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## GENOME-WIDE ASSOCIATION STUDY OF GRAIN QUALITY TRAITS IN SPRING BARLEY COLLECTION GROWN IN KYZYLORDA REGION

Barley, among many other crops, has better resistance to harsh environmental conditions. Still, high temperature, drought, and soil salinity prevent the formation of high-quality grain, especially for the malting and brewing industry. In this study, spring barley accessions were tested in conditions of high-salinity soil of Kyzylorda region and analyzed for major grain quality traits, including protein,  $\beta$ -glucan, and starch contents, plumpness, grain hardness, and extractivity. Based on the results, the list of accessions optimal in the region for malting and the list for livestock feeding was made using the above-mentioned traits. Genome-wide association study revealed 20 marker-trait(s) associations combined into 7 quantitative trait loci (QTLs). Three QTLs demonstrated pleiotropic effect affecting grain protein,  $\beta$ -glucan, and starch content and extractivity, two QTLs were identified for grain protein and starch content and extractivity, one QTL was for grain hardness index, and one QTL for plumpness. Identified pleiotropic QTLs were genetically mapped in the vicinity of known barley flowering genes. QTLs identified in the study, as well as promising accessions, can be integrated into the barley breeding process in stressed conditions of Kyzylorda region.

**Key words:** *Hordeum vulgare* L., malting, livestock feed, genotype  $\times$  environment, molecular markers, single nucleotide polymorphism.

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### Қызылорда облысы жағдайында өсірілген жаздық арпа коллекциясының дән сапасы белгілерінің ассоциацияларын толық геномдық талдау

Арпа – бұл басқа мәдени өсімдіктермен салыстырғанда қоршаған ортаның қолайсыз жағдайларына төзімділігі жоғары дақыл. Алайда, жоғары температура, құрғақшылық және тұзды топырақ жоғары сапалы дәннің қалыптасуына жол бермейді, әсіресе сыра қайнату өнеркәсібі үшін. Бұл зерттеуде жаздық арпа үлгілері Қызылорда облысының жоғары тұздануы жағдайында өсіріліп, дән сапасының негізгі ерекшеліктеріне, оның ішінде, ақуыз құрамына, крахмал,  $\beta$ -глюкан, сығындалғыштығы, дән натурасы және оның қаттылығы бойынша талданды. Алынған нәтижелер негізінде сыра қайнату өнеркәсібі үшін оңтайлы көрсеткіштерге ие үлгілер тізімі, сонымен қатар жем өндірісі үшін де тізбе құрылды. Толық геномдық талдау 7 сандық белгілер локусына (СБЛ) топтастырылған 20 маркер-белгі ассоциацияларын анықтады. Үш СБЛ дәннің құрамындағы ақуызға, крахмалға,  $\beta$ -глюканға және сығындыға плеотропты әсерін көрсетті, екі СБЛ ақуыз, крахмал мен сығынды үшін анықталды. Дәннің қаттылығы мен натурасы үшін, әрқайсысына 1 СБЛ анықталды. Табылған плейотропты СБЛ арпаның белгілі гүлдеу гендеріне жақын орналасты. Осы жұмыс барысында табылған барлық СБЛ, сондай-ақ перспективалы арпа үлгілері Қызылорда облысының стресстік жағдайында арпа селекциясы процесіне интеграциялануы мүмкін.

**Түйін сөздер:** *Hordeum vulgare* L., сыра қайнату, жем өндірісі, генотип  $\times$  орта, молекулалық маркерлер, бір нуклеотидтік полиморфизм.

