Cereal Research Communications 47(4), pp. 593–603 (2019)
DOI: 10.1556/0806.47.2019.37

Use of *H. vulgare* EST Markers, GISH and C-banding to Study Bread Wheat – *H. marinum* subsp. *gussoneanum* (2n = 28) Introgression Lines

N.V. Trubacheeva[1]*, E.D. Badaeva[2], T.S. Osadchaya[1] and L.A. Pershina[1]

[1]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia
[2]Vavilov Institute of General Genetics RAS, Moscow, Russia

(Received 5 April 2019; Accepted 10 July 2019;
Communicated by M. Molnár-Láng)

Wild barley, *Hordeum marinum* subsp. *gussoneanum* (2n = 28) is a valuable source of genes that determine resistance to abiotic stresses. These resistance traits might be transferred to wheat due to the crossability of wild barley with bread wheat. The availability of reliable and rapid methods for the identification of *H. marinum* subsp. *gussoneanum* chromatin in a wheat background would facilitate the development of introgression wheat genotypes. For this purpose, we evaluated the applicability of eighty-seven *H. vulgare* EST markers for studying bread wheat – *H. marinum* subsp. *gussoneanum* substitution and addition lines. Of all of the markers studied, forty-three (49%) were amplified in *H. marinum* ssp. *gussoneanum* and wheat introgression lines. The identification of wild barley chromosomes using EST markers confirmed the GISH and C-banding data. Thus, it was established that the *H. vulgare* EST markers can be successfully used to identify the chromosomes of the *H. marinum* subsp. *gussoneanum* in introgression lines of wheat.

Keywords: barley, *H. marinum* subsp. *gussoneanum*, GISH, C-banding, *H. vulgare* EST markers

Introduction

An important approach for enriching genetic diversity available to *T. aestivum* is the transfer of alien genetic materials from its wild (Gradzielewska 2006; Gill et al. 2011) and cultivated (Ren et al. 2009) relatives. Species of the genus *Hordeum* L., including *H. vulgare* L., are also potential donors of genetic resources for wheat improvement (Rubiales and Moral 2011; Garthwaite et al. 2005). The genus *Hordeum* includes more than 30 species that grow in different climatic areas and is represented by diploid (2n = 2*x* = 14), tetraploid (2n = 4*x* = 28) and hexaploid (2n = 6*x* = 42) taxa. Due to the ability of some barley species to cross wheat, introgression wheat lines were obtained with *H. vulgare* (Molnár-Láng et al. 2014), *H. spontaneum* (Taketa, Takeda 2001), *H. chilense* (Miller et al. 1982), and *H. californicum* (Fang et al. 2014). Moreover, a new Tritordeum grain crop has been developed from *H. chilense* hybrids with bread and durum wheat (Martín et al. 1999).

*Corresponding author; E-mail: natas@bionet.nsc.ru

Sea barley grass *(Hordeum marinum)* is a wild Triticeae species. It is divided by some authors into two species – *H. marinum* Huds. (2x) and *H. gussoneanum* Parl. (2x, 4x) (Blattner 2009). According to other authors, these species are not separate but are subspecies of the grassy annual *H. marinum* Hudson: subsp. *marinum* (2x) and subsp. *gussoneanum* (Parl.) Thell. (2x, 4x) (Bothmer et al. 1991; Bothmer and Komatsuda 2011). The tetraploid subspecies *H. marinum* ssp. *gussoneanum* is considered a segment allopolyploid with two subgenomes, with one belonging to the progenitor of the diploid cytotype *gussoneanum* and the other belonging to the unknown diploid ancestor, the donor of the genome closer to *marinum* (Carmona et al. 2013). *H. marinum* ssp. *gussoneanum* habitats are saline meadows or marshes along the coast, as well as areas remote from the sea: riverbeds, vacant lots and pastures where plants of this species occur as weeds (Bothmer et al. 1991; Bothmer and Komatsuda 2011). Due to their ability to grow under such conditions, accessions with high salt tolerance (Garthwaite et al. 2005; Islam et al. 2007), waterlogging (Garthwaite et al. 2005), and tolerance to combined salinity and waterlogging results in low O_2 concentrations (Malik et al. 2009) were found in species of *H. marinum*. As noted by Garthwaite et al. (2005), the salt tolerance of *H. marinum* is higher than that of other representatives of Triticeae, including such salt-tolerant species as *Thinopyrum elongatum* and *Th. bessarabicum*. This property makes it possible to use *H. marinum* accessions as unique models for studying salt tolerance mechanisms (Garthwaite et al. 2005; Boustani et al. 2017). *H. marinum* ssp. *gussoneanum* (= *H. geniculatum* All., 2n = 4*x* = 28) also showed resistance to drought and severe changes in temperature (Kobylyanskii 1967). In our work, we used accessions of *H. marinum* ssp. *gussoneanum* with a high protein content in seeds (Pershina et al. 2009).

