ABSTRACT

of thesis work entitled «Identification of wheat genotypes resistant to common bunt (*Tilletia spp.*) of adapted to South-East of Kazakhstan» for the Doctor of Philosophy (PhD) in the «8D05101– Biology» education program of Bakirov Serik Bakiruly

The theme of the research: Identification of wheat genotypes resistant to common bunt (*Tilletia spp.*) of adapted to South-East of Kazakhstan.

The purpose of the study: Identification of wheat samples that are resistant to populations of *Tilletia caries* (D.C.) Tul in the Almaty region, as well as the identification of sources of *Bt*-resistance genes.

Objectives of the research:

- Monitor and test the resistance of wheat samples to the pathogen *Tilletia* caries (D.C.) Tul by creating an artificial infectious background in a laboratory and field conditions:
- To identify the source of genes (Bt8, Bt9, Bt10, Bt11, Bt12) resistant to common bunt in wheat samples using molecular PCR methods;
- To calculate biomass indices during different stages of ontogenesis in the selected wheat samples;
- To conduct a structural analysis of resistant and susceptible wheat samples in the collection to determine their economically valuable characteristics;
- To contribute to the production of wheat samples with enhanced resistance and productivity against the common bunt (*Tilletia caries* (D.C.) Tul.) based on the research findings.

Research methods:

To complete the tasks outlined in the research work, the following methods have been applied: One of the most effective methods of inoculation of wheat samples with common bunt spores is the Borghardt–Anpilogova method, in which seeds are infected with fungi spores a few days before planting. In phytopathological approbation of wheat samples infected with the pathogen *Tilletia caries* (D.C.) Tul, V.I. Krivchenko's (1984) scale was used. In order to determine the source of Bt gene resistance to common bunt, the molecular PCR analysis method was applied. The determination of the plant biomass index (NDVI – Normalized Difference Vegetative Index) was performed using the Green Seeker (Trimble Navigation Limited, United States). The determination of the productivity indicators of wheat samples was carried out by structural analysis of characteristics. The obtained data are corrected by mathematical calculations according to the method of B.A. Dospekhova (1985). The statistical processing was performed using Excel and Mini TAB (Anova) 21 software.

The main provisions proposed for defense (proven scientific hypotheses and other new knowledge conclusions):

- Local soft wheat varieties and foreign wheat samples (Bulgaria, Hungary, Romania, CIMMYT) have been tested for resistance to common bunt under natural and artificial infectious conditions.
- Biomass index indicators (NDVI) have been taken into account in the stages of the vegetative development of wheat sources (head, flowering, milk ripeness). NDVI indicators are used to monitor crop health, determine yield potential, identify stressors, and determine the impact of pests and diseases.
- Phenological monitoring was conducted during the growing season of commercial wheat varieties and foreign wheat samples that were allowed to be planted in Kazakhstan to meet Kazakhstan's production needs, and structural analyses of economically valuable characteristics were carried out. Indicators of disease-causing infections of wheat varieties and other characteristics determining yield and quality are directly related to the vegetative stage.
- The resistance of Bt genes to the pathogenic *Tilletia caries* (D.C.) Tul is determined by molecular methods. At the global level, *Bt-1*, *Bt-2*, *Bt-3*, *Bt-4*, *Bt-5*, *Bt-6*, *Bt-7*, *Bt-8*, *Bt-9*, *Bt-10*, *Bt-11*, *Bt-12*, *Bt-13*, *Bt-14* and *Bt-15* are sources of common bunt resistant genes. However, these genes are not equally effective in all regions of the world. Thus, the identification of the relevant gene sources to our region is required.
- Through phytopathological and molecular testing, selective samples of wheat resistant to common bunt were proposed for commercial sowing.