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### Полногеномный анализ ассоциаций признаков качества зерна коллекции ярового ячменя, выращенного в Кызылординской области

Ячмень – это культура, которая в сравнении с другими культурными растениями обладает лучшей устойчивостью к неблагоприятным условиям среды. Тем не менее, высокая температура, засуха и засоленная почва не позволяют формироваться высококачественному зерну, особенно для пивоваренной промышленности. В данной работе образцы ярового ячменя были выращены в условиях высокой засоленности Кызылординской области и проанализированы по основным признакам качества зерна, в т. ч. содержание белка, крахмала, β-глюкана, экстрактивности, натуре зерна и его твердости. На основе полученных результатов был сформирован список образцов с оптимальными показателями для пивоваренной промышленности, а также список для кормопроизводства. Полногеномный анализ выявил 20 ассоциаций маркер-признак сгруппированных в 7 локусов количественных признаков (ЛКП). Три ЛКП показали плеiotропный эффект в отношении содержания в зерне белка, крахмала, β-глюкана и экстрактивности, два ЛКП были обнаружены для содержания белка, крахмала и экстрактивности. Для твердозерности и натуре зерна было идентифицировано по 1 ЛКП. Найденные плеiotропные ЛКП находились в непосредственной близости к известным генам цветения ячменя. Все ЛКП, обнаруженные в ходе данной работы, а также перспективные образцы ячменя могут быть потенциально интегрированы в процесс селекции ячменя в стрессовых условиях Кызылординской области.

**Ключевые слова:** *Hordeum vulgare* L., пивоварение, кормопроизводство, генотип × среда, молекулярные маркеры, однонуклеотидный полиморфизм.

#### Abbreviations

GPC – grain protein content, GBGC – grain β-glucan content, GSC – grain starch content, GPL – grain plumpness, GH – grain hardness index, GEX – grain extractivity.

#### Introduction

Barley (*Hordeum vulgare* L.) is one of the widely produced cereal crops in the world (156.41 million metric tons in 2019 / 2020) [1]. In Kazakhstan, it is the third mostly-cultivated cereal after wheat and rice (18.25 % in 2020) [2]. Barley production in Kazakhstan has three basic directions: for the livestock feeding (43.3 % from total barley production), for the food industry (36.5 %), and for grain depositary or export (20.1 %) [2]. Barley plays an important role in Kazakhstan's economics. The country was the 7<sup>th</sup> largest barley exporter in 2019 / 2020 [1].

In Kazakhstan, barley is annually cultivated in all grain sowing regions. There is a list of highly productive cultivars developed for each particular region and approved by the State register of breeding achievements of the Republic of Kazakhstan [3]. Many regional breeding programs are focused on the development of highly productive cultivars with good grain quality. Although barley grain quality indicators may vary depending on the direction

of use, the content of protein, starch, and extractivity are very important. For example, barley grain used for malting require low protein content (less than 12 % according to the GOST 5060-86 [4]) and high extractivity, while grain for livestock feeding, on the contrary, have to be enriched with protein (more than 13 % according to the GOST 53900-2010 [5]). Most barley quality traits are complex quantitative traits with polygenic control [6]. In addition, there is a great influence of both genotype and environment on barley grain quality, as well as productivity. In this regard, the agricultural industry needs a large assortment of modern barley cultivars oriented on different usage directions for all grain-sowing regions of Kazakhstan.

Akmola and North Kazakhstan regions traditionally are leaders in spring barley production in Kazakhstan [2]. Together these two regions produced 46.6 % of the total barley yield in 2020 [2]. It is explained by high fertility of the soil and optimal climatic conditions in the northern Kazakhstan. At the same time, high ecological and genetic plasticity of barley allows developing cultivars adapted to drought and hot climate of the southern Kazakhstan too. One of the southern regions – Kyzylorda region – produced less than 1 % of the total spring barley in the country in 2020 [2]. The climate of Kyzylorda region is sharply continental; summers are hot and dry; winters are cold with unstable snow cover.

The average annual air temperature is 9.8 ° C. The soil in the region is meadow-boggy, typical for rice growing (the region is a leader in rice production in Kazakhstan), with less than 1 % humus content and chloride and sulfate salinity [7]. High soil salinity in combination with arid land results in poor protein synthesis and inhibited growth processes [8]. Thus, arid climate and barren soil have a great negative influence on both productivity and grain quality of barley in the region.

