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VARIABILITY AND POTENTIAL OF MICROSATELLITE LOCI OF 5HL CHROMOSOME IN BARLEY GENOTYPES OF DIFFERENT ORIGIN

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Aim. To investigate the polymorphism of microsatellite loci of the 5HL chromosome of barley, including those which are location in the area of LT-resistance key genes and close to them, to determine and evaluate the frequence of microsatellite alleles in the genetic material of autumn-sown barley of different origin. **Methods**. The isolation of genomic DNA, microsatellite analysis, PCR amplification, gel electrophoresis, and statistical analysis. We studied 46 barley genotypes of different origin, including 33 winter barley varieties, and 13 varieties of alternative (facultative) growth habit; among these 46 barley genotypes, 21 varieties were developed by PBGI-NCSCI, 25 – of other origin, including 21 from the countries of Central Europe (Czech Republic/CZ – 2; Grabe, Luran; Germany/DE – 4: Skarpia, Majbrit, Cinderella, Highlight), Western Europe (France/FR – 1: Anzhelika; the Netherlands/NL – 1: Gerlach) and Eastern Europe (NGC named after P.P. Lukianenko/RU – 13, 11 winter varieties: Derzhavnyi, Espada, Zhavoronok, Kondrat, Kumach, Meteor, Metaksa, Mikhailo, Platon, Tigr, Khutorok, and 2 winter-and-spring varieties: Putnik, Timofei) and 4 Western-Asian varieties (Syria/SYR – 4: Pamir013/Sonata, Pamir065/Pamir149, CWB-117-77-97, ROHO). **Results.** The allelic polymorphism was studied by 14 microsatellite loci of the 5HL chromosome of barley, including those in the area of genes *Fr-H1, Fr-H2* and close to these regions, the distribution of the identified microsatellite alleles was studied along with their frequencies and genetic diversity in the sampling of 46 collection varieties of autumn-sown barley of different origin. Among the investigated microsatellite areas of the 5HL chromosome of barley, only 50 % of loci in the selected samples of varieties were found to be polymorphic. These were microsatellite loci Bmag0760, GMS061, Bmag0337, UMB702, Bmag0323, Bmag0223, and Bmag0222. We found the dominating alleles and those with reliably lower frequency, and the alleles specific only for some regions. The estimated values of the polymorphism information content (PIC) for the investigated polymorphic microsatellite loci varied between 0.29 (UMB702) – 0.77 (Bmag0223). The diversity index was 0.54 on average. **Conclusions.** The results of the study demonstrated moderate allelic diversity of the investigated microsatellite loci of the 5HL chromosome of barley, which indicates the presence of potential genetic variability of some loci, the possibility of applying them in further studies on determining the effects of specific alleles of each locus and their associations with required economically valuable traits of barley, including resistance to low temperatures (LT-resistance). The potential of polymorphic alleles as markers of frost-resistance traits of autumn-sown barley genotypes is discussed.

Key words: barley (*Hordeum vulgare L.*), 5HL chromosome, SSR-analysis, genetic variability, microsatellite markers, allele frequencies, PIC.

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INTRODUCTION

Barley is one of the most important crops of high economic value for agrarian production in many countries, including Ukraine. The largest barley fields are in Europe and Asia. Almost half of the global production of barley is covered by five extremely powerful producing countries, and Ukraine is among them.

One of the main factors limiting the crop performance and the total agronomic value of barley varieties, including autumn-sown ones, is low-temperature stress, the impact of which has recently increased considerably in different regions of the world. On the one hand, it is caused by climate changes, which occur almost everywhere, both globally and locally. These changes include the insufficient layer of snow cover on winter cereals or its complete absence, the increase in the incidence of abnormal conditions with sharp fluctuations and a large amplitude of temperatures, observed practically in the entire world in winter and spring, etc. (Yarmolska et al, 2022). On the other hand, cultivated barley (*H. vulgare L.*), like many other economically valuable cereals, has suffered a reduction in its genetic diversity during the years-long breeding process. Due to this factor, the sensitivity of commercial varieties to different stresses, including those induced by low temperatures, increases. In its turn, it requires the creation of new, more resistant genotypes and the efficient evaluation of their resistance (Gudzenko and Vasylkiskyi, 2016; Linchevsky and Legkun, 2020).

The selection in terms of frost resistance in field conditions is not always possible and resultative. Currently, classic methods of genetic monitoring in plant breeding are time-consuming, as before. In addition, the conventional methods of evaluating the resistance and selection in field conditions do not always ensure the identification of resistant genotypes. Mainly, this is a consequence of natural reasons, including an insufficient load of the stress factor in the years of cultivation or its complete absence. As for the autumn-sown barley, the evaluation is complicated by the presence of two genetically different forms in it – one of winter type of development and the other of alternative, winter-and-spring, type of development, which have different mechanisms of forming resistance to below-freezing temperatures. It is known that the duration of the vernalization stage is dominant in this aspect for the latter, while in the case of winter-and-spring varieties, the main factor is their response to the number of daylight hours (Stelmakh et al, 2017; Linchevsky and Legkun I, 2020).

Modern plant breeding, including barley, is gradually coming to transformation into a different type using DNA technologies (Varshney et al, 2014; Cobb et al, 2019). DNA markers have acquired great significance in evaluating genotype traits (Lanka et al, 2023; Baidyussen et al, 2024). Due to the application of genomic and molecular strategies in breeding, there are chances to improve the condition of plants under conditions of abiotic stress and enhance barley performance (Akar et al, 2009; Hernandez et al, 2020).

