



ПОКАЗАТЕЛЬ ПРОНИЦАЕМОСТИ КЛЕТОЧНЫХ МЕМБРАН ПРОРОСТКОВ В ОЦЕНКЕ СТРЕССОУСТОЙЧИВОСТИ СОРТОВ ПШЕНИЦЫ

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Представлены результаты исследований применения показателя проницаемости клеточных мембран, определяемого по относительному изменению удельной электропроводности (УЭП) водных вытяжек тканей, проростков сортов яровой пшеницы Новосибирская 18, Новосибирская 44, Сибирская 21 и Омская 18 при совместном действии стрессоров. В модельных лабораторных вегетационных опытах исследована почасовая динамика УЭП водных вытяжек листьев проростков (экспозиция листьев в воде 0,5–4,5 ч) в условиях одновременного действия хлоридного засоления (1,3%) и возбудителя обыкновенной гнили злаков *Bipolaris sorokiniana* Shoem. (5000 конидий на зерно). Установлено достоверное увеличение УЭП в 1,5 раза и скорости выхода электролитов в 2 раза у менее устойчивого сорта Новосибирская 44 по сравнению с более устойчивым Омская 18. Исследована посуточная динамика УЭП 10–16-суточных проростков в условиях последовательного действия гипертермии семян (43 °C), хлоридного засоления (1,3%) и *Bipolaris sorokiniana* Shoem. (5000 конидий на зерно). Установлен протекторный эффект гипертермии у более устойчивого сорта Сибирская 21 (достоверное снижение УЭП до 1,3 раза) по сравнению с вариантом без прогрева семян. У менее устойчивого сорта Новосибирская 18 прогрев семян дестабилизировал состояние клеточных мембран (достоверное увеличение УЭП и скорости выхода электролитов в 1,5 и 1,2 раза соответственно). Экспериментально определены условия, обеспечивающие выявление максимальных различий исследуемых сортов пшеницы: возраст проростков – 10 сут, временной интервал экспозиции образцов в воде – 1,5–4,5 ч. Межсортные различия по относительному изменению значений УЭП в варианте без прогрева семян составляли 1,9 раза и в варианте с прогревом семян – 3,7 раза с достоверностью различий на уровнях $p \leq 0,05$ и $p \leq 0,01$. Межсортные различия по относительному изменению УЭП, установленные на интервале времени экспозиции выхода электролитов 1,5–4,5 ч, составляли 1,50–1,67 раза с достоверностью различий на уровне $p \leq 0,05$. Предложенный подход позволит разработать методику оценки новых генотипов на устойчивость к совместному действию биотических и абиотических стрессоров.

Ключевые слова: сорт, пшеница, устойчивость, стрессоры, проницаемость клеточных мембран, удельная электропроводность

THE INDICATOR OF CELL MEMBRANE PERMEABILITY OF WHEAT SEEDLINGS IN ASSESSING STRESS RESISTANCE OF WHEAT VARIETIES

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The paper presents the results of studies on the use of the permeability index of cell membranes, determined by the relative change in the specific electrical conductivity (EC) of water extracts of

tissues of spring wheat seedlings, varieties Novosibirskaya 18, Novosibirskaya 44, Sibirskaya 21 and Omskaya 18, under the combined action of stressors. In model laboratory vegetation experiments, the hourly dynamics of the EC of water extracts of seedling leaves (exposure of leaves to water for 0.5–4.5 h) was investigated under the simultaneous action of chloride salinity (1.3%) and the causative agent of common rot of cereals *Bipolaris sorokiniana* Shoem. (5000 conidia per grain). It was established that EC increased by 1.5 times and the rate of electrolyte leakage increased twofold in the less resistant variety Novosibirskaya 44 compared to the more resistant Omskaya 18. The daily dynamics of the EC of 10–16-day-old seedlings was studied under the sequential action of seed hyperthermia (43 °C), chloride salinity (1.3%), and *Bipolaris sorokiniana* Shoem. (5000 conidia per grain). The protective effect of hyperthermia was established in the more resistant variety Sibirskaya 21 (a decrease in EC up to 1.3 times) in comparison with the variant without heating the seeds. In the less resistant variety Novosibirskaya 18, heating the seeds destabilized the state of the cell membranes (increase in EC and electrolyte leakage rate by 1.5 and 1.2 times respectively). The conditions that ensure the identification of the maximum differences in the studied wheat varieties were experimentally determined: the age of seedlings 10 days, the time interval of exposure of the samples to water 1.5–4.5 h. Intervarietal differences in the relative change in the EC values in the variant without heating the seeds were 1.9 times and in the variant with heating the seeds – 3.7 times, with the significance of difference at the levels $p \leq 0.05$ and $p \leq 0.01$. Intervarietal differences in the relative change in the EC, established for the time interval exposition of electrolytes leakage of 1.5–4.5 h, were 1.50–1.67 times with the significance of difference at the level of $p \leq 0.05$. The proposed approach will make it possible to develop a methodology for assessing new genotypes for resistance to the combined action of biotic and abiotic stressors.

Keywords: wheat, variety, resistance, stressors, permeability of cell membranes, electrical conductivity

Для цитирования: Гурова Т.А., Свежинцева Е.А., Чесноченко Н.Е. Показатель проницаемости клеточных мембран проростков в оценке стрессоустойчивости сортов пшеницы // Сибирский вестник сельскохозяйственной науки. 2021. Т. 51. № 3. С. 31–43. <https://doi.org/10.26898/0370-8799-2021-3-4>

For citation: Gurova T.A., Svezhintseva E.A., Chesnochenko N.E. The indicator of cell membrane permeability of wheat seedlings in assessing stress resistance of wheat varieties. *Sibirskii vestnik sel'skokhozyaistvennoi nauki* = *Siberian Herald of Agricultural Science*, 2021, vol. 51, no. 3, pp. 31–43. <https://doi.org/10.26898/0370-8799-2021-3-4>

Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

Conflict of interest

The authors declare no conflict of interest.

INTRODUCTION

In crop variety breeding it is necessary to diagnose their resistance to biotic and abiotic stress factors of the environment. Performing such an assessment in the field by a direct method (by yield depression) is a laborious and time-consuming stage. In this regard, the assessment of varieties is carried out in laboratory conditions by indirect methods to change the physiological, biochemical and biophysical parameters, which reflect the process of plant adaptation to stress [1, 2]. The advantages of indirect assessment include high information content, the ability to predict a decrease in pro-

ductivity under stress at the early stages of plant ontogenesis, the independence of diagnostic indicators from environmental conditions, and the possibility of automated assessment [3].

