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Original article

GENOME-WIDE ASSOCIATION STUDY FOR GRAIN QUALITY TRAITS IN SPRING BARLEY COLLECTION GROWN IN NORTH KAZAKHSTAN

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ABSTRACT

Barley is a cereal crop that is grown all over the world. Its grain is used for animal feed, malting, brewing, and food. The quality of barley grain is important, particularly raw starch and protein contents, and it depends on the end-use product. This study looked at a collection of 356 barley accessions from the USA and Kazakhstan grown under conditions of northern Kazakhstan (Karabalyk agricultural experimental station) and genotyped with 1631 polymorphic SNPs markers. The collection was studied for starch (GSC), protein (GPC), cellulose (GCC), and lipids contents (GLC), and for grain test weight (TWL) during two years. Phenotypic analysis demonstrated impact of the year on studied traits and significant associations between grain quality and the yield ($P < 0.01$). Population structure analysis revealed three subclusters in the studied barley collection with the dominance of the USA's barley in two of them. As a result of GWAS, 22 significant QTLs ($P < 0.001$) were identified for the studied grain quality traits including 19 single-trait QTLs, 2 double-trait QTLs, and a one triple-trait QTL. For 16 QTLs, reference quality genes and/or QTLs were found, while the remaining 6 QTLs were presumably novel genetic factors for grain quality traits. As result, these 22 QTLs are expected to be useful for future breeding projects targeting the selection of high grain quality barley cultivars.

Key words: *Hordeum vulgare* L., starch, protein, cellulose, lipids, grain test weight, marker-assisted selection.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a major cereal crop that is fourth most cultivated in the world after corn, wheat, and rice [1]. It is the second most cultivated cereal crop in Kazakhstan after wheat. Barley is a versatile crop that can be used for a variety of purposes [2]. It is an important source of feed for animals (about 70 % of the total barley production), and 20 – 25 % of barley grain is used to make beer, whiskey, and other alcoholic beverages [3]. Barley is also used in the production of bread, pasta, and other foods (5 – 10 %) [4]. However, the quality of the grain, including chemical composition and physical properties, is important for all of these purposes.

The main component of barley grain is carbohydrates. Carbohydrates make up 78 – 84 % of the grain [5]. Starch is the most abundant carbohydrate in barley grain (52 – 72 %), followed by β-glucans (4 – 6 %), pentosans (4 – 8 %), and cellulose (1.5 – 5 %) [5]. In addition to carbohydrates, barley grain also contains proteins, lipids, minerals, vitamins, dietary fibers, and antioxidants [6, 7]. The exact chemical composition of barley grain varies depending on its intended use. For example, barley grain used for malting should have a protein content of 9.5 % to 12.5 % and a starch content of greater than 60 % [8]. Barley grain used for feed or food products, on the other hand, typically has higher protein and lower starch contents [4].

Our study is focused on four important biochemical traits and one physical trait of barley grain: contents of raw starch (GSC, %), raw protein (GPC, %), cellulose (GCC, %), and lipids (GLC, %), and grain test weight per liter (TWL, g/L). The traits of barley grain quality are complex and are controlled by multiple genetic factors. For example, the synthesis of starch is mediated by multiple enzymes, including starch synthases, starch-branching enzymes, and debranching enzyme isoamylase [9, 10]. Barley grain proteins are also complex. About 30-50% of them are hordeins belonging to the prolamin group [11, 12]. Biosynthesis of hordeins is controlled by many genes, but the major ones are *Hor1* (chromosome 1H), *Hor2* (chromosome 1H), and *Hor5* (chromosome 1H) [13]. The remaining proteins in barley grain are albumins, globulins, and glutelin [12]. There are two important genes controlling protein content in barley grain — *HvNAM-1* (chromosome 6H) and *HvNAM-2* (chromosome 2H) [14, 15]. Both of them are homologs of the well-studied wheat gene *NAM-B1* [16]. This gene is a transcription factor of the *NAC* family that is responsible for accelerating senescence and increasing nutrient remobilization from leaves to grains in wheat [16]. The loss of functionality of the *HvNAM-1* in barley is associated with lower GPC [14]. Although *HvNAM-1* and *HvNAM-2* are genes that have been shown to greatly affect GPC in barley grain, they are not very variable [17]. This suggests that other genes and/or loci are likely responsible for the majority of the variation in GPC in barley grain. As for the lipids, barley grain contains linoleic acid (50.7 – 57.9 % of all lipids) followed by smaller proportions of palmitic (18.3 – 27.0 %), oleic (12.2 – 21.2 %), and linolenic (4.3 – 7.1 %) acids [18]. Genetic control of GLC in barley is not clear, but there are some genes controlling this trait. One of them is the *WIN1/SHN1* (chromosome 6H) gene playing an important role in the regulation of lipid biosynthesis pathways [19]. The other one is the *Nud* gene (chromosome 7H, hulled/hulless grain) probably regulating the lipids composition in pericarp epidermis [20]. Synthesis of cellulose in plants is regulated by a large cellulose synthase (*CesA*) gene superfamily [21]. Thus, the biosynthesis of starch, protein, lipids, and cellulose in barley is a complex process that is controlled by many genes, quantitative trait

loci (QTLs), and transcription factors. Although each QTL may only have a small effect on the manifestation of a trait, the plant genome may contain dozens of QTLs that are associated with a particular trait. This means that the total contribution of all of these QTLs can be significant and their joint role may affect the trait greatly.

