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Assessing the Adaptive Mechanisms of Two Bread Wheat (*Triticum aestivum* L.) Genotypes to Salinity Stress

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Abstract: This work deals with the assessment of physiological and biochemical responses to salt stress, as well as the regulation of the expression of the K^+/Na^+ transporter gene-*TaHKT1;5* of two *Triticum aestivum* L. genotypes with contrasting tolerance. According to the observations, salinity stress caused lipid peroxidation; accumulation of soluble sugars and proline; decreased osmotic potential, Fv/Fm value, and K^+/Na^+ ratio; and increased the activity of antioxidant enzymes in both genotypes. In the salt-tolerant genotype, the activity of enzymes, the amounts of soluble sugars and proline were higher, the osmotic potential and the lipid peroxidation were lower than in the sensitive one, and the Fv/Fm value remained unchanged. A comparison of the accumulation of Na^+ and K^+ ions in the roots and leaves showed that the Na^+ content in the leaves is lower. The selective transport of K^+ ions from roots to leaves was more efficient in the salt-tolerant genotype Mirbashir-128; consequently, the K^+/Na^+ ratio in the leaves and roots of this genotype was higher compared with the sensitive Fatima genotype. The semi-quantitative RT-PCR expression experiments on *TaHKT1;5* indicated that this gene was not expressed in the leaf of the wheat genotypes. Under salt stress, the expression level of the *TaHKT1;5* gene increased in the root tissues of the salt-sensitive genotype, while it decreased in the salt-tolerant wheat genotype. The results obtained suggest that the ion status and salt tolerance of the wheat genotypes are related to the *TaHKT1;5* gene activity.

Keywords: wheat; salt stress; MDA; Fv/Fm; antioxidant enzymes; K^+/Na^+ transporters; *TaHKT1;5* gene

1. Introduction

Soil salinity is a serious ecological problem for many countries around the world. The annual increase in the area of saline soils due to secondary salinization processes leads to degradation and desertification of agricultural lands, resulting in their withdrawal from circulation. These processes, in turn, lead to losses in world agricultural production. The negative effect of high concentrations of salts is based on a violation of the osmotic status and ionic homeostasis, as well as being based on the manifestation of the toxic effect of inorganic ions on cellular metabolism [1]. Moreover, salinization causes the generation of reactive oxygen species (ROS), provoking lipid peroxidation, the final product of which is malondialdehyde (MDA) [2]. The accumulation of Na^+ ions, which are toxic to plant

organisms, is especially dangerous. It is assumed that the metabolic toxicity of Na^+ is largely the result of its ability to compete with K^+ for binding sites in key enzymes activated by K^+, disrupting their full functioning. Thus, at a high Na^+/K^+ ratio, many enzymatic processes in the cytoplasm can be disrupted. In particular, the disruption of protein synthesis processes by high concentrations of Na^+ is considered as one of the examples of the toxic effect of Na^+ ions on cellular metabolism [3]. Therefore, all living cells, regardless of their taxonomic affiliation and living conditions, maintain low concentrations of Na^+ ions in the cytoplasm. Many mechanisms have been developed to minimize radial and long-distance Na^+ transport in salt-tolerant plants and to prevent their accumulation at toxic levels in metabolically active tissues. These mechanisms include minimizing the input of Na^+ ions as a result of selective assimilation of ions by root cells, returning Na^+ ions from roots to the rhizosphere, preventing xylem from being loaded with Na^+ ions, removing Na^+ ions from the transpiration flux, etc. Maintaining low levels of Na^+ ions and high levels of the K^+/Na^+ ratio in the cytoplasm of photosynthetic tissues is considered a key factor for the tolerance of higher plants to salt stress [4,5]. Ion transporters, ion channels, and signal molecules are directly involved in maintaining this ratio at optimal levels [6,7].

