ABSTRACT

of the PhD thesis by Kharipzhanova Aidana on the topic:
«The identification and developing control methods against common root rot (agent –*Bipolaris sorokiniana* Shoem.) of cereals (wheat, barley and triticale) in Kazakhstan» submitted for the degree of Doctor of Philosophy (PhD) in the educational program 8D08104 – «Plant Protection and Quarantine»

Relevance of the research topic.

Grain production stands as a cornerstone of Kazakhstan's agricultural economy, significantly contributing to both national food security and export revenues. The country's vast and diverse landscapes, coupled with favorable climatic conditions, create an ideal environment for cultivating a variety of high-quality soft and hard wheat types, alongside other essential cereal crops. Wheat cultivation is widespread across Kazakhstan, with the exception of the arid Mangystau and Atyrau regions. The majority of wheat production, approximately 80%, is concentrated in the fertile regions of Kostanay, Akmola, and North Kazakhstan. Additionally, the Aktobe, East Kazakhstan, Karaganda, and Pavlodar regions contribute between 3 to 5% each to the nation's wheat output, highlighting the geographical diversity of wheat farming across the country.

Global agricultural projections, as reported by FAO experts, indicate that the world population is expected to double by 2050. This demographic surge underscores the critical need to enhance grain production to meet escalating food demands. Despite recent increases in global grain yields, wheat production faces significant challenges, with diseases accounting for roughly 10% of potential yield losses worldwide. These diseases pose a substantial threat to agroecosystems and, consequently, to global food security. In Kazakhstan, plant diseases and pests are primary factors contributing to reduced wheat yields and compromised grain quality. The most prevalent diseases across the country's varied ecological and geographical zones include various rust types and, notably, root rots.

Among these, common root rot emerges as the principal cause of diminished wheat grain yields in Kazakhstan. This disease is primarily caused by the fungus *Bipolaris sorokiniana*. Specifically, the soil fungus *Cochliobolus sativus Drechsler* ex Dastur, also known by its anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker, is recognized as one of the most widespread and destructive pathogens affecting cereal crops like wheat, barley, and triticale within the country. The extensive geographical range of *B. sorokiniana* exacerbates its impact, making it a formidable adversary for grain producers.

The vulnerability of cultivated wheat varieties to root rot significantly hampers productivity, leading to a substantial 35-45% decrease in plant yields. This reduction not only affects the quantity of wheat produced but also its quality, thereby impacting both domestic consumption and export potential. Addressing this challenge requires the adoption of genetically resistant wheat varieties, which stand out as the most effective, economical, and ecologically sustainable method for

controlling root rot. Implementing such resistant varieties can substantially mitigate yield losses and enhance overall crop resilience against common root rot.

However, there have not been any studies in our country focused on investigating the response of wheat varieties to the dominant isolates of root rots. There is limited information available about the pathogenicity and racial structure of these pathogens' populations. Therefore, it is crucial to identify and develop protective measures against wheat root rot caused by *Bipolaris sorokiniana*.

The purpose of this dissertation research is to analyze the prevalence and progression of common root rot, to identify the pathogen *Bipolaris sorokiniana* Shoem. in cereal crops using species-specific primers, and to develop protective measures against wheat root rot in Kazakhstan.

Research objectives.

1. Evaluate the resistance of cereal crop varieties to common root rot in the Almaty region and collect infectious root rot material.
2. Determine the morphological and biological characteristics of root rot in cereal crops.
3. Identify the pathogen of root rot in cereal crops using species-specific primers and conduct a pathogenicity test of *B. sorokiniana* isolates.
4. Assess the harmfulness of root rot on commercial winter wheat varieties in the Almaty region.
5. Evaluate the ability of the fungus *B. sorokiniana* to persist in plant residues of various crops.
6. Analyze the impact of infection and the type of mycotoxins on their content in 15-day-old triticale seedlings.
7. Evaluate the impact of chemical and biological seed treatments on root rot development indices and plant productivity parameters.

Materials and methods of research.

