# Polymorphism of *Sdr* genes regulating seed dormancy in *Triticum persicum* Vav. and *Triticum aethiopicum* Jakubz.

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> Preharvest sprouting of wheat grain, sporadically observed in many regions of cultivation of this crop, leads to deterioration of its food and sowing qualities. Seed dormancy is considered to be the main component of resistance to preharvest sprouting. This physiological state of seeds is regulated by many genes, and it depends heavily on environmental conditions. One of the regulators of seed dormancy in cereals is the Sdr4 gene (Seed dormancy 4), which was first studied in rice. In common wheat, the homologues of this gene (TaSdr-A1 and TaSdr-B1) are also involved in the regulation of seed dormancy. The search for valuable alleles in local varieties and endemic forms is a promising area of research aimed at increasing the resistance of crops to adverse environmental factors. In this study, Sdr genes were sequenced in several accessions of two tetraploid wheat species with limited cultivation areas: Persian wheat (Triticum persicum Vav.) and Ethiopian wheat (Triticum aethiopicum Jakubz.). As a result, the same Sdr-A1 and Sdr-B1 variants that had been found in common wheat were detected in these species. The Persian wheat accessions possessed only the Sdr-A1a allele, while Ethiopian ones, only Sdr-A1b. The analysis of F<sub>2</sub> hybrids obtained from crossing these tetraploid species showed that the Sdr-A1b allele was associated with a lower germination index of grains than Sdr-A1a. This result was inconsistent with earlier association studies. Previously unknown polymorphisms were found in the promoter of the Sdr-B1 gene in the studied accessions. A deletion of 16 nucleotides was detected in the 3'-terminal region of the TraesCS2B02G215200 gene, located on the complementary DNA chain close to the 3'-end of the Sdr-B1 gene. Possible effects of the detected polymorphisms on the expression of Sdr genes are discussed. Key words: preharvest sprouting; tetraploid wheats; markers; interspecific hybridization; sequencing.

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## Полиморфизм генов *Sdr*, регулирующих покой семян у *Triticum persicum* Vav. и *Triticum aethiopicum* Jakubz.

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> Предуборочное прорастание зерна пшеницы, периодически наблюдаемое во многих регионах возделывания этой культуры, приводит к ухудшению его продовольственных и посевных качеств. Покой семян считается основным компонентом устойчивости к предуборочному прорастанию. Это физиологическое состояние регулируется множеством генов и сильно зависит от условий окружающей среды. Один из регуляторов покоя семян злаков – ген Sdr4 (Seed dormancy 4), впервые изученный у риса. У мягкой пшеницы гомологи этого гена, TaSdr-A1 и TaSdr-B1, также участвуют в регуляции покоя семян. Поиск ценных аллелей генов у местных сортов и эндемичных форм считается перспективным направлением исследований, нацеленных на повышение устойчивости сельскохозяйственных культур к неблагоприятным факторам окружающей среды. В настоящем исследовании гены Sdr были секвенированы у нескольких образцов двух тетраплоидных видов пшеницы, имеющих ограниченные ареалы возделывания, – пшеницы карталинской (Triticum persicum Vav.) и пшеницы эфиопской (Triticum aethiopicum Jakubz.). В результате у этих видов были найдены те же варианты аллелей генов Sdr-A1 и Sdr-B1, которые ранее были обнаружены у пшеницы мягкой. При этом у пшеницы карталинской встречается только аллель Sdr-A1a, а у пшеницы эфиопской – аллель Sdr-A1b. При анализе гибридов F<sub>2</sub>, полученных от скрещивания данных тетраплоидных видов, аллель Sdr-A1b был связан с меньшим индексом прорастания зерна, чем аллель Sdr-A1a, что не согласуется с результатами предшествующих ассоциативных исследований. В промоторе гена Sdr-B1 у изучаемых образцов были обнаружены ранее неизвестные полиморфизмы. В З'-конце гена TraesCS2B02G215200, расположенного на комплементарной цепи ДНК близко к 3'-концу гена Sdr-B1,

обнаружена делеция 16 нуклеотидов. Обсуждается возможное влияние найденных полиморфизмов на экспрессию генов Sdr.

Ключевые слова: предуборочное прорастание; тетраплоидные пшеницы; маркеры; межвидовая гибридизация; секвенирование.

#### Introduction

Preharvest sprouting of wheat grains remains a challenge in many regions of cultivation of this crop around the world. It decreases the grain yield and deteriorates the quality of the end products (Olaerts, 2018).

Seed dormancy is the main component of resistance to preharvest sprouting. This special physiological condition of seeds is observed after their maturation and associated with a delay or complete absence of germination even with sufficient moisture. Seed dormancy is controlled by many genes and is highly dependent on environmental conditions. Currently, quantitative trait loci (QTL) associated with resistance to pre-harvest sprouting have been mapped on virtually all wheat chromosomes. The most significant loci have been found on chromosomes 2B, 3A, and 4A. In some of these QTL, candidate genes were identified, including *TaMKK3*, *TaPHS1* (*TaMFT*), *TaVp1*, *Tamyb10*, and *TaSdr* (Nakamura, 2018; Vetch et al., 2018).

