

SSR-BASED ASSESSMENT OF GENETIC DIVERSITY IN A BARLEY COLLECTION FROM KAZAKHSTAN

Genievskaya Yuliya A.¹, Azhgaliev Talgat B.², Abugalieva Saule I.^{1,3}, Turuspekov Yerlan K.^{1,3*}¹ Institute of Plant Biology and Biotechnology, Almaty 050040, Kazakhstan² State Commission for Variety Testing of Agricultural Crops, Astana 020000, Kazakhstan³ al-Farabi Kazakh National University, Almaty 050040, Kazakhstan

* yerlant@yahoo.com

ABSTRACT

Barley (*Hordeum vulgare* L.) is a globally significant cereal crop with a substantial production footprint. As a versatile commodity, it serves as a primary resource for animal feed, malting, and human consumption. Genetic diversity among 49 barley accessions procured from the State Commission for Variety Testing of Agricultural Crops in Kazakhstan was assessed using a set of 21 SSR markers associated with various agronomic traits. The collection exhibited moderate genetic diversity, as indicated by a mean polymorphic information content (PIC) of 0.548 ± 0.153 and a mean Shannon's information index (*I*) of 0.980 ± 0.317 , aligning with previously reported estimates of barley genetic variation in the World. STRUCTURE analysis identified three distinct population clusters ($K = 3$) using Bayesian clustering and DeltaK, which was confirmed by PCoA results. However, neighbor-joining tree analysis revealed a more detailed population structure with six distinct clusters, indicating substantial genetic diversity within the barley collection. Distinct phenotypic variations were observed among identified genetic clusters, implying a strong association between genetic diversity and agronomic traits, including plant height, kernel number per spike, and grain yield. Seven barley accessions (GSI_B_60, GSI_B_82, GSI_B_216, GSI_B_85, GSI_B_96, GSI_B_141, and GSI_B_208) forming a cluster NJ_tree_2 demonstrated the highest mean grain yield of 50.99 g/m². This specific cluster and its constituent accessions exhibit promising potential for development into high-yielding cultivars or as a valuable source of beneficial alleles for barley breeding initiatives.

Key words: *Hordeum vulgare* L., microsatellites, population structure, phenotypic variation.

INTRODUCTION

Barley (*Hordeum vulgare* L.), an important grain with a long history, ranks as the fourth most important cereal crop globally [1]. It serves a multitude of purposes, from animal feed and a key brewing ingredient to a food source in certain regions, such as North Africa, the Middle East, Eastern Europe, Central Asia, and Tibet [2, 3, 4]. With its expansive agricultural landscape, Kazakhstan has emerged as a significant barley producer on the world stage. Annually, the country produces around 1.5 – 2.0 million tonnes of barley grain [5]. The crop contributes significantly to the nation's food security by providing animal feed and direct human consumption [5]. Barley's economic importance in Kazakhstan extends beyond domestic needs. The country exports around 1 million tonnes of barley grain annually [1], boosting its agricultural sector and contributing to overall economic growth. The main sowing areas of barley are concentrated in the northern regions of Kazakhstan, Akmola, North Kazakhstan, and Kostanay [5], which are characterized by a moderate continental climate, black and chestnut soils, which are rich in organic matter, and have good moisture capacity [6]. However, recent weather fluctuations have highlighted the vulnerability of barley production in Kazakhstan [6]. Rising temperatures in Central Asia, especially during the growing season, can lead to heat stress in barley crops. Barley is sensitive to high temperatures during the flowering and grain-filling stages, which can reduce grain size and overall yield [7]. The region is prone to droughts, and climate change is expected to increase the frequency and severity of these events. Barley, relatively drought-tolerant compared to other cereals, may still suffer yield reductions if water stress occurs during key developmental stages [7, 8]. This underscores the need to re-

search and develop hardier cultivars that withstand changing environmental conditions.

Knowledge about the genetic diversity of a local breeding pool can significantly enhance crop breeding programs [9]. The unique genetic traits in local populations are leveraged through this approach, ensuring that new cultivars are well-suited to local conditions and challenges [10]. In plant breeding, genetic diversity in a population is commonly investigated using different types of DNA markers [11]. They help identify genetic variations within and between populations, which can be used for various applications in plant breeding, conservation, and evolutionary biology [12]. The most common DNA markers used in the assessment of genetic diversity and molecular breeding in barley are restriction fragment length polymorphisms (RFLPs) [13, 14], random amplified polymorphic DNA (RAPD) [15], amplified fragment length polymorphisms (AFLPs) [16, 17], simple sequence repeats (SSRs) or microsatellites [18, 19], and single nucleotide polymorphisms (SNPs) [20, 21]. The most interesting one among the above-mentioned markers is SSR. These markers consist of short, repetitive DNA sequences that vary in length between individuals. This high level of polymorphism allows them to distinguish between closely related barley lines, effectively revealing genetic diversity within the breeding pool [22]. Numerous SSR markers have been developed for barley, beginning with initial studies identifying SSRs from genomic libraries and ESTs. These efforts led to the creation of the first SSR maps in the early 2000s, which later evolved into more comprehensive maps by integrating additional markers such as SNPs and RFLPs [23, 24]. By 2010, consensus maps combining data from multiple studies provided more detailed genetic insights into barley [25]. Many SSRs have been linked

to valuable agronomic traits, and SSR maps have been utilized in linkage mapping and genome-wide association studies (GWAS) to identify loci associated with these traits in barley. Notably, a diverse genetic pool provides a wide range of SSR alleles that may confer resistance to various diseases, such as resistance to the barley yellow mosaic virus [26] and leaf rust [27], drought tolerance [28], heat resistance [29], improved barley grain quality traits [30, 31], increased grain productivity [32], and etc. In addition, by identifying unique SSR marker profiles, breeders can distinguish individual barley lines within the breeding pool [33]. SSR markers linked to desirable traits are used in MAS to accelerate the barley breeding process [33]. This allows the selection of lines with specific genetic backgrounds to avoid redundancy.

