

ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (Ph.D.) on the specialty "6D070100-Biotechnology"

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on the topic "Identification of QTL loci of durum wheat (*Triticum durum* Desf.) associated with yield components based on genome-wide association study"

The general description of work. The work is devoted to the identification of quantitative trait loci for the yield components of durum wheat (*Triticum durum* Desf.) using the methodology of genome-wide association study.

The relevance of the topic. Durum wheat (*Triticum durum* Desf.) is one of the important cereals in the World and Kazakhstan (FAO, 2019). A stable increase in the production of high-quality durum wheat, which is a strategic and export grain crop, is one of the important directions for ensuring food security, both globally and domestically (KazakhZerno, 2019). The dissemination of modern knowledge and the use of new technologies in genetics and breeding is an important factor in the success of the implementation of scientific research results in applied science. The world experience in the introduction of molecular genetic technologies in breeding is a positive example for Kazakhstan, which makes it possible to significantly accelerate and modernize the breeding process to create highly productive and high-quality cultivars. In this regard, it is very important to study the genetic resources of durum wheat of Kazakhstan and foreign collection using both traditional breeding methods and modern methodologies of molecular genetics, genomics, and marker-assisted breeding.

The object of the work. 300 cultivars and lines of durum wheat (*Triticum durum* Desf.).

The subject of the study. Analysis of marker-trait associations based on the use of SNP markers associated with economically valuable traits of durum wheat adaptability and yield.

The aim of the work. Identification of quantitative trait loci (QTL) associated with adaptation traits and yield components of durum wheat (*Triticum durum* Desf.) based on the use of phenotyping and high-density genotyping of studied collection and association mapping approach.

The tasks of the work:

1. Phenotypic study of durum wheat collection consisting of 300 foreign and local cultivars and lines in the conditions of the North Kazakhstan and Almaty regions of Kazakhstan.

2. Assessment of the genetic diversity of the durum wheat collection based on the use of SSR- and SNP-markers.

3. Identification of the QTL of durum wheat economically valuable traits based on the use of the association mapping method.

4. Development of KASP-markers using identified SNP-markers of durum wheat economically valuable traits.

Research methods. Agrotechnological work was carried out within the framework of comprehensive research with the North Kazakhstan Agricultural

Experimental Station (NKAES) and Kazakh Research Institute of Agriculture and Crop Production (KRIACP, Almaty region). The following phenological phases (number of days at 50 % trait occurrence) were assessed: seedlings, phase of four leaves, tillering, phase of stem elongation, the appearance of a flag leaf, booting, heading, flowering, maturation. The analysis of durum wheat accessions was carried out according to the following traits: plant height (cm), length of the upper internode (cm), number of productive ears per plant (pcs), length of the main spike (cm), number of grains of the main spike (pcs), weight of grains of the main spike (g), weight of grains per plant (g), weight of 1000 grains (g), yield (g/m²). For genotyping – SSR, SNP, and KASP (*KBiosciences competitive allele specific PCR*) markers, DNA isolation, polymerase chain reaction (PCR), electrophoresis in polyacrylamide gel (PAGE) were used. The search for QTLs was carried out using association mapping method (GWAS). For statistical processing of the obtained data, GenStat, GraphPad, GenAEx 6.5, STRUCTURE 2.3.4, TASSEL 5.0, GAPIT R software packages were used.

The scientific novelty of the research lies in the identification of new informative DNA markers associated with the adaptability and yield components of durum wheat based on the use of modern genomic technologies aimed at increasing the efficiency of breeding research in Kazakhstan. For the first time, based on the use of genome-wide association study (GWAS), the QTLs associated with the yield of durum wheat in the southeast and north of Kazakhstan were identified. 32 QTLs were identified that were not previously found in durum wheat studies, including 8 loci of adaptability traits (heading time, plant height) and 24 loci of yield components (spike length, number of productive spikes, 1000 grain weight). Based on the results of the GWAS, KASP markers have been developed that control the productivity components of durum wheat in the southeast and north of Kazakhstan.

