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# Characterisation of synthetic hexaploid wheat (BBA<sup>u</sup>A<sup>u</sup>A<sup>m</sup>A<sup>m</sup>) and its potential use in wheat breeding

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# Abstract

Free-threshing einkorn wheat (*Triticum sinskajae* A. Filat. et Kurk.) is an excellent source of resistance against wheat diseases and grain quality parameters. A new synthetic hexaploid amphiploid SHW45 (*T. durum* 198-2 × *Triticum sinskajae* acc. 18397) was studied in  $C_{2,7}$  generations and characterised for field growing. Some traits such as a plant height and spike features including fertility and spike fragility revealed a large phenotypic variation. A strong selection for resistance to spikelet dispersal and a number of seeds per selfed spike led to the creation of several lines. They manifested a variety of resistance to powdery mildew at the seedling stage. Three amphiploid lines with diverse phenotypes and a complete resistance to spike brittleness were crossed as females to the wheat cultivar 'Sadovska ranozreika-4'. Viable hybrid seeds are obtained only when the synthetic form is a female parent. F<sub>1</sub> hybrids of amphiploid line 45-10 bore an average of 9 seeds per isolated spike, which was a significantly better yield than that of other hybrids. The lines showed homogeneity in high molecular weight (HMW) glutenins, as subunit pairs Ax2\*+Ay1 and Bx17+By18 are inherited from the *T. sinskajae*, and *T. durum* form 198-2, respectively. In total, 14 F<sub>2</sub>, 22 BC<sub>1</sub>, and 141 F<sub>2</sub>-BC<sub>1</sub> seeds from the crosses with two bread wheat cultivars were planted in the field. The synthetic amphiploid lines might be promising genetic resources for resistance to powdery mildew and grain quality in wheat breeding.

Keywords: Triticum sinskajae, synthetic amphiploid, breeding traits, resistance to powdery mildew, HMW glutenins.

# Introduction

Wheat (*Triticum* sp.) is one of the world's small important crops, and as in many other plant species introducing novel genetic diversity has become increasingly important to achieve a high efficiency in wheat breeding programs. Interspecific hybridisation is a reliable method to transfer useful genetic materials from one species to another one. Diploid wheat species (*T. uraru, T. boeoticum, T. monococcum*, and *T. sinskajae*) from the *Monococcum* Dum. section represent a large genetic variation, as they have two genomes, A<sup>u</sup> and A<sup>b</sup> (Goncharov, 2011; Sharma et al., 2021). Evolutionary, durum wheat (2n=28, BBA<sup>u</sup>A<sup>u</sup>) has received the A genome from *T. urartu* (A<sup>u</sup>A<sup>u</sup>), while *T. boeoticum* donated the A<sup>b</sup> genome to *T. monococcum* (A<sup>b</sup>A<sup>b</sup>) and *T. zhukovski* (GGA<sup>u</sup>A<sup>u</sup>A<sup>b</sup>A<sup>b</sup>) (Sharma et al., 2021).

Among the diploid wheat species, *T. sinskajae* (Sinskaya wheat, 2n=14,  $A^{m}A^{m}$ ) A. Filat. et Kurk. is the youngest taxon discovered in the accession K-20970 of cultivated einkorn wheat in the second half of 20th

century (Filatenko, Kurkiev, 1975). In Russia, T. sinskajae is represented by a single accession K-48993, in many studies (Goncharov et al., 2007; 2020; Kuluev et al., 2018). The authors stated that this species was a natural naked mutant of hulled diploid T. monococcum. Compared to it, Sinskaya wheat has another ear shape, easily oval einkorn spikelets, and a less fragile rachis with additional morphological spike traits (Goncharov et al., 2007). The two species are crossable and produce fertile progenies, which can be used in genetic and breeding experiments (Kurkiev, Filatenko, 2000). To develop early, freethreshing and semi-dwarf einkorn lines, Watanabe (2017) utilised two different genes determining free threshing of einkorn wheat, one sog (soft glume) of T. sinskajae and another sos (soft spike) of T. monococcum. Kuluev et al. (2018) found differences between T. sinskajae and T. monococcum in the variable region of the histone gene H3.2, and the random amplified polymorphic DNA (RAPD) analysis showed the presence of unique polymorphic loci in T. sinskajae.

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Grown in the Eastern Steppe of Ukraine, the cultivated diploid einkorn was more productive than Sinskaya wheat but with less protein content. In *T. sinskajae*, the highest rate of seed protein (21.5%) was observed (Tverdokhleb, 2015). One sample of this species was resistant to leaf rust at two temperatures, but the resistance provided to Helminthosporium leaf blotch was expressed at 22°C temperature only (Kolesova et al., 2022).