The main results of the research:

- The resistance of domestic wheat varieties and foreign wheat samples to the *Tilletia caries* (D.C.) Tul pathogen of Almaty region was tested by the creation of artificial infectious backgrounds on the field. According to phytopathological assessments,12 domestic wheat varieties were highly resistant to common bunt. Of the foreign wheat samples, 6 samples from Bulgaria, 9 samples from Hungary, 8 samples from Romania and 7 samples from Turkey, which obtained from the CIMMYT center showed tolerance for common bunt.
- In the genetics and breeding laboratory of the Institute of Plant Biology and Biotechnology, the resistance of Bt gene to common bunt was molecularly identified in domestic and foreign wheat samples. Upon molecular screening, the *Bt8*, *Bt9*, *Bt10*, *Bt11* and *Bt12* genes resistant to the *Tilletia caries* (D.C.) Tul pathogen were identified from domestic and foreign wheat samples tested for common bunt in an artificial infectious background.
- During the stages of heading, flowering and milk ripening, the biomass index (NDVI) indicators of wheat samples were taken into account. The high biomass index indicators in wheat samples that were domestically allowed to be cultivated in Kazakhstan comprise 12 wheat varieties. Furthermore, 12 Bulgarian, 5 Hungarian, 5 Romanian and 6 Turkish wheat samples were distinguished from foreign wheat samples with high biomass index indicators.

Analysis of the structural characteristics of the wheat sample revealed
domestic wheat varieties with high productivity and common bunt resistance.
Among foreign wheat samples, 7 Bulgarian, 8 Hungarian, 6 Romanian and 6 Turkish wheat samples showed higher economic value.

Justification of the novelty and importance of the obtained results:

- the novelty of the first result is that the resistance of wheat samples to the pathogen *Tilletia caries* (D.C.) Tul was tested within the artificial infectious background. The *significance* of the obtained results showed that 32 percent of domestic and foreign wheat samples were resistant to common bunt. In light of the phytopathological assessment, 12 local wheat varieties have been identified as highly resistant to common bunt. The resistance of common bunt was determined among foreign wheat samples including 6 Bulgarian samples, 9 Hungarian samples, 8 Romanian samples and 7 Turkish samples obtained from the CYMMIT center.
- novelty of the second result for the first time in Kazakhstan, the resistant gene sources to common bunt were determined from wheat samples on the basis of molecular PCR analysis. The significance of the results is that wheat samples protected by the Bt-resistant gene are long-term resistant to disease. The use of resistant wheat samples in agriculture inhibits the use of chemicals in the fight against common bunt diseases and maintains the ecological balance of the environment. PCR analysis in the research showed that a combination of five resistance genes, Bt8, Bt11, Bt9, Bt10 and Bt12, were identified in the Karasai variety. The 4 gene combination was identified as Bt8, Bt9, Bt10, Bt12 in Dinara, Egemen 20, Sultan 2, Kazahstanskaya 16 and Kazahstanskaya 75 varieties, and in (Alatau, Azharly, Bezostaya 1, Zhetysu, Kokbidai, Mereke 75, Naz, Sultan 95, Sanzar 8, Sapaly) 10 varieties 3 gene combinations (Bt8, Bt9, Bt10) were identified. In Kazakhstanee wheat varieties, 14 genes (Akbidai, Adyr, Bulava, Diana, Zhalyn, Krasnovodopadskaya, Karabalykskaya ostistaya, Karlygash, Koksu, Kyzylbidai, Matai, Mironovskaya 808, Steklovidnaya 24, Almaly) were identified as carriers of Bt9 and Bt12. Molecular screening of Romanian wheat samples tested for common bunt revealed that 2 samples (02429GP-1, F08245G1) possessed the Bt9-gene. In the Romanian sample F08034G1, a combination of 4 genes (Bt8, Bt10, Bt11, Bt12) was identified. Following molecular screening, 3 samples (Berény, Petur, Rába) and 4 samples (Ati, Bereny, Koros, Petur) were characterized as Bt10 gene carriers from Hungarian wheat samples. Klara, Demetra, Zlatitsa, Todora, Korona, Milena, Pobeda and Sadovo-1 samples were identified as carriers of the Bt9 gene from Bulgarian wheat samples. Among wheat samples from CIMMYT Center (SAULESKU#26/PARUS//F885K 1.1/SXL/3/BEZOSTAYA1) and (TREGO/BTYSIB//ZARGANA-6/4/AU/CO652337//2*CA8-155/3/) were identified 5 genes (Bt8, Bt11, Bt9, Bt10, Bt12), which showed resistance.
- novelty of the third result biomass index (NDVI) indicators were taken into account in the growth and development phases of wheat samples. The *significance* of the obtained results is revealed in the comparative analysis of the biomass index