The practical significance of the study is associated with the strengthening of domestic breeding programs to increase the yield of durum wheat, based on the use of new genomic technologies. In particular, new informative DNA-markers have been proposed for the breeding organizations of the country for the express analysis of the yield potential of durum wheat cultivars and lines at the early stages of breeding. 30 durum wheat promising cultivars and lines have been identified. DNA-passports of commercial cultivars and promising lines of durum wheat of Kazakhstan were created using two types of DNA markers – SSR and KASP, associated with adaptability and yield traits.

The theoretical significance of this study lies in the identification of QTLs associated with an increase in the yield of durum wheat in the conditions of Kazakhstan. Analysis of local and foreign literature has shown that in Kazakhstan, similar work has not yet been carried out at the molecular genetic level to study the durum wheat genetics, including GWAS. The prospects of a fundamental nature consist in preparing an important platform for developing new strategies to increase the efficiency of the breeding process for durum wheat, including marker-assisted breeding, aimed at creating new competitive cultivars.

The main statements of the defense:

1. Ecologically tested in the northern and southeastern regions of Kazakhstan, cultivars and lines of the durum wheat world collection, ranked according to adaptability and productivity, are valuable sources for breeding programs in Kazakhstan.

2. The 59 quantitative trait loci (QTL) identified as a result of genome-wide association study (GWAS) are associated with 5 important durum wheat adaptability and yield traits (heading time, plant height, spike length, number of productive spikes, 1000 grain weight) using 16425 SNP-markers in two growing zones – in the northern and southeastern grain-growing regions of Kazakhstan.

3. Among the identified QTLs, 32 loci are presumably new, statistically reliably associated with economically valuable traits of durum wheat in Kazakhstan.

4. Genetic passports of durum wheat promising cultivars and lines of Kazakhstan by SSR and SNP markers can be used to identify valuable genotypes and in marker-assisted breeding of durum wheat.

5. Developed a set of KASP-markers associated with economically valuable traits as a way to identify durum wheat (*T. durum* Desf.) breeding-valuable lines.

The main results and conclusions of the work:

1. The world durum wheat collection, consisting of 300 accessions, was first studied in the field conditions of northern and southeastern Kazakhstan in 2018-2020 growing years. Average "yield per square meter" (g/m^2) based on field trials of three years in northern Kazakhstan made it possible to identify 52 accessions of the world collection, which surpassed the yield of the local standard cultivar "Damsinskaya yantarnaya", which indicates the high value of foreign accessions of durum wheat for breeding research in Kazakhstan.

2. According to the phenotypic analysis and analysis of "genotype-environment" (GGE), the most promising accessions in the Almaty region (above or at the level of the check cultivar) were Aghrass-1, Aizen-1, Appio, Berillo, Bronte, Cosmodur, Fiore, Granizo, Lesina, Pedroso, Quadrato, Strongfield, Trinakria, Altayka, Altaiskyi yantar, Kharkovskaya 46. As the most promising (above or at the level of the check cultivar) for the North Kazakhstan region, were AC Navigator, Barcarol, Cappelli, Lloyd, Taganrog, Tiziana, Waskana, G2609, G2607, G2611, G2638, Kustanayskaya 1, Orenburgskaya 10, Saratovskaya 31.

3. Revealed a positive correlation between the plant height and the length of the upper internode ($r = 0.9^{***}$ (KRIACP); $r = 0.8^{***}$ (NKAES)); the number of productive spikes and the weight of grains per plant ($r = 0.7^{***}$ (KRIACP); $r = 0.6^{***}$ (NKAES)); the number of grains per spike and the weight of grains per plant ($r = 0.5^{***}$ according to the data of two regions).

4. Analysis of variance (ANOVA) based on field data from three years made it possible to establish that the contribution of the "environment" factor to the total variance of the traits "heading time", "flowering time", "number of grains of the main spike" and "yield" was statistically significant exceeded the contribution of the factor "genotype", which indicates the importance of taking into account the influence of the environment on the variation of these traits.

