ABSTRACT

of the dissertation for the degreeDoctor of Philosophy (PhD) 6D070100-Biotechnology Doktyrbay Gulina

«Development of new productive mutant wheat lines and molecular-biochemical study»

General characteristics of the work. The dissertation work is devoted to the creation of genetically stable (M_5) mutant lines biofortified with micronutrients with improved productivity components, based on three different varieties of spring wheat of Kazakh selection using different doses of ^{60}Co gamma radiation (100 and 200 Gy), studying the bioavailability of microelements, characterizing morphological grain parameters, correlation between the studied traits, identification of the specificity of the expression of genes involved in Fe homeostasis in the roots and leaves of the created biofortified mutant lines of Fe and Zn.

Relevance of the research thesis. Micronutrient deficiencies are widespread in the diet of the population. Fe and Zn deficiency is especially acute, affecting more than half of the world's population. A lack of microelements is called “hidden hunger” Solving the problem of iron deficiency is one of the priorities of the Ministry of Health of Central Asia and Kazakhstan, as well as states in general.

Wheat (*Triticum aestivum* L.) is the main grain crop for human and animal nutrition, providing 28% of edible dry matter and up to 60% of daily caloric intake in countries. It is also the main source of micronutrients in the daily diet. However, wheat grain has a low content of micronutrients necessary for a balanced diet, especially in varieties of Kazakhstani selection. In this regard, genetic enrichment (biofortification) of wheat grain with micronutrients is necessary. Over the years, intensive wheat breeding programs to create modern high-yielding varieties have led to a decrease in its genetic diversity, accompanied by a simultaneous decrease in the nutritional value of the grain.

Wild emmer wheat, with its higher micronutrient content, has advantages over cultivated wheat. However, additional experiments necessary to reduce the influence of unwanted genes reduce its breeding value.

An important property that determines the quality and nutritional value of wheat is the protein content in the grain, which affects the quality of the final product. In modern wheat varieties, the protein content in the grain is insignificant. At the same time, selection to increase the protein content in grain is difficult due to the negative correlation between productivity and this indicator of grain quality.

Mutagenesis is a powerful tool used to genetically improve wheat and induce a new allele of a gene previously absent from the germplasm pool. Mutagenesis-based mutation selection was widely used to increase productivity, but was not widely used to improve grain quality, especially the content of essential microelements, such as Fe and Zn and the morphological parameters of grains.

Along with biofortification with micronutrients, it is important in the genetic improvement of wheat grain quality to increase their bioavailability by reducing the content of phytic acid (PA), an antinutrient and a powerful metal chelator. In this

regard, the work carried out screening of created genetically stable mutant lines of spring wheat to identify low-phytin genotypes associated with high bioavailability of microelements.

To understand the processes of accumulation of metals, Fe and Zn, in grain, the features of the expression of key genes involved in Fe homeostasis were studied in the roots and leaves of the created mutant lines of spring wheat with a high content of them. It was found that among the genes that provide regulation of Fe uptake, its rate of translocation and accumulation, the highest level of expression was detected in the transcription factor *TabHLH* (13.1 and 30.2 times in the roots of biofortified mutant lines).

The aim of the work: The development of new genetically stable spring wheat M_5 mutant lines to broaden of genetic variability and the molecular biochemical study of the characteristics associated with the nutritional value of the grain.

The tasks of the work:

1. To expand the genetic variability of spring wheat, create new genetically stable M5 mutant lines on the genetic basis of the Kazakh varieties Zhenis, Almaken, Erythrospeperum-35 using 100 Gy and 200 Gy doses of gamma irradiation from a ^{60}Co source.
2. Determine the productivity components of mutant lines and their original varieties and establish a correlation between them.
3. Characterize the original varieties of spring wheat and mutant lines according to the morphometric parameters of the grain.
4. Determine the content of protein, Fe and Zn in the grain of the original spring wheat varieties and mutant lines and identify samples (genotypes) biofortified with micronutrients.
5. To identify the localization of Fe and Zn in the structural elements of grain in the original varieties of spring wheat and biofortified mutant lines using the method of histochemical staining of metals.
6. To analyze the correlations between the content of micronutrients, productivity components and morphometric parameters of grain in the original varieties and mutant lines of spring wheat.
7. Conduct screening for the content of phytic acid (the main antinutrient) in the grain of the original varieties and mutant lines of spring wheat. To identify low-phytin genotypes associated with high bioavailability of microelements.
8. Determine expression of genes involved in Fe homeostasis, such as genes that control the processes of metal absorption, *TaSAMS, TaHAS1, TaHAAT2-B, TaDMAS1-A* and *TaTOM*, genes that determine the regulation of translocation, *TaYSL* and *TaVIT2* , genes responsible for metal accumulation *TaNRAMP* and *TaFer1A-D*, and transcription factor *TabHLH*.

