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COMPARATIVE CHARACTERIZATION AND ADAPTIVE MECHANISMS OF SALT TOLERANCE OF DIFFERENT WHEAT GENOTYPES

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Abstract

The study of adaptive mechanisms of salt tolerance for the identification and selection of resistant wheat genotypes remains an urgent task since the area of lands with high salinity is constantly increasing worldwide. This work was focused on the processes of accumulation and excretion of toxic ions from the roots and leaves of different wheat genotypes and the effect of these ions on the state of plant tissues. It was shown that in the studied varieties of durum wheat, with an increase in salinity, the size of the root system decreased and, as a result, the absorption of toxic Na^+ ions decreased. In soft wheat, ionic conductivity increased and the excretion of Na^+ ions increased too. We compared the manifestations of salt stress caused by high concentrations of NaCl, and the mechanisms of salt tolerance and adaptation to its toxic effect in varieties of different types of wheat in the conditions of the Orenburg region in the field. The initial assessment of salt tolerance of wheat varieties was carried out according to the degree of growth inhibition by sodium chloride. Based on the initial assessment, wheat varieties differing in salt tolerance were selected, i.e., two durum wheat (*Triticum durum* Desf.) varieties Zolotaya and Orenburgskaya 10 and two soft wheat (*Triticum aestivum* L.) varieties Ulyanovskaia 105 and Orenburgskaya 22. The final assessment of the salt tolerance of the selected varieties was carried out in 2022, growing plants in small-scale (1.8 m^2 plots) field trials in 3 repetitions of each variant. To create salt stress, sodium chloride was applied for root watering after emergence and before the tillering phase in the form of a solution (200 mM NaCl). Plants grown without the addition of salts served as control. The adaptive mechanisms of resistance of different wheat genotypes to sodium chloride were studied using biochemical, molecular genetic and light-optical methods of analysis. The defense mechanisms of plants against the action of salt stress include blocking and excretion of Na^+ and Cl^- from the cell cytoplasm, activation of the antioxidant defense system, and an increase in plant tolerance due to various mechanisms of regulation of gene activity. Our results show that the Ulyanovska 105 and Orenburgskaya 22 wheat cultivars retain the selectivity of K+ ions with respect to Na^+ ions in the roots and maintain a higher K^+/Na^+ ratio (4.12 and 4.18, respectively) under stress compared to with varieties Zolotaya and Orenburgskaya 10 (1.26 and 3.75, respectively). The regulation of ion flows is provided by ion transporters. Increased activity of the genes of two classes of *HKT* transporters in the Orenburgskaya 22 variety contributes to a greater excretion of Cl^- and Na^+, and, conversely, in the Zolotaya variety, accumulation of toxic ions occurred, which leads to a decrease in the content of chlorophylls a and b (Chl a and Chl b). The lower Chl a/Chl b ratio in Orenburgskaya 22 than in Zolotaya 22 (2.03 vs. 2.43) indicates a high content of Chl b. This expands the light absorption spectrum, which contributes to an increase in photosynthesis. The difference in the accumulation of reactive oxygen species (ROS) indicates the initiation of different mechanisms of antioxidant defense in different wheat genotypes. Accumulation of ROS products in the roots of Ulyanovskaia 105 and Orenburgskaya 22 cultivars during salinization occurs more intensively in the zones of the cap and

meristem, which is 1.3 times lower compared to the Zolotaya and Orenburgskaya 10 cultivars. In these wheat varieties, ROS products accumulate in all the studied zones, however, in the area of the cap is less than in the other zones. NaCl-sensitive wheat genotypes showed damage in root cells, while damage was minimal in resistant wheat genotypes. Salt stress in sensitive wheat varieties can lead to programmed cell death. The degradation of nucleic acids is a marker of the plant death. According to the degree of DNA degradation, the Zolotaya variety turned out to be the most unstable. The integrated approach used by us in this study made it possible to identify various mechanisms of resistance to salt stress in wheat genotypes. The obtained results can be in demand by breeders and specialists of the agrarian complex.

Keywords: soft wheat, durum wheat, salt stress, ion transporters, chlorophyll, reactive oxygen species, nuclease activity, DNA degradation, programmed cell death

Plants are very plastic and quickly adapt to changing unfavorable environmental conditions. Depending on the mechanisms and speed of adaptation, plants can be divided into tolerant and sensitive to abiotic stress. Sodium chloride salinity is one of the most common and studied abiotic factors [1, 2]. Soil salinization is largely associated with human economic activity and the agricultural practices used (irrigation, use of fertilizers). This is becoming one of the major threats to sustainable agriculture, causing a decrease in plant productivity due to disturbances at the physiological, biochemical and molecular levels. The areas of saline agricultural land tend to constantly increase as a result of secondary salinization. The total area of saline soils in Russia is 66.4 million hectares, or 3.9% of the total land fund [3]. The Orenburg region belongs to an area with a high percentage of saline soils (5.3% of the area of agricultural land). Currently, soil salinity is considered to be the main limiting factor that negatively affects the growth and development of wheat, the most important agricultural crop [4]. High concentrations of Na^+ ions are toxic to cellular metabolism and can inhibit the activity of many important enzymes, cell division and reproduction, cause membrane disorganization and osmotic imbalance, which can ultimately lead to growth inhibition and even plant death [1, 2, 5].