Amphiploids were produced from *H. marinum* (2x)-wheat (Islam et al. 2007) and *H. marinum* ssp. *gussoneanum* (4x)-wheat hybrids (Pershina et al. 1988). Earlier alloplasmic wheat – *H. marinum* subsp. *gussoneanum* addition and substitution lines originating from backcross progenies of wild barley-wheat hybrids and wild barley-wheat amphiploid have been developed. The characterization of the chromosome configurations of the lines was carried out using C-banding, SSR- and GISH-analysis (Trubacheeva et al. 2008; 2009).

The aim of this work was to study the amplification of EST markers of barley *H. vulgare* in the genome of wild barley *H. marinum* ssp. *gussoneanum* and to assess their use for the analysis of the alloplasmic bread wheat – *H. marinum* subsp. *gussoneanum* introgression lines along with GISH analysis and C-banding.

Materials and Methods

Plant material

The accessions of barley *H. marinum* ssp. *gussoneanum* Hudson (2n = 4x = 28), *H. vulgare* cv. Nepolegaushii (2n = 2x = 14), and bread wheat *T. aestivum* cv. Pyrotrix 28 were used for the initial transferability analysis of 87 EST markers (Table 1). The seeds of *H. marinum* ssp. *gussoneanum* as *H. geniculatum*. All were received from the Tashkent

Table 1. PCR analyses showing the presence (+) or absence (–) of EST markers in chromosomes of *H. vulgare* (*H.v.*), *H. marinum* ssp. *gussoneanum* (*H.m.*) and *T. aestivum* (*T.a.*).

H.v. chromosome	Marker name	PCR product							
		H.v.	*H.m.*	*T.a.*	*H. vulgare*	Marker name	*H.v.*	*H.m.*	*T.a.*
1HL	**k00183**	+	+	–	3HS	k0069	+	–	–
1HL	**k0072**	+	+	–	3HS	k0769	+	–	–
1HL	**BAWU17**	+	+	–	**4HL**	**BAWU808**	+	+	–
1HL	**k0033**	+	+	–	**4HL**	**BAWU755**	+	+	–
1HS	**BAWU136**	+	+	–	**4HL**	**BAWU112**	+	+	–
1HS	**k00936**	+	+	*	**4HL**	**BAWU673**	+	+	–
1HL	**BAWU756**	+	*	–	**4HS**	**BAWU505**	+	+	–
1HL	k0007	+	–	–	**4HS**	**k02539**	+	+	–
1HL	k1437	+	–	–	**4HL**	**BAWU670**	+	*	–
1HL	BAWU211	+	–	–	**4HL**	**BAWU152**	+	*	–
1HL	k0097	+	–	–	4HL	BAWU217	+	–	–
1HL	k2305	+	–	–	4HL	k0916	+	–	–
1HL	BAWU162	+	–	–	4HS	BAWU303	+	–	–
1HL	BAWU316	+	–	–	4HS	BAWU852	+	–	–
1HS	k0681	+	–	–	**5HL**	**k2464**	+	+	–
1HS	k4311	+	–	–	**5HS**	**k5024**	+	+	*
1HS	k0647	+	–	–	**5H**	**BAWU315**	+	+	*
1HS	BAWU616	+	+	+	**5H**	**BAWU160**	+	+	*
1H	BAWU12	+	+	+	**5HL**	**k4947**	+	*	–
2HL	**k2692**	+	+	*	**5H**	**BAWU891**	+	*	–
2HS	**k0514**	+	+	*	5HS	k0675	+	–	–
2H	**BAWU288**	+	+	*	5HL	k2569	+	–	–
2H	**BAWU555**	+	+	–	5HL	k3357	+	–	–
2H	**BAWU660**	+	+	–	5H	BAWU30	+	–	–
2HL	**k0932**	+	*	–	5H	BAWU240	+	–	–
2H	BAWU40	+	–	–	5H	BAWU749	+	–	–
2H	BAWU32	+	–	–	**6HL**	**k4898**	+	+	–
2HL	k3376	+	–	–	**6HS**	**k0066**	+	+	*
2HS	k0677	+	–	–	**6HS**	**k1385**	+	+	–
2HS	k0144	+	–	–	**6H**	**BAWU155**	+	+	–
2HS	k4909	+	–	–	**6H**	**BAWU361**	+	+	–
2HL	k5037	+	–	–	6HL	k1220	+	–	–
2HL	k0314	+	–	–	6HS	k1172	+	–	–
2HL	k0132	+	–	–	6HL	k3623	+	–	–
3HL	**k3141**	+	+	–	**7HL**	**BAWU550**	+	+	–
3HL	**k3164**	+	+	–	**7HL**	**k1355**	+	+	–
3HS	**k4656**	+	+	–	**7HL**	**k0277**	+	+	–
3HS	**k5014**	+	+	*	**7HL**	**k3717**	+	+	–
3HS	**k0247**	+	+	–	**7HS**	**k4783**	+	+	–
3H	k0873	+	–	–	**7HS**	**BAWU406**	+	*	–
3HS	k0892	+	–	–	7HS	k3350	+	–	–
3HL	k1383	+	–	–	7HL	k1156	+	–	–