The Sdr4 gene (Seed dormancy 4) was first identified in rice as a candidate gene in one of the major QTL for resistance to preharvest sprouting. Experiments with rice showed that Sdr4 gene expression was reduced in forms mutant for the OsVp1 gene. It was also shown that the Sdr4 gene upregulates the genes associated with seed dormancy (OsDOG1-like) and downregulates the genes associated with germination (OsGA20ox-1, OsEXPB3). The protein encoded by the Sdr4 gene has no similarity to other proteins of known functions, but has a nuclear localization signal (Sugimoto et al., 2010). Bioinformatic analysis suggested the presence of coiled coil and zinc finger domains in its structure (Zhang et al., 2017). Presumably, the SDR4 protein is a specific regulator of seed dormancy, acting as a transcription factor under the control of a more general regulator of seed maturation, the VP1 protein (Sugimoto et al., 2010).

In common wheat, homologues of *OsSdr4* gene – the genes *TaSdr-A1* and *TaSdr-B1*, located on chromosomes 2A and 2B, respectively, were cloned. Point mutations associated with germination index variation were found in the sequences of these genes. The association of *Sdr4* alleles with the germination index was shown both in a comprehensive collection of Chinese wheat varieties and in recombinant inbred lines Yangxiaomai × Zhongyou 9507. It was also shown that the alleles of these genes associated with resistance to sprouting are often present in Chinese and Japanese wheat varieties but nearly nonexistent among Russian ones (Zhang et al., 2014, 2017).

One of the ways to seek valuable alleles associated with resistance to adverse environmental factors is the investigation of wild forms and landraces of the crop. In order to expand the allelic diversity of the *Sdr* genes used in wheat breeding, what is especially important for Russia, we decided to study these genes in two locally cultivated tetraploid wheat species:

Persian wheat (Triticum persicum Vav.) and Ethiopian wheat (Triticum aethiopicum Jakubz.)<sup>1</sup>. Persian wheat is grown in the Caucasus geographical region, including regions of Russia (Dagestan), Georgia, Armenia, and northeastern Turkey. It is characterized by low demands for heat, resistance to fungal diseases, and a long period of afterripening of the grain. However, this species has poor drought tolerance (Dorofeev et al., 1979). Ethiopian wheat is a naked tetraploid species found in mountainous regions of Ethiopia and in the south of the Arabian Peninsula. Ethiopian tetraploid wheat is characterized by an exceptional diversity of botanical forms. However, these forms share some common features that allow them to be merged into a separate species (Dorofeev et al., 1979). Due to the mountainous topography of the original ranges of Persian and Ethiopian wheats, the climatic conditions under which these species developed are very diverse. Some areas of cultivation of these species have a humid climate in which precipitations touch the season of grain ripening. Thus, we may assume that some forms of Persian wheat and Ethiopian wheat carry valuable alleles of genes associated with resistance to preharvest sprouting.

#### Materials and methods

The accessions of Ethiopian wheat collected during a joint Ethiopian–Russian biological expedition in 2012 were obtained from the Vavilov Institute of General Genetics, Russian Academy of Sciences (Badaeva et al., 2018). The accessions of Persian wheat were obtained from the Vavilov All-Russia Institute of Plant Genetic Resources. The information on the country of origin of each accession is presented in Table 1. Wheat accessions were grown in the field on 1-m<sup>2</sup> plots in 2016–2017. Every year they were assessed for resistance to preharvest sprouting by germinating grains collected at full ripeness and calculating the germination index.

To determine the germination index, the grains were manually extracted from the ear and placed in Petri dishes on two layers of filter paper moistened with 8 mL of distilled water. Germination was carried out in the dark at controlled temperature of 24 °C. Sprouted grains were counted and removed daily. The filter paper was moistened as it dried. The germination lasted 7 days. The grains that did not germinate within this time interval were kept in a refrigerator at 4 °C for 3 days to break dormancy; after that, viable seeds were counted. The experiment was performed in six replications. The grains from each ear were placed in a separate Petri dish. The germination index was calculated as:

$$GI = (7 \times n_1 + 6 \times n_2 + 5 \times n_3 + \dots + 1 \times n_7) / (7 \times N),$$

where  $n_1, n_2 \dots n_7$  are the numbers of germinated grains on the first, second and subsequent days up to the seventh day;

<sup>&</sup>lt;sup>1</sup> This article follows the taxonomy of the genus *Triticum* reported in (Dorofeev et al., 1979).