Synthetic hexaploid polyploids are developed by artificially crossing the tetraploid wheat cultivars or their hybrids/advanced breeding lines with different accessions of diploid Triticum and Aegilops species. The obtained F<sub>1</sub> hybrids (2n = 3x = 21) are treated with colchicine for chromosome doubling and production of fertile hexaploid genotypes known as synthetic hexaploid wheats (SHW) (Daskalova et al., 2016a). SHW involving diploid wheat species are often called A-genome amphiploids possessing an enormous variability to biotic and abiotic stresses (Ahmed et al., 2014a; Daskalova et al., 2019). References for SHW with T. sinskajae are very exiquous. To investigate the asymmetry index of some quantitative traits, Chuprina et al. (2021) used as amphiploid PAG-39 (*T. dicoccum*  $\times$  *T. sinskajae*) and Triticum  $\times$  sinskourarticum (T. sinskajae  $\times$  T. urartu). Among the six species in the Compositum section, only T. dimococcum and T. palmovae (syn. T. erebuni Gandil.) include the A<sup>b</sup> genome as the former originated by the T. dicoccum × T. monococcum cross (Schiemann, Staudt, 1958; Goncharov, 2011).

The objectives of this study were to characterise the synthetic amphiploid (*T. durum* 198-2  $\times$  *T. sinskajae*) for its potential application in the wheat breeding program: 1) field performance including germination, winter hardiness, plant and spike morphological traits, 2) crossability to common and durum wheat for the production of hybrids, 3) reactions to powdery mildew races in the seedling stage, and 4) the HMW glutenins spectrum of amphiploid progenies and selected lines compared to their parents.

#### Material and methods

Plant material and field experiment. Two accessions of Triticum sinskajae received from the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany were involved in crosses to tetraploid hybrids and durum cultivars (Daskalova et al., 2016b). After the treatment of  $F_1$  plants with colchicine, only the cross No. 45 (198-2 $F_3/T$ .sinskajae acc. 18397) yielded viable hybrid seeds. The female parent (TD198-2) of the newly obtained synthetic amphiploid (SHW45) was selected from the SHW8A-Tb  $\times$  *T*. durum cv. Progres cross. The parents of SHW8A-Tb are the durum wheat cultivar 'Martondur-3' and Triticum boeoticum acc. 110 (Daskalova et al., 2019). SHW45 was grown in  $C_{2-6}$  generations to study field germination, winter hardiness, and spike fragility and additional traits such as a plant height, a number of spikes per plant, a heading date (counted from 1 May), and a number of seeds per selfed spike (NSSS). Plants were grown during 2016–2021 in a crop rotation field in Varna (43°12'N, 27°54'E, 50 m), Bulgaria. Forage peas were grown as preceding crop, and no fertilizers were applied. During the growing seasons, weeds were controlled manually, and only insecticides were used. The sowing dates were typical of Varna conditions, between 10 and 20 October. Regarding the total precipitation, growing seasons were different (Table 1). Two of them, 2017-2018 and 2020-2021, received almost the same amount of precipitation, 597.3 and 578.8 mm, respectively. The years 2016-2017 and 2018-2019 had the lowest rainfall, 347.8 and 307.3 mm. The coldest months were December and January characterised by average quarterly positive minimum temperatures. Low minimum but positive temperatures favour the normal development of plants.

The seeds were manually planted on single-row plots at 12 seeds per row 1 m long and at an inter-row spacing of 40 cm using a randomised design with two replications. In 2017, the number of seeds sown varied between 24 and 36 per progeny. The evaluation of germination was done in the autumn at the 1–2 leaf stage, and the winter survival was calculated using all available

Quarter / year		2016-2017			2017-2018			2018-2019	
Months	t <sub>max</sub>	t <sub>min</sub>	PP	t <sub>max</sub>	t <sub>min</sub>	PP	t <sub>max</sub>	t <sub>min</sub>	РР
$ \begin{array}{r} 11, 12, 01 \\ 02, 03, 04 \\ 05, 06 \end{array} $	7.0 11.1 23.1	$0.6 \\ 3.8 \\ 15.1$	46.9 49.3 29.7	11.4 12.0 24.6	4.1 4.8 16.6	73.3 64.6 91.9	8.8 12.9 25.3	2.6 4.8 16.6	49.2 20.0 49.9
Total		347.8			597.3			307.3	
Quarter / year		2019-2020	l		2020-2021		_		
11, 12, 01	13.0	8.6	31.1	10.8	4.6	95.1			
02, 03, 04	14.3	5.3	26.4	11.7	3.6	42.0			
05,06	23.5	14.5	91.7	23.4	15.0	83.7			
Total		355.9			578.8				

