2-бөлім

ӨСІМДІКТЕР ФИЗИОЛОГИЯСЫ МЕН БИОХИМИЯСЫ

Section 2

PLANTS PHYSIOLOGY AND BIOCHEMISTRY

Раздел 2

ФИЗИОЛОГИЯ И БИОХИМИЯ РАСТЕНИЙ

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COMPARATIVE STUDY OF *BRACHYPODIUM DISTACHYON* AND KAZAKHSTAN SOFT WHEAT VARIETIES RESISTANCE TO *PUCCINIA RECONDITA*

One of the important issues of the modern world economics is ensuring the quality of food, relevant for Kazakhstan, where the possibility of cultivating soft and hard wheat varieties and high technological quality grain production are among the strategically important tasks of its national security. Biotic and abiotic environmental stress factors may lead to decrease in cereals productivity, and annual loss in the global yield of crops from diseases, according to FAO, is estimated at more than $25 billion, equivalent to 35% of the potential harvest. At the same time, creation of pathogen-resistant varieties of cultivated plants by means of traditional breeding is long-term, and the evolution of pathogens is ahead of the possibilities of practical breeding, resulting in creation of pathogen resistant varieties being late with their introduction into production; for a deeper understanding of the basics and in terms of accelerating processes in the leading countries of the world, model organisms are involved into the process. The aim of this work is to estimate the influence of brown leaf rust on the elements of productivity and protein content in grain of local varieties of soft wheat Kazakhstanskaya 19, Kazakhstanskaya early and new model object Brachypodium distachyon along with their correlation. The results of the comparative study of the impact of biotic stress on the elements of productivity have shown that Puccinia recondita statistically significantly reduces the productivity of all parameters in wheat varieties Kazakhstanskaya 19, Kazakhstanskaya early and model object Brachypodium distachyon. The protein content in the grain of wheat of local breeding varieties Kazakhstanskaya 19, Kazakhstanskaya early and wild cereal Brachypodium distachyon is not changed when infected with brown leaf rust.

Key words: Brachypodium distachyon, Kazakhstani soft wheat varieties, brown leaf rust, resistance, study.

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Brachypodium distachyon және қазақстандық селекциясының жұмсақ бидай сорттарының *Puccinia recondita*-ға салыстырмалы зерттеу

Қазіргі заманғы әлемдік экономиканың маңызды мәселелерінің бірі – азық-түлік өнімдерінің сапалы болуын қамтамасыз ету; жұмсақ және қатты бидай сорттарын өсіру мүмкіндігі және жоғары технологиялық, сапалы дән өндіру – ұлттық қауіпсіздікті қамтамасыз етудің стратегиялық маңызы бар міндеттерінің қатарына жатады. Биотикалық және абиотикалық экологиялық стрестік факторлар астықтық дақылдардың өнімділігінің төмендеуіне алып келуі

мүмкін. Әлемдік азық-түлік және ауыл шаруашылық ұйымының мәліметтері бойынша, әлемде жыл сайынғы дақылдардың әртүрлі аурулардан жоғалтатын өнімі 25 миллиард долларға бағаланады, бұл алынуы мүмкін өнімнің 35%-на парапар. Мәдени өсімдіктердің патогендердің әсеріне төзімді сорттарын дәстүрлі селекция әдістерімен шығару өте ұзақ процесс болып табылады. Патогендердің эволюциясы практикадағы селекцияның мүмкіндіктерінен әлдеқайда ілгері жүреді, соның салдарынан өсімдіктердің патогендердің әсеріне төзімді сорттарын шығару, олардың өндіріске енгізілуінен кешеуілдеп қалады. Мәселенің төркінін тереңірек түсіну үшін және селекция процесін жеделдету үшін әлемнің алдыңғы қатарлы елдерінде модельдік организмдер қолданылады. Берілген еңбектің мақсаты – Brachypodium distachyon өсімдігінің және жұмсақ бидайдың қазақстандық сорттарының Puccinia recondita-ның әсеріне төзімділігін салыстырмалы түрде зерттеу. Биотикалық стресстің өнімділік элементтеріне әсерін салыстырмалы талдау нәтижелері Puccinia recondita Казахстанская 19, Казахстанская раннеспелая сорттарының және модельдік объект Brachypodium distachyon өсімдігінің барлық өнімділік көрсеткіштерін статистикалық сенімді деңгейде төмендететінің көрсетті. Жергілікті селекция нәтижесінде шығарылған Казахстанская 19, Казахстанская раннеспелая және жабайы астық тұқымдас Brachypodium distachyon өсімдігін қоңыр жапырақ татымен жұқтырғанда өсімдіктердің дәндеріндегі нәруыз мөлшері өзгермейді.

Түйін сөздер: Brachypodium distachyon, жұмсақ бидайдың қазақстандық селекциясының сорттары, қоңыр жапырақ таты, төзімділік, зерттеу.

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Сравнительное изучение устойчивости *Brachypodium distachyon* и казахстанских сортов мягкой пшеницы к *Puccinia recondita*

Одними из важных проблем современной мировой экономики являются обеспечение качества продуктов питания; возможность культивирования сортов мягкой и твердой пшеницы и высокотехнологичного качественного производства зерна, это также относится к стратегически важным задачам обеспечения национальной безопасности. Биотические и абиотические экологические стрессовые факторы могут привести к снижению продуктивности зерновых культур, а ежегодная мировая потеря урожайности культур от болезней, по данным Продовольственной и сельскохозяйственной организации, оценивается более чем в 25 миллиардов долларов, что эквивалентно 35% потенциального урожая. Создание патогенно-устойчивых сортов культурных растений посредством традиционной селекции является длительным процессом, а эволюция патогенов опережает возможности практической селекции, из-за чего производство устойчивых к патогенам сортов опаздывает по сравнению с их внедрением в производство; для более глубокого понимания основ и с целью ускорения селекционного процесса в ведущих странах мира привлекаются модельные организмы. Цель данной работы – сравнительное изучение устойчивости Brachypodium distachyon и казахстанских сортов мягкой пшеницы к Puccinia recondita. Результаты сравнительного анализа влияния биотического стресса на элементы продуктивности показали, что Puccinia recondita статистически достоверно снижает все показатели продуктивности у сортов пшеницы Казахстанская 19, Казахстанская раннеспелая и модельного объекта Brachypodium distachyon. Содержание белка в зерне мягкой пшеницы местной селекции Казахстанская 19, Казахстанская раннеспелая и дикого злака Brachypodium distachyon при инфицировании бурой листовой ржавчиной не изменяется.

Ключевые слова: Brachypodium distachyon, сорта мягкой пшеницы казахстанской селекции, бурая листовая ржавчина, устойчивость, исследование.

Introduction

From the ancient times, humanity has been growing cereals, with wheat, corn, and rice currently being able to provide nearly two thirds of the global caloric intake [1]. Under current conditions of growing shortage of wheat, humanity might once again face an acute problem of the food crisis. Annual production of wheat on average is about 600 million tons. It is expected that by 2020 the demand for it may reach more than 840 million tons. Satisfying this need is a rather difficult task, taking into

account the fact that the number of cultivating areas decreases, and wheat yields in most developed countries have already reached the maximum level, for example, in Europe, this number reaches more than 8 tons per hectare [2].