Previously, due to the collaborative projects between Institute of Plant Biology and Biotechnology and breeding organizations from the Ministry of Agriculture, barley accessions from Kazakhstan were studied for morphological traits and yield components in 7 different regions of Kazakhstan [9]. It was shown that some cultivars initially developed for the northern regions, for example, for the Kostanay region, in Kyzylorda region had a higher yield than the cultivars traditionally cultivated here [10]. Part of those local lines and cultivars were additionally studied in Kazakh Research Institute of Rice-growing (KRIRG) for morphological and productivity parameters in order to identify cultivars resistant to abiotic stresses [7], and for protein and starch content [8]. Promising lines from the world barley collection were also selected and recommended for the cultivation in Kazakhstan's Aral region, including Kyzylorda region [11]. Due to a collaborative study between IPBB and KRIRG (2009-2014) a new cultivar Shakhristan was developed and approved for cultivation in 2017 [3]. Nowadays, several barley cultivars with the yield of 2.5-3.5 t/ha, such as Rosava winter, Baishek, Saule, Zhuldyz, Asem, Arna, Shakhristan, and Syr Aruy, are officially approved for mass cultivation in Kyzylorda region [3, 12]. Still, it is possible to increase the yield and grain quality of spring barley in the harsh environment of Kyzylorda region by introducing new early ripening genotypes with good grain quality potential.

Modern crop breeding is an effective combination of traditional breeding and genomic technologies, such as marker-assisted selection (MAS) and genetic modifications (GMO) [13]. One of these technologies, called genome-wide association study (GWAS), has been successfully applied to identify the causative loci used in the breeding of crops for adaptation, productivity, and quality improvement by molecular breeding gene modifications [14-16]. This method is based on the searching of associations between genotypic and phenotypic variability resulting in the identification of molecular markers for the trait(s) [17]. Previously, in Kazakhstan, this approach was effectively used for a wide range of

crop studies: productivity of barley [19-20], wheat [20], and soybean [21], as well as diseases resistance in wheat [22], barley [23], and soybean [24]. Barley collection used in this study was already involved in GWAS analysis for productivity and adaptation [19-20]. However, this study is the first attempt to identify loci in barley genome associated with barley grain quality traits.

## Materials and methods

**Barley collection used for the study.** The barley collection used in this study was composed of two-rowed and six-rowed spring barley cultivars and breeding lines from the USA and Kazakhstan. USA's part of the collection included 557 accessions. Kazakhstan's part included 103 accessions originated from 6 agricultural institutions: 17 accessions from Aktobe Agricultural Experimental Station (AES) (Aktobe region), 15 accessions from Karabalyk AES (Kostanay region), 20 accessions from Karaganda AES (Karaganda region), 16 accessions from Kazakh Research Institute of Agriculture and Plant Industry (Almaty region), 20 accessions from Kazakh Research Institute of Rice-growing (Kyzylorda region), and 15 accessions from Krasnovodopad AES (Turkestan region) [9]. In total, 660 accessions were used for genotyping and phenotyping.

**Phenotyping and genotyping of barley collection.** Cultivation of 660 barley accessions was performed in the field of Kazakh Research Institute of Rice-growing (Kyzylorda region) [9]. Each line was grown in tripled one meter plots at each site. Grains of each accession were analyzed on the content of raw protein (GPC, %),  $\beta$ -glucan (GBGC, %), and starch (GSC, %), plumpness (GPL, g/L), grain hardness (GH, SKCS units), and extractivity (GEX, %) in the laboratory of grain quality (Kazakh Research Institute of Agriculture and Plant Industry). The GPC was determined by near-infrared spectroscopy using calibration equations based on Kjeldahl method [25] using FOSS 1241 and FOSS 2500. The GBGC was determined by the spectrofluorimetric method [26]. The GSC was analyzed using polarimetric method [27]. Also the collection was tested for indicators of plumpness, grain hardness index, and extractivity. GPL levels were determined using methods described in GOST 1084064 [28]; the hardness index (HI) measured by Single Kernel Characterization System 4100 (SKCS 4100; Perten Instruments, Huddinge, Sweden) according to the manufacturer's manual: GEX was calculated using method described in GOST 12136-77 [29]. The

correlations between quality traits and productivity components, such as thousand kernels weight (TKW, g) and yield per m<sup>2</sup> (YM2, g/m<sup>2</sup>) were also analyzed.