Table 1. Quarterly meteorological data for a 5-year experimental period

Average quarterly temperature (°C):  $t_{max}$  – maximum,  $t_{min}$  – minimum; PP – quarterly precipitation (mm)

plants for each line in the beginning of spring regrowth in March. Additionally, the winter hardiness (WH) of plants per row was assessed according to the WH scale (1 - the most sensitive, 9 - the hardiest) (APHA, 2019). Spike fragility (%) as a ratio of plants with ear brittleness and the number of individuals studied was calculated. This trait was assessed at maturity by visualising each plant when the main spike is held by hand with a slight pressing. If the top spikelets disintegrated, the plant was marked as fragile one. The brittleness occurred only at the apical spikelets of the spike, which makes this manual action easy to divide a fragile vs not fragile plant (Daskalova et al., 2019). Every year, seeds from two plants with resistance to spike fragility are used for sowing. *T. aestivum* cultivars 'Sadovska ranozreika-4', 'Lider GT', and line 450-5 and one *T. durum* line 3a4 were involved in a common crossing procedure for hybridisation with lines of SHW45. Crossability was expressed as the ratio of the number of seed sets to the total number of flowers pollinated. The crosses were made under field conditions in 2020 and 2021. Grain plumpness was estimated visually using a four-point scale (poor, satisfactory, good, and excellent) (Bazhenov et al., 2015).

*Protein extraction and SDS-PAGE.* Ten grains from each line were crushed and ground to powder. Extraction of high molecular weight-glutenin subunits (HMW-GS) was performed in four stages (Singh et al., 1991) as described by Doneva et al. (2018). The electrophoresis run on a vertical apparatus in two ways: 1) classical one-dimensional 12% polyacrylamide gel; 2) one-dimensional 12% polyacrylamide gel SDS- PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) with the addition of 4M urea (Lafiandra et al., 1993). Protein fractions were investigated and designated through the universal system for the arrangement and numbering of glutenins (Payne, Lawrence, 1983).

Powdery mildew test. Blumeria graminis f. sp. tritici (Bgt) races were collected from different parts of Bulgaria and selected from single spore isolates. The test for mildew resistance was conducted on plants in the second leaf stage grown in plastic containers. Inoculation methods, incubation conditions, and disease assessment were performed according to Lutz et al. (1992). Each genotype was tested using two replications, each consisting of 8 to 10 individuals. Disease response patterns were recorded ten days after inoculation using a quantitative scale of 0 to 10 (0 - no visible disease)symptoms, 10-100% leaf area covered with sporulating colonies). Three major classes of host reactions were distinguished: r - resistant (0-20% infection level), i - intermediate (21–50% infection), and s - susceptible (>51% infection). Where accessions were heterogeneous for response, they were recorded as 'r,i' or 'i,s' depending on the level of infection. The pathotype virulence on the tested Pm genes was estimated according to Samborski and Dyck (1976). A set of 11 differentials of wheat monogenic lines (Pm1,2,2+6,2+6+?,3a,3b,4,4b,5,7, and *Mld*) was used to calculate the avirulence/virulence formulae for the races applied in SHW45 in the C<sub>2</sub> generation: race 1611-1,2,3a,4,2+6,2+6+?,Mld/; race 3657-1,3a,Mld/; race 7653-3a,4,Mld/; race 7777-/1,2,3a,3b,4,4b,5,7,*Mld*,2+6,2+6+?

Statistical analysis. To determine significant differences (p < 0.05) between the genotypes, the data were statistically evaluated by analysis of variance (ANOVA) using the Develve statistical software (www. develve.net). LSD<sub>0.05</sub> was calculated according to Snedecor and Cochran (1980).

#### **Results and discussion**

*Field performance.* In the C<sub>2</sub> generation, plants were measured for a spike length, a spikelet number, and a seed number per selfed spike (Table 2). All amphiploid progenies yielded spikes longer than the parents. The mean population was 9.1 cm. The progeny 45-2-7 produced the longest spikes (M = 10.1 cm), and the plants of 45-2-10 and 45-4-3 presented the shortest ears (8–8.2 cm). The female parent bore very short spikes (5.7 cm) and did not differ from the pollen parent. The same tendency appeared for the spikelet number. The parents distinguished sharply as TD198-2 expressed the least and the T. sinskajae (TS18397) showed the largest spikelet number (12 vs 29 spikelets). Significant differences among the offspring were observed in NSSS. The average value of the population (27.0) was higher than that of the parents individually. Two families were the most productive among all yielding 36-38 NSSS. Three progenies had seeds above the population mean of 27.0. Comparing the variation on traits, the NSSS varied most (with CV between 21.0 and 61.0).