At the same time, creation of pathogen-resistant varieties of cultivated plants by means of traditional breeding is long-term, and the evolution of pathogens is ahead of the possibilities of practical breeding, because of which production of pathogen resistant varieties is late with their introduction into production. Study on attributes of plant immunity, especially in chosen based on their suitability for research model plants facilitates understanding the mechanisms of plant resistance and improving disease management strategies [3; 4]. Production of high-quality grain in Kazakhstan is an important strategic direction, contributing to stabilization of agriculture, food security of the country and a decent position in the club of grain exporters in the world market [2].

Puccinia recondita Rob. ex Desm f. sp. tritici or wheat brown leaf rust is one of the most common and harmful types of rusts and despite the fact that its harmfulness is somewhat lower than that of stripe and yellow rusts, the loss of grain from this disease for a number of losses over a number of years might be higher than from the other rusts during the same period of time with epiphytotic diseases occurring in Kazakhstan with a frequency of 2-3 times in 10 years with the loss of yield up to 30-50% [5-7]. Losses of the world harvest, only from the brown rust are estimated in the equivalent of $2 billion [8; 9]. The harmfulness of brown rust is manifested in a decrease in plant assimilation, enhancement of transpiration, respiration and biochemical processes, with a complete disturbance of the water balance, causing premature death of the leaves. The disease inhibits synthesis and deposition of starch, as well as protein in the endosperm, resulting in the formation of a frail grain. With severe damage to plants, fewer grains are formed in the ears, they are of poor quality and with a reduced protein content. The shortage of the harvest depends on the pathogenicity of the pathogen, crop resistance, weather conditions and other [7].

Creation of pathogen-resistant varieties of cultivated plants by methods of traditional breeding is long-term, and the evolution of pathogens is ahead of the possibilities of practical breeding, resulting in creation of varieties resistant to pathogens being late with their introduction into the production cycle [10]. An important mean of control is breeding of wheat with application of new, non-traditional techniques, combining the efforts of classical breeders, geneticists, biochemists, physiologists, immunologists and biotechnologists, as only the complex approach may increase the breeding performance. This trend implies, first, identifying the biological signs that provide the best possible adaptation to the natural conditions of the arid zone in order to obtain initial material for breeding of new productive varieties [11].

On the south-eastern part of Kazakhstan primary staple culture is soft wheat, which is currently infested by the brown rust. *Brachypodium* (*B.*) *distachyon* is the only annual wild grass, phylogenetically closely related to the most important cereal crops – wheat, barley, rice, has a number of advantages (a relatively small size of genome, consisting of five chromosomes with a capacity of 272 million base pairs, 15-20 cm in height, ease of cultivation in the laboratory conditions), which makes it a convenient object for fundamental and innovative applied research in the field of cell biology, biochemistry, molecular genetics and agricultural biotechnology; leading scientists from all over the world contributed to the development of its resources and research tools, in particular, the BrachyTAG project with the basic goal of identifying key genes involved in its development, reproduction, bioenergetics, adaptation to environmental factors, including the study of the resistance of cultural grasses to the most harmful and common diseases (rust, septoriosis, fusariosis) [3; 12; 13].

Earlier in a number of works, it was shown that brown rust could infect *B.distachyon*: the formation of pustules, necrotic spots, and indicative of the hypersensitivity response of plant to the action of the pathogen [5]. However, the detailed study of the mechanisms underlying the nonhost resistance in the model object *B. distachyon* to the action of brown rust never performed earlier.

Our scientific group is the first in Kazakhstan, engaged in detailed studies on the use of *B. distachyon* as a model object for studying changes caused by the brown rust pathogen. In the process of completion the research work on the project “Introduction of a new model object Brachypodium distachyon L. into the breeding practice in order to improve the resistance of cereals to biotic environmental factors” (SR No. 0115RK00382), work continued in “Physiological and biochemical mechanisms of nonhost resistance of the model object Brachypodium distachyon L. to brown leaf rust”. Within the framework of the project the influence of *P. recondita* on the activity of a number of stress enzymes in *B. distachyon* (standard resistant line Bd21) and soft wheat (two

varieties with different degrees of resistance – Kaz. (Kazakhstanskaya) early and Kaz. 19) prior and upon the pathogen infection, the composition of the storage proteins in the endosperm of *Bd* and soft wheat were estimated among the rest. Some results of this study are presented in the current paper.

Materials and methods

Seeds of *B. distachyon* L. (Bd21 line) were obtained from the RIKEN BioResource Center (Tsukuba City, Ibaraki, Japan). Wheat varieties were picked on their degree of resistance and susceptibility to *P. recondita*. According to the assessment of Kazakh SRI for Arable Farming and Plant Growing (Almalybak, Almaty region, Kazakhstan) and SRI of Biological Security Issues, MES RK (Gvardeyskyi, Zhambyl region, Kazakhstan) Kaz. 19 variety possesses a resistance to the brown rust (15%), and Kaz. early variety is affected up to 40%. According to the laboratory assessment of the quality of grain by RK state commission for variety testing both varieties meet the requirements of the state standard No.1046-2008 14-day old seedlings of *B. distachyon* and chosen wheat varieties served the material of the study. Prior to growing seeds were soaked for 5 minutes in a weak solution of potassium permanganate at a temperature of 25°C. Treated seeds were washed for 5 minutes in running water, then for 5 minutes three times with sterile water. Seeds were placed in plastic Petri dishes and germinated for 48 hours in a thermostat at a temperature of 25°C. After 48 hours, sprouts were germinated at room temperature at the light. In the process of germination sprouts were watered with chilled boiled water (Figure 1).

Sowing of wheat seeds on plots 1 m wide with a row spacing of 15 cm was carried out annually by hand. 20 seeds of wheat were planted in each row and 12 seeds of Bd21. Seeds were sown every year in the same calendar periods as the control group in order to analyze plants resistance to the leaf brown rust. Bd21 seeds germination was 94%, while wheat seeds one – 97-98%. In the two leaves growth phase, the plants of the experimental variant were inoculated with urediniospores, while control consisted of untreated plants. Kazakhstani population of fungus spores *P. recondita* provided by the SRI of Biological Security Issues, MES RK (Gvardeyskyi, Zhambyl region, Kazakhstan).

A B C

Figure 1 – Growth and development of the model system Brachypodium distachyon.
Note: A – in the laboratory; B – in the field; C – plant material for the morphometric analysis

P. recondita infection of the plants of the experimental sample was carried out in the tillering phase, by inoculation with urediniospores (Figure 2). Inoculum has been activated during 30 min at a temperature of 37-40°C, and it has been watered in a wet chamber during 4 hours. The plants were sprayed with an aqueous suspension of *P. recondita* spores which contented 0.001% of Twin-80, contagion pathogen load was 20 mg/m^2. Plants treatment was carried out after pre-moisturizing in the evening time and high humidity conditions were constructed (by covering with polyethylene film). There were untreated plants in the control. Infection symptoms were registered in 7-9 days. Sustainability to the brown rust of wheat and Bd21 were screened in the field conditions. Immunological analysis was performed according to R.A. McIntosh, where there are 5 types of reaction such as 0 is immune, damage is not observed; R is resistant, the plant ability to resist pathogen action; MR is moderately stable; MS is moderately susceptible; S is susceptible [14].

