 wheat genotypes. They were tested in the field and showed sufficient germination and winter survival with high seed set. Hierarchical cluster analysis divided them in two groups (four in the first, including standard wheat cultivar 'Sadovska ranozreika-4' and six lines in the second cluster). The urea / SDS-PAGE method separated the new lines in two high molecular weight glutenin variants, Glu-A1-2*, Glu-B1-7+8, Glu-D1-4t+10.1t (8 genotypes) and Glu-A1-null, Glu-B1-7+8, Glu-D1-4t+10.1t (1 genotype), displaying equal low molecular weight glutenins. All families expressed two ω-gliadin bands: the gliadin #1 originated from SHW530-1, while #2 was transferred from wheat cultivar 'Albena'. The former one appeared to be the unique gliadin subunit in the genotypes, not expressed in both wheat parents. The incorporated high molecular weight glutenin alleles might serve as new genetic resources for improving the grain quality of modern common wheat.

Key words: bread wheat, gliadins, glutenins, seed storage protein, synthetic hexaploid wheat.

Introduction

Wheat is one of the most widespread crops in the world, grown mainly as a basic source of protein in the human food. Along with the productivity per unit of area, grain quality has recently appeared as very important trait too. About 85% of the total protein contained within seed endosperm is gluten, forming a continuous matrix in dough and confers elasticity and extensibility essential for bread making (Goryunova et al., 2012; Shewry, Tatham, 2016). Glutenins, which are basic decisive factors of gluten elasticity (Shewry, 2009; Ribeiro et al., 2013), are classified as high molecular weight (HMW-GS) and low molecular weight (LMW-GS) glutenin subunits.

HMW-GS are encoded by *Glu-1* loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) on long arm of homoeologous group 1 chromosomes. Each *Glu-1* locus has two linked genes designated as x- and y-type based on differences in their structure (Harberd et al., 1986; Garg et al., 2009). LMW-GS consist of fractions encoded by a multigene family, *Glu-3* loci, covering 30–40 genes located in homoeologous group 1 chromosomes and linked to gliadins (*Gli-1* loci) (Shewry, Tatham, 2016). Allelic variation in HMW-GS composition was found to be correlated with differences in bread-making quality. Therefore, the analysis of glutenins is an important criterion in breeding for grain quality improvement. The intensive breeding process in wheat led to the loss of many favourable alleles in the newly developed cultivars. They possess limited genetic variation as compared to the old cultivars, landraces and wild species (Gul et al., 2015).

Broadening the genetic variability in wheat is the fundamental base in breeding of productive and adaptive cultivars with high grain quality. Genetic resources of wild relatives are considered to have many valuable traits to improve the cultivated *Triticum* species (Qi et al.,

Please use the following format when citing the article:
Doneva S., Daskalova N., Spetsov P. 2018. Transfer of novel storage proteins from a synthetic hexaploid line into bread wheat. Zemdirbyste-Agriculture, 105 (2): 113–122 DOI 10.13080/z-a.2018.105.015

2007; Tiwari et al., 2014). *Aegilops tauschii* (2n = 14, DD), donated the D-genome to hexaploid wheat, covers accessions possessing a wide variation in endosperm proteins, mainly gliadins and glutenins (William et al., 1993; Zhang et al., 2008; Xu et al., 2010). Synthetic hexaploid wheats (SHW) resulting as products of wide hybridization between tetraploid wheat and *Ae. tauschii*, are involved in breeding of SHW-derived lines and wheat cultivars (Plamenov, Spetsov, 2011; Cooper et al., 2012; Li et al., 2014; Tang et al., 2016), and seem to be an excellent vehicle for transferring different HMW-GS present in the D-genome of *Ae. tauschii* for improvement of bread wheat.

Incorporating allelic diversity for *Glu-1* from SHW is preferred due to their promising agronomic features. Xu et al. (2010) investigated synthetic wheats derived by crossing durum cultivar 'Langdon' to 43 *Ae. tauschii* accessions and found seventeen 1Dx and 1Dy combinations encoding by eight novel *Glu-D1* alleles. Forty-four different HMW-GS compositions (22 alleles) were observed in 95 Elite-I SHWs (*Triticum turgidum*/*Aegilops tauschii*) by Rasheed et al. (2012). Bibi et al. (2012) showed a range of D-genome-encoded subunits of the Elite-II SHW subset along with superior glutenin alleles in the B-genome (1Bx7+1By8, 1Bx6+1By8 and 1Bx13+1By16). In our previous study (Daskalova et al., 2016), five subunits at the *Glu-A1* (1.1), *Glu-B1* (14+15, 22) and *Glu-D^{t}1* (2+11, 4+10.1) loci were reported in nine synthetic lines (*T. dicoccum*/*Ae. tauschii*), which have not been presented in the publications for SHW till now. Two of them, the subunit pairs 2+11 and 4+10.1, encoded at the *Glu-D^{t}1* locus, were published for the first time.

The objective of this study was to incorporate the high molecular weight (HMW) glutenin subunit pair 1Dx4+1Dy10.1 from a synthetic hexaploid wheat (SHW) line 530 into winter type of bread wheat and characterize the new SHW-derived lines in the field.