SSR markers were previously used for the assessment of genetic diversity in several crop panels, including bread wheat [34, 35, 36], durum wheat [37, 38, 39], millet [40, 41], soybean [42, 43], chickpea [44, 45], tomato [46], and barley [47, 48]. The current study was focused on the following tasks: (i) to assess the genetic diversity in a collection of 49 barley cultivars provided by the State Commission for variety testing of agricultural crops of the Republic of Kazakhstan using SSR markers associated with various agronomic traits, (ii) to check the genetic stability of these barley accessions using SSR markers, and (iii) to compare variations of phenotypic traits and genetic variability in studied barley accessions. Table 1. The list of barley accessions used for the genotyping

MATERIALS AND METHODS

Barley collection and field assessment

The barley collection, including 49 spring barley accessions (Table 1), was provided by the State Commission for Variety Testing of Agricultural Crops of Kazakhstan.

The field experiments were performed at the Kazakh Research Institute of Agriculture and Plant Growing (KRIAPG, Almalybak) in 2022 and 2023, according to Dospekhov's methodology [49]. Each accession was cultivated in the field within individual one m² plots, with 15 cm spacing between adjacent plots, and conducted in two replications. After harvesting, four key agricultural traits, including plant height (PH, cm), number of kernels per spike (NKS, count), thousand kernels weight (TKW, g), and grain yield per m² (YM2, g/m²) were assessed.

DNA extraction and SSR genotyping

The Genetic experiment was performed in the laboratory of molecular genetics at the Institute of Plant Biology and Biotechnology (IPBB, Almaty). The DNA was extracted from 4-day seedlings of barley accessions in five replicates of each accession using a modified CTAB method [50]. The quality and quantity of the DNA were checked on NanoDrop One spectrophotometer (Thermo Fisher Scientific Inc., USA) and 1% agarose gel electrophoresis.

No.	Accession ID	Growing season	Row-type	No.	Accession ID	Growing season	Row-type
1	GSI_B_45	Winter	2-R	26	GSI_B_199	Spring	2-R
2	GSI_B_60	Spring	2-R	27	GSI_B_208	Spring	2-R
3	GSI_B_61	Spring	6-R	28	GSI_B_216	Winter	2-R
4	GSI_B_66	Spring	2-R	29	GSI_B_224	Spring	2-R
5	GSI_B_67	Spring	2-R	30	GSI_B_231	Spring	2-R
6	GSI_B_79	Spring	2-R	31	GSI_B_232	Spring	6-R
7	GSI_B_81	Spring	2-R	32	GSI_B_233	Spring	2-R
8	GSI_B_82	Spring	2-R	33	GSI_B_234	Spring	2-R
9	GSI_B_83	Spring	2-R	34	GSI_B_235	Spring	2-R
10	GSI_B_85	Spring	2-R	35	GSI_B_254	Spring	2-R
11	GSI_B_90	Spring	2-R	36	GSI_B_258	Winter	2-R
12	GSI_B_91	Spring	2-R	37	GSI_B_265	Winter	2-R
13	GSI_B_96	Spring	2-R	38	GSI_B_266	Winter	2-R
14	GSI_B_105	Spring	2-R	39	GSI_B_277	Spring	2-R
15	GSI_B_106	Spring	2-R	40	GSI_B_294	Spring	2-R
16	GSI_B_109	Spring	6-R	41	GSI_B_295	Spring	2-R
17	GSI_B_111	Spring	2-R	42	GSI_B_296	Spring	2-R
18	GSI_B_126	Spring	2-R	43	GSI_B_305	Spring	2-R
19	GSI_B_134	Spring	2-R	44	GSI_B_321	Spring	2-R
20	GSI_B_136	Spring	2-R	45	GSI_B_325	Spring	2-R
21	GSI_B_140	Spring	2-R	46	GSI_B_334	Spring	2-R
22	GSI_B_141	Spring	2-R	47	GSI_B_343	Spring	2-R
23	GSI_B_182	Spring	2-R	48	GSI_B_357	Spring	2-R
24	GSI_B_183	Spring	2-R	49	GSI_B_367	Spring	2-R
25	GSI_B_184	Winter	2-R				

The genotyping of barley 245 individuals was carried out using 21 SSR markers described in previous studies [33, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61]. These SSRs were chosen for their association with economically valuable traits of barley. PCR conditions (T_a) were optimized for each marker to ensure high efficiency and accuracy (Table 2). The PCR was conducted in a total volume of 20 μ L, containing 20 ng of genomic DNA, 1 U of Taq polymerase, 0.2 mM of each dNTP, 10 pM of each primer, 1.5 mM of MgCl₂, and a standardized 1 \times Taq buffer solution. The amplification protocol included an initial cycle of 3 minutes at 94 °C, followed by 30 cycles of 30 seconds at 94 °C, 30 seconds at the annealing temperature (T_a °C) specified in Table 2, and 30 seconds at 72 °C, with a final extension cycle of 7 minutes at 72 °C.

The PCR products were separated using a QIAxcel Connect System for capillary electrophoresis (QIAGEN, Germany), employing a QIAxcel DNA High-Resolution Kit, QX Alignment Marker (15 bp/3 kb), and QX Size Marker (50 bp/1 kb). Samples were processed using the standard OH500 method with an injection time of 20 seconds.

Analysis of genetic diversity, population structure, and other statistics

Several genetic diversity parameters, including the number of alleles (N_a), number of effective alleles (N_e), Shannon's information index (I), Nei's genetic diversity index (h), and percentage of polymorphic loci (%P), and Polymorphic Information Content (PIC) were used for the assessment of genetic diversity and calculated using GenAlex software (version 6.5) [62]. Based on these values, SSR markers with PIC Table 2. The list of SSR markers used for the amplification.

> 0.5 were classified as highly informative, those with PIC between 0.25 and 0.5 as informative, and markers with PIC < 0.25 as non-informative [63].