One of the main regulators of K^+/Na^+ homeostasis in plant cells under salt stress is the HKT (high-affinity potassium transporters) transporter system, which is active in the plasma membrane. Under salt stress, *HKT* transporters regulate the balance between Na^+ and K^+ ions in the cell. The *HKT* may have two major functions—namely, to take up Na^+ from the soil to reduce the requirement of K^+ when K^+ is a limiting factor and to reduce the accumulation of Na^+ in the leaf by removing Na^+ from the xylem sap and loading Na^+ into the phloem sap [8]. The involvement of these transporters in the return of Na^+ from aboveground organs to the roots through the phloem can play an important role in salt tolerance. The roots can maintain a somewhat constant level of Na^+ ions over time and regulate their level by exporting to the soil. The symporters of the *HKT* family are also involved in organizing long-distance transport in a plant, performing the function of Na^+-unloading of xylem. Due to the work of the symporter, Na^+ is retained in the upper part of the root system and the lower part of the stem, leading to a decrease in the Na^+ content of the leaf, where photosynthesis is performed [8].

Hitherto, numerous functional *HKT* genes have been identified in various plant species. While weakly represented in dicot species genomes (e.g., a single *HKT* gene in *Arabidopsis* and poplar), the *HKT* family comprises more members displaying a large functional diversity in monocots, including in cereals. For instance, rice (*Oryza sativa* L.) possesses 9 *HKT* genes [9], and barley (*Hordeum vulgare*) and wheat have been deduced from Southern blot analyses to possess 5–11 *HKT* genes per genome [10]. Based on phylogenetic and functional analyses, plant *HKT* genes have been divided into two subfamilies [11]. *HKT* subfamily 1 (present in all higher plant species) encodes Na^+-selective transporters, while *HKT* subfamily 2 (monocot specific) encodes systems permeable to both Na^+ and K^+ [12–15]. Experiments have shown that *HKT* genes play an important role in the salt tolerance of various plant species. The important role of *HKT* I transporters under salt stress has been characterized in detail in plant species such as Arabidopsis, wheat, barley, rice, sorghum, and tomatoes, as well as in *Eutrema parvula* and *Eutrema salsuginea*. Disruption of the expression of *HKT* family genes leads to hypersensitivity to Na^+ ions and excessive accumulation of Na^+ ions in the stem and leaves. High expression of *HKT* family genes in roots and leaves under salt stress is associated with rapid restoration of K^+/Na^+ ion balance and exclusion of toxic Na^+ ions—in other words, a protective function. The clarification of tissue-specific expression of these genes and their control mechanisms is considered an important issue to increase the tolerance of plants to salt stress under unfavorable conditions. The molecular mechanisms underlying the osmotic aspects of salt tolerance are not completely clarified. However, *HKT* genes, which play an important role in regulating the ionic status of plants under salt stress, are among the important goals in improving the salt tolerance of plants of agricultural importance.

Bread wheat (*Triticum aestivum* L.) is a strategic product that plays an important role in the national economy of many countries. Currently, soil salinity is one of the adverse factors that negatively affect the growth and development of wheat. Under saline conditions, the quality of grain deteriorates, and productivity decreases [16]. The study of the salt tolerance potential of local wheat genetic resources is one of the important issues in the selection of parental genotypes for future breeding and biotechnological development strategies. Additionally, the study of the response of wheat genotypes to salt stress and the expression of *HKT* genes are important for the determination of their salt tolerance. In bread wheat, *TaHKT1;5* was shown to confer the essential salinity tolerance mechanism associated with the Kna1 locus via shoot Na^+ exclusion and is critical in maintaining a high K^+/Na^+ ratio in the leaves [17].

The study aimed to assess the physiological and biochemical responses to salt stress and expression level of the *TaHKT1;5* gene in two bread wheat genotypes: salt-tolerant Mirbashir 128 (MIR) and salt-sensitive Fatima (FAT). Salt tolerance properties of these genotypes were presented in our previous research [18]. Responses to salt stress of these genotypes were evaluated based on multivariable biochemical and physiological analyses.

