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PHENOTYPIC AND GENOTYPIC EVALUATION OF SYNTHETIC HEXAPLOID WHEAT LINES (AABBDD) FOR GRAIN PARAMETERS UNDER THE CONDITIONS OF WESTERN SIBERIA

I.V. POTOTSKAYA [1], V.P. SHAMANIN[1], S.S. SHEPELEV[1], V.E. POZHERUKOVA[1], A.I. MORGOUNOV[2]

[1]*Stolypin Omsk State Agrarian University*, 1, Institutskaya pl., Omsk, 644008 Russia, e-mail iv.pototskaya@omgau.org (✉ corresponding author), vp.shamanin@omgau.org, sergeyschepelew@mail.ru, ve.pozherukova@omgau.org
[2]*International Maize and Wheat Improvement Center (CIMMYT)*, P.K. 39 Emek, 06511, Ankara, Turkey, e-mail a.morgounov@CGIAR.ORG
ORCID:
Pototskaya I.V. orcid.org/0000-0003-3574-2875
Shamanin V.P. orcid.org/0000-0003-4767-9957
Shepelev S.S. orcid.org/0000-0002-4282-8725
Pozherukova V.E. orcid.org/0000-0001-8429-2167
Morgounov A.I. orcid.org/0000-0001-7082-5655
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Abstract

Wheat is both a vital food crop and an economically important commodity for Russia. Plant breeders are increasingly looking to wild relatives to introduce new genes and alleles to obtain high, stable wheat yields. In this paper, we present the study results for grain morphometric parameters and genotyping with usage of 47 SNP (single nucleotide polymorphisms), including 13 markers assosiated with genes responsible for grain size/weight. For the first time in Western Siberia these data were obtained for synthetic hexaploid wheat genotypes set (the genome AABBDD) in which the genome D was transferred from *Aegilops tauschii*, originated from regions with the highest genetic diversity of this species. The evaluation of 47 synthetic hexaploid wheat lines was performed on the experimental field of Omsk SAU (Omsk city) in 2016-2018. The synthetic lines were created in CIMMYT by crossing durum wheat (*Triticum durum* Desf., AB genome) with different entries of *Aegilops tauschii* Coss. (synonym *Ae. squarrosa*, D genome) from Germaplasm Bank (CIMMYT). Synthetic lines of Kyoto University (Japan) were bred via hybridization of durum wheat variety Langdon (USA) with entries of *Aegilops tauschii* of different ecological origin. After harvesting the structural analysis of yield components, i.e. the number of tillers per plant, number of spikelets per spike, grains per spike, grains per plant, grain weight per spike, grain weight per plant, thousand kernel weight, was carried out. The basic morphometric parameters of grain (area, length, width, perimeter, circularity) were evaluated. Synthetic lines were genotyped with 47 SNP markers using KASP^{TM} technology («LGC Genomics», UK). Synthetic lines were characterized by low variability of grain parameters (Cv = 3.3-6.5 %), higher grain lenght (7.58 mm), area (21.1 mm^2), perimeter (19.7 mm), and thousand kernel weight (34.9-46.7 g) than standard variety Pamyati Azieva. In 2017-2018, the grain yield positively correlated with grain lenght, area, perimeter and negativeley correlated with grain quality indicators (protein and gluten content). An insignificant relationship between the grain lenght and width (r from –0.08 to –0.23) was established. The results indicate that synthetic wheat lines are useful genetic donors for increasing thousand kernel weight due to genes *TaTPP6A*, *TaGW2-6A*, *TaGASR-A1*, *TaGS5-3A*, *TaTGW6*, *TaTGW-7A*, *TaCwi-A1*, *TaGS-D1*, and *TaCKX-D1*. The winter durum wheat varieties, which were used in the creation of synthetic lines, and genes of the A genom mainly contribute to grain size/weight. Synthetic lines from Kyoto University had larger and heavier grains (length 8.22-8.51 mm; thousand kernel weight 43.8-46.4 g) compared to the CIMMYT lines. Hybrid combinations Aisberg/*Ae. squarrosa* (511), Ukr.-Od.1530.94/*Ae. squarrosa* (392), Ukr.-Od.1530.94/*Ae. squarrosa* (1027), and Langdon/*Ae. squarrosa* had the highest thousand kernel weight. Lines No. 8 Ukr.-Od.1530.94/*Ae. squarrosa* (392), No. 37 Ukr.-Od.1530.94/*Ae. squarrosa* (310), No. 44, 46 Ukr.-Od.1530.94/*Ae. squarrosa* (1027), No. 27 Leuc 84693/*Ae. squarrosa* (392), No. 41 Ukr.-Od.1530.94/*Ae. squarrosa* (1027), No. 29

