

ABSTRACT

of the dissertation for the Doctor of Philosophy (Ph.D.) degree
on the specialty «6D060700 – Biology»

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on the topic: «Identification of QTLs for common wheat yield and grain quality traits
on the base of using biparental mapping populations»

The general description of work. The work is devoted to the identification of quantitative trait loci for adaptability, yield components and grain quality of common wheat using biparental mapping populations.

The relevance of topic. Common wheat (*Triticum aestivum* L.) is one of the richest energy and protein sources for humanity. Increasing common wheat production is essential for global food security (Curtis & Halford, 2014). Kazakhstan is one of the largest common wheat producers in the world. The success of the production of common wheat largely depends on the gene pool of cultivars, agricultural techniques and breeding programs aimed at creating highly productive cultivars. Progress in the genetic improvement of cereals over the last century has been based, among other things, on the use of single genes with relatively clear effects on the phenotype. Since yield and grain quality traits are complex with continuous variability and controlled by many genes (loci), the identification and mapping of quantitative trait loci (QTLs), the development of new informative markers for economically valuable traits is very important for breeding and genetic programs. The use of various types of mapping populations and informative DNA markers including KBiosciences competitive allele specific PCR (KASP) markers can be an effective tool for development new highly productive and high-quality cultivars.

The object of the work. Two biparental mapping populations of spring common wheat: 1) 101 doubled haploid lines (DHL) of the mapping population ‘Avalon × Cadenza’, 2) 94 recombinant inbred lines (RIL) of the mapping population ‘Pamyati Azieva × Paragon’. Also, for the validation of KASP markers, 95 cultivars of common wheat, including those approved for use on the territory of the Republic of Kazakhstan, were used.

The subject of the study. Identification and mapping of QTLs associated with traits of adaptability, yield components and grain quality of common wheat using two biparental mapping populations of common wheat.

The aim of the work. To identify and map quantitative trait loci for productivity and grain quality of common wheat using biparental mapping populations.

The tasks of the work.

1. To characterize the genetic maps of two biparental mapping populations of common wheat using DNA markers.

2. To study the phenotypic variability of two biparental mapping populations grown under conditions of northern, central, southern and southeastern Kazakhstan.

3. To study the level of variability of grain quality traits in two mapping populations grown under conditions of northern, central, southern and southeastern Kazakhstan.

4. To identify and map the QTLs of common wheat yield and grain quality components using two biparental mapping populations.

5. To develop informative DNA markers for the most important quantitative trait loci associated with traits of adaptability and yield components of common wheat, to improve the efficiency of common wheat breeding in Kazakhstan.

Research methods. The research involved field experiments, biochemical, molecular genetic, and statistical methods. Field experiments were carried out as a part of complex research in the following organizations: A. F. Khristenko Karaganda Agricultural experimental station, Karabalyk Agricultural experimental station, I. Zhakhayev Kazakh Research institute of rice growing, A.I. Baraev Research and production center for grain farming, North-Kazakhstan Agricultural experimental station and Kazakh Research Institute of Agriculture and Plant growing (KRIAPG). Determination of grain quality was carried out according to State standards of the RK at the laboratory of biochemistry and grain quality of KRIAPG. Molecular genetic studies were carried out using an appropriate material and technical base of the Laboratory of Molecular Genetics at Institute of Plant Biology and Biotechnology (IPBB) of the Ministry of Science and Higher Education of the RK. For the genotyping of common wheat samples, DNA isolation and purification, polymerase chain reaction (PCR), and modern DNA markers were used. QTL mapping was carried out using the QTL Cartographer applied genetic and statistical program Windows QTL Cartographer v2.5 (Wang et al., 2012). The MapChart v2.32 (Voorrips, 2002) and Windows QTL Cartographer v2.5 software was used for the visualization of QTLs on the genetic map. For statistical processing of the obtained data GenStat, GraphPad, SSPS, Rstudio, packages were used.

The scientific novelty of the research is the identification of 1) 89 presumably new QTLs and confirmation of 71 QTLs previously described in other research works associated with the adaptability, yield and grain quality of common wheat under conditions of Kazakhstan, and 2) the development and validation of 12 KASP markers for the identification of promising spring common wheat genotypes. Identification of 864 QTLs associated with traits of adaptability (heading date, period from heading to maturity, plant height), yield components (spike length, number of productive spikes, number of kernel per spike, thousand kernel weight, yield per 1 m²) and grain quality (grain test weight, protein content in the grain, gluten content, vitreousness, kernel hardness , sedimentation in acetic acid). Among them, 160 QTLs were stable, 525 were major QTLs and 89 were presumably new ones. For the first time, QTL identification was carried out using biparental mapping populations ‘Pamyati Azieva × Paragon’ and ‘Avalon × Cadenza’ in 4 regions of Kazakhstan.