Neighbor-joining (NJ) clustering, principal coordinate analysis (PCoA), and Bayesian clustering with Markov chain Monte Carlo (MCMC) were employed for population structure analysis. NJ clustering was performed using PAST 3.19 software [64], PCoA was conducted with GenAlex, and Bayesian clustering was carried out using STRUCTURE [65] with admixture models and correlated allele frequencies. The optimal number of clusters (K) in the population was determined using CLUMPAK [66] and Evanno method [67]. Pairwise genetic distances among barley accessions were calculated using GenAlex and visualized using the Heatmapper web tool [68]. R statistical software [69] was used for the descriptive statistics and ANOVA.

RESULTS

SSR genotyping and genetic diversity in studied barley collection

A total of 21 SSR markers associated with various agronomic traits of barley (Table 2) were used in the analysis. Among them, 20 markers were polymorphic and were used for the analysis of population structure and genetic diversity in the collection of 49 barley cultivars (Table 3).

The number of alleles varied from 2 to 6 per polymorphic locus (Table 3). The *Bmac040* was monomorphic and, therefore, excluded from further analysis. Genetic diversity indices were calculated for all 20 SSR markers (Table 4).

SSR	Chromosome	Pos. (cM)	T_a	References
<i>Bmag872</i>	1H	49.78	55	[51]
<i>HVM20</i>	1H	66.25	55	[33]
<i>HVM36</i>	2H	30.97	55	[52]
<i>EBmac415</i>	2H	117.86	55	[52, 53]
<i>HVM54</i>	2H	122.41	55	[52, 53]
<i>GBM1110</i>	3H	60.27	55	[54]
<i>HVM33</i>	3H	65.50	55	[55]
<i>HVM40</i>	4H	22.40	55	[54]
<i>EBmac788</i>	4H	97.67	60	[54]
<i>Bmag751</i>	5H	42.87	55	[54]
<i>Bmac096</i>	5H	51.10	58	[56]
<i>GBM1506</i>	5H	75.45	55	[54]
<i>Bmac316</i>	6H	4.30	55	[57]
<i>HVM74</i>	6H	62.66	55	[58]
<i>EBmac602</i>	6H	75.42	58	[51]
<i>Bmac040</i>	6H	129.82	58	[59]
<i>HVM04</i>	7H	22.19	55	[60]
<i>GBM1464</i>	7H	53.43	55	[59]
<i>Bmag321</i>	7H	79.24	58	[61]
<i>Bmag013</i>	7H	113.70	58	[54]
<i>Bmag135</i>	7H	147.51	58	[53]
Pos. – position on Barley SSR Consensus Map (cM) [53], T_a – annealing temperature.				

Table 3. Results of SSR-genotyping.

SSR	Chr.	Pos.	Na	SSR size (bp) [53]	Actual sizes (bp)
<i>Bmag872</i>	1H	49.78	4	125	96, 103, 110, 128
<i>HVM20</i>	1H	66.25	2	151	134, 158
<i>HVM36</i>	2H	30.97	6	114	108, 110, 112, 113, 120, 140
<i>EBmac415</i>	2H	117.86	3	247	231, 240, 252
<i>HVM54</i>	2H	122.41	3	159	150, 160, 164
<i>GBM1110</i>	3H	60.27	2	230	236, 241
<i>HVM33</i>	3H	65.50	4	157	160, 162, 165, 167
<i>HVM40</i>	4H	22.40	3	160	145, 152, 162
<i>EBmac788</i>	4H	97.67	3	168	165, 167, 169
<i>Bmag751</i>	5H	42.87	5	189	173, 190, 194, 198, 207
<i>Bmac096</i>	5H	51.10	3	178	172, 173, 181
<i>GBM1506</i>	5H	75.45	2	158	166, 174
<i>Bmac316</i>	6H	4.30	5	135	136, 138, 153, 168, 179
<i>HVM74</i>	6H	62.66	5	162	189, 191, 199, 208, 233
<i>EBmac602</i>	6H	75.42	4	205	210, 225, 230, 235
<i>Bmac040</i>	6H	129.82	1	236	171
<i>HVM04</i>	7H	22.19	5	198	0, 197, 199, 203, 208
<i>GBM1464</i>	7H	53.43	4	160	153, 172, 214, 224
<i>Bmag321</i>	7H	79.24	5	218	213, 218, 222, 226, 230
<i>Bmag013</i>	7H	113.70	4	155	171, 184, 188, 195
<i>Bmag135</i>	7H	147.51	5	161	0, 140, 144, 146, 160
Chr. – chromosome, Pos. – position on Barley SSR Consensus Map (cM) [53], Na – number of alleles.					

Table 4. Genetic diversity of SSR markers

SSR	<i>Na</i>	<i>Ne</i>	<i>I</i>	<i>PIC</i>
<i>Bmag872</i>	4	1.817	0.740	0.450
<i>HVM20</i>	2	1.690	0.598	0.408
<i>HVM36</i>	6	3.851	1.482	0.740
<i>Ebmac415</i>	3	2.084	0.894	0.520
<i>HVM54</i>	3	2.004	0.862	0.501
<i>GBM1110</i>	2	1.167	0.273	0.143
<i>HVM33</i>	4	2.128	0.846	0.530
<i>HVM40</i>	3	2.244	0.940	0.554
<i>Ebmac788</i>	3	1.859	0.813	0.462
<i>Bmag751</i>	5	1.706	0.854	0.414
<i>Bmac096</i>	3	2.746	1.048	0.636
<i>GBM1506</i>	2	1.471	0.500	0.320
<i>Bmac316</i>	5	2.639	1.171	0.621
<i>HVM74</i>	5	2.508	1.092	0.601
<i>Ebmac602</i>	4	2.478	1.037	0.596
<i>HVM04</i>	5	3.531	1.380	0.717
<i>GBM1464</i>	4	3.974	1.383	0.748
<i>Bmag321</i>	5	3.413	1.370	0.707
<i>Bmag013</i>	4	2.464	1.015	0.594
<i>Bmag135</i>	5	3.190	1.299	0.696

Mean	3.850	2.448	0.980	0.548
SD	1.182	0.795	0.317	0.153
<i>Na</i> – number of alleles; <i>Ne</i> – number of effective alleles; <i>I</i> – Shannon’s information index; PIC – polymorphic information content; SD – standard deviation.				