Lang-don/IG 126387 (with exception of *TaGW2*) are recommended as promisis soucers for pyramiding genes *TaCwi-A1*, *TaGASR-A1*, *TaGS5-3A*, *TaGW2*, *TaTGW-7A, TaGS-D1*, and *TaCKX-D1*, which are responsible for grain size and thousand kernel weight. The indentified entries in this studies are valuable souces of combination of genes responsible for grain size and thousand kernel weight for breeding under conditions of Western Siberia region.

Keywords: synthetic wheat, grain size, thousand kernel weight, SNP marker, breeding

A thousand kernel weight is one of the most stable trait of wheat productivity the increase of which is topical for breeding [1, 2]. The grain size and shape are closely related to crop yields and milling properties. Grain of modern wheat varieties is wider in width and shorter in length compared to wild relatives which demonstrate greater variability in grain size and shape. This is due to the effect of domestication and breeding of wheat during which the shape of the grain became more rounded resulting in higher flour yield [3]. Larger grains also have larger germination energy, which favorably affects the increase in yield [4, 5].

Bread wheat (*Triticum aestivum* L.) is a natural allohexaploid with subgenomes A, B and D, of which D subgenome was introduced from *Aegilops tauschii* Coss. Probably only some accessions of *Ae. tauschii* participated in crossing with tetraploid wheat (*Triticum turgidum* L., AABB), which led to the limited polymorphism of genome D present in cultivated wheat [6].

Genetic potential of subgenome D in wheat breeding are beeing mobilized via created synthetic hexaploids or by direct hybridization between *Ae. tauschii* and commercial varieties [7]. Many researchers consider synthetic hexaploid wheat a promising source of economically valuable traits, i.e. a high thousand kernel weight, spike productivity and the number of productive plants per unit area, for impoving cultivated varieties [8]. The thousand kernel weight is under polygenic control. QTLs responsible for this trait are mapped on almost all wheat chromosomes. The thousand kernel weight has a high coefficient of heritability and is closely related to grain length, width, area, and perimeter [9-11].

Increasing wheat yield include molecular methods for identifying genes that control the size and weight of a wheat grain. Their search is complicated by the complex structure and large genome of common wheat [12]. Many genes that control the size, shape and weight of the grains are orthologous genes of those already identified in other crops, the rice, barley, and sorghum. A comparative search for such genes in wheat contributes to the expansion of polymorphism in productivity parameters [13]. *TaGW2* [14], *TaCwi-A1* [15], *TaGaSR7-A1* [16], and *TaTGW6* [17] genes that determine the grain size and thousand kernel weight in wheat were originally identified in the rice genome [18].

Ae. tauschii accessions possess significant genetic and phenotypic variability for grain size and weight compared to bread wheat [11, 19]. Synthetic hexaploid wheat with genome D from various *Ae. tauschii* forms also have a broader polymorphism of loci controlling size, shape and weight of grain [18]. In synthetic wheat hexaploids, the QTLs with a pleiotropic effect on the grain length, width, area, and perimeter were mapped on chromosomes 2DL and 7DS [20]. In a synthetic wheat line of the ITMI collection tested under various ecological conditions of Russian regions QTLs for thousand kernel weight were mapped on chromosomes 4A, 1B, 3B, 1D, and 2D [9]. More recently, when examining this collection in Western Siberia, QTL for thousand kernel weight was mapped on chromosome 6A [21].

In recent years, functional markers habe been devepoled for genes that contribute to wheat productivity, *TaSus2-2B*, *TaCwi-A1*, *TaGW2*, *TaGW2-6B*, *TaCKX6-D1*, *TaGS-A1*, *TaSAP1-A1*, *TaGASR7-A1*, *TaGS-D1*, and *TaTGW6* [12, 22]. Searching for sources of genes that control grain size and weight, and

the DNA markers closely linked to these genes, ensured creation of commercial varieties with genetically determined traits to increase wheat productivity in Western Siberia.

