Creation of starting material for selection of soft wheat by means fungicides

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Abstract. *The effectiveness of the use of fungicides as inducers of biological variability and the creation of a new source material for the selection of spring bread wheat was studied. It has been shown that the disinfectants have both inhibitory and stimulating effects on the development of seedlings. It was found that under the influence of fungicides, the range of variability in morphological characteristics increased several times in conditionally mutant populations as compared with test objects (control). A different reaction of varieties to the action of fungicides was revealed, due to the differences in their genotypes. The "Serebristaya" variety showed a greater lability of indicators in response to the action of fungicides than the "Pavlogradka" variety. Cytological analysis did not reveal chromosome damage and their behavior during mitosis. New morphological types of plants have been identified by plant height; shape, density and color of the spike, the branching of the spike; the appearance of signs of other varieties of wheat. Infrared spectral analysis showed that the accumulation of fungicides was maximum for caryopses with a dormancy period of one and two years after treatment, significantly lower for the root system, while their content in leaves and stems was minimal. The dependence of the residual amount of active substances on the storage period of seeds after treatment, as well as on the concentration (dose) of the fungicide, was revealed.*

Keywords: *wheat, fungicide, cultivar, population, variability, chromosome, selection.*

Introduction

The central place in the sustainable growth of crop productivity belongs to selection, creation and use of new varieties and hybrids of grain crops as an innovative resource of the country's agro-industrial complex [4 - 6, 14]. The most important for global food security is the future productivity of soft wheat [18].

However, in recent years, the genetic base of the main domesticated crops, including wheat, has been narrowing as a result of the use of the same donors of high productivity, disease resistance and grain quality in breeding [9-11]. A decrease in the genetic diversity of wheat varieties during breeding can lead to genetic erosion in the gene pool of Russian wheat and the loss of a significant number of genes or alleles of productivity and resistance to biotic and abiotic stresses [11, 13]. The genetic similarity of varieties bred within the framework of regional breeding programs is much higher than the recommended one, which leads to massive damage to crops by pathogens in vast territories (epiphytotics) due to uniform susceptibility to them [5]. Genetic varietal uniformity of wheat, which until recently was a problem in individual regions, has acquired global proportions. In this regard, research is being carried out all over the world with the aim of expanding the genetic base of wheat at the expense of various sources of its variability [19]. In the breeding process, a special place is occupied by the improvement of economically valuable traits of existing varieties and the breeding of new ones using induced mutagenesis [6]. Currently, work on induced mutagenesis is being carried out in almost all countries of the world. With the skillful use of experimental mutagenesis, it is possible to change the plant beyond recognition and create a variety of breeding material in a fairly short time [7]. For example, the Green Revolution, which made it possible to double the yields of rice and wheat in developing countries, was largely based on the use of induced mutants [12]. However, intensive cross-breeding and selection programs carried out over the past decade and aimed mainly at increasing plant productivity have led to a decrease in the level of genetic diversity of cultivated plants in all their properties. Within a culture, plants become genetically more and more uniform, therefore, crops as a whole are vulnerable to the influence of biotic and abiotic environmental factors [5, 9, 13]. Insufficient adaptability of highly productive varieties is due to a decrease in the level of their resistance to unfavorable environmental factors. The pursuit of the harvest has led to an increase in the vulnerability of plants to stress [11].

To prevent such damage to agriculture, it is necessary to maintain the level of available genetic diversity of the initial material for breeding programs and conduct large-scale research in order to increase the level of genetic variability of the main crops due to different sources of its variability [5, 8, 19]. The induction of genetic diversity using chemical compounds contributes to the creation of new hybrids and varieties on a heterogeneous genetic basis with increased productivity and resistance to biotic and abiotic stressors. The implementation of such programs can lead to the creation of more productive and environmentally sustainable agro-cenoses [5]. It has now been established that, in addition to chemical mutagens, fungicides can be quite effective mutagenic factors for creating the starting material of grain crops [1-4, 6-8, 12].

