ABSTRACT

Dissertation for the degree of Doctor of Philosophy (PhD)
in the educational program "8D05104 – Genetics"
by Dana Omarovna Mynbayeva on the topic:
"Creation of pre-breeding rice resources with genes for blast resistance by the method of marker breeding"

Relevance of the study. Rice (*Oryza sativa* L.) is a vital crop for food security, particularly in Asia. A significant portion of the region's population directly or indirectly relies on rice as a primary source of calories. Rice blast, caused by the phytopathogenic fungus *Magnaporthe oryzae*, is one of the three most dangerous diseases, significantly affecting crop yields worldwide. This disease poses a serious annual threat to rice production, reducing yields by 10-30% in normal years and up to 50% or more during severe epiphytotics. Therefore, the development and introduction of blast-resistant varieties and hybrid lines is a priority in modern rice breeding. The disease develops throughout the growing season and can affect various plant organs, including seedlings, leaves, nodes, and panicles, depending on the time and location of infection. Panicle blast poses the greatest threat to rice production, as it directly affects grain formation and fullness, leading to significant yield losses. Selecting resistant rice varieties through careful selection and judicious application is the most cost-effective. Traditional disease control methods, including fungicide use and agricultural practices, are proving ineffective, especially during severe epidemics. Consequently, there has been a shift toward identifying genes that confer resistance to rice blast (R genes), which is essential for producing reliable varieties. Furthermore, the use of genetic approaches to rice crop protection provides targeted and sustainable solutions that minimize the use of chemicals and enhance crop resistance to various diseases.

Rice is a vital food source for a significant portion of the world's population, including Kazakhstan, providing basic human caloric needs. However, the yield of this key crop is declining under the influence of a number of biotic stresses. Among these, rice blast, caused primarily by the phytopathogenic fungus *Magnaporthe oryzae*, poses a particular threat. In recent years, it has significantly impacted crop production and yields worldwide. The problem is becoming increasingly pressing given global climate change, which facilitates the spread and development of the disease. Significant progress has been made in developing effective methods for combating rice blast in recent decades. However, most existing control measures, including chemical control and agronomic practices, are limited in effectiveness and do not provide a long-term solution, highlighting the need to develop and introduce varieties resistant to this disease. In Kazakhstan, research into rice blast fungal diseases is sporadic and insufficient, primarily focusing on fungal populations during outbreaks. Researchers at the Research Institute of Biological Safety Problems have created a collection of isolates of the Kazakh population of *Magnaporthe oryzae* fungus and provided their morphological and cultural characteristics, but the aggressiveness of the population has not been studied due to the lack of a set of differentiating varieties.

Unfortunately, research into developing resistant varieties has been virtually nonexistent in Kazakhstan. Since the majority of varieties cultivated in Kazakhstan's rice farms are foreign-bred, developing blast-resistant varieties based on domestic, highly productive, regionalized varieties is a pressing issue. This is particularly important for strengthening food security and developing domestic rice breeding. Therefore, the use of modern molecular genetic approaches, particularly marker-assisted selection, is particularly valuable. It accelerates the development of resistant varieties and improves the efficiency of selection when combined with traditional methods.

Research objects. The objects of the study were 35 rice varieties (Oryza sativa L.) of domestic and foreign selection, presented in the IPBB collection: 31 - varieties and lines of foreign selection from the IPBB rice collection kindly provided by breeders from the Federal Scientific Center of Rice (Doctor of Biological Sciences, Professor G.L. Zelensky) and the Don Scientific Center of Agricultural Research (Doctor of Biological Sciences, Professor P.I. Kostylev); 4 varieties of Kazakh selection – Bakanasskii, Fatima, Aisaule, Aru created by scientists of the IPBB (Taranov O.N., Aimakov Zh.Zh., Mamonov L.K., Sedlovsky A.I. Usenbekov B.N., etc.) and the Kazakh Research Institute of Rice Growing named after I. Zhakhaev (Bakiruly K.B., Podolskikh A.N., Karlykhanov T.K., Meirman G.T., Baibosynova S.M.).