Understanding the mechanisms of responses to abiotic stress is important to ensure stable yields. Plants have developed the ability to withstand the effects of stressors at the cellular and tissue levels. Plant resistance to salinity is due to the action of specific and (or) nonspecific mechanisms that support stable metabolism, growth and development in ontogenesis and are associated with sensitivity to one or more types of stress factors, including the osmotic and oxidative stress effects of sodium chloride [6]. These mechanisms include changes in stomatal conductance, hormonal balance, antioxidant defense system, osmotic regulation and removal of toxins, Na^+ and Cl^- from vacuoles, blocking Na^+ transport into the cell, and exclusion of Na^+ from the transpiration stream [7, 8].

The complex control and response to abiotic and biotic stresses is achieved through the coordinated action of multiple genes that are directly or indirectly involved in defense responses in plants [9]. An increase in the activity of certain genes encoding osmolytes, ion channel components, receptors, and some other regulatory signaling factors or enzymes can increase plant tolerance to salt action [10, 11]. Normally, in soil, the water potential in root cells is lower than in the external environment, and the influx of water occurs with the participation of water channel proteins, the so-called aquaporins [12]. In a saline environment, the difference in water potential between soil and root cells is much less than under normal conditions, or even greater, resulting in decreased water uptake or loss of water [13]. As a result, growth inhibition occurs, which ultimately leads to severe damage to plant tissue.

At the cellular level, salt tolerance mechanisms are aimed at reducing the accumulation of Na^+ in the cytoplasm by limiting the penetration of Na^+ into cells, ensuring active transport of Na^+ from cells and movement into cellular compartments, the vacuoles [14]. K^+ ions are preferred for uptake by roots from

the soil, and most plants exhibit a high degree of discrimination between K+ and Na^+. High-affinity K^+ transporters (HKTs) have been reported to be active at the level of the plasma membrane and function as Na^+/K^+ symporters as well as selective Na^+ uniporters [15]. HKTs may have two main functions, i.e., the extraction of Na^+ from soil solution to reduce K^+ demand, and the reduction of Na^+ accumulation in the leaf by removing Na^+ from the xylem and moving Na^+ into the phloem.

For cells, toxicity caused by a high content of Na^+ ions is the predominant ion toxicity; it leads to inhibition of various processes, such as K^+ uptake [9, 10], inactivation of vital enzymes [15], and inhibition of photosynthesis [16]. In the cell, photosynthetic processes are most sensitive to the toxic effects of Na^+, and their disruption is directly related to a reduction in carbon fixation and plant biomass production [16].

As a result of the influence of various unfavorable biotic and abiotic factors (hypoxia, drought, salinity, physical damage at the wound site and infection by pathogens), the content of reactive oxygen species (ROS) in plant tissues increases, but their excess can also lead to damage by oxidative stress [17, 18]. The production and accumulation of ROS occur in the plant during redox metabolism [19, 20]. The plant cell is protected from the toxic effects of ROS by the antioxidant system which controls the ROS concentration [21]. ROS can also act as important signaling molecules that regulate plant defense mechanisms, growth, development, and response to external stress [22].

Many studies have been devoted to the study of chlorophylls, in particular to determining their quantity and ratio, due to the important role of this pigment in plant physiology. Chlorophylls are involved in the absorption and transmission of light energy and the transfer of electrons during photosynthesis. Chlorophyll content can change in response to biotic and abiotic stresses, such as pathogen infection [23] or light stress [24, 25]. Thus, chlorophyll quantification provides important information about the influence of the environment on plant growth [26].

The Orenburg region is located mainly in two soil zones (chernozem and chestnut soils), gray forest soils make up 0.4%. The areas subject to salinity in the region amount, according to various estimates, from 13.9 to 15.0% of the soil fund. In total, solonetz soils here occupy 1971.8 thousand hectares, including 541.5 thousand hectares of arable land (27.5%) [27). Under these conditions, the basis for ensuring stable wheat yields remains the assessment of the ability of varieties to adapt to salt stress conditions, the study of its manifestations in plants and the identification of mechanisms that can effectively resist it.

In this work, the main attention was paid to the accumulation and removal of toxic ions from the roots and leaves of different wheat genotypes and the influence of these ions on plant tissues. We showed that in the studied varieties of durum wheat, with increasing salinity, the size of the root system decreased, and as a result, the absorption of toxic Na^+ ions decreased. In soft wheat, ionic conductivity and the excretion of Na^+ ions increased. It was also noted that in durum wheat varieties, the aboveground part of the plants is more adapted to salinity than the underground part.

The purpose of the work is to compare the manifestations of salt stress caused by high concentrations of NaCl, and the mechanisms of salt tolerance and adaptation to its toxic effects in different wheat varieties under the conditions of the Orenburg region.

Materials and methods. The varieties of soft wheat (*Triticum aestivum* L.) Ulyanovskaya 105 and Orenburgskaya 22 and durum wheat (*Triticum durum* Desf.) Zolotaya and Orenburgskaya 10 were obtained from the collection of the

Federal Scientific Center for Biological Systems and Agrotechnologies RAS (Orenburg, Russia).