The study utilized widely accepted methods in phytopathology and mycology research. The main sources include: A. Chumakov's "Basic methods of phytopathological research" (Moscow, 1974); V. Kotova's "Guidelines for studying the harmfulness of root rot of spring wheat and barley, and methods for calculating losses from the disease" (Leningrad, 1979); N. Naumova's "Analysis of seeds for fungal and bacterial infection" (Moscow, 1978); and the "Guidelines for conducting registration tests of fungicides, seed treatments, and bio preparations in crop production" (Almaty - Akmola, 1997).

The degree of plant infection by root rot was assessed at the tillering stage and full grain maturity according to the Zadoks scale, analyzing 200-300 plants (4-6 samples of 50 plants each). The methodology described in V. Pidoplichko's work was used for analyzing the biological characteristics and pathogenic properties of disease pathogens. Seed germination was determined according to Interstate standard 12038-84, the mass of 1000 seeds according to Interstate standard 12042-80, and disease infection according to Interstate standard 12044-93. Mycotoxin determination was carried out using the method described in Interstate standard 34140-2017 "Food products, feeds, food raw materials; Method for determining

mycotoxins using high-performance liquid chromatography with mass spectrometric detection.

Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method, as described in the Diversity Arrays Technology protocol. Species-specific PCR analyses were performed using the COSA_F/COSA_R primer sets for *B. sorokiniana* for species identification.

For pathogenicity studies, a sterilized mixed substrate of vermiculite, sand, and soil was used, and the inoculum was prepared from a conidial suspension according to the method by Duczek (1985).

Statistical data processing was performed using R-Studio. The non-parametric Mann-Whitney test for independent samples was used for analysis. The significance of the calculations was assessed using P-values. Analysis of variance (ANOVA) was conducted using the Kruskal-Wallis test. Statistical significance was established at $P \leq 0.05$.

The main provisions for defense:

1. Evaluate the resistance of cereal crop varieties to common root rot in the Almaty region and collect infectious root rot material.
2. Determine the morphological and biological characteristics of root rot in cereal crops.
3. Identify the pathogen of root rot in cereal crops using species-specific primers and conduct a pathogenicity test of *B. sorokiniana* isolates.
4. Assess the harmfulness of root rot on commercial winter wheat varieties in the Almaty region.
5. Evaluate the ability of the fungus *B. sorokiniana* to persist in plant residues of various crops.
6. Analyze the impact of infection and the type of mycotoxins on their content in 15-day-old triticale seedlings.
7. Evaluate the impact of chemical and biological seed treatments on root rot development indices and plant productivity parameters.

Description of the main results of the study.

During the research, the degree of infection with root rot of cereal crops at different stages of their development was assessed. During the tillering phase, the disease incidence ranged from 4,5% to 11,0%**,** and in the phase of full ripeness, it reached from 10,5% to 25%**.** The cereal crop varieties least affected by root rots to a moderate extent included Prospective Line 231, Farabi, Run, Idea, TI 17, Nevo, Dokuchaevskiy 9, and Altaiskiy 5, with disease prevalence ranging from 35% to 45% and development at the level of 10-15%**.** The most severely affected varieties were Kazakhstan 10, Steklovidnaya 24, Almaly, Arna, Naz, Zhetysu, Symbat, Zhan, Aidyn 2, Rondo, and Fidelio 5, Valentino with disease prevalence ranging from 45% to 55% and development at the level of 20-25%.

As part of the study, genetic identification of *Bipolaris sorokiniana* fungal isolates extracted from various varieties of wheat and barley grown in Kazakhstan was conducted. From the subcrown internodes and roots of the winter wheat of the Prospective line 231, isolates Kz 8, Kz 12a and Kz 15b were obtained. From the spring wheat variety Kazakhstan 10 isolates Kz 48, Kz 52 and Kz 56 were extracted,

and from the winter wheat Steklovidnaya 24 - Kz 60, Kz 69, Kz 70 and Kz 78b. From the spring barley variety Symbat 14 isolates were obtained (Kz 82, Kz 83, Kz 85, Kz 86, Kz 87, Kz 89, Kz 91, Kz 92, Kz 93, Kz 94, Kz 95, Kz 96, Kz 97, Kz 98), and from the winter barley Aidyn 2 - also 14 isolates (Kz 127, Kz 128, Kz 130, Kz 132, Kz 134a, Kz 137, Kz 139, Kz 141, Kz 144, Kz 145, Kz 147, Kz 148, Kz 149, Kz 153a).