This paper is the first report on grain morphometry and analysis of yield structure elements coupled with genotyping by 47 SNP (single nucleotide polymorphisms) of synthetic hexaploid wheat lines in Western Siberia. As a result, we identified valuable genetic sources for increasing grain size and weight in bread wheat varieties.

Our objective was the phenotypic and genotypic assessment of synthetic wheat lines (AABBDD genome) for grain parameters and the selection of the best samples for breeding in Western Siberia.

Materials and methods. A total of 47 synthetic hexaploid wheat lines were tested in 2016-2018 (experimental field of Omsk State Agrarian University, Omsk). CIMMYT synthetic lines were developed by crossing durum wheat varieties from the Institute of Breeding and Genetics (Odessa, Ukraine) and variety Pandur (Romania) (*Triticum durum* Desf., genome AB) with *Aegilops tauschii* Coss. (syn. *Aegilops squarrosa*, genome D) from Germaplasm Bank (CIMMYT). No cytological control after colchicization of F_1 was applied. Fertile plants with good agronomic traits were selected in F_4 hybrid populations, followed by their reproduction till F_7. The synthetic lines of Kyoto University (Japan) were obtained via hybridization of the durum wheat variety Langdon (USA) with *Aegilops tauschii* accessions of various ecological origins.

In 2016, each line was sown in a 1 m row, at 25-30 seeds per row. A mid-early season variety Pamyati Azieva variety and mid-late season Serebryristaya variety were the standards alternately sown after every 5th test plant in the row. In 2017-2018, each line was sown on a 1.4 m^2 plot, at 25 g seeds per plot. The same standard varieties were alternatively sown after every 10th test plant. Row spacing was 15 cm. Field trials utilized a randomized complete block design with four replicates.

Plant productivity elements (the number of productive stems per plant, the number of productive spikelets per spike, grain number per main spike, per spike, and per plant, grain weight per main spike, grain weight per plant, and thousand kernel weight) were estimated after harvesting. The thousand kernel weight was evaluated by counting and weighing grains from a plant, followed by recalculation per 1000 grains.

In 2016, 20 plants of each line and standards were analyzed, in 2017-2018, 10 plants were collected from the middle row at each plot (in four replicates). Grain morphometric parameters (area, length, width, perimeter, circularity) were assessed with Smart Grain v. 1.2 software (http://www.kazusa.or.jp/phenotyping/smart-grain/index.html). A total of 40 grains per line were examined in 2016, 400 grains in 2017-2018. Synthetic lines were genotyped by KASP™ technology (LGC Genomics, UK) with 47 SNP markers, including 13 markers linked to genes that control grain size and weight.

An Oktopure automated system (Biosearch Technologies, UK) and sbeadex™ plant kit (LGC Genomics, UK) were used for DNA extraction. The final DNA concentration in the samples was normalized to 50 ng/l, and 5 μl aliquotes were added into wells of a 96-well microplate (a repliKator automated station, LGC Genomics, UK). KASP™ genotyping mixture (LGC Genomics, UK) containing 5 μl of KASP Master-Mix and 0.14 1 of KASP Assay Mix (allele-specific primers) were added to each well in an automated mode. PCR was carried out for 1 h 20 min in sealed microplates (a Hydrocycler2™ thermocycler,

LGC Genomics, UK), followed by measurement of FAM/HEX fluorescence (a multifunctional Pherastar reader, BMG Labtech, USA).

Statistical processing comprised calculation of mean (*M*), standard error of the mean (±SEM), and variance analysis. Statistical significance of differences was assessed by the least significant difference at 5 % significance level (LSD_{05}) [27] (Microsoft Excel software). Two-way analysis of variance (ANOVA) was performed for 2 years (2017-2018) with STATISTICA v. 6.0 software (StatSoft, Inc., USA). Variability of traits, as depended on the conditions of the growing season and genotype was assessed by Fisher's *F*-test. Principal component analysis (PCA) was performed using Microsoft R Open 3.3.3 software (https://mran.microsoft.com/download).

Results. Table 1 comprises the list of spring synthetic wheat lines used in the work, table 2 describes SNP markers.