2. Materials and Methods

2.1. Plant Material, Germination Assay, and Stress Conditions

The objects of the present study were two Azerbaijan local wheat (*Triticum aestivum* L.) genotypes: Mirbashir 128 (MIR) and Fatima (FAT). However, as per confirmation of Ibrahimova et al. [18], the variety MIR is salt tolerant and FAT is salt-sensitive. Seeds were surface sterilized with 10% NaOCl for 10 min and then washed three times with sterile distilled water. They were germinated and hydroponically grown in a half-strength Hoagland solution (pH 6.0) in a growth chamber for 10 days at 22 ± 2 °C with 16 h light and 8 h dark photoperiod at a light intensity of 150 μmol/m^2s. For salinity treatment, 10-day-old seedlings were transferred into the half-strength Hoagland solution containing 150 mM NaCl for 10 days. Leaf and root samples from treated and control plants were quick-frozen in liquid nitrogen and stored at −80 °C until further use.

2.2. RNA Isolation and cDNA Synthesis

Root and shoot samples (200 mg) were collected from plants of both wheat genotypes treated as control (0 mM NaCl) and exposed to salt stress using 150 mM NaCl for 10 days. The samples were wrapped in aluminum foil, frozen immediately in liquid nitrogen, and stored at −80 °C until used for RNA extraction in batches. Total RNAs were isolated from 200 mg root and shoot tissue samples using the TRIzol reagent (Invitrogen, TelTest Inc., Friendswood, CA, USA) according to the manufacturer's instructions. To remove contaminating DNA, isolated RNA (10 μg) samples were treated with RNase-free DNase I (Promega, 800 Woods Hollow Road. Madison, WI 53711-5399, USA). The quality of the isolated RNA samples was tested by electrophoresis on 1% agarose gels, and RNA concentration was estimated using a spectrophotometer BioTek Epoch (100 Tigan StreetWinooski, VT 05404, USA). Only those RNA samples showing a 260/280 ratio in the range of 1.9–2.2 and a 260/230 ratio >2.0 were used for cDNA synthesis.

The first-strand cDNA was synthesized using 2 μg total RNA in a 20 μL reaction mixture containing 40 pmoL oligo (dT) 18 primer, 1× reaction buffer with 10 mmol/L dNTP mix, and 200 U of Moloney Murine Leukemia Virus reverse transcriptase enzyme (MBI Fermentas, Hanover, MA, USA). The reaction mixture was incubated for 1 h at 42 °C, followed by heating for 10 min at 70 °C. The first cDNA template was diluted 6-fold and kept at −20 °C for RT-PCR amplification analysis. The first-strand cDNA generated from total RNA, including salt-treated and untreated samples from either the MIR or FAT genotype, was subjected to semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis. To examine the expression of *TaHKT1;5*, RT-PCR was carried out using the gene-specific primers:

Forward: 5′-CTGTCGCTCTTCTGCGCCAT-3′

Reversed: 5′-TTATACTATCCTCCATGCCT-3′

The constitutively expressed actin gene was used as a reference gene. The following primer sequences were used for amplification of actin:

Forward: 5′-CTTGTATGCCAGCGGTCGAACA-3′

Reversed: 5′-CTCATAATCAAGGGCCACGTA-3′

The following thermal cycle conditions were used: 95 °C for 3 min, followed by 94 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s, for a total 40 cycles. Reactions were carried out under the following conditions: the first cycle for 4 min at 94 °C, followed by 35 cycles at 94 °C for 45 s, 58 °C for 45 s, 72 °C for 45 s, and final extension at 72 °C for 5 min.

The band intensity was visualized and photographed by a gel documentation system («UVIPRO», UK). The RT-PCR experiment was repeated three times.

2.3. Measurement of Potassium and Sodium Ions

K^+ and Na^+ were determined using a PFP7 (Jenway, Cole-Parmer, Beacon Road, Stone, Staffordshire, ST15 OSA, England) flame photometer. First, plant samples were combusted according to the method in [19]. A mixture of sulfuric and perchloric acid (3:1) was added to 0.2 g of powdered plant samples and stored for a day. The next day, the test tubes were gradually heated to a temperature of 250–270 °C. After combustion, the bleached solution in the test tube was cooled and used for the determination of sodium and potassium ions. Standard solutions of KCl and NaCl salts were used to construct the calibration curve.