Accessions from Kazakhstan were genotyped using the 9K SNP chip by GoldenGate Illumina at the TraitGenetics company (TraitGenetics GmbH, Gatersleben, Germany). The SNP genotyping data of BOPA1 and BOPA2 (Barley Oligo Pool Assay) sets from Illumina assays [30] for USA's accessions were obtained from Triticeae toolbox ([www.triticeaetoolbox.org](http://www.triticeaetoolbox.org)). In total, 2344 polymorphic SNPs with MAF (minor allele frequency) > 0.05 and with SNP's call rate > 0.90 were selected for GWAS analysis.

**GWAS analysis and statistics.** The genetic structure of 660 studied barley accessions was determined in order to obtain Q-matrix for GWAS analysis. Based on the  $\Delta K$  method [31], the K was set at 3. The Kinship matrix (K-matrix) was obtained using TASSEL 5 software [32]. The Mixed Linear Model (MLM) with K- and Q-matrices was

used for GWAS analysis. The significant associations were selected after the application of a threshold at  $P < 1E-3$ .

Pearson correlation and PCA analysis were performed using R statistical platform [33]. A genetic map with significant SNPs was constructed based on physical positions in barley genome [34] using MapChart 2.32 software [35].

## Results and Discussion

**Grain quality and productivity of barley grown in Kyzylorda.** Analysis of basic barley grain quality traits had showed a wide range of GH and GPL, while GPC, GSC, and GBGC had demonstrated a moderate level of variability (Table 1). Barley productivity measured as yield per m<sup>2</sup> varied greatly from 63.0 g/m<sup>2</sup> (USA's line FEG148-16) and up to 1336.0 g/m<sup>2</sup> (USA's line FEG142-16). Among Kazakhstan's accessions, the best yield was observed for the cultivar Nurinskiy 1 (655.0 g/m<sup>2</sup>) originated from Karaganda AES.

**Table 1** – Grain quality traits of USA's and Kazakhstan's barley grown in Kyzylorda 2010

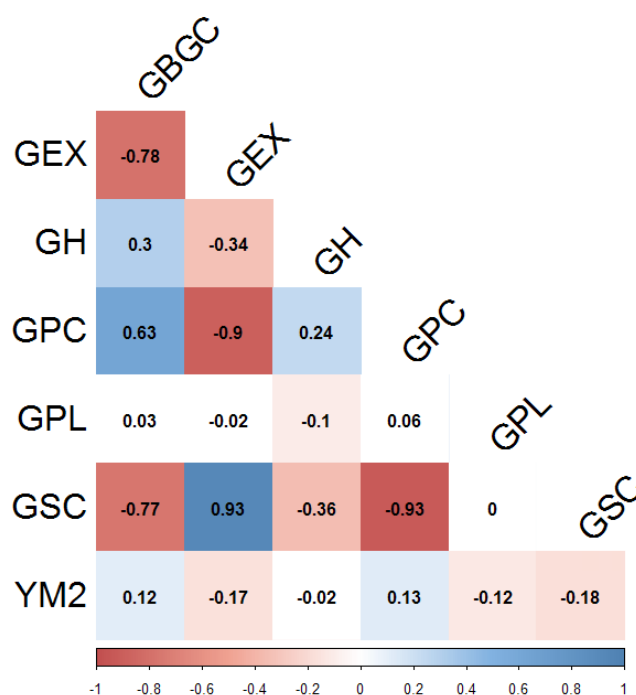
| Quality trait                        | Minimum | Maximum | Mean $\pm$ SD     |
|--------------------------------------|---------|---------|-------------------|
| Grain protein content (GPC)          | 10.2    | 15.5    | 12.6 $\pm$ 0.9    |
| Grain starch content (GSC)           | 50.6    | 57.0    | 54.0 $\pm$ 1.1    |
| Grain $\beta$ -Glucan content (GBGC) | 2.9     | 4.9     | 4.1 $\pm$ 0.2     |
| Grain hardness (GH)                  | 67.0    | 132.0   | 92.8 $\pm$ 8.6    |
| Grain plumpness (GPL)                | 300.0   | 704.0   | 585.4 $\pm$ 38.8  |
| Grain extractivity (GEX)             | 70.2    | 76.2    | 73.4 $\pm$ 1.0    |
| Yield per m <sup>2</sup> (YM2)       | 63.0    | 1336.0  | 599.5 $\pm$ 234.7 |
| Notes: SD – standard deviation.      |         |         |                   |