Materials and methods

Plant materials. Following a technical manual for hybridization in cereals, spikes from common wheat (*Triticum aestivum* L.) cultivar 'Albena' were emasculated and pollinated with fresh pollen of F_1 (Slaveya/SHW530) to create BC_1 plants. All crossing procedures were conducted by one person to keep skill levels. The backcross plants were self-pollinated in a greenhouse and the next two generations were grown in the field. They were maintained through mass selection. The investigation started with seeds obtained from five randomly selected plants (112-1, 112-2, 112-3, 112-4 and 112-5) of a population (BC_1F_4) derived from the cross Albena/Slaveya/SHW530. 'Albena' and 'Slaveya' are bread wheat cultivars released in the Dobrudzha Agricultural Institute, Bulgaria. The total grains analysed in the beginning were 50. Synthetic hexaploid wheat (SHW) 530 was derived by crossing a tetraploid hybrid (45390/45398) to *Aegilops tauschii* accession 19088 (Daskalova et al., 2016). This research showed that SHW530 included two lines (530-1 and 530-2), differing only at the *Glu-B1* locus, and both had a HMW glutenin pair 4t+10.1t. This subunit pair was found to be a novel protein in *T. aestivum*. The high molecular weight glutenin subunits (HMW-GS) composition of SHW530-1 and 530-2 were Glu-A1-1.1, Glu-B1-7+8, Glu-D1-4t+10.1t, Glu-A1-1.1, Glu-B1-22 and Glu-D1-4t+10.1t, respectively.

Soil and climatic conditions. The soil of the experimental site was *Haplic Chernozem* (*CH-ha*). Forage peas preceded and no fertilization and pesticides were applied during the plant growth.

In 2014–2015, the weather conditions were characterized by precipitation level of 490 mm, almost equal to 485 mm in 2015–2016. The temperature in October was optimal for the seed germination in the whole period (Fig. 1). Snow depth on the field amounted to an average 70 mm in December–February of 2016. The minimal temperatures occurred in December and February of the first year reaching −10°C and −12°C in December and January of the second year. The winter conditions in 2014–2016 were different concerning the presence of snow cover and duration of minimal freezing temperatures. More rain fell in the last three months of the second year that accelerated plant development. Concerning the four factors, average minimum temperature (between 0.8–2.4°C), average maximum temperature (21–23.5°C), rainfall (485–490 mm) and snowfall (from 0 to 70 mm in 2015–2016) the meteorological conditions were satisfactorily good for plant germination and growth to harvesting during the whole period.

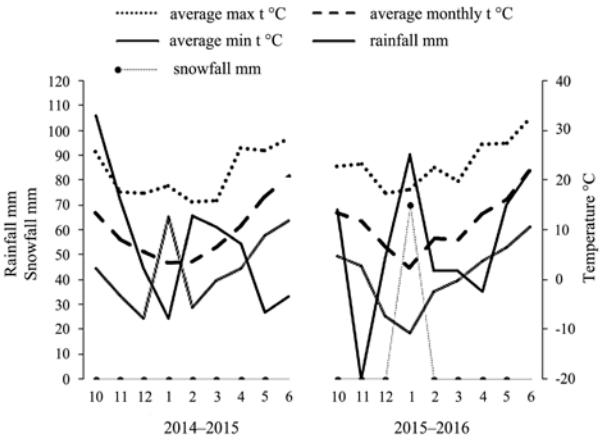


Figure 1. Climatic conditions for 2014–2015 and 2015–2016

Protein extraction and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Ten seeds per plant were crushed and ground to powder. Gliadins were first extracted in 70% ethanol and protein fractions were separated by A-PAGE using 8% polyacrylamide gel under constant 10°C (Khan et al., 1983). Extraction of HMW and low molecular weight (LMW) glutenin subunits was performed in four stages (Singh et al., 1991). The electrophoresis run on a vertical apparatus in two ways: (1) classical one-dimensional 12% polyacrylamide gel and (2) one-dimensional 12%

polyacrylamide gel SDS-PAGE with addition of 4M urea (Lafiandra et al., 1993). Protein fractions were investigated and designated through the universal system for arrangement and numbering of HMW-GS (Payne, Lawrence, 1983) and LMW-GS nomenclature in wheat (Gupta, Shepherd, 1990) by labelling the bands at each subunit as Glu-A1, Glu-B1 and Glu-D1. The allelic variations of LMW-GS at the *Glu-3* loci were registered using the labels Glu-A3, Glu-B3 and Glu-D3. Combined method for LMW-GS and gliadins identification (Jackson et al., 1996) and the catalogue for gliadins recording in common wheat (Metakovsky, 1991) were also applied. 'Bezostaya' and 'Chinese Spring' were the standard cultivars in seed protein analysis.

Grain quality analysis. Crude protein content (%) was determined by the standard Kjeldahl method (N × 5.7). Lysine content in mg was measured per 100 g of dry matter basis according to Musiyko et al. (1976). The sedimentation value (ml) of the whole wheat flour was assessed using the method of Pumpyanskiy (1971).