All isolates were identified as *B. sorokiniana* using specific molecular methods, amplifying a single clear band of 520 base pairs using the COSA_F/COSA_R primers. These data confirm the presence of *B. sorokiniana* in the studied cereal crop varieties, which is crucial for further research and the development of control measures for this pathogen.

The study of the ability of *B. sorokiniana* to persist in plant residues of various crops showed that this pathogen could significantly infect spring barley and spring wheat seedlings, reaching infection levels of up to 100% on the 10th day after inoculation. Oat seedlings did not show statistically significant differences in infection levels between different inoculation levels, remaining at 45-55%. Peas and chickpeas were not infected by *B. sorokiniana* but did show infection by other fungal pathogens.

Introducing rapeseed, chickpeas, peas, and oats into crop rotation in southeastern Kazakhstan may limit the level of *B. sorokiniana* infection in spring barley and spring wheat seedlings, without causing root rot symptoms in rapeseed, peas, and chickpea seedlings.

Research conducted in field and laboratory trials confirmed the effectiveness of various chemical and biological preparations in protecting spring wheat and spring barley from root rot. The Celest top preparation of 312.5 suspension concentrate per 1.0 l/ton, showed high biological efficacy, with wheat achieving 93.3% and barley 87.4%. Other chemical agents also demonstrated significant effectiveness in wheat, ranging from 83.0% to 91.9%, and in barley, from 83.0% to 91.0%. Although biological products, including Fitosporin-M and Sporabacterin, showed some protective activity, they did not achieve high results, with efficacy ranging from 53.1% to 64.7% in wheat and from 62.4% to 64.2% in barley.

Treatment of seeds with chemical preparations significantly increased wheat yield by up to 2.9 c/ha and barley yield by up to 3.0 c/ha compared to the control. Biological preparations also contributed to yield increases, albeit to a lesser extent—up to 0.8 c/ha for wheat and up to 0.7 c/ha for barley.

Economic analysis not only demonstrated the high profitability of using fungicides and biological preparations but also highlighted their significant contribution to increasing agricultural profitability.

Justification of the novelty and importance of the results obtained.

The dissertation aligns with the main directions of the Development Concept of the Agro-Industrial Complex of the Republic of Kazakhstan for 2021-2030, which are aimed, among other things, at the development of biotechnology as an important component of innovation. Within the framework of this research work, genetic identification using specific primers to detect isolates of *B. sorokiniana*, extracted from Kazakhstani varieties of wheat and barley, was conducted for the first time in

Kazakhstan. An assessment of the pathogenic activity of various isolates on the main varieties of cereal crops grown in Kazakhstan was also carried out. The results of the study not only expand knowledge about the biology and epidemiology of *B. sorokiniana* but also contribute to the development of sustainable and effective strategies for protecting cereal crops from this dangerous pathogen in Kazakhstan. This is of great importance for increasing the resilience of agriculture, ensuring food security, and reducing economic losses in the region.

Relevance to scientific development directions or state programs are to secure of grant funding for the project AP19676202 «Identification of sources of resistance to wheat root rot caused by *Bipolaris sorokiniana* and *Fusarium culmorum* using phytopathological and molecular methods» for the period 2023-2025.

Description of the doctoral student's contribution to the preparation of each publication.

Based on the dissertation, 7 scientific works have been published, including: 1 publication in journals recommended by the Committee for Quality Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 3 publications in journals included in the Scopus database, with percentiles of 41; 3 in the proceedings of international scientific conferences. The author directly participated in the development of 3 recommendations.

Scope and structure of the dissertation. The dissertation is presented on 118 pages and consists of an introduction, 4 sections, a conclusion, recommendations for production, acts of implementation in the production of scientific research, and appendices. It contains 25 tables and 30 figures. The list of sources used includes 138 titles, of which 122 are foreign**.**