UDC 68.35.29; 34.15.23

VALIDATION OF KASP ASSAYS ASSOCIATED WITH BARLEY ADAPTATION AND PRODUCTIVITY TRAITS

Genievskaya Yuliya A. [1, 2], **Almerekova Shyryn S.** [1], **Chudinov Vladimir A.** [3], **Turuspekov Yerlan K.** [1, 2], **Abugalieva Saule I.** [1 *]

[1] *Institute of plant biology and biotechnology, Almaty 050040, Kazakhstan*

[2] *al-Farabi Kazakh National University, Almaty 050040, Kazakhstan*

[3] *Karabalyk Agricultural Experimental Station, Nauchnoe 110908, Kostanay region, Kazakhstan*

[*] *absaule@yahoo.com*

ABSTRACT

Barley (*Hordeum vulgare* L.) is an important cereal crop traditionally used in animal feed, malting, and food production. In Kazakhstan, barley is the second most cultivated cereal grain. However, despite the long history of barley cultivation in Kazakhstan, traditional breeding methods prevail here. The introduction of marker-assisted selection (MAS) in the breeding process may improve the adaptation and productivity of local cultivars, as well as help in the development of new ones. For that, validation of 23 kompetitive allele-specific PCR (KASP) assays for adaptation and productivity traits developed using previous GWAS results was performed. The collection of 35 two-rowed barley promising lines was grown at the Kazakh research institute of agriculture and plant growing (KRIAPG, Almaty region, Kazakhstan) in 2021 and studied for 5 adaptation-related (heading time, heading-maturity time, plant height, peduncle length, and spike length) and 5 productivity-related (number of kernels per spike, the weight of kernels per spike, weight of kernels per plant, thousand kernels weight, and yield per m^2) traits. The same collection was genotyped using 23 KASP assays. As a result, 21 KASPs demonstrated a good level of polymorphism (MAF > 0.10 and I > 0.36) in the studied barley collection. Six KASP assays confirmed their associations with adaptation and productivity traits ($P < 0.05$); nine KASPs were associated with other agronomic traits ($P < 0.05$). Nine KASP assays were identified for adaptation traits, one assay was detected for productivity traits and six KASPs were found to be associated with both types of traits. Four KASP assays (*ipbb_hv_9, ipbb_hv_101, ipbb_hv_109,* and *ipbb_hv_110*) confirmed significant ($P < 0.05$) effect of shorter heading-maturity time on higher productivity traits under stress conditions of south-east Kazakhstan. Thus, in this study fifteen out of the studied twenty-one KASP assays were validated for their associations with adaptation and productivity traits and potentially can be included in the barley breeding projects.

Key words: *Hordeum vulgare* L., barley breeding, marker-assisted selection, yield, KASP genotyping.

INTRODUCTION

Spring barley (*Hordeum vulgare* L.) is one of the leading cereal crops occupying 4th place among all cereals in the world after corn, wheat, and rice [1]. In Kazakhstan, barley is the second most cultivated cereal crop [2]. According to the Bureau of National Statistics of Kazakhstan, in 2021, spring barley was the second cereal after wheat by total grain production (2.35 million tons) [2]. Among that, 0.44 million tons (about 18.7 % of the total yield) were produced in the Almaty region, which makes the region economically prospective for barley breeding.

For many years, barley breeding in Kazakhstan was based on using traditional methods, including the selection and hybridization of individuals with favorable traits. However, these methods have several disadvantages. Often, favorable genes are inherited along with unfavorable ones; inheritance of one gene is often accompanied by the loss of another; some genes are linked to each other, which makes it difficult to separate the good one from the bad one. To strengthen desirable hereditary properties, it is necessary to increase the homozygosity of a new cultivar taking years. The development of modern biotechnological approaches including molecular DNA markers may help to improve accuracy and reduce the time of breeding processes. One of these approaches is marker-assisted selection (MAS) successfully used worldwide for crop improvement [3, 4]. The basic principle of MAS is the identification of associations between a DNA marker and a linked gene controlling the trait of interest. These marker-trait associations (MTAs) are used for the search and development of new cultivars and breeding lines. DNA markers are usually used for the assessment of genetic diversity, genes, and quantitative trait loci (QTL) mapping, selection of promising breeding material, loci introgression, and gene pyramiding [5, 6]. The most common type of DNA markers involved in MAS for the last 10 years is single nucleotide polymorphism (SNP). These markers are numerous and can be found on all chromosomes throughout the plant genome [7]. In addition, now, the detection of SNPs is relatively simple and accelerated by arrays, next- and third-generation sequencing technology, and MALDI-TOF mass spectrophotometry [8].

SNPs found in the barley genome are widely used for gene mapping genes and QTLs associated with economically valuable traits. This type of markers was used for association mapping via genome-wide association study (GWAS) of the resistance to biotic [9-11], abiotic stresses [12-14], adaptation traits [15-17], yield components [17-19], and grain quality traits of barley [20, 21]. All of these QTLs together with known genes potentially may be involved in the barley breeding process aimed at yield improvement. The big advantage of SNP markers is the possibility to convert them into markers for kompetitive allele-specific PCR or KASP. KASP is a high-throughput assay for the rapid genotyping of breeding material by markers associated with economically valuable traits listed above [22]. The official website of the National Institute of Agricultural Botany (NIAB) (www.niab.com/mas/species/type/3/Barley) provides information about 25 KASP assays developed for spike row number, grain pigmentation, seasonal type,

plant height, lodicule disposition, and other important barley traits, as well as genotyping data for many barley cultivars. KASP assays were also developed for the resistance to greenbug [23], powdery mildew [24], leaf rust [25], seed dormancy regulation [26], α-amylase activity [27], β-glucan content in the grain [28], and other valuable traits of barley.

In Kazakhstan, the MAS approach in barley breeding is not fully developed and traditional methods still prevail. However, there are works on GWAS of barley grown in Kazakhstan with SNP markers identified for key adaptation and productivity traits [18, 19]. According to Y. Xu and coauthors [29], proper genetic markers for MAS should be significantly associated with traits. One of the ways to test MTAs is the validation – genotyping of new germplasm material with markers and association test. SNPs from previous barley GWAS studies [18, 19] were used for the transformation of them into convenient KASP assays. The current study was aimed at validation of KASP assays using the collection of promising barley lines in the Almaty region.

MATERIALS AND METHODS

Barley collection and field experiment

Studied spring two-rowed barley collection included 35 promising lines ($F_{>8}$) obtained from Karabalyk agricultural experimental station (KAES, Kostanay region, Kazakhstan). The collection was grown in the field of the Kazakh research institute of agriculture and plant growing (KRIAPG, Almaty region, Kazakhstan) under natural non-irrigated conditions in 2021. Each individual barley line was sowed in one m^2 plot in two independent random replications. The collection was studied for 5 adaptation-associated traits: heading time (HT, days), heading-maturity time (HMT, days), plant height (PH, cm), peduncle length (PL, cm), and spike length (SL, cm). As well as for 5 productivity-associated traits: number of kernels per spike (NKS, pcs), the weight of kernels per spike (WKP, pcs), the weight of kernels per plant (WKP, g), thousand kernels weight (TKW, g), and grain yield per m^2 (YM2, g/m^2). Together with the studied collection, the check cultivar 'Asem' was grown for comparative analysis. For further validation, the average values of two repetitions were used.

KASP genotyping and statistics

Total genomic DNA was extracted from 4-days individual seedlings of studied barley collection using the CTAB extraction protocol [30]. SNP markers identified in previous GWAS studies of barley [18, 19] were transformed into KASP assays using sequences obtained from the Triticeae Toolbox (https://barley.triticeaetoolbox.org). Two forward allele-specific primers and one reverse common primer were designed using SNP sequences and the LGC Genomics tool (www.biosearchtech.com). Positions and associated traits for five 23 KASP assays are provided in Table 1. The KASP assays were designed by LGC Genomics and the PCR was carried out in accordance with the manufacturer's protocol with three repetitions for each barley line. KlusterCaller software (https://

Table 1 – The list of KASP assays used for the analysis.