2.4. Measurement of Leaf Water Potential

After freezing in liquid nitrogen and subsequent thawing, osmotic potential (ΨS) was measured by the psychrometric method using a microvoltmeter Wescor HR-33 with measuring chamber C-52 (Wescor Inc., 370 West 1700 South Logan, UT 84321, USA).

2.5. Determination of Malondialdehyde

Malondialdehyde (MDA) was determined through the thiobarbituric acid (TBA) reaction, described earlier [20]. The 500 mg of leaves was homogenized in 2 mL of 5% trichloroacetic acid (TCA). Homogenate was centrifuged for 10 min at 10,009 g. The supernatant was added to 4 mL of a medium containing 0.5% TBA and 20% TCA, and the mixture was kept in the water bath at 95 °C for 30 min. Then the sample was transferred into the ice bath, and after cooling, it was precipitated at 1000 g for 15 min. MDA was determined by measuring the optical density of the last supernatant at wavelengths of 532 and 600 nm.

2.6. Enzyme Extraction and Activity Determination

An amount of 1.0 g of the leaves was ground in liquid nitrogen and homogenized by adding 0.05 M Na_2HPO_4/NaH_2PO_4 buffer (pH 7.0). The mixture was precipitated at 16,000 g for 20 min at 4 °C. The obtained supernatant was used to determine the activity of enzymes. The activity of ascorbate peroxidase (APX) was determined by the spectrophotometric method at 290 nm, based on the decrease in the optical density due to the oxidation of ascorbic acid in the presence of H_2O_2 (Nakano Asada). The reaction medium consisted of 50 mM phosphate buffer (pH 7.0) containing 0.5 mM ascorbic acid (AsA), 0.1 mM H_2O_2, 0.1 mM EDTA, and 0.3 mL enzyme extract. The optical density was determined within 30 s immediately after the addition of H_2O_2. The calculations were performed according to the following formula:

$$A = \Delta D290 \times V/\varepsilon \times b$$

where, ΔD—change of optical density in 30 s; V—volume of the cuvette; b—volume of the sample under study; ε—molar extinction coefficient ($\varepsilon = 2.8\ mM^{-1}cm^{-1}$).

The activity of catalase was determined based on a decrease in the optical density at 240 nm, due to the decomposition of H_2O_2 [19]. The reaction medium consisted of 50 mM phosphate buffer (pH 7.0) containing 15 mM H_2O_2 and 0.3 mL enzyme extract.

The reaction was initiated after the addition of H_2O_2, and the change in the optical density was determined within 1 min. The molar extinction coefficient was assumed to be 39.4 $mM^{-1}cm^{-1}$.

Chlorophyll *a* fluorescence (Fv/Fm value) was measured by a portable non-modulated fluorimeter Plant Efficiency Analyser (Handy PEA; Hansatech Instruments, Kings Lynn, UK) [21].

2.7. Determination of Proline and Soluble Sugars

The Bates method [22] was used to determine proline in plants. Pre-mixed Ninhydrin (1.25 g) and cold crystalline acetic acid (30 mL) were dissolved in 20 mL of 6 M phosphoric acid. The mixture was kept at 4 °C for a max of 24 h. The homogenization of 0.5 g of the plant material was performed in 10 mL of 3% sulfosalicylic acid. The obtained homogenate was filtered through two layers of Whatman paper, and 2 mL of ninhydrin and 2 mL of acetic acid were added. Then, the mixture was boiled at 100 °C for 1 h. A water–ice mixture was used to stop the reaction immediately after boiling. After adding 4 mL of the toluene solution, the mixture was shaken for 15–20 s and stored at room temperature until the two-phase system was fully formed. The optical density of the organic-toluene phase containing chromophore was measured at 520 nm using a Hitachi 557 spectrophotometer (Hitachi High-Tech Corporation, 1-14 Matsuyama 1-chome, Naha-shi, Okinawa 900-0032, Japan). The toluene solution was taken as standard. The calibration curve was used to find the proline concentration. Calculations were performed on a fresh weight basis.