+ Amplification; – No amplification; *Different band size polymorphism between *H. marinum* ssp. *gussoneanum* and bread wheat or between *H. vulgare* and *H. marinum* ssp. *gussoneanum*.

Table 2. Chromosome constitutions of bread wheat-*H. marinum* ssp. *gussoneanum* introgression lines determined by GISH, C-banding and EST-analysis

Lines	Chro-mosome number	Chromosome constitution according to analysis of			EST markers
		GISH	C-banding	EST-analysis	
L-49	42	40w+2H^{mar}	7H^{mar}(7D) disomic substitution	7H^{mar}	k4783, BAWU550, k1355, k0277, k3717, BAWU406
L-48	42	40w+2H^{mar}	7H^{mar}(7B) disomic substitution	7H^{mar}	k4783, BAWU550, k1355, k0277, k3717, BAWU406
L-32	40+2t	40w+2tH^{mar}	7H^{mar}L(7D) ditelosomic substitution	7H^{mar}L	k1355, k277, k3717
L-28	42	36w+6H^{mar}	1H^{mar}(1B)	1H^{mar}	k0936, k0033, k0072, k0183, BAWU17, BAWU136, BAWU756
			5H^{mar}(5D)	5H^{mar}	k2464, BAWU315, BAWU160, k5024, k4947, BAWU891
			7H^{mar}(7D)	7H^{mar}	k4783, BAWU550, k1355, k0277, k3717, BAWU406
L-52	42+2t	42w+2tH^{mar}	7H^{mar}S ditelosomic addition	7H^{mar}S	k4783, BAWU406
L-38	42+2t	42w+2tH^{mar}	7H^{mar}L ditelosomic addition	7H^{mar}L	k1355, k0277, k3717, BAWU550
L-53	44	42w+2H^{mar}	7H^{mar} disomic addition	7H^{mar}	k4783, BAWU550, k1355, k277, k3717, BAWU406
L-26	40+4t	40+2tw+2tH^{mar}	7H^{mar}L ditelosomic addition	7H^{mar}L	k1355, k0277, k3717, BAWU550
JI-65	46	42w+4H^{mar}	Not studied	1H^{mar}	k0936, k0033, k0072, k0183, BAWU17, BAWU136, BAWU756
				4H^{mar}	BAWU505, BAWU673, BAWU808, BAWU755, BAWU112,k02539, BAWU152
JI-66	46	42w+4H^{mar}	Not studied	1H^{mar}	k0936, k0033, k0072, k0183, BAWU17, BAWU136, BAWU756
				7H^{mar}	k4783, BAWU550, k1355, k277, k3717, BAWU406
L-503 (F)	54	42w + 12H^{mar}	Not studied	1H^{mar}, 2H^{mar}, 3H^{mar}, 4H^{mar}, 6H^{mar}, 7H^{mar}	All markers except markers for the 5H^{mar}

w – wheat chromosome;
t – telocentric chromosome.