Greenhouse and field experiment. The first selected plants (BC_1F_5) by glutenin composition were grown in an unregulated greenhouse in two different places, a plot and pots, following the designation of the five randomly selected spikes from the cross (numbers from 1 to 5). Four plants were transplanted in a pot with a volume of 4 kg soil from the field. Simultaneously, plants from the same generation were set out in a row of the plot, at a distance of 5 and 40 cm, between plants and rows, respectively. Wheat progenies (BC_1F_6) were grown in a crop rotation field in Varna (43°12′ N, 27°54′ E, 50 m), Bulgaria, during 2014–2016. The sowing dates were typical of Varna conditions, between 10–20 October. The seeds were manually planted in single-row plots at 20 seeds per 1 m long row and at an inter-row spacing of 40 cm using randomized design with two replications, along with wheat parents. The evaluation of germination was done in autumn at 1–2 leaf stage and the winter survival was calculated using all available plants for each line in the beginning of vegetation (15 March–15 April). Wheat cultivar 'Sadovska ranozreika-4'was sown as a standard at regular intervals every 25 rows. Pureline selection was applied in the sense that individual plants with the desired HMW glutenin pair 4t+10.1t were selected, their progeny were evaluated and the best one-three plants in derived progeny for grain number and kernel weight per main spike were used to obtain the next generation (Baenziger, DePauw, 2009). Each family obtained from a selected plant consisted of 20 seeds, sown in two replications.

Statistical analysis. The data were statistically evaluated by analysis of variance to determine significant differences ($p < 0.05$) between wheat genotypes with Tukey test using software *Assistat*, version 7.7 beta (Silva, Azevedo, 2016). To estimate the effect of the new fraction pair from *Glu-D1* locus on the qualitative indices (protein, lysine and sedimentation value), the statistical package *SPSS 17.0* was used to compare the differences between the selected genotypes and the mid-parent value of wheat cultivars 'Albena' and 'Slaveya'. The methods of descriptive statistics and the *t*-measure (criterion of *Student*) were applied. Hierarchical cluster analysis was performed of the wheat lines using *IBM SPSS Statistics*, version 22.0 (IBM Corp., USA). This type of clustering identifies relatively homogeneous groups of cases based on selected characteristics. An algorithm is used that starts with each case in a separate cluster and combines clusters until only one is left. Differences between genotypes are represented by Euclidean distances.

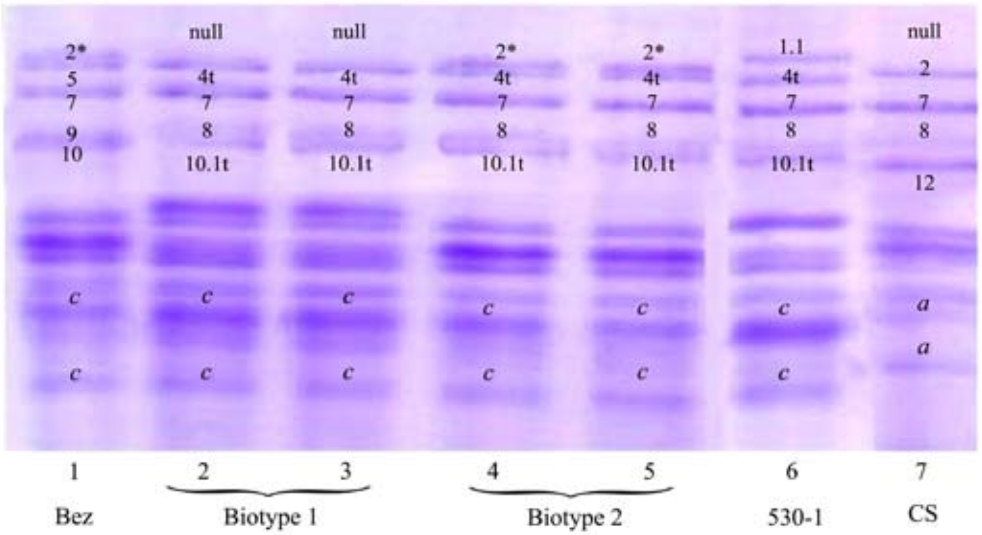
Results and discussion

Development of wheat lines with new high molecular weight (HMW) glutenins. Overall, six biotypes for HMW-GS were found (Table 1, Fig. 2): 1) null (*Glu-A1*), 7+8 (*Glu-B1*), 4t+10.1t (*Glu-D1*); 2) 2* (*Glu-A1*), 7+8 (*Glu-B1*), 4t+10.1t (*Glu-D1*); 3) null (*Glu-A1*), 7+8 (*Glu-B1*), 5+10 (*Glu-D1*); 4) 2* (*Glu-A1*), 7+8 (*Glu-B1*), 5+10/4t+10.1t (*Glu-D1*); 5) null (*Glu-A1*), 7+8 (*Glu-B1*), 5+10/4t+10.1t (*Glu-D1*); and 6) 2* (*Glu-A1*), 7+8 (*Glu-B1*), 5+10 (*Glu-D1*).

