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Development and characterization of a line with substitution of chromosome 4B of wheat *Triticum aestivum* L. on chromosome 4H^{mar} of wild barley *Hordeum marinum* ssp. *gussoneanum* (4*x*)

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Abstract. Introgressive hybridization is the main method of broadening the genetic diversity of bread wheat. Wild barley *Hordeum marinum* ssp. *gussoneanum* Hudson (2*n* = 4*x* = 28) has useful agronomical traits, such as high resistance to stress factors, that could be a potential source of new genes for bread wheat improvement. This study aimed to evaluate the possibility of introgression of *H. marinum* chromosomes into the genome of bread wheat using an incomplete amphiploid *H. marinum* ssp. *gussoneanum* (4*x*)–*T. aestivum* (Pyrotrix 28) (2*n* = 54) carrying the cytoplasm of wild barley. For this purpose, we crossed the line of bread wheat variety Pyrotrix 28 with an incomplete amphiploid, and then selected cytogenetically stable 42-chromosome plants with a high level of fertility in hybrid progeny. Genomic *in situ* hybridization (GISH) revealed a pair of *H. marinum* chromosomes in the genome of these plants. C-banding analysis confirmed that bread wheat chromosome 4B was replaced by wild barley chromosome 4H^{mar}. SSR markers *Xgwm368* and *Xgwm6* confirmed the absence of chromosome 4B, and EST markers *BAWU808* and *BAW112* identified chromosome 4H^{mar} in the genome of the isolated disomic wheat-barley substitution line. The study of this line showed that the substitution of chromosome 4B with chromosome 4H^{mar} resulted in a change of some morphological traits. It included intense anthocyanin coleoptile coloration, specific for *H. marinum*, as well as a lack of purple coloration of the ears in the leaf sheath, specific for Pyrotrix 28. Line 4H^{mar}(4B) showed increased performance for several traits, including plant height, number of spikes and tillers per plant, spikelet and grain number in the main spike, grain number per plant, but it had decreased values of 1000-grain weight compared to wheat. Cytogenetic stability and fertility of line 4H^{mar}(4B) indicated a high compensation ability of barley 4H^{mar} for wheat chromosome 4B and confirmed their homeology.
Key words: *Hordeum marinum* ssp. *gussoneanum*; bread wheat; wheat-barley substitution line 4H^{mar}(4B).

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Получение и изучение линии с замещением хромосомы 4B пшеницы *Triticum aestivum* L. на хромосому 4H^{mar} дикого ячменя *Hordeum marinum* ssp. *gussoneanum* (4*x*)

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Аннотация. Интрогрессивная гибридизация является основным методом расширения генетического разнообразия мягкой пшеницы. В качестве источника новых генов для мягкой пшеницы может служить дикий ячмень *Hordeum marinum* ssp. *gussoneanum* Hudson (2*n* = 4*x* = 28), который характеризуется высокой устойчивостью к стрессовым факторам. Настоящая работа посвящена изучению возможности использования неполного амфиплоида *H. marinum* ssp. *gussoneanum* (4*x*)–*T. aestivum* (Пиротрикс 28) (2*n* = 54), носителя цитоплазмы дикого ячменя, в качестве источника хромосом *H. marinum* для их интрогрессии в геном мягкой пшеницы. С этой целью получены гибриды между линией сорта мягкой пшеницы Пиротрикс 28 (далее П28) и неполным амфиплоидом,