The mean number of alleles per locus (*Na*) in the studied barley collection was 3.850, while the mean number of effective alleles (*Ne*) was 2.448, with a range of 1.167 – 3.974 alleles per locus. Shannon’s information index (*I*) values for polymorphic loci ranged from 0.273 to 1.482 (mean *I* = 0.980). As for the PIC values, they ranged from 0.143 to 0.748 (mean PIC = 0.548). Among 20 polymorphic SSRs, one locus was non-informative with relatively low PIC value

(*GBM1110*), five loci were informative (*GBM1506*, *HVM20*, *Bmag751*, *Bmag872*, and *Ebmac788*) with PIC values from 0.320 to 0.462, and the remaining 14 loci were highly informative demonstrating PIC values from 0.501 to 0.748 for the studied barley collection.

The largest polymorphism was observed for SSRs *HVM36* on chromosome 2H and *GBM1464* on chromosome 7H, with PIC values of 0.740 and 0.748, respectively (Table 4).

Table 5. Genetic diversity of barley accessions used in the study

ID	<i>Na</i>	<i>Ne</i>	<i>I</i>	<i>h</i>	%P
GSI_B_45	1.70 ± 0.19	1.52 ± 0.17	0.37 ± 0.10	0.23 ± 0.06	50.00
GSI_B_60	1.10 ± 0.07	1.07 ± 0.05	0.06 ± 0.04	0.04 ± 0.03	10.00
GSI_B_61*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_66*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_67*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_79*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_81	1.05 ± 0.05	1.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_82*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_83	1.05 ± 0.05	1.05 ± 0.05	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_85	1.60 ± 0.11	1.28 ± 0.05	0.30 ± 0.06	0.19 ± 0.04	60.00
GSI_B_90	1.75 ± 0.12	1.60 ± 0.11	0.46 ± 0.07	0.31 ± 0.05	70.00
GSI_B_91	1.50 ± 0.15	1.26 ± 0.06	0.26 ± 0.06	0.17 ± 0.04	50.00
GSI_B_96*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_105*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_106	1.05 ± 0.05	1.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_109	1.05 ± 0.05	1.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_111	2.05 ± 0.15	1.83 ± 0.13	0.60 ± 0.08	0.39 ± 0.05	80.00
GSI_B_126*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_134	1.30 ± 0.11	1.19 ± 0.07	0.17 ± 0.06	0.11 ± 0.04	30.00
GSI_B_136	1.05 ± 0.05	1.05 ± 0.05	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_140	1.45 ± 0.14	1.28 ± 0.10	0.24 ± 0.07	0.15 ± 0.05	40.00
GSI_B_141	1.05 ± 0.05	1.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_182	1.60 ± 0.11	1.31 ± 0.06	0.31 ± 0.06	0.20 ± 0.04	60.00
GSI_B_183	1.55 ± 0.11	1.26 ± 0.05	0.28 ± 0.06	0.18 ± 0.04	55.00
GSI_B_184	1.20 ± 0.09	1.14 ± 0.07	0.12 ± 0.06	0.08 ± 0.04	20.00
GSI_B_199	1.05 ± 0.05	1.05 ± 0.05	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_208	1.05 ± 0.05	1.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_216	1.20 ± 0.09	1.18 ± 0.06	0.10 ± 0.05	0.07 ± 0.03	20.00
GSI_B_224	1.60 ± 0.11	1.31 ± 0.06	0.31 ± 0.06	0.20 ± 0.04	60.00
GSI_B_231	1.50 ± 0.12	1.44 ± 0.10	0.33 ± 0.08	0.23 ± 0.05	50.00
GSI_B_232	1.05 ± 0.05	1.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_233	1.15 ± 0.08	1.14 ± 0.08	0.10 ± 0.06	0.07 ± 0.04	15.00

GSI_B_234	1.15 ± 0.08	1.12 ± 0.07	0.09 ± 0.05	0.06 ± 0.04	15.00
GSI_B_235	1.60 ± 0.13	1.42 ± 0.12	0.34 ± 0.08	0.22 ± 0.05	55.00
GSI_B_254	1.55 ± 0.11	1.42 ± 0.10	0.34 ± 0.07	0.23 ± 0.05	55.00
GSI_B_258	1.35 ± 0.13	1.21 ± 0.10	0.18 ± 0.07	0.11 ± 0.04	30.00
GSI_B_265	1.05 ± 0.05	1.05 ± 0.05	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_266	1.10 ± 0.07	1.05 ± 0.03	0.05 ± 0.03	0.03 ± 0.02	10.00
GSI_B_277	1.10 ± 0.10	1.09 ± 0.09	0.05 ± 0.05	0.03 ± 0.03	5.00
GSI_B_294	1.10 ± 0.07	1.07 ± 0.05	0.06 ± 0.04	0.04 ± 0.03	10.00
GSI_B_295	1.05 ± 0.05	1.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	5.00
GSI_B_296	1.10 ± 0.07	1.09 ± 0.06	0.07 ± 0.05	0.05 ± 0.03	10.00
GSI_B_305	1.80 ± 0.16	1.59 ± 0.14	0.44 ± 0.09	0.29 ± 0.05	65.00
GSI_B_321	1.70 ± 0.15	1.41 ± 0.09	0.36 ± 0.07	0.23 ± 0.05	60.00
GSI_B_325	2.05 ± 0.20	1.75 ± 0.16	0.54 ± 0.10	0.34 ± 0.06	70.00
GSI_B_334	1.35 ± 0.11	1.19 ± 0.06	0.18 ± 0.06	0.12 ± 0.04	35.00
GSI_B_343	1.05 ± 0.05	1.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.03	5.00
GSI_B_357*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
GSI_B_367*	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Mean	1.26 ± 0.02	1.17 ± 0.01	0.14 ± 0.01	0.09 ± 0.01	23.47 ± 15.42
* – genetically uniform accessions; N_a – number of alleles; N_e – number of effective alleles; I – Shannon’s information index; h – Nei’s genetic diversity index; %P – percentage of polymorphic loci per type.					