No. <sup>1</sup>	VIR catalog accession <sup>2</sup>	Accession number, site code <sup>3</sup>	Country of origin	Botanical variety <sup>5</sup>
		Т. р	ersicum	
1	k-13382	_	Georgia	rubiginosum
2	k-13989	-	Armenia	rubiginosum
3	k-13768	-	»	persicum
4	k-1694	-	Georgia	persicum
5	k-26828	-	Russia <sup>4</sup>	persicum
6	k-13938	-	»	osseticum
7	k-7106	-	Georgia	persicum
8	k-6429	-	»	stramineum
9	k-47794	-	Russia <sup>4</sup>	rubiginosum, stramineum
10	k-49456	-	Canada <sup>4</sup>	stramineum
		T. aet	thiopicum	
11	-	7480, 28a	Ethiopia	albiviolaceum
12	-	7610, 47a	»	nigriviolaceum, pseudoschimperi
13	-	7655, 50b	»	harraricum
14	_	7634, 49a	»	purpureum
15	_	7567, 40b	»	rufescens
16	-	7719, 58b	»	brownii
17	_	7417, 21a	»	nigrimarginatum
18	_	7589, 42b	»	pseudorubescens

### **Table 1.** The country of origin and botanical varieties of Persian and Ethiopian wheat accessions used in the study

<sup>1</sup> Numerical designation in this study; <sup>2</sup> Vavilov All-Russia Institute of Plant Genetic Resources; <sup>3</sup> in the joint Ethiopian–Russian biological expedition in 2012; <sup>4</sup> breeding or research material; <sup>5</sup> identified using (Dorofeev et al., 1979).

*N* is the total number of viable grains taken for germination. The germination index ranges from 0 to 1, and the deeper is the seed dormancy, the lower the index (Walker-Simmons, 1988).

In 2016, interspecific crosses of some Persian wheat and Ethiopian wheat accessions were made, including crosses *T. aethiopicum* no.  $15 \times T.$  persicum no. 5 and *T. aethiopicum* no.  $15 \times T.$  persicum no. 8. In 2017, F<sub>1</sub> hybrids were grown in the field, and in the winter of 2017–2018, F<sub>2</sub> hybrids were grown in a greenhouse. To estimate the germination index, half of the grains harvested from each F<sub>2</sub> plant were germinated in a Petri dish (single replication).

DNA was extracted from dried leaves of plants by the P.J. Doyle method (1991). Primers for amplification of the *SDR-A1* (SDR-AF, SDR-AR) and *Sdr-B1* (SDR-BF, SDR-BR) genes, including parts of their promoters, were selected specifically for wheat subgenomes A and B on the basis of wheat genome sequences IWGSC RefSeq v1.0 (Alaux et al., 2018) using Primer BLAST (NCBI).

The sequences of all primers used in the study are shown in Table 2. PCR was performed in a SimpliAmp Thermal Cycler (Applied Biosystems). The concentrations of the reaction mixture components were as follows:  $1 \times$  buffer supplied with the polymerase (including 2.5 mM MgCl<sub>2</sub>), 0.2 mM of each dNTP, 10 % v/v dimethyl sulfoxide, 1 µm of each of the forward and reverse primers, 0.05 U/µL *Taq*-polymerase (Sileks), 0.01 U/µL *Pfu*-polymerase (Sileks), and 4 ng/µL template DNA. The addition of dimethyl sulfoxide to the reaction mixture is needed due to the high content of GC base pairs (bp) in amplified DNA fragments. The PCR program was conducted as follows: predenaturation 94 °C, 5 min; 45 cycles 94 °C, 30 s; 57 °C, 45 s; and 72 °C, 2 min; postextension 72 °C, 5 min. Prior to sequencing, the PCR products were purified with a Cleanup Mini Kit (Evrogen).

The sequencing was carried out with a BrilliantDye Terminator v3.1 Cycle Sequencing Kit (Nimagen), SimpliAmp Thermal Cycler (Applied Biosystems) and 3130xl Genetic Analyzer (Applied Biosystems). The end primers (used for PCR) as well as additional primers SDR-2AF, SDR-2AR (for the *Sdr-A1* gene) and SDR-3BF, SDR-3BR (for the *Sdr-B1* gene) were used for the sequencing reaction (Zhang et al., 2014). The nucleotide sequences were aligned with GeneDoc v.2.7 software. The nucleotide sequence of the *Sdr-B1* promoter was analyzed with the PlantCARE online service (Lescot et al., 2002).