а затем среди потомков гибридов проведен отбор цитогенетически стабильных 42-хромосомных растений с высоким уровнем фертильности. С использованием GISH-анализа обнаружено наличие пары хромосом *H. marinum* в геноме этих растений. По результатам С-окрашивания хромосом установлено, что у этой гибридной линии произошло замещение хромосомы 4В мягкой пшеницы на хромосому дикого ячменя 4H^{mar}. С помощью хромосом-специфичных SSR-маркеров *Xgwm368* и *Xgwm6* подтверждено отсутствие хромосомы 4В мягкой пшеницы, а с применением EST-маркеров *BAWU808* и *BAW112* – наличие хромосомы 4H^{mar} в геноме выделенной дисомной пшенично-ячменной линии. Изучение этой линии показало, что замещение хромосомы 4В мягкой пшеницы на хромосому дикого ячменя 4H^{mar} привело к изменению ряда признаков: сильно выраженной антоциановой окраске колеоптиле, характерной для дикого ячменя *H. marinum*, а также отсутствию пурпурной окраски ушек у основания листьев, которая проявляется у линии сорта пшеницы П28. По высоте растений, числу стеблей и колосьев в растении, числу колосков и зерен в главном колосе, а также числу зерен в растении линия 4H^{mar}(4B) превосходит родительскую линию П28, а по массе 1000 зерен ей уступает. Цитогенетическая стабильность и фертильность линии 4H^{mar}(4B) указывают на высокую компенсационную способность хромосомы 4H^{mar} ячменя по отношению к хромосоме 4В мягкой пшеницы и гомеологию между этими хромосомами.
Ключевые слова: *Hordeum marinum* ssp. *gussoneanum*; мягкая пшеница; пшенично-ячменная замещенная линия 4H^{mar}(4B).

Introduction

Introgressive hybridization is the main method of broadening the genetic diversity of bread wheat. Identification of new genetic resources for agronomically important traits, such as biotic and abiotic stress resistance, bread-making quality, increased content of microelements in grain and others is an important task (Molnár-Láng, Linc, 2015; Hao et al., 2020). The species of the genus *Hordeum* L., including annual and perennial barley grasses, as well as grain-type species, are considered a potential source to introgress genes for these traits (Garthwaite et al., 2005; Rubiales, Moral, 2011).

The progamous and embryonic incompatibility is strongly displayed in wheat × barley hybridizations and intergeneric hybrids were produced after the technique to overcome these difficulties had been developed (Kruse, 1973). To date, substitution, addition and translocation lines were developed by hybridization between bread wheat and *H. vulgare* (Molnár-Láng et al., 2014), *H. spontaneum* (Taketa, Takeda, 2001), *H. chilense* (Rey et al., 2015), *H. californicum* (Fang et al., 2014). Alloplasmic lines (*H. vulgare*)-*T. aestivum* were developed and used in breeding to create promising forms and cultivars of wheat (Pershina et al., 2018). In addition, a new grain crop, tritordeum, was produced from hybridization between durum wheat (*Triticum durum*) and a wild barley, *H. chilense* (Martín et al., 1999; Martín, 2017).

The ability to cross with wheat is also shown by two subspecies of the sea barley complex *H. marinum*: herbaceous annuals *H. marinum* Hudson ssp. *marinum* (2*x*) and *H. marinum* Hudson ssp. *gussoneanum* (Parl.) Thell. (2*x*, 4*x*) (Pershina et al., 2004; Islam et al., 2007). Due to their high resistance to salinity and waterlogging, these species are able to grow in saline meadows and marshes along sea coasts (Garthwaite et al., 2005; Islam et al., 2007). At the same time, salinity tolerance in *H. marinum* was estimated to be higher than in other species of Triticeae (Garthwaite et al., 2005). In addition, *H. marinum* ssp. *gussoneanum* (=*H. geniculatum* All.) (2*n* = 28) is resistant to drought and sudden temperature changes (Kobylyanskiy, 1967), and samples with a high protein content in seeds were also isolated (Pershina et al., 2009).