#	KASP assay	Chromosome	Associated traits with references
1	*ipbb_hv_5*	1H	NKS [18]
2	*ipbb_hv_6*	3H	HT, HMT, PH, PL, NKS, TKW, YM2 [18]
3	*ipbb_hv_7*	6H	HT, HMT, PH, PL, NKS, TKW, YM2 [18]
4	*ipbb_hv_9*	1H	HT [19]
5	*ipbb_hv_10*	2H	NKS [19]
6	*ipbb_hv_11*	7H	TKW [19]
7	*ipbb_hv_101*	4H	HT, PH, PL, TKW [18]
8	*ipbb_hv_102*	5H	PH (unpublished)
9	*ipbb_hv_103*	6H	TKW, YM2 (unpublished)
10	*ipbb_hv_104*	1H	HT, PH, TKW, YM2 [18]
11	*ipbb_hv_105*	4H	PL, NKS (unpublished)
12	*ipbb_hv_106*	7H	NKS (unpublished)
13	*ipbb_hv_107*	6H	NKS (unpublished)
14	*ipbb_hv_108*	2H	SL (unpublished)
15	*ipbb_hv_109*	5H	YM2 [18]
16	*ipbb_hv_110*	5H	PH, YM2 (unpublished)
17	*ipbb_hv_111*	6H	NKS (unpublished)
18	*ipbb_hv_113*	5H	TKW [18]
19	*ipbb_hv_114*	5H	YM2 (unpublished)
20	*ipbb_hv_115*	1H	SL [18]
21	*ipbb_hv_116*	1H	HT, HMT, PH, PL, TKW, YM2 [18]
22	*ipbb_hv_128*	7H	HMT, PH, PL, NKS, TKW, YM2 [18]
23	*ipbb_hv_134*	2H	HT, HMT, PH, PL [18]

Notes: HT – heading time, HMT – heading-maturity time, PH – plant height, PL – peduncle length, NKS – number of kernels per spike, TKW – thousand kernels per spike, YM2 – yield per m^2

www.biosearchtech.com) was used to visualize the KASP genotyping results. A t-test in the IBM SPSS Statistics v 27.0 (https://www.ibm.com/products/spss-statistics) was applied to determine the significant differences ($P < 0.05$) between the means of studied adaptation and productivity traits in two allelic groups for each KASP assay.

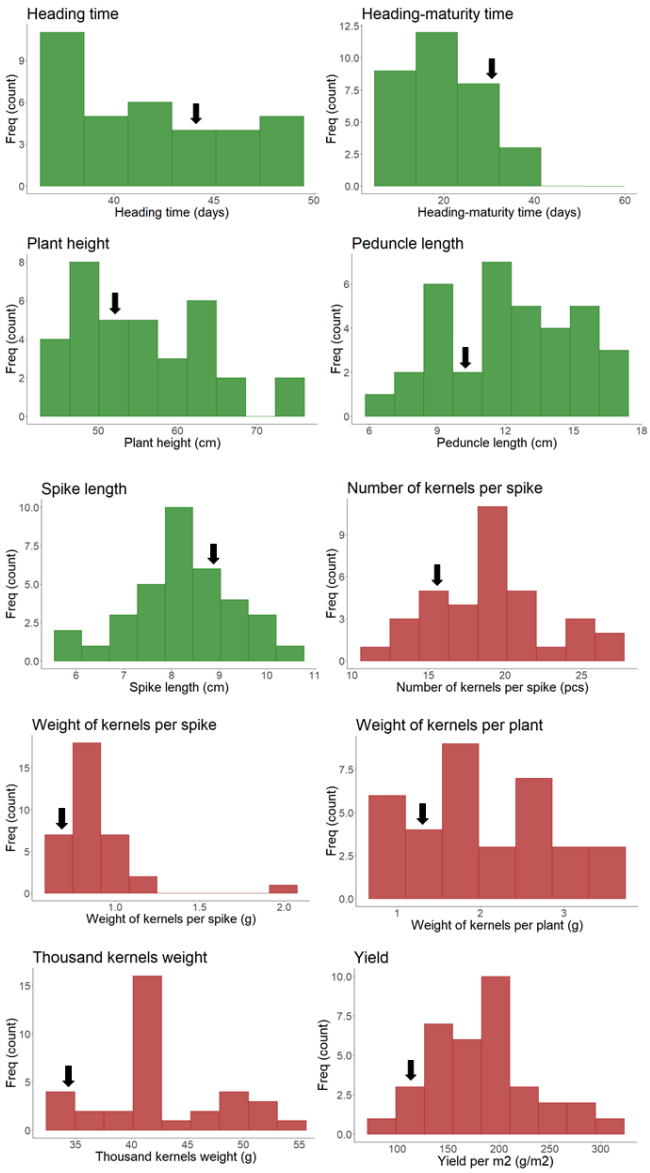

Figure 1 – Distribution of adaptation (green) and productivity (red) traits in studied two-rowed barley collection. The arrows denote spring barley check cultivar 'Asem' originated and developed in KRIAPG for south-east Kazakhstan.

RESULTS

Adaptation and productivity traits in studied barley collection

The collection of 35 two-rowed barley promising lines was grown and studied for 10 key agronomic traits including 5 adaptation and 5 productivity traits. For all studied traits, a wide range of variability was observed (Figure 1). HT was between 38 and 49 days, while HMT ranged from 7 to 36 days. PH varied from 45.3 to 75 cm, PL was from 6.7 to 17 cm, and SL was between 5.7 and 10.3 cm. As for the productivity traits, NKS was observed between 11.7 and 27 pcs, WKS – from 0.6 to 1.9 g, WKP – from 1 to 3.6 g, TKW varied from 33.6 to 54.3 g, and YM2 – from 72.2 to 297 g/m^2 (Figure 1). Generally, for adaptation traits (HT, HMT, PH, PL, and SL), there were lines with larger and smaller values than the check cultivar. At the same time, according to productivity traits (NKS, WKS, WKP, TKW, and YM2), the major part of the collection was more productive than the check cultivar (Figure 1).

KASP genotyping

In total, 23 KASP assays were used for validation of previously identified MTAs for productivity and adaptation traits. The collection of 35 barley promising lines was genotyped with these KASP assays (Figure 2).

Among them, two KASP assays demonstrated a low level of polymorphism and minor allele frequency (MAF) < 0.05, therefore they were excluded from further analysis (Table 2). Other 21 assays showed good segregation, MAF from 0.11 to 0.50, I (Shannon's Information Index) > 0.355, and were used as genotypes for the t-test. Among studied barley lines, heterozygous individuals were detected by 17 KASP assays and were excluded from analysis as well.

Associations between KASP markers and adaptation traits in studied barley collection

For the validation, values of 5 adaptation traits studied in the collection of 35 barley promising lines and their genotyping results were used in the t-test. The results of the t-test are summarized in Table 3.