The international project, which aimed to study the barley genome, led to the elaboration of genetic maps with molecular markers that became available for analysis. The use of microsatellite markers is an advantage as compared to previous approaches, which were based on other types of molecular markers. The microsatellite analysis proved its significance and convenience both as a method to study genetic diversity (Struss and Plieske, 1998; Kolesnyk et al, 2015) and as a method of assortment in marker-assisted selection (Hasan N et al., 2021; Ghomi et al, 2023) due to its high informative value, codominant inheriting, and multiallele nature of this type of DNA markers (Kelkar et al, 2010).

The first genetic maps of barley, containing SSR-markers (Ramsay et al, 2000; Li et al, 2003), including EST-SSR (Pillen et al, 2000; Varshney et al, 2007) were developed at the beginning of the century and improved by other researchers (Zhang et al, 2014; Jo et al, 2017). The high density SSR consensus map, developed based on 775 marker loci of SSR obtained from genomic DNA (Varshney et al, 2007), is still used to provide the programs of molecular selection of barley with the best selection choices in terms of marker quality with higher probability of polymorphism in the relevant chromosomal interval. At present, the alleles of polymorphic microsatellite loci from different areas of the genome are widely used as molecular markers for genotype identification (Kolesnyk et al, 2015). While detecting the association with the required traits, these markers may be involved with the purpose of additional rapid selection of genotypes as well, namely the ones resistant to unfavorable stressful cultivation conditions, and, which is especially relevant, it does not require the simulation of the stressful background (Jan et al, 2022; Adhikari et al, 2022; Ghomi et al, 2023). Therefore, it is reasonable to expand the area of the evaluation criteria and the possibility of determining the differences between genotypes for further selection and forecasting of LT-resistance of varieties using modern approaches, envisaging modern marker-assisted DNA technologies, including microsatellite analysis. For targeted search for DNA markers, including microsatellite markers, suitable for the evaluation and selection of genotypes with specific traits, including LT-resistance, it is wiser to analyze the regions of the genome containing genetic determinants of these traits.

Up to now, there have been many various studies on the representatives of the *Triticeae* tribe to find functional associations between some DNA regions and frost-resistance traits; the main determinants of LT-resistance were found and localized (Reinheimer et al, 2004; Tondelli et al, 2006; Lei et al, 2019; Ahres et al, 2020; Ji et al, 2021). Yet, despite the achievements in the technology of molecular markers (Fiust and Rapacz, 2020), a relatively small number of loci having a major impact on the ability of plants to survive under stress have been identified. The genomic and, to a lesser degree, functional studies, including the ones on barley, determined the regions of the genome associated with frost resistance (Fisk et al, 2013; Visioni et al, 2013; Rizza et al, 2016). The most consistent region was found on the long arm of the 5H chromosome where most genes, depending on the effect of low and below-freezing temperatures, are located (Reinheimer et al, 2004; Dhillon et al, 2017; Guerra et al, 2022). The associations between DNA loci (STS, RAPD, SNP, microsatellite), including the 5H chromosome, and frost resistance/sensitivity were studied on several samplings of varieties and genotype populations to be further used in the marker-assisted selection. The statistically relevant positive association between a series of marker loci of the 5HL chromosome and the tolerance of barley genotypes to below-freezing temperatures, evaluated in laboratory and field conditions, was found (Toth et al, 2004; Rapacz et al, 2010; Rizza et al, 2016). These marker loci are suggested for the evaluation of the resistance of genotypes to abiotic stresses, including low-temperature stress in laboratory conditions (Fiust and Rapacz, 2020; Hasan et al, 2021; Ghomi et al, 2024). At the same time, the impact of different loci related to frost resistance, and their contribution to the cumulative result may vary depending on the genetic material and environmental conditions.

The study aimed to investigate the polymorphism of microsatellite loci of the 5HL chromosome of barley, including the ones in the region of the key genes of LT-resistance, evaluation, and analysis of the frequency of microsatellite alleles in the collection genotypes of autumn-sown barley of different origin. The application of molecular and genetic methods of analysis to obtain potential microsatellite markers envisages further enhancing the efficiency of the identification and selection of genotypes with required traits in the breeding programs, including the southern regions of Ukraine.

MATERIALS AND METHODS

Plant material. The collection varieties of autumn-sown barley (winter type and facultative type of development) of the Department of General and Molecular Genetics, PBGI-NCSCI, were used as material for the study. A total of 46 genotypes of different origins were studied, including 33 winter barley varieties and 13 alternative facultative types of development. These samples included 21 varieties, developed by PBGI-NCSCI, the south of Ukraine/UKR (10 winter varieties: Akademichnyi, Zymran, Manas, Metelytsia, Oksamyt, Roman, Selena Star, Trudivnyk, Barvinok, Odeskyi 170, and 11 winter-and-spring varieties: Aboryhen, Ivenhoe, Valkiriia, Deviatyi Val, Dostoinyi, Odeskyi 46, Osnova, Rosava, Snihova Koroleva, Taina, Taman), 25 – bred by other originators, including 21 from the countries of Central Europe (Czech Republic/CZ – 2; Grabe, Luran; Germany/DE – 4: Skarpia, Majbrit, Cinderella, Highlight), Western Europe (France/FR – 1: Anzhelika; the Netherlands/NL – 1: Gerlach) and Eastern Europe (NGC named after P.P. Lukianenko/RU – 13, 11 winter varieties: Derzhavnyi, Espada, Zhavoronok, Kondrat, Kumach, Meteor, Metaksa, Mikhailo, Platon, Tigr, Khutorok, and 2 winter-and-spring varieties: Putnik, Timofei) and 4 Western-Asian varieties (Syria/SYR – 4: Pamir013/Sonata, Pamir065/Pamir149, CWB-117-77-97, ROHO).