There are two main ways to identify QTLs in plants: interval mapping (IM) and genome-wide association studies (GWAS) [22]. IM uses a population of lines that have been generated by crossing two parent lines. By looking at how markers and trait alleles segregate together in this population, researchers can identify linked markers that are likely to be associated with the trait of interest [23]. IM has been used to identify QTLs for several barley grain quality traits, such as protein content [24, 25], starch content [26], acid detergent fiber content, [26] and grain plumpness and test weight [27]. However, the efficiency of IM is limited by the genetic diversity of the parents used to develop the mapping population and by the small number of recombination events that occur per chromosome per generation [28]. In contrast, GWAS take advantage of larger genetic diversity and many recombination events in natural populations [29]. GWAS also considers haplotype segregation and linkage disequilibrium (LD) to identify markers associated with the trait of interest [29]. This method is now routinely applied for mapping QTLs of barley yield components [30, 31], resistance to biotic and abiotic stress factors [32, 33, 34], and grain quality traits [35]. Thus, GWAS can be applied in a large population to identify markers associated with the trait of interest and provide insights into that trait's genetic architecture.

In this study, we studied a collection of 406 spring barley accessions for two years under conditions of northern Kazakhstan — major barley-growing region in the country. Previously, in Kazakhstan, several GWAS studies in barley collections were performed for the identification of QTLs associated with yield-related traits, stem rust and powdery mildew resistance, and some grain quality traits. Thus, the main purpose of our study was to identify new QTLs associated with important grain quality traits using GWAS.

MATERIALS AND METHODS

Barley collection and its genotyping

A collection of 356 spring two-row barley accessions included cultivars and lines from the USA (n = 267) and Kazakhstan (n = 89). The American part of the collection was obtained from the US Barley Coordinated Agricultural Project (CAP) [36] and has been previously used in the various GWAS works [31, 33, 37]. The Kazakhstan's part of the collection included cultivars and promising lines from 6 breeding institutions [31]. Both parts of the collection have been described earlier [31]. The accessions from Kazakhstan were genotyped using the Illumina GoldenGate 9K SNP chip at the TraitGenetics Company (TraitGenetics GmbH, Gatersleben, Germany) [31]. Dr. T. Blake provided genotyping data and seed material of the US accessions. The SNP genotyping data for barley accessions from Kazakhstan and the USA were compared and merged into one file. The file was filtered by the minor allele frequency (MAF) and SNP call rate: SNPs with MAF < 0.05 and accessions with missing data > 0.1 were removed from the experiment. In total, 1631 polymorphic SNPs and 356 barley accessions met all criteria and were selected for further analysis. The genetic positions of SNP according to the Illumina iSelect2013 (cM) and physical positions according to the Barley 50k iSelect SNP Array (bp) were obtained from the Triticeae toolbox [38].

Field experiment, assessment of grain quality traits, and statistics

The collection was grown in the field of Karabalyk Agricultural Experimental Station (KAES, Kostanai region, northern Kazakhstan, 53°51'07»N 62°06'12»E) in 2020 and 2021. Each accession was grown in 1 m^2 individual plots in a rainfed field with 15 cm spaces between neighboring plots. Two replications were evaluated per year in a nearest neighbor randomized complete block design (nn-RCBD) with randomly assigned barley accessions. The field experiment design was standardized for both years of the experiment. The seed material of each accession was collected and sent to the laboratory of grain quality at the LLP "Kazakh Research Institute of Agriculture and Plant Growing" (Almaty region, Kazakhstan). The grains were studied for five grain quality traits: the grain contents of raw starch (GSC, %), raw protein (GPC, %), raw cellulose (GCC, %), and raw lipids (GLC, %) and the grain test weight per liter (TWL, g/L). GSC, GPC, GCC, and GLC were measured using an NIRS DS2500 Grain Analyzer (FOSS, Hillerød, Denmark) with manufacturer's calibration. TWL was determined in g/L according to the GOST 10840-2017 "Grain. Method for determination of hectolitre weight" [39]. For a better understanding of the relationships between grain quality and the yield of barley, the collection was studied for thousand kernel weight (TKW, g), and grain yield per m^2 (YM2, g/m^2) as well. Clean grains from each individual plot were weighed in g for YM2. TKW was measured as a mass of 100 random grains in g multiplied by 10. Frequency distribution histograms were constructed using ggplot2 package for R v4.2.1. Pearson correlation analysis was performed using R v4.2.1 statistical platform [40] and RStudio v2022.07.1 software [41].