Plot tests were carried out in the central zone of the Orenburg Province (experimental field of the Federal Scientific Center for Biological Systems and Agrotechnologies RAS, 1.8 m^2 plots, 3 plots per treatment). The soil of the plots is southern carbonate chernozem, loamy solonetz. The humus horizon is 45-55 cm, the pH of the soil solution is close to neutral (6.8-7.0). Humus content in the arable layer is 3.5-4.2%, total nitrogen from 0.2 to 0.6%, available phosphorus 1.5-2.5 mg/100 g of soil, exchangeable potassium 30-40 mg/100 g soil. The crop predecessor was fallowing soil. The varieties were sowed on May 3, 2022 using a CH-16 seeder (Russia) at a viable seed rate of 4.5 million/ha. The effect of salt exposure on wheat was studied by root watering of seedlings with a solution of sodium chloride (200 mM, 100 ml of solution per seedling) from the stage of full germination to the tillering (3 times within 14 days). Control wheat samples were watered. During this period, there was an excess of the average daily air temperature with insufficient precipitation (33% of the norm). The content of productive moisture in the experimental plot at the beginning of the growing season was sufficient and remained without significant changes until the tillering stage. Thirty plants in 3 replicates were collected at tillering stage for morphometric, biochemical, and cytological analyses.

The proportion of dead cells in shoots was determined during the tillering stage by intravital staining with a 0.5% aqueous solution of trypan blue for 5 min. The samples were washed 3 times with running water, and the staining was visualized by light microscopy (Olympus BX51 microscope, Olympus Corporation, Japan; ×10 objective; images were made with a Color View II digital camera, Germany).

For intravital visualization of ROS in root cells at the tillering stage, an aqueous solution of Carboxy-H2DFFDA (Thermo Fisher Scientific, USA) was used according to protocol [28]. The preparations were analyzed (an Olympus BX51 fluorescence microscope, ×10 objective, λ = 490 nm). Fluorescence intensity was measured using a Color View II digital camera (Cell program, Soft Imaging System, Germany). The images were analyzed and processed in the ImageJ program (https://imagej.softonic.ru).

Chlorophyll was extracted from crushed aboveground biomass (shoot samples, 500 mg each) with 80% acetone for 30 min. The optical density of the solution was measured (a SmartSpec Plus spectrophotometer, Bio-Rad, USA). The content of chlorophylls was quantified using formulas [29]:

$$\text{Chl a, mg/ml} = 11.63 \times A665 - 2.39 \times A649;$$

$$\text{Chl b, mg/ml} = 20.61 \times A_{649} - 5.18 \times A_{665}.$$

Total protein from shoots and roots (1 g of test material each) was extracted with 50 mM Tris-HCl buffer (pH 7.5) containing 0.8 M sucrose and 0.35 M NaCl (for 1 h at 25 °C). The amount of protein was determined using the Bradford method. The OD_{595} of the extract was measured and the concentration was calculated by a calibration curve constructed with bovine serum albumin (Serva, USA).

Nuclease activity was determined spectrophotometrically (SmartSpec Plus spectrophotometer, Bio-Rad, USA) at λ = 260 nm, using a solution of thymic DNA (Reakhim, Russia) (A_{260} = 1.0 in 0.5 M acetate) as a substrate buffer, pH 5.0). The reaction was carried out at 37 °C for 2 hours. A unit of activity was taken to be the amount of enzyme that caused an increase in the optical density of the solution by 0.01 units [30].

DNA was isolated from plant roots at the tillering stage (2 g each) according to the protocol of OOO Syntol (Russia) and visualized using electrophoresis in a 1.2% agarose gel.

RNA was isolated separately from shoots and roots of wheat seedlings at the tillering stage (100 mg each) using the RNA-Extran reagent kit as per the manufacturer's protocol (OOO Syntol, Russia). The RNA concentration in the resulting preparations was measured (an IMPLEN nanophotometer, IMPLEN, USA).

cDNA was obtained by standard methods using a set of reagents for reverse transcription according to the protocol (OOO Synthol, Russia). Information on the primary structure of the *HKT* genes was taken from the NCBI database (http://www.ncbi.nlm.nih.gov). Primers for the transcripts of these genes were selected with the NCBI Primer-BLAST online service (https://www.ncbi.nlm.nih.gov/tools/primer-blast) and synthesized at OOO Synthol LLC (Russia), the TaHKT1;4 is 5′-ATT CAG GCA ACA CCT AAT CAT GC-3′ and 5′-GCA TCA CAA GAA TGA GGA TGA GC-3′; TaHKT2;1 is 5′-TAT GTG ATG AGT CGC AGC TTG AA -3′ and 5′-GCA ACA AGA GGC CTG AAT TCT TT-3′.

Real-time PCR (RT-PCR) was performed in a CFX 96 Real-Time System thermal cycler (Bio-Rad, USA). The RT-PCR mode was the same for all samples: 95 °C, 5 min for polymerase activation, then 45 cycles 94 °C, 30 s, 58 °C, 30 s, 72 °C, 30 s. RT-PCR was performed in 3 replicates for each sample and in 3 analytical replicates. The level of relative gene expression was calculated using a calibration curve constructed with PCR products that were obtained with primers to the *GaPDh* gene encoding the glyceraldehyde-3-phosphate dehydrogenase protein, taken as a reference (5′-GCC CCA GAG GAG TGT TCA AA-3′ and 5′-AAA ATG TGA GCC GCT AAG CC-3′).

The efficiency of RT-PCR was calculated by the formula:

$$E, \% = (10^{-1/s} - 1) - 100,$$

where s is the slope of the dependence of the decimal logarithm of Ct values on cDNA concentration. The efficiency of RT-PCR with primers for the studied genes was 95-96%.

To analyze the ion content in the leaves and roots of wheat at the tillering stage, cell walls were destroyed (100-300 mg in 25 ml of deionized water) (ultrasonic disintegrator, NPP Sapphire, Russia; 35 kHz for 30 min at 40 °C). The resulting suspension was filtered through a 0.45 μm Millipore membrane (Millipore, USA). Samples were analyzed using an ITAN ionometer (NPP Tomanalit, Russia). The ion concentrations (mg/l) in the samples were determined by a calibration curve. The electrolyte concentrations in the samples was measured by the electrical conductivity of the solution (an Expert-002 conductometer, Econix LLC, Russia) [31].