Molecular and genetic analysis. The genomic DNA of barley varieties was extracted from 3 seedlings of each variety using cetyltrimethylammonium bromide (CTAB), according to (Syvolap et al, 2014). The samples of the isolated DNA from individual plants of each variety were combined in the mixture and investigated by PCR analysis.

Specific primers (**Table 1**) to microsatellite loci of the 5HL chromosome of barley were used to conduct PCR (Beaubien and Smith, 2006; Varshney et al, 2007).

PCR amplification was conducted on thermocycler T100 ™ Bio-Rad (USA). The reaction mixture for PCR had the following composition: 1×PCR-buffer for *Taq*-polymerase (50 mM KCl, 20 mM tris-HCl, pH 8.4 (25 °C), 2 mM $MgCl_2$, 0.01 % Tween-20), 0.2 mM of each dNTP (deoxynucleoside triphosphates), 0.25 µM of each primer. The mixture with a volume of 10 µl contained 50 ng of DNA and 0.2 units of *Taq*-polymerase.

The conditions of PCR-amplification for microsatellite loci according to (Balvinskaja et al, 2001; Syvolap et al, 2004): 45 cycles; initial denaturation: 94 °C –

Table 1. The sequences of primers for PCR amplification of MS loci alleles

Name	Sequences (5′→3′) of primers	
	forward (F)	reverse (R)
Bmag812a	atagttctttcaggaccaatg	gtcatatggatctccaaagag
Bmag0222	tgctactctggagtggagta	gaccttcaactttgccttata
Bmag0223	ttagtcaccctcaacggt	cccctaactgctgtgatg
Bmag0323	tttgtgacatctcaagaacac	tgacaaacaaataatcacagg
Bmac0337	acaaagagggagtagtacgc	gacccatgatatatgaagatca
Bmag0760	gtgatacatcaagatcgtgc	tccccaacaccagtatcta
HvLOXC	caaacacctccgaccacacg	catgcaccggcacaactctc
UMB702	cagcatccatcagcaatgaa	catgtttggcttcttcgtcc
GBM1166	ctcgaagatgaagacggagg	cccacccaatgttcctgtag
GMS061	cacctgttccgtcccgtc	aacctctttttatccctcgc
GBM1227	ggtcatcatacatacgctgctg	gggtggtgtaggaggaggat
Bmag0387	cgatgaccattgtattgaag	ctcatgttgatgtgtggttag
Bmag0357	cttctacatcatccttgttgc	atgatcattgtattgaagagca
Bmag0113a	ggaatcttctggaacgtc	ttaagaagatcattgtattgaaga

3 min, then all cycles: 94 °C – 1 min; annealing: 55 °C – 1 min, elongation: 72 °C – 2 min. Final elongation: 72 °C – 5 min.

The products of the amplification reaction were fractioned by standard electrophoresis in 1×TBE with further visualization according to (Struss and Plieske, 1998; Syvolap et al, 2014). The molecular mass of the amplification products was determined using markers DNALadder M50 and M1000.

The DNA profiles were registered and processed using the Samsung digital minicamera and relevant software.

Analysis and statistical processing of data. The average number of alleles per locus was estimated as the mean arithmetic (X_m) from the total number of alleles detected in the locus.

The frequency of the incidence was determined for alleles of polymorphic microsatellite loci; it was defined as a share (in %) of a specific allele regarding the total number in the sampling of genotypes, investigated by this locus.

The PIC value for polymorphic microsatellite loci was calculated using the simplified formula (Powell et al, 1996):

$$PIC = 1 - \sum p_{ij}^2,$$

where p_i – the phenotypic frequency of the allelic variant for each *j* of the microsatellite locus.

The diversity index for the investigated loci was calculated as the mean arithmetic (Xm) of PIC values for each locus.

The statistical processing of the obtained results was performed using standard methods according to (Rokitskiy, 1973) and the Data Analysis package of Microsoft Excel.

Some varieties were genotyped by several microsatellite loci in the previous study (Balvinska et al, 2023), and the results of allele characterization of these varieties were used in this study.

RESULTS

The evaluation of variability and the analysis of allele frequencies. We studied the presence of DNA polymorphism and identified alleles by 14 microsatellite loci of the 5HL chromosome in 46 varieties of autumn-sown barley varieties of different origins. The range of the sizes of the detected alleles in the investigated DNA samples varied within 105–280 bp and was notable for microsatellites, as noted in the publications (Varshney et al, 2007; Jo et al, 2017). The allele polymorphism among the investigated genotypes was detected by seven out of 14 microsatellite loci (Bmag0760, GMS061, Bmag0337, UMB702,

Bmag0323, Bmag0223, and Bmag0222). No polymorphism was found by the other analyzed loci in the investigated genetic material of barley varieties. For instance, only one allele variant was detected by loci GBM1166 (165 bp), GBM1227 (210 bp), HvLOXC (182 bp), Bmag0113a (153 bp), Bmag0357 (146 bp), Bmag0387 (123 bp), Bmag0812 (190 bp). The number of alleles by polymorphic loci varied from 2 to 6 (**Table 2**) and in total amounted to 25 with an average value of 3.6 alleles per polymorphic locus. The total number of all the identified variants of allele with the consideration of non-polymorphic ones was 32, and the average number of alleles per locus was 2.3.

