The relationship of cell membrane permeability of wheat seedlings with resistance of the variety

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Abstract. The article presents the results of studies on the possibility of using the indicator of the permeability of cell membranes, estimated by the specific electrical conductivity of water infusions of seedling tissues in assessing the stress resistance of wheat varieties. In laboratory vegetation experiments, the smallest changes in the value of the relative index of electrical conductivity and the rate of release of electrolytes in seedlings of resistant varieties of spring wheat were found under the combined action of hyperthermia of seeds (43 °C), chloride salinity (1.3 %) and the causative agent of common rot of cereals Bipolaris sorokiniana Shoem. (5000 conidia per grain). The methodological techniques of conductometric measurements were experimentally determined, which ensure obtaining the maximum intervarietal differences under the combined action of stressors. The proposed approach will make it possible to assess new genotypes for resistance to biotic and abiotic stressors in breeding, crop production and biotechnology, accelerate the selection of breeding material and adjust agricultural technologies.

1. Introduction
In breeding, when creating varieties, it is required to diagnose their resistance to biotic and abiotic stress factors of the environment. In the field, performing such an assessment is time-consuming step. Therefore, the assessment of varieties in laboratory conditions is used by indirect methods to change a number of physiological, biochemical and biophysical parameters, which reflect the process of plant adaptation to stress.

It is known that the main plant responses to abiotic and biotic factors are associated with the occurrence of oxidative stress, which leads to disruption of the structure and functioning of cell membranes and, as a consequence, inhibition or complete cessation of growth [1,2].

The most important property of biological membranes is its permeability. It determines the stability of tissues, cells and cell organelles and characterizes the static genetically determined potential resistance of the genotype [3,4]. Therefore, changes in membrane permeability for electrolytes is one of the criteria for plant resistance to biotic and abiotic stressors. The higher the plant resistance, the less the structure and properties of membranes are disturbed and the less the output of electrolytes from tissues into the environment decreases [5].

The permeability of cell membranes is determined by the conductometric method by the release of electrolytes from plant tissues. The results of studies on changes in the permeability of cell membranes under the action of low [6], high [7] temperatures, drought [8], and pathogen infection [9] have been obtained.
Previously, the possibility of taking into account changes in the permeability of cell membranes, assessed by the specific electrical conductivity (EC) of seedling tissues, as a diagnostic indicator for assessing the relative resistance of spring wheat and barley varieties to chloride salinity and the causative agent of common rot of cereals, and the development of diagnostic methods on this basis [10, 11].

The aim of the research is to experimentally establish the informativeness of the indicator of changes in 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 salinity and hyperthermia.

2. Conditions, materials and methods
The studies were carried out in laboratory conditions (vegetation experiment - water crops) on seedlings of zoned varieties of soft spring wheat bred by the Siberian Scientific Research Institute of Plant Production and Breeding – the branch of the Institute of Cytology and Genetic (Krasnooobsk) - Novosibirskaya 18, Novosibirskaya 44, Sibirskaya 21 and by the Siberian Scientific Research Institute of Agriculture (Omsk) - Omskaya 18.

To reveal the relationship between changes in the permeability of cell membranes of seedlings and the resistance of varieties to the combined action of the pathogen of common rot *B. sorokiniana* Shoem. (abbr. *B. sorokiniana*). Preliminarily, the resistance of the varieties was assessed in laboratory conditions by growth parameters, changes in dry and wet biomass of seedlings and the development of disease on seedlings.

Hourly dynamics of changes in the specific electrical conductivity (EC) of leaf infusions - the kinetics of electrolyte release for 0.5–4.5 hours was recorded in seedlings of varieties Omskaya 18 (relatively resistant variety) and Novosibirskaya 44 (relatively unresistant variety).

Experimental options:
- control;
- *B. sorokiniana* infection with 5000 conidia per grain + chloride salinity of 1.3 %.

The daily dynamics of electrical conductivity was measured in 10–16 day old seedlings of the varieties Sibirskaya 21 (relatively resistant) and Novosibirskaya 18 (relatively unresistant).

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

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 minutes in hot water on a water bath at a temperature of +43 °C. After cooling, the seeds were laid out 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. Infection of seeds 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 hours, respectively, illumination of 20000 lx (day), temperature 22 °C and humidity 60 %.

To measure the specific electrical conductivity (EC), water infusion of plant tissues were prepared from the first leaves of 10-day-old seedlings in accordance with the technique [12].
After filtration of the water infusion their electrical conductivity was measured using an edge EC conductometric device, HANNA Instruments (Germany).

The response of the cultivar was determined by the relative change in the measured parameters of seedlings after exposure of plants to stressors.

The experiments were repeated 4-6 times. A representative sample - 200 seedlings in each variant of the experiment. The experimental data were mathematically processed using a statistical program. The average error did not exceed 3-5 %. Three series of experiments were carried out. Correlation and regression analysis were carried out to reveal the relationship between the recorded parameters of varieties and their resistance.

3. Results and discussion

3.1. Study of the hourly dynamics of electrolyte output

The study of the kinetics of the release of electrolytes depending on the duration of exposure of leaf tissue in water is an important methodological technique for establishing the time of maximum intervarietal differences in assessing the stress resistance of varieties.

The results of studying the kinetics of the release of electrolytes depending on the duration of exposure of leaf tissue in water - the hourly dynamics of changes in the specific electrical conductivity (EC), of wheat varieties are presented in figure 1.