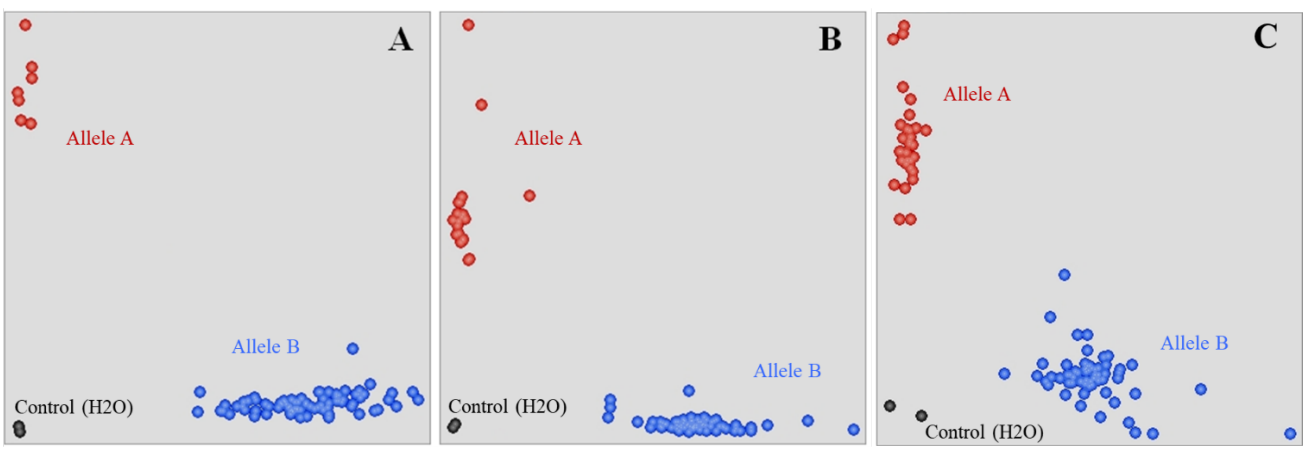

Figure 2 – Fragment of genotyping results. KASP segregation plots of ipbb_hv_6 (A), ipbb_hv_116 (B), and ipbb_hv_128 (C).

Table 2 – Polymorphism information for 23 KASP assays in the population of barley promising lines.

#	KASP assay	N	MAF	Ne	I
1	*ipbb_hv_5*	34	0.26	1.637	0.578
2	*ipbb_hv_6*	34	0.12	1.262	0.362
3	*ipbb_hv_7*	35	0.03	1.059	0.130
4	*ipbb_hv_9*	32	0.38	1.882	0.662
5	*ipbb_hv_10*	33	0.21	1.502	0.517
6	*ipbb_hv_11*	32	0.50	2.000	0.693
7	*ipbb_hv_101*	33	0.42	1.955	0.682
8	*ipbb_hv_102*	33	0.18	1.424	0.474
9	*ipbb_hv_103*	35	0.03	1.059	0.130
10	*ipbb_hv_104*	32	0.41	1.932	0.675
11	*ipbb_hv_105*	32	0.47	1.992	0.691
12	*ipbb_hv_106*	32	0.25	1.600	0.562
13	*ipbb_hv_107*	32	0.19	1.438	0.483
14	*ipbb_hv_108*	35	0.34	1.820	0.643
15	*ipbb_hv_109*	31	0.29	1.701	0.602
16	*ipbb_hv_110*	34	0.32	1.778	0.630
17	*ipbb_hv_111*	31	0.29	1.701	0.602
18	*ipbb_hv_113*	31	0.42	1.949	0.680
19	*ipbb_hv_114*	31	0.13	1.290	0.385
20	*ipbb_hv_115*	32	0.28	1.679	0.594
21	*ipbb_hv_116*	35	0.49	1.998	0.693
22	*ipbb_hv_128*	35	0.11	1.254	0.355
23	*ipbb_hv_134*	35	0.43	1.960	0.683

Notes: N – number of individuals. MAF – minor allele frequency. Ne – number of effective alleles. I – Shannon's Information Index.

Table 3 – T-test results for adaptation traits. P-values are presented.

#	KASP	MAF	HT	HMT	PH	PL	SL
1	*ipbb_hv_5*	0.26	0.586	0.307	0.450	0.796	0.486
2	*ipbb_hv_6*	0.12	**0.033**	0.676	0.260	0.123	0.594
3	*ipbb_hv_9*	0.38	0.931	**0.041**	**0.038**	0.068	0.670
4	*ipbb_hv_10*	0.21	**0.005**	0.588	0.767	0.369	0.965
5	*ipbb_hv_11*	0.50	0.546	0.603	0.275	0.279	0.939
6	*ipbb_hv_101*	0.42	0.404	**0.038**	0.234	0.162	0.341
7	*ipbb_hv_102*	0.18	0.325	**0.004**	**0.001**	**0.003**	**0.037**
8	*ipbb_hv_104*	0.41	0.184	**0.004**	**4.10E-05**	0.065	0.120
9	*ipbb_hv_105*	0.47	0.593	0.315	**0.0171**	0.423	0.269
10	*ipbb_hv_106*	0.25	**0.040**	0.844	0.075	**0.002**	0.202
11	*ipbb_hv_107*	0.19	0.845	**0.009**	**0.004**	0.746	0.408
12	*ipbb_hv_108*	0.34	0.494	0.926	0.772	0.487	0.912
13	*ipbb_hv_109*	0.29	0.892	**0.039**	0.092	0.694	0.613
14	*ipbb_hv_110*	0.32	0.095	**0.008**	0.052	0.713	0.630
15	*ipbb_hv_111*	0.29	0.822	0.057	**0.006**	0.702	0.332
16	*ipbb_hv_113*	0.42	0.397	0.399	0.430	0.319	0.615
17	*ipbb_hv_114*	0.13	0.932	0.811	0.818	0.407	0.814
18	*ipbb_hv_115*	0.28	0.544	0.183	0.284	0.655	0.523
19	*ipbb_hv_116*	0.49	**0.046**	**0.014**	**7.30E-06**	**0.016**	**0.046**
20	*ipbb_hv_128*	0.11	0.363	0.542	0.599	0.108	0.838
21	*ipbb_hv_134*	0.43	0.750	0.106	0.064	**0.034**	0.732

Notes: MAF – minor allele frequency. P values < 0.05 are highlighted in bold.

Among 21 polymorphic KASPs, 14 assays demonstrated significant associations (P < 0.05) with studied adaptation traits. Assay *ipbb_hv_6* was significantly associated with HT (P < 0.05); *ipbb_hv_9* – with HMT and PH (P < 0.05); *ipbb_hv_10* – with HT (P < 0.05); *ipbb_hv_101* – with HMT (P < 0.05); *ipbb_hv_102* – with HMT (P < 0.01), PH (P < 0.01), PL (P < 0.01), SL (P < 0.05); *ipbb_hv_104* – with HMT (P < 0.01) and PH (P < 0.0001); *ipbb_hv_105* – with PH (P < 0.05); *ipbb_hv_106* – with HT (P < 0.05) and PL (P < 0.01); *ipbb_hv_107* – with HMT (P < 0.01) and PH (P < 0.01); *ipbb_hv_109* – with HMT (P < 0.05); *ipbb_hv_110* – also with HMT (P < 0.01); *ipbb_hv_111* – with PH (P < 0.01); *ipbb_hv_116* – with all traits – HT (P < 0.05), HMT (P < 0.05), PH (P < 0.00001), PL (P < 0.05), SL (P < 0.05); and *ipbb_hv_134* – with PL (P < 0.05) (Table 3). In total, 8 assays were associated with one adaptation trait, 4 assays were identified for two traits, 1 assay demonstrated association with 4 traits, and one assay – with all 5 traits. By the traits, the largest number of assays were observed for HMT (8 assays) and for PH (7 assays). For SL only two assays were detected.

Associations between KASP markers and productivity traits in studied barley collection

All 21 KASP assays were used for the validation of their association with productivity traits. For these traits, 6 out of 21 KASP assays demonstrated significant associations (P < 0.05). General information about associations is provided in Table 4.