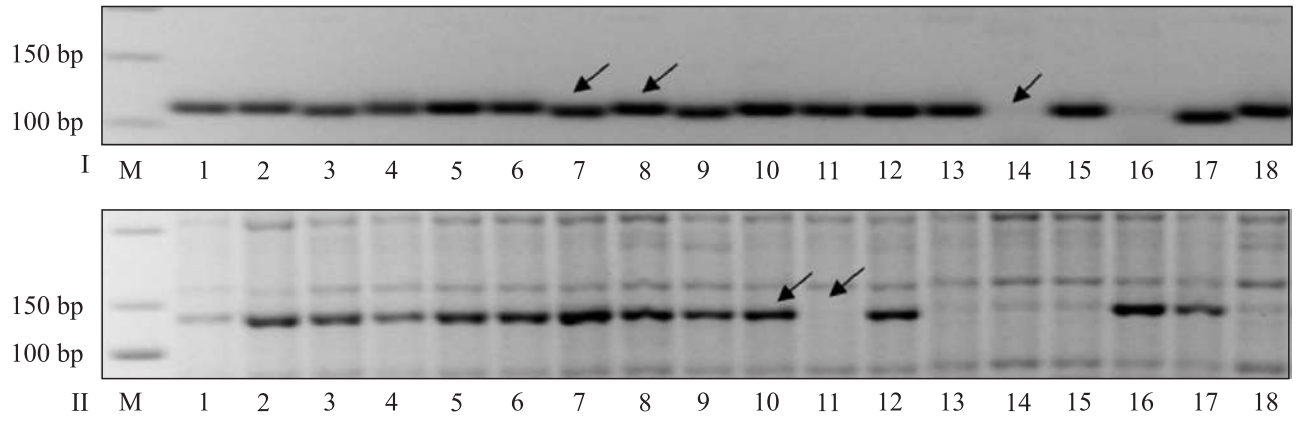
The highest allelic variability for the investigated sampling of barley genotypes was found at the locus Bmag0222. Among six allele variants of this locus, the allele of 150 bp was found to be the most common. The frequency for the allele of 150 bp was 52.3 ± 7.53 % from the total number of the investigated varieties (Table 2).

This is significantly 34.1–47.8 % ($t_{observed}$ = 3.58–5.87 at $t_{0.05}$ = 2.04–2.06) higher than the frequencies of other alleles by Bmag0222, which did not have considerable differences among themselves. The allele of 150 bp was detected in 23 investigated varieties of different origins, including 12 varieties, bred in the south of Ukraine (PBGI-NCSCI), 8 varieties from other European countries, and 3 varieties from Syria (Table 2). The alleles of 145 and 180 bp of this locus had rather low frequency (4.5 ± 3.13 %) and occurred only in two genotypes each.

Table 2. The variability, frequencies and distribution of alleles of polymorphic microsatellite loci of the 5HL chromosome in the sampling of barley varieties of different origins

Locus	N[1]	Alleles, bp	n[2]	Allele frequency p ± Sp3, %	PIC	n(n1)[2] in samples from different countries						
						UKR[2]	RU	DE	CZE	NL	FR	SYR
Bmag0222	44	145	2	4.5 ± 3.13	0.67	–	2	–	–	–	–	–
		150	23	52.3 ± 7.53		12	6	1	–	1	–	3
		155	7	12.5 ± 4.98		6(3)	–	–	1	–	–	–
		160	9	18.2 ± 5.82		3(2)	3	2	1	–	–	–
		165	4	8.0 ± 4.09		1(1)	–	–	–	–	–	1
		180	2	4.5 ± 3.13		0	–	1	–	–	1	–
Bmag0223	45	127	7	14.4 ± 5.23	0.77	–	6	–	1(1)	–	–	–
		150	12	26.7 ± 6.59		6	1	3	1	1	–	–
		160	13	27.8 ± 6.68		7	3	–	1(1)	–	–	2
		170	10	22.2 ± 6.20		5	2	1	–	–	1	1
		180	4	8.9 ± 4.24		2	1	–	–	–	–	1
Bmag0323	46	148	8	9.8 ± 4.38	0.54	3(3)	2(2)	1	2(2)	–	–	–
		155	6	10.9 ± 4.59		2	2(1)	–	1(1)	–	–	1
		160	31	65.2 ± 7.02		14(2)	9	3	–	1	1	3
		165	8	14.1 ± 5.13		5(1)	2(1)	–	1(1)	–	–	–
Bmag0760	46	105	4	8.7 ± 4.15	0.44	4	–	–	–	–	–	–
		110	33	71.7 ± 6.64		17	11	2	1	–	–	2
		null-allele	9	19.6 ± 5.85		–	2	2	1	1	1	2
GMS061	46	135	10	21.8 ± 6.09	0.65	4	2	2	–	–	1	3
		140	18	39.1 ± 7.19		1	6	2	–	–	–	1
		145	18	39.1 ± 7.19		7	5	–	2	1	–	–
Bmag0337	45	130	33	65.6 ± 7.08	0.45	11(4)	11(1)	3(1)	2	1	1	4(1)
		145	19	34.4 ± 7.08		13(4)	3(1)	2(1)	–	–	–	1(1)
UMB702	44	null-allele	8	17.4 ± 5.59	0.29	19	8	4	2	1	–	4
		280	38	82.61 ± 5.59		2	5	–	–	–	1	–

Note: [1] N – total number of investigated varieties; [2] n – number of varieties–carriers of this allele (total), (n1) – including population (or heterogeneous) varieties with this allele. [3] $p \pm Sp$ – allele frequency and its standard error.