The correlational analysis had demonstrated strong positive relations between GEX and GSC. The concentration of  $\beta$ -Glucan was moderately positively associated with GPC and weakly associated with GH. GH was also positively associated with GPC. GSC and GEX were negatively correlated with GBGC, GH, and GPC. As for the GPL, a weak negative correlation was observed for GH only. Barley productivity (YM2) had positive associations with GBGC and GPC and negative correlations with GEX, GSC, and GPL.

**Promising barley accessions for Kyzylorda region.** The lists of promising lines and cultivars for

livestock feeding (Table 2) and for malting (Table 3) were made using the indicators of grain quality and productivity in Kyzylorda region 2010.

The list of accessions that had demonstrated promising levels of grain quality and yield for livestock feeding included 6 cultivars and breeding lines from Kazakhstan and 6 breeding lines from the USA (Table 2). All selected accessions had showed a high GPC level and good productivity; however, GPL close to the GOST was observed for USA's accessions only. Accessions from Kazakhstan exceeded GOST's requirement for GPL by 10% on average.



**Figure 1** – Pearson correlation among grain quality traits and productivity.

Red color denotes negative correlation, blue color – positive.

Color intensity increases with the increasing of significance ( $P < 0.05$ ).

**Table 2** – Promising accessions for livestock feeding according to grain quality traits and productivity of barley in Kyzylorda region

| #  | Barley accessions  | GPC (%)        | GPL (g/L)  | YM2 (g/m <sup>2</sup> ) |
|----|--|----------------|------------|-------------------------|
|    | <b>Livestock feeding (GOST R 53900-2010, first class)</b>    | <b>&gt; 13</b> | <b>590</b> | <b>-</b>                |
| 1  | Accession-1 (USA)  | 15.1           | 592        | 546                     |
| 2  | Accession-2 (USA)  | 15.0           | 598        | 314                     |
| 3  | Accession-3 (KAZ, Kazakh Research Institute of Rice-growing) | 15.4           | 661        | 566                     |
| 4  | Accession-4 (USA)  | 14.5           | 597        | 770                     |
| 5  | Accession-5 (KAZ, Kazakh Research Institute of Rice-growing) | 14.5           | 669        | 555                     |
| 6  | Accession-6 (KAZ, Krasnovodopad AES)                         | 14.5           | 665        | 449                     |
| 7  | Accession-7 (USA)  | 14.5           | 592        | 392                     |
| 8  | Accession-8 (USA)  | 15.5           | 512        | 528                     |
| 9  | Accession-9 (USA)  | 15.5           | 590        | 369                     |
| 10 | Accession-10 (KAZ, Aktobe AES)                               | 14.6           | 658        | 396                     |
| 11 | Accession-11 (KAZ, Krasnovodopad AES)                        | 14.8           | 627        | 418                     |
| 12 | Accession-12 (KAZ, Krasnovodopad AES)                        | 14.8           | 655        | 347                     |

The list of barley accessions that are promising for malting according to their grain quality and yield performance included 13 breeding lines from the USA only (Table 3). All of them had protein concentration less than 12% as required by GOST, but their average GEX was 3.3 % lower than the recommended level.