Assay *ipbb_hv_9* was associated with NKS (P < 0.01) and TKW (P < 0.05); *ipbb_hv_101* – with NKS (P < 0.05); *ipbb_hv_109* – with WKP (P < 0.01); *ipbb_hv_110* – with NKS (P < 0.05); *ipbb_hv_115* – NKS (P < 0.05) and TKW (P < 0.05); *ipbb_hv_134* – with TKW (P < 0.05) (Table 4). Among KASPs significantly associated with productivity traits, two were found to be related to two traits and four were identified for one trait each. By traits, for NKS there were 4 assays, for TKW – 3 assays, and for WKP only one assay was detected. For WKS and YM2 there were no significant associations observed.

Assays *ipbb_hv_9*, *ipbb_hv_101*, *ipbb_hv_109*, *ipbb_hv_110,* and *ipbb_hv_134* were associated with both adaptation and productivity traits demonstrating significant effects (Table 5).

Genotype 'A:A' of *ipbb_hv_9* negatively affected HMT, PH, and TKW, but positively affected NKS. Genotype 'A:A' of *ipbb_hv_101* demonstrated the positive effect of HMT, but the negative on NKS. Genotype 'A:A' of *ipbb_hv_109* decreased HMT, but increased WKP. Genotype 'A:A' of *ipbb_hv_110* had a positive effect on HTM but it was negative for NKS. And finally, genotype 'A:A' of *ipbb_hv_134* affected positively both PL and TKW.

DISCUSSION

One of the top priorities in MAS is the validation of MTAs and/or QTLs identified in association and linkage mapping studies [31]. The validation of such genotype-phenotype associations is an essential step for the application of MAS in practice. From a technical point of view, it is very important to test these associations by using cost-effective, informative, and reliable types of DNA markers, such as KASP assays. For this purpose, previously identified MTAs [18, 19] were

Table 4 – T-test results for productivity traits. P-values are presented.

#	KASP	MAF	NKS	WKP	WKS	TKW	YM2
1	*ipbb_hv_5*	0.26	0.113	0.927	0.163	0.063	0.876
2	*ipbb_hv_6*	0.12	0.454	0.783	0.386	0.158	0.110
3	*ipbb_hv_9*	0.38	**0.008**	0.508	0.699	**0.015**	0.133
4	*ipbb_hv_10*	0.21	0.196	0.456	0.216	0.084	0.905
5	*ipbb_hv_11*	0.50	0.074	0.084	0.133	0.138	0.905
6	*ipbb_hv_101*	0.42	**0.012**	0.567	0.577	0.216	0.054
7	*ipbb_hv_102*	0.18	0.055	0.951	0.483	0.054	0.260
8	*ipbb_hv_104*	0.41	0.191	0.331	0.129	0.491	0.667
9	*ipbb_hv_105*	0.47	0.051	0.318	0.908	0.413	0.682
10	*ipbb_hv_106*	0.25	0.332	0.597	0.149	0.502	0.085
11	*ipbb_hv_107*	0.19	0.235	0.503	0.414	0.578	0.993
12	*ipbb_hv_108*	0.34	0.642	0.696	0.340	0.616	0.155
13	*ipbb_hv_109*	0.29	0.353	**0.004**	0.171	0.543	0.903
14	*ipbb_hv_110*	0.32	**0.037**	0.187	0.823	0.338	0.250
15	*ipbb_hv_111*	0.29	0.659	0.374	0.606	0.587	0.534
16	*ipbb_hv_113*	0.42	0.852	0.783	0.613	0.391	0.079
17	*ipbb_hv_114*	0.13	0.639	0.321	0.554	0.615	0.988
18	*ipbb_hv_115*	0.28	**0.028**	0.823	0.235	**0.024**	0.977
19	*ipbb_hv_116*	0.49	0.091	0.149	0.082	0.367	0.385
20	*ipbb_hv_128*	0.11	0.533	0.493	0.254	0.992	0.905
21	*ipbb_hv_134*	0.43	0.140	0.786	0.764	**0.013**	0.184

Notes: MAF – minor allele frequency. P values < 0.05 are highlighted in bold.

Table 5 – Significant (P < 0.05) effect of genotypes on adaptation and productivity traits in the studied barley breeding lines.

ipbb_hv_9	**Genotype**	**N**	**Mean**	**SD**	**Effect (%)**
HMT (days)	A:A	20	18.30	7.88	-38.65
	B:B	12	29.83	12.67	+38.65
PH (cm)	A:A	20	52.75	7.23	-9.42
	B:B	12	58.24	6.31	+9.42
NKS (pcs)	A:A	20	20.51	3.63	+16.58
	B:B	12	17.11	2.48	-16.58
TKW (g)	A:A	20	40.82	5.05	-10.80
	B:B	12	45.76	5.60	+10.80
ipbb_hv_101	**Genotype**	**N**	**Mean**	**SD**	**Effect (%)**
HMT (days)	A:A	19	25.53	13.24	+34.55
	B:B	14	16.71	8.59	-34.55
NKS (pcs)	A:A	19	17.82	3.52	-14.98
	B:B	14	20.96	3.11	+14.98
ipbb_hv_109	**Genotype**	**N**	**Mean**	**SD**	**Effect (%)**
HMT (days)	A:A	22	18.09	11.41	-35.14
	B:B	9	27.89	11.55	+35.14
WKP (g)	A:A	22	2.31	0.83	+32.47
	B:B	9	1.56	0.47	-32.47
ipbb_hv_110	**Genotype**	**N**	**Mean**	**SD**	**Effect (%)**
HMT (days)	A:A	23	25.78	10.09	+39.68
	B:B	11	15.55	6.85	-39.68
NKS (pcs)	A:A	23	17.89	3.18	-13.32
	B:B	11	20.64	3.95	+13.32
ipbb_hv_134	**Genotype**	**N**	**Mean**	**SD**	**Effect (%)**
PL (cm)	A:A	15	13.27	2.64	+15.15
	B:B	20	11.26	2.68	-15.15
TKW (g)	A:A	15	45.37	5.63	+10.09
	B:B	20	40.79	4.71	-10.09

transformed into KASP assays and used for the validation of their associations in different barley germplasm. In the current study, the collection of promising two-rowed barley lines demonstrated wide ranges of both adaptation and productivity traits (Figure 1), as well as good polymorphism by studied KASP assays (Table 2). Values of five key productivity traits were higher in the collection than in the check cultivar 'Asem' (Figure 1). While adaptation traits in the majority of the collection were similar to the check cultivar. Nine KASP assays were identified for adaptation traits exclusively, one assay was identified for productivity traits and six KASPs were found to be associated with both types of traits (Figure 3).

In total, the validation of six KASP assays can be considered successful, since their associations with adaptation and productivity traits identified in GWAS [18, 19] were confirmed in the current study (Tables 1, 3, 4). Six KASPs did not associate with any agronomic traits in the studied barley promising lines collection (Tables 3 and 4). Nine KASP assays demonstrated associations with other agronomic traits not-mentioned in previous works [18, 19] (Tables 3 and 4). The largest number of significant associations among all traits was observed for HT, HMT, and PH – key adaptation traits. It is known, that heading time- and flowering-associated gene groups, such as *PpdH*, *HvFT*, *HvCO*, and others, as well as QTLs, have a great effect on almost all agronomic traits of barley including yield [32]. In the current study, four KASP assays (*ipbb_hv_9*, *ipbb_hv_101*, *ipbb_hv_109*, and *ipbb_hv_110*) demonstrated associations with HMT and productivity traits NKS, WKP and TKW (Tables 3 and 4). Presumably, genes and/or QTLs similar to the above-mentioned heading time genes may affect these productivity traits. Interestingly, the alleles of these four KASP assays decreased HMT at the same time increasing the productivity of barley lines (NKS and WKP) (Table 5). It may be explained by the fact that grain filling during HMT in southeast Kazakhstan coincides with periods of drought and high-temperature stress reducing crop yield [33]. The short duration of HMT appeared to contribute to increased stress tolerance and yield, as was observed for wheat earlier [34]. A short to medium grain filling period appeared to be desirable in environments where the growing season included severe stress [35]. The largest effect on productivity was detected for the genotype 'A:A' of *ipbb_hv_109* increasing WKP by 32.47 % (1.56 g for 'B:B' and 2.31 g for 'A:A') and decreasing HMT by 35.14 % (from 27.89 days for 'B:B' to 18.09 days for 'A:A') (Table 5). In previous GWAS work, this SNP was associated with other important produc-