Biotype 1 was identified in 20 seeds (homogeneous, 40%), biotype 2 in 23 seeds (20-homogeneous + 3-heterogeneous, 46%), biotype 3 in 2 seeds (homogeneous, 4%), biotype 4 in 3 seeds (heterogeneous, 6%), biotype 5 in 1 seed (heterogeneous, 2%) and biotype 6 in 1 seed (homogeneous, 2%). Homogeneous (46 seeds) and heterogeneous (4 seeds) bands occurred

Table 1. Glutenins in BC_1F_5 seeds of five randomly selected spikes from the cross Albena/Slaveya/SHW530-1

Initial plant / seed No.	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	Biotype
1/1, 2, 3, 4, 5, 6, 7, 8, 9, 10	null	7+8	4t+10.1t	*c*	*b*	*c*	1
2/1, 2, 3, 4, 5, 6, 7, 8, 9, 10	2*	7+8	4t+10.1t	*c*	*b*	*c*	2
3/1, 4	null	7+8	5+10	*c*	*b*	*c*	3
3/2, 5, 10	2*	7+8	5+10, 4t+10.1t	*c*	*b*	*c*	4
3/3, 9, 6	2*	7+8	4t+10.1t	*c*	*b*	*c*	2
3/7	null	7+8	5+10, 4t+10.1t	*c*	*b*	*c*	5
3/8	2*	7+8	5+10	*c*	*b*	*c*	6
4/1, 2, 3, 4, 5, 6, 7, 8, 9, 10	null	7+8	4t+10.1t	*c*	*b*	*c*	1
5/1, 2, 3, 4, 5, 6, 7, 8, 9, 10	2*	7+8	4t+10.1t	*c*	*b*	*c*	2
Albena	null	7+8	5+10	*f*	*b*	*c*	
Slaveya	2*	7+9	5+10	*c*	*b*	*c*	
SHW530-1	1.1	7+8	4t+10.1t	*b*	*b*	*c*	



1 Bez – 'Bezostaya', 7 CS – 'Chinese Spring'

Figure 2. High- and low molecular weight glutenin (*Glu-D3*) composition in BC_1F_5 seeds expressed by 12% SDS-PAGE analysis

only in the *Glu-D1* locus. Seeds with a subunit pair 4t+10.1t in the homogeneous state were 43 (86%) and they differed only in the *Glu-A1* locus (null or 2* band). One subunit pair, 7+8 in *Glu-B1*, was found in all seeds investigated (Table 1). Regarding the LMW glutenins, all seeds expressed allele *c* in the *Glu-A3* and allele *c* in the *Glu-D3*. The former one was derived from the wheat cultivar 'Slaveya' and the latter from any one of the three parents (Table 1).

All seeds from biotypes 1 and 2 were germinated in Petri dishes. From them, 15 (Table 2) and 18 (data not shown) plants were grown and harvested in the plot and pots in the greenhouse, respectively. Between 1 to 3 spikes per plant were isolated before flowering. Morphological traits varied among individuals, especially grain number per isolated spike, ranging from 9 to 48 seeds. Six plants were selected as ancestors for testing the progenies on field conditions (denoted as G38, G39, G42, G44, G45 and G46), based on their grain number and seed weight per isolated spike (Table 2).

The shortest plant (height – 65 cm), harvested in the greenhouse, produced 29 seeds under spike isolation and was ancestor of G38. Another three plants were chosen from the materials grown in pots to provide next offspring designated as G40, G41 and G43. Ten seeds (BC_1F_6) from each selected plant harvested in the greenhouse, excepted plant No. 2/1 plot with 6 seeds, were analysed for glutenins and gliadins (Table 3). All seeds showed identical HMW-GS composition 1Ax2*-1Bx7+1By8-1Dx4t+1Dy10.1t, excluding those from plant No. 1/2 plot with difference at *Glu-A1* (expression of 1Axnull). LMW glutenins were the same, *Glu-A3c*, *Glu-B3b* and *Glu-D3c* alleles.

Table 2. First selected plants (BC_1F_5) grown in the greenhouse plot during 2014

Initial plant / No.	Plant height cm	Isolated spike number	Spike length cm	Number of spikelets	Grain number in isolated spikes	Grain weight in isolated spikes g	Number of spikes per plant	Designated genotype number BC_1F_6 seeds
1/1	81	1	9.5	30	22	0.34	1	
1/2	87	2	10.0	22	29	0.82	3	46
1/3	91	1	10.0	21	9	0.21	2	
2/1	65	1	7.6	25	29	1.22	4	38
2/2	89	1	10.6	25	44	1.67	4	39
2/3	83	1	7.8	21	18	0.64	4	
2/4	70	1	9.6	22	37	1.41	4	42
2/5	85	2	10.5	23	25	0.86	3	
2/6	80	1	11.7	27	46	1.82	3	
4/2	102	2	11.3	32	95	3.79	6	44
4/3	111	2	11.2	26	93	3.64	6	
4/4	110	3	13.2	28	52	1.48	8	
4/5	104	2	12.2	24	43	1.96	5	
5/1	94	2	10.5	34	93	2.09	7	45
5/3	87	3	10.3	30	52	1.64	8	
Range	65–111		7.6–13.2	21–34	9–48	0.2–1.9	1–8	

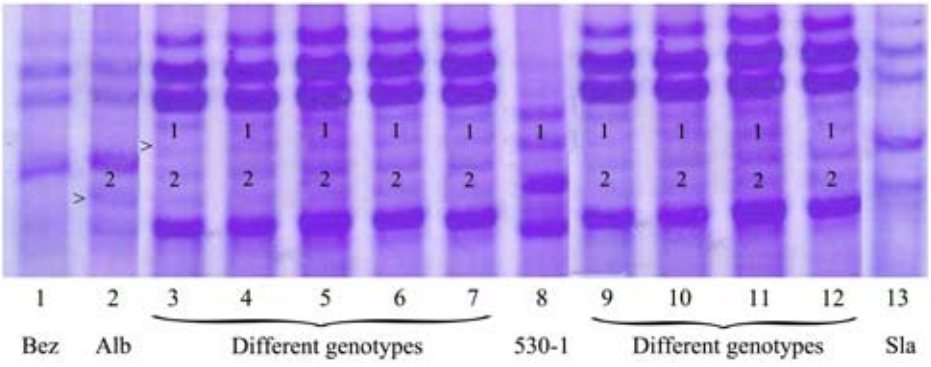
Note. Spike length and number of spikelets are measured on the heaviest spike isolated.