Results of Kazakhstan's and USA's barley phenotyping in Kyzylorda region had demonstrated a wide range for studied grain quality traits. One of the crucial barley quality traits for both malting and livestock feeding is protein. Generally, barley lines from the USA used in this study expectedly show lower GPC (Table 3), since the majority of them are

malting-oriented. As for Kazakhstan's accessions, promising lines were observed only for livestock feeding with relatively high GPC (Table 2). It is explained by the specialization of these accessions for the livestock. At the same time, the combination of accessions from Kazakhstan and the USA provided a good range for the GWAS.

For malting and brewing, low GPC has to be combined with low GBGC [36] and high level of GSC providing high GEX. Accessions grown in Kyzylorda region had demonstrated a strong positive correlation between GPC and GBGC, as well as the strong negative correlation between GPC and GSC / GEX (Figure 1). GH is another important trait for malting quality. Usually, GH declines with the increasing GSC and decreasing GPC [37]. Usually, the good grain quality of barley is associated with low productivity and low grain yield [38]. The same correlation was observed in the current study,

where high YM2 was associated with increased GPC, while high levels of GSC / GEX were negative correlated with the yield (Figure 1). Still, barley cultivars with high GPC and YM2 are a great raw material for livestock feeding [39]. GPL levels had showed absence of correlation with other quality traits except for weak negative correlation with GH (Figure 1), which confirms the small impact of GPL on other grain and malting qualities [40]. Generally, 6 grain quality traits of barley grown in Kyzylorda region had demonstrated good variability for GWAS analysis. In the studied collection, the majority of accessions from the USA had good grain quality levels for malting and brewing, while accessions from Kazakhstan fitted requirements for livestock feeding. The list of USA's accessions from table 3 is good candidates for the introduction into potential malting barley breeding in Kyzylorda region.

**Table 3** – Promising accessions for malting according to grain quality traits and productivity of barley in Kyzylorda region

| #  | Barley accessions                        | GPC (%)        | GEX (%)        | YM2 (g/m <sup>2</sup> ) |
|----|--|----------------|----------------|-------------------------|
|    | <b>Barley for malting (GOST 5060-86)</b> | <b>&lt; 12</b> | <b>&gt; 78</b> | <b>-</b>                |
| 1  | Accession-13 (USA)                       | 10.9           | 75.8           | 493                     |
| 2  | Accession-14 (USA)                       | 10.9           | 73.8           | 439                     |
| 3  | Accession-15 (USA)                       | 10.8           | 75.6           | 669                     |
| 4  | Accession-16 (USA)                       | 10.8           | 75.4           | 420                     |
| 5  | Accession-17 (USA)                       | 10.7           | 75.9           | 383                     |
| 6  | Accession-18 (USA)                       | 10.7           | 75.1           | 490                     |
| 7  | Accession-19 (USA)                       | 10.7           | 75.5           | 758                     |
| 8  | Accession-20 (USA)                       | 10.6           | 75.5           | 840                     |
| 9  | Accession-21 (USA)                       | 10.2           | 76.2           | 421                     |
| 10 | Accession-22 (USA)                       | 11.0           | 75.7           | 342                     |
| 11 | Accession-23 (USA)                       | 11.0           | 75.5           | 408                     |
| 12 | Accession-24 (USA)                       | 11.0           | 75.0           | 515                     |
| 13 | Accession-25 (USA)                       | 11.0           | 75.4           | 482                     |

**Marker-trait associations (MTAs) identified for barley grain quality traits.** In total, 20 MTAs for studied grain quality traits were identified on 6 out of 7 barley chromosomes (Table 4). Their positions on the barley physical map are demonstrated in Figure 2.