Figure 1. Spikes from (a) wheat Pyrotrix 28; (b) 7H^{mar} (7D) disomic substitution line L-49; (c) 7H^{mar}L ditelosomic addition line L-38; (d) line L-503(F)

Botanic Garden, Uzbekistan (code number according to the Catalogue is 46773). Wheat cv. Pyrotrix 28 was included as a control because this cultivar was used to produce barley-wheat hybrids *H. marinum* ssp. *gussoneanum* × *T. aestivum* and their amphiploids (Pershina et al. 1988). The plant material used in this study consisted of ten alloplasmic (with cytoplasm of *H. marinum*) wheat-barley introgression lines (Table 2, Fig. 1). The lines L-28, L-32, L-38, and L-49 were developed and characterized previously (Trubacheeva et al. 2008; 2009). The lines L-48, L-52, L-53, L-65, and L-66 are developed from the plants of F_4–F_6 generations of the hybrid combination L-503(F) × wheat cv. Pyrotrix 28. The line L-503(F) (2n = 54) is a progeny of cytogenetically unstable barley-wheat amphiploid *H. marinum* ssp. *gussoneanum* (4x) × *T. aestivum* cv. Pyrotrix 28 (6x) (2n = 68–70) (Fig. 1d).

Genomic in situ *hybridization (GISH) and C-banding*

Genomic *in situ* hybridization was carried out as described by Schubert et al. (2001). Total genomic DNA was isolated from young frozen leaf tissue of barley *H. marinum* ssp. *gussoneanum* and wheat *T. aestivum* following the phenol–chloroform method described by Edwards et al. (1991). The barley DNA was labelled with biotin (biotin-5-dUTP, Roche, Mannheim, Germany) by nick translation. The C-banding technique was performed according to the protocol described by Badaeva et al. (1994).

Analysis of EST markers

A set of 87 EST-SSR markers developed by Nasuda et al. (2005) and Hagras et al. (2005) and uniformly distributed across the *H. vulgare* chromosomes were tested for amplifica-

tion of *H. marinum* ssp. *gussoneanum* DNA. PCR conditions followed a touch-down protocol as described in Nasuda et al. (2005) and Hagras et al. (2005). Amplified products were separated in 1.5% agarose gels, stained with ethidium bromide and photographed in ultraviolet light. A complete list of the markers used is given in Table 1. Primer sequences and PCR conditions were identical to those described in the original references.

Results

Transferability of barley EST markers

The transferability of 87 *H. vulgare* EST markers, which were from all seven homoeologous groups of barley, to the chromosomes of *H. marinum* ssp. *gussoneanum* was examined (Table 1). The amplification patterns of three EST markers were not reliable because of faint bands. We found that 40 markers, i.e., a ratio of 48%, did not amplify fragments in *H. marinum* ssp. *gussoneanum*. Two markers (BAWU12, BAWU616) amplified a fragment of the same size in *H. vulgare*, *H. marinum* ssp. *gussoneanum* and *T. aestivum* and hence could not be used. These 42 markers were not applicable for analysing lines with chromosomes of *H. marinum* ssp. *gussoneanum*. Seven primer pairs showed polymorphism between *H. vulgare* and *H. marinum* ssp. *gussoneanum* and did not amplified in *T. aestivum*. Thirty-six EST markers, i.e., a ratio of 41%, showed a clear single band of the same size in *H. vulgare* and *H. marinum* ssp. *gussoneanum* but failed to amplify or amplified a fragment of different sizes in wheat. Thus, these 43 EST markers of *H. vulgare* were transferable to *H. marinum* ssp. *gussoneanum* and would be useful in identifying *H. marinum* ssp. *gussoneanum* chromosomes in bread wheat backgrounds. In Table 1, these markers are highlighted in bold. For 35 EST markers out of these 43, localization on the short or long arm of *H. vulgare* chromosomes was known.

Characterization of wheat – H. marinum *ssp*. gussoneanum *introgression lines by GISH and C-banding*

GISH was performed to reveal the chromosome configuration and the presence of *H. marinum* ssp. *gussoneanum* chromosomes in alloplasmic bread wheat – *H. marinum* subsp. *gussoneanum* lines (Table 2). It was established that all studied lines carry *H. marinum* ssp. *gussoneanum* chromosomes and are either substitution or addition lines. In the L-503(F) line (2n = 54), 12 wild barley chromosomes were added to 42 wheat chromosomes (42w + 12H^{mar}) (Fig. 2a). Among the previously unexamined lines obtained using L-503(F), we found a disomic substitution line with 2n = 40w + 2H^{mar} (L-48), a ditelosomic addition line with 2n = 42w + 2tH^{mar} (L-52) and a disomic addition line with 2n = 42w + 2H^{mar} (L-53) (Fig. 2b). A pair of wheat telocentric chromosomes and a pair of *H. marinum* ssp. *gussoneanum* telocentric chromosomes (2n = 40w + 2tw + 2tH^{mar}) were identified in the L-26 line. The lines L-65 and L-66 were the multiple addition lines carrying two pairs of *H. marinum* ssp. *gussoneanum* chromosomes (2n = 42w + 4H^{mar}). The previously obtained introgression lines maintained the chromosomal configuration in the series of self-