The theoretical significance of this study is the identification of QTL associated with adaptability, yield and grain quality of common wheat using biparental genetic mapping involving recombinant inbred lines and doubled haploid lines populations under conditions of various agro-climatic zones of common wheat cultivation.

The practical significance of this study is the identification of 40 promising recombinant inbred lines and 15 doubled haploid lines in two mapping populations that exceed check cultivars by several traits of grain yield and quality. These lines are included in the breeding process at the Karabalyk Agricultural experimental station and A.I. Baraev Research and production center for grain farming. A preliminary set of 12 KASP markers associated with individual yield and grain quality traits has been

confirmed for use at the early stages of the breeding process, for genotyping and certification of common wheat cultivars.

The main statements of the defense:

1. It has been established that, two genetic maps of biparental doubled haploid ($A \times C$, 3647 DNA markers) and recombinant inbred (' $PA \times P$ ', 4595 SNP markers) mapping populations have high resolution for QTL mapping for the adaptability, yield and grain quality traits.

2. 40 recombinant inbred lines and 15 doubled haploid lines identified in the phenotypic assessment of two common wheat mapping populations, according to a set of yield and grain quality traits, were selected as valuable genotypes for breeding programs for the improvement of wheat in four regions of Kazakhstan.

3. 160 stable quantitative trait loci identified among 525 major loci in QTL-analysis of two mapping populations and associated with the traits of adaptability, yield and grain quality, identified in QTL analysis of two mapping populations, provide an important information on the localization of genetic factors controlling complex traits.

4. 89 loci identified in two mapping populations and associated with traits of adaptability, yield and grain quality are presumably new.

5. A set of KASP markers associated with traits of adaptability and yield components of common wheat has been developed. An effectiveness of 12 KASP markers, significantly associated with economically valuable traits, for the identification of valuable genotypes of spring common wheat was confirmed.

The main results and conclusions of the work:

1. The research characterizes genetic maps of two common wheat biparental mapping populations: 1) 94 recombinant inbred lines of 'Pamyati Azieva \times Paragon' (' $PA \times P$ ') 2) 101 doubled haploid lines of 'Avalon \times Cadenza' (' $A \times C$ '). Comparison of genetic maps of two biparental mapping populations showed greater total length of the genetic map ' $A \times C$ ' (3246.9 cM) than the ' $PA \times P$ ' (2723.9 cM). Both mapping populations (' $A \times C$ ' with 3647 DNA markers and ' $PA \times P$ ' with 4595 SNP markers) having 222 common SNP markers were used for QTL mapping for the traits of adaptability, yield, and grain quality.

2. Analyzing two mapping populations, both in terms of yield and grain quality traits, 63 recombinant inbred lines of ' $PA \times P$ ' exceeded check cultivars by yield per 1 m² (YM2) at A.I. Barayev research and production center for grain farming (38 RILs), North Kazakhstan Agricultural Station (6 RILs), and KRIAPG (19 RILs). Two lines of ' $PA \times P$ ' ($PA \times P$ -01 and $PA \times P$ -05) demonstrated high YM2 at all three regions. Also, among the ' $A \times C$ ' mapping population grown in four regions of Kazakhstan, 61 the most productive doubled haploid lines were identified under the conditions of Karabalyk Agricultural Station (9 DHL), Karaganda Agricultural Station (22 DHL), Kazakh Research Institute of Rice Growing (26 DHL) and KRIAPG (4 DHL). Two lines $A \times C$ -52 and $A \times C$ -55 showed the highest yield under three conditions at once (north, center, south), which indicates a high genetic and breeding potential of these genotypes.

3. As a result of grain quality traits analysis in the mapping population ' $PA \times P$ ' grown in the North Kazakhstan region, a wide range of distributions by classes was noted for the following characteristics: grain size, grain protein content, gluten content, and kernel hardness. For grain quality traits of the ' $A \times C$ ' doubled haploid population,

among four study regions a wide range of distribution by class by grain size was noted. In terms of grain quality, 35 valuable and 12 strong recombinant-inbred lines were identified in the 'PA×P' population and 12 valuable and 3 strong doubled haploid lines were selected in the 'A×C' population. Based on the analysis results of yield and grain quality traits, 40 recombinant-inbred lines ('PA×P') and 15 doubled haploid lines ('A×C') were identified as the most valuable genotypes for increasing the efficiency of spring common wheat breeding in Kazakhstan.