Objects of the study: New genetically stable (M_5) 90 mutant lines of spring wheat, created on the genetic basis of the varieties Zhenis, Almaken, Erythrosperum-35 and the original varieties.

Methods of the study: Atomic absorption spectrometry, infrared spectrometry, WinRHIZO image analysis system, PCR.

The scientific novelty of the study. For the first time, mutant lines were created

by expanding the genetic variability of spring wheat based on Kazakhstani varieties Zhenis, Almaken and Erythrospermum-35, adapted to local conditions, by physical mutagenesis of gamma irradiation in doses of 100 and 200 Gy for genetic improvement. Lines with improved productivity components such as the weight of grains of the main ear, the weight of grains of one plant and the weight of 1000 grains were identified.

-It was established for the first time that the created mutant lines of spring wheat were characterized by improved morphometric parameters of the grain compared to the original varieties. Grain area, and to a lesser extent its length, are the most phenotypically variable traits.

-For the first time, the biofortification ability of micronutrients (protein, Fe and Zn) in grain was revealed in a number of new mutant lines of spring wheat, which was accompanied without a decrease in productivity components and morphometric parameters of the grain.

-For the first time, it was discovered, based on histochemical staining to identify the localization of Fe and Zn in the grain, that Fe and Zn are concentrated in the greatest quantities in the aleurone layer of biofortified mutant lines.

-For the first time, in biofortified Fe and Zn mutant lines, the features of organ-specific expression of key genes regulating Fe homeostasis were studied, including genes involved in metal uptake, *TaSAMS, TaHAS1, TaHAAT2-B, TaDMAS1-A* and *TaTOM*, genes providing translocations *TaYSL* and *TaVIT2*, metal accumulation genes *TaNRAMP* and *TaFer1A-D*, and transcription factor *TabHLH*. The highest level of expression of the transcription factor *TabHLH* (13.1 and 30.2 times) was detected in the roots of the line.

Scientific and practical significance of the work: The effectiveness of induced physical mutagenesis for expanding genetic diversity and improvement with the aim of creating new promising mutant wheat lines based on Kazakhstani spring wheat varieties has been shown. An integrated approach has been developed to identify new mutant lines characterized by improved components of productivity, morphological parameters and nutritional value of grain.

A close correlation has been established between the nutritional value of grain (protein, Fe and Zn content) and morphological parameters in new mutant lines biofortified with micronutrients.

The genetically stable biofortified by micronutrients mutant lines of spring wheat were introduced into breeding programs of Kazakh Research Institute of Agriculture and Plant Growing, JSC Kaz Agro Innovation.

Basic statements for the defence:

New genetically stable M_5 mutant lines created on the basis of the Kazakh varieties Zhenis, Almaken and Erythrosperum-35 using gamma radiation doses of 100 Gy and 200 Gy were characterized by improved productivity components, such as the number and weight of grains in the main ear, the weight of plant grains and the weight 1000 grains, compared to the original varieties.

Mutant lines with improved morphological parameters of grain (area, length and width) compared to the original varieties were identified. The most phenotypically variable traits are grain area and length.

The biofortification ability of micronutrients (protein content, Fe and Zn) was

revealed in new mutant lines without reducing productivity components and morphological parameters of grains.

Mutant lines with a low content of phytic acid (the main antinutrient) in grains have been identified, which is associated with their high bioavailability of microelements.

In order to understand the mechanisms of metal accumulation in grain, genotype-dependent and organ-specific expression of key genes involved in iron homeostasis were studied and identified in the Erythrosperum-35 variety lines biofortified by Fe and Zn content. The expression of homologous genes *TaSAMS*, *TaHAS1* and *TaDMAS* significantly increased in the roots of the studied mutant lines by 2.1–4.7 times compared to the parent variety. The combined expression of *TaYSL* and *TaVIT2* was also detected in the roots of mutant lines by 1.3–2.7 times. Genes encoding intracellular Fe transport and genes responsible for accumulation, *TaNRAMP* and *TaFer1A-D*, were also significantly upregulated in roots and leaves. respectively. The highest degree of expression was found for the transcription factor, *TabHLH*, which was expressed 13.1- and 30.2-fold in the roots of the mutant lines.