A B C

Figure 2 – *Puccinia recondita* spores preparation to the germination and infection the plants in a field condition.
Note: A – spores germination; B – treatment; C – pustules on plants

The identification of morphometric features was carried out in accordance with I. Ermakov conventional methods (2007).

The analysis of the contamination by *P. recondita* spores was carried out on from 8th to 11th days after inoculation according to generally accepted procedure [6].

A grain analyzer of protein and humidity based on the Near Infrared Spectroscopy (NIR) method, GrainAZX-50 portable grain analyzer, Zeltex (USA) was used to determine grain protein content in wheat and Bd21, automatic calibration of the instrument was carried out by software. 25 grains were assessed to determine the protein content of wheat, and 50 grains of *B. distachyon*. Protein content is % to dry weight of grain. The total protein content of the leaves (mg/ml) was estimated by a microbiuretic method using Benedict reagent at a wavelength of 330 nm [15; 16]. Estimation of free proline in leaves was carried out by L. Bates method (1973).

Extraction of the storage proteins of endosperm, gliadins, and glutenins. The glutenins were fractionated with a buffer solution (pH 6.8). Separation of glutenins in an alkaline condition was carried out according to U. Laemmli (1970), in acidic – according to Poperelya (1989). High molecular weight glutenin subunits (HMGS) were identified by comparing the electrophoregrams of the analyzed sample with the spectrum of assay varieties according to the catalog. Gliadin extraction was performed with 70% ethanol. Electrophoresis was performed in polyacrylamide gel in glycine acetate buffer pH 3.1. Gels were fixed in 10% trichloroacetic acid, stained with 0.2% Coomassie R-250. Gliadin components were recorded by their electrophoretic mobility in the gel within α, β, γ and ω subfractions. Analysis of seed storage proteins was carried out in an alkaline (sodium dodecyl sulphate electrophoresis) and acidic systems according to G. Galili (1983).

For estimation of superoxide dismutase (SOD), samples were placed on the gels in 20% glycerol and electrophoresis was performed at 80 to 120 V (two mA/tube) until the bromphenol blue marker dye had swept through most of the gel. Protein was stained by immersion of the gels in 0.2% amido black in 7% acetic acid for 1 hour followed by destaining in 7% acetic acid. SOD was localized by soaking the gels in 2.45 x 10^{-3} M nitro blue tetrazolium for 20 min, followed by an immersion, for 15 min, in a solution containing 0.028 M tetramethylethylenediamine, 2.8 x 10^{-5} M riboflavin, and 0.036 M potassium phosphate at pH 7.8. The gels were then placed in small dry test tubes and illuminated for 5 to 15 min. During illumination, the gels became uniformly blue except at positions containing superoxide dismutase. Illumination was discontinued, when maximum contrast between the achromatic zones and the general blue color had been achieved. The gels were then photographed [17].

For estimation of nitrogen metabolism enzymes (GDG) leaves (200 mg) were chilled for 1 hour and then crushed with 2 ml extraction medium, containing 0-2 M Tris-buffer (pH 8.0), 0.1 M KH8PO4 and 1mM mercaptoethanol in a chilled mortar. The homogenate was filtered. The filtrate was centrifuged at 4 °C at 3500 rpm/min for 20 min. The supernatant was used as the enzyme source. The reaction was initiated by adding the enzyme extract to 0.5 ml of incubation mixture at 30°C. The assay mixture in a total volume of 1.5 ml contained 10 ml of 0.2 M Tris-buffer (pH 8.0), 0.2 ml of 0.2 M 2-oxoglutarate, 0.1 ml of 50 mM NADH and 0.2 ml of 1.5 M NH_4Cl. After 4 min, the reaction was terminated by adding 0-5 ml of 2,4-dinitrophenyl-

hydrazine (01 per cent in 2 n HC1) to 0-5 ml of reaction mixture after which 0-5 ml of benzyl alcohol (solvent specific for 2-oxoglutarate) was added. The contents were mixed for 2 min, and were then centrifuged at low speed (1500 rpm/min^{-1}) to separate the aqueous and the alcoholic fractions, 0-5 ml of 10 per cent Na_2CO_3 was then added to the latter and was mixed thoroughly. At denned time intervals (5 min), 2.5 ml of 1 n NaOH solution was added to each sample. A red to orange-red color developed, the optical density of which was measured at 420 nm [18].

Statistic processing of the results was done by Excel and according to P. Rokickij (1973).

Results of research

Crop yield is the criterion for assessing the quality of a variety. It depends on various factors. The following indicators are important elements of the crop structure: the number of productive stems from the unit area, the length of the ear, the number of spikelets in the ear, the number of grains in the ear, the mass of grains from one ear and the mass of 1000 grains. The productivity of plants is determined by a different combination of quantitative characteristics, which in turn are the result of a complex interaction of the genotype and environmental conditions. The mass of 1000 grains in grams reflects the amount of substance contained in the grain, its density and size. It is also an indicator of the quality of seed material taken into account in determining the seeding rate, largely determines its germination and viability [19].

The control over the yield formation gives a possibility to determine and compare the elements due to them the potential for productivity can be, to identify the critical stages in plants organogenesis during which reducing the elements of potential productivity passes, and to determine which elements of productivity are most stable under unfavorable conditions [20]. The analysis of morphogenesis contribution to productivity using the method of structural analysis of ripened plants according to the elements of shoot productivity is most promising because of its informative impact [21]. Spike productivity is a complex trait, which depends on the interaction of a number of features, such as length of shoot and ear, the number of spikelets in spike, number of grains per spike and thousand-grain weight [22].

We analyzed the features of crop formation in soft wheat and *B. distachyon* under the action of *P. recondita.* During the structural analysis, the indices of the productivity elements were estimated, which determine the main components of the crop: productive bushiness, plant height, length of the main spike, number of grains of the main spike, the mass of grain from the main spike and mass of 1000 grains. The results of the morphometric analysis revealed that the pathogen exerted an inhibitory effect on the parameters of the elements of productivity in wheat and *B. distachyon* (Table 1).

Table 1 – Effect of *Puccinia recondita* pathogen on productivity elements in spring wheat varieties Kazakhstanskaya 19 and Kazakhstanskaya early differing in rust tolerance and *B. distachyon*

Variant	Plant height, cm	Productive bushiness, number	Main spike			1000-grain weight, g
			Spike length, cm	Grain number	Grain weight, g	
Kaz. 19						
C	124.61±0.40	5.50±0.51	10.48±0.43	46.58±0.50	2.81±0.33	45.86±0.49
E	119.23±0.49**	3.18±0.38**	8.38±0.37**	40.28±0.45**	1.31±0.32***	31.64±0.30***
Kaz. early						
C	126.38±0.48	5.68±0.47	10.24±0.49	42.40±0.65	3.03±0.23	43.41±0.58
E	108.16±0.35***	3.15±0.36***	8.28±0.44*	37.08±0.47***	1.52±0.47*	35.44±0.41***
B. distachyon						
C	21.63±0.46	14.44±0.65	2.94±0.33	51.04±0.68	0.22±0.01	4.01±0.27
E	12.01±0.45***	5.84±0.37***	1.66 ±0.21**	45.16±0.47***	0.16±0.01***	2.80±0.18**
C – control, E – experiment; * under P < 0.05; ** under P < 0.01; *** under P < 0.001 comparative to control						