![Figure 1. Electrical conductivity of water infusion of wheat leaves depending on the duration of exposure of leaf tissue in water (conidial suspension of **B. sorokiniana** +sodium chloride 1.3 %): 1 – Omskaya 18 (relatively resistant), 2 – Novosibirskaya 44 (relatively unresistant).](image)

The release of electrolytes in seedlings of both varieties in the control variants was practically the same. In seedlings of the relatively resistant cultivar Omskaya 18, the electrolyte yield stabilized at a lower level of the the specific electrical conductivity (EC) value compared to the relatively unstable variety Novosibirskaya 44. The rapid release of electrolytes in the first 30-90 minutes of incubation of the samples (exposure time 0.5-1.5 h) changed further with a weaker but stable yield 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 (figure 2).

The first section (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 [13].

The second section (exposure time 1.5-4.5 h), with a smaller slope of the linear approximation than in the first section (0.5-1.5 h), reflects the functional activity of the plasmalemma, its resistance to diffuse penetration of electrolytes.
Figure 2. Linear dependence of the relative change in the specific electrical conductivity of water infusion of wheat leaves on the duration of exposure of leaf tissue in water: 1 - Omskaya 18; (relatively resistant); 2 – Novosibirskaya 44 (relatively unresistant).

Consequently, the time interval that shows the release of electrolytes from the free space (apoplast) and cytoplasm (through the plasmalemma) and indirectly indicates the state of the plasmalemma is 1.5-4.5 hours. The indicator of the relative the specific electrical conductivity (EC) value for the unresistant variety Novosibirskaya 44 significantly exceeds (with an exposure of 1.5 hours, 1.5 times) the indicator of the relative change in the specific electrical conductivity (EC) for the resistant variety Omskaya 18. The slope of the linear approximation (the rate of change in the electrolyte yield) for the unstable variety Novosibirskaya 44 significantly exceeds (more than 2 times) the rate of electrolyte release in seedlings of resistant variety Omskaya 18.

Thus, the maximum intervarietal differences were revealed in the time interval of 1.5 ... 4.5 h, that is, the exposure time of the samples in water, as we have established experimentally, should be at least 1.5 hours. At shorter exposure times, electrolytes are released only from the free space (apoplast), and an increase in exposure time more than 4.5 hours leads to an unjustified increase in the duration of the sample evaluation procedure and a decrease in analysis productivity. The more resistant variety Omskaya 18 has a lower relative change in the EC and the rate of electrolyte release. The smaller the relative change in the specific electrical conductivity (EC), the more resistant the variety in the group of the studied varieties for resistance to the combined effect of the conidial suspension of \(B.\ sorokiniana\) (5000 conidia per grain) and 1.3% chloride salinity.

3.2. Study of the daily dynamics of the release of electrolytes

The diurnal dynamics of changes in the specific electrical conductivity (EC) of water infusions was studied in 10–16 day old seedlings of wheat variety Sibirskaya 21 (relatively resistant) and Novosibirskaya 18 (relatively unstable) under the combined action of stressors - a conidial suspension of \(B.\ sorokiniana\) and chloride salinity without preliminary heating and with heating of the seeds.

Analysis of the obtained experience data on the study of daily dynamics and the corresponding approximating quadratic dependences, revealed the following features (figure 3).
Figure 3. Dependence of the relative change in the specific electrical conductivity of water infusion 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 warming; 2 – Novosibirskaya 18 variety, with seed warming; 3 – Sibirskaya 21, without seed warming; 4 – Sibirskaya 21, with seed warming.

In the variant without heating the seeds, the indicator of the relative change in the specific electrical conductivity (EC), in seedlings of the Novosibirskaya 18 variety over the entire range of studies exceeds the indicator of the EC for the resistant variety Sibirskaya 21 (almost twice on the 10th day of seedling cultivation). Preliminary heating of seeds followed by the action of two stressors *B. sorokiniana* and chloride salinity led to the destabilization of cell membranes and an increase in the specific electrical conductivity of water infusion seedlings of variety Novosibirskaya 18 (relatively unresistant), and in all days of seedling cultivation.

In both varieties, the difference in the relative change in the specific electrical conductivity (EC), in the variants with and without heating the seeds is maximal in 10 day old seedlings and is 1.5 times (variety Novosibirskaya 18) and 1.3 times (variety Sibirskaya 21). Intervarietal differences are also maximal in 10-day-old seedlings and are 1.9 times in the variant without heating of the seeds and 3.7 times in the variant with heating of the seeds.

Thus, to obtain the maximum varietal differences under the combined action of hyperthermia (43 °C), conidial suspension of *B. sorokiniana*, and chloride salinization, it is sufficient to cultivate seedlings up to 10 days of age.

4. Conclusion
The dependence of the permeability of cell membranes of seedlings of 4 spring wheat cultivars on their resistance to the combined action of seed hyperthermia (43 °C), chloride salinity (1.3%), and the causative agent of common rot of cereals *Bipolaris sorokiniana* Shoem. was determined. (5000 conidia per grain), which establishes the smallest changes in the yield of electrolytes in resistant varieties.

Analytical expressions have been obtained that confirm the experimentally established methodological methods of conductometric measurements, ensuring maximum varietal differences
under the action of hyperthermia of seeds (+43 °C), conidial suspension of *B. sorokiniana* and chloride salinity: seedlings age - 10 days; the time interval of exposure of samples in water is 1.5...4.5 hours.

The obtained experimental data and analytical dependences indicate the possibility of using the conductometric method to assess the resistance of wheat varieties to the complex action of stressors in breeding and crop production.

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