

ABSTRACT

of the dissertation work of Geniyevskaya Yuliya
titled: “**Genome-wide association study for grain quality traits of spring barley in Kazakhstan**” submitted for the academic degree “Doctor of Philosophy”
(Ph.D.), education program 8D05101 – “Biology”

The relevance of research. Barley (*Hordeum vulgare* L.) is one of the important grain crops in the world and Kazakhstan. A stable increase in the production of high-quality barley grain, used for feed and food production, malting, and brewing, is important for ensuring food security worldwide and within the country. Modern breeding science and the usage of new molecular genetic technologies in the process of cultivar development jointly may provide a push for the development of the industry. The world experience of introducing molecular genetic technologies into breeding programs significantly accelerates crop improvement and saves appropriate resources and money. However, in Kazakhstan, the introduction of molecular genetic technologies into breeding is still insufficient. In this regard, it is very important to study the barley genetic resources from Kazakhstan and foreign collections using both traditional breeding methods and modern methodologies of molecular genetics, genomics, and marker-assisted selection (MAS), as well as to develop new molecular tools for local barley breeders.

Research objects: 1) a world collection consisting of 406 cultivars and lines of two-row spring barley from the USA, Kazakhstan, Europe and Africa; 2) a collection of two-row and six-row spring barley, consisting of 658 samples from the USA and Kazakhstan; 3) 34 promising lines of spring barley ($F_{>8}$) provided by the Karabalyk Agricultural Experimental Station.

Research subjects. Quantitative trait loci (QTLs) and Kompetitive allele-specific PCR (KASP)-type markers associated with grain quality traits of barley.

The goal of research. Identification of QTLs associated with traits of grain quality using spring barley grown in Kazakhstan.

Tasks of research.

1. To assess the variability of six traits of grain quality in collections of spring barley grown in 4 regions of Kazakhstan.
2. To study the genetic diversity and characterize the genetic structure of spring barley collections, including accessions from the USA, Kazakhstan, Europe, and Africa, using single nucleotide polymorphism (SNP) markers.
3. To identify quantitative trait loci for six traits of grain quality using spring barley collections and genome-wide association study (GWAS) methodology.
4. To develop new KASP-type DNA markers associated with barley grain quality traits and to confirm their effectiveness.
5. To select promising barley lines for breeding using new KASP-type markers for grain quality traits.

Research methods. In this work, molecular genetic methods, field methods, methods for assessing grain quality traits, as well as statistical methods were used. Molecular genetic methods included isolation and purification of DNA, SNP- and

KASP-genotyping (including polymerase chain reaction – PCR). Field experiments were carried out in collaboration with Kazakh Research Institute of Agriculture and Plant Growing (Almalybak, Almaty region), Karabalyk Agricultural Experimental Station (Nauchnoe, Kostanay region), A.F. Khristenko Karaganda Agricultural Experimental Station (Tsentralnoe, Karaganda region), and I. Zhakhaev Kazakh Research Institute of Rice Growing (Kyzylorda city, Kyzylorda region) during several years. Analysis of grain quality was made in the laboratory of grain quality at the LLP “Kazakh Research Institute of Agriculture and Plant Growing”. The list of grain quality traits under the study included grain raw protein content (GPC, %), the content of raw starch (GSC, %), lipids (GLC, %), and cellulose (GCC, %) in the grain, grain extractivity (EX, %) using the Near Infrared Spectroscopy (NIRS) DS2500 Grain Analyzer (FOSS, Denmark), and the grain test weight per liter (TWL, g/L) using the GOST 10840-2017. Statistical methods included GWAS using GAPIT package for R and Multiple Loci Mixed Model (MLMM), population structure analysis using principal component analysis (PCA), and neighbor-joining (NJ) clustering, Bayesian Markov chain Monte Carlo (MCMC) approach with admixture and correlated allele frequency models in STRUCTURE software. Other statistics involved analysis of variance (ANOVA), Pearson correlation tests, t-tests, and the visualization of their results using packages for RStudio. In 2023, the Ph.D. applicant completed a scientific internship on the research topic at the John Innes Centre, Norwich, United Kingdom, under the supervision of a foreign scientific supervisor.

The scientific novelty of the research includes the identification of new QTLs associated with barley grain quality traits using modern genomic technologies to improve breeding research efficiency in Kazakhstan. For the first time, the genetic profile of barley cultivars from Kazakhstan using SNP markers was compared with foreign cultivars. QTLs associated with barley grain quality traits were identified using GWAS and collections harvested in northern, central, south-eastern, and southern Kazakhstan conditions. In total, 64 QTLs for six grain quality traits of barley were identified. Among them, 11 novel QTLs identified for GSC, GPC, GCC, GLC, EX, and TWL have never been described in the literature before, including 3 QTLs for multiple traits that were stable across environments. Fourteen stable QTLs were identified in two or more environments, exhibiting similarities with barley genes and/or QTLs documented in the literature. Using the GWAS results, 28 KASP-type markers have been developed for these important barley grain quality traits. Eight of them were successfully validated for their associations with GPC, GSC, EX, and TWL and recommended for traditional breeding and MAS of barley in Kazakhstan.