Estimation of winter hardiness was initiated from the  $C_3$  generation. Most of the offspring showed 90– 97% surviving plants, a little over the population mean

Table 2. Variation (minimum-maximum) of amphiploid traits in C<sub>2</sub> and C<sub>3</sub> generations under field conditions

Progenies /		C <sub>2</sub> generation				C <sub>3</sub> generation	l	
parents	SL	SN	NSSS	NSS	NLP	NLP%	PH	PFS
SHW45	8.1-10.1	19-27	19-38	24-36	19-35	70–98	77–93	7–75
Mean	9.1	22.6	27.0	200*	178*	89.0	83.0	47.0
CV	12-20	10-22	21-61	-	3–6	-	6-15	-
TD198-2	5.7	12	21	20	17	85.0	74	0
TS18397	6.3	29	14	20	18	90.0	137	0
LSD <sub>0.05</sub>	1.6	4.1	11.0				8.5	

CV - coefficient of variation; SL - spike length, SN - spikelet number, NSSS - number of seeds per selfed spike, NSS - number of seeds sown, NLP - number of live plants at the beginning of spring, NLP% - number of live plants in percent, PH - plant height, PFS - plants with fragile spike, \* - total (NSS, NLP)

of 89.0% (Table 2). Sinskaya wheat performed also very well, exhibiting the survival of 90.0%. Progenies had a different plant height varying from 77 to 93 cm. Two families included plants in height above the population mean of 83.0 cm. The values of SHW progenies were between both parents, 74 and 137 cm.

Spike fragility was observed in the C<sub>2</sub> generation to be a problem for the morphological analysis, especially for the seed set assay after harvesting. In the C<sub>3</sub> generation, 47.0% of the harvested plants gave brittle spikes. The variation for this trait was very large, from 7.0% to 75.0%. Three progenies performed better indicating values under the population mean for spike fragility. This trait was primarily controlled by genes located on the homoeologous group 3 chromosomes. All wild wheats had a brittle rachis leading to shattering of either the whole spike or individual spikelets (Sood et al., 2009). In addition to the Q gene, three loci Br1, Br2, and Br3 governed the rachis character. In tetraploid wheats, recessive alleles of two of them (Br2 and Br3)determined non-brittle rachis. Species of T. monococcum, T. dicoccum, and T. timopheevii possessed a specific type of brittle rachis being disarticulated with the slightest mechanical pressure at maturity (Konopatskaia et al., 2016). The presented phenotypic diversity on spike traits and plant height in  $C_{2-3}$  generations allowed us to select eight SHW45 lines: 45-1, 45-2, 45-2a, 45-3, 45-6, 45-7, 45-8, and 45-10.

The field germination and spike brittleness of SHW45 were studied in 2019–2021. In all, 113 seeds were sown in autumn of 2018, and 81 germinated (71.7%), from them 70 plants (86.4%) reached maturity (Table 3). After harvesting, plants with spike fragility counted 23, or 32.9%. The line 45-2a yielded plants with the most fragile spikes and fell off. The population mean for brittleness was assessed as 3.0 plants. Three lines showed plants with spike fragility (PSF) under the mean of 3.0. Among them, the line 45-1 was distinguished as resistant to spike brittleness (PSF = 0). In 2020, field germination was determined as 88.4%, a little higher than in the previous year.

From the harvested plants, 11 were with fragile spikes leading to the population mean of 1.6 plants (Table 3). The PSF values of three (45-1, 45-8, and 45-10) lines were smaller than 1.6, and among them the line 45-1 exhibited again resistance to ear brittleness. All amphiploid lines in the  $C_6$  generation showed a very high resistance to spikelet disarticulation, but two of them (45-1 and 45-8) seemed to have a full resistance to spike fragility. The amphiploid plants expressed a very good winter hardiness receiving estimates between 7 and 9 as well as the parents (data not shown). Phenotypically,

Lino		20	19			2020			2021	
Line	NSS	NSG	NPH	PSF	NSS	NSG	PSF	WH	NPH	PSF
SHW45	12-24	7–20	6-19	0–7	12-24	10-21	0-3	7–9	9-12	0-1
Total	113	81	70	23	103	91	11		54	3
%		71.7	86.4	32.9		88.4	12.1			5.6
Mean	14	10	9	3	15	13	1.6	8	10.8	0.6*

*Table 3.* Field performance of SHW45 lines after selecting plants with resistance to spike fragility of  $C_{5-7}$  generations in 2019–2021

NSS – number of seeds sown, NSG – number of seeds germinated, NPH – number of plants harvested, PSF – plants with spike fragility; WH – winter hardiness assessment key;  $* - p \le 0.05$ 

the SHW45 lines resembled common wheat yielding long ears with extended awns similar to those of durum wheat (Figure 1). Spikelets were arranged along the stem in a straight line. The plant height varied from 68 (line 45-1) to 82 (line 45-10) cm with a seed set ranging between 21 (line 45-1) and 35 (line 45-6) NSSS. Some differences between the lines, e.g., seed size and plumpness (Figure 2) as well as between them and wheat cultivar cultivar 'Sadovska ranozreika-4' (SR-4) were significantly demonstrated (Table 4). Four SHW45 lines were crossed as females to common wheat in 2020 (Table 5). The seed set ranged from 36.8% to 69.1% with an average of 56.3%. Hybrid grains had a good phenotype (GP=b and b-c groups). Two crosses were made when the 'Sadovska ranozreika-4' was a female parent that bore only six seeds. These hybrid seeds did not germinate. In 2021, three (45-1, 45-6, and 45-8) lines were involved as pollinators in the crosses to line SR-4. The average crossability was 35.3% producing 41 hybrid grains that failed to germinate again.