Table 3. Glutenins in BC_1F_6 seeds yielded in 2014 from 9 initial selected wheat genotypes

Initial plant / seed No.	*Glu-A1*	*Glu-B1*	*Glu-D1*	Genotype No.
2/1 plot /1–6	2*	7+8	4t+10.1t	38
2/2 plot /1–10	2*	7+8	4t+10.1t	39
2/1 pot /1–10	2*	7+8	4t+10.1t	40
2/2 pot /1–10	2*	7+8	4t+10.1t	41
2/4 plot /1–10	2*	7+8	4t+10.1t	42
4/1 pot /1–10	2*	7+8	4t+10.1t	43
4/2 plot /1–10	2*	7+8	4t+10.1t	44
5/1 plot /1–10	2*	7+8	4t+10.1t	45
1/2 plot /1–10	null	7+8	4t+10.1t	46

Note. Alleles in *Glu-A3*, *Glu-B3* and *Glu-D3* are *c*, *b* and *c*, respectively; in *Gli-A1*, *Gli-B1* and *Gli-D1* – allele *b* only.

Gliadins were investigated in seeds obtained from the selected plants in the greenhouse. All progenies expressed identical alleles: *b* (*Gli-A1*), *b* (*Gli-B1*) and *b* (*Gli-D1*) (Tables 3–4), as the gliadin pattern of cultivar 'Slaveya'. The other wheat parent 'Albena' possessed the alleles *b* in *Gli-B1* and *Gli-D1*, and *f* in *Gli-A1* (data not shown). Furthermore, all the nine families expressed two ω-gliadin bands, pointed in the Figure 3. The gliadin #1 originated from SHW530-1, while #2 was transferred from 'Albena' wheat. The former one might be the unique gliadin subunit in the new lines, not expressed in both wheat parents. All identified *Gli* alleles in the six loci were alike *b*.



Note. The sign > points the ω-gliadin position; 1 Bez – 'Bezostaya', 2 Alb – 'Albena', 13 Sla – 'Slaveya'.

Figure 3. Gliadins in BC_1F_6 seeds produced from nine wheat genotypes

Characterization in laboratory and field conditions. Three plants with the highest yield from each genotype in 2015 were checked for the seed storage proteins (three BC_1F_7 seeds per plant) and found again two biotypes, differing only at *Glu-A1* locus, 2*-7+8-4t+10.1t and null-7+8-4t+10.1t. The latter biotype was expressed only by G46 (Table 4). LMW glutenins in *Glu-A3* and *Glu-D3* were manifested by allele *c* and the gliadins in the three loci, as previously was shown, by allele *b* only. Seed storage protein pattern in BC_1F_7 seeds, harvested in the field, was as the same as in BC_1F_6 seeds, yielded from 9 initial selected plants matured in the greenhouse.

Means of nine traits in all genotypes grown in two years (2015 and 2016) in the field were compared to standard cultivar 'Sadovska ranozreika-4' (SR-4). Lines were equalled to this cultivar and expressed no differences in between, on number of spikes, spike weight and grain yield per plant (Table 5).

Genotypes 38 and 41 showed decreased performance for winter survival and field germination, respectively. The earliest headed genotypes (G43 and G46) differed from G42 by six days. G46 was the highest (108 cm) and G45 the shortest (94 cm) genotype. Big differences were observed among lines in grain number

Table 4. Glutenins and gliadins in BC_1F_7 seeds, harvested in 2015

Genotype	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-B3*
38	2*	7+8	4t+10.1t	*b*
38[a]	2*	7+8	4t+10.1t	*b*
39	2*	7+8	4t+10.1t	*b*
39[a]	2*	7+8	4t+10.1t	*b*
40	2*	7+8	4t+10.1t	*b*
40[a]	2*	7+8	4t+10.1t	*b*
41	2*	7+8	4t+10.1t	*b*
41[a]	2*	7+8	4t+10.1t	*b*
42	2*	7+8	4t+10.1t	*b*
42	2*	7+8	4t+10.1t	*b*
43	2*	7+8	4t+10.1t	*b*
43[a]	2*	7+8	4t+10.1t	*b*
44	2*	7+8	4t+10.1t	*b*
44[a]	2*	7+8	4t+10.1t	*b*
45	2*	7+8	4t+10.1t	*b*
45[a]	2*	7+8	4t+10.1t	*b*
46	null	7+8	4t+10.1t	*b*

Note. [a] – seeds analysed from the second replication; in *Glu-A3* and *Glu-D3* allele *c* was found in all lines, while in *Gli-A1*, *Gli-B1* and *Gli-D1* – allele *b* only; BC_1F_8 seeds, harvested in 2016, showed again the same glutenin and gliadin composition.