Population structure, linkage disequilibrium, and the GWAS

The population structure was determined for 356 accessions using 1631 polymorphic SNPs. Principal component analysis (PCA), neighbor-joining (NJ) clustering method, and clustering with a Bayesian Markov chain Monte Carlo (MCMC) approach based on admixture and correlated allele frequency models (covariance or Q-matrix) were used for the estimation of population structure. The PCA was calculated and visualized using RStudio v2022.07.1 software. An NJ tree was generated using TASSEL v5.2.84 software [42]. MCMC clustering was performed using STRUCTURE v2.3.4 software [43] with the K-value set from 1 to 10, the burn-in period to 100,000, the number of MCMC replications after each burn to 100,000, and the iteration number to 3. The ΔK method of the STRUCTURE HARVESTER v0.6.94 web-based program [44] was used to determine the K-value. The Q-matrix was generated based on the K-value. To correct for the effects of population substructure in the GWAS, both kinship (K-matrix) and covariance (Q-matrix) were used in the mixed linear model (MLM). The GWAS was performed using the GAPIT v3 package [45] for RStudio v2022.07.1. P-value < 1E−03 was chosen as a criterion for significant associations.

RESULTS

Grain quality traits

Barley collection was assessed for 5 grain quality traits (GSC, GPC, GCC, GLC, and TWL) in the field of KAES during two seasons (2020 and 2021). Assessment results revealed differences in quality traits between two years of experiment (Figure 1).

In 2020, average GSC values in the collection were significantly higher than in the next year – 60.97 ± 1.06 % in 2020 vs 50.58 ± 1.86 % in 2021, while average GPC was, on the opposite, lower in 2020 (13.16 ± 0.40 %) than in 2021 (15.93 ± 1.03 %) (Figure 1). The differences between two years for GCC, GLC, and TWL were not that large. However, average GCC and GLC values were greater in 2020 (4.88 ± 0.39 % and 2.74 ± 0.30 % vs 4.17 ± 0.69 % and 2.03 ± 0.31 % in 2021, respectively) (Figure 1). As for TWL, in 2020, its average value was 599.58 ± 27.07 g/L, which is lower than 665.21 ± 25.17 g/L in 2021 (Figure 1). The range of values was wide for all studied traits: 57.23 % – 62.66 % of GSC in 2020 and 43.83 % – 54.84 % in 2021; 11.95 % – 14.35 % of GPC in 2020 and 10.05 % – 18.55 % in 2021; 3.24 % – 5.97 % of GCC in 2020 and 2.02 % – 7.69 % in 2021; 1.70 % – 3.80 % of GLC in 2020 and 0.60 % – 2.75 % in 2021; 479.5 g/L – 681.0 g/L of TWL in 2020 and 583.5 g/L – 734.0 g/L in 2021. For all traits, normal or close to normal distribution was observed (Figure 1).

Correlation analysis showed stable negative correlations between GSC and GPC and between GCC and TWL in both years of experiment (Figure 2).

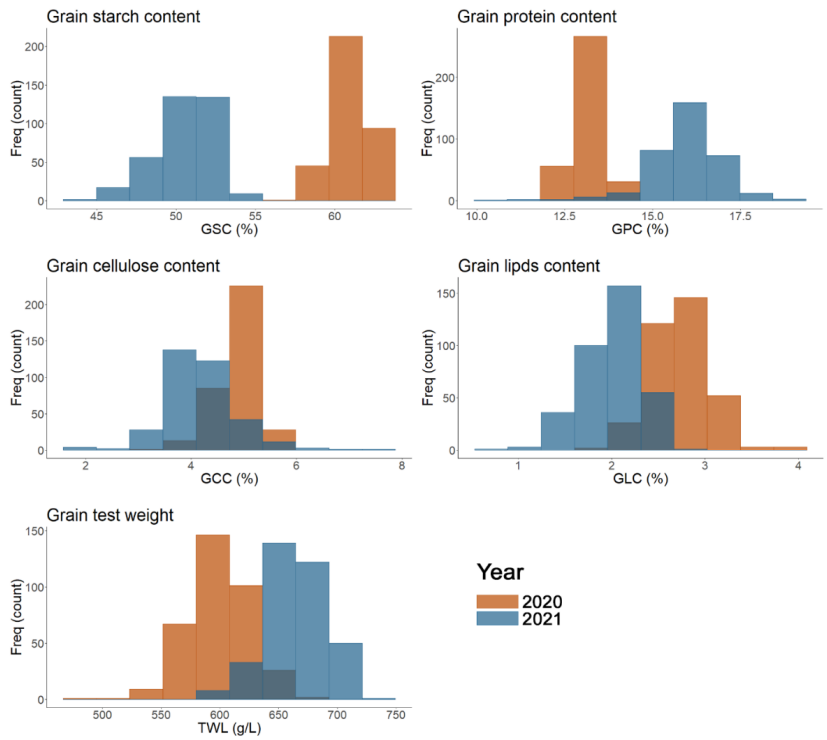

Figure 1. Distribution of barley grain quality traits in a barley collection studied for two years under conditions of KAES.