4. Based on the results of QTL analysis among 'PA×P' mapping population, 468 QTLs were identified for 14 traits of adaptability, yield and grain quality, of which 229 were major QTLs, 68 were stable QTLs, and 38 QTLs were presumably novel. As a result of QTL analysis among the 'A×C' doubled haploid population, 92 stable and 296 major QTLs were found among 482 identified loci for 13 economically valuable traits. Among the 92 stable loci, 51 QTLs are presumably novel ones. Comparing the QTLs found in two mapping populations ('PA×P' and 'A×C'), it was found that 6 loci were common in two populations for the following traits: plant height, spike length, number of productive spikes, 1000 kernels weight positioned on chromosomes 1A, 2D, 5A (3 QTL), and 6A. Thus, as a result of the QTL analysis of two mapping populations, a total of 864 QTLs were identified, of which 160 were stable QTLs, 525 QTLs were the major loci, 89 QTLs were presumably novel and 71 QTLs were known previously in other studies.

5. Based on the QTL analysis of two mapping populations, a preliminary set of KASP markers was developed. It consists of 17 SNP markers successfully transformed into KASP markers. According to the results of genotyping, only 12 out of 17 KASP markers were polymorphic for 95 common wheat accessions. Based on the results of validation of these 12 KASP markers, a statistically significant difference was revealed between groups of samples under two conditions with different alleles for 6 KASP markers: *ipbb_ta_169* (yield per 1 m²), *ipbb_ta_171* (peduncle length, number of productive spikes, number of grains per spike, 1000 kernels weight), *ipbb_ta_184* (heading time, vegetation period), *ipbb_ta_185* (number of grains per spike, yield per 1 m²), *ipbb_ta_197* (heading time, vegetation period, peduncle length), and *ipbb_ta_199* (number of grains and length of the spike). A patent for a utility model based on a set of 6 effective KASP markers was registered.

Connection with the plan of main scientific works. The dissertation work was carried out in the project AP08855387 "Nested association mapping for gene discovery and deployment for improvement of yield, quality, and disease resistance in bread wheat" by the Ministry of Education and Science of the Republic of Kazakhstan (now Ministry of Science and Higher Education) for 2020-2022, AP14871383 "Development of KASP markers to facilitate construction of competitive bread wheat, durum wheat, and barley cultivars in Kazakhstan", and the project "Creation of new DNA markers of drought resistance of spring wheat grown in the conditions of Northern Kazakhstan" under the budget program of the Ministry of Agriculture of the RK number BR06249219 "Selection and seed production of drought-resistant, productive, high-quality varieties of spring wheat on the basis of classical methods of selection and modern approaches of biotechnology for the conditions of Northern Kazakhstan" for 2018-2020 Scientific supervisor of projects was candidate of biological sciences, professor Turuspekov Y.

Approbation of work. The results of the dissertation were reported and published at international scientific and practical conferences: international congress «VII Congress of the Vavilov Society of Geneticists and Breeders, dedicated to the 100th anniversary of the Department of Genetics of St. Petersburg State University, and associated symposiums» (Russia, 2019), the VI International Conference PlantGen2021 (Russia, 2021). International scientific conference for students and young scientists «Farabi Alemi» (Kazakhstan, 2019-2021); International Conference on Veterinary, Medicine, Agriculture and Life Science (Türkiye, 2022), IV International Congress of Plant Breeding (Türkiye, 2022). The main results of the dissertation work were annually reported for the scientific and technical council of the faculty «Biology and biotechnology», at KazNU's «Biodiversity and bioresources» department meetings, scientific seminars of the laboratory of molecular genetics, at young scientists conferences of Academic Council of «Institute of plant biology and biotechnology». The results of the dissertation work were partially included in the Report on the research work of projects BR06249219, AP08855387, and AP14871383.

Publications. The main content of the dissertation is reported in 16 scientific publications, including 5 articles in international peer-reviewed journals with an impact factor indexed at Scopus and Web of Science; 3 articles in journals from the list of publications recommended by the Committee for Control in the Sphere of Education and Science (CCSES) of the Ministry of Science and Higher Education of the Republic of Kazakhstan for the publication of the main results of scientific activity; 1 patent (utility model), 7 abstracts in the materials of international and local conferences including 4 foreign.

The personal contribution of PhD-student to the preparation of each publication includes collection of data on the subject of research, performing the theoretical and experimental research, including analysis, interpretation and presentation of the results, and preparation of manuscripts for publications.

The volume and structure of the dissertation. The dissertation is presented on 152 pages and includes following paragraphs: designations and abbreviations, introduction, literature review, materials and methods, results and discussions, conclusion, list of references including 230 items, 179 of which are English. The dissertation work contains 41 tables, 40 pictures, and 6 supplementary materials.