For the most part, the diagnostic methods used make it possible to assess genotypes for resistance to one acting stressor. However, in natural conditions, plant organisms are exposed to a complex of environmental stress factors in various combinations and doses at different stages of plant development. Therefore, it is important to conduct research on the resistance of crops to stressful influences, simulating their cumulative effect [4]. In Siberia, among other stressors limiting the yield, soil salinity, com-

mon root rot, and elevated air temperatures should be taken into account [5].

The main plant responses to abiotic and biotic factors are associated with the occurrence of oxidative stress, which disrupts the structure and function of cell membranes. This leads to complete cell death, inhibition or arrest of growth [6, 7]. The biological membrane serves as a primary protective barrier and provides a nonspecific component of adaptation mechanisms under the action of stress factors [8].

The most important property of biological membranes is permeability. It determines the stability of plant tissues and cells and characterizes the static, genetically determined potential resistance of the genotype [9, 10]. The higher the resistance of plants, the less the structure and properties of membranes are disturbed, and the release of electrolytes from plant tissues into the external environment decreases [11]. Changes in membrane permeability for electrolytes is one of the criteria for plant resistance to biotic and abiotic stressors. The reason for the increase in the permeability of cell membranes under stress may be the H^+ / Ca^{2+} ratio in the membranes, a decrease in the level of SH-groups, the formation of defective regions in lipids, and an increase in the activity of endogenous phospholipases¹.

The permeability of cell membranes is determined by the conductometric method by measuring the specific electrical conductivity of electrolytes released through damaged cell membranes from plant tissues and organs into distilled water, which can be a diagnostic indicator of the stress resistance of the variety.

The results of studies on changes in the permeability of cell membranes under the action of low temperatures on plants [12, 13], drought [14, 15], infection with a pathogen [16, 17], biologically active substances [18, 19], heavy metals [20] were obtained.

The method also turned out to be in demand in studying the response of plants to the separate and combined action of stressors: low temperature and cadmium ions [21], heat shock and water deficit [22], unfavorable temperature and heavy metals [23], salinity and zinc [24].

Earlier, we experimentally proved the possibility of taking into account the change in the permeability index of cell membranes, assessed by the specific electrical conductivity (EC) of seedling tissues. On the basis of this, diagnostic methods have been developed to assess the resistance of spring wheat and barley varieties to chloride salinization and the causative agent of common rot of cereals^{2, 3}.

The aim of the research is to evaluate the specific electrical conductivity of aqueous extracts of leaves as an indicator of the permeability of cell membranes of seedlings to determine the stress resistance of wheat varieties to the combined action of the causative agent of root rot of cereals, chloride salinization, and hyperthermia of seeds.

MATERIAL AND METHODS

The experimental work was carried out in the laboratory for the study of physical processes in agrophytocenoses of the Siberian Institute of Physics and Technology, SFNCA RAS. The studies were carried out in laboratory conditions (vegetation experiment - water crops) on seedlings of zoned varieties of soft spring wheat Novosibirskaya 18, Novosibirskaya 44, Sibirskaya 21 of the Siberian Research Institute of Plant Production, a branch of the ICG SB RAS, and Omsk 18 of the selection of the Omsk ASC.

The study of the permeability index of seedling cell membranes was carried out to assess the resistance of varieties to the combined action of the common rot pathogen *Bipolaris sorokiniana* Shoem. (*B. sorokiniana*), chloride sa-

¹Chirkova T.V. Physiological foundations of plant resistance: a textbook. SPb.: Publishing house S.-Pb. University, 2002.244 p.

²Patent RU 2446671 IPC A01G7 / 00, A01H1 / 04. The method for determining the relative resistance of soft spring wheat varieties to chloride salinity / T.A. Gurova, V.Yu. Berezina, N.S. Kutserubova. Publ. 10.04.2012.

³Patent RU 2625027 IPC A01C12N 1/14, A01H 5/12. The method for determining the relative resistance of soft spring wheat varieties to the causative agent of common root rot of cereals / T.A. Gurova, V.V. Alt, O.S. Lugovskaya. Publ. 11.07.2017.

linization and hyperthermia of seeds. The hourly and daily dynamics of the EC of the extracts of seedlings leaves of wheat cultivars differing in their resistance to these stressors was studied. Preliminarily, the resistance of varieties was assessed in laboratory conditions according to growth parameters, changes in dry and wet biomass, and development of the disease on seedlings [25].

The hourly dynamics of changes in the leaf extracts EC (kinetics of electrolyte release after exposure of leaf tissue in distilled water for 0.5–4.5 h) was recorded in 10-day-old seedlings of varieties Omskaya 18 (relatively stable) and Novosibirskaya 18 (relatively unstable). The stressors acted simultaneously.

Experiment options:

- control (stress-free seeds),
- infection of seeds with *B. sorokiniana* (5000 conidia per grain) + chloride salinity of 1.3%.

The daily dynamics of EC was recorded in 10–16 day old seedlings of varieties Sibirskaya 21 (relatively resistant) and Novosibirskaya 18 (relatively unstable). A sequential action of stressors was used - preliminary heating of seeds, followed by infection with *B. sorokiniana* and chloride salinization.

Experiment options:

- control (seeds without heating) and heating of seeds at 43 °C;
- seeds without heating + infection with *B. sorokiniana* (5000 conidia per grain) + chloride salinity 1.3%;
- seed heating + *B. sorokiniana* infection (5000 conidia per grain) + chloride salinity 1.3%.

The levels of stress loads (conidial suspension of *B. sorokiniana* 5000 conidia per grain, concentration of sodium chloride (NaCl) 1.3% and temperature 43 °C) were determined by us in specially conducted vegetation experiments. These levels make it possible to differentiate wheat varieties of Siberian selection when as-

sessing their resistance to these stress factors (see footnotes 2 and 3) [26].

Wheat seeds were pre-sterilized with 96% ethyl alcohol for 2 min, followed by three rinsing with distilled water. The seeds were heated for 20 min in hot water in a water bath according to the VIR⁴ method. After cooling, the seeds were placed in Petri dishes with moistened filter paper and germinated in a thermostat at 22 °C for three days. Simultaneously, the soaked seed samples were germinated without heating. Seed infection was carried out in the germination phase (on the third day of cultivation) with a conidial suspension of a mixture of medium pathogenic isolates of *B. sorokiniana* prepared on 0.1% aqueous agar (one drop per grain). Then the plants were grown in a roll culture on tap water (control option) and sodium chloride (*B. sorokiniana* infection + chloride salinization option) in a Biotron-8 climatic chamber with a day-night photoperiod of 16 and 8 h, respectively, illumination 20,000 lx (day), temperature 22 °C and humidity 60%.