1. Origin of tested synthetic hexaploid wheat lines (AABBDD)

Combination	Numer	*Aegilops tauschii*	
		origin	subspecies
CIMMYT synthetic lines			
Aisberg/*Ae. squarrosa* (369)	5	Mazenderan, Iran	*tauschii*
Aisberg/*Ae. squarrosa* (511)	5	Unknown	Unknown
Leuc. 84693/*Ae. squarrosa* (409)	1	Dagestan, Russia	*tauschii*
Ukr.-Od.952.92/*Ae. squarrosa* (1031)	4	Zanjan, Iran	*tauschii*
Ukr.-Od.1530.94/*Ae. squarrosa* (310)	3	Jilan, Iran	*strangulata*
Ukr.-Od.1530.94/*Ae. squarrosa* (392)	2	Shamakhi, Азербайджан	*tauschii*
Ukr.-Od.1530.94/*Ae. squarrosa* (458)	3	Unknown	Unknown
Ukr.-Od.1530.94/*Ae. squarrosa* (629)	3	Mazenderan, Iran	*strangulata*
Ukr.-Od.1530.94/*Ae. squarrosa* (1027)	10	Mazenderan, Iran	*tauschii*
Pandur/*Ae. squarrosa* (223)	1	Jilan, Iran	*tauschii*
Pandur/*Ae. squarrosa* (409)	1	Dagestan, Russia	*tauschii*
Kyoto University synthetic lines			
Langdon/Ku-20-9	1	Babulsar, Iran	*strangulata*
Langdon/Ku-2075	1	Babulsar, Iran	*strangulata*
Langdon/Ku-2088	1	Sari, Iran	*strangulata*
Langdon/Ku-2092	1	Babulsar, Iran	*strangulata*
Langdon/Ku-2093	1	Babulsar - Chalus, Iran	*strangulata*
Langdon/Ku-2097	1	Babulsar, Iran	*typica*
Langdon/Ku-2105	1	Baklava, Iran	*typica*
Langdon/IG 48042	1	Jammu Kashmir, India	Unknown
Langdon/IG 126387	1	Ashgabat, Turkmenistan	Unknown

Note. KU — Plant Germ-Plasm Institute, Faculty of Agriculture, Kyoto University, IG — International Center for Agricultural Research in the Dry Areas (ICARDA).

2. SNP markers associated with grain size/weight genes of synthetic wheat (AABBDD)

Gene	Chromosome	Marker	Reference
TaCwi-A1	2A	Cwi-A1_SNP	[15]
TaSus2-2A	2A	TaSus2_2A_20_SNP[a]	[23]
TaTGW6-A1	3A	TaTGW6_SNP	[22]
TaGS5-3A	3A	GS5_2334_ SNP	[24]
TaTPP-6A	6A	TPP-6A	[25]
TaGW2-6A	6A	TaGW2_ SNP	[14]
TaGASR-A1	7A	TaGASR_IND	[22]
TaTGW-7A	7A	TGW7A_985	[30]
TaSus2-2B	2B	TaSus2_2B _SNP[a]	http://www.cerealsdb.uk.net
TaSus1-7B	7B	TaSus1_7B_2932_IND[a]	[26]
TaCKX-D1	3D	CKX-D1_IND	[22]
TaCwi-5D	5D	CWI5D_SNP[a]	[22]
TaGS-D1	7D	TaGS-D1_SNP	[22]

Note. Letter [a] means absence of the marker amplification in synthetic lines.

In May 2016, air temperatures were elevated with the absence of precipitation, which led to strong early spring drought. In the second part of the growing season, the weather was moderately warm with heavy rainfall (hydrothermal coefficient HTC = 1.8), favorable for high leaf and stem rust infection, which

resulted in producing smaller and shrunker grain. In 2017, an early summer drought typical of the southern forest-steppe of Western Siberia occurred in the first part of the growing season (HTC = 0.5). During the heading—ripening period, moisture conditions were favorable for grain filling and ripening. In 2018, the weather was cool with a large amount of precipitation during formation of generative organs (HTC = 1.2), with a positive effect on formation of large and completed grains.

CIMMYT and Kyoto University synthetic lines with six durum wheat varieties and various *Ae. tauschii* accessions in pedigree were significantly polymorphic in grain size and weight, which indicats unexploited potential for breeding for high productivity. Meteorological conditions during the growing season significantly influenced the formation of morphometric parameters of grains (Table 3).