pollinated generations. Thus, L-28 carried three pairs of *H. marinum* ssp. *gussoneanum* chromosomes and had a chromosomal configuration of 2n = 36w + 6H^{mar}; L-49 was a disomic wheat-barley substitution line (2n = 40w + 2H^{mar}); L-32 was a ditelosomic substitution line (2n = 40w + 2tH^{mar}); L-38 was a ditelosomic addition line (2n = 42w + + 2tH^{mar}). Table 2 shows the chromosome constitutions of wheat-*H. marinum* introgression lines.

C-banding confirmed the number of chromosomes in the studied lines and determined the types of substitutions in the alloplasmic wheat-barley substitution lines. C-banding also showed the prevalence of barley chromosome 7H^{mar} or its arms in the obtained wheat-barley substitution and addition lines. The following types of substitutions have been identified: 7H^{mar}L (7D) (L-32) (Fig. 2c), 7H^{mar} (7D) (L-49), 7H^{mar} (7B) (L-48) (Fig. 2d), and the L-28 line contained three chromosome substitutions – 1H^{mar} (1B), 5H^{mar} (5D) and 7H^{mar} (7D) (Table 2). A pair of telocentric chromosomes for the long arm of chromosome 7H^{mar} were identified in lines L-26 (Fig. 2e) and L-38, and a pair of telocentric chromosomes for the short arm of 7H^{mar} was identified in line L-52. The line L-53 was a disomic addition line with 42 chromosomes of wheat and a pair of chromosomes 7H^{mar} (Fig. 2f).

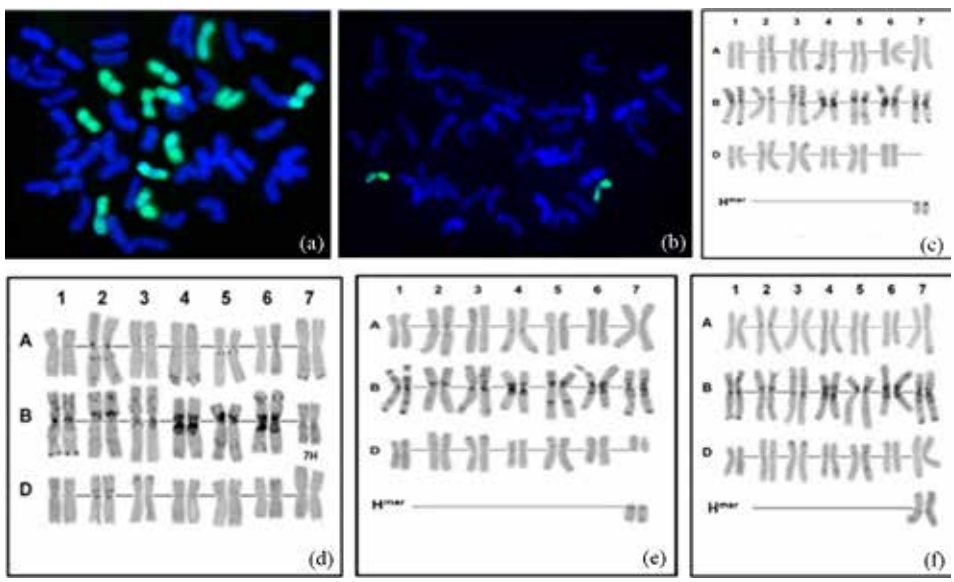

Figure 2. Genomic *in situ* hybridization with *H. marinum* ssp. *gussoneanum* genomic DNA (green) probes to mitotic metaphase chromosomes and C-banding. (a) L-503(F) (42w + 12H^{mar}); (b) and (d) 7H^{mar} (7B) disomic substitution line L-48; (c) 7H^{mar}L (7D) ditelosomic substitution line L-32; (e) 7H^{mar}L (7D) ditelosomic addition line (in wheat genetic background 40 + 7DS//) L-26; (f) 7H^{mar} disomic addition line L-53

Characterization of bread wheat – H. marinum *ssp*. gussoneanum *introgression lines using EST markers*