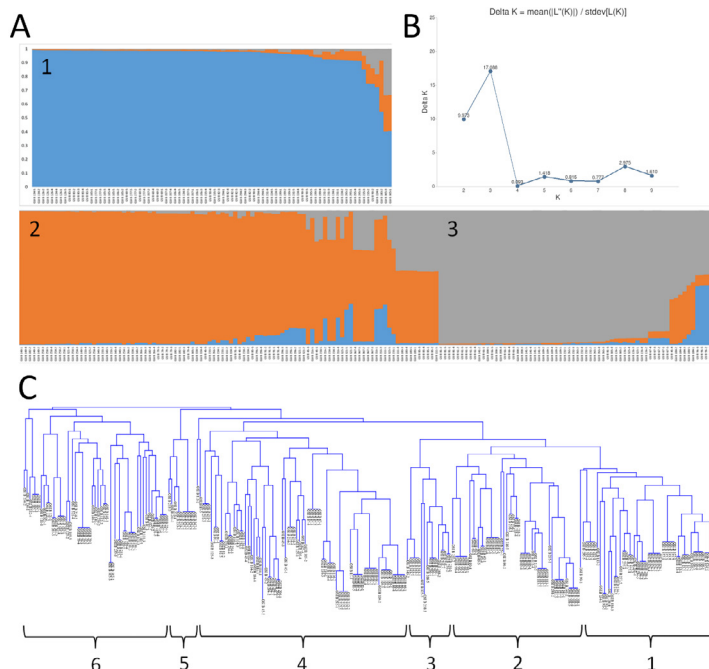


Figure 1. Population structure of 49 barley accessions. A. Distribution of barley accessions by clusters at K = 3. B. Delta K plot. C. Neighbor-joining (NJ) tree.

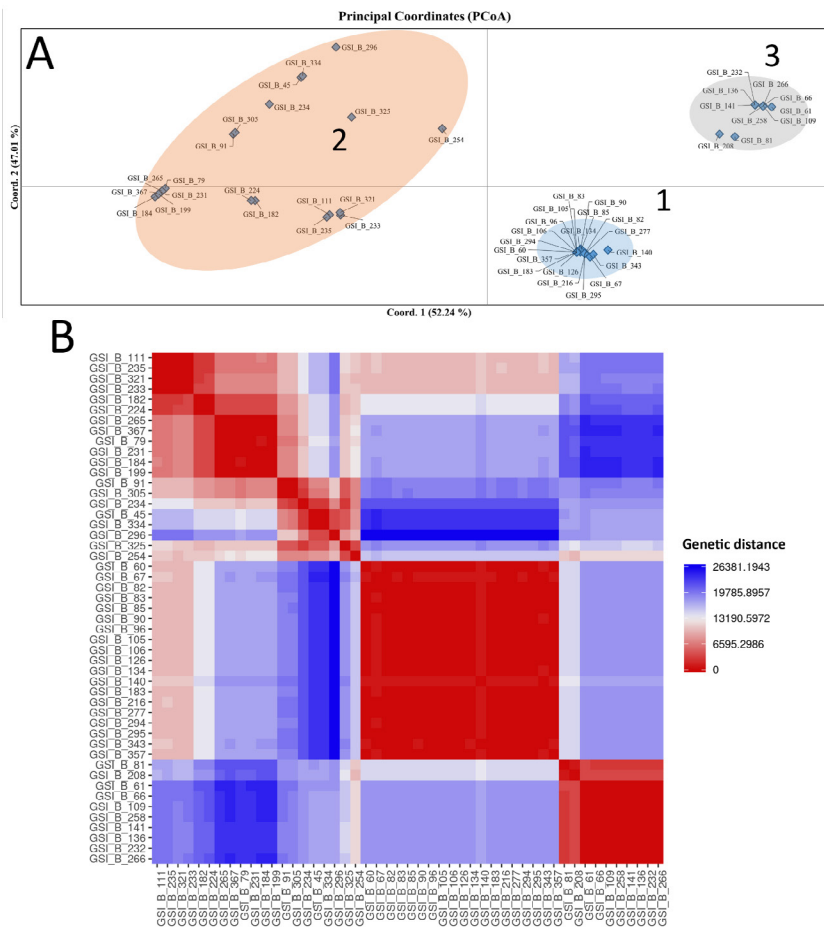


Figure 2. Genetic distance-based population structure of barley collection. A. Principal coordinate analysis (PCoA) plot. B. Pairwise heatmap of genetic distances.

Genetic stability of barley accessions

In order to assess the genetic stability of barley accessions, genetic diversity indices were also calculated for each individual accession (Table 5).

The percentage of polymorphic loci per accession varied from 0 to 80.00 % with mean values of 23.47 %. Based on the results of SSR genotyping, 10 out of 49 barley accessions demonstrated genetic uniformity with zero diversity. The largest genetic diversity was observed for accession GSI_B_111, followed by GSI_B_90 and GSI_B_305 with 80 %, 70 %, and 65 % of polymorphic loci, respectively. The mean number of alleles (*Na*) per barley accession was 1.26 and varied from 1.00 to 2.05 alleles. The number of effective alleles (*Ne*)

was 1.00 – 1.83, with a mean value of 1.17. Shannon’s information index (*I*) varied from 0.00 to 0.60 with a mean value of 0.14. Finally, the mean Nei’s genetic diversity index (*h*) in the studied collection was 0.09.

Population structure of barley collection

Results of the Bayesian clustering with MCMC estimated that *K* = 3 was the optimum for the studied barley population, with 3 clusters formed (Figures 1A, 1B).