The anthrone–sulphuric acid method was used to determine sugars [23]. An amount of 10 mL of 80% ethanol was added to 0.1 g of dried leaves, and the obtained mixture was shaken for 24 h. After the precipitation of the homogenate at 5000× *g* for 10 min, 2.5 mL of anthrone was added to the supernatant (0.5 mL) and heated in a water bath at 40 °C for 30 min. To prepare the anthrone reagent, 0.2 g anthrone was dissolved in 100 mL of concentrated H_2SO_4. The optical density of the mixture was measured at 625 nm using a Hitachi 557 spectrophotometer (Hitachi High-Tech Corporation, 1-14 Matsuyama 1-chome, Naha-shi, Okinawa 900-0032, Japan) after cooling. The calibration curve was constructed using sucrose.

2.8. Statistical Analysis

Data were analyzed by partitioning the total variance, and treatment means were compared with the help of R package "stats" (version 2.15.3) "R" at the 5% level of significance [24].

3. Results

CAT and APX are key enzymes for the detoxification of ROS in the cell. These enzymes, especially APX, perform the conversion of H_2O_2 to water in the cell cytosol, mitochondria, and peroxisomes, as well as in the apoplastic space. Given the high affinity of CAT and APX for H_2O_2, we preferred to study them. The activities of CAT and APX increased in leaves of the studied genotypes treated with 150 mM NaCl (Tables 1 and A2). The APX activity increased 2.1-fold and 1.6-fold in the MIR and FAT genotypes, respectively. Similarly, the CAT activity increased 1.7-fold in the MIR genotype and 1.3-fold in the FAT genotype. Likewise, the soluble sugars and proline content were found to be 1.7- to 4.1-fold higher in MIR genotype, and 1.2- to 1.9-fold higher in FAT genotype, respectively, as compared with the control (Table 1).

Table 1. Effect of 150 mM NaCl on APX (µmoL/mg protein), CAT (µmoL/mg protein) activity in leaves of the salt-tolerant Mirbashir-128 and salt-sensitive Fatima wheat genotypes after 10 days of salt treatment.

Salt Levels (mM NaCl)	Wheat Variety	APX (µmol/mg Protein)	CAT (µmol/mg Protein)	Soluble Sugars (mg/g DW)	Proline (µmol/g FW)
0	MIR	1.03a	0.63a	36b	4.97b
	FAT	0.99a	0.61a	39b	5.07b
150	MIR	2.22a	1.10a	60a	20.38a
	FAT	1.63b	0.83a	48a	9.63a
F-test		**	**	**	**
CV (%)		5.47	6.77	3.09	4.73

MIR, Mirbashir 128; FAT, Fatima; **, significant at 1% level of probability. Within each trait, means followed by the same lowercase letter(s) in a column or in a row followed by the same capital letter(s) indicate significant differences ($p \leq 0.05$) according to DMRT.

MDA is a product of lipid peroxidation and is considered an indicator of oxidative damage. The amount of MDA increased in the leaves of plants exposed to 150 mM NaCl, as shown in Figure 1 and Table A1. The MDA difference between the two genotypes affected by 150 mM NaCl was obvious. In the salt-sensitive genotype FAT, MDA increased 2.8-fold compared with the control plants, whereas the MIR genotype showed a 1.9-fold increase.