Seven SNPs associated with the different number of traits were positioned on all barley chromosomes, except chromosome 4H. Chromosome 6H contained 2 trait-associated SNPs. Several markers had demonstrated multiple associations. Markers

11\_20971 (1H), 11\_21505 (3H), and 12\_31509 (6H) were associated with 4 quality traits: GPC, GSC, GBGC, and GEX. Markers 11\_21414 (2H) and 11\_21103 (7H) were associated with 3 traits: GPC, GSC, and GEX. Marker 12\_10408 on chromosome 5H was associated with GPL only, and marker 12\_10199 on chromosome 6H only with GH. All 20 MTAs had demonstrated significant associations at  $P < 0.001$  (Table 4). The total phenotypic variation explained by MTA ( $R^2$ ) varied from 1.9 to 3.4% (Table 4).

**Table 4** – Marker-trait associations for barley grain quality traits identified in Kyzylorda in 2010

| #  | Trait | SNP      | Chr. <sup>1</sup>    | Pos. (cM) <sup>1</sup> | Pos. (bp) <sup>2</sup> | P-value  | R2 (%) | All. | Effect  |
|----|-------|----------|----------------------|------------------------|------------------------|----------|--------|------|---------|
| 1  | GEX   | 11_20971 | U (1H <sup>2</sup> ) | U                      | 496,660,040            | 1.04E-04 | 2.9    | A    | -1.035  |
| 2  | GEX   | 11_21414 | 2H                   | 158.15                 | 761,624,420            | 4.73E-04 | 2.3    | A    | -0.733  |
| 3  | GEX   | 11_21505 | 3H                   | 87.24                  | 580,635,994            | 2.17E-04 | 2.3    | A    | -0.869  |
| 4  | GEX   | 12_31509 | 6H                   | 55                     | 203,509,034            | 1.29E-05 | 3.2    | A    | 1.021   |
| 5  | GEX   | 11_21103 | U (7H <sup>2</sup> ) | U                      | 582,767,743            | 7.59E-04 | 1.9    | A    | -0.718  |
| 6  | GBGC  | 11_20971 | U (1H <sup>2</sup> ) | U                      | 496,660,040            | 4.17E-04 | 3.0    | A    | 0.202   |
| 7  | GBGC  | 11_21505 | 3H                   | 87.24                  | 580,635,994            | 5.48E-04 | 2.3    | A    | 0.168   |
| 8  | GBGC  | 12_31509 | 6H                   | 55                     | 203,509,034            | 1.44E-04 | 2.8    | A    | -0.184  |
| 9  | GH    | 12_10199 | 6H                   | 45.44                  | 66,485,252             | 1.28E-04 | 2.5    | A    | 8.655   |
| 10 | GPC   | 11_20971 | U (1H <sup>2</sup> ) | U                      | 496,660,040            | 1.29E-04 | 2.8    | A    | 0.932   |
| 11 | GPC   | 11_21414 | 2H                   | 158.15                 | 761,624,420            | 2.62E-04 | 2.4    | A    | 0.685   |
| 12 | GPC   | 11_21505 | 3H                   | 87.24                  | 580,635,994            | 3.94E-04 | 2.1    | A    | 0.764   |
| 13 | GPC   | 12_31509 | 6H                   | 55                     | 203,509,034            | 2.10E-05 | 3.1    | A    | -0.915  |
| 14 | GPC   | 11_21103 | U (7H <sup>2</sup> ) | U                      | 582,767,743            | 4.78E-04 | 2.1    | A    | 0.677   |
| 15 | GSC   | 11_20971 | U (1H <sup>2</sup> ) | U                      | 496,660,040            | 4.20E-05 | 3.2    | A    | -1.208  |
| 16 | GSC   | 11_21414 | 2H                   | 158.15                 | 761,624,420            | 3.24E-04 | 2.4    | A    | -0.822  |
| 17 | GSC   | 11_21505 | 3H                   | 87.24                  | 580,635,994            | 1.21E-04 | 2.5    | A    | -1.002  |
| 18 | GSC   | 12_31509 | 6H                   | 55                     | 203,509,034            | 7.34E-06 | 3.4    | A    | 1.165   |
| 19 | GSC   | 11_21103 | U (7H <sup>2</sup> ) | U                      | 582,767,743            | 3.32E-04 | 2.2    | A    | -0.843  |
| 20 | GPL   | 12_10408 | 5H                   | 102.06                 | 547,771,176            | 4.74E-05 | 3.0    | A    | -51.508 |

Notes: <sup>1</sup> – Position according to 9K GoldenGate Illumina; <sup>2</sup> – Physical position according to Morex 2016 map. Chr. – chromosome; Pos. – position; R2 – phenotypic variation explained by MTA; All. – effective allele.