The first cluster included 17 out of 245 samples (49 accessions in 5 replications) belonging to 28 accessions. The second cluster included 98 samples belonging to 20 accessions, while the third cluster included 65 samples of 13 ac-

Table 6. Mean values of 4 agronomic traits in barley collection by genetic clusters

Cluster	PH (cm)	NKS (count)	TKW (g)	YM2 (g/m ²)
Bayesian_1	62.86 ± 4.09	20.16 ± 2.97	42.15 ± 6.33	38.33 ± 12.71
Bayesian_2	65.28 ± 6.19	25.38 ± 2.99	43.09 ± 5.14	44.46 ± 9.43
Bayesian_3	63.51 ± 3.78	21.28 ± 4.43	45.70 ± 5.42	48.29 ± 12.95
NJ_tree_1	62.97 ± 2.88	21.85 ± 2.77	45.70 ± 5.94	47.35 ± 10.75
NJ_tree_2	67.58 ± 0.99	18.46 ± 1.57	45.06 ± 3.01	72.13 ± 12.28
NJ_tree_3	32.98 ± 1.02	26.11 ± 2.01	32.36 ± 1.12	8.67 ± 2.23
NJ_tree_4	40.02 ± 4.67	32.48 ± 9.75	35.48 ± 5.78	30.39 ± 9.96
NJ_tree_5	47.45 ± 0.41	40.09 ± 0.47	48.88 ± 0.74	50.99 ± 2.95
NJ_tree_6	65.49 ± 6.27	20.38 ± 2.50	42.71 ± 5.55	48.40 ± 7.44
PCoA_1	63.39 ± 4.02	19.79 ± 3.33	43.30 ± 6.17	36.73 ± 11.82

PCoA_2	65.88 ± 5.89	27.97 ± 2.70	42.10 ± 5.00	50.50 ± 10.88
PCoA_3	62.96 ± 3.94	22.26 ± 9.65	44.23 ± 5.74	52.06 ± 11.88
Collection's mean	63.97 ± 4.86	22.47 ± 7.87	43.71 ± 5.95	44.00 ± 11.45
PH – plant height, NKS – number of kernels per spike, TKW – thousand kernels weight, YM2 – grain yield per m ² , PCoA – principal coordinate analysis, NJ – Neighbor-joining clustering				

Table 7. P-values of factors in ANOVA

Factor (clusters)	PH	NKS	TKW	YM2
Bayesian	0.9350 ^{ns}	0.0058 ^{**}	0.0820 ^{ns}	0.5360 ^{ns}
PCoA	0.5130 ^{ns}	0.0006 ^{***}	0.0545 ^{ns}	0.3640 ^{ns}
NJ tree	0.0279 [*]	0.3060 ^{ns}	0.0668 ^{ns}	0.0104 [*]
PH – plant height, NKS – number of kernels per spike, TKW – thousand kernels weight, YM2 – grain yield per m ² , PCoA – principal coordinate analysis, NJ – Neighbor-joining clustering. * – P < 0.05, ** – P < 0.01, *** – P < 0.001, ^{ns} – not significant				

cessions (Figure 1A). NJ tree clustering revealed the presence of 6 clusters in the studied barley collection (Figure 1C). The first cluster included 49 samples of 11 barley accessions, and the second cluster included 45 samples of 11 accessions. The third cluster included 16 samples of 4 accessions. The largest 4th cluster was made up of 75 samples of 19 barley accessions, and the smallest 5th cluster included 10 samples of only 2 accessions. The last 6th cluster included 50 samples of 15 accessions.

Two Principal coordinate analysis (PCoA) axes explained that 99.25 % of total genetic variability resulted in 3 clusters along the X-axis (Figure 2A). PCoA allowed us to divide the accessions into three groups with 19, 20, and 10 barley accessions, respectively (Figure 2A).

A pairwise matrix of genetic distances among 49 studied barley accessions showed the presence of 3 clusters corresponding to the same clusters found in PCoA (Figure 2B).

Comparison of agronomic traits among genetic clusters

The mean values of PH, NKS, TKW, and YM2 were calculated for each genetic cluster for Bayesian clustering with MCMC, PCoA, and NJ tree formed as a result of population structure analysis (Table 6).

The largest mean PH values were observed for clusters Bayesian_2, NJ_tree_2, NJ_tree_6, and PCoA_2; the smallest mean PH was found for genetic clusters NJ_tree_3, NJ_tree_4, and NJ_tree_5. Significant impact (P < 0.05) of clusters on PH, as factors of ANOVA, was revealed for NJ tree clusters (Table 7).

The greatest mean NKS values were found for clusters NJ_tree_4 and NJ_tree_5, while the smallest NKS values were observed for clusters NJ_tree_2 and PCoA_1 (Table 6). ANOVA revealed a significant impact of Bayesian and PCoA clusterizations (P < 0.01 and P < 0.001, respectively) on NKS

(Table 7). The largest TKW was revealed for the cluster NJ_tree_5 (Table 6); however, none of the clusterization methods was significant for TKW (Table 7). For YM2, clusterization via the NJ method was significant (P < 0.05) (Table 6), and the highest YM2 was observed for the cluster NJ_tree_2 (Table 7).

Thus, the genetic clusterization of barley accessions via Bayesian clustering and PCoA was mostly explained by differences in NKS values, while NJ clusterization was conditioned by differences in PH and YM2 among studied accessions.