Primers for creating new markers were designed with Primer BLAST (NCBI). The PCR volume was 25  $\mu$ L. The final concentration of the components of the mixture was similar to that mentioned above, but without the addition of *Pfu*-polymerase. SDR-5BF and SDR-SNP-BR primers were used to detect the single nucleotide polymorphism at position (-11) of the *Sdr-B1* gene. The PCR program was as follows: 95 °C, 1 min; 40 cycles 95 °C, 45 s; 65 °C, 45 s; 72 °C, 45 s; postextension 72 °C, 2 min. The PCR products were digested with *Psp*CI restriction endonuclease (SibEnzyme) without pretreatment. The reaction mixture included a buffer solution

Name	Sequence 5'-3'	Use	
SDR-AF	ACAACAGGATGATACAGGGGAC	Amplification of the entire gene, sequencing	
SDR-AR	AGGGAGTATAATATAATTTTGCCATCT		
SDR-BF	ACCCCTACGCATTTATACAGACT		
SDR-BR	CTGTCGTGCAATATGAGCTAGAA		
SDR-2AF*	CGTCGGCAGACATCGACTCC	Sequencing	
SDR-2AR*	GAAGCTCACTAGCTCAGAACACGC		
SDR-3BF*	CGTCAGCAGACTTCGACTCGC		
SDR-3BR*	CAAGAAGCTCACTATCTCAGAACACAA		
SDR-SNP-AF	ACCATCCACGTCGAATCCATC	Amplification of marker fragments	
SDR-SNP-AR	CTTGGTTTCGCACGCAGCTC		
SDR-5BF*	CGCCTACGTGTCGGCCC		
SDR-SNP-BR	CACGGCCTGTTCTGCAGGTG		
SDR-B1-InDel-F	ATGTACAAAAGCCTAGCCACAGA		

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\* Primers designed in (Zhang et al., 2014). Other primers are of our own design.

supplied with the endonuclease (buffer B, SibEnzyme) and bovine serum albumin (BSA) at recommended concentrations  $(1\times)$  plus 1 U/µL restrictase. The volume of the reaction mixture was 10 µL. The reaction was carried out at 37 °C for 2 h.

To identify the deletion located 449-464 bp downstream from the stop codon of the Sdr-B1, the primers SDR-B1-InDel-F and SDR-BR were used. The PCR program was as follows: 95 °C, 2 min; 35 cycles 95 °C, 30 s; 58 °C, 30 s; 72 °C, 30 s; postextension 72 °C, 2 min.

The SDR-SNP-AF and SDR-SNP-AR primers were used to detect a single nucleotide polymorphism at position 643 of the Sdr-A1 gene. The PCR program was as follows: 95 °C, 1 min; 10 cycles 95 °C, 45 s; 66 °C (with a temperature decrease by 0.4 °C per cycle), 45 s; 72 °C, 45 s; 35 cycles 95 °C, 45 s; 62 °C, 45 s; 72 °C, 45 s; postextension 72 °C, 2 min. The PCR products were cut with Ple19I restriction endonuclease (SibEnzyme) without pre-treatment. The reaction mixture consisted of the buffer solution, supplied with endonuclease (buffer Y, SibEnzyme) at the concentration  $1\times$ , and  $0.1 \text{ U/}\mu\text{L}$ restrictase. The volume of the reaction mixture was 10 µL. The reaction was carried out at 37 °C for 2 h.

The electrophoresis of the PCR and restriction products was carried out in 1.5 % agarose gel in TBE buffer supplemented with ethidium bromide. The results were visualized using a GelDoc XR+ Gel Documentation System (Bio-Rad).

The statistical analysis of the data was carried out using Statistica 6.0 software. Before the analysis of variance, germination index values were transformed according to the formula:  $\arcsin(\sqrt{x})$ . In the Tables 1–3 and Figures 1–4, back transformed mean values are presented. The nontransformed germination index values were used in the Mann-Whitney test.

#### Results

The tested wheat accessions differed significantly from each other in terms of germination index both in 2016 and in 2017 (Table 3). The lowest germination indices averaged over the two years were recorded in accessions nos. 2, 4, 8, and 10 of Persian wheat and accession no. 15 of Ethiopian wheat. The highest germination index was found in Ethiopian wheat accessions nos. 13, 16, and 18. On the average, Persian wheat showed a lower germination index than Ethiopian (p < 0.05).

The Ethiopian wheat accessions nos. 15 and 16 and Persian wheat accessions nos. 5 and 8 were chosen for sequencing the Sdr-A1 and Sdr-B1 genes, as these accessions showed the most contrasting germination indices. The resulting sequences were deposited into the GenBank database under accession numbers MK396766, MK396767, MK396768, and MK396769.

Sequencing of the Sdr-A1 gene in the mentioned accessions showed that they differed in the same single nucleotide polymorphism that had been found in common wheat (Zhang et al., 2017). The nucleotide A, characteristic of the Sdr-A1b allele, was found in Ethiopian wheat accessions nos. 15 and 16 at position 643 from the start-codon, and the nucleotide G, characteristic of the Sdr-Ala allele, was found in Persian wheat accessions nos. 5 and 8. Apart from that, the Sdr-A1 gene sequences of the two studied wheat species did not differ from each other, and they matched the reference sequence of the common wheat genome of the cultivar Chinese Spring.