The **subject** of the study is genetic resistance of rice to blast caused by *Magnaporthe oryzae*, as well as methods of traditional breeding, haploid technology, and marker-assisted selection (MAS) for the development of hybrids and lines.

The topic of the dissertation: Creation of pre-breeding rice resources with genes for blast resistance by the method of marker breeding.

The **aim** of the study is to evaluate domestic and foreign-bred rice varieties from the IPBB collection for the presence of genes controlling the resistance trait to *Magnaporthe oryzae* and to create new resistant hybrids and lines using traditional breeding methods, haploid technology, and MAS.

To achieve this goal, it was necessary to solve the following **tasks**:

1. Conduct an analysis and screening of domestic and foreign rice varieties from the IPBB collection for the presence of resistance genes to Magnaporthe oryzae using a molecular genetic approach using *Pi* complex genes;
2. Introduce *Pi* complex resistance genes to blast into high-yielding rice varieties from Kazakhstan and pyramid them using marker-assisted selection.
3. Select resistant genotypes to the pathogen using molecular SSR markers.
4. Evaluate promising hybrids for resistance to the *Magnaporthe oryzae* population in an infectious nursery using an artificial infectious background.
5. Create dihaploids from F_2 hybrids using a biotechnological method and evaluate lines and promising F_2 and F_3 hybrids resistant to the pathogen for key economically valuable traits.

Research methods. DNA extraction methods, hybridization using pneumatic castration (three-channel pneumatic castrator), the TVELL plant pollination

method, polymerase chain reaction with SSR markers, phytopathological screening, haploid technology, and structural analysis of crop element assessment.

Scientific Novelty. A large number of fungal diseases are known to occur in rice, but they remain understudied in Kazakhstan. No studies have been conducted on domestically released rice varieties for the presence of blast resistance genes, and no collection of differentiated varieties with various resistance genes to *Magnaporthe oryzae* has been created. Studies of fungal diseases of rice are sporadic, due to outbreaks of this disease. For the first time in Kazakhstan, research has been conducted to develop baselines for breeding new varieties immune to the phytopathogen *Magnaporthe oryzae* using marker-assisted selection methods. The novelty lies in the development and achievement of the following conclusions:

For the first time in Kazakhstan, research was conducted to create original hybrids resistant to the phytopathogen *Magnaporthe oryzae* using marker-assisted selection methods.

Donor varieties of resistance genes *Pi-1, Pi-ta, Pi-2, Pi-33, Pi-40, Piz-t*, and *Pi-54*, which control resistance to *Magnaporthe oryzae*, were selected for introgression and gene pyramiding into Kazakh rice varieties.

Hybrid generations F_1, F_2, and F_3 were obtained by introducing and pyramiding *Magnaporthe oryzae* resistance genes into high-yielding rice varieties released in Kazakhstan.

Pre-breeding materials characterized by high yields and increased resistance to the Kazakh population of *Magnaporthe oryzae* were created.

Practical significance. Twenty-seven new original hybrids were developed based on highly productive Kazakh regionalized varieties (Bakanasskii, Fatima, Aisaule, and Aru). These hybrids carry blast resistance genes. Seven of these lines are pyramidal, meaning they contain combinations of two or three resistance genes (*Pi-1*, *Pi-33*, and *Pi-ta*). The dihaploid F_2 line Aisaule/7668 (*var. italica Alef*) carrying two genes (*Pi-1* and *Pi-ta*) for resistance to *Magnaporthe oryzae* was obtained.