5. The collection, consisting of 29 spring durum wheat cultivars from Kazakhstan, Russia, and Ukraine (including those included in the State Register of

the Republic of Kazakhstan) and 15 lines of the Karabalyk agricultural station was characterized using 9 polymorphic SSR-markers. Genetic passports of cultivars and promising lines of durum wheat have been created for 9 SSR-markers, which can be used both for selection of pairs for crossing, in marker breeding, and for the protection of breeders' rights.

6. Based on the phylogenetic analysis of the durum wheat collection using 16425 polymorphic SNP-markers, wheat cultivars of Kazakhstan (Gordeiforme 254, Kostanayskaya 12, Kostanayskaya 52, Chernokolosaya 20) formed one joint group with cultivars of Russia (Almaz, Omskyi Rubin, Saratovskaya 31), Ukraine (Kharkovskaya 46) and the USA (Langdon).

7. Using the durum wheat collection for the GWAS analysis, 59 stable QTLs were identified for 5 traits (heading time, plant height, spike length, number of productive spikes, 1000 grain weight).

8. Comparison of the 59 identified QTLs with previously published durum wheat GWAS studies revealed 32 presumably new (previously unknown) QTLs.

9. Twenty durum SNP markers identified by GWAS analysis were converted to KASP-markers. This set of KASP-markers associated with various economically valuable traits can be recommended for use in breeding programs.

10. The validation of 20 KASP markers on 44 durum wheat accessions was carried out. Statistical significance with the studied traits was shown by 5 KASP markers: *ipbb_td_106* (number of productive spikes), *ipbb_td_107* (number of productive spikes), *ipbb_td_116* (spike length), *ipbb_td_117* (spike length), *ipbb_td_119* (1000 grain weight).

Connection with the plan of the main scientific works. The dissertation was carried out within the framework of the project "QTL mapping of agronomic traits of durum wheat *Triticum durum* Desf. based on genome-wide association study" (State registration number: 0118RK00250, 2018-2020) on the budget program of the Ministry of Education and Science of the Republic of Kazakhstan 217 "Development of Science", subprogram 102 "Grant financing of scientific research" for 2018-2020. Priority: 4. Life and health sciences. Sub-priority: 4.1 Basic and applied research in the field of biology. – Physiological, biochemical and molecular-genetic mechanisms of plant, animal and human life, their adaptation to biotic and abiotic factors of the environment.

Approbation of work. The research results were reported at the international scientific and practical conferences "Biotechnology in crop production, animal husbandry and veterinary medicine" (Russia, 2018), "From Seed to Pasta" (Italy, 2018), the international congress "VII Congress of the Vavilov Society of Geneticists and breeders dedicated to the 100th anniversary of the Department of Genetics, St. Petersburg State University, and associated symposia "(Russia, 2019), "VI International Farabi Readings" (Kazakhstan, 2019). The main results of the dissertation were heard at the scientific seminars of the faculty "Biology and biotechnology", at meetings of the department "Biotechnology" of "al-Farabi Kazakh National University", scientific seminars of the molecular genetics laboratory and Scientific Council of "Institute of Plant Biology and Biotechnology".

The results of the dissertation are included in the Report on research work on project AP055131328 (2018, 2019, 2020).

Publications. The main content of the dissertation is published in 11 printed works, including 2 articles in publications indexed in the Web of Science or Scopus databases; 1 short communication indexed in the Scopus database; 2 articles in republican scientific journals included in the list of KOKSON MES RK, 1 patent (utility model), 5 abstracts in materials of international conferences held in the far abroad (Italy), near abroad (Russia) and the Republic of Kazakhstan.

The personal contribution of the PhD student to the preparation of each publication consisted in performing the bulk of theoretical and experimental research, including analysis, interpretation, and presentation of the results obtained, and preparing manuscripts of publications.

The volume and structure of the dissertation. The dissertation is presented on 111 pages and consists of designations and abbreviations, an introduction, a review of literature, materials and methods, results and discussions, a conclusion, a list of used sources of 248 titles. Contains 33 tables, 30 figures, 3 appendices.