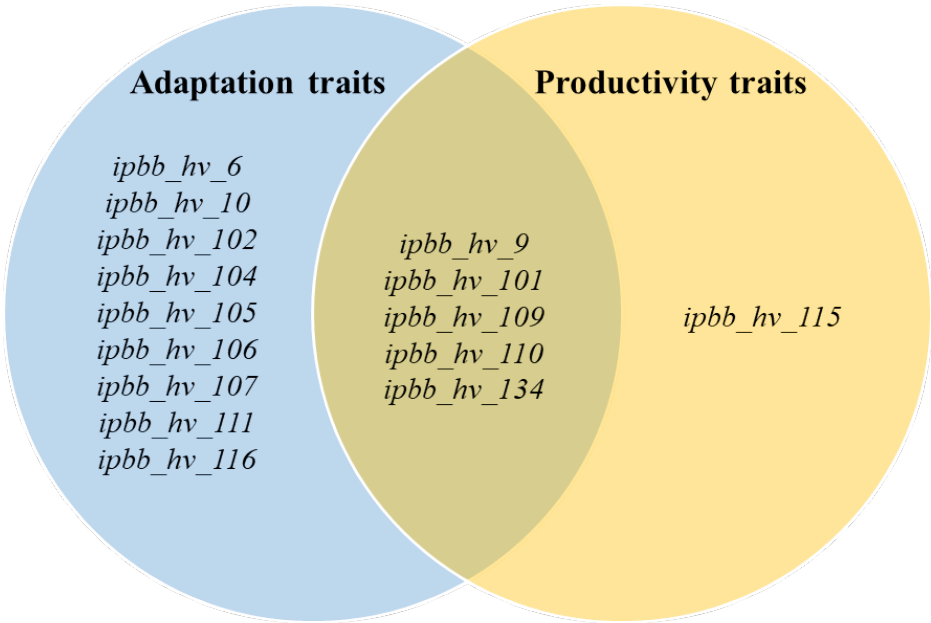

Figure 3 – KASP assays identified for adaptation (blue), productivity (yellow), and for both groups of traits (intersection).

tivity traits – YM2 [18]. Thus, KASP assays *ipbb_hv_9*, *ipbb_hv_101*, *ipbb_hv_109*, and *ipbb_hv_110* confirmed their significance in the studied collection of barley promising lines and are presumably novel genetic factors that can be successfully used in barley breeding in drought regions of Kazakhstan. Other assays significantly associated with agronomic traits confirmed in this study also may be included in the breeding process as a part of MAS.

CONCLUSIONS

In the present study, the collection of 35 two-rowed barley promising lines was used for the genotyping and phenotyping, and further validation. For that, 23 KASP assays for adaptation and productivity traits were developed using the result of previous GWAS studies. Among them, 21 KASPs demonstrated a good level of polymorphism (MAF > 0.100 and I > 0.355) in the studied barley collection. Six KASP assays confirmed associations with adaptation and productivity traits, nine KASPs demonstrated associations with other agronomic traits not-mentioned in previous works, and six KASPs had no significant associations. Nine KASP assays were identified for adaptation traits, one assay was for productivity traits and six KASPs were found to be associated with both types of traits. Four KASP assays (*ipbb_hv_9*, *ipbb_hv_101*, *ipbb_hv_109*, and *ipbb_hv_110*) revealed genotypes conditioning significant ($P < 0.05$) effect of shorter heading-maturity time on higher productivity traits under stress conditions of south-east Kazakhstan. Thus, fifteen KASP assays significantly associated with agronomic traits confirmed in this study ($P < 0.05$) can be included in the barley breeding activities.

FUNDING

The study was supported by the grant AP08052804 “Development and validation of KASP arrays efficiency for key productivity and grain quality traits in two-rowed spring barley” from the Ministry of Education and Science of the Republic of Kazakhstan.

LITERATURE

1. Statista Portal. Available at: https://www.statista.com/ (Accessed 28.08.2022).

2. Бюро национальной статистики Агентства по стратегическому планированию и реформам Республики Казахстан. Официальный сайт: https://stat.gov.kz/ (От 28.08.2022).

3. Varshney, R.K., Mahendar, T., Aggarwal, R.K., Börner, A. Genic molecular markers in plants: development and applications // Genomics-assisted crop improvement. - 2007. - P. 13-29.

4. Abdi, S., Dwivedi, A., Kumar, S., Bhat, V. Development of EST-SSR markers in *Cenchrus ciliaris* and their applicability in studying the genetic diversity and cross-species transferability // Journal of Genetics. - 2019. - Vol. 98. - № 4. - P. 1-16.

5. Леонова, И.Н. Молекулярные маркеры: использование в селекции зерновых культур для идентификации, интрогрессии и пирамидирования генов // Вавиловский журнал генетики и селекции. - 2014. - Т. 17. - № 2. - С. 314-325.

6. Lister, D.L., Jones, H., Jones, M.K., O’Sullivan, D.M., Cockram, J. Analysis of DNA polymorphism in ancient barley herbarium material: validation of the KASP SNP genotyping platform // Taxon. - 2013. - Vol. 62. - № 4. - P. 779-789.

7. Mammadov, J., Aggarwal, R., Buyyarapu, R., Kumpatla, S. SNP markers and their impact on plant breeding // International journal of plant genomics. - 2012. - Vol. 2012. - P. 1-12.

8. Morgil, H., Can Gercek, Y., Tulum, I. Single Nucleotide Polymorphisms (SNPs) in plant genetics and breeding // The recent topics in genetic polymorphisms. - 2020. - doi:10.5772/intechopen.91886.

9. Czembor, J.H., Czembor, E., Suchecki, R., Watson-Haigh, N.S. Genome-Wide Association Study for Powdery Mildew and Rusts Adult Plant Resistance in European

Spring Barley from Polish Gene Bank // Agronomy. - 2021. - Vol. 12. - № 1. - P. 7.

10. Gyawali, S., Chao, S., Vaish, S.S., Singh, S.P., Rehman, S., Vishwakarma, S.R., Verma, R. P.S. Genome wide association studies (GWAS) of spot blotch resistance at the seedling and the adult plant stages in a collection of spring barley // Molecular breeding. - 2018. - Vol. 38. - № 5. - P. 1-14.

11. Gyawali, S., Mamidi, S., Chao, S., Bhardwaj, S. C., Shekhawat, P.S., Selvakumar, R., et al. Genome-wide association studies revealed novel stripe rust resistance QTL in barley at seedling and adult-plant stages // Euphytica. - 2021. - Vol. 217. - №. 1. - P. 1-18.

12. Zhou, G., Broughton, S., Zhang, X.Q., Ma, Y., Zhou, M., Li, C. Genome-wide association mapping of acid soil resistance in barley (*Hordeum vulgare* L.) // Frontiers in plant science. - 2016. - Vol. 7. - P. 406.

13. Mwando, E., Han, Y., Angessa, T.T., Zhou, G., Hill, C.B., Zhang, X.Q., Li, C. Genome-Wide Association Study of Salinity Tolerance During Germination in Barley (*Hordeum vulgare* L.) // New Insights into Salinity Sensing, Signaling and Adaptation in Plants. - 2021. - Vol. 11. - P. 118.