The electrophoretic profiles of alleles, found by microsatellite loci Bmag0760 (I), UMB702 (II). 1–18 – DNA samples of barley varieties. M – molecular weight marker DNALadder50bp. The arrows indicate the profiles of different alleles by loci (Bmag0760 – 105 bp, 110 bp; UMB702 – 280 bp) and variants with null-allele

Contrary to the previous one (Bmag0222), there was another ratio of allele frequencies by locus Bmag0223, their distribution among the investigated genotypes of barley was more even. Reliable differences were found between two most common alleles within the sampling, 150 and 160 bp, which generally were found in 25 varieties of different origin with the frequency of 26.7 ± ± 6.59 and 27.8 ± 6.68 %, respectively, on the one hand, and the least common allele of 180 bp with the incidence of 8.9 ± 4.24 %, ($t_{observed}$ = 2.27 and 2.39 at $t_{0.05}$ = 2.12 and 2.11, respectively) on the other hand, which was found in four varieties, including two Ukrainian ones and two of other origin.

The ratio of frequencies, found by locus Bmag0323, was similar to that of Bmag0222. The frequency of the allele of 160 bp, most commonly found by this locus, which occurred in a total of 31 varieties from different regions, was 65.2 ± 7.02 %. This is by 51.1–55.4 % ($t_{observed}$ = 5.88–6.70 at $t_{0.05}$ = 2.03–2.11) higher than the incidence of three other alleles of this locus, the values of which were within 9.8–14.1 % and did not differ much. These alleles were found among the varieties from Ukraine and other countries from eastern and central parts of Europe. It should be noted that the allele of 148 bp, except for one variety from Germany, was found in heterogeneous (population) varieties of different origins (Table 2).

In the sampling of genotypes, investigated by loci GMS061 and Bmag0760, three alleles were detected in each, by loci Bmag0337 and UMB702 – two in each, and genotypes with null-allele were found by the latter and Bmag0760 (**Figure**).

Among the diversity of allele variants of locus GMS061, the most common alleles were those of 140 and 145 bp. In general, these alleles were found in 36 investigated varieties in the equal ratio with the frequency of 39.1 ± 7.19 % per each allelic variant, which was 17.3 % higher than the other allele of 135 bp, whose frequency was 21.8 ± 6.09 %. By locus Bmag0760, the most common allele had 110 bp, its carriers were 33 genotypes of different origin, including 17 varieties, bred in Ukraine. The incidence of this allele of 110 bp was 71.7 ± 6.64 %. Two other alleles (105 bp and null-allele) of locus Bmag0760 did not differ in their incidence (d=10.9 ± 7.17 %) and were reliably inferior to the incidence of the most common allele of 110 bp by 65.0 % and 52.1 % ($t_{observed}$ = 8.30 and 5.89, respectively, at $t_{0.05}$ = 2.02 in both cases). It should be noted that the allele of 110 bp was present in the varieties from five different countries, and the allele of 105 bp – only in Ukrainian varieties. At the same time, no carrier of null-allele was found among Ukrainian varieties.

In addition to locus Bmag0760, by which we found 9 genotypes with null-allele, the latter variant occurred in 8 other varieties by EST-locus of UMB702. Another allele of 280 bp was found in most (38 out of 46) samples by locus UMB702. The frequency of the allele of 280 bp was 82.6 ± 5.59 %, which considerably, by 65.2 % ($t_{observed}$ = 8.24 at $t_{0.05}$ = 2.02), exceeded that for null-allele.

At the locus Bmag0337, two allele variants of 130 bp and 145 bp were detected, and they were rather asymmetrically distributed among the investigated varieties in the general sampling. Two latter alleles differed considerably among themselves in terms of their frequency, 65.6 ± 7.08 and 34.4 ± 7.08 %, respectively ($t_{observed}$ = 3.17 at $t_{0.05}$ = 2.02).

The PIC value for the investigated microsatellite loci, calculated based on the allele frequencies, varied for polymorphic microsatellites within 0.29–0.77 (Table 2). The highest polymorphism index (0.77) was observed for locus Bmag0223. The lowest value of this index (0.29) was found for locus UMB702. The diversity index for this sampling of genotypes was 0.54 on average.

DISCUSSION

A long process of barley breeding led to an increase in the number of varieties and a simultaneous reduction of genetic diversity, which resulted in higher sensitivity of varieties to the impact of different unfavorable ecological conditions, including the effect of below-freezing temperatures. In its turn, it requires the creation of new, more resistant genotypes with better adaptability to conditions in specific regions of cultivation (Visioni et al, 2013). The study and evaluation of molecular and genetic diversity of the barley genome, for instance, its specific, including microsatellite, sites, may be a relevant source of information to further use individual loci and their alleles, associated with relevant traits or their combinations for the analysis of genotypes in breeding programs. The studies on barley in this direction are still a matter of large-scale research and have been conducted for several decades already (Akar et al, 2009; Sallam et al, 2020; Fricano et al, 2021). During this period, there have been many studies on mapping microsatellites (Ramsay et al, 2000; Varshney et al, 2007) and investigation of basic collections of barley genetic material from different countries or regions based on microsatellite analysis (Zhang et al, 2014; Jo et al, 2017; Elakhdar et al, 2018; Lawati et al, 2021). Microsatellite markers are also suggested for the evaluation of the resistance to abiotic stresses, including the effect of low temperatures and other factors in laboratory conditions (Fiust and Rapacz, 2020; Hasan et al, 2020; Baidyussen et al, 2024).