Table 5. Agronomic traits of 9 wheat genotypes, grown in 2015 (BC_1F_6 plants) and 2016 (BC_1F_7 plants)

Geno-type	FG	WS	HD	PH	NSP	SWP	GNP	GYP	TKW
38	100.0 a	89.5 b	208.1 ab	96 c	6.25 a	15.0 a	267 abcd	12.1 a	45.2 b
39	100.0 a	100.0 a	207.5 ab	102 abc	7.85 a	20.2 a	289 abc	13.9 a	48.2 ab
40	100.0 a	95.0 ab	208.5 ab	95 c	5.81 a	16.9 a	216 d	11.4 a	51.8 ab
41	85.0 b	95.0 ab	210.3 ab	103 abc	8.15 a	22.9 a	316 a	16.7 a	49.0 ab
42	100.0 a	95.0 ab	210.5 a	99bc	6.01 a	18.4 a	250 bcd	12.7 a	51.6 ab
43	95.0 ab	100.0 a	204.5 b	99bc	7.14 a	19.6 a	298 ab	14.6 a	49.4 ab
44	95.0 ab	100.0 a	206.5 ab	101 abc	5.85 a	17.7 a	243 bcd	13.1 a	54.6 a
45	100.0 a	100.0 a	209.5 ab	94 c	6.02 a	16.7 a	244 bcd	11.9 a	50.2 ab
46	100.0 a	100.0 a	204.5 b	108 ab	6.03 a	16.4 a	228 cd	11.8 a	54.0 ab
SR-4	98.4 ab	98.4 a	208.5 ab	110 a	7.95 a	19.6 a	326 a	14.8 a	46.5 ab
SMD	13.7	8.5	5.81	8.97	5.17	9.2	61.6	6.6	9.2

Note. FG – field germination (%), WS – winter survival (%), HD – heading date (days), PH – plant height (cm), NSP – number of spikes per plant, SWP – spike weight per plant (g), GNP – grain number per plant, GYP – grain yield per plant (g), TKW – 1000-kernel weight (g); SR-4 – 'Sadovska ranozreika-4', a winter wheat standard cultivar, SMD – significative minimum difference ($p < 0.05$); values in a column followed by different letters are significantly different.

per plant. Based on this trait, two groups could be formed: 1) G38, G39, G41, G43 and cultivar SR-4 with grain number per plant between 267–326, and 2) G40, G42, G44, G45 and G46 ranging from 216 to 250 seeds. All genotypes gave awned spikes (like 'Albena' wheat) with red coloured glumes, except for G45 which bore white glumes (as both wheat parents).

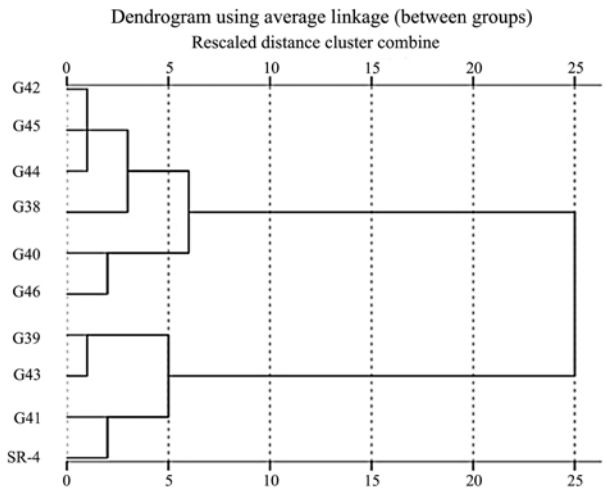
Hierarchical cluster analysis was performed on the nine studied characters to obtain a specific classification of the 10 wheat lines (including SR-4). Differences between genotypes are represented by Euclidean distances. Figure 4 showed a dendrogram of hierarchical cluster analysis using the between-group linkage method. As a result, the genotypes were divided in two clusters when the Euclidean distance of the dendrogram was 10. Cluster I incorporated 4 lines (G39, G41, G43 and SR-4) and cluster II – the rest 6 (G38, G40, G42, G44, G45 and G46). This type of clustering resembled the groups formed by grain number per plant, as stated above, except for G38 which produced 267 seeds per plant and was classified to cluster II. Research on a chromosome number and detailed morphological features in the genotypes is in progress.

Table 6 provides the mean values of crude protein, lysine and sedimentation value in the selected genotypes compared to the mid-parent value of the wheat cultivars 'Albena' and 'Slaveya'. The average mean of the nine families exceeded the mid-parent value in the investigated period. Regarding the protein, the differences were 2.7% and 2.9% for the first and second year, respectively, being largely significant (t_{exp} = 2.90 and 3.50). The average crude protein of the genotypes was 14.05% against 11.25% for the mid-parent value. This positive tendency was very well expressed in the sedimentation value. Its mean varied from 71.4 in 2015 to 65.8 ml in 2016, as compared to 50.2 and 51.4 ml of the mid-parent value, respectively. Lysine content of the investigated lines expressed the same advance over the wheat parents as for protein and sedimentation capacity.