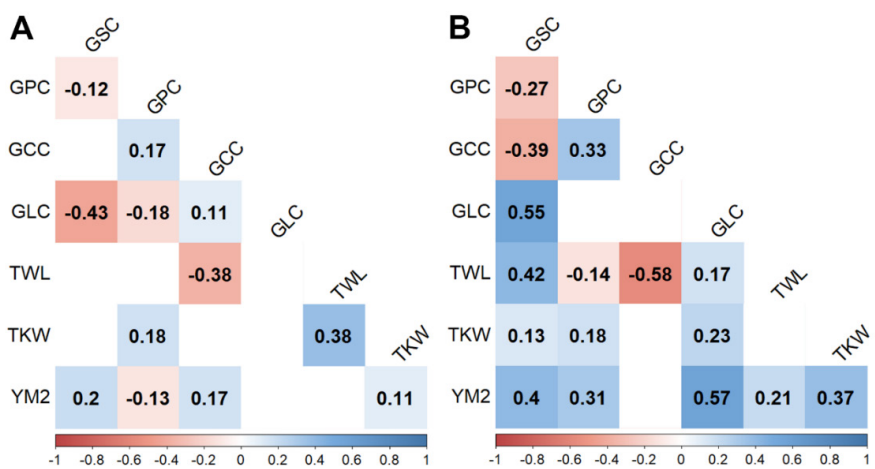

Figure 2. Pearson correlation coefficients (r) among five barley grain quality traits and two yield components in 2020 (A) and 2021 (B) in the field of KAES. Cells with P < 0.05 are highlighted in color. Red color is a negative correlation, blue color – positive. Color intensity increases with the decreasing of P-value.

Stabe positive correlation in two years was observed for pairs GPC/GCC, GPC/TKW, and GSC/YM2 (Figure 2). In 2020, positive correlation with YM2 was observed for GSC and GCC, while with GPC YM2 was correlated negatively (Figure 2A). In 2021, YM2 had demonstrated positive correlation with GSC, GPC, GLC, and TWL (Figure 2B). TKW was positively correlated with GPC and TWL in 2020 (Figure 2A) and with GSC, GPC, and GLC in 2021 (Figure 2B).

Population structure in the studied barley collection

Accessions of two origins were used in the study resulting in a strong population structure influencing GWAS. Clustering with NJ method revealed presence of three clusters in the studied barley collection (Figure 3A). The largest Cluster 1 included 189 accessions from the USA and 86 accessions from Kazakhstan. Cluster 2 included 50 accessions from the USA and only one accession from Kazakhstan. The smallest Cluster 3 included 28 accessions from the USA and two accessions from Kazakhstan.

On the PCA plot, barley accessions from Kazakhstan and accessions from the USA were subdivided into two groups by the X-axis (22.8 %) (Figure 3B). However, accessions from two origin groups were not strictly separated, but smoothly transition into each other along the X-axis. Delta-K plot demonstrated the peak of ΔK at K = 3 (Figure 3C) suggesting the presence of three clusters in the studied barley collection. STRUCURE barplot for K = 3 showed almost equal distribution of accessions among three clusters (Figure 3D). More detailed analysis of these clusters revealed dominance of the USA's accessions in the cluster K 1 (99 %) and the cluster K 3 (98 %) (Figure 3E). Cluster K 2 contained almost all accessions from Kazakhstan representing 78 % of all accessions in this cluster and the remaining 22 % were from the USA (Figure 3E). The result of STRUCTURE analysis for K = 3 were used in GWAS in the form of covariance (Q) matrix in order to prevent influence of population structure on the results.

GWAS and identification of novel QTLs

GWAS was separately performed using two-year phenotypic data (2020 and 2021) for each trait. Manhattan plots and QQ plots are presented in Figure 4.

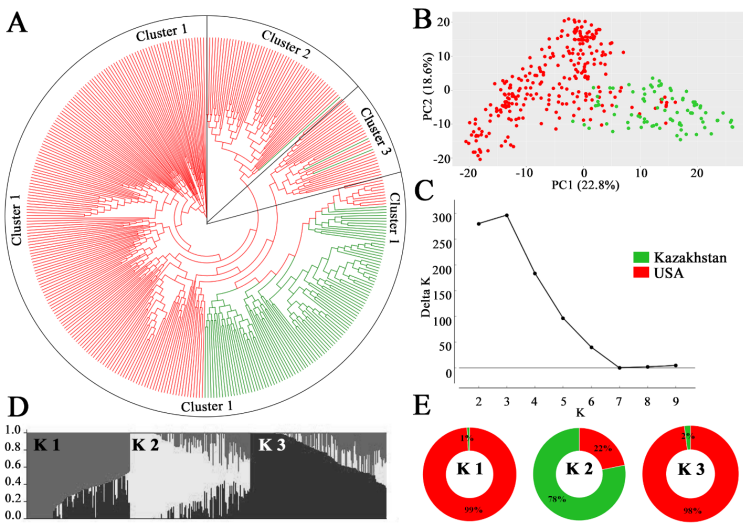

Figure 3 – Population structure: Neighbor-joining tree (A), PCA (B), delta K plot (C), barplot for K = 3 (D), and distribution of accessions from Kazakhstan and the USA by three clusters (E).