14. Borrego-Benjumea, A., Carter, A., Zhu, M., Tucker, J.R., Zhou, M., Badea, A. Genome-wide association study of waterlogging tolerance in barley (*Hordeum vulgare* L.) under controlled field conditions // Frontiers in plant science. - 2021. - Vol. 11. - P. 1717.

15. Bai, Y., Zhao, X., Yao, X., Yao, Y., An, L., Li, X., et al. Genome wide association study of plant height and tiller number in hulless barley // PLoS ONE. - 2021. - Vol. 16. - № 12. - P. e0260723.

16. Maurer, A., Draba, V., Jiang, Y., Schnaithmann, F., Sharma, R., Schumann, E., et al. Modelling the genetic architecture of flowering time control in barley through nested association mapping // BMC genomics. - 2015. - Vol. 16. - № 1. - P. 1-12.

17. Xu, X., Sharma, R., Tondelli, A., Russell, J., Comadran, J., Schnaithmann, F., et al. Genome-Wide Association Analysis of Grain Yield-Associated Traits in a Pan-European Barley Cultivar Collection // The plant genome. - 2018. - Vol. 11. – № 1. - P. 170073.

18. Genievskaya, Y., Almerekova, S., Sariev, B., Chudinov, V., Tokhetova, L., Sereda, G., Ortaev, A., Tsygankov, V., Blake, T., Chao, S., Sato, K., Abugalieva, S., Turuspekov, Y. Marker–trait associations in two–rowed spring barley accessions from Kazakhstan and the USA // PLoS ONE. - 2018. - Vol. 3. - № 10. - P. e0205421.

19. Almerekova, S., Sariev, B., Abugalieva, A., Chudinov, V., Sereda, G., Tokhetova, L., Ortaev, A., Tsygankov, V., Blake, T., Chao, S., Genievskaya, Y., Abugalieva, S., Turuspekov, Y. Association mapping for agronomic traits in six–rowed spring barley from the USA harvested in Kazakhstan // PLoS ONE. - 2019. - Vol. 14. - № 8. - P. e0221064.

20. Szűcs, P. Blake, V.C., Bhat, P.R., Chao, S., Close, T.J., Cuesta-Marcos, A., et al. An integrated resource for barley linkage map and malting quality QTL alignment // The Plant Genome. – 2009. - Vol. 2. - № 2. DOI: 10.3835/plantgenome2008.01.0005.

21. Li, M., Geng, L., Xie, S., Wu, D., Ye, L., Zhang, G. Genome-wide association study on total starch, amylose and amylopectin in barley grain reveals novel putative alleles // International journal of molecular sciences. - 2021. - Vol. 22. - № 2. - P. 553.

22. Majeed, U., Darwish, E., Rehman, S.U., Zhang, X. Kompetitive allele specific PCR (KASP): a singleplex genotyping platform and its application // Journal of Agricultural Science. - 2019. - Vol. 11. - № 1. - P. 11-20.

23. Xu, X., Mornhinweg, D., Bernardo, A., Li, G., Bian, R., Steffenson, B.J., Bai, G. Characterization of *Rsg2. a3*: A new greenbug resistance allele at the *Rsg2* locus from wild barley (*Hordeum vulgare* ssp. *spontaneum*) // The Crop Journal. - 2022. DOI: 10.1016/j.cj.2022.01.010.

24. Gupta, S., Vassos, E., Sznajder, B., Fox, R., Khoo, K.H., Loughman, R., et al. A locus on barley chromosome 5H affects adult plant resistance to powdery mildew // Molecular Breeding. – 2018. - Vol. 38. - № 8. - P. 1-10.

25. Jost M., Singh D., Lagudah E., Park R. F., Dracatos P. Fine mapping of leaf rust resistance gene *Rph13* from wild barley // Theoretical and Applied Genetics. - 2020. - Vol. 133. - № 6. - P. 1887-1895.

26. Yan, L., Jiang, C., Cai, Y., Pan, Y., Xu, R., Luan, H., et al. Evaluating the genetic effects of seed dormancy regulatory genes *Qsd1* and *Qsd2* in a global collection of cultivated barley (*Hordeum vulgare* ssp. *vulgare*) with functional kompetitive allele-specific PCR markers // Plant Breeding. - 2021. - Vol. 140. - № 5. - P. 827-834.

27. Cu, S.T., March, T.J., Degner, S., Eglinton, J.K. Identification of novel alleles from wild barley for the improvement of alpha-amylase and related malt quality traits // Plant Breeding. - 2016. - Vol. 135. - № 6. - P. 663-670.

28. Houston, K., Russell, J., Schreiber, M., Halpin, C., Oakey, H., Washington, J. M., et al. A genome wide association scan for (1, 3; 1, 4)-β-glucan content in the grain of contemporary 2-row Spring and Winter barleys // BMC genomics. - 2014. - Vol. 15. - № 1. - P. 1-15.

29. Xu, Y. B. Developing marker-assisted selection strategies for breeding hybrid rice // Plant breeding reviews. - 2003. - P. 73-174.

30. Doyle, J.J., Doyle, J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue // Phytochemical bulletin. - 1987. - Vol. 19. - № 1. - P. 11-15.

31. Riaz, A., Kanwal, F., Börner, A., Pillen, K., Dai, F., Alqudah, A. M. Advances in genomics-based breeding of barley: molecular tools and genomic databases // Agronomy. - 2021. - Vol. 11. - № 5. - P. 894.

32. Alqudah, A.M. Sharma, R., Pasam, R.K., Graner, A., Kilian, B., Schnurbusch, T. Genetic dissection of photoperiod response based on GWAS of pre-anthesis phase duration in spring barley // PLoS One. - 2014. - Vol. 9. - № 11. - P. e113120.

33. Наурозбаева, Ж.К. Климатические особенности распределения осадков на территории южного и юго-восточного Казахстана в теплый период // Вестник КазНУ. Серия географическая. - 2010. - Т. 31. - № 2. - С. 108-113.

34. Bruckner, P.L., Frohberg, R C. Rate and Duration of Grain Fill in Spring Wheat 1 // Crop Science. - 1987. - Vol.

27. - № 3 - P. 451-455.

35. Passarella, V.S., Savin, R., Slafer, G.A. Breeding effects on sensitivity of barley grain weight and quality to events of high temperature during grain filling // Euphytica. - 2005. - Vol. 141. - № 1. - P. 41-48.

REFERENCES

1. Statista Portal. Available at: https://www.statista.com/ (Accessed 28.08.2022).

2. Agency for Strategic planning and reforms of the Republic of Kazakhstan Bureau of National statistics. Available at: https://stat.gov.kz/ (Accessed 28.08.2022).

3. Varshney, R.K., Mahendar, T., Aggarwal, R.K., Börner, A. Genic molecular markers in plants: development and applications // Genomics-assisted crop improvement. - 2007. - P. 13-29.

4. Abdi, S., Dwivedi, A., Kumar, S., Bhat, V. Development of EST-SSR markers in *Cenchrus ciliaris* and their applicability in studying the genetic diversity and cross-species transferability // Journal of Genetics. - 2019. - Vol. 98. - No. 4. - P. 1-16.

5. Leonova, I.N. Molekulyarnye marker: ispolzovanie v selekcii zenovyh kultur dlya identifikacii, introgressii i piramidirovaniya genov [Molecular markers: use in crop breeding for identification, introgression and gene pyramiding]. // Vavilov Journal of Genetics and Breeding.- 2014. - Vol. 17. - No 2. - P. 314-325.

6. Lister, D.L., Jones, H., Jones, M.K., O'Sullivan, D.M., Cockram, J. Analysis of DNA polymorphism in ancient barley herbarium material: validation of the KASP SNP genotyping platform // Taxon. - 2013. - Vol. 62. - No 4. - P. 779-789.

7. Mammadov, J., Aggarwal, R., Buyyarapu, R., Kumpatla, S. SNP markers and their impact on plant breeding // International journal of plant genomics. - 2012. - Vol. 2012. - P. 1-12.