Table 6. Crude protein, lysine and sedimentation value of the selected wheat genotypes compared to the mid-parent values of cultivars 'Albena' and 'Slaveya'

Quality characters	Year	Genotype	Mean ± SD	VC%	t_{exp}	*P*
Crude protein %	2015	G	14.2 ± 0.5	2.2	2.90	0.005
		MP	11.5 ± 0.8	5.5		
	2016	G	13.9 ± 0.5	4.1	3.50	0.001
		MP	11.0 ± 1.0	6.2		
Lysine mg per 100 g DM	2015	G	293.2 ± 7.7	4.2	2.01	0.05
		MP	270.0 ± 9.3	8.6		
	2016	G	278.8 ± 9.7	6.9	2.71	0.01
		MP	251.1 ± 8.7	10.8		
Sedimentation value ml	2015	G	71.4 ± 2.5	10.6	3.20	0.005
		MP	50.2 ± 7.2	12.3		
	2016	G	65.8 ± 2.8	12.9	3.56	0.001
		MP	51.4 ± 4.8	13.3		

Note. Lysine content is reported on a dry matter (DM) basis; G – nine selected wheat genotypes, MP – mid-parent value of the two wheat cultivars, SD – standard deviation, VC% – coefficient of variation, %, t_{exp} – experimental *t* value, *P* – probability value.



Note. The abscissa indicates the Euclidean distances and the ordinate expresses the new wheat genotypes.

Figure 4. Dendrogram of hierarchical cluster analysis of the genotypes based on 9 quantitative characters

The variation coefficients of the three parameters showed that the new progenies performed better in the field than the wheat parents. The variation was weaker on crude protein (2.2% and 4.1% contrasted to 5.5% and 6.2% for the mid-parent value) and larger on the sedimentation value (10.6% and 12.9% to 12.3% and 13.3%, respectively).

Grain protein content (GPC) is a major factor determining end-use quality in wheat, and genetic improvement to attain higher protein content is a key topic in wheat breeding. GPC has been hindered by a high environmental effect and a complex genetic system governing this trait. A Japanese hard red winter wheat cultivar 'Yumechikara' with a high GPC showed three-year mean variation from 12.5% to 16.1%. Unlike *Gpc-B1* derived from tetraploid wheat, a single major QTL (*QGpc.2B-yume*) had no negative effects on yield-component-related traits and could be useful in wheat to increase grain protein content (Terasawa et al., 2016). The information presented here describes the selected genotypes as potential genetic resources for improving the grain quality due to the positive influence of the Glu-D1-4t+10.1t subunit pair on crude protein, lysine and sedimentation value.

We used the urea / SDS-PAGE method to identify new glutenin subunits and monitor their incorporation into common wheat. Xu et al. (2010) and Rasheed et al. (2012) performed the same procedure to detect new HMW glutenin compositions in synthetic wheats. In this experiment, the subunit pair 4+10.1 was inherited from the locus *Glu-D^{t}1* of SHW530-1 due to the presence of bands 7+8 at *Glu-B1* in contrast to synthetic line 530-2 possessing a subunit 22. The subunit null in the *Glu-A1* was transferred from the wheat parent 'Albena', and protein unit 2* – from the wheat 'Slaveya'. In the locus *Glu-B1*, the protein pair 7+8 could be donated either from 'Slaveya' or the synthetic line 530-1.

The introgressed alleles from synthetic wheats have contributed a number of valuable traits such as more spikes per plant, grains per spike, larger grains and higher grain yield potential to the new bred wheat cultivars (Plamenov, Spetsov, 2011; Li et al., 2014). The genomic region linked to Xbarc1183 in 'Chuanmai-42' provided by SHW is a candidate locus that could play an extremely important role in the further development of high-yielding wheat cultivars (Li et al., 2011). The developed 'Shumai-969' expressed a new HMW-GS composition (Dtx3.1 and Dty11) for good quality and a new *Ppd-D1* allele for early heading that were transferred from SHW-L1. Using the SHW-derived lines as breeding sources, 12 new wheat cultivars were developed (Chen et al., 2012). Chromosome engineering of whole or partial segments of chromosomes derived from wheat relatives through chromosome addition, substitution or translocations has been shown to exert significant effects in wheat breeding, on disease resistance, salt tolerance, protein content, and others important crop traits. Wang et al. (2010) investigated 107 wheat alien chromosome addition lines for the *P* efficiency at the seedling stage. Nine addition lines conferred high *P* efficiency, seven lines conferred high *P* uptake, and 39 lines displayed high *P* utilization capacity. In some cases, alien substituted chromosomes in wheat also served as sources for improving the grain quality. For example, the 1S^{l}/1B substitution in 'Chinese Spring' leads to a significant improvement for dough and bread-making quality due to the introgression of two novel HMW-GS 1S^{l}x2.3* and 1Sy16* (Wang et al., 2013). Six near-isogenic lines of the wheat cultivar 'Saratovskaya 29' carrying marker genes derived from different species were studied to have pleiotropic effects on quantitative traits related to spike productivity (Arbuzova et al., 2010). Rye (*Secale cereale* L.) chromosome 1RS harboured multiple genes including *Lr26*, *Sr31*, *Yr9* and *Pm8* conferring disease resistance and tolerance to abiotic stresses. Deleterious effects of the rye translocation on bread making quality have urged to search for other rye and related wheat species with broader genetic diversity (Tahir et al., 2014).