Table 4. Breeding traits of SHW45 lines compared to the common wheat cultivar 'Sadovska ranozreika-4'

Line	Plant	Number of spikes	Heading	Number of seeds
SR-4	height	per plant	date	per selfed spike
45-1	$68 c \pm 9.5$	$5.3 b \pm 2.1$	23 a ± 1.1	$21 c \pm 8.6$
45-6	$81 b \pm 3.3$	$5.4 b \pm 0.5$	$21 a \pm 0.6$	$35 b \pm 11.0$
45-10	$82 b \pm 8.9$	$7.7 \ a \pm 0.7$	$21 a \pm 1.3$	$29 \text{ bc} \pm 11.2$
SR-4	98 a ± 1.8	7.8 a ± 1.2	$13 b \pm 0.6$	74 a ± 7.0

*Note.* Values in a column followed by different letters are significantly different; average value  $\pm$  SD.



*Figure 1.* Spikes of SHW45 and the parents: TD198-2, 45-6, 45-1, and TS18397



*Figure 3.* Spikes of SHW45 and its F1 (SHW45 × SR-4): 45-6, F1131, F1132, F1133, and SR-4

A cross between the line 45-8 and the durum line 3a-4 yielded 35 seeds (crossability of 40.7%) with a variation of seed plumpness (b-d). Surprisingly, they performed a high germination ability, and 30 hybrid plants were transplanted in the field. A sample of 10 seeds from each cross-combination (SHW45  $\times$  *T. aestivum*) germinated (78.0%), and 23 F<sub>1</sub> plants matured in 2021. Plants from



*Figure 2.* Seed morphology: 1 – TD198-2, 2 – TS18397, 3 – 45-1, 4 – 45-6, and 5 – 45-10



*Figure 4.* HMW-GS composition of SHW45 in C2 generation and its parents: 1 – 'Bezostaya 1' (standard), 2–4 – different progenies, 5 – TD198-2, 6 – TS18397

two crosses suffered from hybrid necrosis, as these of  $F_1(45-7/450-5)$  headed and yielded lean spikes with shrivelled seeds. From the  $F_1(45-10/450-5)$ , only three seeds germinated and necrotised at the tillering stage. The *Ne1-Ne2* necrosis system was the best known in wheat caused by two complementary genes of *Ne1* (5BL) and *Ne2* (2BS) occurring in a single genotype (Kishii, 2019).

Cro	SS	NFP	NSO	SS	GP	PSG	GS	NPH
				2020				
45-7 × 4	450-5 <sup>1</sup>	42	29	69.1	b	10	10	8 <sup>3</sup>
45-10 ×	450-5	38	14	36.8	b	10	3	0 <sup>3</sup>
45-1 ×	SR-41	38	24	63.2	b	10	8	8
45-6 ×	SR-4	36	18	50.0	b-c	10	8	8
45-10 ×	< SR-4	36	22	61.1	b-c	10	10	7
Tot	al	190	107	56.3		50	39 (78%)	31
SR-4 ×	45-1	28	6	21.4	a-b	6	0	
SR-4 ×	45-6	26	0	0				
Tot	al	54	6	11.1				
				2021				
SR-4 ×	45-1	32	6	18.8	b	6	0	
SR-4 $\times$	45-6	52	9	17.3	b-c	9	0	
SR-4 $\times$	45-8	32	26	81.3	b	26	0	
Tota	al	116	41	35.3		41	0	
45-8 ×	3a-4 <sup>2</sup>	86	35	40.7	b-d	35	33	

Table 5. Crossability	y of SHW45 lines to	wheat and the viabilit	y of hybrid seeds

 $^{1}$  – *T. aestivum*,  $^{2}$  – *T. durum*;  $^{3}$  – hybrid necrosis; NFP – number of flowers pollinated, NSO – number of seeds obtained, SS –seedset in %, GP – grain plumpness (a – excellent, b – good, c – satisfactory, d – poor), PSG – planted seeds for germination, GS – germinated seeds, NPH – number of plants harvested

Two amphiploids, *Triticum durum-Haynaldia villosa* and *Hordeum chilense-Triticum durum*, were crossed reciprocally with 11 bread wheat accessions. Out of 44 cross combinations, 18 crosses showed a severe necrosis, 8 showed a moderate one, and the remaining 18 were found to have a weak necrosis (Lata, Sethi, 1998). The degree of necrosis in the hybrids varied greatly due to the multiple allelism of *Ne1* and *Ne2* genes. However, this phenomenon is more frequent and complex in wheat × *Aegilops* crosses.