Amphiploids carrying wild barley cytoplasm were produced from hybrids between *H. marinum* ssp. *gussoneanum* (4*x*) and *T. aestivum* (Pershina et al., 2004) and between *H. marinum* (2*x*) and *T. aestivum* (Islam et al., 2007). The salt- and waterlogging tolerances of amphiploids were intermediate to those of their parents (Islam et al., 2007; Malik et al, 2009) and reduced grain yield was observed due to the negative influence of the *H. marinum* cytoplasm. To eliminate this cytoplasm effect, it is necessary to produce euplasmic hybrid genotypes, in which wheat is the maternal parent. However, the use of *H. marinum* ssp. *gussoneanum* as a pollinator when crossing with wheat is extremely difficult due to the limited amount of pollen in the small flowers of *H. marinum.*

This paper presents the results of using the incomplete amphiploid *H. marinum* ssp. *gussoneanum–T. aestivum* (2*n* = 54) with wild barley cytoplasm as a source of *H. marinum* chromosomes for their introgression into the bread wheat genome. The euplasmic wheat-barley disomic substitution line 4H^{mar}(4B) isolated among the progenies of these hybrids was also studied.

Materials and methods

Plant material. The line formed from one plant of the wheat variety Pyrotrix 28 (maternal parent, P28) was crossed with individual plants of an incomplete amphiploid L-503 *H. marinum* ssp. *gussoneanum–T. aestivum* (P28) (2*n* = 54). The incomplete amphiploid isolated from the progeny of a cytogenetically unstable amphiploid *H. marinum* ssp. *gussoneanum–T. aestivum* (2*n* = 68–70) carried 42 wheat chromosomes and 12 *H. marinum* chromosomes, with the exception of 5H^{mar} (Trubacheeva et al., 2019).

Plants were grown from seeds set in two hybrid combinations (P28 × 503 plant (p)5) and (P28 × 503 plant (p)10), and their progenies were studied in F_2–F_6 generations. Each generation was formed from the seeds of the most productive plant of the previous generation. Plants were characterized by seed set in the main spike, dividing them according to the level of fertility into groups: CS – completely sterile (no seeds); PS – partially sterile (1–9 seeds); PF – partially fertile (10–19 seeds); F – fertile (20–30 seeds); FF – fully fertile (more than 30 seeds). Starting from F_2, the number of chromosomes was counted in plants. The chromosome composition in metaphase I (MI) was determined in euploid

($2n$ = 42) hybrid plants F_5 (P28 × 503p10). Cytogenetically stable euploid plants were studied to identify *H. marinum* chromosomes. Hybridization and the study of the hybrid progeny were carried out in a hydroponic greenhouse.

The isolated wheat-barley substitution line 4H^{mar}(4B) was studied in the field. The plants were grown on plots 1 m wide, 20 plants per row, with a distance of 25 cm between rows. The control was the parental line P28. Each genotype was grown in three rows arranged in a randomized order. The lines were evaluated according to morphological traits. During harvesting, plant height, tiller number, number of productive spikes, main spike length, spikelet number per main spike, grain number per main spike and per plant were estimated. Statistical analyses were conducted in Microsoft Excel 2007. Single-factor analysis and the calculation of the least significant difference were used (Dospekhov, 1985).

Cytological analysis, genomic *in situ* hybridization and C-banding. Analysis of chromosome number was performed according to the standard Felgen preparation method using root tips of plants grown in a hydroponic greenhouse. The MI chromosome configuration was examined in pollen mother cells (PMCs) using the 2 % acetocarmine smear method. GISH was performed according to previously published protocols (Trubacheeva et al., 2019). Total genomic *H. marinum* ssp. *gussoneanum* DNA was labelled by nick translation with biotin-16-dUTP and used as a probe. The signals were observed and captured using AxioImagerM1 (Zeiss, Germany) fluorescence microscope. The work was performed at the Collective Center for Microscopic Analysis of Biological Objects (ICG SB RAS, Novosibirsk, Russia). C-banding was performed according to a previously published protocol (Badaeva et al., 1994).

DNA marker analysis. Two expressed sequence tag (EST-PCR) markers, *BAWU808* and *BAW112* (Trubacheeva et al., 2019), were used to identify chromosome 4H^{mar} of wild barley in the genome of the isolated line. The absence of wheat chromosome 4B in the substitution line 4H^{mar}(4B) was confirmed using chromosome-specific SSR markers *Xgwm368* and *Xgwm6* (Röder et al., 1998). The primer sequences were reported in (Trubacheeva et al., 2019. Amplifications were carried out using a Bio-Rad T-100 Thermal Cycler PCR. Amplification products were resolved in 2 % w/v agarose gels and visualized with ethidium bromide. Gel images were captured using a gel documentation system Gel Doc XR+ (Bio-Rad, USA).