Concerning the LMW glutenins in this experiment, all genotypes expressed allele *c* in the *Glu-A3* and allele *c* in the *Glu-D3*. The former one was derived from the 'Slaveya' wheat, and the latter from any one of the three parents (Tables 1 and 3). Gliadin patterns are genotype-specific, reproducible and not dependent on growing field and laboratory conditions. Utebayev et al. (2016) found 48 alleles of six gliadin-coding loci in 43 cultivars of spring wheat from Northern Kazakhstan. The alleles *Gli-A1f*, *Gli-A2p*, *GliB1e*, *Gli-B2d*, *Gli-D1a* and *Gli-D2e* had maximal frequencies in each locus. In our study, gliadins were equal in all genotypes, *Gli-A1b*, *Gli-B1b* and *Gli-D1b*, and identical to that of 'Slaveya' parent. The other wheat cultivar 'Albena' possessed the alleles *b* in *Gli-B1* and *Gli-D1*, and *f* in *Gli-A1*. So, the gliadins in the genotypes were inherited from the two wheat cultivars and/or the synthetic parent.

Conclusions

1. Pure line selection based on the presence of Glu-D1-4t+10.1t in high molecular weight glutenin subunits (HMW-GS) deriving from a synthetic hexaploid wheat (SHW) line 530-1 along with the grain number and seed weight per plant of selected BC_1F_{5-7} individuals, led to the creation of 9 wheat genotypes.

2. They were tested in the field and showed adequate germination and winter survival with very good seed set. Hierarchical cluster analysis based on nine studied traits divided genotypes in two groups: G39, G41 and G43 in the first, including standard cultivar 'Sadovska ranozreika-4' (SR-4), and G38, G40, G42, G44, G45 and G46 in the second cluster.

3. The new lines expressed two HMW glutenin variants, 2*-7+8–4t+10.1t (8 genotypes) and null-7+8–4t+10.1t (1 genotype). The incorporated HMW glutenin subunit alleles might serve as new genetic resources for quality improvement in wheat due to their positive influence on crude protein, lysine and sedimentation value.

4. The genotypes expressed additionally two ω-gliadin bands: the gliadin #1 originated from SHW530-1, while #2 was transferred from cultivar 'Albena' wheat parent. The former protein band appeared to be the unique gliadin fraction in the lines, not expressed in both wheat parents, which could increase their breeding potential later.

Acknowledgments

We thank the Dobrudzha Agricultural Institute for providing facilities to carry out the crosses, selection and biochemical studies on synthetic hexaploid wheats and their derivatives.

Received 26 08 2017
Accepted 06 04 2018

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ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 105, No. 2 (2018), p. 113–122
DOI 10.13080/z-a.2018.105.015

Naujų atsarginių baltymų perkėlimas iš sintetinės heksaploidinės linijos į paprastąjį kvietį

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Santrauka

Kryžminimo kombinacijoje tarp sintetinio heksaploidinio kviečio (SHW) 530-1 (*Triticum dicoccum* / *Aegilops tauschii* acc. 19088) (2n = 42, $BBA^uA^uD^tD^t$) ir paprastojo kviečio (*T. aestivum* L.) dviejų veislių ‘Albena’ ir ‘Slaveya’ (2n = 42, BBA^uA^uDD) buvo taikyta grynų linijų atranka pagal Glu-D1-4t+10.1t alelį didelio molekulinio svorio gliuteninų subvienetuose. BC1F5-7 kartoje atrinkti augalai, pasižymėję grūdų skaičiumi bei sėklų svoriu ir turintys didelio molekulinio svorio gluteninų subvienetų porą, buvo panaudoti 9 kviečių genotipams sukurti. Jie buvo tirti lauko sąlygomis, gerai sudygo bei peržiemojo ir užmezgė daug sėklų. Taikant klasterinę analizę jie buvo suskirstyti į dvi grupes (keturias linijas), iš jų kviečių standartinė veislė ‘Sadovska ranozreika-4’ yra pirmajame klasteryje, o šešios linijos – antrajame. Taikant urėjos / SDS-PAGE metodą naujosios linijos buvo suskirstytos į du didelio molekulinio svorio gliuteninų variantus: Glu-A1-2*, Glu-B1-7+8 bei Glu-D1-4t+10.1t (8 genotipai) ir Glu-A1-null, Glu-B1-7+8 bei Glu-D1-4t+10.1t (1 genotipas), rodančius vienodus mažo molekulinio svorio gliuteninus. Visos šeimos parodė dvi ω-gliadino juostas: gliadinas #1 išskirtas iš SHW 530-1, o #2 buvo perkeltas iš kviečių veislės ‘Albena’. Pirmasis – unikalus gliadino subvienetas genotipuose, kuris nebuvo pasireiškęs abiejose tėvinėse linijose. Inkorporuoti didelio molekulinio svorio gliutenino aleliai gali būti naujais genetiniais ištekliais siekiant pagerinti šiuolaikinių paprastųjų kviečių grūdų kokybę.

Reikšminiai žodžiai: duoniniai kviečiai, gliadinai, gliuteninai, sėklų atsarginiai baltymai, sintetiniai heksaploidiniai kviečiai.