In other Persian wheat and Ethiopian wheat accessions that were characterized in our study by the germination index alleles of the Sdr-A1 gene were determined using the molecular marker previously developed by Y. Zhang et al. (2017), based on PCR and endonuclease cleavage. All studied Persian wheat accessions possessed only the Sdr-A1a allele, whereas all Ethiopian wheat accessions possessed only Sdr-A1b.

We also analyzed the effects of different alleles of the Sdr-A1 gene on seed dormancy in  $F_2$  of two interspecific crosses between Persian and Ethiopian wheat accessions. A molecular marker developed by Y. Zhang et al. (2017), involves the amplification of a DNA fragment of 1294 bp. Due to the fact that the amplification of such large DNA fragments, especially those containing GC-rich sites, with Taqpolymerase is often difficult, we decided to reselect primers for the marker of this single-nucleotide polymorphism so that the resulting amplicon be as short as possible. Primers SDR-SNP-AF and SDR-SNP-AR specific for subgenome A (see Table 2) were designed to yield an amplicon of 499 bp. The obtained PCR product was cleaved with restrictase Ple19I. The

No.	Alleles of the genes		l=D=l*	Germination	Germination index**		
	Sdr-A1	Sdr-B1	indei	2016	2017	Mean	
			Т. р	persicum			
1	а	а	230	0.45 <sup>cd</sup>	0.19 <sup>bc</sup>	0.31 <sup>abc</sup>	
2	а	а	230	0.17 <sup>ab</sup>	0.12 <sup>abc</sup>	0.14 <sup>a</sup>	
3	а	b	214	0.62 <sup>d</sup>	0.35 <sup>cd</sup>	0.48 <sup>abcde</sup>	
4	а	b	214	0.18 <sup>ab</sup>	0.08 <sup>ab</sup>	0.13ª	
5	а	b	214	0.63 <sup>de</sup>	0.57 <sup>def</sup>	0.60 <sup>abcde</sup>	
6	а	b	214	0.38 <sup>c</sup>	0.36 <sup>cd</sup>	0.37 <sup>abcd</sup>	
7	а	b	214	0.27 <sup>abc</sup>	0.21 <sup>bc</sup>	0.24 <sup>ab</sup>	
8	а	b	214	0.13ª	0.10 <sup>ab</sup>	0.11ª	
9	а	а	230	0.80 <sup>ef</sup>	0.19 <sup>bc</sup>	0.49 <sup>abcde</sup>	
10	а	b	214	0.31 <sup>bc</sup>	0.03 <sup>a</sup>	0.14 <sup>a</sup>	
			T. aei	thiopicum			
11	Ь	а	230	0.60 <sup>d</sup>	0.68 <sup>efg</sup>	0.64 <sup>abcde</sup>	
12	Ь	а	230	0.85 <sup>f</sup>	0.75 <sup>efg</sup>	0.80 <sup>bcde</sup>	
13	Ь	b	230	1.00 <sup>g</sup>	0.83 <sup>fgh</sup>	0.96 <sup>e</sup>	
14	Ь	b	230	0.44 <sup>cd</sup>	0.35 <sup>cd</sup>	0.39 <sup>abcde</sup>	
15	b	b	214	0.16 <sup>ab</sup>	0.15 <sup>abc</sup>	0.15 <sup>a</sup>	
16	b	b	230	0.90 <sup>f</sup>	0.97 <sup>h</sup>	0.94 <sup>de</sup>	
17	b	b	230	0.45 <sup>cd</sup>	0.55 <sup>de</sup>	0.50 <sup>abcde</sup>	
18	b	b	214	0.89 <sup>f</sup>	0.85 <sup>gh</sup>	0.87 <sup>cde</sup>	
<i>p</i> -values	of differences b	etween acces	sions (F test)	< 0.001	< 0.001	< 0.001	

**Table 3.** Alleles of the Sdr-A1 and Sdr-B1 genes and germination indices in T. persicum and T. aethiopicum accessions

\* Marker of the deletion close to the Sdr-B1 gene; amplicon sizes (bp) are shown; \*\* The germination index values marked in each column

with the same letters do not differ significantly according to Tukey's test.

PCR product of the *Sdr-A1a* allele was not cleaved, and that of *Sdr-A1b* yielded fragments of 387 and 112 bp (Fig. 1, *a*). Test of the new marker on the Persian wheat and Ethiopian wheat accessions showed a complete coincidence of the results of the analysis with the results obtained with the previously developed marker. In addition, the new primers significantly improved the reliability of amplification.

A total of 66  $F_2$  plants *T. aethiopicum* no. 15 × *T. persicum* no. 5 were analyzed with the new marker. Of them, 15 plants were homozygous for the *Sdr-A1a* allele, 11 were homozygous for *Sdr-A1b*, and the other 40 were heterozygous. The segregation in  $F_2$  matched the Mendelian 1:2:1 ratio ( $\chi^2 = 4.125$ ; p = 0.178). As the statistical distribution of the germination index in  $F_2$  was far from normal, we used the nonparametric Mann–Whitney U test to estimate the association of the index with plant genotypes. Only homozygous forms were used in these calculations. According to the results of the statistical test, the *Sdr-A1* gene had no significant effect on the germination index in  $F_2$  of the *T. aethiopicum* no. 15 × *T. persicum* no. 5 cross (p = 0.09), but plants with the *Sdr-A1b* allele tended to possess lower indices.

