Agronomic performance of introgression lines derived from a cross of common wheat with synthetic hexaploid amphiploid 530-1

Селскостопанска характеристика на интрогресивни линии, получени от кръстоска на обикновена пшеница със синтетичен хексаплоиден амфиплоид 530-1

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ABSTRACT

This study characterized six introgression lines obtained from crossing two winter bread wheat varieties Albena and Slaveya with the D-genome synthetic hexaploid wheat 530-1. Phenotypically, the lines differed from the parents, as five of them expressed red spike color versus line 45-5 producing new coloration, identified as striped or banded glume pigmentation. All lines were taller, with longer ears and spikelets, later in heading time and less productive in grain yield than both wheat parents. They showed some positive traits in the field: optimal test weight (78–80 kg/hl), high thousand kernel weight (48–51 g), low sprouted seeds (0.3–1.5%), and high falling number (314-350 s). Two lines (38-7 and 46-7) exceeded the parents in grain protein content. In the competitive variety trial, Albena showed significantly higher sprouted grains (4.1%) and extreme measurements for a percentage of germination (99.0%) and germination index (83.0%) in 2022. The data identified Albena as a wheat variety with pre-harvest sprouting susceptibility. Of the lines, only 45-5 stood out with the lowest germination values and appeared as a promising genotype with resistance to pre-harvest sprouting. The introgression lines exerted useful genetic diversity for the studied traits and could serve as resources for quality improvement in wheat breeding programs.

Keywords: bread wheat, alien introgression, D^t-genome synthetic hexaploid wheat, breeding traits, physicochemical properties, pre-harvest sprouting

РЕЗЮМЕ

Това изследване характеризира шест интрогресивни линии, получени от кръстосване на два сорта зимна, хлебна пшеница Албена и Славея със синтетичната хексаплоидна пшеница 530-1, притежаваща генома Dt. Фенотипно, линиите се различават от родителите, тъй като пет от тях дават червен цвят на класа, срещу линия 45-5, която формира ново оцветяване на глумите, характеризирано като пигментация на ивици. Всички линии са по-високи, с по-дълги класове и класчета, по-късно се развиват и дават по-нисък добив на зърно от двамата родители. Те показват някои положителни признаци при полски условия: оптимален хектолитър (78–80 kg/hl), висока маса на 1000 семена (48–51 g), нисък процент покълнали семена (0.3–1.5%) и високо число на падане (314–350 s). Две линии (38-7 и 46-7) надвишават родителите по съдържание на протеин в семената. В конкурсен сортов опит Албена показва значително по-висок процент покълнали зърна (4.1%) и много високи стойности на

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преджътвено покълване (99.0%) и индекс на кълняемост (83.0%) през 2022 г. Данните идентифицират Албена като сорт чувствителен към преджътвено покълване на семената. От линиите само номер 45-5 се откроява с най-ниски стойности на кълняемост, които я определят като перспективен генотип с устойчивост към преджътвено покълване. Интрогресивните линии проявяват известно генетично разнообразие по отношение на изследваните признаци и могат да служат като изходен материал за подобряване на качеството в селекцията на обикновената пшеница.

Ключови думи: хлебна пшеница, чужда интрогресия, синтетична хексаплоидна пшеница с генома Dᵗ, селекционни признаци, физикохимични свойства, преджътвено покълване

INTRODUCTION

Increasing the genetic variability of common wheat (*T. aestivum* L.) using distant or closely related species has been associated with many important traits for plant and seed improvement (Kishii, 2019; Zhou et al., 2021). Alien introgression is usually understood as the integration of genetic material of one species, mainly a wild strain, into the genome of cultivated plants (Adonina et al., 2021). Introgressive hybridization is one of the methods to enlarge the variation in cultivated crops for the successful creation of hybrids and varieties.

When applying wide hybrids in breeding, changes affecting genes for glume color, seed quality, and resistance to biotic and abiotic stresses are very often obtained in one or several wheat chromosomes. Successful wheat breeding is a puzzle that needs to combine agronomic performance with grain quality (Venske et al., 2019), all of which are complex traits contributed by manifold components. Breeding progress in bread wheat is based on the utilization of registered variation within the species gene pools such as GP-1, 2, 3, and 2/3 (Hao et al., 2020; Bo et al., 2022). In GP-2, *Aegilops tauschii* (2n = 14, DD) shares one of the three bread wheat chromosome sets, as a close relative and donor of the D genome.

Following the domestication and breeding of wheat, there is limited variation at the *Glu-D1* locus in the D-genome with only two alleles (*a* coding 2+12, and *d* coding 5+10) found in the vast majority of bread wheat throughout the world (Li et al., 2021a). According to Shewry et al. (1992), size separation of HMW-GS makes it possible to identify 3 (called *a*, *b* and *c*), 11 (*a* to *k*) and 6 (*a* to *f*) allelic forms at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively. Up to now, 14 alleles at *Glu-D1* locus of

bread wheat have been resided (Dai et al., 2020; López-Fernández et al., 2021), among which alleles *c* and *v* coded 4+12 and 2.1+10.1 subunits, respectively.