Theoretical significance of the study. Fundamental aspects of molecular genetics in rice breeding, including the development of new pathogen-resistant varieties bred in Kazakhstan using modern methods, are examined. The theoretical significance of the study includes new initial pre-breeding materials obtained using traditional hybridization and MAS methods for further use in domestic rice breeding programs. All selected hybrids have potential for further study and the development of rice varieties resistant to *Magnaporthe oryzae*. A literature review revealed that similar studies have not been conducted in Kazakhstan. The results obtained in this study can serve as a basis for developing new strategies in rice breeding using MAS, aimed at creating competitive rice varieties that are significant for the domestic market.

Key points of the dissertation submitted for defense:

35 domestic and foreign rice varieties were tested for the presence of resistance genes to *Magnaporthe oryzae* using SSR markers. The selected donors were used to introduce and pyramid the resistance genes into high-yielding Kazakh

rice varieties to create hybrids. Fifty-four hybrids carrying blast genes in their genotype were created by gene transfer into high-yielding regionalized Kazakh varieties.

27 promising F_2 hybrid rice lines carrying *Magnaporthe oryzae* resistance genes were selected using MAS. 6 hybrids were identified (7698/Aisaule *var. suberythroseros* Kan., Aru/7702 *var. erythroceros* Koern., Bakanasski/7667 var. subjanthoseros Brsch., Bakanasski/7667 *var. vulgaris* Koern., Bakanasski/7701 *var. vulgaris* Koern., Aisaule/7664 *var. italica* Alef.), which contain 2 genes of resistance to blast (*Pi-1*, *Pi-ta*), 1 hybrid Aisaule/7689 *var. zeravschanica* Brsch contain 3 genes of resistance to blast (*Pi-1*, *Pi-33* and *Pi-ta*) and 16 F_3 hybrids containing 1 resistance gene (*Pi-1*). Phytopathological screening identified 37 F_2 hybrids with varying degrees of resistance (from weak to high) to the fungal disease population.

Pre-breeding productive hybrids were developed for domestic programs to develop blast-resistant rice varieties and a dihaploid F_2 line.

Connection with the main research plan. The research was conducted in the Laboratory of Plant Physiology and Biochemistry of the Institute of Plant Biology and Biotechnology of the Kazakh Ministry of Higher Education of the Republic of Kazakhstan as part of project AP14869300 "Improving rice resistance to *Magnaporthe oryzae* using marker-assisted selection (MAS)" for 2022-2024, funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan.

Research work approval. The research results were published and presented at the international scientific conferences "Breeding, Seed Production, Cultivation Technology and Processing of Agricultural Crops", dedicated to the 90th anniversary of the Federal Scientific Center of Rice (Russia, 2021) and "Ecological Genetics and Population Health: Achievements and Prospects", Proceedings of the international scientific and practical conference dedicated to the 80th anniversary of Doctor of Biological Sciences, Professor, Academician of the National Academy of Sciences of the Higher School of the Republic of Kazakhstan and the International Academy of Sciences of the International Academy of Sciences of the Higher School of Agriculture A.B. Bigaliyev (Kazakhstan, 2023). The main results were presented at meetings of the Department of Molecular Biology and Genetics of Al-Farabi KazNU, scientific seminars of the Laboratory of Plant Physiology and Biochemistry of the Institute of Biology and Biotechnology (IPBB), and at the IPBB Academic Council during hearings of project reports. The results of the dissertation work are included in the annual reports on research work under project AP14869300 (2022-2024).

Publications. The main results of the dissertation are reflected in 13 published works, including four articles in journals included in the second quartile of the Scopus database; two articles in journals included in the list of the Committee for the Study of Higher Education of the Ministry of Higher Education of the Republic of Kazakhstan; one monograph; and six abstracts in the proceedings of international conferences.

The applicant's **personal contribution** consists of theoretical preparation and development of the research program, formulation of objectives, and implementation of scientific experiments and trials, as well as synthesis and analysis of the results. Specifically, the applicant was directly involved in conducting scientific research, collecting, processing, and analyzing experimental data, validating the results, and preparing and publishing research materials in scientific journals, including journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan and the Scopus database. The applicant is the responsible executor of the project within which the research was conducted.