Selfed  $F_1$  spikes yielded 175 (12.8% seed set)  $F_2$  grains with a variation of 1 to 17 seeds per spike (Table 6, Figure 3). The plants of  $F_1133$  (45-10/SR-4) had a significantly higher fertility (MNSS = 9) than those

of  $F_1132$  (45-6/SR-4) (MNSS = 4.17), which confirmed genetic differences between the female synthetic parents. A sample of 25 seeds was planted for germination and 14  $F_2$  plants were transplanted in the field. BC<sub>1</sub> seeds were produced from four crosses. More seeds were obtained in the crosses when  $F_1$  hybrids were females compared to the reciprocal combinations (37>13 seeds) (Table 6). The seed sets between the cross-combinations were very different: 44.1% (pollen from SR-4) vs 19.1% (pollen from  $F_1$  hybrids). In case when pollen of the wheat cultivar 'Lider GT' was used for backcrossing, the hybrid seeds had a good phenotype (a-b group) but failed to germinate or hybrid necrosis occurred (data not shown). We tried to obtain hybrid seeds when open flowers in hybrid plants

*Table 6.* Self-fertility of three F, hybrids and the volume of hybrid seeds

Hybrid	NSS	TNS	Min–Max	MNSS	S SD		GP	PSG	GS	TF
F.131	176	44	1-17	6.29	5.28		a-b	11	6	5
$F_{1}^{1}132$	296	50	1-11	4.17	2.52		a-c	5	4	4
F.133	211	81	1 - 14	9.00	4.95		b-d	9	6	5
Total	683 <sup>1</sup>	175		3.97				25	16	14
LSD <sub>5%</sub>		(12.8%)							(64.0%)	
	Cross	NFP	NSO	SS	SG	GP	PSG	GS	TF	
F,131	$-8 \times SR-4$	40	19	47.5	BC,	a-c	10	5	5	
F,132	$2-1 \times SR-4$	44	18	40.9	$BC_1$	b-c	10	8	8	
1		84	37	44.1	1		20	13	13	
SR-4	× F,133-4	36	7	19.4	BC,	а	7	4	4	
SR-4	$\times F_{1}^{1}131-8$	32	6	18.8	$BC_1$	а	6	5	5	
	1	68	13	19.1	1		13	9	9	
F,131-5	$(OP) \times SR-4$	27	28	51.9	F <sub>2</sub> -BC <sub>1</sub>	с	28		17	
F,132-8	$(OP) \times SR-4$	27	40	74.1	$F_2^2$ -BC <sub>1</sub>	с	40		25	
F1133-1 (0	ÔP) × Lider G	T 76	73	48.0	$F_{2}^{2}-BC_{1}^{1}$	a-c	73		39	
,	,	130 <sup>1</sup>	141	54.2	2 1		141		81 (57.5)	

OP – open flowers pollinated by two wheat spikes; NSS – number of spikelets per spike, TNS – total number of seeds, Min–Max – minimum and maximum for the number of seeds per spike, MNSS – mean number of seeds per spike, SD – standard deviation, GP – grain plumpness (a – excellent, b – good, c – satisfactory, d – poor), PSG – planted seeds for germination, GS – number of germinated seeds, TF – transplanted to field, NFP – number of flowers pollinated, NSO – number of seeds obtained, SS – seedset in %, SG – seed generation; <sup>1</sup> – number of spikelets per spike assuming two pollinated flowers per spikelet

(not emasculated) are pollinated by wheat spikes. In this case ( $F_2$ -B $C_1$  group), the seed set (mean = 54.2%) was better than self bagging of  $F_1$  spikes (mean = 12.8%) and backcrossing (means = 44.1% and 19.1%). All 141 seeds in the  $F_2$ -B $C_1$  group sown in the field resulted in 81 plants at the 2–3 leaf stage. Further data are needed to make basic conclusions in this turn.

**Resistance to powdery mildew in seedlings.** Three progenies in the  $C_2$  generation were estimated for the reaction to four Bgt races (Table 7). They showed a different response: the family 45-2-3 exhibited resistance to all powdery mildew pathotypes as TS18397 did. The progeny 45-2-4 was susceptible to the race 7777 and expressed resistance to race 1611. This family showed a variable response (r,s) to the other two strains. The progeny 45-2-9 revealed a better resistance to Bgt races than 45-2-4 as showing resistance to the race 7777. The female parent TD198-2 was susceptible to the pathotypes used.