To measure the EC, the first leaves of the seedlings were removed, washed with tap water, and dried with filter paper. An average sample was prepared in each variant. For this purpose, cuts were made from the middle part of the leaves with a blade, they were placed in a bag made of nylon cloth, washed with distilled water, and then dried with filter paper. Samples of plant tissue were placed in a glass cup with distilled water at a certain tissue - water ratio. The cups were kept under illumination conditions for at least 1.5 h. The specific electrical conductivity of aqueous extracts of leaves was measured on an edge EC conductometric device, HANNA Instruments (Germany). The response of the cultivar was determined by the relative change in the UEP of water extracts of the leaves of seedlings after exposure of plants to stressors. The smaller the change in the parameter, the more resistant the variety⁵.

⁴Diagnostics of plant resistance to stress effects: guidelines / ed. G.V. Udovenko. L., 1988.228 p.

⁵Gurova T.A., Denisjuk S.G., Lugovskaya O.S., Svezhintseva E.A., Mineev V.V. Methodological provisions for early diagnosis of the resistance of spring wheat and barley to the combined action of stressors. Novosibirsk: SFSCA RAS, 2017.62 p. ностью различий на уровне $p \leq 0,05$.

Analytical repetition of experiments is 6-8 times, biological - 3 times. Representative sample - 200 seedlings in each variant of the experiment. The experimental data were mathematically processed using standard statistical programs. To determine the significance of differences in mean values, the Student's t-test was used. Regression analysis was carried out to reveal the relationship between the recorded parameters of varieties and their resistance. The average error did not exceed 3–5%. Three series of experiments were carried out.

RESULTS AND DISCUSSION

A study of the hourly dynamics of the release of electrolytes. One of the most essential requirements for a diagnostic method is its differentiating ability. It is determined by the reliability of the assessment of the resistance of closely related objects (varieties of the same crop, plants from the same varietal population) to various stress effects. Therefore, the choice of regimes (conditions) that would provide the greatest amplitude of the manifestation of varietal differences is necessary in the experiment.

The study of electrolyte release kinetics depending on the duration of leaf tissue exposure to water is an important methodology for establishing the time of maximum inter-varietal differences in the assessment of variety stress tolerance. The EC measurements showed a different pattern of electrolyte release from leaf tissue of wheat seedlings into distilled water under simultaneous action of chloride salinity and cereal root rot pathogen (see Fig. 1).

The release of electrolytes in seedlings of both varieties in the control variants did not differ significantly. In the experimental variant, the electrolyte yield in seedlings of the relatively resistant cultivar Omskaya 18 stabilized at a lower level of the EC value compared to the relatively unstable cultivar Novosibirskaya 44. The rapid release of electrolytes in the first 0.5–1.5 h of exposure of the samples to distilled water was replaced by a weaker one, but with a stable output over the next three hours (exposure time 1.5–4.5 h). Thus, on the curve of the total electrolyte yield, two sections can be

distinguished, characterizing the time intervals of various processes (see Fig. 2).

Section 1 of the graph (exposure time 0.5–1.5 h) characterizes the release of electrolytes from the apoplast, in which ions move in accordance with the laws of diffusion and adsorption [27]. Section 2 of the graph (exposure time 1.5–4.5 h) differs in a smaller angle of inclination of the linear approximation than section 1, and reflects the functional activity of the plasma membrane, its resistance to diffusive penetration of electrolytes.

Consequently, the time interval for the release of electrolytes from the free space (apoplast) and cytoplasm (through the plasmalemma), which indirectly indicates the state of the plasmalemma, is 1.5–4.5 hours. The indicator of the relative change in the EC for the unstable variety Novosibirskaya 44 significantly exceeds (with an exposure of 1.5 h - 1.5 times) the indicator of the relative change in the EC for the resistant cultivar Omskaya 18. The slope of the linear approximation (the rate of electrolyte release) for the unstable cultivar Novosibirskaya 44 significantly exceeds (more than 2 times) the rate of electrolyte release in seedlings of the resistant cultivar Omskaya 18. Intervarietal differences established by exposure times 1.5; 3.0 and 4.5 hours were 1.5; 1.66 and 1.67 times, respectively, with the significance of differences at the level of $p \leq 0.05$.

Thus, the maximum differences between the studied varieties of spring wheat were revealed in the time interval of the experiment, 1.5–4.5 h. The time of exposure of the samples in water was established (at least 1.5 h). With a shorter exposure period, electrolytes are released only from the free space (apoplast). An exposure time of more than 4.5 hours increases the duration of the sample evaluation procedure and reduces the quality of analyzes. The more resistant cultivar Omskaya 18 has the smallest indicators of the relative change in the EC and the rate of release of electrolytes. The smaller the relative change in EC, the more resistant the cultivar to the combined effect of the conidial suspension of *B. sorokiniana* (5000 conidia per grain) and 1.3% chloride salinity.