For the analysis of the lines, 43 EST markers, which amplified in *H. marinum* ssp. *gussoneanum*, were used. The markers of all chromosomes were amplified, except for $5H^{mar}$, in the L-503(F) line ($42w + 12H^{mar}$). All markers specific for *H. vulgare* chromosomes 1H, 5H and 7H were successfully amplified in the wheat-barley substitution line L-28 with three wild barley chromosomes $1H^{mar}$, $5H^{mar}$, and $7H^{mar}$ (Table 2). In lines L-49 and L-48, which were disomic for the $7H^{mar}$(7D) and $7H^{mar}$(7B) substitutions, respectively, as well as in the L-53 addition line for $7H^{mar}$, five markers for 7H were amplified (k4783, BAWU550, k1355, k0277, k3717). Amplification of a marker, k4783, localized in the short arm of 7H, was observed in the L-52 line carrying the $7H^{mar}S$ telocentric chromosome, as well as in the lines with the whole $7H^{mar}$ chromosomes (L-49, L-48, L-28, L-53, L-66) (Fig. 3). All markers for 7HL (k1355, k0277, k3717, BAWU550) were amplified in the lines L-38 and L-26 carrying the $7H^{mar}L$ telocentric chromosomes, as well as in L-49, L-48, L-28, L-503 (3) and L-66 lines with the whole $7H^{mar}$ chromosomes. In line L-65 with chromosomes $1H^{mar}$ and $4H^{mar}$ and in line L-66 with chromosomes $1H^{mar}$ and $7H^{mar}$, markers located in the homoeologous chromosomes of *H. vulgare* were also amplified.

Figure 3. Example of a PCR amplification profile used for identifying chromosome $7H^{mar}S$ with EST marker k4573. 1 – *H. vulgare,* 2 – *H. marinum* ssp. *gussoneanum,* 3 – *T. aestivum,* 4 – $7H^{mar}$ (7D) disomic substitution line L-49; 5 – $7H^{mar}$ (7B) disomic substitution line L-48; 6 – $1H^{mar}$(1B), $5H^{mar}$(5D), $7H^{mar}$(7D) multiple substitution line L-28, 7 – $7H^{mar}S$ ditelosomic addition line L-52, 8 – $7H^{mar}$ disomic addition line L-53, 9 – $1H^{mar} + 7H^{mar}$ multiple addition line L-66

Discussion

The aim of this work was to study the amplification of *H. vulgare* EST markers in wild barley *H. marinum* subsp. *gussoneanum* and to assess the possibility of their use for the analysis of the introgression lines of bread wheat. There is a lack of molecular markers for wild species such as *H. marinum* subsp. *gussoneanum*, and the transfer of markers to them from related crop species is a feasible method for genetic analysis (Hagras et al. 2005; Castillo et al. 2008). Therefore, EST markers of *H. vulgare* were used because cultivated barley is a closely related species of wild barley. Eighty-seven markers were studied, of which 41% amplified a fragment of the same size in both barley species, indicating a conserved nature of the EST sequences. These markers are expected to be valuable in identifying *H. marinum* subsp. *gussoneanum* chromosomes in introgression lines of wheat. The applicability of using *H. vulgare* EST markers to identify the chromosomes of wild barley *H. chilense*, as well as other cereal species, in the wheat genome has been

shown in works (Hagras et al. 2005; Castillo et al. 2008). At the same time, the absence of amplification products in *H. marinum* ssp. *gussoneanum* with 48% of the barley markers used indicates that the genomes of the two barley species have undergone significant changes in evolution. *H. vulgare* and *H. marinum* subsp. *gussoneanum* are phylogenetically distant from each other, and they are classified as different subgenera of the genus *Hordeum* (Blattner 2009). Despite the morphological similarities of the *H. marinum* group with some other barley species, chromosomal homology was not found, which may indicate the presence of a separate genome designated as "X" (Bothmer et al. 1991) or Xa (Blattner 2009). This genome is common for two diploid subspecies, subsp. *marinum* and subsp. *gussoneanum* (Bothmer et al. 1991). The accession of *H. marinum* subsp. *gussoneanum* used in our work is tetraploid. Tetraploid cytotype demonstrated a high degree of homology between the chromosomes of its two genomes (Komatsuda et al. 2001).