Results

Identification of euploid plants among the progeny of hybrids (P28 × L-503)

As a result of crossing the line P28 with incomplete amphiploid L-503 in four hybrid combinations, shriveled seeds set with a frequency of 3.1 to 6.6 %, from which F_1 plants were grown (Table 1).

The shriveled seeds set in F_1 plants germinated in the hybrid (P28 × 503p5) with a frequency of 13.9 %, and in the hybrid (P28 × 503p10) with a frequency of 55.5 %. Starting from the second generation, the fertility level was studied in the progeny of these hybrids, estimated by the number of seed set per main spike, and the number of chromosomes was analyzed (Table 2).

In the second generation of the hybrid combination (P28 × 503p5), partially fertile (33 %) plants ($2n$ = 46), as well as sterile (45 %) and partially sterile (22 %) plants were observed, among which 46- and 48-chromosome plants were identified. The third generation, derived from a partially fertile plant ($2n$ = 46), also consisted of sterile (31 %), partially sterile (44 %) and partially fertile (25 %) plants. The most productive in F_3 was a 44-chromosome plant, among the progeny of which along with sterile (18 %), partially sterile (41 %) and partially fertile (27 %), fertile (14 %) plants were also identified in F_4. Among the progeny of the fourth generation, 42- and 44-chromosome plants were found. In F_5 and F_6 generations derived from fertile 42-chromosome plants, in addition to euploids ($2n$ = 42), aneuploids ($2n$ = 41; $2n$ = 43) were also identified. At the same time, segregation into plants with different levels of fertility, including sterile ones, was still observed (see Table 2). Fully fertile plants were obtained only in F_6 (6 %).

When selecting for productivity, the hybrid combination (P28 × 503p10) had a more accelerated formation of 42-chromosomal cytotypes with a high level of fertility. Fertile 46-chromosome plants (20 %) were already identified in F_2 along with sterile (13 %), partially sterile (27 %) and partially fertile (49 %) plants. The remaining groups of plants were represented by cytotypes with chromosome numbers $2n$ = 46, 46 + t, 46 + 2t.The progenies of the 46-chromosome plant in F_3 were sterile (6 %), partially sterile (38 %), partially fertile (25 %), and 42-chromosome fertile (31 %) plants. In F_3, in addition to euploids, aneuploids were found, including those with telocentric chromosomes (($2n$ = 43, 43 + t, 44 + t, 44 + 2t).

Among 26 F_4 plants derived from a 42-chromosome plant of the third generation, fertile plants prevailed (62 %), including plants with full fertility (11 %). In total, in F_4 of (P28 × 503p10), completely sterile (8 %) plants and plants with a low level of fertility (partially sterile – 11 % and partially fertile – 8 %) only accounted for less than one third. The remaining plants were fertile (62 %) and fully fertile (11 %). In F_4 cytotypes with $2n$ = 41, 42, 42 + t were identified among the studied plants. As a result of the selection of the most productive 42-chromosome plants, the fifth and sixth generations were represented only by fertile plants (in F_5 – 70 %, and in F_6 – 16 %) and fully fertile plants (in F_5 – 30 %, and in F_6 – 84 %) (see Table 2).

In order to select meiotically stable 42-chromosome plants for further work, three lines derived from individual plants of the P28 × 503p10 hybrid from F_6 were characterized by chromosome configuration at the first meiotic metaphase (MI). Study of meiosis in pollen mother cells (PMC) has shown a high cytological stability of euploid lines. All the studied plants of the line (P28 × 503p10) F_6 p1 and the major part of the plants of the line (P28 × 503p10) F_6 p2 (79 %) and the line (P28 × 503p10) F_6 p3 (89 %) had 21″ bivalents (Table 3). The identified violations are associated with the presence of univalents (20″ + 2′), including those involving telocentric chromosomes (21″ + 2t′).