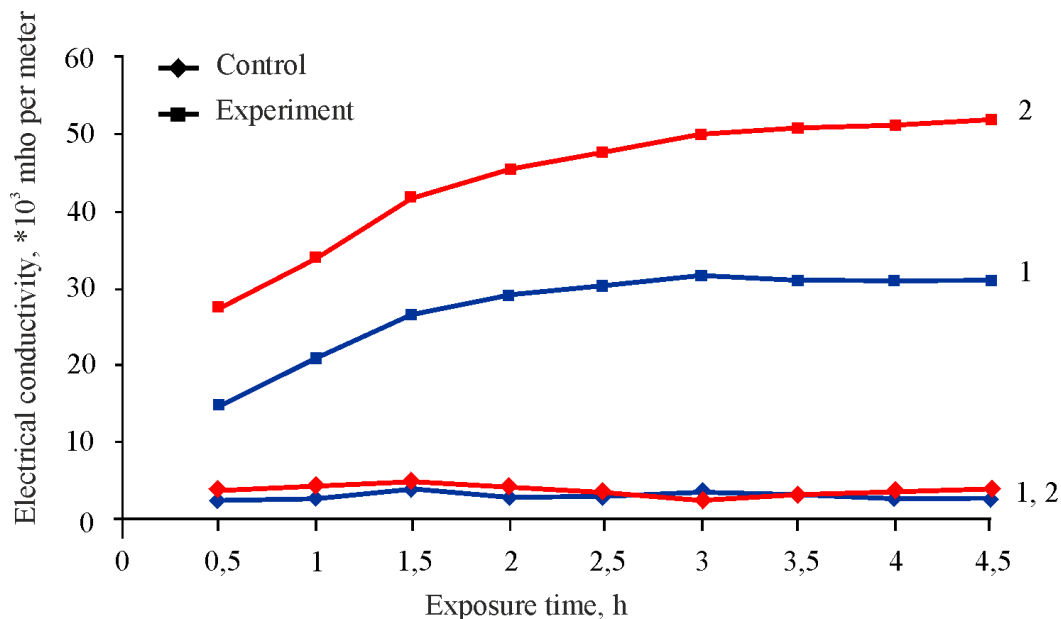


Рис. 1. Удельная электропроводность водных вытяжек листьев пшеницы в зависимости от длительности экспозиции листовой ткани в воде (стрессоры – конидиальная суспензия *B. sorokiniana* – 5000 конидий на зерно + хлорид натрия 1,3%): 1 – сорт Омская 18 (относительно устойчивый), 2 – сорт Новосибирская 44 (относительно неустойчивый). Различия с контролем достоверны на уровне $p \leq 0,01$

Fig. 1. Electrical conductivity of water extracts of wheat leaves depending on the duration of exposure of leaf tissue to water (stressors – conidial suspension of *B. sorokiniana* – 5000 conidia per grain + sodium chloride 1.3%): 1 – variety Omskaya 18 (relatively resistant), 2 – variety Novosibirskaya 44 (relatively non-resistant). Differences with control are reliable at the level of $p \leq 0.01$

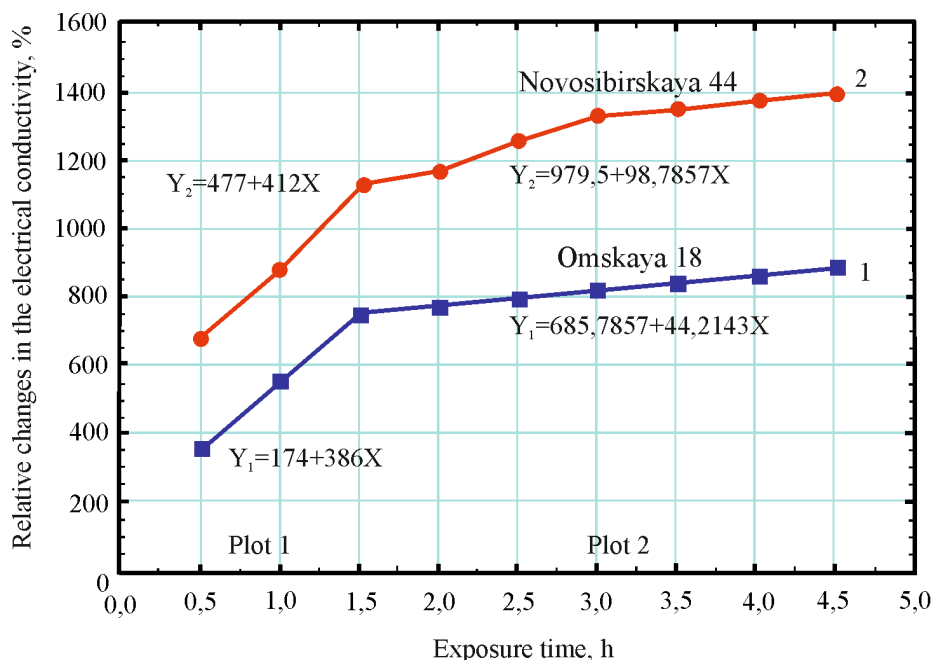


Рис. 2. Линейная аппроксимация относительного изменения удельной электропроводности водных вытяжек листьев пшеницы от длительности экспозиции листовой ткани в воде: 1 – сорт Омская 18 (относительно устойчивый); 2 – сорт Новосибирская 44 (относительно неустойчивый)

Fig. 2. Linear approximation of the relative change in the electrical conductivity of water extracts of wheat leaves on the duration of exposure of leaf tissue to water: 1 – variety Omskaya 18 (relatively resistant); 2 – variety Novosibirskaya 44 (relatively non-resistant)

A study of the daily dynamics of the release of electrolytes. The daily dynamics of the leaf extracts EC was studied in 10–16-day old seedlings of wheat cultivars Sibirskaya 21 (relatively resistant) and Novosibirskaya 18 (relatively unstable). The experiment was carried out without preliminary heating and with heating of seeds under the combined action of the following stressors: conidial suspension of *B. sorokiniana* and sodium chloride. The primary experimental data are presented in Table 1. The results obtained are approximated graphically in the form of quadratic dependences (see Fig. 3). The coefficients of quadratic equations for each variant of the experiment, respectively, are presented in Table 2. Analysis of the obtained experimental data on the study of daily dynamics and the corresponding approximating quadratic dependences made it possible to reveal the following features.

The value of the relative change in the EC sharply increases in 12-day-old seedlings with a subsequent increase in the indicator during cultivation for 16 days in both varieties

in the variants with and without heating the seeds (see Table 1 and Fig. 3). In the variant without heating the seeds, the indicator of the relative change in the EC in seedlings of the Novosibirskaya 18 variety over the entire range of studies reliably ($p \leq 0.05$) exceeds the EC indicator for the resistant cultivar Sibirskaya 21 (almost 2 times on the 10th day of seedling cultivation). Preliminary heating of seeds followed by the action of two stressors (*B. sorokiniana* and sodium chloride) led to the destabilization of cell membranes and a significant ($p \leq 0.05$) increase in the EC of seedlings of Novosibirskaya 18 variety (relatively unstable). In the cultivar Sibirskaya 21, the seeds heating stimulated the formation of protective and adaptive reactions, which was reflected in a decrease in the EC for the entire period of cultivation of seedlings of this cultivar. One of the possible reasons for such a combined tolerance in seedlings of the Sibirskaya 21 cultivar is the ability of plants to adapt existing cross reactions under the action of stressors [28].