In addition to hexaploid wheat, *Aegilops tauschii* (Coss.), the diploid ancestor of the D-genome, possessed many unique *Glu-D1* alleles. Kumar et al. (2019) listed *Aegilops* species explored for high molecular weight glutenins, indicating subunit 4D in *Ae. tauschii* and SHW line, and subunit 10.1D in several *Ae. tauschii* accessions. The coding gene for the 1Dy10.1t subunit was isolated and characterized by Zhang et al. (2006). The author found this subunit in combination as $5^{*t} + 10.1^t$ in *Ae. tauschii* accession TD81 (Zhang et al., 2008). Delorean et al. (2021) discovered 45 haplotypes in *Glu-D1* of *Ae. tauschii*, relative to the two predominant haplotypes in wheat. By now, over 37 SDS-PAGE *Glu-D1* alleles have been named in this species. High number of HMW-GS were reported to highlight the potential use of synthetic hexaploid wheats for transferring new allelic variation into bread wheat for broadening the genetic base of quality traits (Rasheed et al., 2012; Tariq et al., 2018).

Cox et al. (2017) compared two approaches for introgression of germplasm from *Ae. tauschii* into common wheat: production of amphiploids, known as synthetic hexaploid wheat (SHW) between a tetraploid form and this species, and direct hybridization between the wheat-6x and the wild relative. It has become clear that introgressing multiple segments from an SHW parent could remarkably enhance important traits including seed quality and spike yield of wheat (Li et al., 2018; Hao et al., 2019).

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The present study characterized a set of introgression lines selected from a cross of common wheat with the D^t-genome synthetic hexaploid amphiploid 530-1, for several agronomic traits including yield-related indices and seed germination in field conditions over a three-year period in the North-Eastern region of Bulgaria, and compared to their bread wheat parents.

MATERIALS AND METHODS

Plant materials, soil and climatic conditions

Several common wheat (*Triticum aestivum* L.) lines were selected from a cross Albena/Slaveya/SHW530-1. The synthetic hexaploid wheat 530-1 was derived by crossing a tetraploid wheat hybrid to an *Aegilops tauschii* accession (Daskalova et al., 2016). Albena and Slaveya are bread wheat winter cultivars released in the Dobrudzha Agricultural Institute (DAI)-General Toshevo, Bulgaria.

Six wheat genotypes in BC_1F_9 generation (38-7, 39a-5, 40-3, 41a-6, 45-5, and 46-7) among the nine selected introgression lines (Doneva et al., 2018), together with Albena and Slaveya varieties were planted in October according to a completely randomized block design in DAI with two (2020) and four replications in 2021 and 2022. The harvested grain from the competition trial (CT) served to measure the grain yield and five other indices: test weight, thousand kernel weight, grain protein content, sprouted grain, and falling number (Table 1). Moisture content was determined in each test sample according to ISO 712:2009. Analysis of physicochemical and alveograph properties was practiced matching the ISO standards.

All plots were treated in the same manner by standard local agricultural practice to keep them free of diseases and weeds. Plots had 12 rows, 8 m long and spaced at

Table 1. Units of measurement and standards

Symbol	Trait	Unit of measurement	Standard
TW	Test weight	Kg/hl	ISO 7971-3:2019
TKW	Thousand kernel weight	Gram (g)	ISO 520:2010
SG	Sprouted grains	Percent (%)	BS EN 15587:2018
GPC	Grain protein content	Percent (%)	ISO 20483:2013
FN	Falling number	Second (s)	ISO 3093:2009
PG	Percentage of germination	Percent (%)	
GI	Germination index	Percent (%)	
M	Moisture content	Percent (%)	ISO 712:2009
GY	Grain yield	t/ha	
PH	Plant height	Centimeter (cm)	
FG	Field germination	Percent (%)	
HD	Days after 01 May	Number	
SL	Spike length	Centimeter (cm)	
SLN	Spikelet number per spike	Number	
NSS	Number of seeds per spike	Number	
GWS	Grain weight per spike	Gram (g)	

11 cm. They were seeded at 500 seeds per square meter. Sowing and harvesting were done by machine and grain yield was calculated in t/ha. Disinfection of seeds with the commercial product RAXIL 060 FS (tebuconazole 60 g/l), applied in a standard dose of 50 ml/100 kg of seeds for semi-wet disinfection, consumable working solution 2 l/100 kg of seeds; loading date - there is one treatment with an insecticide (commercial product DECA EC, deltamethrin) against enemies and a fungicide for rust and powdery mildew species (product AMISTAR 25 SK, azoxystrubin). Pesticides used during the growing season are in doses according to their authorization for use. The application of N, P and K was 100, 40 and 20 kg/ha respectively which was used as the main fertilizer and 40% of other nitrogen fertilizer was applied at the shoot stage. The main fertilizer is NPK 20:10:10 (complex solid inorganic fertilizer with macroelements, manufactured by Neochim AD code: 35 – 03), and the spring feeding is realized with commercial product YaraBela™ SULFAN™, which is a specialized granular fertilizer. Its granules are covered with sulphur, which prevents losses of nitrogen resulting from the take-off of ammonia in the atmosphere.