The relevance of research was determined by three main problems that have emerged by now in the world community:

1). *The threat to the food security of all mankind due to the massive destruction of crops by pathogens over large areas, causing enormous damage to the harvest of grain crops.*

In recent years, along with traditional diseases (brown rust, septoria, dust smut, etc.), the threat of stem rust epiphytoties has increased, the loss of grain from which can reach 50% and more. Periodically recurring disease epiphytotics are one of the main reasons for the instability of the wheat yield. This situation is caused by the lack of resistant varieties that can contain the massive damage to crops by stem rust [13]. Along with this, there was a threat of penetration into Russia from the countries of the Middle East through Central Asia of a new race of stem rust Ug 99, capable of destroying up to 100% of the crop. The emergence and spread of this race has caused great concern in the world scientific community. The Borlaug Global Wheat Rust Initiative was created to tackle rust diseases that threaten the food security of the entire planet (www.globalrust.org). The creation of wheat varieties resistant to rust diseases is the most effective way to protect the crop [13].

2). *Genetic uniformity of wheat varieties, which is the main reason for massive damage to crops by pathogens.*

The method of induced mutagenesis plays a huge role in increasing genetic diversity and improving economically valuable traits of existing varieties and breeding new ones [9]. However, there is an opinion that chemical mutagens used for this purpose have largely exhausted their capabilities [15], therefore, a wide search for new inducers of variability is being carried out. It has now been established that pesticides, including fungicides [6 - 8, 16, 17], can be such mutagenic factors for the creation of the initial material for grain crops.

3). *The ecological situation undergoing anthropogenic stress as a result of human economic activity.*

Chemical compounds used by humans need to be screened for mutagenic, cytotoxic and other damaging activities for organisms, as well as to monitor their environmental pollution. For this, it is necessary to study their effect on cellular structures, on the possibility of causing undesirable changes (mutations) in genotypes, the ability to accumulate in cells, tissues and other structures of organisms, to be included in food chains and the general ecological cycle [1]. On the other hand, the mutagenic activity of pesticides can be used to increase the genetic diversity of cultivated plants, in the gene pools of which the erosion of gene complexes and individual loci of adaptability to aggressive environmental factors has recently been observed [2–4].

Purpose of the study

To study the effects of fungicides on the biological traits and properties of wheat plants, including:

- influence on the cellular structures of wheat plants, morphology and behavior of chromosomes in the cell cycle (mitosis);

- to establish the change in the level of intrapopulation variability in experimental wheat populations, which can be used in the selection of biotypes with new properties;

- to determine the residual amount of active components of fungicides in vegetative organs and seeds of wheat in order to determine the food, feed and environmental safety of the use of chemical compounds in grain production, as well as - varietal specificity in the cumulative capacity of these substances.

- to create a source material for practical breeding in order to search for donors of economically valuable traits for obtaining new varieties of wheat with a complex of traits of increased productivity and adaptive properties.

Material and methods

In 2017–2019, pre-sowing treatment of wheat seeds of varieties Serebristaya and Pavlogradka with fungicides of a new generation was carried out: AltSil: (active ingredient - tebuconazole); Alcazar: (active ingredients - difenoconazole + cyproconazole); Comfort: (carbendazim); Terrasil: (tebuconazole). Each of the fungicides was used in two concentrations: at a dose recommended for grain production (n) and a doubled dose (2n) to enhance the morphogenesis process and assess the damaging effect on the cellular and organismic systems of wheat plants [2–4].

Germination of seeds was carried out in rolls, observing the methods and requirements of GOST for seed organizations. A total of 36 variants of the experiment were laid, 60 seeds in each roll (fig. 1). The test objects were seedlings of the Pavlogradka and Serebristaya spring soft wheat varieties, the seeds of which were not exposed to fungicides.

Fig. 1. Laying seeds for germination in rolls.

Results and discussion

During the period of joint work from 2013 to 2021 a scientific project "Study of the effects of chemical compounds on soft wheat plants" is being carried out, developed on the basis of an agreement on scientific cooperation between the Pedagogical and Agrarian Universities of the city of Omsk and included in the list of innovative projects of universities.

In order to identify the presence or absence of chromosomal aberrations in the mitotic phases of mutants obtained by us under the influence of fungicides, we studied the meristematic zones of division of the primary root of wheat seedlings in 2017-19. A cytological study of conditional mutants and their initial forms showed that no morphological abnormalities of chromosomes and their behavior were found in all phases of mitosis [2]. In laboratory conditions, a morphophysiological study of wheat seedlings, the effect of dressing agents on the sowing quality of seeds, depending on the dose of the reagent and the dormancy period of seeds after treatment, was carried out. A multidirectional effect of various disinfectants and their consumption rates on the morphometric parameters of seedlings was established: fungicides AltSil and Alkasar inhibited the development of sprouts of the Pavlogradka variety (fig. 2), reducing the average values of the trait by 15.4-23.5%, while stimulating the development of the main root (fig. 3, 4).