A comparative analysis of the effect of the pathogen on wheat genotypes shows a significant decrease in the indices of quantitative traits in infected plants (Table 1). Structural analysis of main spike traits such as "length", "number of grains" and "grain weight" revealed that length of the main spike is 2.94±0.33 cm in control, while experiment's significantly decreases to 1.66±0.21 cm. The trait "number of grains" is reduced by 12%. The grain weight of the main spike of *B. distachyon* under pathogen influence decreased by 28%. The main indicator of productivity elements "1000 grains weight" significantly decreases by 31% (4.01 ± 0.27 g – control and 2.80 ± 0.18 g – experiment). Wheat grains are frail, especially in the Kaz. early, e.g. the weight of grain from the main spike of Kaz. early in control plants – 3.03±0.23 g, after infection – 1.52±0.47 g, 1000 grains weight in the control is 43.41±0.58 g, and after infection with the pathogen – 35.44±0.41 g.

Symptoms of infection were recorded on the 7th day, which on the upper side of leaves, less on leaf vaginas as in the form of brown pustules (uredinia) of 0.5-2.0 mm in diameter were manifested.

Immunological analysis results are presented in Table 2. Immunological evaluation revealed maximum resistance to brown rust in B. *distachyon* plants at primary and secondary screening (67MR and 12MS, respectively). The Kaz. early characterized by moderate resistance to rust during at primary screening showed relatively high susceptibility at adult stage (60-70S). The Kaz. 19 variety with relative resistance at secondary screening was moderately susceptible.

Under contamination with a pathogen, grain protein content did not change in Kaz. 19 and Kaz. early varieties (Table 3).

Table 2 – Immunological screening of *B. distachyon* and wheat plants for resistance to *P. recondita*

Variants of experiments	Bd21	Kaz. early	Kaz. 19
	Degree of damage		
Control	75R	53MR	83MR
Treatment by *Puccinia recondita*	Primary screening		
	67MR	40MS	39MR
	Secondary screening		
	12MS	65S	45MS
R – resistant type of the reaction; MR – moderately stable; MS – moderately susceptible; S – susceptible			

Table 3 – *P. recondita* influence on protein content in wheat and *B. distachyon* grain

Variants of experiments	Protein content in grain, %
Kaz.19	
control	15.28±0.04
experiment	15.16±0.05
Kaz.early	
control	15.22±0.11
experiment	15.12±0.08
B.distachyon	
control	14.96±0.13
experiment	14.66±0.11

The pathogen also did not affect grain protein content in Bd21 (14.96±0.13% – control, and 14.66±0.11% – experiment).

The functional properties of storage proteins are of great importance in nutrition and in industrial transformation. The spectra of glutenins and gliadins are reliable genetic characteristics of any variety; they allow determining the nature of the inheritance of alleles of the initial and hybrid forms.

Analysis of storage proteins in seeds of *B. distachyon* and wheat was carried out in alkaline (SDS-Na electrophoresis) and acidic systems. Comparative analysis of the composition of storage proteins in a grain of healthy plants (control – c) and grain of plants infected with the pathogen (experiment – o) showed the following. By the composition of the high-molecular-weight glutenin subunits (HMW-GS), Kaz. early has a 2* subunit encoded by the *Glu1A* locus, 7+9 subunits encoded by the *Glu1B* locus and 5+12 subunits encoded by the *Glu1D* locus. The composition of HMW-GS in Kaz. 19-2*; 7*+9; 5+10. It is these subunits, which contribute to the potential baking quality, are highly ranked and the total quality assessment for these varieties for glutenin is 9 points. The effect of the pathogen on HMW-GS is not significantly expressed; however, the intensity of the bands in the gliadin and HMW-GS zone is diminished (shown on Figure 3).

In the spectrum of wheat prolamin (gliadins) fractionated in the acidic system, changes in the accumulation of components under the influence of the pathogen are clearly noted. The prolamin spectrum of wheat is usually divided into α, β-, γ- and ω-bands. In the experimental variants of both wheat varieties, the intensity of manifestation of the component ω 9 (on Figure 9, B indicated by an arrow) is considerably weaker in comparison with the control samples.

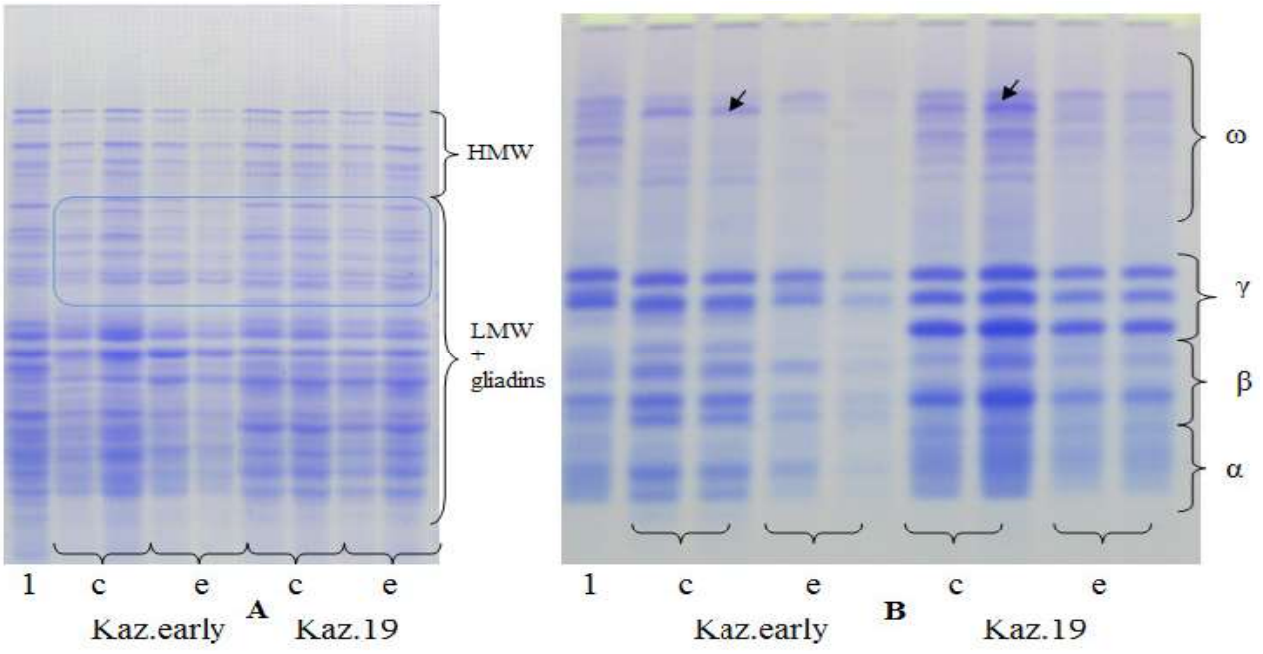

Figure 3 – Spectrum of storage proteins in soft wheat in alkaline (A) and acidic (B) electrophoretic systems.
Note: 1– reference variety of winter soft wheat Bogarnaya 56, c – control, e – experiment,
HMW – high molecular weight, LMW – low molecular weight

It is known that slow-moving components ω of the wheat zone (8 and 9) are controlled by *D* genome and affect the baking parameters. It can be assumed that the damage to plants by the pathogen negatively affects the quality. The weakening of the intensity of the components in the experimental samples was also noted for the gliadin bands – α, β-, γ. The data obtained shows that the pathogen causes changes in the accumulation of a number of components of the storage proteins in wheat seeds.