To measure the field germination of visually selected healthy seeds, four lines (39a-5, 40-3, 41a-6, and 45-5) plus the two wheat parents, were grown in a micro trial (a quarter of a square meter) in two replications at the same seed rate as in CT, in DAI and TU-Varna for three years.

Here, all operations were performed by hand. Several traits were studied: field germination, plant height, heading date, spike length spikelet number, number of seeds per spike, and grain weight per spike (Table 1). Additionally, glume and coleoptile color were visually determined.

Two spikes were harvested from each plot in DAI (43°42′ N, 28°2′ E, 230 m) and Technical University (TU)–Varna (43°12′ N, 27°54′ E, 50 m) at physiological maturity characterized by loss of green color from the spike, during 2022. The evaluation of pre-harvest sprouting (PHS) resistance used the procedure of Li et al. (2021b). Shortly, the spikes were air-dried for 2 days at room temperature, hand threshed and stored in a refrigerator at -20 °C

to maintain dormancy until phenotyping. Seeds were sterilized with 1% of NaClO for 20 min, followed by rinses with sterile water. Twenty healthy seeds in a replicate of 4 in total from each line were incubated with distilled water at 20 °C for 7 days. Based on the germination data, two measurements were calculated to characterize the PHS resistance: percentage of germination (PG) and germination index (GI), following the formulas:

PG = (seeds germinated/total seeds) × 100;

GI = (7 × n_1 + 6 × n_2 + 5 × n_3 + 4 × n_4 + … + 1 × n_7/7 x total grains) × 100

where n_1, n_2, … n_7 is the number of seeds germinated on the first, second, and subsequent days until the seventh day (DePauw et al., 2012; Li et al., 2021b). The two indices (PG and GI) were separately calculated for both locations, DAI and DAI + TU (averaged data).

The soil in DAI and TU was haplic chernozem and claysilt, respectively. Regarding rainfall, the three growing seasons in DAI were different. The second year (2020/2021) had the most favorable conditions, where the high rainfall (639 mm) was associated with lower monthly temperatures and high relative humidity (Mean = 77.7%), especially when compared to the first year (Table 2). The conditions of the third year were similar to those of the 2019/2020 season but with lower average temperatures and the highest humidity among the growing seasons. For TU-Varna, the climatic conditions in 2022 were slightly worse compared to DAI-General Toshevo: less precipitation in the three months (May, June and July) combined with significantly higher average monthly temperatures (32.6 > 19.5 °C) and lower relative humidity. However, taking into account the minimum and maximum temperatures, and precipitation, the meteorological factors were satisfactorily good for plant germination and growth to harvesting during the whole period.

Statistical analysis

All chemical analyses were performed in three replicates and the results were statistically analyzed. To determine significant differences ($P < 0.05$) between

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Table 2. Climatic conditions

Month	Average montly t °C	Average minimum t °C	Average maximum t °C	Average relative humidity %	Rainfall mm
2019/2020 in DAI – General Toshevo					
November	11.7	5.4	14.2	80	35.4
December	5.2	3.4	7.8	81	21.8
January	1.8	-2	6	88	2.8
February	5.1	0.3	10.8	80	28.1
March	8	3.2	13.5	71	28.3
April	10	3,7	17.2	62	5.8
May	15.4	10.6	21.8	67	48
June	19.6	15.1	26.1	74	192.2
July	22.6	16.8	29.6	67	2.4
Mean	11.0	6.3	16.3	74.4	365*
2020/2021					
November	6.3	2.8	10.7	86	26
December	5.3	3.2	8.3	91	74.4
January	3	-0.1	7.2	89	109.7
February	4	-0.1	9.2	84	13.2
March	4.2	-0.1	8.8	78	22.2
April	8.8	4.2	14.3	74	44.6
May	15.8	10.9	22.1	71	63.6
June	11.3	8.8	14.7	55	255.6
July	22.8	17.8	29.2	71	29.7
Mean	9.1	5.3	13.8	77.7	639*
2021/2022					
November	8.3	5.2	12.9	86	16.0
December	4.0	0.6	7.7	89	86.5
January	1.6	-1.7	5.8	82	23.7
February	4.3	0.6	9.3	79	20.7
March	2.8	-1.4	7.2	74	31.6
April	10.9	5.4	16.9	75.6	76.1
May	15.8	7.5	22.1	74.1	30.7
June	21.4	14.5	23.7	79.4	60.5
July	21.1	16.6	25.9	65.4	10.8
Mean	10.0	5.2	13.8	78.3	356.6*
TU - Varna, 2022					
May	30.5	4.9	16.1	73	31
June	33.5	13.5	21.7	72	46
July	33.7	15.5	24.1	64	15.5
Mean (TU)	32.6	11.3	20.6	69.7	92.5*
Mean (DAI)	19.4	12.9	23.9	73.0	102*