Fig. 2. Left photo: unequal effect of the influence of fungicides AltSil and Alkazar and their concentrations on the development of seedlings (in the center of the row - Pavlogradka variety, control).

Right photo: Comfort stimulated the development of seedlings (to the left of the control, it is in the center); Terrasil inhibited their development at both doses (to the right of the control).

Fig. 3. Variety Pavlogradka (control). The third day after soaking the seeds.

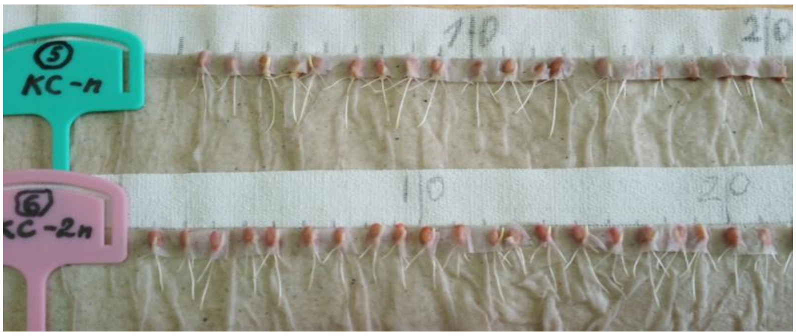

Fig. 4. Seeds of the Pavlogradka variety after treatment with the Comfort fungicide: stimulating effect on the growth of primary roots in both concentrations.

Similar tendencies manifested themselves in all other variants of the experiment. In all of the studied wheat samples (36 variants of the experiment), an increase in the range of phenotypic variability was observed with the appearance of several new classes of distribution of the values of the traits "sprout length" "and the length of the central root" of plants.

It was found that under the action of fungicides the range of variability along the sprout length increased several times in conditionally mutant populations (Cv = 34 - 43%) of the Serebristaya variety, while in the control it remained at a low level (Cv = 6.5%). The intrapopulation variability also significantly increased in the mutants of the Pavlogradka variety (Cv: 24-37%) as compared to the control (Cv = 7.2%). However, the range of variability in them had a narrower range than in the mutants of the Serebristaya variety (fig. 6). The variability of root length in both cultivars showed greater stability under the influence of all fungicides than the sprout length. According to this trait, the mutants of the Serebristaya variety (Cv = 20.4-34.2%) had greater variability, in the control - 8.0%; in the Pavlogradka variety - 14.5-18.7 and 10.0%, respectively.

It has been established that dressing agents have a different effect on the growth and development of plants for different varieties of wheat. The Pavlogradka cultivar is more sensitive to the action of the Terrasil fungicide than the Silvery wheat cultivar. In the case of Comfort, a similar trend can be noted (fig. 5, 6). Under the effect of Comfort on the Pavlogradka variety, this fungicide in both doses caused the splitting of the first leaf of the seedlings, which may indicate an inhibitory effect on the formation of leaf mesophyll.

Fig. 5. Seed sprouts of Pavlogradka variety - control (without treatment).

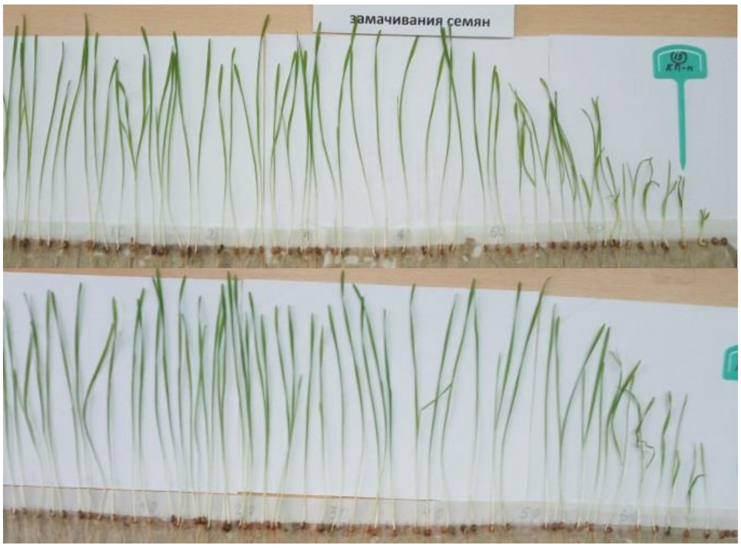

Fig. 6. Development of Pavlogradka seedlings under the influence of Comfort:
at dose n - top row; 2n - bottom row
(unfinished student's attempt at constructing a variation series in nature)