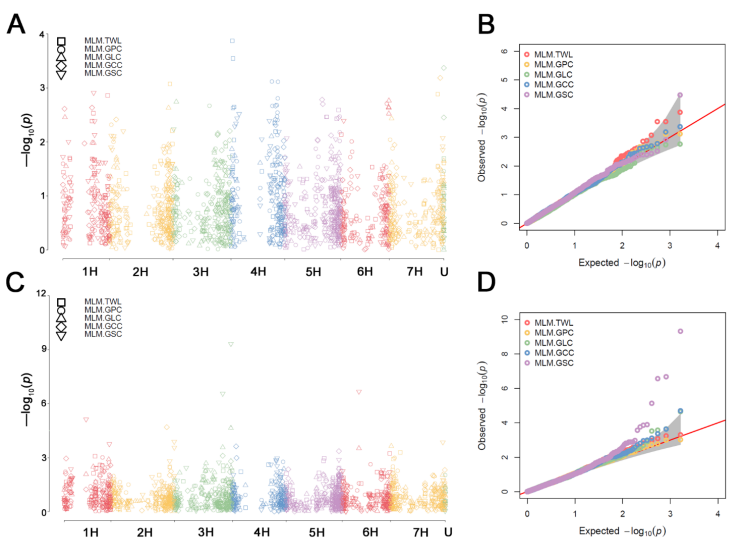

Figure 4 – Results of GWAS analysis: Manhattan plot (A) and QQ-plot (B) in 2020, Manhattan plot (C) and QQ-plot (D) in 2021.

Neighboring SNPs associated with the same trait and with R^2 values (LD) > 0.1 were merged into one QTL. In total, 29 SNPs were identified for the studied grain quality traits and 20 out of 22 QTLs and their SNPs (Table 2). For 16 QTLs, reference quality genes and/or QTLs were found (Table 2).

Table 1. Quantitative trait loci (QTLs) identified for five grain quality traits in the studied barley collection.

QTL #	Trait	Marker	Chromo-some	Position (Barley 50K, bp)	2020					2021				
					P-value	P-value (FDR)	PVE (%)	Alelle	Effect	P-value	P-value (FDR)	PVE (%)	Alelle	Effect
1	GSC	11_11336	1H	261773377	ns	ns	ns	ns	ns	7.36E-06	0.003	0	G	0.80
2	TWL	12_30350	1H	368821886	ns	ns	ns	ns	ns	8.25E-04	0.385	0.15	A	10.72
3	GPC	12_31464	1H	459030191	ns	ns	ns	ns	ns	9.57E-04	0.687	1.25	T	0.59
4	GSC	11_21068	1H	540586569	ns	ns	ns	ns	ns	1.74E-04	0.041	0.68	A	0.45
5	GCC	11_10214	2H	672009637	ns	ns	ns	ns	ns	2.00E-05	0.033	38.73	A	0.36
6	GSC	12_10739	2H	708561161	3.35E-05	0.055	0.55	A	0.52	ns	ns	ns	ns	ns
7	TWL	11_10383	2H	723653266	8.43E-04	0.345	0.97	G	10.89	ns	ns	ns	ns	ns
8	GSC	11_20681	2H		ns	ns	ns	ns	ns	1.28E-04	0.037	1.84	G	0.84
9	GCC	11_21505	3H	580635994	ns	ns	ns	ns	ns	7.73E-04	0.299	0.96	A	0.29
	GLC				ns	ns	ns	ns	ns	2.66E-04	0.122	0.74	G	0.13
	GSC				ns	ns	ns	ns	ns	2.72E-07	0.0001	0	G	1.09
10	GLC	11_10935	3H	678512385	ns	ns	ns	ns	ns	2.24E-05	0.037	49.08	A	0.16
	GSC				ns	ns	ns	ns	ns	4.79E-10	0.0001	25.19	A	1.42
11	TWL	12_10562	4H	15522510-23601422	1.34E-04	0.154	1.79	A	15.70	ns	ns	ns	ns	ns
12	GCC	11_10793	4H	45245669	ns	ns	ns	ns	ns	2.32E-04	0.189	1.08	G	0.28
13	GPC	11_20020	4H	489816721	7.60E-04	0.501	0.59	C	0.15	ns	ns	ns	ns	ns
14	GPC	11_10846	4H	563098102	7.69E-04	0.501	0.89	G	0.12	ns	ns	ns	ns	ns
15	GCC	11_20324	5H	632384040	ns	ns	ns	ns	ns	4.29E-04	0.233	0.68	A	0.33
16	GLC	12_31509	6H	203509034	ns	ns	ns	ns	ns	3.00E-04	0.122	0.53	A	0.13
	GSC				ns	ns	ns	ns	ns	2.11E-07	0.0001	0	A	1.10
17	TWL	11_11187	6H	573487209	ns	ns	ns	ns	ns	5.74E-04	0.385	0.46	A	10.59
18	GLC	11_11031	7H	8172607	ns	ns	ns	ns	ns	2.26E-04	0.122	0.67	G	0.12
19	TWL	12_30496	7H	116658838	ns	ns	ns	ns	ns	5.00E-04	0.385	0.45	A	8.91
20	GCC	12_30362	7H	611405335	6.51E-04	0.531	0.57	C	0.16	ns	ns	ns	ns	ns
21	GSC	12_10543	7H	626516365	ns	ns	ns	ns	ns	1.38E-04	0.037	1.68	G	0.65
22	GCC	11_21191	UN	0	4.26E-04	0.531	0.45	A	0.17	ns	ns	ns	ns	ns