We genotyped 83  $F_2$  plants in the *T. aethiopicum* no. 15 × × *T. persicum* no. 8 intercross. Of these, 25 were identified to have the *Sdr-A1a* allele, 15 – *Sdr-A1b*, and 43 were heterozygotes. The segregation generally corresponded to the Mendelian 1:2:1 ( $\chi^2 = 2.98$ ; p = 0.284); however, as in the cross described above, the proportion of homozygotes in

the *Sdr-A1b* allele was slightly lower than expected. In this cross, the relationship between the germination index and the marker of the *Sdr-A1* gene was significant (p = 0.035 by the Mann–Whitney U test). The plants with *Sdr-A1b* had a lower grain germination index than plants with *Sdr-A1a*. The same trend remained when data from the two F<sub>2</sub> populations were bulked into one sample (p = 0.012 by the Mann–Whitney U test) (Fig. 2).

The sequencing of the *Sdr-B1* gene in Persian wheat accessions nos. 5 and 8 and Ethiopian wheat accessions nos. 15 and 16 showed that they did not differ from each other in the coding region of this gene. In the gene promoter, accession no. 16 had two single-nucleotide substitutions, while accessions nos. 5, 8, and 15 had a dinucleotide insertion CT compared to the reference sequence of Chinese Spring wheat (Fig. 3, *a*). The previously known variant of the single nucleotide polymorphism at position (-11) from the start codon characteristic for the *Sdr-B1b* allele (Zhang et al., 2014) was found in the 5'-noncoding region of the gene in all the four wheat accessions.

We also optimized the marker intended for the identification of *Sdr-B1* gene alleles by redesigning the primers to obtain a shorter PCR product (see Fig. 1, *b*). The alleles of the *Sdr-B1* gene, differing by the single-nucleotide polymorphism at position (-11), showed no statistically significant correlation with the germination index in the studied collection of the two wheat species (p = 0.9 by Fisher's F test).

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Near the 3'-end of the *Sdr-B1* gene, the DNA fragment sequenced in our study partially overlaps the 3'-untranslated region of the *TraesCS2B02G215200* gene, lying close nearby on the complementary DNA chain and supposedly encoding the 18-kDa subunit of NADH ubiquinone oxidoreductase. In the 3'-untranslated region of *TraesCS2B02G215200*, accessions nos. 5, 8, and 15 have a 16-nucleotide deletion, which is absent from the sequence of accession no. 16 and from the reference sequence of common wheat, cultivar Chinese Spring (see Fig. 3, *b*).

Due to the fact that this 16-nucleotide deletion can serve as an easy-to-use linked marker of *Sdr-B1* alleles, we designed flanking primers (SDR-B1-InDel-F and SDR-BR) for its identification. Electrophoresis of the PCR products obtained with these primers makes it possible to identify two variants of the DNA fragments with sizes 230 and 214 bp. There is also an additional fragment of larger size (about 266 bp), probably amplified from chromosome 2A (Fig. 4). A test of the marker with the collection of Persian wheats showed that the presence of the 230-bp fragment coincided with the presence of the *Sdr-B1a* allele, which had been associated with a low germination index in previous studies. However, this pattern was broken among the Ethiopian wheat accessions. In general, in the two wheat species, the accessions showing the shorter marker fragment (214 bp) had a low germination index on the average (0.35), while the accessions with the longer fragment (230 bp) had high (0.57). However, this correlation between the presence of the deletion and the germination index in the tested collection was of low statistical significance (p = 0.1 according to the Fisher F test for the two-year means).

#### Discussion

Earlier, researchers had noted that Persian wheat possessed good resistance to preharvest sprouting (Dorofeev et al., 1979). This was confirmed by our results. For two years of our experiments, the average germination index of Persian wheat was lower than that of Ethiopian wheat.

In previous studies, the *Sdr-A1a* and *Sdr-B1a* alleles were associated with resistance, while *Sdr-A1b* and *Sdr-B1b* were associated with susceptibility to preharvest sprouting (Zhang, 2014, 2017). In all the Persian wheat accessions tested we found the *Sdr-A1a* allele, while all, Ethiopian wheat accessions that generally less resistant to sprouting, had solely *Sdr-A1b*. Thus, it could be assumed that the *Sdr-A1a* allele contributes to the resistance to preharvest sprouting in Persian wheat.