Bgt/progenies	s in C <sub>2</sub>	1611		3657		7653		7777	
45-2-3		r		r		r		r	
45-2-4		r		r,s		r,s		s	
45-2-9		r		r,s		r,s		r	
TD198-2	2	s		Ś		Ś		s	
TS18397	7	r		r		r		r	
Bgt/lines in C <sub>5</sub>	1116	1611	1613	1671	3611	3613	3637	3653	3657
45-1	r	_	_	s	_	r	S	r	_
45-2	r	r	s	r,s	r	r	S	r	i
45-2a	S	r,i	i	S	r	S	S	S	s
45-3	S	-	-	r	-	r	S	r	-
45-6	r	r	r	r,s	r	r	r	r	r
45-7	S	r	i	S	i	S	S	S	i
45-8	r	r	r	r,s	r	r	r	r	r
45-10	r	r	r	r	S	r	S	r	r
TD198-2	S	S	s	S	S	S	S	S	s
TS18397	r	r	i	r	i	r	S	r	r
	3673	3677	3713	3753	7617	7637	7771	7773	7777
45-1	r	r	_	r	_	r	_	-	s
45-2	r	r	r	S	r	S	r	r	r
45-2a	S	S	r	S	r,s	S	r	r	s
45-3	r	S	-	r	_	S	-	_	r,s
45-6	r	r	r	r	r	r	r	r	r
45-7	S	S	r	S	r	S	r	r	s
45-8	r	r	r	r	r	r	r	r	r
45-10	r,s	S	i	r	r	S	r	i	r,s
TD198-2	S	S	ş	s	S	S	S	S	s
<u>TS18397</u>	r	r	i	r	r	S	r	r,s	r

*Table 7.* Resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) (Bgt) races in the seedling stage of three SHW45 progenies and lines

In the C<sub>5</sub> generation, eight amphiploid lines showed a different reaction to 18 Bgt races (Table 7). The most resistant lines 45-6 and 45-8 expressed the same response: variable r,s performance to race 1671 and resistance to the other 17 races. The line 45-2a was distinguished revealing susceptibility to 11 Bgt races, while the lines 45-3 and 45-7 showed the same reaction to four and ten strains, respectively. The different spectrum of resistance to the Bgt races showed the genetic diversity between the SHW45 lines. The race 3637 attacked strongly the amphiploid lines, excepting 45-6 and 45-8. The female parent revealed susceptibility to all races, while the male parent expressed resistance to 12 strains , susceptibility to 2, intermediate to 3 and variability to race 7773.

The results indicated that the resistance of SHW45 to Bgt has been derived from T. sinskajae acc. 18397. Goncharov et al. (2020) studied 108 artificial wheat amphiploids, and among them *T. sinskourarticum* and PAG-39 (*T. dicoccum*  $\times$  *T. sinskajae*) showed leaf rust resistance. The comparative analysis of A-genome amphiploids (2n = 6x = 42, AABBAA) and their durum parents identified the putative source of yellow rust resistance (Ahmed et al., 2014b). This resistant amphiploid collection was also important because of carrying resistance from both sources, i.e., durum and A genome diploid accessions, and the isolate used to screen against stripe rust carried the latest gene virulence information. Data for powdery mildew resistance at the seedling stage were obtained by Rafique et al. (2012) for 104 accessions of amphiploids (T. turgidum  $\times$  T. urartu and T. turgidum  $\times$  T. monococcum). The selected durum and einkorn lines were used in bread wheat improvement to transfer the useful properties of einkorn into the cultivated hexaploid wheat via 'bridge-crossing' (Megyeri et al., 2011).

High-molecular-weight glutenin spectrum. TS18397 displayed A1x2\*+A1y1 vs null-B1x17+B1y18 of the female parent (Figure 4, Table 8). The subunit A1x2\* of SHW45 looked the same as in 'Bezostaya-1', but it originated from *T. sinskajae*. The components 17+18 came from the female parent TD198-2. Early report on T. monococcum and one accession of T. sinskajae described one x-type and one y-type HMW-GS. For the last species, an allele o at *Glu-A*  $l^m$  was determined (Saponaro et al., 1995). Later, Alvarez et al. (2006) detected three allelic variants (alleles a, b and c) for the HMW-GS located between the subunits 1 and 2\* in the Spanish cultivated einkorn wheat. More than five different intersubunit combinations in the Turkish cultivated einkorn wheat (T. monococcum ssp. monococcum) landraces were found, and among them Glu-A1x2\*+Glu-A1y1 had the highest frequency of approximately 31% (San et al., 2015). Li et al. (2016) detected eight Glu-Alx alleles in the cultivated diploid wheat, and five from them were novel alleles. We succeeded to transfer the same A1x2\*+A1y1 subunit pair from T. boeoticum into four durum wheat progenies through the amphiploid SHW8A-Tb. Most of the selected durum genotypes possessed  $\gamma$ -gliadin45, relating to a good end-use quality (Daskalova et al., 2019). Thus, the A<sup>b</sup> genome from diploid *Triticum* species offers new possibilities to enrich the wheat genetic basis.

