Transferability of barley EST markers used for analysis *T. aestivum – H. marinum* subsp. *gussoneanum* introgression lines

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Abstract: We evaluated the applicability of seventy-eight *H. vulgare* EST markers for studying bread wheat-*H. marinum* subsp. *gussoneanum* substitution and addition lines. Of all the markers studied, thirty-six (46%) 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 *H. marinum* subsp. *gussoneanum* in introgression lines of wheat.

Key words: EST markers; introgression alloplasmic lines; wild barkey.

1. Introduction

Wild relatives have been employed successfully in common wheat breeding programmes to introgress agronomically important genes (Ceoloni et al., 2014). Sea barley (*Hordeum marinum* subsp. gussoneanum, $2n = 28$) has potentially useful traits such as resistance to abiotic stresses, including high salt tolerance (Garthwaite et al., 2005), waterlogging (Garthwaite et al., 2005), and tolerance to combined salinity and waterlogging resulting in low O_2 concentrations (Malik et al., 2009). These resistance traits may have been transferred to wheat due to the crossability of wild barley with bread wheat. Chromosomes from *H. marinum* could be introduced into common wheat through wheat-barley hybrids and backcrossing to wheat (Pershina et al., 2009; Trubacheeva et al., 2009). Introgressed segments can be assessed by *in situ* hybridisation, which readily distinguishes *H. marinum* chromosomes from those of wheat (Trubacheeva et al., 2009). Molecular markers capable of detecting small segments of *H. marinum* chromatin in a wheat background would also enhance the use of this wild species to increase wheat genetic resources. The aim of this work was to study the amplification of EST markers of barley *H. vulgare* in the genome of the wild barley *H. marinum* ssp. *gussoneanum* and to assess their use for detecting barley chromatin segments in the alloplasmic bread wheat-*H. marinum* subsp. *gussoneanum* introgression lines.

2. Materials and methods

The accessions of the 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 78 EST markers. Alloplasmic (with the cytoplasm of *H. marinum*) wheat-barley introgression lines were studied using GISH, C-banding and EST analysis. A set of 78 EST-SSR markers developed by Hagras et al. (2005) and uniformly distributed across the *H. vulgare* chromosomes were tested for amplification of *H. marinum* ssp. *gussoneanum* DNA. PCR conditions followed a touch-down protocol as described by Hagras et al. (2005). Amplified products were separated in 1.5 % agarose gels, stained with ethidium bromide and photographed in ultraviolet light.

3. Results and discussion

The transferability of 85 *H. vulgare* EST markers, which were from all the seven homoeologous groups of barley, to the chromosomes of *H. marinum* ssp. *gussoneanum* was examined. Forty-two markers, i. e., 49 %, did not amplify fragments in *H. marinum* ssp. *gussoneanum*. Two markers were not polymorphic between *H. vulgare*, *H. marinum* ssp. *gussoneanum* and *T. aestivum* and therefore could not be used. These 44 markers were not applicable for analysing lines with chromosomes of *H. marinum* ssp. *gussoneanum*. Forty-one EST markers, i.e., 48%, 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 41 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.

The presence of barley chromosomes in the alloplasmic lines was detected using GISH analysis and C-banding of chromosomes. 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. It was established that all the lines studied carry *H. marinum* ssp. *gussoneanum* chromosomes and are either substitution or addition lines. We found a disomic substitution line with $2n = 40w + 2H^{mar}$, a ditelosomic addition line with $2n = 42w + 2tH^{mar}$ and a disomic addition line with $2n = 42w + 2H^{mar}$ (Figure 1, a). In the line derived from incomplete amphiploid ($2n = 54$), 12 wild barley chromosomes were added to 42 wheat chromosomes ($42w + 12H^{mar}$) (Figure 1, *b*). Two lines were multiple addition lines carrying two pairs of *H. marinum* ssp. *gussoneanum* chromosomes ($2n = 42w + 4H^{mar}$) and one line was a multiple substitution line ($2n = 36w + 6H^{mar}$). C-banding confirmed the number of chromosomes in the lines studied and determined the types of substitutions in the alloplasmic wheat-barley substitution lines.