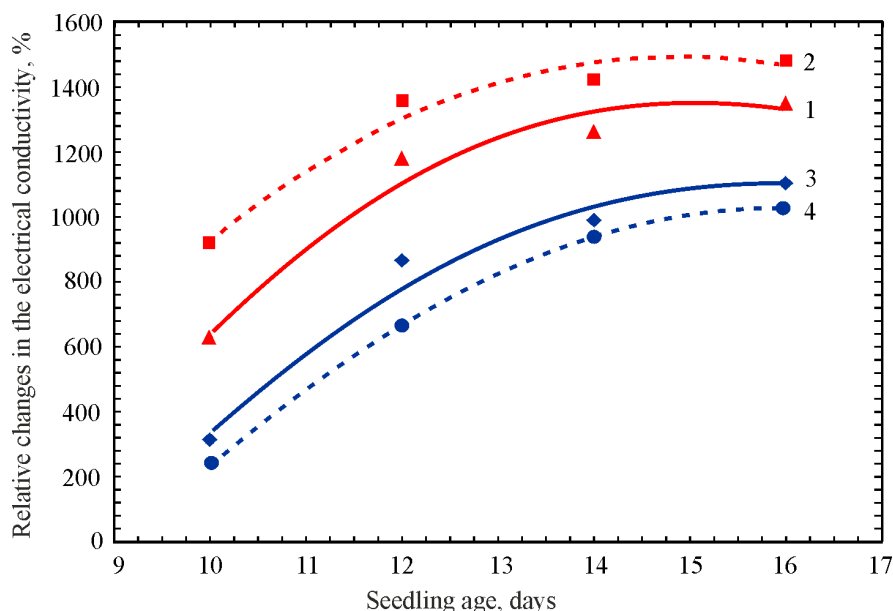


Рис. 3. Зависимость относительного изменения УЭП от возраста проростков при совместном действии стрессоров (конидиальная суспензия *B. sorokiniana* + хлорид натрия 1,3%): 1 – сорт Новосибирская 18, без прогрева семян; 2 – сорт Новосибирская 18, с прогревом семян; 3 – сорт Сибирская 21, без прогрева семян; 4 – сорт Сибирская 21, с прогревом семян

Fig. 3. Dependence of the relative change in EC on the age of seedlings under the combined action of stressors (conidial suspension of *B. sorokiniana* + chloride salinity 1.3%): 1 – Novosibirskaya 18, without seed heating; 2 – Novosibirskaya 18 variety, with seed heating; 3 – Sibirskaya 21, without seed heating; 4 – Sibirskaya 21, with seed heating

Табл. 1. Изменение удельной электропроводности сортов пшеницы в зависимости от возраста проростков при действии комплекса стрессоров, УЭП $\times 10^{-3}$ См/м

Table 1. Changes in the specific electrical conductivity of wheat varieties depending on the age of seedlings under the action of a complex of stressors, EC $\times 10^{-3}$ Cm/m

Variety	Option	Seedling age, days			
		10	12	14	16
Without heating the seeds					
Novosibirskaya 18	Control	3,5 ± 0,01	3,5 ± 0,05	3,1 ± 0,03	3,0 ± 0,05
	Conidial suspension <i>B. sorokiniana</i> + sodium chloride	25,5 ± 0,5*	44,7 ± 0,5*	42,2 ± 0,3*	43,5 ± 0,5*
Sibirskaya 21	Control	5,1 ± 0,05	4,1 ± 0,06	4,0 ± 0,01	4,1 ± 0,07
	Conidial suspension <i>B. sorokiniana</i> + sodium chloride	21,4 ± 0,7*	39,6 ± 1,1*	40,4 ± 0,9*	49,2 ± 1,2*
Heating up seeds at 43 °C					
Novosibirskaya 18	Control	3,8 ± 0,03	3,8 ± 0,02	2,9 ± 0,03	3,0 ± 0,03
	Conidial suspension <i>B. sorokiniana</i> + sodium chloride	38,7 ± 1,0*	55,4 ± 1,2*	44,1 ± 0,8*	47,4 ± 0,9*
Sibirskaya 21	Control	6,9 ± 0,08	4,8 ± 0,04	4,2 ± 0,03	4,0 ± 0,02
	Conidial suspension <i>B. sorokiniana</i> + sodium chloride	23,8 ± 0,5*	36,8 ± 0,7*	43,6 ± 0,8*	45,4 ± 1,1*

* Differences with the control are significant at the significance level of $p \leq 0.01$.

In both cultivars, the difference in the relative change in the EC in the variants with and without heating the seeds was maximal in 10-day-old seedlings - 1.5 times (Novosibirskaya 18) and 1.3 times (Sibirskaya 21) with the significance of differences at the level of $p \leq 0, 05$. Intervarietal maximum differences were also recorded in 10-day-old seedlings; in the variant without heating the seeds, they were 1.9 times and in the variant with heating the seeds, 3.7 times, with the significance of differences at the level of $p \leq 0.05$.

One of the important characteristics of the process is the rate of its growth. To characterize the rate of release of electrolytes, an analysis of linear functions (derivatives of approximation dependences) was carried out, which made it possible to reveal a number of features (see Table 2).

The maximum electrolyte yield was achieved at a seedling age of about 15.5 days for all variants of experiments (14.86; 14.95; 15.67; 16.25 days), and in the Novosibirskaya 18 variety - a day earlier than in the Sibirskaya 21 variety. The slope of the function, which characterizes the rate of change in the rate of release of electrolytes, is also noted to be higher in the Novosibirskaya 18 variety, which indicates a greater

instability of cell membranes in this variety under the combined action of stressors.

Thus, under the conditions of the sequential action of stressors, a protective effect of hyperthermia was established in the more resistant cultivar Sibirskaya 21 (a significant decrease in EC up to 1.3 times) as compared with the variant without heating the seeds. In the less resistant variety Novosibirskaya 18, heating the seeds destabilized the state of the cell membranes (a significant increase in the EC and the rate of electrolyte release by 1.5 and 1.2 times, respectively).

CONCLUSIONS

1. The informativity of leaf aqueous extracts EC as an indicator of change in cell membrane permeability of seedlings has been experimentally established to determine stress tolerance of wheat varieties to the combined action of cereal root rot, chloride salinity and seed hyperthermia pathogen.

2. The dependence of resistibility of the EC of leaf extracts of four varieties of spring wheat to simultaneous and sequential combined action of seed hyperthermia (43 °C), chloride salinity (1.3%) and common cereal rot pathogen *B. sorokiniana* (5000 conidia per grain) was

Табл. 2. Аналитические выражения аппроксимационных функций зависимости относительного изменения УЭП от возраста проростков**Table 2.** Analytical expressions of the dependence approximation functions of EC relative change on the age of seedlings

Option	Analytic expression		Maximum abscissa X_m , days	Angle factor of function Y_1
	Function Y , EC	Derivative Y_1		
<i>Novosibirskaya 18 variety</i>				
Seeds without heating + Conidial suspension <i>B. sorokiniana</i> + sodium chloride	$Y_1 = -28,8125X^2 + 861,275X - 5080,45$	$-57,6250X + 861,275$	14,95	-57,6
Seeds with heating + Conidial suspension <i>B. sorokiniana</i> + sodium chloride	$Y_2 = -23,5625X^2 + 700,675X - 3713,65$	$-47,1250X + 700,675$	14,86	-47,1
<i>Sibirskaya 21 variety</i>				
Seeds without heating + Conidial suspension <i>B. sorokiniana</i> + sodium chloride	$-22,3125X^2 + 699,475X - 412,050$	$-44,6250X + 699,475$	15,67	-44,6
Seeds with heating + Conidial suspension <i>B. sorokiniana</i> + sodium chloride	$-20,3188X^2 + 660,353X - 4327,895$	$-40,6376X + 660,353$	16,25	-40,6

determined. The smallest changes in the EC value and electrolyte release rate were recorded in seedlings of relatively resistant varieties of spring wheat Omskaya 18 and Sibirskaya 21.