indicators of wheat samples at the stage of heading, flowering, and milking. In these periods, high biomass index indicators have a direct positive impact on plant productivity. Therefore, if the biomass index is low in these three periods, plant productivity will be low. During field research, domestic wheat samples that allowed to be cultivated for production needs, such as Zhetysu, Raminal, Nureke Krasnovodopadskaya 25, Egemen 20, Karasai Reke Prezident Mereke 70, Manshuk Kazahstanskaya 16, Arai, Akmola 3, Shortandinskaya 2012, Shortandinskaya 95 and Kazahstanskaya10, were noted to have high biomass index indicators. The NDVI indicated that among foreign wheat samples 12 Bulgarians (Demeifa, Antonovka, Neda, Karat, Svilena, Dragana, Enola, Kristi Merilin, Laska, Korona, Boryana), 5 Hungary (Békés, Kalász, Göncöl, Szemes, Rába), 5 Romanians (F08245G1, 02429GP-1, F06659G-1, F06393GP10, F07270G2) and 6 Turkish (338-K1-1//ANB/.../ZARGANA-4), (TX87V1613/... *3/AMI//BUC/CHRC), (338-K1-1/...*3/AMI//BUC/CHRC), #26/.../3/BEZOSTAYA1), (SAULESKU (TREGO/...//2*CA8-155/3/...), (TREGO/JGR 8W/4/AGRI/NAC//...) had a high biomass index indicators.

- novelty of the fourth result Analysis of the structural features of wheat samples and determination of economic value indicators. The significance of the obtained results is that the characteristics that affect wheat productivity are the length of the plant, the length of the spikes, the number of spikes, the number of grains in the largest spikes, the weight of the grain in the largest spikes, and the weight of 1000 grains. During structural characteristics analysis, the correlation link between spike number and the grain weight of the main spike, along with the grain weight of 1000 grains was determined. The structural characteristics of wheat samples were analyzed, and 12 domestic wheat varieties Zhetysu, Sapaly, Raminal, Dinara, Karasai, Kyzylbidai, Naz, Mereke 75, Kazahstanskaya 25, Zhenis, Astana and Kazahstanskaya 10 were identified as a high productive and resistant to common bunt. According to the economic value analysis of foreign wheat samples, 7 Bulgarian (Demeifa, Aiika, Karat, Todora, Enola, Geya-1, Sadova 1) wheat samples, 8 Hungarian (Békés, Körös, Mentor, Pilis, Petur, Vitorlás, Rege, Rába) wheat samples and 6 Romanian (PARTENER, F08347G8, F06659G-1, F08126G1, F08034G1, F07270G2) wheat samples were considered as a high quality. And the wheat samples from CYMMIT center, those are distinguished by a high productivity indicator (SUNR30 /...ZARGANA-3), (KATEA-1/...*CA8-155/3/F474S1-1.1), (KATEA-1//.../KAUZ), (338 -K1-1/... MERCAN-1), (338-K1-1/...WELS-2) and (SAULESKU...BEZOSTAYA1).
- the novelty of the fifth result Wheat samples identified as resistant to common bunt (Tilletia caries (D.C.) Tul and selected for high productivity in the Almaty oblast were offered for cultivation on farms. The *significance* of the results was that the Heti, Egemen 20, Karasai, Kishilbidai, Naz, Almaly, Mereke 75, Zhalyn, Kazakh 16 and Dinara domestic varieties with high performance and

resistance to common bunt were recommended to be planted in the fields of the agricultural company "MENEN" located in the Sarkant region of the Almaty oblast.