* Total precipitation; Source: NIMH Varna (2022); Technical University of Varna – IVARNA50 (2023)

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the genotypes, the data were statistically evaluated by analysis of variance (ANOVA) and multi-correlation matrix using the Develve statistical software (Pauw, 2012). $LSD_{5\%}$ was calculated according to Snedecor and Cochran (1980). Two-way ANOVA in Excel was applied to obtain the source and level of variation on the traits investigated.

RESULTS AND DISCUSSION

Breeding traits

Field germination is an important feature for obtaining the required plant density. There were no significant differences in this trait, although some of the genotypes (lines 40-3 and 41a-6) gave a low level of germination as Albena did (72%, Table 3). The Slaveya variety showed the highest germination rate in the micro trial. Plant height sharply distinguished the new lines from the parents. Their height varied from 101 to 109 cm, the highest being 39a-5 (109 cm) and the lowest being 45-5 (101 cm) with a proven difference between them.

On average, the genotypes exceeded the parents by nearly 13 cm. The introgression lines (ILs) were significantly later in heading than the parents. The genotypes 40-3 and 45-5 headed 8 days later than Albena and almost 4 days later than Slaveya.

Significant differences were also found in other characteristics such as spike length and number of spikelets per spike. The largest spike was produced by 39a-5 and 41a-6, and the shortest – was by 45-5 (9.7 cm), which was almost equal to the parent ears (9.4 and 10.2 cm). The same trend was observed in the number of spikelets, where line 45-5 had the minimum expression of the trait.

The situation is different when measuring the fertility of plants. All four lines set the same number of seeds per spike as Albena did (NSS = 43–46 seeds) (Table 3). Slaveya was the most productive, and its spike bore 52 seeds, an average of 7 seeds per spike more than the introgression genotypes. The data for GWS showed a predominance of the lines (2.6 – 2.3 g) in relation to the parents (2.2 – 2.3

g). Probably, the smaller number of seeds generated the formation of heavier seeds.

The experimental lines in this study were created by selecting plants containing the high-molecular-weight glutenin subunits Glu-D1 4t+10.1t along with the high seed number and grain weight per spike in each generation (Doneva et al., 2018). Dhaliwal et al. (1990) published the first report on the isolation of a complete set of Aegilops squarrosa D-genome monosomic additions in a pure durum background. Only the monosomic addition 1D from the seven developed addition lines had red glume color with a compact head shape.

The glume color is in important taxonomic discriminator in wheat and is associated with the crop's adaptability (Khlestkina et al., 2006). The genes controlling the glume color are located in the homoeologous group 1, including Rg-A1, Rg-B1 and Rg-D1 in common wheat and its related species (Kozub et al., 2016; Song et al., 2020). Multiple alleles were reported for Rg-A1 and Rg-D1. Alleles Rg-A1b and Rg-A1c from T. aestivum control red and black glume coloration, respectively, whereas Rg-D1b (Ae. tauschii) and Rg-D1c (bread wheat) control red and smokey gray colors, respectively (Pshenichnikova et al., 2005). In this study, 5 of the six ILs had red glumes (Figure 1), except for line 45-5. The phenotype in line 45-5 was described as a striped glume color (Table 3, Figure 2), which was different from the spike color phenotypes published to date. Gorafi et al. (2018) scored black glume in multi synthetic derivatives as a qualitative trait for two environments in Sudan. Genome-wide association analysis showed one QTL at the short arm of chromosome 1D different from those alleles reported previously indicating that black glume in this population was controlled by a new allele at the same locus. In wheat, the ability to accumulate anthocyanins in the coleoptile is inherited and controlled by the Rc (red coleoptile) genes (Khlestkina et al., 2011; Wang et al., 2016). In the synthetic wheat T. durum × Ae. tauschii, and IL Chinese Spring-Ae. tauschii 7D (Pestsova et al., 2006) the anthocyanin pigmentation of the coleoptile was controlled by Rc-D1 (Khlestkina et al., 2011).