8. Morgil, H., Can Gercek, Y., Tulum, I. Single Nucleotide Polymorphisms (SNPs) in plant genetics and breeding // The recent topics in genetic polymorphisms. - 2020. - doi:10.5772/intechopen.91886.

9. Czembor, J.H., Czembor, E., Suchecki, R., Watson-Haigh, N.S. Genome-Wide Association Study for Powdery Mildew and Rusts Adult Plant Resistance in European Spring Barley from Polish Gene Bank // Agronomy. - 2021. - Vol. 12. - No 1. - P. 7.

10. Gyawali, S., Chao, S., Vaish, S.S., Singh, S.P., Rehman, S., Vishwakarma, S.R., Verma, R. P.S. Genome wide association studies (GWAS) of spot blotch resistance at the seedling and the adult plant stages in a collection of spring barley // Molecular breeding. - 2018. - Vol. 38. - No 5. - P. 1-14.

11. Gyawali, S., Mamidi, S., Chao, S., Bhardwaj, S. C., Shekhawat, P.S., Selvakumar, R., et al. Genome-wide association studies revealed novel stripe rust resistance QTL in barley at seedling and adult-plant stages // Euphytica. - 2021. - Vol. 217. - №. 1. - P. 1-18.

12. Zhou, G., Broughton, S., Zhang, X.Q., Ma, Y., Zhou, M., Li, C. Genome-wide association mapping of acid soil resistance in barley (*Hordeum vulgare* L.) // Frontiers in plant science. - 2016. - Vol. 7. - P. 406.

13. Mwando, E., Han, Y., Angessa, T.T., Zhou, G., Hill, C.B., Zhang, X.Q., Li, C. Genome-Wide Association Study of Salinity Tolerance During Germination in Barley (*Hordeum vulgare* L.) // New Insights into Salinity Sensing, Signaling and Adaptation in Plants. - 2021. - Vol. 11. - P. 118.

14. Borrego-Benjumea, A., Carter, A., Zhu, M., Tucker, J.R., Zhou, M., Badea, A. Genome-wide association study of waterlogging tolerance in barley (*Hordeum vulgare* L.) under controlled field conditions // Frontiers in plant science. - 2021. - Vol. 11. - P. 1717.

15. Bai, Y., Zhao, X., Yao, X., Yao, Y., An, L., Li, X., et al. Genome wide association study of plant height and tiller number in hulless barley // PLoS ONE. - 2021. - Vol. 16. - No 12. - P. e0260723.

16. Maurer, A., Draba, V., Jiang, Y., Schnaithmann, F., Sharma, R., Schumann, E., et al. Modelling the genetic architecture of flowering time control in barley through nested association mapping // BMC genomics. - 2015. - Vol. 16. - No 1. - P. 1-12.

17. Xu, X., Sharma, R., Tondelli, A., Russell, J., Comadran, J., Schnaithmann, F., et al. Genome-Wide Association Analysis of Grain Yield-Associated Traits in a Pan-European Barley Cultivar Collection // The plant genome. - 2018. - Vol. 11. – No. 1. - P. 170073.

18. Genievskaya, Y., Almerekova, S., Sariev, B., Chudinov, V., Tokhetova, L., Sereda, G., Ortaev, A., Tsygankov, V., Blake, T., Chao, S., Sato, K., Abugalieva, S., Turuspekov, Y. Marker–trait associations in two–rowed spring barley accessions from Kazakhstan and the USA // PLoS ONE. - 2018. - Vol. 3. - No 10. - P. e0205421.

19. Almerekova, S., Sariev, B., Abugalieva, A., Chudinov, V., Sereda, G., Tokhetova, L., Ortaev, A., Tsygankov, V., Blake, T., Chao, S., Genievskaya, Y., Abugalieva, S., Turuspekov, Y. Association mapping for agronomic traits in six–rowed spring barley from the USA harvested in Kazakhstan // PLoS ONE. - 2019. - Vol. 14. - No 8. - P. e0221064.

20. Szűcs, P. Blake, V.C., Bhat, P.R., Chao, S., Close, T.J., Cuesta-Marcos, A., et al. An integrated resource for barley linkage map and malting quality QTL alignment // The Plant Genome. – 2009. - Vol. 2. - No 2. DOI: 10.3835/plantgenome2008.01.0005.

21. Li, M., Geng, L., Xie, S., Wu, D., Ye, L., Zhang, G. Genome-wide association study on total starch, amylose and amylopectin in barley grain reveals novel putative alleles // International journal of molecular sciences. - 2021. - Vol. 22. - No 2. - P. 553.

22. Majeed, U., Darwish, E., Rehman, S.U., Zhang, X. Kompetitive allele specific PCR (KASP): a singleplex genotyping platform and its application // Journal of Agricultural Science. - 2019. - Vol. 11. - No 1. - P. 11-20.

23. Xu, X., Mornhinweg, D., Bernardo, A., Li, G., Bian, R., Steffenson, B.J., Bai, G. Characterization of *Rsg2. a3*: A new greenbug resistance allele at the *Rsg2* locus from wild barley (*Hordeum vulgare* ssp. *spontaneum*) // The Crop Journal. - 2022. DOI: 10.1016/j.cj.2022.01.010.

24. Gupta, S., Vassos, E., Sznajder, B., Fox, R., Khoo,

K.H., Loughman, R., et al. A locus on barley chromosome 5H affects adult plant resistance to powdery mildew // Molecular Breeding. – 2018. - Vol. 38. - No 8. - P. 1-10.

25. Jost M., Singh D., Lagudah E., Park R. F., Dracatos P. Fine mapping of leaf rust resistance gene *Rph13* from wild barley // Theoretical and Applied Genetics. - 2020. - Vol. 133. - No6. - P. 1887-1895.

26. Yan, L., Jiang, C., Cai, Y., Pan, Y., Xu, R., Luan, H., et al. Evaluating the genetic effects of seed dormancy regulatory genes *Qsd1* and *Qsd2* in a global collection of cultivated barley (*Hordeum vulgare* ssp. *vulgare*) with functional kompetitive allele-specific PCR markers // Plant Breeding. - 2021. - Vol. 140. - No 5. - P. 827-834.

27. Cu, S.T., March, T.J., Degner, S., Eglinton, J.K. Identification of novel alleles from wild barley for the improvement of alpha-amylase and related malt quality traits // Plant Breeding. - 2016. - Vol. 135. - No 6. - P. 663-670.

28. Houston, K., Russell, J., Schreiber, M., Halpin, C., Oakey, H., Washington, J. M., et al. A genome wide association scan for (1, 3; 1, 4)-β-glucan content in the grain of contemporary 2-row Spring and Winter barleys // BMC genomics. - 2014. - Vol. 15. - No 1. - P. 1-15.

29. Xu, Y. B. Developing marker-assisted selection strategies for breeding hybrid rice // Plant breeding reviews. - 2003. - P. 73-174.

30. Doyle, J.J., Doyle, J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue // Phytochemical bulletin. - 1987. - Vol. 19. - No 1. - P. 11-15.

31. Riaz, A., Kanwal, F., Börner, A., Pillen, K., Dai, F., Alqudah, A. M. Advances in genomics-based breeding of barley: molecular tools and genomic databases // Agronomy. - 2021. - Vol. 11. - No 5. - P. 894.

32. Alqudah, A.M. Sharma, R., Pasam, R.K., Graner, A., Kilian, B., Schnurbusch, T. Genetic dissection of photoperiod response based on GWAS of pre-anthesis phase duration in spring barley // PLoS One. - 2014. - Vol. 9. - No. 11. - P. e113120.