The main results of research and conclusions:

1. As a result of expanding the genetic diversity of spring wheat based on the Kazakh varieties Zhenis, Almaken, Erythrosperum-35 by treating them with gamma radiation doses of 100 Gy and 200 Gy from a ^{60}Co source, new genetically stable M_5 mutant lines were created.

2. Based on improved productivity traits, such as the number and weight of grains in the main ear, plant grain weight and 1000 grain weight, 100- and 200 Gy mutant lines were identified (11.1%), plant grain weight and 1000 grain weight 19 mutant lines (21.1%) higher compared to the original varieties.

3. The area of the grain, to a lesser extent, its length, the most phenotypic variable characteristics. The increase in grain length and width in mutant lines was in the range of 7.6-34.9%, and 11.8-34.4%, respectively, compared to those of the original varieties. To generate an improvement in all morphological parameters of grain, the most effective dose was 200 Gy.

4. The protein content in grain of a number of mutant lines increased by 3.4-16.9% compared to the original varieties. Mutant lines biofortified by the content of Fe and Zn in the grain were identified. The range of metal content in grain was 46.4-111.3 mg/kg for Fe and 50.6-106.2 mg/kg for Zn. The maximum value of Fe in mutant wheat grain was detected in mutant germplasm weighing 200 Gy.

5. Histochemical analysis of biofortified ones in terms of Fe and Zn content revealed their high levels in the aleurone and endosperm of the grain.

6. Increase in grain Fe concentration in Almaken 100 Gy-treated lines was related to significantly positive correlation with productivity components, such as 1000-grain weight ($r^2 = 0.15$, $P < 0.01$) and grain weight per plant, ($r^2 = 0.30$, $P < 0.001$). Accumulation of Fe and Zn concentrations in Zhenis and Almaken 200 Gy-dosed mutant lines, respectively, is determined by grain area. A significant correlation was detected between grain Fe and Zn concentrations at high irradiation dose in Zhenis, and Eritrospermum-35 mutant ($r^2 = 0.22$, $P < 0.01$) and at 100 Gy dose in Almaken and Eritrospermum-35 ($r^2 = 0.15$ and $r^2 = 0.42$, $P < 0.01$, respectively) which indicates that metals accumulation may be controlled by the same loci.

7. Significant genetic variability was revealed among mutant lines in the content of phytic acid (PA, the main antinutrient) in grain. It was found that in Almaken mutant lines a, the content of phytic acid decreased by 1.1-5.8 times. It was established that the mutant lines were characterized by a wide range of variability in the molar ratio of FA:Fe and FA:Zn, 1.14–14.5 and 0.9–13.0, respectively. The bioavailability of trace elements in Fe and Zn biofortified mutant lines increased by 1-8, 7 and 1.1-7.9 times, respectively.

8. Organ-specific expression of genes involved in iron homeostasis in Fe and Zn biofortified mutants lines were revealed. The expression of homologous genes *TaSAMS, TaHAS1* and *TaDMAS1-A* of wheat increased significantly (2.1-4.7 times) in the roots of the studied mutant lines compared to the original variety, the expression of *TaYSL* and *TaVIT2* increased by 1.3-2.7 times. The highest degree of expression was detected for the transcription factor *TabHLH* in the roots of mutant lines (13.1- and 30.2-fold).

Connection of study with basic scientific program. The thesis was performed within the projects 074/GF "Development and study of mutant wheat lines for identification of breeding-valuable forms and new genes alleles control- ling key adaptive properties" (2012-2014), (State Registration No. 012PK00581) and IAEA National TC project KAZ/5003" Increasing Micronutrient Content and Bioavailability in the Wheat Germplasm by Means of an Integrated Approach "(2012-2015).

Publications and personal contribution of the author.

The main content of the dissertation is reflected in 32 published works, including 2 articles in an international journal with an impact factor cited in Scopus; 8 articles in republican scientific journals of the Committee for Control in Education and Science of the Republic of Kazakhstan, 2 articles in the international scientific books, 2 articles in the international scientific journals, 18 theses presented at international conferences and symposiums.

The volume and structure of the dissertation. The text of the dissertation consists of 126 pages, including notations and abbreviations, introduction, literature review, materials and research methods, research results and their analysis, conclusion, bibliography. The number of literature used is 200. The results and data obtained during the experiment are presented in 50 figures and 26 tables.