The *Bt8*, *Bt9*, *Bt10*, *Bt11* and *Bt12* effective genes were identified by molecular screening of foreign wheat samples tested for common bunt resistance: 02429GP-1, F08245G1, F08034G1, Ati, Bereny, Koros, Petur, Berény, Petur, Rába, Klara, Demetra, Zlatitsa, Todora, Korona, Milena, Pobeda and Sadovo-1 samples were presented to the Genetic Fund Department of the "Kazakh Agriculture and Plant Breeding Research Institute" LLP. It was included in the hybridization program as a donor resistant to common bunt disease of wheat.

Coherence with the directions of development of science or state programs:

The research work is consistent with the state programme for the development of education and science in the Republic of Kazakhstan from 2020 to 2050, the state programme "Digital Kazakhstan".

Description of the contribution of doctoral students to the preparation of each publication (the contributions of the author of the dissertation are expressed in percentages of the total text)

The 11 scientific articles published on the subject of the thesis. 2 articles were published in publications in scientific journals listed in Scopus and WoS international databases, 4 articles in publications in the field of science and higher education approved by the Quality Assurance Committee of the Ministry of Education and Culture of the Republic of Kazakhstan, 2 articles in scientific journals of Kazakhstan, and 3 articles in publications of materials from international scientific and practical conferences held in the Republic of Kazakhstan. All publications are based on research.

Publications in scientific journals listed in Scopus, WoS international database:

1. Screening of wheat genotypes for the presence of common bunt resistance genes // Saudi Journal of Biological Sciences. – 2021. – Volume 28, Issue 5. – P. 2816 – 2823. (Percentile – 86 (Q2), (Bakirov S.B. 70%), (Co-authored by: Madenova A., Sapakhova Z., Galymbek K., Yernazarova G., Kokhmetova A. 30%).

This article presents information on the identification of sources of Bt genes that have demonstrated resistance to common bunt in both domestic and foreign wheat samples authorized for cultivation in Kazakhstan.

2. Searching for resistance sources to wheat common bunt (*Tilletia caries* (D.C.) // The Bulletin The national academy of sciences of the republic of Kazakhstan. – Almaty, NAS RK. – Vol. 1, №389.– P. 50 –57. (Bakirov S.B. 60%), (Co-authored by: A.K. Madenova, A.M. Kokhmetova, M.N. Atishova, Zh.S. Keishilov 40%).

The article presents data on the evaluation of the resistance of Hungarian wheat samples to *Tilletia caries* (D.C.) Tul in an artificially created infectious environment in the Almaty region of Kazakhstan.

Publications in the field of science and higher education approved by the Quality Assurance Committee of the Ministry of Education and Culture of the Republic of Kazakhstan (QAC):

3. Determination of resistance of wheat samples to brown rust (*Puccinia triticina Eriks*) population. "Science and Education" Zhangir Khan scientific-practical magazine of BKATU. - 2022. - №. 1. - P.152-162. (Bakirov S.B. 65%), (Co-author: Galymbek K., Madenova A., Amangeldyovna Z., 35%).

The article presents findings on the resistance of Kazakhstani wheat varieties to the population of Turkish brown rust during their growth period.

4. Identification the sources of resistance genes to common bunt from samples of romanian wheat // Bulletin of the Korkyt Ata Kyzylorda University. − 2022. - № 2 (61). − P. 114-122. (Bakirov S.B. 70%), (Co-authored by: Galymbek K., Madenova A.K., Safarova N.S., Kulzhanova D.K. 30%).

The article reports on the assessment of the resistance of Romanian wheat samples to the pathogen Tilletia caries (D.C.) Tul, under artificially created infectious conditions in the Almaty region, with the calculation of biomass index indicators.