DISCUSSION

Genetic diversity of barley accessions from the State Commission for variety testing of agricultural crops of Kazakhstan

As a result of this work, 49 barley accessions were used for SSR genotyping and field trials in KAES. The collection mostly included 2-R spring barleys as the prevailing barley type for cultivation in Kazakhstan [5]. Genotyping revealed moderate average genetic diversity (PIC = 0.548, I = 0.980) for 21 SSRs associated with important agronomic traits (Table 4). Our findings align with previous studies, which also report moderate to high genetic diversity in barley collections based on SSR markers. For instance, a study by Jilal et al. [70] on a barley landrace collection observed similar genetic diversity levels, with PIC values ranging from 0.27 to 0.83 and an average PIC of 0.54, closely matching our average PIC of 0.548 (Table 4). Moreover, another study by Kolodinska Brantestam et al. [71] on Nordic and Baltic barley varieties found PIC values between 0.39 and 0.78, further corroborating SSRs like *GBM1464* and *HVM36* showing high genetic diversity (Table 4). Additionally, a study by Varshney et al. [25] reported an average PIC of 0.49 across various SSR markers, which is slightly lower but comparable to our findings. This consis-

tency across different geographical regions underscores the utility of SSR markers in assessing genetic diversity and identifying valuable alleles for barley breeding.

The largest genetic diversity was observed for SSRs *GBM1464*, *HVM36*, *HVM04*, and *Bmag321* ($PIC > 0.700$, $I > 1.300$) associated with protein content in the grain, fusarium head blight resistance, gene *brh1* (brachytic spike), and gene *dsp1* (dense spike), respectively (Table 2). All of these phenotypic traits are associated with barley spikes and grains, suggesting a large allelic variation of genes involved in spike morphology, disease resistance, and grain quality in the barley collection studied. This barley collection can be a source for potential donors of alleles associated with desirable traits for breeding programs [72].

The other important results of the current study are values of polymorphic loci percentages indicating genetic uniformity of studied barley accessions (Table 5). Our study revealed significant variations in the percentage of polymorphic loci per barley accession, ranging from 0 to 80.00 %, with a mean of 23.47 % (Table 5). Ten out of 49 barley accessions exhibited genetic uniformity, showing zero diversity: *GSI_B_61*, *GSI_B_66*, *GSI_B_67*, *GSI_B_79*, *GSI_B_82*, *GSI_B_96*, *GSI_B_105*, *GSI_B_126*, *GSI_B_357*, and *GSI_B_367* (Table 5). Our findings of polymorphic loci percentages are comparable to those reported in other studies. For instance, in a study on barley accessions from Tibet, the percentage of polymorphic loci ranged from 40% to 70%, with an average of 55% [73]. Similarly, research on Nordic and Baltic barley cultivars showed polymorphic loci percentages ranging from 30% to 75% [71]. These variations highlight the genetic diversity present in different barley collections globally. The observation of genetic uniformity in 10 accessions aligns with findings from Varshney et al. [25], who reported that certain barley accessions from their study exhibited minimal genetic diversity, indicating high genetic uniformity. Genetic uniformity can be attributed to intensive breeding practices aimed at maintaining specific desirable traits [74]. The current study comprehensively assesses the genetic diversity in 49 barley accessions. The results are consistent with those from other regions of the World, demonstrating moderate genetic diversity and identifying accessions with high allelic variation. This information is crucial for future barley breeding programs aimed at enhancing traits such as disease resistance and grain quality.

Population structure in studied barley collection

Three optimal clusters ($K = 3$) were identified by Bayesian clustering with MCMC (Figure 1A and 1B) and confirmed by PCoA (Figure 2A), while six distinct clusters were revealed by NJ tree clustering (Figure 1C), confirming significant genetic diversity within the collection. Accessions with high genetic variability ($\%P \geq 50\%$), as well as genetically uniform barley accessions with $\%P = 0\%$, were distributed among all clusters without any particular pattern (Table 5). In the current study, ANOVA based on four important agronomic traits showed that the genetic clustering of barley accessions using Bayesian clustering with MCMC and PCoA methods was primarily influenced by variations in NKS values, while NJ clustering was determined by differences in PH and YM2 among the studied accessions (Table 7).

Previously, the population structure of barley cultivars and

breeding lines in Kazakhstan was studied using 5636 polymorphic SNPs and suggested that Kazakh barley samples consisted of five subclusters in three major clusters explained mainly by the originating breeding organization, while the majority of cultivars from Kazakhstan were grouped in a separate subcluster with a common ancestral node [75]. In our study, on the opposite, the clusterization of barley accessions was conditioned mainly by phenotypic traits (PH, NKS, and YM2) and the allelic variations that underlie them.

ANOVA revealed a significant impact ($P < 0.05$) of NJ tree clusters on PH. This is consistent with the findings of Pasam et al. [76], who also reported significant variation in plant and spike morphology and region of origin across different genetic clusters in barley populations, emphasizing the role of genetic differentiation in phenotypic expression. The cluster *NJ_tree_5*, including two 2-R spring barley accessions *GSI_B_233* and *GSI_B_367*, demonstrated the mean NKS value of 40.09 counts and the mean TKW of 48.88 g (Table 6). The highest mean grain yield was observed for the cluster *NJ_tree_2* (Table 6) including 7 accessions: *GSI_B_60*, *GSI_B_82*, *GSI_B_216*, *GSI_B_85*, *GSI_B_96*, *GSI_B_141*, and *GSI_B_208*. This cluster and these accessions can be used as highly productive cultivars or as a source of alleles in barley breeding programs.

CONCLUSION

The genetic diversity of 49 barley accessions from the State Commission for Variety Testing of Agricultural Crops of Kazakhstan was assessed using genotyping results with 21 SSR, revealing a moderate genetic diversity ($PIC = 0.548$ and $I = 0.980$). The highest genetic diversity was observed in SSR markers associated with key agronomic traits, highlighting the collection's potential as a source of valuable alleles for breeding programs. Furthermore, significant phenotypic differences were noted among the genetic clusters identified, indicating that genetic diversity in this collection is strongly linked to important agronomic traits such as plant height, number of kernels per spike, and grain yield.