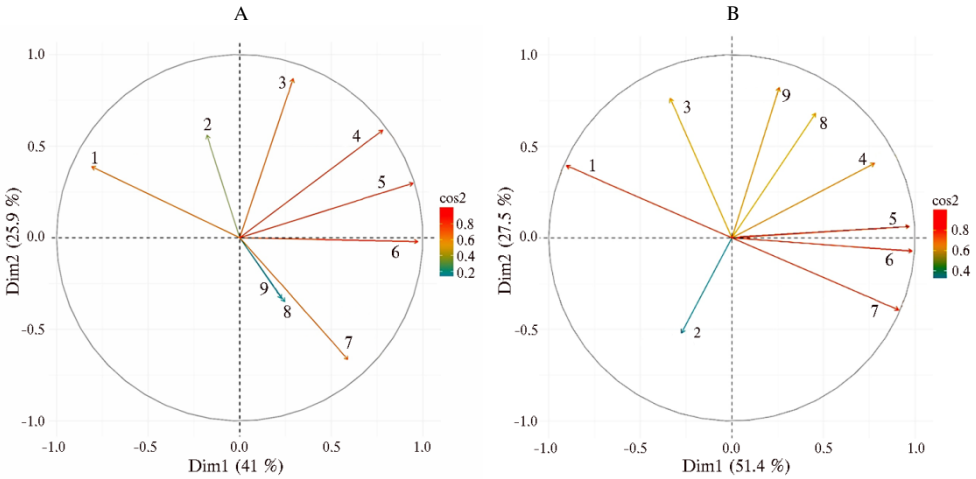
3. Main morphometric parameters of grain amd thousand kernel weight of hexaploid synthetic wheat lines (AABBDD) over years of research (experimental field of Omsk SAU)

Trait	Year	*M*±SEM	Min-max	Pamyati Azieva (standard), *M*±SEM	*Cv*, %	$F_{observed}$	
						genotype	year
Grain length, mm	2016	7.07±0.07	5.66-8.50	6.45±0.04	7.4	4.29**	203.30**
	2017	7.31±0.06	6.48-8.59	6.48±0.03	6.5		
	2018	8.37±0.07	7.43-9.44	7.68±0.02	5.7		
	2016-2018	7.58±0.06	6.74-8.51	6.87±0.03	5.4		
Grain width, mm	2016	2.93±0.03	2.10-3.30	3.29±0.03	8.1	1.47	264.90**
	2017	3.42±0.02	3.10-3.66	3.29±0.02	3.8		
	2018	3.79±0.02	3.48-4.05	3.83±0.01	3.1		
	2016-2018	3.38±0.02	3.04-3.60	3.47±0.02	3.3		
Grain area, mm^2	2016	18.80±0.35	15.90-22.50	15.9±0.17	7.8	2.35**	142.80**
	2017	20.50±0.21	14.20-25.50	15.90±0.14	12.1		
	2018	23.90±0.21	21.00-28.20	22.10±0.14	6.0		
	2016-2018	21.10±0.19	17.90-23.60	18.00±0.15	6.5		
Grain perimeter, mm	2016	18.60±0.15	16.80-21.40	16.60±0.17	3.5	5.87**	53.10**
	2017	19.20±0.15	18.20-20.50	16.80±0.13	5.6		
	2018	20.90±0.13	19.00-22.90	19.50±0.16	4.4		
	2016-2018	19.70±0.14	18.20-21.80	17.70±0.15	4.9		
Grain circularity	2016	0.680±0.004	0.63-0.69	0.730±0.005	2.7	0.27	0.56
	2017	0.690±0.003	0.63-0.75	0.720±0.003	4.1		
	2018	0.690±0.003	0.60-0.75	0.730±0.001	3.8		
	2016-2018	0.690±0.004	0.63-0.75	0.730±0.003	3.8		
Thousand kernel weight, g	2016	34.90±2.10	23.30-44.50	33.20±0.59	14.5	1.68*	146.50**
	2017	45.70±2.37	33.00-57.40	42.10±0.91	11.6		
	2018	46.70±1.66	37.60-53.70	44.70±0.66	8.4		
	2016-2018	42.40±1.39	35.70-51.30	39.40±0.72	7.7		

*, ** Variance is statistically significant at $p < 0.05$ and $p < 0.01$, respectively.