Table 1. Seed set per spike, number of grains in F_1 plants and their germination in hybrid combination (P28 × L-503)

Hybrid combination	Number and frequency* (%) of hybrid grains in a pollinated spike	Number of		Germination of grains, %
		F_1 plants studied	grains in F_1 plant	
P28 × 503p5	1 (3.3)	1	129	13.9
P28 × 503p9	1 (3.1)	1	2	0
P28 × 503p10	2 (6.6)	2	18	55.5
P28 × 503p22	1 (4.0)	1	0	–

* From the number of pollinated flowers.

Table 2. Fertility level and number of chromosomes in F_1–F_6 of the hybrid combination *T. aestivum* (P28) (2*n* = 42) × (*H. marinum* ssp. *gussoneanum* × P28) (2*n* = 54)

Generation	Number of plants analysed	Number and frequency (%) of plants					Number of chromosomes
		FS 0	PS (1–9)#	PF (10–19)#	F (20–30)#	FF (more 30)#	
P28 × 503p5							
F_2	18	8 (45 %)	4 (22 %)	6 (33 %)	0	0	46*, 48
F_3	16	5 (31 %)	7 (44 %)	4 (25 %)	0	0	44*, 46
F_4	22	4 (18 %)	9 (41 %)	6 (27 %)	3 (14 %)	0	42*, 44
F_5	28	5 (18 %)	6 (21 %)	8 (29 %)	9 (32 %)	0	41, 42*, 43
F_6	32	2 (6 %)	4 (13 %)	10 (31 %)	14 (44 %)	2 (6 %)	41, 42, 43
P28 × 503p10							
F_2	15	2 (13 %)	4 (27 %)	6 (40 %)	3 (20 %)	0	46*, 46 + t, 46 + 2t
F_3	16	1 (6 %)	6 (38 %)	4 (25 %)	5 (31 %)	0	42*, 43, 43 + t, 44 + t, 44 + 2t
F_4	26	2 (8 %)	3 (11 %)	2 (8 %)	16 (62 %)	3 (11 %)	41, 42*, 42 + t
F_5	30	0	0	0	21 (70 %)	9 (30 %)	42*, 42 + t, 42 + 2t
F_6	38	0	0	0	6 (16 %)	32 (84 %)	42, 42 + t

Number of grains in the main spike; * the most productive cytotypes used to form the next generation.

Table 3. Characteristics of meiosis in lines derived from the hybrid combination (P28 × 503p10) F_6

Line	Number of plants examined	Number of plants and chromosome configuration at MI		
		21″	20″ + 2′	21″ + t″
(P28 × 503p10) F_6 p1	18	18 (100 %)	–	–
(P28 × 503p10) F_6 p2	19	15 (79 %)	4 (21 %)	–
(P28 × 503p10) F_6 p3	18	16 (89 %)	–	2 (12 %)

Identification of the wheat–barley disomic substitution line 4H^{mar}(4B)

At the next stage, plants with a 21″ chromosome configuration were included in the work. Using GISH analysis, it was shown that they carry a pair of *H. marinum* chromosomes (Fig. 1).

This result indicates that during self-pollination of the progeny of the hybrid (P28 × 503p10) and the production of euploids, a pair of *H. marinum* chromosomes introgressed into the genome of bread wheat. C-banding identified a pair of chromosomes 4H^{mar} and the absence of a pair of chromosomes 4B in the isolated line indicating that it was disomic for the substitution 4H^{mar} (4B) (Fig. 2, *a*). A telocentric chromosome for the arm of chromosome 4H was identified in a line with 2*n* = 40 + t chromosomes (see Fig. 2, *b*).