Table 3. Breeding traits of four introgression lines grown for three years in a micro trial

Line / WPs	FG	PH	HD	SL	SLN	NSS	GWS	Glume color	Coleoptile color
39a-5	80	109[a]	12.3c	11.3[ab]	19.9[ab]	45[b]	2.6[a]	Red	White
40-3	73	104[b]	14.7[a]	10.8[bc]	19.8[ab]	45[b]	2.4[ab]	Red	Colored
41a-6	72	104[b]	13.3[bc]	11.6[a]	20.5[a]	45[b]	2.5[ab]	Red	White
45-5	78	101[b]	14.2[ab]	9.7[de]	19.2[bc]	43[b]	2.3[b]	Striped	Colored
Albena	72	91[c]	6.1[e]	9.4[e]	17.7[d]	46[b]	2.2[b]	White	White
Slaveya	84	94[c]	10.6[d]	10.2[cd]	18.9[c]	52[a]	2.3[b]	White	White
LSD$_{5\%}$	ns	3.8	1.1	0.63	0.79	4.4	0.25		

WPs: wheat parents, FG: field germination in %, PH: plant height in cm, HD: heading date counting from 01 May, SL: spike length in cm, SLN: spikelet number, NSS: number of seeds per spike, GWS: grain weight per spike.

$LSD_{0.05}$: least significant difference in the level of signification $P < 0.05$. The different characters or different groups of characters mean that there are statistically significant differences.

Figure 1. Spike color of Albena, 39a-5, 40-3, 41a-6, 45-5 and Slaveya

Figure 2. Spike colour of line 45-5 compared to Slaveya

Coleoptile coloration was also different as compared to wheat parents. The coleoptiles of 38-7 and 45-5 were evaluated as strong coloration, 40-3 as weak, and 41a-6 as absent or very weak (Figure 3).

Heading/flowering time is largely controlled by major loci, namely *Vrn-1* and *Ppd-1*, in common wheat. In *Ae. tauschii*, several QTLs for heading time have been found (Koyama et al., 2018). The chromosomal regions transferred from the parental synthetic wheat lines to the four Japanese wheat cultivars shortened wheat heading

dates and were dependent on the genetic background (Takumi et al., 2020).

The strong negative effect of the synthetic chromosome 5D on flowering time was detected, as the substitution line 5D had 14 days delay in flowering compared to Chinese Spring. The verbalization sensitivity of the 5D substitution line was coupled with an increased tillering. Due to that and the late flowering most of the other traits, especially fertility and grain weight per ear, were negatively influenced (Pestsova et al., 2001). We

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observed the same tendency in our ILs. All of them were late in HD and bore significantly fewer seeds per spike than Slaveya did (Table 3).

Figure 3. Coleoptile colour: Albena (white), 38-7 (colored), 40-3 (weak colored), 41a-6 (absent) and 45-5 (colored)

Genome-wide association analysis for HD in two environments revealed three QTLs on the short arms of chromosomes 2A, 2B and 2D and two on the long arms of chromosomes 5A and 5D (Börner et al., 2015; Gorafi et al., 2018). Nguyen et al. (2013) reported several QTLs on chromosomes 1D, 6D and 7D in F_2 populations involving four of the primary synthetic hexaploid wheat used to develop the synthetic derivative population. A set of *Triticum aestivum - Aegilops tauschii* introgression lines was developed (Pestsova et al., 2001, 2006). The highest number of recombination events was detected for chromosome 5D and the lowest number for chromosome 1D. Two genomic regions appeared to have favorable alleles derived from the *Ae. tauschii* segment. A positive effect of synthetic chromosome 7D was found for grain weight per ear and thousand-grain weight (Pestsova et al., 2001). In the micro trial, lines 39a-5, 40-3 and 41a-6 had lower seed numbers but showed higher GWS values than both parents. This could be due to several reasons: (1) a smaller number of kernels in the ear resulted in heavier seeds; (2) the tillering number was not examined, and this trait might be very important.

Ae. tauschii contributed favorable alleles for plant height, ear length and spikelet per spike (Pestsova et al., 2006). Three positive synthetic QTLs for spike length on chromosomes 2A and 5A, and one for spikelet number on chromosome 2D were inherited by Shumai 830, a wheat variety obtained with SHW-L1 (Hao et al., 2019). Three of the four ILs grown in the micro trial produced tall plants with long spikes, and possibly other genetic changes, which are unknown at present. Shumai 830 also harbored QTL, mapping to chromosomes 2A, 2D and 5A, which acted to increase both the length of the spike and the number of spikelets per spike (Yu et al., 2014; Hao et al., 2019).