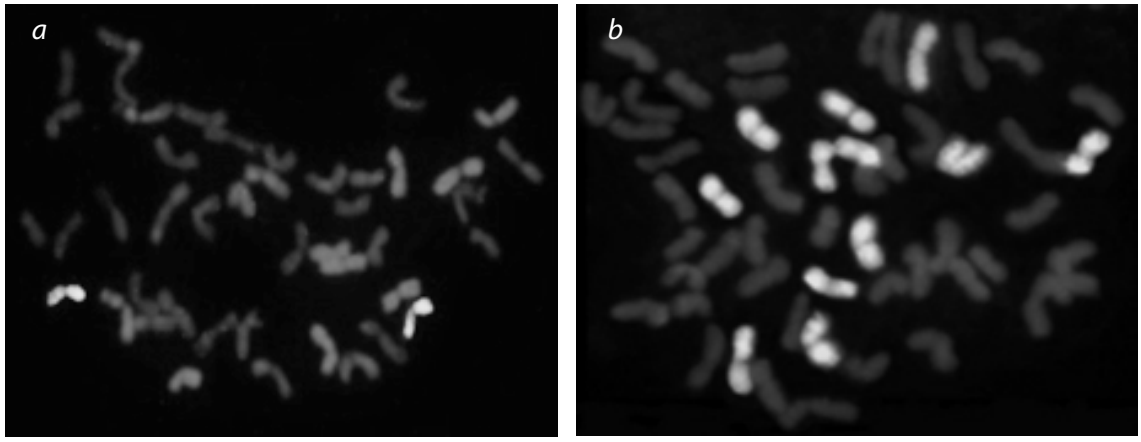

Figure 1. Genomic *in situ* hybridization with *H. marinum* ssp. *gussoneanum* genomic DNA (green) probes to mitotic metaphase chromosomes.

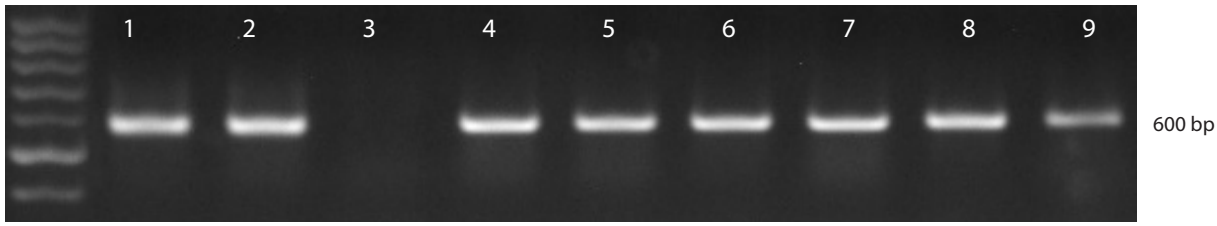

Figure 2. Example of a PCR amplification profile used for identifying chromosome 7H^{mar}S with EST marker k4573. 1, *H. vulgare*; 2, *H. marinum* ssp. *gussoneanum*; 3, *T. aestivum*; 4, 7H^{mar} (7D) disomic substitution line; 5, 7H^{mar} (7B) disomic substitution line; 6, 1H^{mar}(1B), 5H^{mar}(5D),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

The following types of substitutions have been identified: 7H^{mar}L (7D), 7H^{mar} (7D), 7H^{mar} (7B), and one line contained three chromosome substitutions, 1H^{mar} (1B), 5H^{mar} (5D) and 7H^{mar} (7D). We identified the addition lines with a pair of telocentric chromosomes for the long arm of chromosome 7H^{mar}, a pair of telocentric chromosomes for the short arm of 7H^{mar} and the line with 42 chromosomes of wheat and a pair of chromosomes 7H^{mar}.

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). Therefore, EST markers of *H. vulgare* were used because cultivated barley is a closely related species of wild barley. The transferable EST markers of all chromosomes were amplified, except for 5H^{mar}, in the line with 42w + 12H^{mar}. All markers specific for *H. vulgare* chromosomes 1H, 5H and 7H were successfully amplified in the wheat-barley substitution line with three wild barley chromosomes, 1H^{mar}, 5H^{mar}, and 7H$^{mar.}$ In the lines that were disomic for the 7H^{mar}(7D) and 7H^{mar}(7B) substitutions, as well as in the addition line for 7H^{mar}, five markers for 7H were amplified (Figure 2).

In the line with chromosomes 1H^{mar} and 4H^{mar} and with chromosomes 1H^{mar} and 7H^{mar}, markers located in the homoeologous chromosomes of *H. vulgare* were also amplified. Thus, according to the results of EST analysis, transferable markers of chromosomes 1H, 4H, 5H and 7H are localized on the homoeologous chromosomes of wild barley *H. marinum*. Localization of EST markers on homoeologous chromosomes in related species was also demonstrated in (Hagras, 2005), where it was found that 90 % of the studied EST markers of cultivated barley are localized on homoeologous *H. chilense* chromosomes. The authors explain this by the fact that EST sequences have a unique character in the genome and are highly conserved. At the same time, the absence of amplification products in *H. marinum* ssp. *gussoneanum* with 54 % of the *H. vulgare* markers used indicates that the genomes of the two barley species have undergone significant changes in evolution. These two species of barley are known to be phylogenetically distant and belong to different subgenera of the genus Hordeum (Blattner 2015).

4. Conclusion

In our work, a combination of cytogenetic and molecular genetic approaches were used for characterization of bread wheat-*H. marinum* ssp. *gussoneanum* introgression lines. The results showed that transferable *H. vulgare* EST markers can be successfully used to identify the chromosomes of *H. marinum* ssp. *gussoneanum*.

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Conflict of interest. The authors declare no conflict of interest.