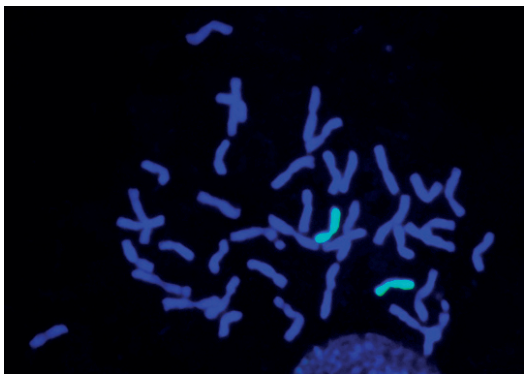

Fig. 1. GISH image of a wheat–barley substitution line. Barley chromosomes are in green, wheat chromosomes are in blue.

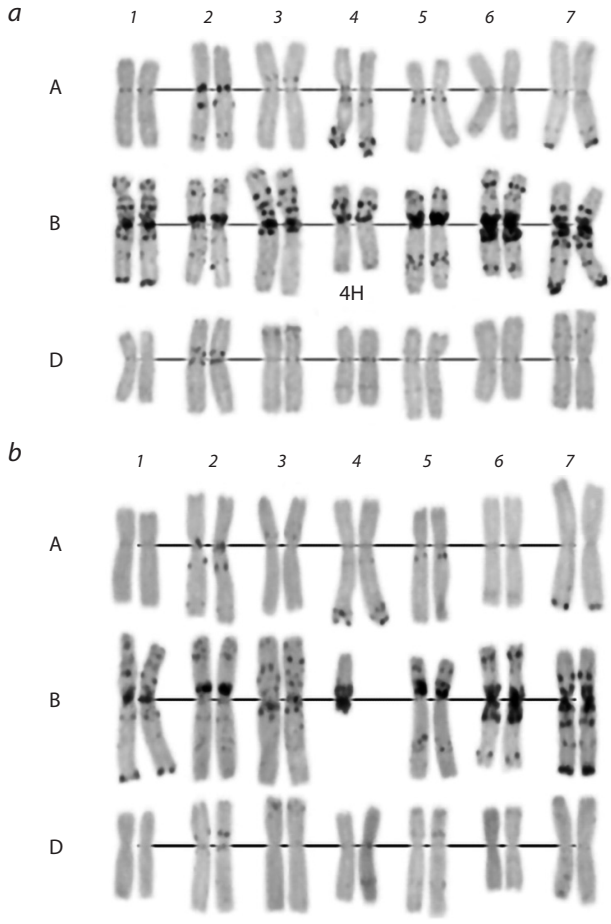

Fig. 2. C-banding of the 4H^{mar}(4B) (2*n* = 42) (*a*) and 4H^{mar} L (2*n* = 40 + t) (*b*) substitution lines.

Molecular analysis confirmed the presence of chromosome 4H^{mar} and the absence of chromosome 4B in the genome of disomic wheat-barley lines isolated from self-pollinated progeny of the hybrid combination P28 × 503p10. Molecular markers were used to confirm both the absence of chromosome 4B (Fig. 3, *a*) and the presence of chromosome 4H^{mar} (see Fig. 3, *b*) in isolated substitution lines. The DNA of the parent line P28 and *H. marinum* was used as a control.

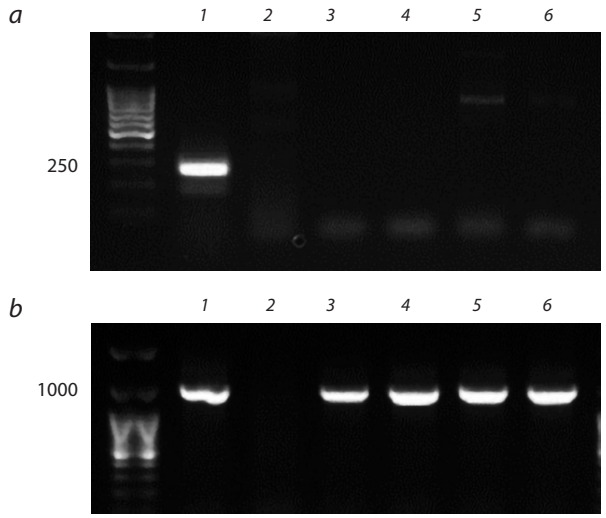

Fig. 3. PCR amplification of SSR marker *Xgwm368* (*a*) and EST marker *BAWU808* (*b*).

a: *1* – *T. aestivum*; *2* – *H. marinum*; *b*: *1* – *H. marinum*; *2* – *T. aestivum*; *3–6* – plants of line 4H^{mar}(4B). The numbers indicate the fragment length in bp.