Notes: FDR – false discovery rate; PVE – phenotypic variance explained; GSC – grain starch content; GPC – grain protein content; GLC – grain lipids content; GCC – grain cellulose content; TWL – grain test weight; UN – unknown chromosome; ns – non-significant.

designated as 22 QTLs (Table 1). Among these QTLs, 7 loci were significant in 2020, and 15 QTLs were found in 2021 (Table 1). The number of QTLs by the traits was as follows: 19 single-trait QTLs (5 QTLs for GSC, 5 QTLs for GCC, 5 QTLs for TWL, 3 QTLs for GPC, and one QTL for GLC), 2 double-trait QTLs (two QTLs for GSC/GLC), and one triple-trait QTL (GSC/GLC/GCC). P-values of significant QTLs ranged from 4.79E-10 to 9.57E-04 with phenotypic variance explained (PVE) values ranging from 0 % to 49.08 % (Table 1). False discovery rate (FDR) values ranged from 0.0001 to 0.6870.

Genetic position of all QTLs identified in the current study were compared with the reference barley genome. As a result, protein-coding candidate genes of barley were identified for

DISCUSSION

Field performance and grain quality traits in the studied barley collection

Barley collection was assessed by five grain quality traits (GSC, GPC, GCC, GLC, and TWL) for GWAS and by two yield-related traits (YM2 and TKW) for better understanding of their relationships with grain quality. Data obtained for quality traits in 2020 and 2021 showed adequate ranges and a sufficient amount of phenotypic variation across two years (Figure 1). However, in 2021, in Kazakhstan, vegetation period was relatively drier and hotter than in 2020 than in 2021 [37], which resulted in greater amount of protein and shortage of starch in barley grain. On the average, GSC value was

Table 2. The list of candidate genes and reference QTLs for identified grain quality loci.

QTL	Trait	Marker	Chromosome	Position (Barley 50K, bp)	Gene (EnsemblPlant)	Protein	Reference gene/QTL
1	GSC	11_11336	1H	261773377	HORVU.MOREX.r3.1HG0039740.1	Ras-related protein Rab-18	QTL_Q2 [46]
2	TWL	12_30350	1H	368821886	HORVU.MOREX.r3.1HG0052410.1	GTPase family protein	-
3	GPC	12_31464	1H	459030191	HORVU.MOREX.r3.1HG0065520.1	KH domain-containing protein	QTl1_GPC [30], [37]
4	GSC	11_21068	1H	540586569	HORVU.MOREX.r3.1HG0088310.1	RING/U-box superfamily protein	*Adh2* [47]
5	GCC	11_10214	2H	672009637	HORVU.MOREX.r3.6HG0579560.1	Kinase family protein	*Vrs1* [38], QTL_Q10 [46]
6	GSC	12_10739	2H	708561161	HORVU.MOREX.r3.4HG0400740.1	Cell division cycle protein 48-like protein	[37]
7	TWL	11_10383	2H	723653266	HORVU.MOREX.r3.2HG0202670.1	Plasma membrane ATPase	[37]
8	GSC	11_20681	2H	760725228-761624420	-	-	QTL_Q11 [46]
9	GCC GLC GSC	11_21505	3H	580635994	HORVU.MOREX.r3.3HG0295530.1	UDP-glycosyltransferase	-
10	GLC GSC	11_10935	3H	678512385	HORVU.MOREX.r3.3HG0322450.1	Transmembrane protein, putative (DUF247)	QTL_Q13 [46]
11	TWL	12_10562	4H	15522510-23601422	-	-	[37]
12	GCC	11_10793	4H	45245669	HORVU.MOREX.r3.4HG0342540.1	Gibberellin-regulated protein 1	QTL_Q14 [46]
13	GPC	11_20020	4H	489816721	HORVU.MOREX.r3.4HG0386900.1	Plant protein 1589 of Uncharacterized protein function	*DTDP* [48],
14	GPC	11_10846	4H	563098102	HORVU.MOREX.r3.4HG0396540.1	GDP-mannose transporter	-
15	GCC	11_20324	5H	632384040	HORVU.MOREX.r3.5HG0523160.1	Proteasome subunit beta type	*Dhn9* [47]
16	GLC GSC	12_31509	6H	203509034	HORVU.MOREX.r3.6HG0579560.1	Kinase family protein	*Adh2* [47], QTL_Q24 [46], QGpc6H.45 [49]
17	TWL	11_11187	6H	573487209	HORVU.MOREX.r3.6HG0629330.1	ABC1-like kinase	-
18	GLC	11_11031	7H	8172607	HORVU.MOREX.r3.7HG0639310.1	Gamma-gliadin	*WAXY* [47]
19	TWL	12_30496	7H	116658838	HORVU.MOREX.r3.7HG0670020.1	Ribonucleoside-diphosphate reductase small chain	*CO1* [47], QTL22_SC [30]
20	GCC	12_30362	7H	611405335	HORVU.MOREX.r3.7HG0732610.1	DNA polymerase alpha subunit B	-
21	GSC	12_10543	7H	626516365	HORVU.MOREX.r3.7HG0738240.1	Mg-protoporphyrin IX chelatase	QTL_Q30 [46]
22	GCC	11_21191	UN	0	HORVU.MOREX.r3.4HG0391070.1	Chaperone protein DnaJ	-

Notes: GSC – grain starch content; GPC – grain protein content; GLC – grain lipids content; GCC – grain cellulose content; TWL – grain test weight; UN – unknown chromosome.