Chromosome 5HL is known to contain key genes controlling LT- and frost resistance (Visioni et al, 2013; Dhillon et al, 2017; Fatemi et al, 2022). This study reports genetic variability of 14 microsatellite loci of barley in chromosome 5HL, including those in the region of genes *Fr-H1*, *Fr-H2,* and close to them (Beaubien et al, 2006; Varshney et al, 2007; Rapacz et al, 2010), and presents the data about the distribution and frequencies of the identified microsatellite alleles and genetic diversity in the investigated sampling of 46 autumn-sown barley varieties of different origin. We found the dominating alleles and those with the reliably lower frequency, and the alleles, specific only to some regions.

Among the investigated microsatellites of the 5HL chromosome of barley, only 50 % of loci in the selected samples of varieties were found to be polymorphic. These were microsatellite loci Bmag0760, GMS061, Bmag0337, UMB702, Bmag0323, Bmag0223, and Bmag0222. No polymorphism was found for other investigated loci. We expected higher allelic diversity for most investigated loci, as we mainly chose the microsatellites with a high PIC value as per scientific literature and considerable allelic variability. For instance, as per several authors, locus Bmag812 can show up to 7 alleles and is recommended for use in MAS for frost resistance (Varshney et al, 2007; Rapacz et al, 2010). Unfortunately, scientific publications do not report which alleles of this locus may have diagnostic relevance in terms of frost resistance. In our previous study using 35 genotypes from the collection of autumn-sown barley (winter and winter-and-spring varieties), we found only one allele by locus Bmag812 (Balvinska et al, 2020; Balvinska et al, 2023). At the same time, Banjarey et al (2017) reported that in the varieties of Indian origin, only two alleles were identified by locus Bmag812, and the PIC value was rather low (0.28). The same authors identified allelic polymorphism by locus Bmag0387 (Banjarey et al, 2017). According to other scientific sources (Beaubien and Smith, 2006; Baidyussen et al, 2024), more than two alleles were demonstrated by loci Bmag0357 and Bmag0387. Contrary to other authors, we did not observe polymorphism by these loci and found only one allelic variant inherent to all genotypes. At the same time, the absence of allelic diversity by locus HvLOX was detected both in this sampling of varieties and other presented publications (Baidyussen et al, 2024). A total of 32 various allelic variants were identified by the investigated 14 MC-loci of barley in the range of sizes corresponding to microsatellites. Seven loci of 5HL chromosome, which were found to be polymorphic, demonstrated the presence of 25 alleles.

The number of alleles for polymorphic loci in this study fluctuated from 2 (Bmag0337, UMB702) to 6 (Bmag 0222) with an average value of 3.6 alleles per polymorphic locus. This is similar to the results mentioned in scientific literature (Ferreira et al, 2016). In the study of barley from different regions of Iran and China and Egypt, Mohammadi S et al (2020) found a relatively high level of genetic variability with an average number of 4,64 alleles per locus.

Six (the highest value) allelic variants were identified in the investigated sampling of varieties by locus Bmag0222. The PIC value was 0.67, which coincided with the one, reported by Tomka et al (2017) while investigating the sampling of 24 genotypes of different types of development and European origin. Previously, Czech researchers (Svobodova et al, 2007) obtained similar results when, in 30 varieties of different origins, they found 8 alleles of a similar range of sizes and PIC values. The researchers from India (Bandjarey et al, 2017) demonstrated a smaller number (3) of alleles by this locus on another genetic material of barley. The authors from China (Hua et al, 2014) found a considerably higher number (15) and private alleles while investigating genotypes of multicolor barley, which could have been possible due to the analysis of a substantially larger number of collection samples (277). Generally, it demonstrated a possible potential in terms of genetic variability by this microsatellite region in genotypes bred in specific regions.

The values for incidence of the identified alleles of locus Bmag0222 fluctuated from 4.5 to 52.3 %. The allele of 150 bp, dominant in the analyzed general sampling, occurred with a frequency of 52.3 ± 7.53 % in the representatives of different regions. For instance, the carriers of this allele were found to be 12 (27 %) Ukrainian varieties, bred by PBGI-NCSCI in different years; most of them were winter-and-spring varieties, and 11 (25 %) varieties of some other origin, including the ones, bred in Europe and Syria (Table 2). The frequencies of other alleles of the locus were within 8.0––18.2 % from the total sampling, did not have considerable differences, and were reliably inferior to those for the most common allele of 160 bp – by 34.1––47.8 % ($t_{observed}$ = 3.58–5.87 at $t_{0.05}$ = 2.04–2.06). Alleles of 155 bp and 165 bp mainly occurred in Ukrainian varieties, for instance, in the composition of varieties with more than one genotype by this locus. As for varieties of another origin, the allele of 155 bp occurred in one variety from Czech Republic, and the allele of 165 bp in a variety from Syria. Alleles of 145 and 180 bp (144 and 178) had an insignificant presence in the sampling (4.5 ± 3.13 %) and did not occur among Ukrainian varieties.

The polymorphic microsatellite loci, by which two (UMB702, Bmag0337), three (Bmag0760), and four (Bmag0323) alleles were found, similar to locus Bmag0222, had one of the allelic variants with considerable incidence, which exceeded the incidence for others. These were the allele of 160 bp of locus Bmag0323 with a frequency of 65.2 ± 7.02 %, the allele of 130 bp of locus Bmag0337 with a frequency of 65.6 ± 7.15 %, alleles of 110 bp (Bmag0760) and 280 bp (UMB702), the frequencies of which amounted to 71.7 ± 6.64 % and 82.61 ± 5.59 %, respectively. The frequencies of other alleles of each of these loci did not have significant differences. By several loci (UMB702, Bmag 0223, Bmag0760), we found private alleles, whose carriers were only the representatives of a specific region.