All EST markers specific for *H. marinum* ssp. *gussoneanum* amplified in the lines carrying chromosomes of wild barley. Thus, in the L-28 line, in which the presence of chromosomes $1H^{mar}$, $5H^{mar}$, and $7H^{mar}$ was determined by C-banding, markers of *H. vulgare* chromosomes 1H, 5H, and 7H were amplified. The line L-503(F) (2n = 54) is a progeny of wheat-barley amphiploid and, according to GISH analysis, carries 12 *H. marinum* subsp. *gussoneanum* chromosomes added to wheat genome. Using EST markers, in this line, we revealed the presence of all chromosomes of wild barley, except for the $5H^{mar}$ chromosome (Table 2). The progenies of hybrid L-503(F) × cv. Pyrotrix 28 according to C-banding carry two pairs of chromosomes of *H. marinum* ssp. *gussoneanum*, $1H^{mar}$ and $4H^{mar}$ (L-65) and $1H^{mar}$ and $7H^{mar}$ (L-66). The use of EST markers revealed amplification of 1H and 4H chromosome markers in L-65 and 1H and 7H in L-66. Thus, according to the results of analysis of the introgression lines, the transferred *H. vulgare* EST markers for the 1H, 4H, 5H and 7H chromosomes localized in the homoeologous chromosomes of *H. marinum* ssp. *gussoneanum*. The localization of *H. vulgare* EST markers in the same homoeologous group of *H. marinum* ssp. *gussoneanum* indicates the conservation of homology between the two species.

In our work, bread wheat – *H. marinum* ssp. *gussoneanum* introgression lines were characterized through the combination of cytogenetic and molecular genetic approaches. Genomic *in situ* hybridization was used to determine the wild barley chromosomes in the wheat genetic background. C-banding established the types of chromosome substitution or addition in these lines. A comparison of the results of C-banding and EST analysis showed that *H. vulgare* EST markers can be used to identify the DNA of *H. marinum* subsp. *gussoneanum* in introgression lines of wheat.

Acknowledgements

This research was supported by Project No. 0324-2019-0039, RFBR Grant No. 17-04-01738 and the Centre of Microscopic Analysis of Biological Objects, ICiG SO RAN.

References

Badaeva, E.D., Badaev, N.S., Gill, B.S., Filatenko, A.A. 1994. Intraspecific karyotype divergence in *Triticum araraticum*. Plant Syst. Evol. **192**:117–145.

Blattner, F.R. 2009. Progress in phylogenetic analysis and a new infrageneric classification of the barley genus *Hordeum* (Poaceae: Triticeae). Breeding Sci. **59**:471–480.

Bothmer, R. von, Jacobsen, N., Baden, C., Jorgensen, R.B., Linde-Laursen, I. 1991. An ecogeographical study of the genus *Hordeum*. In: 2nd ed. IBPGR, Rome, p. 127.

Bothmer, R. von, Komatsuda, T. 2011. Barley original and related species. In: Barley: Production, Improvement, and Uses. Ed. By Steven E. Ullrich, Wiley-Blackwell, pp. 14–62.

Boustani, A., Fatehi F., Azizinezhad, R. 2017. The Proteome response of *"Hordeum marinum"* to long-term salinity stress. Cereal Res. Commun. **45(3)**:401–410.

Carmona, A., Friero, E., Bustos, A. de, Jouve, N., Cuadrado, A. 2013. The evolutionary history of sea barley *(Hordeum marinum)* revealed by comparative physical mapping of repetitive DNA. Ann. Bot. **112**:1845–1855.

Castillo, A., Budak, H., Varshney, R.K., Dorado, G., Graner, A., Hernández, P. 2008. Transferability and polymorphism of barley EST-SSR markers used for phylogenetic analysis in *Hordeum chilense*. BMC Plant Biol. **8(1)**:97–10.

Edwards, K., Johnstone, C., Thompson, C. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR Analysis. Nucl. Acids Res **19**:1349.

Fang, Y., Yuan, J., Wang, Z., Wang, H., Xiao, J., Yang, Z. 2014. Development of *T. aestivum* L. – *H. californicum* alien chromosome lines and assignment of homoeologous groups of *Hordeum californicum* chromosomes. J. Genet. Genomics **41**:439–477.

Garthwaite, A.J., von Bothmer, R., Colmer, T.D. 2005. Salt tolerance in wild Hordeum species is associated with restricted entry of Na+ and Cl- into the shoots. J. Exp. Bot. **56(419)**:2365–2378.

Gradzielewska, A. 2006. The genus *Dasypyrum* – part 2. *Dasypyrum villosum* – a wild species used in wheat improvement. Euphytica **152**:441–454.

Gill, B.S., Friebe, B.R., White, F.F. 2011. Alien introgressions represent a rich source of genes for crop improvement. Proc. Natl. Acad. Sci. USA **108(19)**:7657–7658.