Genotypic differences between synthetic lines on the studied traits were reliable at 5% significance level, with the exception of the width and circularity of the grain. On average, for 2016-2018, synthetic lines had low variability of grain morphometric parameters (*Cv* = 3.3-6.5%) and higher average values of the length, area, perimeter of the grain, and thousand kernel weight than the standard (Pamyat Aziev variety) (see Table 3). The circularity indirectly characterizes the grain shape, i.e. the higher it is, the grain closer approaches in shape to the ball and, accordingly, the higher the flour output [28]. In general, the grain of the synthetic lines had a smaller width and circularity compared to standard Pamyati Azieva.

In 2017-2018, synthetic lines had a grain width comparable to the standard under weak development of rust diseases. The analysis of principal components (PCA) based on the correlation matrix revealed the relationship between the synthetic line yields and grain morphometric parameters (Fig.).



Analysis of principal components (PCA) for 47 synthetic hexaploid wheat lines (AABBDD) shows the relationship of yield with grain morphomentic parameters, protein content and gluten content: A — 2017, B — 2018; 1 — cyrcularity, 2 — yield, g/m^2, 3 — grain wigth, mm, 4 — grain area, mm^2, 5 — grain perimeter, mm, 6 — grain length, mm, 7 — grain lengh to grain width ratio, 8 — gluten content, %, 9 — protein content, %; cos2 — vector of initial variables indicating the significance of the principal component (experimental field of Omsk SAU).

The first main component (Dim1) contributed 41.0% to the total phenotypic variability of the studied parameters in 2017 and 51.4% in 2018. The contribution of the second component (Dim2) was 25.9 and 27.5%, respectively. A two-dimensional graphical representation of the results of PCA analysis revealed a closer relationship between yield and width and shape of the grain (circularity) in 2017 and less close in 2018. This is due to the fact that under favorable moisture conditions in 2018, the number of productive tillers made a greater contribution to the yield.

In both years of research, there was a positive correlation of yield with the grain length, area, perimeter and a negative relationship with grain quality indicators (protein and gluten content). We also established an insignificant relationship between the length and width of the grain (r from –0.08 to –0.23), which is consistent with data from other works [18, 19]. Consequently, the selection of synthetic lines with a wider and rounded grain can reveal high yilding synthetic wheat genotypes for breeding.

Assessment of polymorphism at the loci responsible for grain size and weight with 13 SNP markers revealed the amplification of 9 markers in the genotypes of synthetic forms. The occurrence of the identified loci in the studied synthetic lines was different: 11.1% for *TaTTP6A*, 25.3% for *TaGW2-6A*, 30.6% for *TaGASR-A1*, 30.6% for *TaGS-D1*, 55.6% for *TaGS5-3A*, 66.7% for *TaTGW6*, 72.2% for *TaTGW-7A*, 77.8% for *TaCwi-A1*, and 100% for *TaCKX-D1*. As compared to the standards (Pamyati Azieva and Serebristaya), the synthetic lines have *TaGW2*, *TaTPP6A*, and *TaTGW6* loci. Table 4 presents the best synthetic lines with a combination of 4-7 genes of *TaCwi-A1*, *TaTGW6*, *TaGASR-A1*, *TaGS5-3A*, *TaTPP-6A*, *TaGW2*, *TaTGW-7A*, *TaGS-D1*, and *TaCKX-D1*, selected by the morphomentic parameters and thousand kernel weigh.

Phenotypic estimates of the hexaploid synthetic wheat lines showed that the studied morphometric characteristics of grain in the lines identified over 3 years were higher than the standards, except for the width of the grain. Lines No. 8 Ukr.-Od.1530.94/*Ae. squarrosa* (392), No. 37 Ukr.-Od.1530.94/*Ae. squarrosa* (310), No. 44 and No. 46 Ukr.-Od.1530.94/*Ae. squarrosa* (1027) had higher

thousand kernel weight and two lines exceeded the Serebryristaya standard in the grain width. Japanese breeding lines No. 29 Langdon/IG 126387 и No. 63 Langdon/Ku-2092 also had a higher thousand kernel weight and a longer grain length (see Table 4).