According to the study of the distribution of alleles by locus Bmag0323, the carriers of the dominant allele of 160 bp were representatives of different regions, including 30.4 % (14) varieties of southern Ukrainian breeding in different years, 15 genotypes from other European countries and three from Syria which made up a total of 39.1 %. The frequencies of three other alleles of locus Bmag 0323 (148, 155, 165 bp) were within 9.8–14.1 % and reliably ($t_{observed}$ =5.88–6.70 at $t_{0.05}$ = = 2.03–2.11) inferior to the allele of 160 bp, by 51.1–– 55.4 %, in terms of their frequency. Only the varieties of European breeding were carriers of alleles of 148 and 165 bp. The allele of 155 bp was present in six varieties, including one from Syria, three Ukrainian, and two more European varieties. By loci Bmag0760 and UMB702, we found samples with null allele, whose frequencies were considerably (by 52.1 and 65.2 %, respectively) lower than the frequencies of the most common alleles of these loci, 110 bp (Bmag0760) and 280 bp (UMB702).

The allele of 110 bp, common for the varieties in the general sampling, from locus Bmag0760, occurred in the representatives bred in different regions, except for France and the Netherlands, which had a null allele by this locus. In the prevailing majority, the carriers of the allele of 110 bp were Ukrainian varieties, bred by PBGI-NCSCI (81.0 %), and 86 % were representatives of another eastern European country. At the same time, no carrier of null-allele, occurring in genotypes from other regions, was detected in all the investigated Ukrainian barley varieties by this locus. In general, null-alleles were carried by 9 varieties, including seven of European breeding and three from western Asia (Syria). The allele of 105 bp was found only in several varieties, bred by PBGI-NCSCI, which may be considered specific for this distribution. The allele of 130 bp of locus Bmag0337 was determined in the investigated barley varieties of all regions. At the same time, most (13 out of 21) varieties of Ukrainian breeding (PBGI-NCSCI) had another allele of this locus – 145 bp; it also occurred in four population varieties, which had two different alleles by this locus each. In general, by locus

Bmag0337, genetic heterogeneity was observed in 7 genotypes of different origins, which was 15 % from the sampling of the investigated varieties.

38 varieties, bred in different European countries, except France, and all four genotypes from Syria were found to be carriers of the allele of 280 bp by EST-locus UMB702. This allele was identified in most (19) Ukrainian varieties. The rest of the investigated genotypes had a null allele, which generally occurred in 8 varieties of only European breeding. As known from scientific literature (Beaubien KA, Smith KP, 2006), EST-locus UMB702 is localized in the 5HL chromosome (bin 10–11), i.e. within the limits of *Fr-H1* (bin11) and *Fr-H2* (bin9-10). This locus comes from the transcribed area; it is very similar to the gene of photosystem II complex (NP_565786.1 LHB1B2), involved in binding chlorophyll in *A. thaliana* (Varshney RK et al., 2007). There are also data, demonstrating that the locus sequences were obtained while sequencing mRNA of genotypes, resistant to *Blumeria graminis f.sp. hordei*, which had the gene *Mla13*. There is information that among genes of resistance to this disease, found effective in the investigated barley varieties, the presence of gene *Mla13* provides an additional effect against the populations of the disease agent (Young et al, 2004). Since this locus may be related to genotypes with gene *Mla13*, ensuring a higher resistance to mildew and impacting the regulation of photosystem II type in terms of binding chlorophyll, the alleles of this locus may reflect the association with the state of plant immunity, and on the background of higher or, on the contrary, poorer immunity in combination with alleles of other genes (Steffenson et al, 2009) have an indirect association with maintaining the level of resistance to unfavorable conditions, including frost resistance or effect on its formation. Additional studies are required to investigate the rate of this association or its presence.

Locus Bmag0223 is characterized by sufficient diversity of alleles and the fact that it is localized close to gene *Fr-H2* (Rapacz et al, 2010), which attracts specific attention. Contrary to the abovementioned microsatellites, another ratio of allelic frequencies was observed by loci Bmag0223. In the case of relatively even distribution of alleles, reliable differences were found only between common alleles of 150 and 160 bp with the incidence of 26.7 ± 6.59 and 27.8 ± 6.68%, respectively, on the one hand, and the allele of 180 bp (8.9 ± ± 4.24 %), on the other hand, ($t_{observed}$ = 2.27 and 2.39 at $t_{0.05}$ = 2.12 and 2.11, respectively), which was found to be least common. Among five present polymorphic allelic variants of locus Bmag0223, one of the common alleles of 160 bp was generally present in 14 varieties, 50 % of which were of Ukrainian breeding. Another common allele of 150 bp was found in six Ukrainian varieties, including four winter-and-spring varieties, and four genotypes from other European countries, including three from Germany and one from Czechia. A less common allele of 180 bp with a significantly lower frequency than that for alleles of 150 and 160 bp, by 17.8 and 18.9 %, respectively, occurred only in four varieties, including two from Ukraine. The carriers of the allele of 170 bp of locus Bmag0223 with an incidence of 22.2 ± 6.20 were 10 varieties from different regions, including five, bred by PBGI-NCSCI. In addition, one allele of this locus, of 127 bp, was present only in six genotypes (prevailing majority) of the National Grain Center named after P. P. Lukianenko, along with another allele in the composition of one Czech variety. Thus, alleles 150 bp and 160 bp of this locus, as the most common ones, and the allele 127 bp, mostly present in the varieties from a specific area, may be of breeding value for some regions and should be kept in the breeding process along with other specific and valuable genotypes.