The mean values of the parameters (M) and their standard deviations (±SD) were calculated. Statistical processing was carried out using Statistica 6.0 (StatSoft, Inc., USA) and STATAN (Statanly Technologies, Russia) programs. The significance of differences was determined by Student's t-test at $p < 0.05$.

Results. As the NaCl content in the soil increased, the total biomass of 14-day plants of Orenburgskaya 22 and Ulyanovskaya 105 varieties decreased (up to 20%), while in the Zolotaya variety it increased (up to 30%) (Table 1; data for 30 plants are shown in 3 replicates in each variant at the tillering stage).

Sodium chloride had different effects on root length and shoot height in different wheat genotypes. The varieties of soft wheat in terms of shoot height showed greater tolerance to salinity, while the varieties of durum wheat turned out to be more sensitive to sodium chloride, especially the Zolotaya variety (a decrease in shoot height by 25% compared to varieties of soft wheat, for which this value was 15%). The root system of the Zolotaya variety also turned out to be the most sensitive (reduction in root length by 30%) compared to other studied varieties of both durum and soft wheat, in which we recorded a decrease in root length by 15-20% (see Table 1).

1. Morphometric parameters of plant at the tillering stage (14 days) **of the studied wheat varieties under chloride salinity** (200 mM NaCl) (N = 3, n = 30, M±SD; experimental field of the FSC RAS, Orenburg Province, 2022)

Variety	Option	Total raw biomass, g	Stem height, cm	Root length, cm
Soft wheat (*Triticum aestivum* L.)				
Ul'yanovskaya 105	Control	6,61±0,33[a]	69,6±3,48[a]	15,6±0,78[a]
	NaCl	5,58±0,28[b]	57,6±2,88[d]	13,4±0,67[c]
Orenburgskaya 22	Control	5,42±0,27[c]	61,5±3,07[c]	14,5±0,72[b]
	NaCl	4,54±0,23[e]	51,4±2,57[f]	11,1±0,55[d]
Hard wheat (*Triticum durum* Desf.)				
Zolotaya	Control	4,89±0,24[d]	64,8±3,24[b]	13,1±0,65[c]
	NaCl	3,67±0,18[f]	49,1±2,45[g]	9,2±0,46[f]
Orenburgskaya 10	Control	5,50±0,27[b]	61,5±3,07[c]	13,2±0,66[c]
	NaCl	4,45±0,22[e]	54,2±2,71[e]	10,6±0,53[e]

[a-g] Different letters mean that the average values of the indicator for the options in the column are statistically significantly different by Student's t-test at $p < 0.05$ (letter designations are assigned in descending order of the M value)

K^+ ions are necessary for the regulation of water-salt balance in plants [32]. In all wheat varieties presented in Table 2, the K^+ content in leaf tissues was more than 2 times higher than this indicator in roots. In the studied varieties, the content of K^+ ions in the leaf increased under salt stress. The exception was the variety Ulyanovskaya 105 in which we noted a decrease in the amount of K^+ in the leaf. In the root, under salt stress, an increase in the content of K^+ ions was observed only in the varieties Orenburgskaya 22 and Orenburgskaya 10.

Our data demonstrate a limited supply of Na^+ to wheat roots, followed by transport of the ion to the shoots and its removal through the leaves to maintain acceptable Na^+ levels. These results are consistent with data previously obtained by other investigators [33]. Because the roots are in direct contact with the soil and absorb nutrients, higher accumulation of Na^+ occurred in the roots compared to the control.

A comparison of the Na^+ distribution between leaf and root tissues under salt stress revealed the accumulation of Na^+ in higher concentrations in the leaf (except for the Zolotaya variety) (see Table 2) which indicates that the leaf serves as the main Na^+ accumulator. Under salt stress, a significant amount of Na^+ was transported from the leaf to the root. In the salinity-sensitive variety Zolotaya, there was probably no outflow of excess Na^+ from the leaves and no restriction on the entry of Na^+ into the root.

Our results show that common wheat genotypes retained selectivity for K^+ over Na^+ and maintained a higher K^+/Na^+ ratio under salt stress, while durum wheat genotypes did not exhibit this ability, especially the salinity-sensitive variety Zolotaya. With an increase in the concentration of Cl^- in the soil solution, the accumulation of Cl^- in the roots of durum wheat varieties occurred 2 times more intensely; smaller amounts were noted in soft wheat, especially in the Orenburgskaya 22 variety. The Zolotaya variety accumulated most Cl^- ions in the leaves. According to our data, the Zolotaya variety should be considered sensitive to the action of Cl^- ions. We believe that this variety has impaired mechanisms for the removal of ions from the xylem into root vacuoles [33], as well as the outflow of excess Na^+ and Cl^- ions from the leaves [33].

The electrical conductivity value is proportional to the concentration of electrolytes and characterizes the accumulation of the sum of ions ($K^+/Na^+/Cl^-$) in plant tissues. With high electrical conductivity, a generally larger number of ions accumulate in tissues. For example, in the Ulyanovskaya 105 variety, the electrical conductivity of leaf tissues was the highest and was determined by the highest concentration of K+ ions. The same was noted in the roots. In the Zolotaya variety, under salt stress, electrical conductivity in the leaves and roots increased, but this was due to the accumulation of Cl- ions in these organs.