4. Grain morphometric parameters and 1000-grain weight in the best studied lines of hexaploid synthetic wheats (AABBDD) (*M*±SEM, experimental field of Ovsk SAU, 2016-2018)

Line No.	Variety, line	Gene number	Grain				Circularity	Thousand kernel weigh, g
			lenghth, mm	wigth, mm	area, mm^2	perimeter, mm		
8	Ukr.-Od.1530.94/ *Ae. squarrosa* (392)	7	6.94±0.04	3.55±0.03^{b}	20.70±0.33^{ab}	18.40±0.14^{ab}	0.750±0.004^{ab}	43,80±1,87^{a}
24	Aisberg/ *Ae. squarrosa* (511)	5	8.00±0.06^{ab}	3.40±0.03	22.50±0.18^{ab}	20.70±0.15^{ab}	0.660±0.003	45,40±2,79^{ab}
27	Leuc 84693/ *Ae. squarrosa* (409)	6	7.87±0.05^{ab}	3.37±0.02	22.00±0.32^{ab}	20.60±0.16^{ab}	0.670±0.003	43,30±3,00^{a}
37	Ukr.-Od.1530.94/ *Ae. squarrosa* (310)	7	7.24±0.07	3.54±0.02^{b}	21.60±0.44^{ab}	18.80±0.12^{ab}	0.730±0.002^{b}	43,50±2,92^{a}
41	Ukr.-Od.1530.94/ *Ae. squarrosa* (1027)	6	7.39±0.06^{ab}	3.43±0.03	20.90±0.41^{ab}	19.80±0.18^{ab}	0.710±0.004	46,80±3,02^{ab}
44	Ukr.-Od.1530.94/ *Ae. squarrosa* (1027)	7	7.52±0.04^{ab}	3.48±0.03	21.90±0.21^{ab}	19.90±0.16^{ab}	0.700±0.002	43,70±1,98^{a}
46	Ukr.-Od.1530.94/ *Ae. squarrosa* (1027)	7	7.64±0.04^{ab}	3.60±0.02	22.70±0.42^{ab}	20.10±0.18^{ab}	0.710±0.003	51,30±2,66^{ab}
29	Langdon/IG 126387	6	8.22±0.06^{ab}	3.04±0.03	21.90±0.29^{ab}	20.70±0.16^{ab}	0.650±0.002	43,80±1,87^{a}
63	Langdon/Ku-2092	4	8.41±0.06 ^{ab}	3.39±0.03	23.30±0.33^{ab}	21.30±0.17^{ab}	0.660±0.004	46,40±3,17^{ab}
65	Langdon/Ku-2105	4	8.51±0.05^{ab}	3.39±0.03	23.60±0.24^{ab}	21.80±0.14^{ab}	0.640±0.004	44,70±3,04^{ab}
Pamyati Azieva (standard)		4	6.87±0.03	3.47±0.02	18.00±0.15	17.70±0.15	0.730±0.003	39.40±0.72
Serebristaya (standard)		4	6.89±0.04	3.33±0.02	17.90±0.27	17.20±0.17	0.720±0.003	40.30±1.31
LSD_{05}			0.47	0.21	2.14	0.73	0.01	3.62

$^{a, b}$ Differences between synthetic lines and standards (Pamyati Azieva and Serebristaya) are statistically signiificant at $p < 0.05$.

We have identified 7 loci (*TaCwi-A1*, *TaGASR-A1*, *TaGS5-3A*, *TaGW2*, *TaTGW-7A*, *TaGS-D1*, *TaCKX-D1*) in lines No. 8 Ukr.-Od.1530.94/*Ae. squarrosa* (392), No. 37 Ukr.-Od.1530.94/*Ae. squarrosa* (310), No. 44 and No. Ukr.-Od.1530.94/*Ae. squarrosa* (1027), and 6 loci we identified in lines No. 27 of Leuc84693/*Ae. squarrosa* (409), No. 41 Ukr.-Od. 1530.94/*Ae. squarrosa* (1027), and No. 29 Langdon/IG 126387 (except *TaGW2*). These lines are recommended for wheat selection as sources of a combination of genes that are responsible for grain size and thousand kernel weight. Several paperes report the possibility of increasing the size and weight of grain in spring bread wheat varieties using sources of similar loci, *TaCwi-A1* (29), *TaGW2* (14), *TaGASR-A1* (22), *TaGS5-3A* [24], TaTGW-7A [30]. However, these loci cannot be deemed crucial until further phenotyping of synthetic lines in specific soil and climatic conditions. Since many of the identified loci that control plant productivity traits are insignificant or minor, it is necessary to identify key loci that determine the formation of a quantitative trait, although the contribution of such loci is certainly determined by the limiting environmental factor [9, 31].