However, the analysis of the germination index in connection with plant genotypes in  $F_2$  showed a relationship inconsistent with the hypothesis proposed above: plants with the *Sdr-A1b* allele produced grain with a lower germination index than plants with *Sdr-A1a*. Heterozygous plants showed an intermediate value of the germination index between the homozygotes. There may be several explanations for the different direction of the connection of plant genotypes with the germination index observed in this case. First, the effect of the *Sdr-A1* alleles on seed dormancy may be strongly dependent on the conditions of plant growth. The  $F_2$  plants were grown in a greenhouse, while the original wheat accessions were grown in the field. Second, it can be explained by the interaction of the *Sdr-A1* gene with other genes, which may invert the effects of the alleles in these crosses. Third, it may be associated with a mutation in a closely linked regulatory DNA sequence (enhancer or silencer), which was not covered by the sequencing of the *Sdr-A1* gene.

Analysis of the promoter of the *Sdr-B1* gene using the online service PlantCARE showed that it contains mainly *cis*-regulatory elements associated with the response to light, abscisic acid, methyl jasmonate, and anoxia, as well as elements responsible for expression in meristem. The presence of such regulatory elements in the promoter of the *Sdr-B1* gene can explain to some extent the known enhancement of seed dormancy in wheat and other relative cereals with the presence of light or abscisic acid and with oxygen shortage in contrast to the weakening of seed dormancy in response to methyl jasmonate (Walker-Simmons, 1988; Hoang et al., 2014; Xu et al., 2016). Probably, the CT dinucleotide insertion in the *Sdr-B1* promoters of accessions nos. 5, 8, and 15 does not affect the structure of *cis*-regulatory elements. In accession no. 16, the single-nucleotide substitutions detected involve two regulatory sequences. The nucleotide mutation G(-454)C leads to the appearance of an additional element responsible for the reaction to methyl jasmonate and



**Fig. 1.** The new markers of single nucleotide polymorphisms (SNP) in *Sdr* genes of wheat.

a - the marker of SNP A643G in the Sdr-A1 gene. An example of electrophoresis of the PCR products obtained with primers SDR-SNP-AF/SDR-SNP-AR and cut with endonuclease Ple19I: aa - homozygote for Sdr-A1a allele, bb - homozygote for Sdr-A1b allele, ab - heterozygote. The sizes of the products are given in base pairs (bp); b - the marker of SNP A(-11)G in the 5'-untranslated region of the Sdr-B1 gene. An example of electrophoresis of the PCR products obtained with primers SDR-5BF/SDR-SNP-BR and cut with endonuclease PspCI: bb - homozygote for Sdr-B1b, aa - homozygote for Sdr-B1a, ab – heterozygote. The fragment of 343 bp corresponds to the intact PCR product, which remains as a result of low efficiency of cutting off the short 57-bp fragment. M – DNA ladder M-100 (Syntol)



**Fig. 2.** Germination indices of the grains collected from  $F_2$  plants with different *Sdr-A1* genotypes: aa – homozygotes for *Sdr-A1a* allele, bb – homozygotes for *Sdr-A1b* allele, ab – heterozygotes.

The means are calculated from the combined data of two crosses no.  $15 \times no. 5$  and no.  $15 \times no. 8$ . Error bars show 95 % confidence intervals.