The reaction of *B. distachyon* plants to the infection was studied on the spectrum of storage proteins fractionated in both systems (Figure 4).

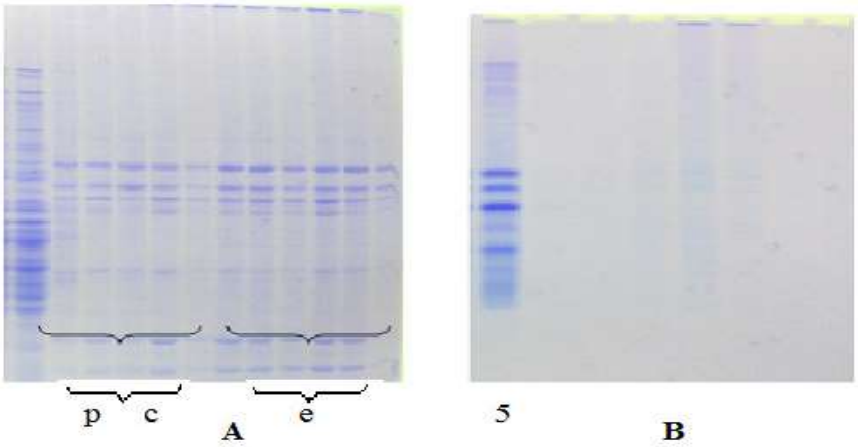

Figure 4 – Electrophoregrams of storage proteins of *B. distachyon* in alkaline (A) and acidic (B) systems.
Note: p – prolamines, c – control, e – experiment

The spectrum of storage proteins of *B.distachyon* seeds, obtained by SDS Na-electrophoresis (Figure 4, A), does not have slowly moving HMW-GS analogous to wheat grains, barley glutenins. Components in the middle part of the gel, apparently, are not prolamines, because when fractionated in an acidic system, these proteins are not detected or present in traces (Figure 4, B).

Also in the spectrum of the wild grass, there are fast-moving proteins that belong in wheat to the albumin-globulin fraction. In the spectrum of *B. distachyon* storage proteins, there is no decrease in the intensity of manifestation of the components of the protein spectrum in the experiment relatively to control. The reduction of the intensity of expression in the spectrum of storage proteins of B. distachyon is not observed in the experimental samples in comparison to control. Soluble proteins, including lectins, due to their physicochemical properties, play an important role in plant adaptation. Accumulation of lectins is closely related to the induction of the host plant stability. Interaction of lectins with carbohydrate components of phytopathogens can trigger, in response to microbial infection, a chain of protective reactions in the host plant [23-25].

A comparative study of soluble protein content in the leaves of *B. distachyon* and wheat seedlings showed that its content in *B. distachyon* leaves (0.464 ± 0.03 mg/ml) statistically reliably exceeds its content in soft wheat by 40-42%, in the roots, such difference varies within the limits of 19-36% (Table 4).

Table 4 – Comparative study of soluble protein content in leaves and roots of soft wheat and *B. distachyon* seedlings before and after infection with the pathogen

Object of study	Content of soluble protein, mg/ml	
	in leaves	in roots
before infection with the pathogen		
B. distachyon	0.464±0.03	0.11±0.003
Kaz. 19	0.186±0.01***	0.07±0.01**
Kaz. early	0.195±0.01***	0.09±0.01
after infection with the pathogen		
B. distachyon	0.452±0.01	0.10±0.01
Kaz. early	0.210±0.01***	0.09±0.01
Kaz. 19	0.172±0.02***	0.06±0.02
* – under P<0.005, ** – under P<0.01, *** – under P<0.001 as compared to control		

The data obtained (Table 4) also shows alterations in the protein content in the roots of wheat and *B. distachyon* after infection with *P. recondita*. The decrease in protein content in Kaz. 19 is 14.3%, and in *B. distachyon*, 9.1%. At the same time, the Kaz. early shows an increase of 12.5%. It is known that suppressors of the fungus, inhibit plant resistance to infection and the protective biosynthetic activity of damaged tissues, immunosuppressors are natural antagonists of elicitors and induce in plants a susceptibility to diseases. It can be proposed that the reaction of plants to the influence of the phytopathogen is associated with a decrease in the rate of formation of protective reactions and inhibition of the biosynthetic activity in affected tissues, including activation of soluble proteins [26-28].

In plants, free proline accumulates in response to various abiotic and biotic stresses: water deficiency, salinization, pathogen infection, etc. [29-31]. According to the results of the experiment, the proline content of *B. distachyon* control plants is almost two times lower than that in soft wheat varieties. After infection with *P. recondita,* the content of free proline in the leaves of seedlings in Kaz. 19 variety statistically increases by 43% in comparison with control, in Kaz. early by 59%, and in *B. distachyon* – 30% (Table 5).

A change in the activity of enzymes of metabolism is one of the significant criteria changes the genetic apparatus exposed to mutagens. In this regard, of particular interest is the study of the activity of several key enzymes of antioxidant system in mutant genotypes as compared to the initial variety. The study of enzymatic activity makes it possible to judge the intensity of the metabolism of the plant body, and more reliably estimate the vitality of these mutant genotypes.

SOD is a metalloenzyme that performs a key function in the mechanism of protection against oxidative stress in all aerobic organisms. Catalyzes the decomposition of O_2^- to O_2 and H_2O_2. The study of SOD activity shows that before the infection with the pathogen, its content in the leaves of the studied wheat varieties (control) equals to 18-29 μg protein/min, whereas, in *B. distachyon*, this indicator is lower. Plant infection results in an increase in enzyme activity of 37 and 24% relatively to control in wheat varieties, Kaz. early and Kaz. 19, while *B. distachyon* does not show significant changes in enzyme activity. Activation of SOD with an increase in the effect of superoxide radicals on plants presumably provides protection of plant cells and tissues against oxidative damage.

The study of SOD activity showed that before the pathogen was damaged, its content in the leaves of the studied wheat varieties (control) was 18-29 μg of protein/min, whereas in *B. distachyon* this indicator was lower (Figure 5). Infection of plants led to an increase in enzyme activity by 37 and 24% relative to the control in wheat varieties Kaz. early and Kaz. 19, while *B. distachyon* showed no significant changes in the activity of the enzyme. Activation of SOD while increasing the effects of superoxide radicals on plants presumably protects plant cells and tissues from oxidative damage.