Our paper shows the first rusults of phenotyping performed in Western Siberia for grain morphometric parameters of synthetic hexaploid wheat lines with D genome of *Ae. tauschii* subspecies, originating from regions with the greatest genetic diversity of this species, i.e. from the southwestern coast of the Caspian Sea (*Ae. tauschii* ssp. *tauschii*), and from Azerbaijan and the northern provinces of Iran, Golestan and Mazenderan (*Ae. tauschii* ssp. *strangulata*) [32, 33].

Our findings indicate a high genetic diversity of synthetic wheat lines as genetic sources for increasing size and weight of the grain of bread wheat varieties in Western Siberia. The largest polymorphism of thousand kernel weigh and

the main morphometric characteristics of the grain is noted in hybrids combinations Aisberg/*Ae. squarrosa* (369), Ukr.-Od.1530.94/*Ae. squarrosa* (1027) (Iran, Mazenderan) and Langdon/*Ae. squarrosa* (Iran, Turkmenistan, and India). Aisberg/*Ae. squarrosa* (511), Ukr.-Od.1530.94/*Ae. squarrosa* (392), Ukr.-Od.1530.94/*Ae. squarrosa* (1027) combinations and synthetic lines created on the basis of durum wheat variety Langdon had maximum thousand kernel weight values.

The involvement of Ukr.-Od.1530.94, Aisberg (Ukraine), and Langdon (USA) durum winter wheat varieties in crossing contributed to higher genetic diversity of synthetic lines in terms of grain size and weight, as it is confirmed by larger grain size and higher 1000-grain weight of synthetic lines compared to the standards, together with detection of *TaGW2*, *TaTPP6A*, and *TaTGW6* loci. We have identified *TaCKX-D1* and *TaGS-D1* genes that control these traits [12, 18] in D subgenome introduced into the synthetic lines from *Ae. tauschii*. In the future, we plan to assess polymorphism of genome D in these synthetic lines for kernel size/weight genes with expanded set of SNP markers.

The positive correlation revealed in synthetic lines between the yield and the grain parameters indicates the prospect of selection forms with wider rounded grain and greater endosperm volume, therefore, having a larger weight, which is confirmed by data from other studies [18, 19].

For the success of valuable introgressions from synthetic wheat forms during hybridization with commercial varieties, their pre-selection study is of no small importance. Yan et al. [11] note a negative correlation between the thousand kernel weight and the productive spikes number per plant, and also the grain number per spike. Therefore, valuable introgressions from synthetic hexaploids should be investigated both for the loci that control grain size and for their effect on yield and its components in different genotypes of bread wheat. Kyoto synthetic lines produced a larger and heavier grains compared to the CIMMYT lines, but were less adaptable and productive under conditions of Western Siberia. The lines No. 8 Ukr.-Od. 1530.94/*Ae. squarrosa* (392), No. 37 Ukr.-Od.1530.94/*Ae. squarrosa* (310) and No. 44, No. 46 Ukr.-Od.1530.94/*Ae. squarrosa* (1027) (*TaCwi-A1*, *TaGASR-A1*, *TaGS5-3A*, *TaGW2*, *TaTGW-7A*, *TaGS-D1* and *TaCKX-D1*), No. 27 Leuc 84693/*Ae. squarrosa* (409), No. 41 Ukr.-Od.1530.94/*Ae. squarrosa* (1027), No. 29 Langdon/IG 126387 (except *TaGW2*) can be recommended for pyramiding of the grain size/weight determining genes in wheat breeding.

Thus, grain size phenotyping and genotyping of hexaploid synthetic wheat lines perforemed in Western Siberia, indicates their significance as sources of grain size and thousand kernel weight gene combinations for new commercial varieties ensuring high and stable yield in the region. Assessment of the main grain morphometric parameters, their variability under the influence of the environmental factors, and the relationship of these traits with yield revealed low variability of grain morphometric parameters (Cv = 3.3-6.5%), However, grain average length (7.58 mm), area (21.1 mm^2), perimeter (19.7 mm), and thousand kernel weight (34.9-46.7 g) are higher compared to the standards. In the synthetic lines nine loci determining grain size and thousand kernel weight, the *TaTTP6A*, *TaTGW2-6A*, *TaGASR-A1*, *TaGS5-3A*, *TaTGW6*, *TaTGW-7A*, *TaCwi-A1*, *TaGS-D1*, and *TaCKX-D1* are identified.

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