3. When studying the hourly dynamics of the electrolyte release (exposure of seedling leaves in water for 0.5–4.5 h) with the simultaneous action of *B. sorokiniana* (5000 conidia per grain) and chloride salinity (1.3%), there was a significant 1.5-fold increase in the relative change of the EC and a 2-fold increase in the electrolyte release in the less resistant variety Novosibirskaya 44 compared to the more resistant variety Omskaya 18.

4. In a study of the daily dynamics of electrolyte release in 10–16-day-old seedlings under the sequential action of seed hyperthermia (43° C), *B. sorokiniana* (5000 conidia per grain) and chloride salinity (1.3%), the protective effect of hyperthermia was established in a more resistant cultivar Siberian 21. There was a significant ($p \leq 0.05$) decrease in the relative change in EC by 1.3 times compared with the option without seeds heating. In the less resistant variety Novosibirskaya 18, the seeds heating destabilized the

state of cell membranes, which led to a significant ($p \leq 0.05$) increase in the relative change in the EC and the rate of electrolyte release by 1.5 and 1.2 times, respectively.

5. Analytical expressions have been obtained, confirming the experimentally established methodological procedures of conductometric measurements, providing the maximum intervarietal differences: the age of seedlings - 10 days; the time interval of exposure of the samples in water was 1.5–4.5 h. The intervarietal differences in the variant without heating the seeds were 1.9 times and in the variant with heating the seeds - 3.7 times with the reliability of differences at levels $p \leq 0.05$ and $p \leq 0.01$. Intervarietal differences established at the time interval of exposure to the release of electrolytes 1.5–4.5 h were 1.50–1.67 times with the significance of differences at the level of $p \leq 0.05$.

СПИСОК ЛИТЕРАТУРЫ

1. Гончарова Э.А., Еремин Г.В., Гасанова Т.А. Экспресс-методы оценки стрессоустой-

- чивости сельскохозяйственных культур и стратегия их диагностики для селекции // Доклады Российской академии сельскохозяйственных наук. 2015. № 5. С. 21–24.
2. Карманенко Н.М. Адаптация зерновых культур к стрессовым факторам: монография. М.: Издательство ВНИИА, 2014. 160 с.
 3. Полонский В.И. Оценка функционального состояния растений: продукционные, селекционные и экологические аспекты: монография. Красноярск: Красноярский государственный аграрный университет, 2014. 408 с.
 4. Suzuki N., Rivero R.M., Shulaev V., Blumwald E., Mittler R. Abiotic and biotic stress combinations // *New Phytologist*. 2014. Vol. 203. N 1. P. 32–43.
 5. Гурова Т.А., Осипова Г.М. Проблема сопряженной стрессоустойчивости растений при изменении климата в Сибири // *Сибирский вестник сельскохозяйственной науки*. 2018. Т. 48. № 2. С. 81–92. DOI: 10.26898/0370-8799-2018-2-11.
 6. Ahmed I., Dai H., Zheng W., Gao F., Zhang G., Sun D., Wu F. Genotypic differences in physiological characteristics in the tolerance to drought and salinity combined stress between Tibetan wild and cultivated barley // *Plant Physiology and Biochemistry*. 2013. Vol. 63. P. 49–60.
 7. Zhong-Guang L., Ming G. Mechanical stimulation-induced cross-adaptation in plants: An overview // *Journal Plant Biology*. 2011. Vol. 54. P. 358–364. DOI: 10.1007/s12374-011-9178-3.
 8. Валитова Ю.Н., Хабибрахманова В.Р., Белкина А.В., Ренкова А.Г., Минибаева Ф.В. Липидный профиль корней пшеницы при действии мембранотропных агентов // *Биологические мембраны: Журнал мембранной и клеточной биологии*. 2020. Т. 37. № 6. С. 466–476.
 9. Грищенко Н.Н., Лукаткин А.С. Определение устойчивости растительных тканей к абиотическим стрессам с использованием кондуктометрического метода // *Поволжский экологический журнал*. 2005. № 1. С. 3–11.
 10. Терлецкая Н.В. Проницаемость клеточных мембран как показатель устойчивости растений к абиотическим стрессам // *Известия Национальной академии наук Республики Казахстан*. 2009. № 2. С. 60–64.
 11. Demidchik V., Straltsova D., Medvedev S.S., Pozhvanov G.A., Sokolik A., Yurin V. Stress – induced electrolyte leakage: the role of K⁺- permeable channels and involvement in programmed cell death and metabolic adjustment // *Journal of Experimental Botany*. 2014. Vol. 65 (5). P. 1259–1270. DOI: 10.1093/jxb/eru 004.
 12. Астахова Н.В., Попов В.Н., Селиванов А.А., Бурханова Е.А., Алиева Г.П., Мошков И.Е. Реорганизация ультраструктуры хлоропластов при низкотемпературном закаливании растений арабидопсиса // *Физиология растений*. 2014. Т. 61. № 6. С. 790–798.
 13. Danielle Fiebelkorn, David Horvath & Mukhlesur Rahman. Genome-wide association study for electrolyte leakage in rapeseed/canola (*Brassica napus*) // *Molecular Breeding*. 2018. Vol. 38. P. 129.
 14. Поминов А.В., Дьячук Т.И., Кибкало И.А., Хомякова О.В., Акинина В.Н. Оценка засухоустойчивости растений сортообразцов тритикале мировой коллекции ВИР им. Н.И. Вавилова // *Аграрный вестник Юго-Востока*. 2016. № 1–2. С. 38–40.
 15. Аллагулова Ч.Р., Авальбаев А.М., Федорова К.А., Шакирова Ф.М. Снижение степени индуцируемых засухой окислительных повреждений в растениях пшеницы при обработке донором NO // *ЭКОБИОТЕХ*. 2020. Т. 3. № 2. С. 181–186.
 16. Hatsugai N., Katagiri F. Quantification of Plant Cell Death by Electrolyte Leakage Assay // *Bio-protocol*. 2018. Vol. 8(5). P. 2758. DOI: 10.21769/BioProtoc.2758.
 17. Imanifard Z., Vandelle E., Bellin D. Measurement of hypersensitive cell death triggered by avirulent bacterial pathogens in *Arabidopsis* // *Methods in Molecular Biology*. 2018. Vol. 1743. P. 39–50. DOI: 10.1007 / 978-1-4939-7668-3_4.
 18. Якунина А.В., Сеницына Ю.В., Крутова Е.К., Веселов А.П. Изучение влияния пирабактина на урожайность растений гороха посевного *Pisum sativum* L. и анализ изменений перекисного окисления липидов и выхода электролитов как возможных факторов этого воздействия // *Известия высших учебных заведений. Поволжский регион. Естественные науки*. 2020. № 4 (32). С. 3–12.
 19. Безрукова М.В., Кудоярова Г.Р., Лубянова А.Р., Масленникова Д.Р., Шакирова Ф.М. Влияние 24-эпибрассинолида на водный об-