Hagras, A.A., Kishii, M., Sato, K., Tanaka, H., Tsujimoto, H. 2005. Extended application of barley EST markers for the analysis of alien chromosomes added to wheat genetic background. Breed Sci. **55**:335–341.

Islam, S., Malik, A.I., Islam, A.K.M.R., Colmer, T.D. 2007. Salt tolerance in a *Hordeum marinum – Triticum aestivum* amphiploid, and its parents. J. Exp. Bot. **58**:1219–1229.

Kobylyanskii, V.D. 1967. Biological characters of wild barley species in relation to aims of breeding. Biolog. Zh. Armenia **20**:41–51.

Komatsuda, T., Salomon, B., Bryngelsson, T., Bothmer, R.V. 2001. Phylogenetic analysis of *Hordeum marinum* Huds. based on nucleotide sequences linked to the vrs1 locus. Plant Syst. Evol. **227**:137–144.

Malik, A.I., English, J.P., Colmer, T.D. 2009. Tolerance of *Hordeum marinum* accessions to O_2 deficiency, salinity and these stresses combined. Ann. Bot. **103(2)**:237–248.

Martín, A., Álvarez, J.B., Martín, L.M., Barro, F., Ballesteros, J. 1999. The development of tritordeum: a novel cereal for food processing. J. Cereal Sci. **30**:85–95.

Miller, T.E., Reader, S.M., Chapman, V. 1982. The addition of *Hordeum chilense* chromosomes to wheat. In: Broertjes, C. (ed.) Proc Int Symp Eucarpia. Induced Variability Plant Breed. Pudoc, Wageningen, pp. 79–81.

Molnár-Láng, M., Linc, G., Szakács, É. 2014. Wheat–barley hybridization: the last 40 years. Euphytica **195**:315–329.

Nasuda, S., Kikkawa, Y., Ashida, T., Islam, A.K.M.R., Sato, K., Endo, T.R. 2005. Chromosomal assignment and deletion mapping of barley EST markers. Genes Genet. Syst. **80**:357–366.

Pershina, L.A., Numerova, O.M., Belova, L.I., Devyatkina, E.P., Shumny, V.K. 1988. Fertility in barley × wheat hybrids *H. geniculatum* All. × *T. aestivum* L., their regenerants and hybrid progeny of backcrosses to *T. aestivum*. Cereal Res. Commun. **16**:157–163.

Pershina, L.A., Devyatkina, E.P., Belova, L.I., Trubacheeva, N.V., Arbuzova, V.S., Kravtsova, L.A. 2009. Features of alloplasmic wheat–barley substitution and addition lines (*Hordeum marinum* subsp. *gussoneanum*) – *Triticum aestivum*. Russ. J. Genet. **45**:1223–1229.

Ren, T., Yang, Z., Yan, B., Ren, Z. 2009. Development and characterization of a new 1BL.1RS translocation line with resistance to stripe rust and powdery mildew of wheat. Euphytica **169(2)**:207–213.

Rubiales, D., Moral, A. 2011. Resistance of *Hordeum chilense* against loose smuts of wheat and barley (*Ustilago tritici* and *U. nuda*) and its expression in amphiploids with wheat. Plant Breeding **130(1)**:101–103.

Schubert, I., Fransz, P.F., Fuchs, J., Jing, J.H. 2001. Chromosome painting in plants. Methods Cell Sci. **23**:57–69.

Taketa, S., Takeda, K. 2001. Production and characterization of a complete set of wheat–wild barley (*Hordeum vulgare* ssp. *spontaneum*) chromosome addition lines. Breed Sci. **51**:199–206.

Trubacheeva, N.V, Badaeva, E.D., Adonina, I.G., Belova, L.I., Devyatkina, E.P., Pershina, L.A. 2008. Construction and molecular and cytogenetic analyses of euploid (2n = 42) and telocentric addition (2n = 42 + 2t) alloplasmic lines (*Hordeum marinum* subsp. *gussoneanum*) – *Triticum aestivum.* Russ. J. Genet. **44**:67–73.

Trubacheeva, N.V., Efremova, T.T., Badaeva, E.D., Kravtsova, L.A., Belova, L.I., Devyatkina, E.P., Pershina, L.A. 2009. Production of alloplasmic and euplasmic wheat-barley ditelosomic substitution lines $7H^{1}L^{mar}$(7D) and analysis of the 18S/5S mitochondrial repeat in these lines. Russ. J. Genet. **45**:1438–1443.