As for locus GMS061, localized close to the region of *Fr-H1* (*Vrn-H1*), a relatively even distribution was observed in it too. The most common alleles of locus GMS061 of 140 bp and 145 bp, found among the investigated varieties in an even number with the frequency of 39.1 ± 7.19 % each, had the advantage of 17.3 % over another allele of this locus of 135 bp in terms of incidence.

As found in this study, the PIC values for microsatellite loci varied within 0.29 (UMB702) – 0.77 (Bmag0223), which reflected the presence of microsatellite areas with a different level of polymorphism (from low to high) in the sampling of barley varieties, there were also loci with no polymorphism. The determined PIC index (0.54) on average demonstrates not high but also not low genetic variability of microsatellite loci of the 5HL chromosome in the sampling of the investigated barley varieties.

CONCLUSIONS

The SSR analysis was used to evaluate the genetic variability of 14 microsatellite regions of the 5HL chromosome, located in the area and close to the key genes of frost resistance of barley, *Fr-H1,* and *Fr-H2*. This study determined the number and range of microsatellite alleles, evaluated their frequencies and distribu-

tion, and genetic diversity in the sampling of 46 varieties of autumn-sown barley of different origins. Polymorphism was observed in 50 % of investigated loci. Genetic variability was found by loci Bmag 0222, Bmag 0223, Bmag0323, Bmag0337, Bmag0760, GMS061, and UMB702. In general, 14 investigated microsatellite loci of the 5HL chromosome demonstrated the presence of 32 alleles, 25 of which were polymorphic. The most common alleles, whose frequency was considerably higher than that of others, were determined by polymorphic loci based on the distribution of alleles and evaluation of frequencies, along with private alleles, occurring only in specific regions. Though the varieties of Ukrainian breeding did not acquire some alleles as compared to varieties of another origin, they also had allelic differences and private alleles, inherent only to the representatives of the southern Ukrainian region. On average, there was the presence of not high, in terms of other microsatellite areas of the barley genome, but moderate variability of the microsatellite fraction of 5HL chromosome, which demonstrates the potential of some loci and the possibility of using their alleles in further studies on determining the associations with the required resistance traits of genotypes.

Adherence to ethical principles. No experiments, described in this article, involved the use of animals.

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Варіабельність та потенціал мікросателітних локусів 5HL хромосоми у генотипів ячменю різного походження

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Мета. Дослідити поліморфізм мікросателітних локусів хромосоми 5HL ячменю, в тому числі тих, що містяться в області ключових генів НТ-стійкості та близько до них, визначити та оцінити частоту зустрічальності мікросателітних алелів в генетичному матеріалі ячменю різного походження. **Методи**. Виділення геномної ДНК, мікросателітний аналіз, ПЛР-ампліфікація, гель-електрофорез, статистичний аналіз. Досліджували 46 генотипів ячменю різного походження. з яких 33 сорти ячменю озимого та 13 альтернативного (дворучки) типу розвитку, серед яких – 21 сорт селекції СГІ-НЦНС, 25 – інших оригінаторів, в тому числі 21 – з країн Центральної (Чехія/CZ – 2: Grabe, Luran; Німеччина/DE – 4: Skarpia, Majbrit, Cinderella, Highlight), Західної (Франція/FR – 1: Анжеліка; Нідерланди/NL – 1: Gerlach) та Східної (НЦЗ ім. П.П. Лук›яненка/RU – 13, 11 озимих: Дєржавний, Еспада, Жаворонок, Кондрат, Кумач, Мєтєор, Мєтакса, Міхайло, Платон, Тігр, Хуторок та 2 дворучки: Путнік, Тімофєй) Європи та 4 західноазіатських сорти (Сирія/SYR – 4: Pamir013/Sonata, Pamir065/Pamir149, CWB-117-77-97, ROHO). **Результати.** Досліджено алельний поліморфізм за 14 мікросателітними локусами хромосоми 5HL ячменю, в тому числі розташованих в області генів Fr-H1, Fr-H2 та близько до цих регіонів, вивчено розподіл ідентифікованих мікросателітних алелів, їх частоти та генетичне різноманіття у виборці 46 колекційних сортів ячменю осіннього посіву різного походження. З досліджених мікросателітних ділянок 5HL хромосоми ячменю на обраній виборці сортів лише 50 % локусів виявились поліморфними. Такими є мікросателітні локуси Bmag0760, GMS061, Bmag0337, UMB702, Bmag0323, Bmag0223 та Bmag0222. Виявлено домінуючі алелі і ті, частота яких достовірно поступалася за перші, алелі, що є приватними для окремих регіонів. Розраховані значення індексу поліморфності (PIC) для досліджених поліморфних мікросателітних локусів варіювали у межах 0,29 (UMB702) –0,77 (Bmag0223). Індекс різноманіття в середньому становив 0,54. **Висновки.** За результатами дослідження спостерігається не високе по відношенню до інших важливих ділянок генома, але помірне алельне різноманіття досліджених мікросателітних локусів 5HL хромосомі ячменю, що, свідчить про наявність потенціалу щодо генетичної мінливості окремих локусів та їх алелів, можливості застосування таких у подальших дослідженнях щодо визначення ефектів тих чи інших алелів кожного з локусів та їх асоціацій з необхідними господарсько цінними ознаками ячменю, зокрема НТ-стійкості. Обговорюється потенціал поліморфних алелів як маркерів ознак морозостійкості генотипів ячменю осіннього строку посіву.

Ключові слова: ячмінь (*Hordeum vulgare L.*), хромосома 5HL, SSR-аналіз, генетична варіабельність, мікросателітні маркери, частоти алелів, PIC.

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