мен отличающихся по засухоустойчивости сортов пшеницы при осмотическом стрессе // Физиология растений. 2021. Т. 68. № 2. С. 161–169.

20. Sánchez-Viveros G., González- Mendoza D., Alarcón A. Copper Effects on Photosynthetic Activity and Membrane Leakage of *Azolla filiculoides* and *A. caroliniana* // International Journal of Agriculture & Biology. 2010. Vol. 12. P. 365–368.
21. Репкина Н.С., Таланова В.В., Тумов А.Ф., Букарева И.В. Реакция растений пшеницы (*Triticum aestivum* L.) на раздельное и совместное действие низкой температуры и кадмия // Труды Карельского научного центра Российской академии наук. 2014. № 5. С. 133–139.
22. Хохлова Л.П., Валиулина Р.Н., Мидер Д.Р., Акберова Н.И. Термостабильность мембран и экспрессия генов низкомолекулярных белков теплового шока при действии на растения повышенных температур и водного дефицита // Биологические мембраны. 2015. Т. 33. № 1. С. 59–71.
23. Лукаткин А.С., Старкина М.И. Влияние тидиазурина на устойчивость проростков огурца к стрессовым факторам // Агрохимия. 2009. № 10. С. 31–38.
24. Pandey S. K., Sujata Upadhyay, Zaffar Mehdi Dar, Hemantaranjan, A and Sri-vastava, J.P. Effect of electrolyte leakage activity on mungbean leaf under induced salinity and zinc levels // International Journal of Current Research. 2017. Vol. 9. Is. 06. P. 52438–52440.
25. Гурова Т.А., Свежинцева Е.А., Чесноченко Н.Е. Адаптация сортов пшеницы при гипертермии, хлоридном засолении и инфицировании *Bipolaris sorokiniana* Shoem. // Сибирский вестник сельскохозяйственной науки. 2020. № 6. С. 12–25. DOI: 10.26898/0370-8799-2020-6-2.
26. Гурова Т.А., Луговская О.С., Свежинцева Е.А. Адаптивные реакции проростков пшеницы, дифференцирующие сорта при гипертермии // Сибирский вестник сельскохозяйственной науки. 2019. Т. 49. № 3. С. 31–40. DOI: 10.26898/0370-8799-2019-3-4.
27. Коваль С.Ф., Шаманин В.П. Растение в опыте: монография. Омск: Издательство ИЦиГ СО РАН, ОмГАУ, 1999. 204 с.
28. Naghmeh Nejat, Nitin Mantri. Plant Immune System: Crosstalk Between Responses to Biotic and Abiotic Stresses the Missing Link in Understanding Plant Defence // Current Issues

in Molecular Biology. 2017. Vol. 23. P. 1–16. DOI: 10.21775/cimb.023.001.

REFERENCES

1. Goncharova E.A., Eremin G.V., Gasanova T.A. Express-methods of evaluation of stress resistance of cultivated crops and strategy for their diagnosis for breeding. *Doklady Rossiiskoi akademii sel'skokhozyaistvennykh nauk = Proceedings of the Russian Academy of Agricultural Sciences*, 2015, no. 5, pp. 21–24. (In Russian).
2. Karmanenko N.M. *Adaptation of crops to stress factors*. Moscow, Izdatel'stvo VNIIA, 2014, 160 p. (In Russian).
3. Polonskii V.I. *Assessment of the functional state of plants: production, breeding and ecological aspects*. Krasnoyarsk, Krasnoyarskii gosudarstvennyi agrarnyi universitet, 2014, 408 p. (In Russian).
4. Suzuki N., Rivero R.M., Shulaev V., Blumwald E., Mittler R. Abiotic and biotic stress combinations. *New Phytologist*, 2014, vol. 203, no. 1, pp. 32–43.
5. Gurova T.A., Osipova G.M. The problem of combined stress resistance of plants under climate change in Siberia. *Sibirskii vestnik sel'skokhozyaistvennoi nauki = Siberian Herald of Agricultural Science*, 2018, vol. 48, no. 2, pp. 81–92. (In Russian). DOI: 10.26898/0370-8799-2018-2-11.
6. Ahmed I., Dai H., Zheng W., Gao F., Zhang G., Sun D, Wu F. Genotypic differences in physiological characteristics in the tolerance to drought and salinity combined stress between Tibetan wild and cultivated barley. *Plant Physiology and Biochemistry*, 2013, vol. 63, pp. 49–60.
7. Zhong-Guang L., Ming G. Mechanical stimulation-induced cross-adaptation in plants: An overview. *Journal Plant Biology*, 2011, vol. 54, pp. 358–364. DOI: 10.1007/s12374-011-9178-3.
8. Valitova Yu.N., Khabibrakhmanova V.R., Belkina A.V., Renkova A.G., Minibaeva F.V. The lipid profile of wheat roots treated with membranotropic agents. *Biologicheskie membrany: Zhurnal membranoi i kletchnoi biologii = Biochemistry (Moscow) supplement. Series A: Membrane and Cell Biology*, 2020, vol. 37, no. 6, pp. 466–476. (In Russian).
9. Grishenkova N.N., Lukatkin A.S. A conductometric technique to estimate the plant tissue