Yield-related traits

The six ILs possessed the same pair of high-molecular-weight proteins, *Glu-D1*-4t+10.1t, and possibly different combinations of LMW-GS and gliadins. Earlier we found that most of the checked progenies in BC_1F_7 had the protein constitution of 2* – 7+8 – 4t+10.1t in *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively, but one family expressed null in *Glu-A1* (Doneva et al., 2018). Six traits were measured for ILs and parents grown in competition trials at DAI (Table 4). Regarding the TW, three lines fell into one group (M = 80–81 kg/hl) together with both parents. With the lowest value was line 40-3 with 77 kg/hl and with the highest - 38-7, 41a-6 and 46-7. By TKW, the lines were differentiated into two groups: one with a variation of 49 – 51 g, and the second with 46 – 48 g involving Albena (M = 46.0 g). Slaveya bore the smallest seeds of all in the trial (M = 42.8 g). The variation in GPC among ILs was much larger than in both parents. Lines 46-7 and 38-7 had the highest grain protein content, of 12.0% and 11.7%, respectively. Another two genotypes expressed the lowest values of GPC on the level of both parents. Surprisingly, line 38-7 exerted the largest variation of 11.4 % on this trait (data not shown), which was two times greater than that of Slaveya.

Mohamed et al. (2022) evaluated the relationship between protein content and grain yield for the multiple synthetic derivatives (MSD) lines. Although the negative

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relationship between grain protein content and grain yield is well-known, the authors showed no relationship between the two traits in MSD lines.

Moreover, most of the lines had higher or lower protein content with comparable grain yield values to the recurrent parent. The protein content varied between 11.0% and 21.8%: 69 lines were significantly higher, 20 lines were significantly lower, and 15 were comparable to wheat cultivar Norin 61. Thus, the authors separated the lines into three categories (A), (B), and (C) based on significant differences in protein content. As compared to our results, GPC ranged very little, from 10.7% to 12.0%.

Improvement of TKW is considered to be an important step for further increasing the yield potential in various parts of the world (Rasheed et al., 2014; Yang et al., 2022). The major contribution made by SHW-L1 to the high yield potential of three Shumai varieties relates to improvements in TGW (thousand-grain weight). The three Shumai varieties reached maturity as early as the conventional varieties but produced heavier grains (Hao et al., 2019). The signature trait of Shumai 830 - the ability to form heavy spikes - was achieved by combining high TGW with a large number of grains per spike. This tendency was observed in our ILs because they were selected for the presence of a pair of HMW-GS accompanied by high NSS and GWS. Three lines (38-7, 39a-5 and 41a-6) gave significantly better values, ranging from 49.4 – 51.0 g,

than the Albena parent with TKW of 46.0 g (Table 4). The rest 3 lines exerted significantly better values than the Slaveya parent, displaying 42.8 g for this trait. The final results showed the breeding success of selected ILs lines carrying the *Glu-D1* pair 4t+10.1t with higher TKW values than the mean of both parents.

Significant variation in grain yield and its component traits have been reported for SHWs and ILs obtained (Dreccer et al., 2007; Ogbonnaya et al., 2013). Interesting results for seed weight compared to bread wheat and TKW of up to 67 g have been reported in Mexico (Calderini and Reynolds, 2000; Cooper et al., 2013). Tang et al. (2014) evaluated three ILs and five bread wheat cultivars consecutively for 3 years under field conditions in Sichuan, China. The new lines showed on average an 11.5 % or 951 kg/ha yield increase. This yield gain was mainly attributed to increases in both grain number per m^2 (5.7%) and TKW (5.9%).

In this study, the ILs were less productive in GY (mean = 7.3 t/ha) than the wheat parents (mean = 8.35 t/ha) which is a 12.6% decrease (Table 4). The reason could be in the type of selection toward the individuals containing the specific pair of HMW-GS in each self-generation after BC_1F_5 (Doneva et al., 2018). The best yielding lines were 39a-5, 40-3 and 41a-6 showing not significantly lower GY than Albena variety.

Table 4. Yield-related traits for lines and parents in DAI for 3 years

Lines	TW	TKW	GPC	SG	FN	GY
38-7	79[abc]	49.4[a]	11.7[ab]	0.6[b]	338[ab]	6.9[c]
39a-5	78[bc]	49.8[a]	10.8[c]	0.3[b]	350[a]	7.5[bc]
40-3	77[c]	48.2[b]	10.7[c]	0.5[b]	339[ab]	7.6[bc]
41a-6	80[abc]	51.0[a]	11.1[bc]	0.7[b]	337[ab]	7.5[bc]
45-5	78[bc]	45.8[b]	11.1[bc]	0.7[b]	314[ab]	7.3[bc]
46-7	80[abc]	48.3[ab]	12.0[a]	1.5[b]	275[b]	6.8[c]
Albena	81[a]	46.0[b]	11.3[bc]	4.1[a]	197[c]	7.9[ab]
Slaveya	80[abc]	42.8[c]	11.0[c]	0.6[b]	319[ab]	8.8[a]
LSD[5%]	2.4	2.8	0.67	1.5	72	0.94

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Williams and Sorrells (2014) conducted QTL mapping in populations derived from SHW and identified several loci on 3D and 6D chromosomes that underpin grain-related traits, including TKW (Börner et al., 2015). Several authors reviewed the current status of synthetic-derived wheat cultivars (Chuanmai 28, 42, 43, 47, 64, 104) released in Southwestern China (Li et al., 2014; Yang et al., 2022). This rise was mainly associated with an increase in seed weight, number of spikes per m^2, and the number of seeds per spike (Cooper et al., 2013; Aberkane et al., 2020).