33. Naurozbaeva Zh.K. Climatic features of the distribution of precipitation in the territory of southern and southeastern Kazakhstan during the warm period. // Journal of Geography and Environmental Management. - 2010. - Vol. 31. - No 2. - P. 108-113.

34. Bruckner, P.L., Frohberg, R C. Rate and Duration of Grain Fill in Spring Wheat 1 // Crop Science. - 1987. - Vol. 27. - No 3 - P. 451-455.

35. Passarella, V.S., Savin, R., Slafer, G.A. Breeding effects on sensitivity of barley grain weight and quality to events of high temperature during grain filling // Euphytica. - 2005. - Vol. 141. - No 1. - P. 41-48.

ВАЛИДАЦИЯ KASP-МАРКЕРОВ, АССОЦИИРОВАННЫХ С ПРИЗНАКАМИ АДАПТИВНОСТИ И УРОЖАЙНОСТИ ЯЧМЕНЯ

Гениевская Ю.А. [1, 2], **Альмерекова Ш.С.** [1], **Чудинов В.А.** [3], **Туруспеков Е.К..** [1, 2], **Абугалиева С.И.** [1 *]

[1] *Институт биологии и биотехнологии растений, Алматы 050040, Казахстан*

[2] *Казахский Национальный Университет им. аль-Фараби, Алматы 050040, Казахстан*

[3] *Карабалыкская сельскохозяйственная опытная станция, Научное 110908, Костанайская область, Казахстан*

[*] *absaule@yahoo.com*

АННОТАЦИЯ

Ячмень (*Hordeum vulgare* L.) является важной зерновой культурой, традиционно используемой в кормопроизводстве, пивоварении и пищевой промышленности. В Казахстане ячмень занимает второе место по производству среди зерновых. Тем не менее, не смотря на долгую историю ячменеводства в Казахстане, здесь используются преимущественно традиционные методы селекции. Интродукция маркер-опосредованной селекции (МОС) в процесс может помочь улучшить адаптацию и прдуктивность метсных сортов, а также разработать новые. Для этого была проведена валидация 23 kompetitive allele-specific PCR (KASP) маркеров, связанных с признаками адаптации и продуктивности и созданных по результатам предыдущих работ GWAS. Коллекция, включающая в себя 35 перспективных двурядных линий ячменя, была выращена в поле Казахского научно-исследовательского института земледелия и растениеводства (КазНИИЗиР, Алматинская область, Казахстан) в 2021 г. и изучена по 5-и признакам адаптации (время колошения, время от колошения до созревания, высота растения, длина верхнего междоузлия и длина колоса) и по 5-и признакам продуктивности (число зерен в колосе, масса зерен с колоса, масса зерен с растения, масса 1000 зерен и урожайность на м^2). Эта же коллекция была генотипирована по 23 KASP-маркерам. В результате 21 маркер показал хороший уровень полиморфизма (частота минорного аллеля > 0,10, индекс Шенона > 0,36) в изученной коллекции ячменя. Шесть KASP-макреров подтвердили свою ассоциацию с признаками адаптации и продуктивности ($P < 0,05$); девять маркеров были ассоциированы с другими агрономическими признакми ($P < 0,05$). Девять KASP-маркеров были идентифицированы для признаков адаптации, один маркер – для признаков продуктивности и шесть маркеров были ассоциированы сразу с двумя группами признаков. Четыре KASP-маркера (*ipbb_hv_9, ipbb_hv_101, ipbb_hv_109* и *ipbb_hv_110*) подтвердили значимый эффект ($P < 0.05$) укороченного периода между колошением и созреванием на высокую продуктивность в стрессовых условиях юго-востока Казахстана. Таким образом, была успешно проведена валидация 15 из 21 изученного KASP-маркера для признаков адаптации и урожайности. Данные маркеры могут быть включены в селекционные проекты.

Ключевые слова: *Hordeum vulgare* L., селекция ячменя, маркер-опосредованная селекция, урожайность, KASP-генотипирование.

АРПАНЫҢ БЕЙІМДЕЛУ ЖӘНЕ ӨНІМДІЛІГІ БЕЛГІЛЕРІМЕН БАЙЛАНЫСҚАН KASP-МАРКЕРЛЕРІН ВАЛИДАЦИЯЛАУ

Гениевская Ю.А. [1, 2], **Альмерекова Ш.С.** [1], **Чудинов В.А.** [3], **Туруспеков Е.К..** [1, 2], **Абугалиева С.И.** [1*]

[1] *Өсімдіктер биологиясы және биотехнологиясы институты, Алматы 050040, Қазақстан*

[2] *әл-Фараби атындағы Қазақ ұлттық университеті, Алматы 050040, Қазақстан*

[3] *Қарабалық ауылшаруашылық тәжірибелік станциясы, Научное 110908, Қостанай облысы, Қазақстан*

[*] *absaule@yahoo.com*

ТҮЙІН

Арпа (*Hordeum vulgare* L.) – дәстүрлі түрде мал азығы ретінде, сыра қайнату және тамақ өнеркәсібінде қолданылатын маңызды дәнді дақыл болып табылады. Қазақстанда арпа дәнді дақылдар арасында өнімі бойынша екінші орында. Дегенмен, Қазақстанда арпа өсірудің ұзақ тарихына қарамастан, мұнда дәстүрлі өсіру әдістері басым. Процесске маркер-жанама селекцияны (МЖС) енгізу жергілікті сорттардың бейімделуін және өнімділігін жақсартуға, сондай-ақ жаңа сорттарды жасауға көмектеседі. Бұл үшін бейімделуі және өнімділік белгілерімен байланысты және GWAS зерттеулерінің нәтижелері негізінде алынған 23 kompetitive allele-specific PCR (KASP) маркерлері валидацияланды. Арпаның 35 перспективті екі қатарлы линиясын қамтитын коллекция 2021 жылы Қазақ егіншілік және өсімдік шаруашылығы ғылыми-зерттеу институтының (ҚазЕӨШҒЗИ, Алматы облысы, Қазақстан) егістік алқабында өсіріліп, 5 бейімделу белгісі (масақтану уақыты, масақтанудан пісіп жетілуге дейінгі уақыт, өсімдік биіктігі, жоғарғы буын аралық ұзындығы және масақ ұзындығы) және 5 өнімділік белгісі бойынша (бір масақтағы дән саны, бір масақтағы дән салмағы, бір өсімдіктегі дән дән салмағы, 1000 дән салмағы және м^2 өнім) зерттелді. Осы коллекция 23 KASP-маркерлері бойынша генотиптелді. Нәтижесінде, зерттелген арпа коллекциясында 21 маркер полиморфизмнің жақсы деңгейін көрсетті (минорлық аллель жиілігі > 0,10, Шенон индексі > 0,36). KASP-маркердің алтауы бейімделу және өнімділік белгілерімен байланысын растады ($P < 0,05$); тоғыз маркер басқа агротехникалық белгілермен байланысқандығы ($P <0,05$) айқындалды. Бейімделу белгілері үшін тоғыз KASP-маркері анықталды, өнімділік белгілері үшін бір маркер және алты маркер бірден екі белгілер тобымен байланысты болғандығы айқындалды. Төрт KASP-маркерлері (*ipbb_hv_9, ipbb_hv_101, ipbb_hv_109* және *ipbb_hv_110*) Қазақстанның оңтүстік-шығысындағы стресс жағдайында жоғары өнімділікке масақтану мен пісіп жетілу арасындағы қысқартылған кезеңнің айтарлықтай әсерін растады ($P < 0.05$). Осылайша, зерттелген 21 KASP-маркерлерінің 15 бейімделу және өнімділік белгілері үшін валидация сәтті жүргізілді. Анықталған маркерлерді селекциялық жобараларға қосуға болады.

Түйінді сөздер: *Hordeum vulgare* L., арпа селекциясы, маркер-жанама селекция, өнімділік, KASP-генотиптеу.