2. Ion concentration and electrical conductivity in leaves and roots of the studied wheat varieties at the tillering stage (14 days) **under chloride salinity** (200 mM NaCl) ($N = 3$, $n = 5$, M±SD; experimental field of the FSC RAS, Orenburg Province, 2022)

Variety, plan organ	K^+, mg/g sample	Na^+, mg/g sample	K^+/Na^+	Cl^-, mg/g sample	Electrical conductivity, μSm
Soft wheat (*Triticum aestivum* L.)					
Ul'yanovskaya 105:					
leaf					
control	15.37±0.77[a]	1.65±0.08[e]	9.31[b]	8.38±0.42[f]	813.38±40.67[b]
NaCl	11.85±0.59[d]	2.03±0.10[c]	5.84[f]	11.96±0.60[c]	660.50±33.02[d]
root					
control	6.17±0.31[f]	0.93±0.05[i]	6.63[d]	5.08±0.25[j]	398.73±19.94[h]
NaCl	5.36±0.27[g]	1.30±0.06[f]	4.12[k]	9.24±0.46[e]	384.75±19.20[h]
Orenburgskaya 22:					
leaf					
control	12.03±0.60[c]	1.26±0.06[g]	9.55[a]	9.11±0.45[e]	802.80±40.14[b]
NaCl	14.30±0.71[b]	1.98±0.10[c]	7.22[c]	13.35±0.67[b]	797.44±39.87[c]
root					
control	5.38±0.27[g]	1.03±0.05[h]	5.22[h]	5.57±0.28[i]	316.56±15.83[i]
NaCl	6.64±0.33[e]	1.59±0.08[e]	4.18[l]	7.92±0.40[g]	300.35±15.02[i]
Hard wheat (*Triticum durum* Desf.)					
Zolotaya:					
leaf					
control	9.86±0.49[e]	1.36±0.07[f]	7.25[c]	9.18±0.46[e]	562.34±28.12[e]
NaCl	12.08±0.60[c]	1.91±0.09[c]	6.32[e]	19.63±0.98[a]	949.33±47.47[a]
root					
control	4.82±0.24[h]	1.20±0.06[g]	4.02·	5.06±0.25[j]	386.05±19.30[h]
NaCl	4.64±0.23[i]	3.67±0.18[a]	1.26[o]	10.28±0.51[d]	468.56±23.43[f]
Orenburgskaya 10:					
leaf					
control	11.37±0.57[d]	2.27±0.11[b]	5.01[i]	8.50±0.42[f]	765.93±38.30[c]
NaCl	12.48±0.62[c]	2.22±0.11[b]	5.62[g]	13.92±0.70[b]	825.31±41.26[b]
root					
control	4.81±0.24[h]	1.01±0.05[h]	4.76[j]	3.71±0.18[k]	308.55±15.43[i]
NaCl	6.53±0.33[e]	1.74±0.09[d]	3.75[n]	7.44±0.37[h]	420.20±21.01[g]

[a–o] Different letters mean that the average values of the indicator for the options in the column are statistically significantly different by Student's t-test at $p < 0.05$ (letter designations are assigned in descending order of the M value).

In general, K^+ is preferred for root uptake from the soil, and most plants exhibit a high degree of K^+/Na^+ discrimination for their uptake. The genes of the *HKT* family (K^+ and Na^+ ion transporters in plants) are divided into two subfamilies. The *HKT1* subfamily is found in all higher plants. The genes of this subfamily encode selective ion transporters; the genes of the *HKT2* subfamily are transporters of both ions (K^+ and Na^+) [11, 14].

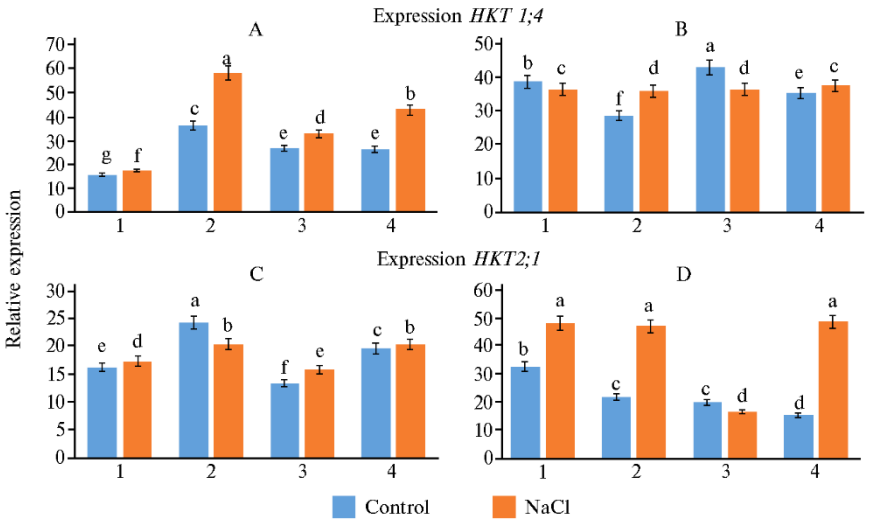

Fig. 1. Expression of the K^+ and Na^+ ion transporter genes *HKT 1;4* and *HKT2;1* in leaves (A and C) **and roots** (B and D) **of the studied varieties of common wheat *Triticum aestivum* L.** (1 — Ulyanovskaya 105, 2 — Orenburgskaya 22) **and durum wheat *Triticum durum* Desf.** (3 — Zolotaya, 4 — Orenburgskaya 10) **at the tillering stage** (14 days) **with chloride salinity** (200 мМ NaCl) (N = 3, n = 5, M±SD; experimental field of the FSC RAS, Orenburg Province, 2022).
[a-g] Different letters mean that the average values of the indicator for the options in the column are statistically significantly different by Student's t-test at $p < 0.05$ (letter designations are assigned in descending order of the M value).