10.39 % higher and GPC was 2.77 % lower in 2020 (Figure 1). The difference in GCC and GLC between two years was not that large – 0.71 % for both traits (Figure 1). An average TWL value was 65.63 g/L higher in 2021 than in 2020 (Figure 1). All of that supports the effect of poor water supply and heat stress on spring barley grain quality observed in south-eastern Kazakhstan [37] and previously in literature [50, 51, 52]. At the same time, heat stress and its effect on GSC resulted in lower grain yield – YM2 was positively correlated with GSC (Figure 2). On the other hand, higher GPC was associated with higher TKW (Figure 2). Thus, heat and water deficiency stress may lead to lower GSC and, as a consequence, to lower YM2, at the same time increasing GPC and an individual grain weight (TKW). The similar situation was observed previously for this barley collection studied for grain quality traits under conditions of Almaty region [37]. Our results support this hypothesis.

Generally, high phenotypic diversity in the studied barley collection provides a solid basis for a robust and accurate GWAS analysis.

Genetic structure of the studied barley collection

The population structure in the studied collection may significantly influence the GWAS results [53]. Therefore, analysis of genetic structure in the studied population is an essential step of the GWAS [53]. For instance, several studies suggest that growth habit, spike morphology, and geographical origin are primary factors affecting the search for MTAs

in diverse barley collections [54, 55]. Since the collection we used in our study contained two-row spring accessions only, the geographical origin was one of the primary factors probably affecting the population substructure. However, NJ dendrogram (Figure 3A) and ΔK graph (Figure 3C) suggested 3 clusters in the studied collection. Distribution of samples among three clusters of NJ dendrogram was uneven and did not fully coincide with the geographic origin (Figure 3A). On the STRUCTURE barplot (Figure 3D), on the opposite, distribution of accessions among three clusters was almost even, but, also did not correspond to the origin (Figure 3E). On the PCA plot, accessions did not form separate clusters according to their origin, but rather made smooth transition from the USA to Kazakhstan along the x-axis (Figure 3B). All of that suggests genetic closeness of studied barley accessions from two countries, as well as possible common breeding history, which had already been suggested before [31, 56]. Thus, analysis of the population structure by three methods revealed the presence of clustering, however, it was not clearly determined by the geographical origin of the accessions. The generated covariance matrix (Q) reflected the genetic differences among origin groups and was applied in the GWAS.

Grain quality QTLs and their candidate loci

QQ plots in the GWAS for all traits demonstrated good fitting to the model with minimal deviation from the line suggesting the correct compensation of the population structure effect (Figure 4B and 4D). In our study, 6 out of 22 identified QTLs had P-value smaller than Bonferroni correction at $p < 3.07E{-}05$ and FDR at $p < 0.05$, while the remaining 16 QTLs were significant at $p < 0.001$ (Table 1). In total, 7 loci were significant in 2020, and 15 QTLs were found in 2021 without matching between years (Table 1), which may confirm the large effect of the environment. Nonetheless, the significance of these QTLs demonstrates their important role in the manifestation of studied quality traits.

For 7 QTLs, there were candidate genes associated with adaptation and/or grain quality of barley (Table 2). For example, *Adh2* is a member of the barley ADH gene family participating in protection against hypoxic stress after flooding, during seed development, and in aerobic metabolism in pollen [57]. The remaining candidate genes were *Vrs1* (row type [58]), *DTDP* and *WAXY* (starch metabolism in the grain [59, 60]), and *Dhn* (response to drought, low temperature, and salinity [61]).

In addition to matches with candidate genes, candidate QTLs for grain quality traits from GWAS and QTL-mapping reports were also detected (Table 2). The largest number of similar loci, as expected, were found in our previous works on barley grain quality traits in different regions of Kazakhstan [37, 46]. At the same time, as there were no matching genetic positions in literature for six QTLs (Table 2), these loci can likely be considered novel genetic factors for controlling grain quality traits.

The genetic position of QTLs associated with the location of genes and QTLs from previously published reports confirming the high reliability of the data in the current GWAS. Along with novel QTLs, it is expected that this data will be useful for future breeding projects targeting the selection of promising barley cultivars with high grain quality both in Kazakhstan and in the World.

CONCLUSIONS

The collection of 356 barley accessions from the USA and Kazakhstan was grown under conditions of Karabalyk agricultural experimental station and genotyped with 1631 polymorphic SNPs markers. The grain of studied barley collection was assessed by GSC, GPC, GCC, GLC, and TWL for two years. Phenotypic data demonstrated impact of the year on studied traits and significant associations between grain quality and the yield ($P < 0.01$), in particular in pairs GPC/GCC, GPC/TKW, and GSC/YM2. Population structure analysis via STRUCTURE revealed three subclusters in the studied barley collection with the dominance of the USA's accessions in two of them. PCA plot and NJ tree showed segregation between accessions from the USA and Kazakhstan, but with a small admixture among two groups of origin. Twenty-two significant QTLs ($P < 0.001$) were identified for the studied grain quality traits including 19 single-trait QTLs (5 QTLs for GSC, 5 QTLs for GCC, 5 QTLs for TWL, 3 QTLs for GPC, and one QTL for GLC), two double-trait QTLs (two QTLs for GSC/GLC), and one triple-trait QTL (GSC/GLC/GCC). For 16 QTLs, candidate barley quality genes and/or QTLs were found, while the remaining 6 QTLs were presumably novel genetic factors. Together, these 22 QTLs are useful tools for breeding projects on the selection of high grain quality barley cultivars.