Table 5 – Free prolin content in seedlings of wheat and *B. distachyon* under *P.recondita* action

Variants of experiments	Free prolin content in wheat and *B. distachyon*, mg/g	
	leaves	roots
B.distachyon		
control	0.23±0.02	0.17±0.02
experiment	0.30±0.01*	0.21±0.01
Kaz.19		
control	0.39±0.005	0.18±0.01
experiment	0.56±0.03**	0.25±0.01*
Kaz.early		
control	0.47±0.02	0.12±0.01
experiment	0.75±0.03***	0.19±0.02*
* – under P<0.005, ** – under P<0.01, *** – under P<0.001 as compared to control		

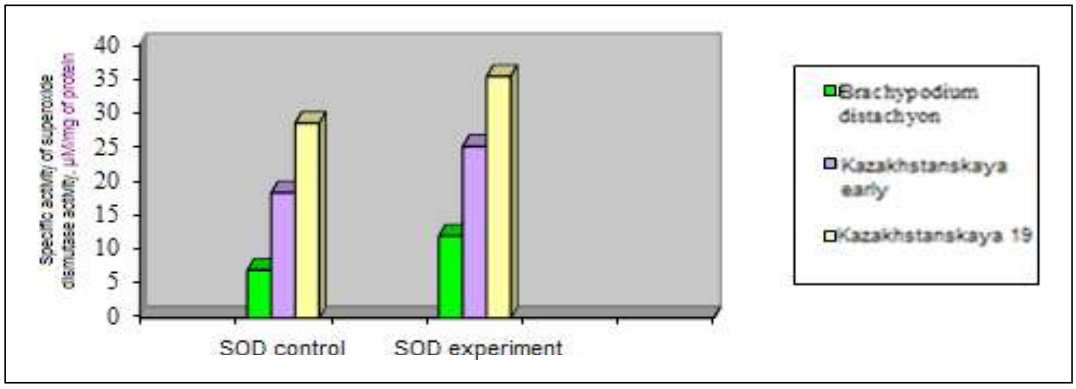

Figure 6 – Comparative analysis of SOD activity before and after the pathogen attack

The enzymes of nitrogen metabolism are of great importance in the processes of plant metabolism. Among the enzymes of nitrogen metabolism, the most important are enzymes of the metabolism of the amino acid of the nitrogen exchange – glutamate, which ensures the assimilation of mineral nitrogen and the synthesis of all amino acids. It is known that the level of enzyme complex of malate dehydrogenase and glutamate oxaloacetate aminotransferase (EC MDG-GOAT) activity plays an important role in the detoxification of protein degradation products, and its reduction promotes an increase in the synthesis of aspartate, which is necessary for the synthesis of purines and other valuable metabolites.

The results of the experiment show that the activity of nitrogen exchange enzymes MDG-GOAT and glutamate dehydrogenase (GDG) in wheat varieties before treatment with the pathogen exceeds its activity in *B. distachyon* by 3 and 2 times, respectively. GDG activity in *Brachypodium* before the infection with the pathogen is 31.59 ± 0.04 μM/mg protein, in Kaz. early is 73.46 ± 0.01 μM/mg protein, and in Kaz. 19 – 78.63 ± 0.01 μM/mg protein. In turn, the activity of EC in Kaz. 19, as more resistant to leaf rust, is significantly higher than in the susceptible variety Kaz. early (329.23 ± 0.01 and 267.12 ± 0.01 μM/mg protein, respectively). Since EC MDG-GOAT participates in a nontoxic pathway of catabolism of glutamate, it can be assumed that the high activity of EC promotes plant resistance to rust.

The analysis of the results (Table 6) showed that under the action of the pathogen, there is no significant increase in the activity of GDG in *B. distachyon* as compared to control (4%), in the susceptible variety of Kaz. early, the activity of GDG increased by 6.4%, in the case of the resistant variety Kaz. 19 enzyme activity increases by 5.7%. In turn, there was an increase in the activity of MDG-GOAT in *B. distachyon* by 4.7%, in Kaz. 19 – 6.4%, and in Kaz. early – 10%. It is known for genetically-selection works that wheat is of interest with reduced activity of GDG since this enzyme releases toxic ammonia destroying biological membranes as a result of the oxidative deamination reaction. The level of EC MDG-GOAT activity plays an important role in the detoxification of the protein degradation products formed within the processes of biotic and abiotic stresses.

Plants with reduced activity of GDG and high MDG-GOAT survive better under conditions of infection and retain high productivity, which may be justified by increased resistance to adverse effects of biotic and abiotic stress factors of the environment in *B. distachyon* as a wild grass.

It is known that malate dehydrogenase (MDG) plays a central role in changing metabolic processes when adapting plants to the environment.

According to the Table 7, in *B. distachyon*, the activity of MDG after infection with the pathogen slightly decreases to 27.17±0.01 μM/mg of protein. Wheat varieties show an increase in the activity of MDG relatively to control: 20% for Kaz. early and 17% for Kaz. 19, and equals to 47.94 ± 0.02 and 42.66±0.02 μM/mg, respectively.

It is known that ADH plays a central role in the anaerobic metabolism of plants. As is known, the enzyme ADH in plant cells is necessary to maintain equilibrium in the system of ethanol-acetaldehyde. It is known that an increase in the concentration of ethanol and acetaldehyde is often accompanied by a decrease in the germination of seeds while maintaining their high viability [32].

Table 6 – Comparative study of specific activity of nitrogen exchange enzymes in leaves of soft wheat and *B. distachyon* seedlings before and after the pathogen infection

Variants of the experiment	Specific activity of enzymes, μM/mg of protein	
	GDG	EC MDG-GOAT
B. distachyon		
Control	31.59±0.04	110.22±0.03
Upon infection with *P.recondita*	32.98±0.02***	115.49±0.03***
Kaz. early		
Control	73.46±0.01	267.12±0.01
Upon infection with *P.recondita*	78.16±0.01***	239.40±0.01***
Kaz. 19		
Control	78.63±0.01	329.23±0.01
Upon infection with *P.recondita*	83.14±0.01***	350.34±0.01***
*** – under P<0.001 as compared to control		

The activity of ADH in *B. distachyon* after infection with the pathogen decreases by 6.8% – 82.61 ± 0.02*** μM/mg protein. The activity of ADH in Kaz. early decreases by 4.38% and amounts to 480.19 ± 0.02 ** μM/mg of protein, while in Kaz. 19 it increases by 2.3% and equals to 530.36 ± 0.02 ** μM/mg of protein. It should be noted that the activity of alcohol dehydrogenase (ADH) in *B. distachyon* is quite low – 5.8 times less than in wheat plants and equals to 88.71 ± 0.01 μM/mg of protein. ADH activity in Kaz. early and Kaz. 19 is close to 502.18 ± 0.01 and 518.40 ± 0.01 μM/mg of protein, respectively.