а

		-454	
т.	carth 5	CTGCCAACGT <u>G</u> ACCCCACCCCACGCCCCATGAATGGAGCGGCAGCCAGCCA	240
т.	aeth $\overline{1}6$	CTGCCAACGTCACCCCACGCCCCATGAATGGAGCGGCAGCCAGC	240
т.	aest_CS	CTGCCAACGTGACCCCACCCCACGCCCCATGAATGGAGCGGCAGCCAGC	240
т.	carth 5	GCGGGACGCGCCACGGGGGACGAGGCGACACGAAGCGAGCAGGCGCGGGGGG	300
т.	aeth $\overline{1}6$	GCGGGACGCCCCCGGGGGACGAGGCGACGCGAGCGGGGGG	300
т.	aest_CS	GCGGGACGCGCCACGGGGGACGAGGCGACACGAAGCGAGCGGGGGG	300
т.	carth 5	GATCGCGCCGCGCATGCAACGGGCCTCTCGCGCCCCGTGCCCGCCC	360
т.	aeth $\overline{1}6$	GATCGCGCCGCGCATGCAACGGGCCTCGCGCCTCCCCGTGCCCGCCCGCCCGTCCGGT	358
т.	aest_CS	GATCGCGCCGCGCATGCAACGGGCCTCGCGCCTCCCCGTGCCCGCCCGCCCGTCCGGT	358
т.	carth_5	GCCTCCCCCACTCCGTCACACGCCTGACGCCCCTCTCACTGGACGCCACTGGAATCCAC	420
т.	aeth_16	GCCTCCCCCCACTCCGTCACACGCCTGACGCCCCTCTCACTGGACGCCACTGGAATCCAC	418
т.	aest_CS	GCCTCCCCCACTCCGTCACACGCCTGACGCCCCTCTCACTGGACGCCACTGGAATCCAC	418
		-226	400
т.	carth_5	AGTCCTCTCCCTCCAAAGCAGCGCGCCCCGCGACTCGCCTCCGCCTACGTGTCGGCCCCG	480
т.	aeth_16	GTCCTCTCCCTCCAAAGCAGCGCGCCCCGCGACTCGCCTCCGCCTACGTGTCGGCCCCG	480
т.	aest_CS	AGTCCTCTCCCTCCAAAGCAGCGCCCCCGCGACTCGCCTCCGCCTACGTGTCGGCCCCG	478
т.	carth_5	TCCCCGCCCGCTCGCCCACGTACCCCGCGCCTCGTTCCCACGTGCCCCTCCCT	540
т.	aeth_16	TCCCCGCCCGCTCGCCCACGTACCCCGCGCCTCGTTCCCACGTGCCCCTCCCT	538
т.	aest_CS	TCCCCGCCCGCTCGCCCACGTACCCCGCGCCTCGTTCCCACGTGCCCCTCCTCTGCGCG	540
т.	carth 5	CATCCGATTGGCCGCCCACGCCTTCTTAAGCCGGCACGGCACCGGGACCCAACGCCGTGC	600
т.	aeth $\overline{1}6$	CATCCGATTGGCCGCCCACGCCTTCTTAAGCCGGCACCGGGACCCAACGCCGTGC	598
т.	aest_CS	CATCCGATTGGCCGCCCACGCCTTCTTAAGCCGGCACGGCACCGGGACCCAACGCCGTGC	598
_		-11 <u>CSD (<i>Sdr-B1</i>)</u>	
т.	carth_5	ACTCCGTCCACCCCCGTCAGCAGACTTCGACTCGCCGCGTGCACGCCATGGCCATGGTGCA	660
т.	aeth_16	ACTCCGTCCACCCCGTCAGCAGACTTCGACTCGCCCGCGTGCACGCAATGGCCATGGTGCA	658
т.	aest_CS	ACTCCGTCCACCCCGTCAGCAGACTTCGACTCGCACGTGCACGCAATGCCCATGCTGCA	658
		3'UTR ( <i>TraesCS2B02G215200</i> )	
т.	carth 5	TAGCCAAGAAATTCTGTGTGAAGGGGAATTTTACAAGCATTTTG	2084
т.	aeth 16	TAGCCAAGAAATTCTGTGTGAAGGGGAATTTTACAGGCAAGGCTAATAACAAGCATTTTG	2098
т.	aest CS	TAGCCAAGAAATTCTGTGTGAAGGGGAATTTTACAGGCAAGGCTAATAACAAGCATTTTG	2098

b

### Fig. 3. The alignment of nucleotide sequences containing polymorphisms identified in the Sdr-B1 gene neighborhood.

a – the promoter and 5'-end of the Sdr-B1 gene of Ethiopian wheat no. 16, Persian wheat no. 5, and the reference sequence of chromosome 2B of wheat variety Chinese Spring (CS). Numerals above the lines designate the distance from the start codon. UTR is the-untranslated region. CSD is the-protein-coding sequence of the gene; b - alignment of the 3' untranslated region of the TraesCS2B02G215200 gene, located on the complementary DNA chain next to the SDR-B1 gene (TraesCS2B02G215300), containing a 16-nucleotide deletion.



Fig. 4. The marker of the deletion close to the 3'-end of the SDR-B1 gene in Triticum persicum accessions (1-10), T. aethiopicum accessions (11-18), and T. aestivum cultivar Chinese Spring (CS).

M - the DNA ladder M-100 (Syntol). The marker fragments are 230 and 214 base pairs (bp). The fragment of 266 bp is a byproduct amplified from chromosome 2A.

the disruption of the light-sensitive G box, and the mutation A(-226)G disrupts the light-sensitive GT-1 binding element. All these changes in the promoter may weaken, although slightly, the expression of the Sdr-B1 gene and, accordingly, can be among the causes for the weak seed dormancy in accession no. 16.

#### Conclusion

The CAPS-marker-identified single-nucleotide polymorphism A(-11)G in the 5'-non-coding region of the Sdr-B1 gene showed no statistically significant correlation with the germination index in the studied wheat accessions. At the same time, on the same plant material, we found a weak (p = 0.1)correlation between the germination index and the deletion in the 3'-untranslated region of another gene lying close on the complementary DNA chain. Presumably, this deletion affects the expression of the Sdr-B1 gene through the mechanism of RNA interference, as the genes TraesCS2B02G215200 and Sdr-B1 (TraesCS2B02G215300) lie on DNA strands complementary to each other, being separated by a very small gap, about 200 bp. Even if the deletion of 16 nucleotides near the *Sdr-B1* gene actually does not influence its expression, it can be used as a convenient supplementary marker to discriminate the alleles of this gene in specific crosses without using endonucleases.

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