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ОЦЕНКА ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ КАЗАХСТАНСКОЙ КОЛЛЕКЦИИ ЯЧМЕНЯ С ИСПОЛЬЗОВАНИЕМ SSR-МАРКЕРОВ

Гениевская Юлия А.¹, Ажгалиев Талгат Б.², Аbugалиева Сауле И.^{1,3}, Туруспеков Ерлан К.^{1,3*}¹ Институт биологии и биотехнологии растений, Алматы 050040, Казахстан² Государственная комиссия по сортоиспытанию сельскохозяйственных культур, Астана 020000, Казахстан³ Казахский национальный университет им. аль-Фараби, Алматы 050040, Казахстан

* yerlant@yahoo.com

АННОТАЦИЯ

Ячмень (*Hordeum vulgare* L.) – это значимая зерновая культура, имеющая высокую производственную значимость во всем мире. Будучи достаточно распространенным, ячменное зерно служит основным источником для производства кормов для животных, пивоварения и прямого употребления в пищу человеком. Генетическое разнообразие 49 образцов ячменя, полученных от Государственной комиссии по сортоиспытанию сельскохозяйственных культур Казахстана, было оценено с использованием набора из 21 SSR-маркера, ассоциированного с различными хозяйственно ценными признаками. Изученная коллекция показала умеренное генетическое разнообразие, на что указывает среднее индекс PIC равный $0,548 \pm 0,153$ и средний индекс Шеннона (I) равный $0,980 \pm 0,317$, что соответствует ранее опубликованным оценкам генетической изменчивости ячменя в мире. Помимо этого, анализ структуры популяции в программе STRUCTURE позволил выявить три отдельных кластера ($K = 3$) с использованием байесовской кластеризации и величины DeltaK, что было также подтверждено результатами анализа главных координат (PCoA). Однако анализ древа, построенного по методике Neighbor-joining (NJ), выявил более подробную структуру популяции с шестью отдельными кластерами, что указывает на значительное генетическое разнообразие в изученной коллекции ячменя. Среди идентифицированных генетических кластеров наблюдались отчетливые фенотипические вариации, что предполагает сильную связь между генетическим разнообразием и агрономическими характеристиками, включая такие признаки как: высота растения, количество зерен в колосе и урожайность зерна. Семь образцов ячменя (GSI_B_60, GSI_B_82, GSI_B_216, GSI_B_85, GSI_B_96, GSI_B_141 и GSI_B_208), образующие кластер NJ_tree_2, продемонстрировали самую высокую среднюю урожайность зерна в размере $50,99 \pm 2,95$ г/м². Данный кластер и входящие в него образцы являются многообещающими и несут в себе потенциал для создания и производства высокоурожайных сортов, а также в качестве источника важных аллелей для селекции ячменя.

Ключевые слова: *Hordeum vulgare* L., микросателлитные маркеры, структура популяции, фенотипическая изменчивость.

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SSR-МАРКЕРЛЕРІН ҚОЛДАНУ НЕГІЗІНДЕ ҚАЗАҚСТАНДЫҚ АРПА КОЛЛЕКЦИЯСЫНЫҢ ГЕНЕТИКАЛЫҚ АЛУАНТҮРЛІЛІГІН БАҒАЛАУ

Гениевская Юлия А.¹, Ажгалиев Талгат Б.², Аbugалиева Сауле И.^{1,3}, Туруспеков Ерлан К.^{1,3*}¹ Өсімдіктер биологиясы және биотехнологиясы институты, Алматы 050040, Қазақстан² Ауылшаруашылық дақылдарын сынау жөніндегі мемлекеттік комиссия, Астана 020000, Қазақстан³ ал-Фараби атындағы Қазақ ұлттық университеті, Алматы 050040, Қазақстан

* yerlant@yahoo.com

ТҮЙІН

Арпа (*Hordeum vulgare* L.) – дүние жүзінде жоғары өндірістік маңызы бар дәнді дақыл болып табылады. Арпа дәні кең таралған болғандықтан, мал азығын өндіру, сыра қайнату және адам тұтынуының негізгі көзі болып табылады. Қазақстанның ауылшаруашылық дақылдарының сорттарын сынау жөніндегі мемлекеттік комиссиядан алынған 49 арпа үлгісінің генетикалық алуантүрлілігін әртүрлі экономикалық құнды белгілермен байланысты 21 SSR маркерлерінің жиынтығы арқылы бағаланды. Зерттелетін жинақ орташа генетикалық алуантүрлілігін көрсетті, бұл орташа PIC индексі $0,548 \pm 0,153$ және Шеннонның орташа индексі (I) $0,980 \pm 0,317$, бұл әлемдегі арпаның генетикалық өзгергіштігінің бұрын жарияланған бағалауларына сәйкес келеді. Сонымен қатар, STRUCTURE популяция құрылымының талдауы Байес кластерін және DeltaK көмегімен үш түрлі кластерді ($K = 3$) анықтады, бұл сонымен қатар негізгі координаталық талдау (PCoA) арқылы расталды. Алайда, Neighbor-joining (NJ) әдісі бойынша салынған ағаш талдауы зерттелген арпа коллекциясында алты бөлек кластерді анықтады, бұл айтарлықтай генетикалық алуантүрлілігін көрсетеді. Анықталған генетикалық кластерлер арасында айқын фенотиптік вариациялар байқалды, бұл генетикалық алуантүрлілігі мен агротехникалық белгілердің, соның ішінде өсімдіктің биіктігін, бір масақтағы дәндердің санын және астық өнімділігін қоса алғанда, тығыз байланысын көрсетеді. NJ_tree_2 кластерін құрайтын жеті арпа үлгісі (GSI_B_60, GSI_B_82, GSI_B_216, GSI_B_85, GSI_B_96, GSI_B_141 және GSI_B_208) орташа дән шығымдылығын

50,99 ± 2,95 г/м² көрсеткен. Бұл кластер және оған кіретін үлгілер жоғары өнімді сорттарды жасау және өндіру үшін, сондай-ақ арпаны селекциялауға қажетті маңызды аллельдер көзі ретінде әлеуетке ие.

Түйін сөздер: *Hordeum vulgare* L., микросателлитті маркерлер, популяция құрылымы, фенотиптік өзгергіштік.