Falling number is the metric used by grain elevators and grain buyers to evaluate the level of sprouting damage and the quality of grain (Martinez et al., 2018). When PHS occurs, the FN of wheat grain decreases to a value generally below 300, due to the large quantity of α-amylase produced during grain germination. PHS resistance differences among experimental lines tested in the field were small, which resulted in non-significant correlations with FN (Gerard et al., 2022).

Thus, many of the developed experimental durum lines had higher FN values (>300) than the three durum cultivars used as checks (<300), with some lines displaying higher values than the donor parent. Five ILs expressed FN values >300 and formed one group with Slaveya (M=319). Line 46-7 and Albena parent had the lowest FN values with distinguishing high values for SG (Table 4). FN showed a strong positive correlation with GY (Table 5A), but non-significant associations with PG and GI in 2022 (Table 5B). In line with these results, a weak or non-significant correlation between FN and PHS resistance has been previously reported (Singh et al., 2014; Martinez et al., 2018).

Table 5. Multiple correlations between six traits of the introgression lines grown for 3 years (A) and between eight indices measured in 2022 (B)

A					
	TKW	GPC	SG	FN	GY
TW[1]	0.832*	-0.600*	-0.590*	0.775*	0.653*
TKW		-0.665*	-0.518*	0.742*	0.592*
GPC			0.592*	-0.814*	-0.685*
SG				-0.873*	-0.672*
FN					0.670*

B							
	TKW	GPC	SG	FN	GY	PG	GI
TW	0.352	0.965*	-0.704*	0.242	-0.676**	-0.654*	-0.699*
TKW		0.223	-0.279	0.198	-0.456	0.282	0.201
GPC			-0.551**	0.204	-0.533	-0.743*	-0.781*
SG				-0.118	0.612*	0.330	0.382
FN					-0.002	-0.119	-0.230
GY						0.239	0.224
PG							0.980*

[1] for symbols see Table 1, * $P < 0.05$.

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Grain yield and grain protein content are important factors affecting the economic value of common wheat. However, the well-documented negative relationship between grain protein content and grain yield is still a major challenge to producing lines that combine high yield and high protein content and hence good quality (Zhao et al., 2020; Mohamed et al., 2022). Our study showed the significant influence of the year, genotype, and their interaction on the trait variation (Table 6). The three factors had a statistically significant impact on the yield-related characters, including the grain yield. On this trait, the effect of the genotype was stronger than the interaction.

Pre-harvest sprouting

Sprouted seeds were examined in the harvested grain from the competition trials. Unexpectedly, Albena showed a drastic difference from all others with 4.1% germinated seeds. Line 46-7 was in second place with 1.5% sprouted grains. The remaining ILs showed a value close to 0.6% of Slaveya. Actually, Albena surpassed the lines, including Slaveya, about 7 times (Table 4). The statistical analysis revealed significant differences ($P <$ 0.05) among the ILs (Table 7). As expected, the Albena variety showed the greatest percentage of germination (M = 99%) followed by 39a-5 and Slaveya (M = 85%). Line 45-5 exerted the lowest value of germination (12.5%) at DAI with the medium value of standard error. The same trend was observed in the averaged data (DAI+TU). Albena stood out again with the highest percentage of sprouted seeds, followed by 41a-6 and Slaveya.

Line 45-5 was noteworthy for its PHS resistance (11.0% seed germination). The germination index also distinguished the lines. Albena expressed the highest value of 83%, followed by 39a-5 and 41a-6 (Table 7). The smallest GI belonged to line 45-5, 4.3% at DAI, and 3.8% at DAI+TU.

Pre-harvest sprouting (PHS) is a critical factor affecting wheat-producing regions characterized by rainfall and high humidity combined with cool temperatures at harvest (Gerard et al., 2022). The authors obtained 16 experimental durum lines through the use of interspecific introgression. Common wheat line RL4137 was one of the parents with PHS resistance and served as check-in trials with the new durum ILs. Averaged over two environments, this line showed 15.0% and 6.1% for PG and GI, respectively. The best IL EDL_13 expressed the two measurements as 17.0% and 7.0%. The lowest value of GI (5.6%) was expounded by EDL_14 (Gerard et al., 2022). Among our ILs, 45-5 impressed with the lowest PG and GI values, 11.0% and 3.8%, respectively, at the DAI+TU location (Table 7).