Fig. 4. Coleoptile (*a*); ears at the base of the leaf (*b*).

1 – line 4H^{mar}(4B); *2* – line P28.

Characterization of the disomic wheat-barley substitution line 4H^{mar}(4B)

Plants of the line 4H^{mar}(4B) differed phenotypically from the parental line P28. Such traits included pronounced anthocyanin coloration of the coleoptile, which is typical for the wild barley *H. marinum* (Fig. 4, *a*), as well as an absence of purple coloration of the ears at the base of the leaves (see Fig. 4, *b*).

Substitution of wheat chromosome 4B for wild barley chromosome 4H^{mar} resulted in the development of viable plants, in which the values of some quantitative traits were higher than those of the parent line P28. The plant height and yield-related traits in the line 4H^{mar}(4B) were shown to be significantly different from those of the parental line (Table 4). Thus, the values of plant height, tiller and spike number per plant, spike length, spikelet number per spike, grain number per spike and per plant of the line 4H^{mar}(4B) were significantly higher than those of P28, while its thousand-kernel weight was less than that in the P28. Figures 5 and 6 show spikes and grains, respectively.

Table 4. Agronomic characteristics of the wheat–barley substitution line 4H^{mar}(4B)

Genotype	Plant height, cm	Tiller number	Spike number	Spike length	Spikelet number per spike	Grain number per spike	Grain number per plant	Thousand-kernel weight
4H^{mar}(4B)	133.63*	9.11*	5.6*	11.9*	22.3*	63.0*	236*	32.51*
П28	123.73	4.5	3.8	8.3	17.0	43.9	138.3	39.77
HCP_{05}	3.63	2.2	0.8	0.94	0.8	4.56	41.32	3.44

* $p < 0.05$.

Fig. 5. Spikes of lines: *1* – P28; *2–4* – 4H^{mar}(4B).

Fig. 6. Grains of line P28 (*1*); line 4H^{mar}(4B) (*2*).

Discussion

Wild relatives of bread wheat and their wheat synthetic derivatives are a valuable resource for introgressive hybridization (Davoyan et al., 2012; Molnár-Láng, Linc, 2015; Li et al., 2018). Tritordium is used in hybridization with wheat to transfer *H. chilense* chromosomes into the wheat genome (Martin, 2017), and triticale is used to transfer rye chromosomes (Shchapova, Kravtsova, 1990).

Earlier in our work, amphiploid *H. marinum* ssp. *gussoneanum–T. aestivum* (2*n* = 70) wild barley cytoplasm was used as a maternal parent in crosses with bread wheat to obtain alloplasmic disomic wheat–barley substitution lines 7H^{mar}(7B), 7H^{mar}(7D), 7H^{mar}L(7D), as well as ditelosomic addition lines 2*n* = 42 + 2t (7H^{mar}L) (Pershina et al., 2004; Trubacheeva et al., 2019). In this work, individual plants of the incomplete amphiploid *H. marinum* ssp. *gussoneanum–T. aestivum* (2*n* = 54) with wild barley cytoplasm were used as pollinators when crossing with the line P28 to introduce the genetic material of *H. marinum* into the euplasmic genetic background of bread wheat. The frequency of hybrid seed set was low, but some of the F_1 hybrid plants were viable, and two F_1 hybrids (P28 × 503p5) and (P28 × 503p10) set seeds that germinated.