The study's practical significance is related to strengthening local breeding programs in Kazakhstan to increase barley grain quality using new genomic technologies. Twenty-eight KASP-type markers were developed based on the results of GWAS (patent for utility model #6643). Eight of them had successfully passed validation and were recommended for the breeding and MAS of barley in Kazakhstan. Using these markers, 4 promising barley lines with good grain quality for malting and

3 lines for feed production were identified. These lines may be recommended for breeding new high-quality malting and animal-feeding cultivars. Together with the Karabalyk Agricultural Experimental Station, a high-quality productive cultivar of spring barley “Atlet” was developed using classical and molecular breeding methods.

The theoretical significance of this study includes novel and confirmed QTLs for six important grain quality traits of barley identified using phenotypic data from field trials in northern, central, south-eastern, and southern Kazakhstan. All of them have a potential for further investigation and possible identification of candidate genes. Literature analysis showed that previously, in Kazakhstan, GWAS for barley grain quality traits and population analysis involving barley from Kazakhstan have never been done before. Results obtained in this study provide the platform for developing new strategies to enhance the efficiency of the barley breeding process, including MAS, aimed at developing new competitive cultivars for the internal and external markets.

The main provisions of the dissertation submitted for defense:

1. Wide range of phenotypic variability of six grain quality traits in spring barley collections, including samples from the USA, Kazakhstan, Europe and Africa, grown in 4 regions of Kazakhstan over several years of field experiments, meets the necessary GWAS criteria. For each region, accessions with the highest quality have been selected and recommended for use in breeding programs aimed at improving barley quality in accordance with the types of the end product.

2. The high genetic diversity and population structure of barley collections revealed via SNP genotyping meet the GWAS criteria.

3. The genetic similarity of barley samples from Kazakhstan with samples from Africa, Europe and Western Asia identified using analysis principal coordinates, confirms the usage of cultivars from these regions in barley breeding in Kazakhstan.

4. As a result of GWAS, 64 highly significant QTLs ($P < 3.14E-05$) were identified for the content of protein, starch, cellulose, lipids, grain test weight and extractivity, including 14 stable, 8 presumably new and 3 presumably new stable QTLs. All 64 QTLs is a source of new knowledge and practical significance for marker-assisted selection.

5. A set of 28 KASP-type markers was developed for the most significant QTLs ($P < 1.00E-6$) associated with the content of protein, starch, cellulose, lipids, grain test weight and extractivity of barley.

6. Eight KASP type markers confirmed significant associations ($P < 0.05$) with the content of protein, starch, grain test weight and extractivity found in GWAS were successfully used to identify promising barley lines and patented.

7. Newly developed KASP markers were used for the identification of 3 barley lines as a promising source for the breeding of food and feed cultivars, and 4 lines – for brewing and malting.

8. Together with the Karabalyk agricultural experimental station, a high-quality and productive cultivar of spring barley “Atlet” was developed using classical and molecular breeding methods.

Relation to the plan of main scientific works. The dissertation was performed

within grant No. AP08052804 “Development and validation of KASP-type markers efficiency for key productivity and grain quality traits in two-rowed spring barley” for 2020 – 2022 and the Scientific Technical Program BR18574149 “Development of highly productive cultivars and lines of agricultural crops based on innovative biotechnologies” (Task 2: Development of a highly productive cultivar of spring barley based on a combination of classical and molecular breeding methods) for 2023 – 2024 from the Ministry of Science and Higher Education of the Republic of Kazakhstan.

The personal contribution of a Ph.D. student to the preparation of each publication includes collecting data on the subject of research, performing the bulk of theoretical and experimental research, including analysis, interpretation, and presentation of the results, and preparing manuscripts for publication.

Approbation of research work. The results of the research were reported and published at international scientific and practical conferences “VI PlantGen” (Russia, 2021) and “Farabi Alemi” (Kazakhstan, 2022). The main results of the dissertation were reported annually at the scientific and technical council of the Faculty of Biology and Biotechnology, at meetings of the Department of Molecular Biology and Genetics of al-Farabi KazNU, scientific seminars of the Laboratory of Molecular Genetics at the Institute of Plant Biology and Biotechnology (IPBB), and the conferences of young scientists of IPBB. The results of the dissertation work were included in the annual Research Reports on the project AP08052804 (2020–2022) and Scientific Technical Program BR18574149 (2023–2024).

Publications. The main content of the thesis is presented in 12 publications, including 3 articles in journals with Q1 in the Web of Science database; 4 articles in local scientific journals included in the list of Committee for Quality Assurance in the Sphere of Education of the Ministry of Education of the Republic of Kazakhstan, 1 patent (utility model), 2 abstracts in the materials of international conferences in the foreign country (Russia) and the Republic of Kazakhstan, 1 monography, and 1 methodological recommendation.