Among the varieties in the study of Gerard et al. (2022), AC Avonlea was the most sensitive wheat variety to PHS showing 95.0% and 51.3% for the PG and GI, respectively.

From the data in 2022, Albena parent had 99% and 83% for the two measurements, which defined it as a potentially sensitive wheat variety. In contrast, line 45-5 stood out as bread wheat with resistance to PHS. More data are needed to draw a final conclusion.

Table 6. Two-way ANOVA analysis of yield-related traits for three years (F*ex*)

Source of variation	df	TW	TKW	GPC	SG	FN	GY
Year	2	5166.2***	649.9***	3852.2***	696.5***	5624.9***	107.3***
Genotype	5	279.2***	91.7***	579.6***	64.5***	265.5***	4.6**
Interaction	10	17.3***	30.1***	172.4***	90.3***	157.2***	2.8*
Total	35	314.5	356.9	32.02	58.1	251858.2	45.7

F*ex*: F experimental; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

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Table 7. Pre-harvest sprouting of four introgression lines grown in the 2022 season at two locations

| Line/WP | Percentage of germination | | | | Germination index | | | |
| | D A I | | D A I + T U | | D A I | | D A I + T U | |
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
39a-5	86[ab]	4.3	83[b]	4.4	41[b]	2.3	37[b]	3.0
40-3	78[b]	8.5	75[b]	6.2	37[bc]	4.8	35[b]	3.9
41a-6	83[ab]	1.5	86[b]	2.4	38[bc]	2.6	42[b]	3.4
45-5	12.5[d]	3.2	11[c]	2.4	4.3[d]	1.9	3.8[c]	1.4
Albena	99[a]	1.3	99a	0.8	83[a]	2.6	82[a]	2.6
Slaveya	85[ab]	7.3	84[b]	5.1	33[bc]	4.3	34[b]	3.1
LSD$_{5\%}$	16.6		13.0		9.9		8.7	

WP: wheat parents, M: mean, SE: standard error

In durum wheat, higher PHS resistance was combined with improved FN, TW, and GPC, which are important traits in defining durum field performance and quality (Singh et al., 2014). QTLs for dormancy index were detected on chromosomes 6DL and 6DS, respectively (Lohwasser et al., 2005; Imtiaz et al., 2008). Most of the QTL for germination per se clustered on chromosome 1DS whereas chromosome 7DS harbored loci controlling the development of normal seedlings. Sprouting was controlled in synthetic derivative lines by QTL on group 6 chromosomes (Gatford et al., 2002; Yang et al., 2014). Kaur et al. (2022) described the genes and QTLs for PHS tolerance in synthetic recombinant inbred lines.

Here, the two measurements were used in a correlation between the 6 traits in DAI for 2022 (Table 5B). They showed significantly negative relations with TW and GPC, and non-significant associations with FN. The PG and GI phenotypic values exhibited a high and significant correlation with each other (r = 0.980).

CONCLUSIONS

Four ILs and their wheat parents were included in a micro trial at two locations, DAI-General Toshevo and TU-Varna, to examine several traits including field germination, plant height, heading time, spike characteristics, glume and coleoptiles coloration. The lines differed sharply from the parents in plant height, date of heading, and spike characteristics such as length, number of spikelets, fertility, and glume color. They formed seeds in ears similar to Albena, but significantly less than the Slaveya variety. Line 45-5 was the most distinctive with its characteristic glume-striped coloring.

Six physicochemical properties were studied in field trials including grain yield. The lines diverged both from each other and from the parents. The differences were the smallest in TW, SG and FN, and the largest in TKW, GPC and grain yield. Two of them, 38-7 and 46-7 showed the highest seed protein, 11.7% and 12.0%, respectively, in combination with high FN and low values of SG. FN significantly correlated with TW and TKW, but negatively with GPC and SG. In 2022, two measurements (PG and GI) were added to get the multiple correlations for the six introgression lines. Here, FN did not show any significant relationships, but grain yield correlated significantly with SG (r = 0.612), and PG and GI were negatively associated with GPC. All traits were influenced by the year, genotype and their interaction at P < 0.001, except yield where the factors were arranged to show the most significant influence of the year, followed by the genotype and their interaction.

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Germination data supported Albena parent as a susceptible wheat cultivar to pre-harvest sprouting, while line 45-5 was distinguished by the lowest germination percentage and germination index in 2022, 11.0% and 3.8%, respectively. This genotype appeared to be an interesting wheat line for future research. For most of the investigated characters and especially for the expressed high protein in combination with the high falling number and low germination index, the studied introgression lines are valuable genetic materials for improving the seed quality in wheat breeding.

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