The analysis of the obtained data revealed differences in the process of formation and the rate of cytological stabilization between the progeny of hybrids (P28 × 503p5) and (P28 × 503p10). This process was slower in the hybrid combination (P28 × 503p5) compared to the combination (P28 × 503p10). Thus, the progeny of 42-chromosome plants of the hybrid (P28 × 503p5) in the F_5 and F_6 segregated in plants with different levels of fertility, including completely sterile ones. In F_6, only half of 32 plants were fertile and fully fertile. In the hybrid combination (P28 × 503p10), on the contrary, only fertile or fully fertile plants were obtained in the fifth and sixth generations already. In addition, in the progeny of the hybrid (P28 × 503p10), in contrast to (P28 × 503p5), barley telocentric chromosomes appeared. Such results are consistent with the data of the authors who emphasized the uniqueness of the progeny of each hybrid grain as a source of diversity in the development of wide hybrid derivatives (Shchapova, Kravtsova, 1990).

As follows from the results obtained, the process of stabilization of karyotypes of 42-chromosome plants in the progeny of the (P28 × 503p10) F_6 hybrid was accompanied by the substitution of a pair of wheat chromosomes for a pair of *H. marinum* chromosomes. It was shown using GISH, C-banding and molecular analysis that chromosome 4B was substituted by chromosome 4H^{mar} in cytogenetically stable euploid plants. In addition, it was found that telocentrics also belong to the chromosome 4H^{mar}.

The type of wheat-barley substitution was also confirmed by the morphological traits that were exhibited in the line 4H^{mar}(4B). The absence of purple coloration of the ears at the base of the leaves in the line 4H^{mar}(4B) indicates the absence of wheat chromosome 4B, since this trait is controlled by the *Ra2* gene located on this chromosome (Melz, Thiele, 1990) and exhibited in P28. The smaller grain in the substitution line 4H^{mar}(4B) can be associated both with the absence of chromosome 4B, which affects the grain size and shape in wheat (Rahman et al., 2020), and with the presence of chromoso-

me 4H^{mar}, because *H. marinum* belongs to small-seeded barley grasses (Bothmer et al., 1991).

The line 4H^{mar}(4B) had a clear phenotypic marker specific for *H. marinum*. This is anthocyanin coleoptile coloration, which is absent in the parent line P28 and was previously found in the alloplasmic wheat–barley substitution line 7H^{mar}(7D) (Khlestkina et al., 2011). The accumulation of anthocyanin in vegetative organs is positively related to resistance to stress factors, and in wheat, ability to accumulate anthocyanins in the coleoptile is controlled by the *Rc* (red coleoptile) genes (Khlestkina et al., 2011). In this regard, the line 4H^{mar}(4B) may be useful for future studies of resistance to abiotic stresses, because *H. marinum* contains resistance genes (Garthwaite et al., 2005; Islam et al., 2007; Malik et al., 2009).

It has been established that homoeologous group 4 chromosomes of other barley species also possess genes that could be used for wheat breeding. For instance, chromosome 4H^{ch} of *H. chilense* contains genes for resistance to *Septoria tritici* and salt stress (Said, Cabrera, 2009), and chromosome 4H of *H. vulgare* was able to increase water use efficiency associated with drought tolerance of a wheat substitution line (Molnár et al., 2007). The line 4H^{mar}(4B) obtained in our work may have a potential for breeding, since it is characterized by high yield. Thus, the values of the number of spikes, the length of the spike, the number of spikelets per spike, the number of grains per and per plant in the 4H^{mar}(4B) line are higher than those in the line P28. These results, as well as the cytogenetic stability of the line 4H^{mar}(4B), indicated homoeology and high ability of barley 4H^{mar} chromosome to compensate for wheat 4B.

Conclusion

Thus, the efficiency of using the incomplete amphiploid (*H. marinum* ssp. *gussoneanum–T. aestivum*) (2*n* = 54) with wild barley cytoplasm to transfer *H. marinum* chromosomes into euplasmic genetic background of bread wheat has been shown.

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