Table 7 – Activity of enzymes (μM/mg of protein) of energy metabolism in leaves of soft wheat and *B. distachyon* seedlings before and after the infection with the pathogen

Variants of the experiment	Specific activity of enzymes, μM/mg of protein	
	MDG	ADH
B. distachyon		
Control	30.24±0.02	88.71±0.01
Upon infection with *P.recondita*	27.17±0.01***	82.61±0.02***
Kaz. early		
Control	39.62±0.02	502.18±0.01
Upon infection with *P.recondita*	47.94±0.02***	480.19±0.02***
Kaz. 19		
Control	36.16±0.01	518.40±0.01
Upon infection with *P.recondita*	42.66±0.02***	530.36±0.02***
*** – under P<0.001 as compared to control		

Conclusion

Comparative study of molecular genetic and biochemical features of the model of wild cereal *B.distachyon* with related cereal grains enables us to understand mechanisms of resistance and increase of resistance of wheat plants to both abiotic and biotic factors. A change in the activity of enzymes of metabolism is one of the significant criteria changes the genetic apparatus exposed to

mutagens. In this regard, of particular interest is the study of the activity of several key enzymes of nitrogen and energy metabolism in mutant genotypes as compared to the initial variety, which makes it possible to judge the intensity of the metabolism in plant, and more reliably estimate the vitality of these mutant genotypes. The increased interest to the composition of storage proteins in wheat is associated with functional significance of specific proteins in determination of the baking properties. Refinement of genetic control and the identification of new and rare protein subunits, detected in the course of studying the collection and breeding material is necessary for a reliable assessment of samples, as well as for the expansion of the genetic basis of cultivars created by examining the value of genotypes with specific variants of alleles and their inclusion in the selection process. As such, the results of morphometric study show that the pathogen exerted an inhibitory effect on the parameters of the elements of productivity in wheat and *B. distachyon*. Besides, the activity of nitrogen exchange enzymes MDG-GOAT and glutamate dehydrogenase in wheat varieties before treatment with the pathogen exceeds its activity in *B. distachyon* by 3 and 2 times, respectively; the activity of MDG after infection with the pathogen slightly decreases to 27.17±0.01 μM/mg of protein. Plants with reduced activity of GDG and high MDG-GOAT survive better under conditions of infection and retain high productivity, which may be justified by increased resistance to adverse effects of biotic and abiotic stress factors of the environment in *B. distachyon* as a wild grass.

References

1 Tilman D., Balzer C., Hill J., Befort B. Global food demand and the sustainable intensification of agriculture // Proc. Natl. Acad. Sci. USA. – 2011. – Vol. 108. – P. 20260–20264.

2 Food and Agriculture Organization of the United Nations. Country fact sheet on food and agriculture policy trends: Kazakhstan. – 2017. – P. 1-6.

3 Figueroa M., Castell-Miller C., Li F., Hulbert S., Bradeen J. Pushing the boundaries of resistance: insights from Brachypodium-rust interactions // Front Plant Sci.. – 2015. – Vol. 6. – P. 1-11.

4 Müller B., Grossniklaus U. Model organisms – a historical perspective // J Proteomics. – 2010. – Vol. 73. – P. 2054–2063.

5 Ayliffe M., Singh D., Park R., Moscou M., Pryor T. Infection of Brachypodium distachyon with selected grass rust pathogens // MPMI. – 2013. – Vol. 26. – P. 946–957.

6 Койшибаев М. Болезни зерновых культур. – А.: Бастау. – 2002. – С. 367.

7 Кочоров А., Сагитов А., Аубакирова А., Алишеров Ж. Распространение ржавчинных болезней пшеницы в Казахстане // Национальная академия микологии. – 2013. – С. 163-165.

8 Неттевич Э. Рождение и жизнь сорта. – М.: Московский рабочий. – 1978. – С. 175.

9 Bushnell W., Roelfs A. The Cereal Rusts: origins, specificity, structure, and physiology. – Academic Press, Inc. – 1984. – P. 546.

10 Mullins E. Engineering for disease resistance: persistent obstacles clouding tangible opportunities // Pest Manag Sci. – 2015. – Vol. 71. – P. 645-651.

11 Shulembayeva K., Chunetova Zh., Dauletbayeva S., Tokubayeva A., Omirbekova N., Zhunusbayeva Zh., Zhussupova A. Some results of the breeding and genetic studies of common wheat in the south-east of Kazakhstan // Int J Biol Chem. – 2014. – Vol. 8. – P. 6-10.

12 Draper J., Mur L., Jenkins G., Ghosh-Biswas G., Bablak P., Hasterok R., Routledge A. Brachypodium distachyon: a new model system for functional genomics in grasses // Plant Physiol. – 2001. – Vol. 127. – P. 1539-1555.

13 Peraldi A., Beccari G., Steed A., Nicholson P. Brachypodium distachyon: a new pathosystem to study Fusarium head blight and other Fusarium diseases of wheat // BMC Plant Biol. – 2011. – Vol. 11. – P. 1-14.

14 McIntosh R., Wellings C., Park R. Wheat Rusts: An atlas of Resistance Genes. – Australia: CSIRO. – 1995. – P. 200.

15 Бейли Д. Методы химии белка. – М.: Иностр. лит. – 1965.– 284 с.

16 Srivastava G.C. Modern Methods in Plant Physiology. – India: New India Publishing. – 2015. – P. 149.

17 Rothe G.M. Electrophoresis of Enzymes: Laboratory Methods. – Germany: Springer. – 2011. –328 p.

18 Mathur M., Singh V.K., Mukerji D., Mathur S.N. Colorimetric estimation of glutamate dehydrogenase in leaf tissue of Vigna mungo (L.) Hepper // Ann. Bot. – 1983. – P. 105-110.

19 McKenzie E., Lees E. Glutamate dehydrogenase activity in developing soybean seed: isolation and characterization of three forms of the enzyme // Arch Biochem Biophys. – 1981. – Vol. 212. – P. 290-297.

20 Calderini D., Dreccer M., Slafer G. Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends // Plant Breed. – 1995. – Vol. 114. – P. 108-112.

21 Морозова З. Методология использования закономерностей морфогенеза колосовых злаков в селекции: научно-методическое пособие. – М.: МАКС Пресс. – 2013. – С. 365.

22 Натрова З., Смочек Я. Продуктивность колоса зерновых культур. – М.: Колос. – 1983. – С. 45.

23 Шакирова Ф.М. Неспецифическая устойчивость растений к стрессовым факторам и ее регуляция. – Уфа: Гилем. – 2001. – 159 с.

24 Молодченкова О., Адамовская В., Цисельская Л., Сагайдак Т. Выделение и свойства лектинов клеточных стенок из проростков пшеницы при инфицировании грибными патогенами и действии салициловой кислоты // Вестник Харьковского национального аграрного университета серия биология. – 2012. – Т. 26. – С. 54-60.

25 Белава В., Зеленый С., Панюта О. Экспрессия генов лектина и дефенсина у сортов пшеницы Мироновская 808 и Roazon при инфицировании Pseudocercosporella herpotrechoides // Biopolimers and Cell. – 2010. – Т. 26. – С. 45-50.

26 Hare P., Cress W., Staden J. Prolin synthesis and degradation: a model for elucidating stress related signal transduction // J Exp Bot. – 1999. – Vol. 50. – P. 413-434.

27 Кузнецов В., Шевякова Н. Пролин при стрессе: биологическая роль, метаболизм, регуляция // Физиол. раст. – 1999. – Т. 46. – С. 321–336.

28 Ozgur R., Uzilday B., Sekmen A., Turkan I. Reactive oxygen species regulation and antioxidant defence in halophytes // Funct. Plant Biol. – 2013. – Vol. 40. – P. 832–847.

29 Qamar A., Mysore K., Senthil-Kumar M. Role of proline and pyrroline-5-carboxylate metabolism in plant defense against invading pathogens // Front Plant Sci. – 2015. – Vol.6. – P. 1-9.