An increase in the expression of the selective K^+ ion transporter gene *HKT1;4* should be accompanied by a decrease in K^+ accumulation. The lowest level of *HKT1;4* expression was observed in the Ulyanovskaya 105 variety in both leaves and roots (Fig. 1). As can be seen from the data presented in Table 2, the Ulyanovskaya 105 variety has the highest K^+ content in both organs. In the Orenburgskaya 22 variety, the K^+ concentration is not the lowest, but the expression level of *HKT1;4* was the highest (see Fig. 1, Table 2). This fact is probably due to the fact that ion exchange in all organs (not only in leaves and roots) in the Orenburgskaya 22 variety is more intense than in other wheat varieties. Class II transporters, as we have already noted, do not have selectivity and are capable of transporting both K^+ and Na^+. The highest *HKT2;1* expression occurred in the Orenburgskaya 22 variety in the leaf, the lowest in the Orenburgskaya 10 variety. These data on the expression of the *HKT2;1* genes are consistent with the accumulation of Na^+ ions in the leaf that we observed.

Probably, the observed level of *HKT1;4* expression is due to the need for intensive transport of K^+ ions (for example, to other plant organs). High activity of the *HKT1;4* expression in the Ulyanovskaya 105 variety leads to the removal of K^+ ions from the roots. The expression level of *HKT1;4* in the Zolotaya variety is also one of the highest, which is consistent with a decrease in the content of K^+ ions in the roots of this variety compared to that in the Orenburgskaya 10 and Orenburgskaya 22 varieties. In the Orenburgskaya 22 variety, salt stress leads to a significant increase in expression of the *HKT1;4* in leaves, but the K^+ ions

concentration also increases. It is possible that class I transporters exhibit selectivity towards K^+ ions only under normal conditions. Under salt stress and an excess of Na^+ ions, transporters of this class also begin to actively move toxic Na^+ ions. It is interesting to note that under salt stress in all studied wheat varieties, the level of the *HKT2;3* expression in the roots is almost the same. Thus, in the Orenburgskaya variety, 22 class II ion transporters are the most active, and this wheat variety is subject to the least toxicity by Na^+ ions, they are more actively removed from the xylem. On the contrary, the greatest accumulation of Na^+ ions in the leaf occurred in the Orenburgskaya 10 variety.

Green plants contain two main types of chlorophylls, Chl a and Chl b which are noncovalently associated with membrane proteins [34]. Chl a is part of the reaction center of the antenna array which contains the main proteins that bind Chl a to carotenoids [34]. Salt stress, resulting from excess Na^+ and Cl^- ions, leads to a decrease in the content of chlorophylls and carotenoids, leaf necrosis and a decrease in metabolic functions in the cell, including photosynthesis [26, 35]. Analysis of Chl a and Chl b content allows us to quantify damage to the photosynthetic apparatus caused by abiotic stress [35].

3. Content of chlorophylls a and b in leaves of the studied wheat varieties at the tillering stage (14 days) **with chloride salinity** (200 мМ NaCl) (N = 3, n = 5, M±SD; experimental field of the FSC RAS, Orenburg Province, 2022).

Variety	Option	Content		Chl a/Chl b
		Chl a	Chl b	
Soft wheat (*Triticum aestivum* L.)				
Ulyanovskaya 105	Control	11.82±0.59[d]	6.87±0.34[c]	1.72[c]
	NaCl	6.37±0.32[g]	4.45±0.22[d]	1.43[d]
Orenburgskaya 22	Control	17.37±0.87[a]	10.37±0.52[a]	1.67[c]
	NaCl	15.33±0.77[b]	7.54±0.38[b]	2.03[b]
Твердая пшеница (*Triticum durum* Desf.)				
Zolotaya	Control	7.38±0?37[f]	2,89±0,14[e]	2,55[a]
	NaCl	3.52±0.18[h]	1,45±0,07[f]	2,43[a]
Orenburgskaya 10	Control	12.67±0.63[c]	10,01±0,50[a]	1,26[e]
	NaCl	7.96±0.40[e]	6,76±0,34[c]	1,18[e]

[a-h] Different letters mean that the average values of the indicator for the options in the column are statistically significantly different by Student's t-test at $p < 0.05$ (letter designations are assigned in descending order of the M value).

We observed a decrease in Chl a content under salt stress in all studied wheat genotypes (Table 3). The most pronounced changes occurred in the Orenburgskaya 22 and Zolotaya varieties. The variety Orenburgskaya 22 was characterized by the largest amount of Chl a, and the variety Zolotaya by the smallest. Under salt stress, the Chl a content in the Orenburgskaya 22 variety decreased 1.1 times, and in the Zolotaya variety 2.1 times. It should be noted that the Chl a/Chl b ratio in the Zolotaya variety was higher than in the Orenburgskaya 22 variety, although the content of Chl a and Chl b for the Orenburgskaya 22 variety turned out to be higher than for the Zolotaya variety. Under salt stress, in the Orenburgskaya 22 variety the Chl a/Chl b ratio increased compared to the control while in the Zolotaya variety it remained virtually unchanged. The fact that the Chl a/Chl b value in the Orenburgskaya 22 variety is lower than in the Zolotaya variety indicates a high content of Chl b which expands the light absorption spectrum, contributing to increased photosynthesis [35].