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ПОЛНОГЕНОМНЫЙ АНАЛИЗ АССОЦИАЦИЙ ПРИЗНАКОВ КАЧЕСТВА ЗЕРНА В КОЛЛЕКЦИИ ЯРОВОГО ЯЧМЕНЯ, ВЫРАЩЕННОЙ НА СЕВЕРЕ КАЗАХСТАНА

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АННОТАЦИЯ

Ячмень – зерновая культура, которую выращивают во всем мире. Его зерно используется при производстве кормов, в пивоварении и пищевой промышленности. Качество зерна ячменя крайне важно и может варьироваться в зависимости от конечного продукта. В частности, содержание в зерне сырого крахмала и белка. Данная работа посвящена изучению коллекции 356 образцов ячменя из США и Казахстана, выращенной в условиях северного Казахстана (Карабалыкская сельскохозяйственная опытная станция) и генотипированой по 1631 SNP маркеру. Коллекция была изучена по содержанию в зерне крахмала (GSC), белка (GPC), клетчатки (GCC) и жиров (GLC), а также натуре зерна (TWL) в течении двух лет. Фенотипический анализ показал влияние года на изученные признаки, а также позволил выявить значимые ассоциации между качеством зерна и урожайностью ($P < 0.01$). Анализ структуры популяции выявил три субкластера в изученной коллекции ячменя с превалированием образцов из США в двух из них. По результатам полногеномного анализа ассоциаций, было идентифицировано 22 локуса количественных признаков (ЛКП) ($P < 0.001$) для изученных признаков, в том числе 19 ЛКП для одного признака, 2 ЛКП для двух признаков и 1 ЛКП для трех признаков. Для 16 ЛКП были найдены референтные гены и/или ЛКП, а оставшиеся 6 ЛКП являются новыми генетическими факторами для признаков качества зерна. Таким образом, эти 22 ЛКП могут быть полезны для будущих селекционных проектов, нацеленных на отбор сортов с высоким качеством зерна.

Ключевые слова: *Hordeum vulgare* L., крахмал, белок, клетчатка, жиры, натура зерна, маркер-опосредованная селекция.

ҚАЗАҚСТАННЫҢ СОЛТҮСТІГІНДЕ ӨСІРІЛГЕН ЖАЗДЫҚ АРПА КОЛЛЕКЦИЯСЫНЫҢ ДӘН САПАСЫНЫҢ БЕЛГІЛЕРІ АССОЦИАЦИЯЛАРЫН ТОЛЫҚ ГЕНОМДЫҚ ТАЛДАУ

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АННОТАЦИЯ

Арпа – барлық әлемде өсірілетін дәнді дақыл. Оның дәні жем-шөп өндірісінде, сыра қайнатуда және тамақ өнеркәсібінде қолданылады. Арпа дәнінің сапасы өте маңызды және соңғы өнімге байланысты өзгеруі мүмкін. Атап айтқанда, дәндегі шикі крахмал мен ақуыздың мөлшері. Бұл жұмыс Солтүстік Қазақстан (Қарабалық ауыл шаруашылығы тәжірибе станциясы) жағдайында өсірілген және 1631 SNP маркері бойынша генотиптелген АҚШ пен Қазақстаннан алынған арпаның 356 үлгісін зерттеуге арналған. Коллекция дәндегі крахмал (GSC), ақуыз (GPC), жасұнық (GCC) және май (GLK) және дән натурасы (TWL) бойынша екі жыл ішінде зерттелді. Фенотиптік талдау жылдың зерттелген белгілерге әсерін көрсетті, сонымен қатар астық сапасы мен өнімділік арасындағы маңызды байланыстарды анықтады ($P < 0.01$). Популяция құрылымын талдау зерттелген арпа коллекциясындағы үш субкластерді анықтады олардың екеуінде АҚШ үлгілері басым. Ассоциацияларды толық геномдық талдау нәтижелері бойынша зерттелген белгілер үшін 22 сандық белгі локустары (СБЛ) ($P < 0.001$) анықталды, оның ішінде бір белгі үшін 19 СБЛ, екі белгі үшін 2 СБЛ және үш белгі үшін 1 СБЛ. 16 СБЛ үшін референтті гендер және/немесе СБЛ табылды, ал қалған 6 СБЛ дән сапасы белгілері үшін жаңа генетикалық факторлар болып табылады. Осылайша, бұл 22 СБЛ жоғары сапалы астық сорттарын таңдауға бағытталған болашақ селекциялық жобалар үшін пайдалы болуы мүмкін.

Түйін сөздер: *Hordeum vulgare* L., крахмал, ақуыз, жасұнық, майлар, дән натурасы, маркер-жанама селекциясы.