Twenty MTAs identified in this study were combined into 7 QTLs (Figure 2). Five out of those 7 QTLs had demonstrated pleiotropic effect since they were associated with several traits. It may indicate the presence of strong pleiotropic genes in these loci or in close regions. Grain quality traits are highly influenced by major heading and flowering genes. For example, SNP 11\_20971 (GPS, GSC, GBGC, GEX) on the chromosome 1H was previously identified as associated with adaptability traits (time of heading and grain maturity, plant height) and with thousand kernels weight [18], which is also quality trait. Heading time genes *HvCMF6a* [41], *HvCMF6b* [41] and *Esp1L / HvELF3 / eam8* [42] are located in the region close to SNP 11\_20971 and may be linked. The SNP 11\_21414 (GPS, GSC, GEX) on the chromosome 2H was also previously mentioned as associated with the time of heading and grain maturity, plant height, and peduncle length [18]. Heading time gene *HvAP2* located on 126.7 cM [43] may be linked with this SNP; however, the distance between them is relatively large (about 31 cM) [44]. The SNP 11\_21505 (GPS, GSC, GBGC, GEX) on

the chromosome 3H was also earlier described as highly pleiotropic associated with both yield and adaptability traits [18]. This SNP is probably linked with the heading time gene *HvCMF1* [41]. The next pleiotropic SNP is 12\_31509 (GPS, GSC, GBGC, GEX) on the chromosome 6H reported as associated with yield and adaptability traits [18] is more likely linked with the group of heading and flowering genes densely located in this region of the chromosome [44]. The last pleiotropic SNP 11\_21103 (GPS, GSC, GEX) on the chromosome 7H previously associated with yield and adaptability traits [18] may be connected with flowering gene *HvCO6* [44] located in this chromosome region. Thus, SNPs with the pleiotropic effects described above and associated with highly correlated grain quality traits GPS, GSC, GBGC, and GEX are most likely linked with known heading / flowering genes of barley. The mixed effect (negative and positive) on different traits may be used for more effective breeding of barley for either livestock feeding or malting. For example, SNP 12\_31509 may help to increase GSC and GEX, but decrease GPC and GBGC, which is good for malting barley.







The last two QTLs were single-trait associated (Table 4). The only SNP for GPL was 12\_10408 on chromosome 5H (Figure 2). The flowering gene *HvPRR95* is located in 5cM apart from this SNP [41, 44], and they may be linked. The SNP 12\_10199 on chromosome 6H was the only one identified for GH in this study (Table 4). There is no information about barley quality-related genes or QTLs located in this region in the literature.

Results obtained in this study give a basis for the development of new barley cultivars, as well as for the integration of germplasm with good grain quality from the USA. A number of promising USA accessions may be used in crosses with local standard cultivars. MTAs for the studied grain quality traits can be used in MAS for the identification of promising genotypes.

## Conclusion

Grain quality traits studied in this work had demonstrated a wide ranges of variability. Acces-

sions from the USA showed good level of quality for malting, while accessions from Kazakhstan had quality levels better for livestock feeding. Two lists of promising barley accessions for malting (13 accessions) and for livestock feed (12 accessions) in Kyzylorda region were formed. In the course of GWAS analysis, 20 MTAs combined into 7 QTLs for grain quality traits were identified. Three QTLs demonstrated pleiotropic effect affecting grain protein,  $\beta$ -glucan and starch content and extractivity, two QTLs were identified for grain protein and starch content and extractivity, one QTL was for grain hardness and one QTL for plumpness.

## Funding

This 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 Republic of Kazakhstan.

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