Limited photosynthesis inhibits plant growth. This leads to an increase in the content of reactive oxygen species (ROS) due to their excess production and functional imbalance of protective mechanisms [19]. Increased ROS accumulation leads to decreased net Na^+ influx into roots, decreased xylem Na^+ load, and K^+ retention in roots, with subsequent increased salinity tolerance [36]. ROS are inevitable by-products of aerobic metabolism and important

signaling molecules involved in the regulation of many physiological processes associated with plant growth and development, but excess ROS causes lipid oxidation, leading to membrane damage, protein degradation, enzyme inactivation, base modification and DNA breaks, leading to mutation and ultimately programmed cell death [37].

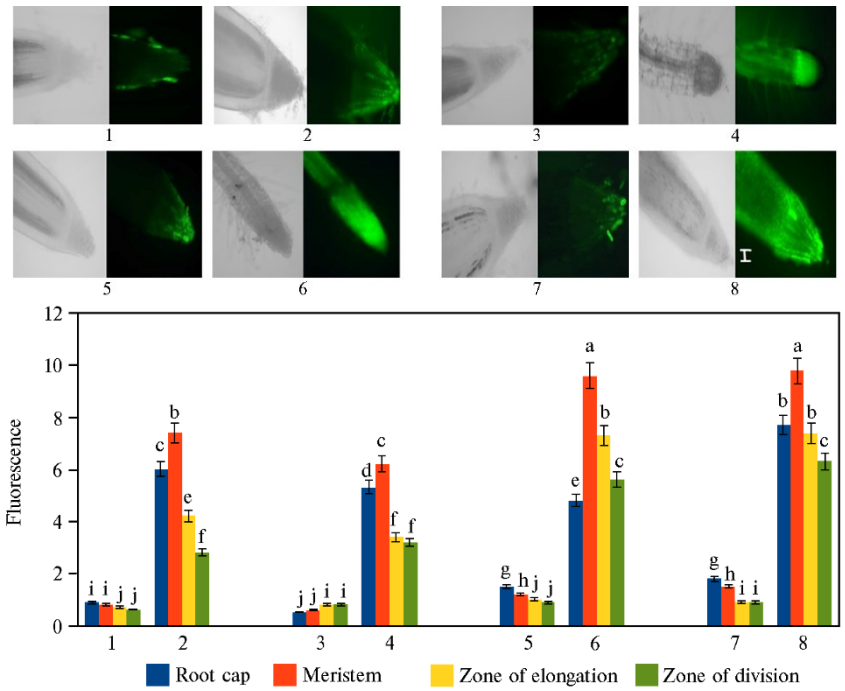

Fig. 2. Distribution of cells containing and not containing reactive oxygen species (ROS$^+$ and ROS$^-$, respectively) **in the root zones of the studied varieties of common wheat *Triticum aestivum* L.** (1, 2 — Ulyanovskaya 105, 3, 4 — Orenburgskaya 22) **and durum wheat *Triticum durum* Desf.** (5, 6 — Zolotaya, 7, 8 — Orenburgskaya 10) **at the tillering stage** (14 days) **with chloride salinity** (200 mM NaCl) (2, 4, 6, 8) compared to the control (1, 3, 5, 7) ($N = 3$, $n = 5$, $M \pm SD$; experimental field of the FSC RAS, Orenburg Province, 2022). Scale bar: 400 μm; fluorescent microscope Olympus BX51 (Olympus Corporation, Japan; for intravital visualization of ROS, an aqueous solution of Carboxy-H2DFFDA, Thermo Fisher Scientific, USA was used).

[a-j] Different letters mean that the average values of the indicator for the options in the column are statistically significantly different by Student's *t*-test at $p < 0.05$ (letter designations are assigned in descending order of the *M* value)

Staining the roots with a fluorescent dye to vizualize ROS showed that under salt stress, ROS were detected in the tissues of many root zones, but the intensity of staining in the cells of different zones varied. We studied the distribution of cells with increased levels of ROS in different root zones (Fig. 2). In the studied varieties of bread wheat, the accumulation of ROS products under salinity was more intense in the zones of the cap and meristem, but was 1.3 times lower than in durum wheat varieties. In durum wheat, ROS products accumulated in all studied zones, but in the root cap their content was lower than in other zones. Moreover, in durum wheat, the increase in ROS content compared to the control occurred to the greatest extent in the cells of the epidermis and cortex and to a lesser extent in the zone of the central cylinder (see Fig. 2). The fact that epidermal and root cortex cells are most transcriptionally active under salinity conditions has also been reported [38]. According to reports, the induction of transcriptional activity in the tissues of the inner layers of the root under the influence of salt stress indicates the spatial regulation of this signaling pathway [38, 39].

We observed the most intense fluorescent staining in the roots of the Zolotaya variety plants. This accumulation of ROS in root cells under the influence of salinity indicates a disruption of ROS homeostasis in these cells and tissues, which can provoke programmed cell death (PCD). Soft wheat varieties turned out to be more resistant to salinity than durum wheat varieties (see Fig. 2).