- stability to abiotic stresses. *Povolzhskii ekologicheskii zhurnal = Povolzhsky Journal of Ecology*, 2005, no. 1, pp. 3–11. (In Russian).
10. Terletskaia N.V. Permeability of cell membranes as an indicator of plant resistance to abiotic stress. *Izvestiya Natsional'noi akademii nauk Respubliki Kazakhstan = Reports of the National Academy of Sciences of the Republic of Kazakhstan*, 2009, no. 2, pp. 60–64. (In Russian).
11. Demidchik V., Straltsova D., Medvedev S.S., Pozhvanov G.A., Sokolik A., Yurin V. Stress – induced electrolyte leakage: the role of K⁺-permeable channels and involvement in programmed cell death and metabolic adjustment. *Journal of Experimental Botany*, 2014, vol. 65 (5), pp. 1259–1270. DOI: 10.1093/jxb/eru 004.
12. Astakhova N.V., Popov V.N., Selivanov A.A., Burakhanova E.A., Alieva G.P., Moshkov I.E. Reorganization of chloroplast ultrastructure associated with low-temperature hardening of Arabidopsis plants. *Fiziologiya rastenii = Russian Journal of Plant Physiology*, 2014, vol. 61, no. 6, pp. 790–798. (In Russian).
13. Danielle Fiebelkorn, David Horvath & Mukhlesur Rahman. Genome-wide association study for electrolyte leakage in rapeseed/canola (*Brassica napus*). *Molecular Breeding*, 2018, vol. 38, pp. 129.
14. Pominov A.V., D'yachuk T.I., Kibkalo I.A., Khomyakova O.V., Akinina V.N. Evaluation of drought resistance of samples of plants of triticale varieties of the world collection of All-Union Research Institute of Plant Breeding named after Vavilov N.I. *Agrarnyi vestnik Yugo-Vostoka = Agrarian Bulletin of the South-East*, 2016, no. 1–2, pp. 38–40. (In Russian).
15. Allagulova Ch.R., Aval'baev A.M., Fedorova K.A., Shakirova F.M. NO-induced reduction of oxidative stress level in wheat plants under drought conditions. *EKOBIOTEKH = ECO-BIOTECH*, 2020, vol. 3, no. 2, pp. 181–186. (In Russian).
16. Hatsugai N., Katagiri F. Quantification of Plant Cell Death by Electrolyte Leakage Assay. *Bio-protocol*. 2018, vol. 8(5), p. 2758. DOI: 10.21769/BioProtoc.2758.
17. Imanifard Z., Vandelle E., Bellin D. Measurement of hypersensitive cell death triggered by avirulent bacterial pathogens in Arabidopsis. *Methods in Molecular Biology*, 2018, vol. 1743, pp. 39–50. DOI: 10.1007 / 978-1-4939-7668-3_4.
18. Yakunina A.V., Sinitsyna Yu.V., Krutova E.K., Veselov A.P. The study of the pirabactin effect on the yield of *Pisum sativum* L. and analysis of changes in lipid peroxidation and electrolytes leakage as possible factors of this effect. *Izvestiya vysshikh uchebnykh zavedenii. Povolzhskii region. Estestvennye nauki = University Proceedings. Volga Region. Natural Sciences*, 2020, no. 4 (32), pp. 3–12. (In Russian).
19. Bezrukova M.V., Kudoyarova G.R., Lubyanova A.R., Maslennikova D.R., Shakirova F.M. Influence of 24-Epibrassinolide on water exchange of wheat varieties various in dry resistance under osmotic stress. *Fiziologiya rastenii = Russian Journal of Plant Physiology*, 2021, vol. 68, no. 2, pp. 161–169. (In Russian).
20. Sánchez-Viveros G., González- Mendoza D., Alarcón A. Copper Effects on Photosynthetic Activity and Membrane Leakage of *Azolla filiculoides* and *A. caroliniana*. *International Journal of Agriculture & Biology*, 2010, vol. 12, pp. 365–368.
21. Repkina N.S., Talanova V.V., Titov A.F., Bukareva I.V. Wheat response to separate and combined impact of low temperature and cadmium. *Trudy Karelskogo nauchnogo tsentra Rossiiskoi akademii nauk = Transactions of Karelian Research Centre of Russian Academy of Sciences*, 2014, no. 5, pp. 133–139. (In Russian).
22. Khokhlova L.P., Valiulina R.N., Mider D.R., Akberova N.I. Membrane thermostability and gene expression of small-heat shock protein in wheat shoots exposed to elevated temperatures and water deficiency. *Biologicheskie membrany = Biochemistry (Moscow) supplement. Series A: Membrane and Cell Biology*, 2015, vol. 33, no. 1, pp. 59–71. (In Russian).
23. Lukatkin A.S., Starkina M.I. Effect of thidiazuron on the resistance of cucumber seedlings to stress factors. *Agrokimiya = Agricultural Chemistry*, 2009, no. 10, pp. 31–38. (In Russian).
24. Pandey S.K., Sujata Upadhyay, Zaffar Mehdi Dar, Hemantaranjan A., Sri-vastava J.P. Effect of electrolyte leakage activity on mungbean leaf under induced salinity and zinc levels. *International Journal of Current Research*, 2017, vol. 9, is. 06, pp. 52438–52440.
25. Gurova T.A., Svezhintseva E.A., Chesnochenko N.E. Adaptation of wheat varieties to hyperthermia, chloride salinity and *Bipolaris sorokiniana* Shoem. infection. *Sibirskii vestnik sel'skokhozyaistvennoi nauki = Siberian Herald*

- of Agricultural Science*, 2020, no. 6, pp. 12–25. (In Russian). DOI: 10.26898/0370-8799-2020-6-2.
26. Gurova T.A., Lugovskaya O.S., Svezhintseva E.A. Adaptive reactions of wheat seedlings differentiating varieties under hyperthermia. *Sibirskii vestnik sel'skokhozyaistvennoi nauki* = *Siberian Herald of Agricultural Science*, 2019, vol. 49, no. 3, pp. 31–40. (In Russian). DOI: 10.26898/0370-8799-2019-3-4.
27. Koval' S.F., Shamanin V.P. *Plant in an experiment*. Omsk, ITsiG SO RAN Publ., OmGAU, 1999, 204 p. (In Russian).
28. Naghmeh Nejat, Nitin Mantri. Plant Immune System: Crosstalk Between Responses to Biotic and Abiotic Stresses the Missing Link in Understanding Plant Defence. *Current Issues in Molecular Biology*, 2017, vol. 23, pp. 1–16. DOI: 10.21775/cimb.023.001.

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Дата поступления статьи / Received by the editors 7.04. 2021
Дата принятия к публикации / Accepted for publication 10.06.2021
Дата публикации / Published 26.07.2021