Plants of generations M-1, M-2 and M-3 were grown under field conditions on the experimental field of the Omsk Agrarian University with selection in M-2 and M-3 of the most valuable biotypes for further use in practical breeding and genetic research. Were identified morphological types of plants on the following grounds: plant height; shape, density and color of the ear, branching of the ear; the appearance of signs of other varieties of wheat. In the next generation (M-3), caryopses from the main spike of the selected plants were sown in families in the first breeding nursery (SP-1); phenological observations were carried out over them during the growing seasons of 2018-19. A structural analysis of these plants was carried out according to quantitative characteristics of productivity according to the generally accepted method, a statistical analysis of the data was carried out, the results of research in scientific articles and conference proceedings were summarized [3, 4, 16, 17].

Infrared (IR) spectral analysis was carried out to determine the residual amount of active ingredients of the fungicides used in different parts of wheat plants. IR absorption spectra were recorded on an FT-801 FT-IR spectrometer (Simeks, Russia) in the range of 500-4000 cm^{-1} using a disturbed total internal reflection (DTIR) attachment. For each sample (leaves, roots, wheat seeds), 3 measurements were carried out, after which the results were averaged. The ZaIR 3.5 software was used to identify compounds and process spectral images.

It was shown that when analyzing wheat samples treated with fungicides (carbendazim, tebuconazole, difenoconazole + cyproconazole), absorption bands characteristic of the functional groups of benzimidazoles and triazoles are observed in the IR spectra. It was revealed that the accumulation of fungicides was maximum for caryopses treated with fungicides with a dormant period of one and two years after treatment, significantly lower for the root system, while their content in leaves and stems was minimal. The dependence of the residual amount of active substances on the storage period of seeds after treatment, as well as on the concentration (dose) of the fungicide, was revealed.

Conclusions

1.The multidirectional effect of various disinfectants and their consumption rates on the morphometric parameters of seedlings was established: fungicides AltSil and Alkasar inhibited the development of seedlings of the Pavlogradka variety, reducing the average values of the trait by 15.4-23.5%, but stimulated the development of the main root. The lag in the development of seedlings of the Serebristaya variety was more significant (by 18.5-30.2%) than in the plants of the Pavlogradka variety. The development of the root of the Serebristaya variety was inhibited by both fungicides; its growth lag averaged 10-16 mm.

2. The effect of the fungicides used on the level of population variability was revealed: the coefficients of variability of the sprouts of mutant lines of the Pavlogradka cultivar in comparison with the control (Cv = 10.1%) increased to 21.2%, and the lines of the Serebristaya cultivar (Cv = 10.3%) - up to 25.6%.

The coefficients of variability of the root length of mutant lines of Pavlogradka cv. In comparison with the control (Cv = 11.4%) increased to 13.2%, and the lines of Serebristaya cv. (Cv = 7.9% - 13.5%) with a single dose of fungicides. At a double dose, these parameters changed in different directions, both in the direction of decreasing and in the direction of increasing the coefficients of variability. The values of the coefficients of variability of the traits of the Serebristaya variety varied in a wider range than that of the Pavlogradka variety.

3. Cytological analysis did not reveal damage to chromosomes and their behavior in mitosis.

4. The morphological types of plants have been identified according to the following features: plant height; shape, density and color of the ear, branching of the ear; the appearance of signs of other varieties of wheat.

5. It was found that the accumulation of fungicides was maximum for caryopses treated with fungicides with a dormancy period of one and two years after treatment, significantly lower for the root system, while their content in leaves and stems is minimal. The dependence of the residual amount of active substances on the storage period of seeds after treatment, as well as on the concentration (dose) of the fungicide, was revealed.

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