5. Testing Romanian wheat samples for resistance to severe black soot and determination of biomass index // Science Herald of the Kazakh Agrotechnical University named after S. Seifullin. – 2022. - №. 2(113). - P. 217-227 (Bakirov S.B. 70%), (Co-author: Galymbek K., Madenova A.K., Safarova N.S., Amangeldinova M.E., Kalidilda A.M. 30 %).

The article provides data on the influence of *Tilletia caries* (D.C.) Tul pathogens on the biomass index of Romanian wheat samples and their economic value.

6. Identification of germplasm of wheat resistant to common blight (Tilletia caries (DC.) Tul). "Science and education" are a scientific and practical journal of the Zhangir Khan State Technical University. No. 3-3 (68), 2022. P 105-113; (Bakirov S.B. 70%), (Co-authored by: Galymbek K., Madenova A.K., Kadir A. 30%).

The article examines the testing of 25 wheat varieties and 23 effective *Bt*-isogenic lines for their resistance to the *Tilletia caries* (D.C.) Tul pathogen in the Almaty region.

Publications in scientific journals of Kazakhstan:

7. Resistance of Hungarian wheat lines to *Tilletia caries* (D.C.) Tul population of Almaty region. "Research, results", - Almaty, 2021. - № 1. - P. 184-193. (Bakirov S.B. 70%), (Co-authors: Madenova A.K., Galymbek K., Kadir A., Sabdenaliyeva G.M. 30%).

The article presents data on phytopathological assessment of the resistance of Hungarian wheat samples to common bunt.

8. Testing the resistance of wheat samples to the pathogen *Tilletia caries* (D.C.) Tul // Reports of the National Academy of Sciences of the Republic of Kazakhstan.

– 2022. – Part 1, № 34.- P. 12-20 (Bakirov S.B. 65%), (Co-author: Galymbek K., Madenova A. K., Akan K., Safarova N. S. 35%).

The article examines resistance of wheat varieties and isogenic lines to the *Tilletia caries* (D.C.) Tul pathogen with comparative results.

Publications of materials from international scientific and practical conferences held in the vicinity of foreign countries:

9. Identification of wheat hermoplasma resistant to common bunt (*Tilletia caries* (D.C.) // Doctor of Pedagogical Sciences, professor Shildebayev Zhumadil Baidildauly, dedicated to his 75th anniversary "30th anniversary of the independence of Kazakhstan: topical issues of biological and environmental education in secondary and higher schools (innovation and experience)" international scientific and practical conference, Almaty, 2021. - P. 374-376; (Bakirov SB 65%), (Co-author: Akan K., Galymbek K., Madenova A. K., Safarova N. S. 35%).

This article presents results on the resistance of foreign wheat samples to the common bunt disease in the Almaty region.

10. New races and patterns of virulence of isolates of common bunt // Proceedings of the international scientific-practical conference "State and future of the industrial innovative development of the agro-industrial complex of the Republic of Kazakhstan" dedicated to the 70th anniversary of the Semey Institute of Veterinary Medicine and the 80th anniversary of Doctor of Veterinary Sciences, Professor Tokaev Zeynolla Kalymbekul. - Semey, 2022. - P. 212-215 (Bakirov S.B. 80%), (Co-author: Galymbek K., Madenova A.K., 20%).

The article describes the common bunt disease of wheat and its new races, which are very dangerous, and provides methods for pathogen prevention.

11. Wheat common bunt (Tilletia caries (D.C.) Tul) disease // Collection of materials of the international scientific and practical hybrid conference "Topical issues of the brain and modern practice of biological education", organized in honor of the 80th anniversary of the birth of Zhunusbekkyzy, Honorary Professor of the Kazakh Public Women's Pedagogical University Kozhantayeva Zhenis. - Almaty, 2023. - P. 51–54 (Bakirov S.B. 100%).

The article provides information on the morphological features of common bunt teliospores of wheat and indicates the area of distribution of common bunt.