30 Cecchini N., Monteoliva M., Alvarez M. Proline dehydrogenase contributes to pathogen defense in Arabidopsis // Plant Physiol. – 2011. – Vol. 155. – P. 1947-1959.

31 Barker A., Pilbeam D. Handbook of Plant Nutrition. – CRC Press. – 2015. – P. 773.

32 Kodde J., Buckley W., de Groot C., Retiere M., Zamora A., Groot S. A fast ethanol assay to detect seed deterioration // Seed Sci Res. – 2011. – P. 1-8.

References

1 Ayliffe M., Singh D., Park R., Moscou M., Pryor T. (2013) Infection of Brachypodium distachyon with selected grass rust pathogens. MPMI, vol. 26, pp. 946–957.

2 Barker A., Pilbeam D. (2015) Handbook of Plant Nutrition. CRC Press, p. 773.

3 Bailey D. (1965) Metody himii belka [Techniques in protein chemistry]. M.: Inostr. Lit, p. 284.

4 Belava V., Zelenyj S., Panjuta O. (2010) Jekspressija genov lektina i defensina u sortov pshenicy Mironovskaja 808 i Roazon pri inficirovanii seudocercosporella herpotrechoides [Expression of lectin and defensin genes in wheat varieties Mironovskaya 808 and Roazon with infection of Pseudocercosporella herpotrechoides]. Biopolimers and Cell, vol. 26, pp. 45-50.

5 Bushnell W., Roelfs A. (1984) The Cereal Rusts: origins, specificity, structure, and physiology. Academic Press, Inc., p. 546.

6 Calderini D., Dreccer M., Slafer G. (1995) Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends. Plant Breed., vol. 114, pp. 108-112.

7 Cecchini N., Monteoliva M., Alvarez M. (2011) Proline dehydrogenase contributes to pathogen defense in Arabidopsis. Plant Physiol., vol. 155, pp. 1947-1959.

8 Draper J., Mur L., Jenkins G., Ghosh-Biswas G., Bablak P., Hasterok R., Routledge A. (2011) Brachypodium distachyon: a new model system for functional genomics in grasses. Plant Physiol., vol. 127, pp. 1539-1555.

9 Figueroa M., Castell-Miller C., Li F., Hulbert S., Bradeen J. (2015) Pushing the boundaries of resistance: insights from Brachypodium-rust interactions. Front Plant Sci., vol. 6, pp. 1-11.

10 Food and Agriculture Organization of the United Nations (2017) Country fact sheet on food and agriculture policy trends: Kazakhstan, pp. 1-6.

11 Hare P., Cress W., Staden J. (1999) Prolin synthesis and degradation: a model for elucidating stress related signal transduction. J Exp Bot, vol. 50, pp. 413-434.

12 Kojshibaev M. (2002) Bolezni zernovyh kul'tur [Diseases of grain crops]. A.: Bastau, p. 367.

13 Kochorov A., Sagitov A., Aubakirova A., Alisherov Zh. (2013) Rasprostranenie rzhavchinnyh boleznej pshenicy v Kazahstane [Distribution of wheat rusts in Kazakhstan]. Nacional'naja akademija mikologii, pp. 163-165.

14 Kodde J., Buckley W., de Groot C., Retiere M., Zamora A., Groot S. (2011) A fast ethanol assay to detect seed deterioration. Seed Sci Res, pp. 1-8.

15 Kuznecov V., Shevjakova N. (1999) Prolin pri stresse: biologicheskaja rol', metabolizm, reguljacija [Proline under stress: biological role, metabolism, regulation]. Fiziol rast, vol. 46, pp. 321-336.

16 Mathur M., Singh V.K., Mukerji D., Mathur S.N. (1983) Colorimetric estimation of glutamate dehydrogenase in leaf tissue of Vigna mungo (L.) Hepper. Ann. Bot., pp. 105-110.

17 McIntosh R., Wellings C., Park R. (1995) Wheat Rusts: An atlas of Resistance Genes. Australia: CSIRO, p. 200.

18 McKenzie E., Lees E. Glutamate dehydrogenase activity in developing soybean seed: isolation and characterization of three forms of the enzyme. Arch Biochem Biophys, vol. 212, pp. 290-297.

19 Molodchenkova O., Adamovskaja V., Cisel'skaja L., Sagajdak T. (2012) Vydelenie i svojstva lektinov kletochnyh stenok iz prorostkov pshenicy priinficirovanii gribnymi patogenami i dejstvii salicilovoj kisloty [Extraction and properties of cell wall lectins from infected with fungal pathogens and affected by salicylic acid wheat germs]. Vestnik Har'kovskogo nacional'nogo agrarnogo universiteta serija biologija, vol. 26, pp. 54-60.

20 Morozova Z. (2013) Metodologija ispol'zovanija zakonomernostej morfogeneza kolosovyh zlakov v selekcii: nauchno-metodicheskoe posobie [Methodology of using the morphogenesis of spiked cereals patterns in breeding: scientific-methodological manual]. M.: MAKS Press, p. 365.

21 Mullins E. (2015). Engineering for disease resistance: persistent obstacles clouding tangible opportunities. Pest Manag Sci, vol. 71, pp. 645-651.

22 Müller B., Grossniklaus U. (2010) Model organisms – a historical perspective. J Proteomics, vol.

23 Natrova Z., Smochek Ja. (1983) Produktivnost' kolosa zernovyh kul'tur [Productivity of the ear of cereals]. M.: Kolos, p. 45.

24 Nettevich Je. (1978) Rozhdenie i zhizn' sorta [Birth and life of a variety]. M.: Moskovskij rabochij, p. 175.

25 Ozgur R., Uzilday B., Sekmen A., Turkan I. (2013) Reactive oxygen species regulation and antioxidant defence in halophytes. Funct Plant Biol, vol. 40, pp. 832–847.

26 Peraldi A., Beccari G., Steed A., Nicholson P. (2011) Brachypodium distachyon: a new pathosystem to study Fusarium head blight and other Fusarium diseases of wheat. BMC Plant Biol., vol. 11, pp. 1-14.

27 Qamar A., Mysore K., Senthil-Kumar M. (2015) Role of proline and pyrroline-5-carboxylate metabolism in plant defense against invading pathogens. Front Plant Sci, vol. 6, pp. 1-9.

28 Rothe G.M. (2011) Electrophoresis of Enzymes: Laboratory Methods. Berlin: Springer, p.328.

29 Shakirova F. (2001) Nespecificheskaja ustojchivost' rastenij k stressovym faktoram i ee reguljacija [Plants nonspecific resistance to stress factors and its regulation]. Ufa: Gilem, p. 159.

30 Shulembayeva K., Chunetova Zh., Dauletbayeva S., Tokubayeva A., Omirbekova N., Zhunusbayeva Zh., Zhussupova A. (2014) Some results of the breeding and genetic studies of common wheat in the south-east of Kazakhstan. Int J Biol Chem, vol. 8, pp. 6-10.

31 Srivastava G.C. Modern Methods in Plant Physiology. – India: New India Publishing. – 2015. – P. 149.

32 Tilman D., Balzer C., Hill J., Befort B. (2011) Global food demand and the sustainable intensification of agriculture. Proc. Natl. Acad. Sci. USA, vol. 108, pp. 20260–20264.