The sununit 1Bx17+1By18 pair can also be of use in breeding as these allelic forms are related to a better breadmaking performance than 1Bx6+1By8 and 1Bx20 (Ma et al., 2005). Similarly, the 1Ax2\* and 1Ax1 subunits provide an improved food quality variation compared to *1Ax-null* allele (Jiang et al., 2017). López-Fernández et al. (2021) established 14 different alleles at

Table 8. HMW glutenin subunits of SHW45 lines in C<sub>2</sub> generation seeds and parents

Progeny / parent	Glu-A1	Glu-B1
45-2-1	A1x2* + A1y1	B1x17+B1y18
45-2-7 45-4-3	$\frac{A1x2^{*} + A1y1}{A1x2^{*} + A1y1}$	B1x17+B1y18 B1x17+B1v18
TD198-2	null	B1x17+B1y18
TS18397	A1x2* + A1y1	-

*Note.* The three lines (45-1, 45-6, and 45-10) in  $C_2$  plants seeds had the same profile as in the  $C_2$  generation.

the Glu-B1 locus in the 189 Spanish landraces analysed, among which the allele *i* coding for HMW-GS 17+18 was found at a low frequency of 1.59%. The same tendency for this allele was described by Dai et al. (2020) who found it in only spring forms of Chinese Xinjiang wheat landraces and historical cultivars.

#### Conclusions

1. The synthetic hexaploid amphiploid (Triticum durum form 198-2 × T. sinskajae acc. 18397) SHW45 showed a large phenotypic variation on the plant height and spike characteristics in the  $C_{2-3}$  generation. A strong selection was applied over a five-year period to minimise the spike brittleness and increase the plant fertility.

2. Three lines with resistance to spike fragility have been selected: the line 45-1 had the shortest stem (68 cm) and bore shrivelled seeds, the line 45-6 was the most fertile with 35 seeds per spike, and the line 45-10 was the highest (82 cm) yielding thinner seeds with a good grain plumpness.

3. The synthetic lines expressed field germination and winter hardiness as high as its parents and could be classified as winter forms. They expressed a different spectrum of resistance to powdery mildew, and two of them showed resistance to 17 races out of total 18 pathotypes tested.

4. The amphiploid easily crossed with common wheat and one durum line yielding the 35-56% seed set, but when it is a pollen parent in crossing to bread wheat, the hybrid seeds did not germinate. Viable F<sub>2</sub> and BC<sub>1</sub> seeds were obtained from the crosses with the wheat cultivar 'Sadovska ranozreika-4' as a male parent. The cytoplasm of the newly hybrid plants originated from T. durum.

5. SHW45 expressed the high-molecularweight glutenin fraction A1x2\*+A1y1 inherited from the T. sinskajae acc. 18397, and the subunit pair B1x17+B1y18 derived from the T. durum form 198-2. This spectrum, along with the powdery mildew resistance at the seedling stage, increased the significance of SHW45 as a potential source for bread wheat improvement.

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# Sintetinių heksaploidinių kviečių (BBA<sup>u</sup>A<sup>u</sup>A<sup>m</sup>A<sup>m</sup>) apibūdinimas ir jų panaudojimas kviečių selekcijoje

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## Santrauka

Vienagrūdžiai kviečiai (*Triticum sinskajae* A. Filat. et Kurk.) yra puikus atsparumo kviečių ligoms didinimo ir grūdų kokybės gerinimo šaltinis. Naujas sintetinis heksaploidinis amfiploidas SHW45 (*T. durum* 198-2 × *Triticum sinskajae* acc. 18397) buvo ištirtas  $C_{2-7}$  kartose ir rekomenduotas auginti lauko sąlygomis. Kai kurios jo savybės, pavyzdžiui, augalų aukštis ir varpų ypatybės, taip pat daigumas ir varpų trapumas, parodė didelę fenotipų įvairovę. Griežta atranka pagal atsparumą varpučių išsisklaidymui ir grūdų kiekį varpoje leido sukurti keletą linijų. Jos išsiskiria įvairiapusu atsparumų netikrajai miltigei daigų auginimo tarpsniu. Trys visiškai atsparios varpų trapumui amfiploidų linijos su įvairiais fenotipais kaip moteriškas komponentas buvo sukryžmintos su kviečių veisle 'Sadovska ranozreika-4'. Daigios hibridų sėklos gaunamos tik tada, kai kryžminimo metu sintetinė forma yra imama kaip motininis komponentas. Amfiploido linijos 45-10 F<sub>1</sub> kartos hibridai varpoje turi vidutiniškai 9 sėklas, o tai yra žymiai geresnis derlius nei kitų hibridų. Šios rūšys pasižymi didelės molekulinės masės gliuteninų vienarūšiškumu, nes subvienetų poros Ax2\*+Ay1 ir Bx17+By18 yra paveldėtos atitinkamai iš *T. sinskajae* ir *T. durum* formos 198-2. Lauke iš viso buvo pasėtos 14 F<sub>2</sub>, 22 BC<sub>1</sub> ir 141 F<sub>2</sub>-BC<sub>1</sub> sėklos, gautos sukryžminus dvi duoninių kviečių veisles. Sintetinių amfiploidų linijos gali būti perspektyvūs kviečių atsparumo netikrajai miltligei didinimo ir grūdų kokybės gerinimo genetiniai ištekliai.

Reikšminiai žodžiai: *Triticum sinskajae*, sintetinis amfiploidas, auginimo ypatybės, atsparumas netikrajai miltligei, HMW gliuteninai.