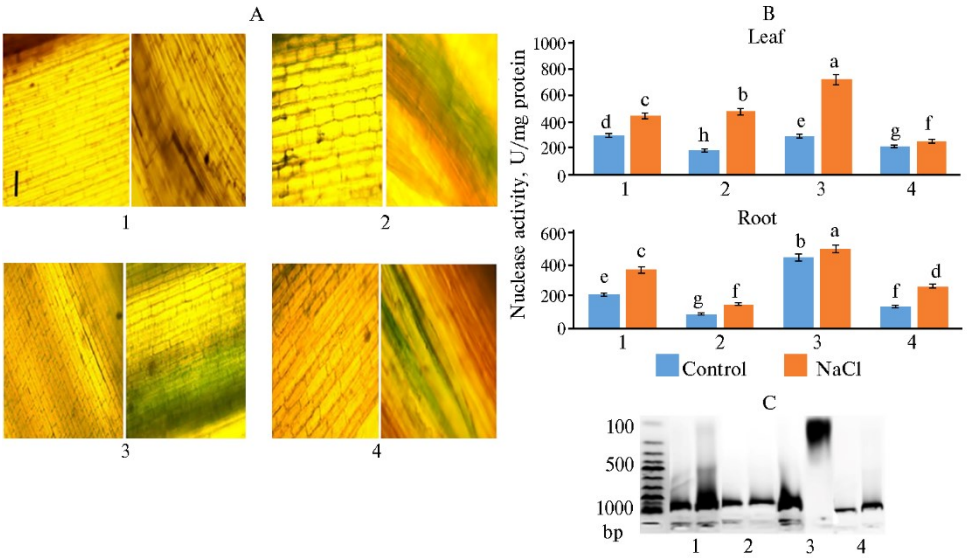

Fig. 3. Viability of coleoptile cells (A, trypan blue staining)**, nuclease activity in leaves and root**s (B, left — control, right — salt stress) **and electrophoretic separation of DNA from root cells in 1.2% agarose gel** (C) **in the studied varieties of soft wheat *Triticum aestivum* L.** (1 — Ulyanovskaya 105, 2 — Orenburgskaya 22) **and durum wheat *Triticum durum* Desf.** (3 — Zolotaya, 4 — Orenburgskaya 10) **at the tillering stage** (14 days) **with chloride salinity** (200 mM NaCl) (N = 3, n = 5, M±SD; xperimental field of the FSC RAS, Orenburg Province, 2022). Scale bar: 400 μm (Olympus BX51 fluorescence microscope, Olympus Corporation, Japan; trypan blue staining). DNA molecular weight marker (100-1000 bp) (OOO Synthol, Russia).
[a-j] Different letters mean that the average values of the indicator for the options in the column are statistically significantly different by Student's t-test at $p < 0.05$ (letter designations are assigned in descending order of the M value).

To assess cell viability under salt stress, we stained seedling coleoptiles with trypan blue (Fig. 3). In the control, there were practically no visible changes in staining, but under salt stress, cell damage turned out to be quite severe (darker staining) and varied depending on the wheat variety. Thus, in the Zolotaya variety, more than 50% of the cell area was damaged due to salinity, while in the Orenburgskaya 10 varieties this figure was no more than 40%, in Orenburgskaya 22 20%, in Ulyanovskaya 105 25%. Thus, under the influence of high concentrations of NaCl, the viability of coleoptile cells in the varieties Orenburgskaya 22 and Ulyanovskaya 105 is higher than in the varieties Zolotaya and Orenburgskaya 10, which are more sensitive to salinity (see Fig. 3).

The degradation of nucleic acids at the final stages of plant ontogenesis (during death) is massive [40, 41]. Figure 3, B shows DNA electrophoresis data from the roots of four wheat varieties grown under different conditions. Control samples of all varieties are characterized by the presence of high molecular weight DNA, while its degradation occurred under salt stress. The Zolotaya variety showed the highest degree of degradation that is consistent with the asseed total nuclease activity (see Fig. 3, B), which in the Zolotaya variety turned out to be the highest in both leaves and roots.

Thus, according to biometric indicators, the varieties of soft wheat turned out to be the most resistant to the action of high concentrations of NaCl in the

soil. The durum wheat variety Zolotaya showed the greatest sensitivity to salt stress. One of the mechanisms for increasing plant tolerance is the removal of toxic ions from the cytoplasm of plant cells. The increased activity of the genes of ion transporters of two classes of NKT in the Orenburgskaya 22 variety contributes to a greater excretion of Cl^- and Na^+ ions and, conversely, in the Zolotaya variety there is an accumulation of toxic ions [42]. It is accompanied by a decrease in the content of chlorophylls a and b and an increase in the content of ROS; as a result, damage to root tissue increases, especially in the Zolotaya and Orenburgskaya 10 varieties. The difference in the accumulation of ROS products indicates the activation of different antioxidant defense mechanisms in different wheat genotypes. The greatest accumulation of ROS products in roots under the influence of NaCl is observed in durum wheat varieties, especially in the Zolotaya variety, which initiates programmed cell death.

So, our results showed that different wheat genotypes have developed different mechanisms of adaptation to salt stress. An increase in soil salinity led to a decrease in the size of the root system in durum wheat varieties, which was accompanied by a decrease in the absorption of toxic Na^+ ions. Common wheat varieties increased ionic conductivity and the excretion of Na+ ions, especially the Orenburgskaya 22 variety. The Ulyanovskaya 105 variety had the highest content of K^+ ions which create a barrier to the penetration of Na^+ ions. The aboveground part of plants in durum wheat varieties is more adapted to salinity than the underground part. This is supported by our data on the content of chlorophylls, in particular on the Chl a/Chl b ratio, indicating an increase in photosynthesis. To deepen our understanding of the mechanisms of adaptation to salinity in different genotypes, we plan to use additional methods for analyzing the processes that shape the response to salt stress depending on the stage of plant development, and to expand the range of studied wheat varieties.

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