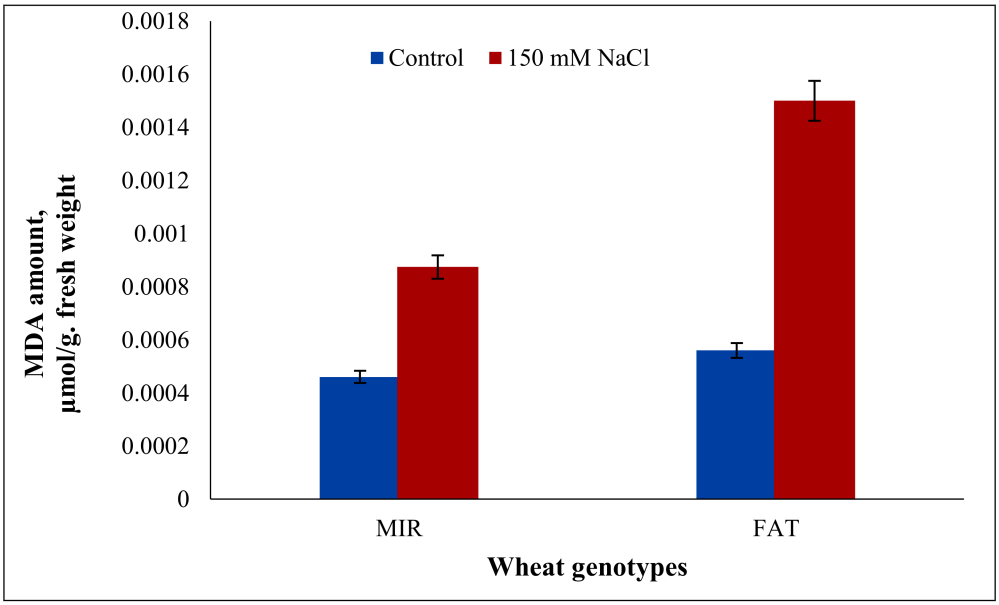

Figure 1. Effect of 150 mM NaCl on the MDA concentration in leaves of the salt-tolerant Mirbashir-128 (MIR) and salt-sensitive Fatima (FAT) genotypes after 10 days of salt treatment. The values of standard error (±SE) in each bar were calculated for each treatment from three replications.

In the present study, the osmotic potential ($\psi\pi$) decreased markedly in both wheat genotypes under salinity (Figure 2 and Table A1), whereas, the FAT genotype manifested a more negative potential compared with MIR.

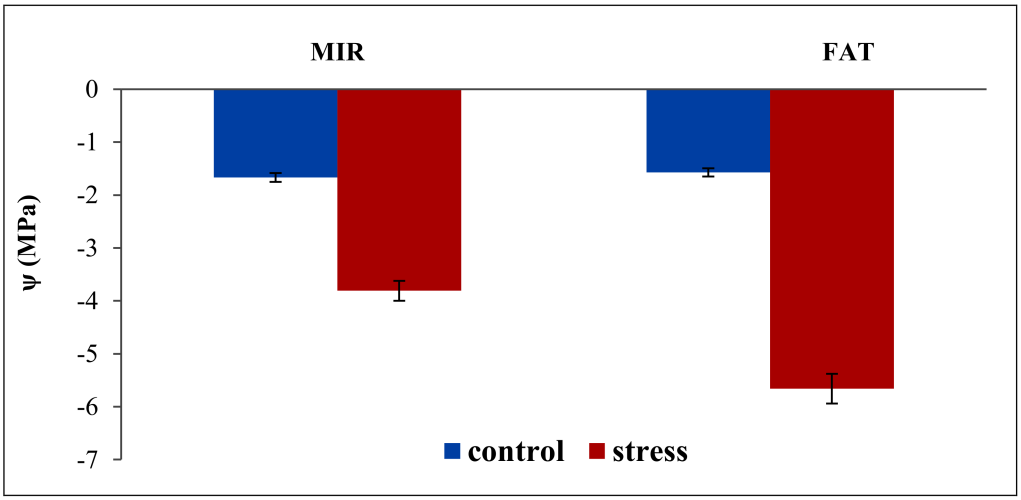

Figure 2. Effect of 150 mM NaCl on osmotic potential (ψπ) in the leaves of the salt-tolerant Mirbashir 128 (MIR) and salt-sensitive Fatima (FAT) wheat genotypes after 10 days of salt treatment. The values of standard error (±SE) in each bar were calculated for each treatment from three replications.

We did not observe a significant difference between the variants of the MIR genotype, and the Fv/Fm parameter decreased from 0.81 to 0.80 under the effect of NaCl (Figure 3 and Table A1). In the FAT genotype, the difference between the variants increased, and the value of the parameter decreased from 0.81 to 0.78.

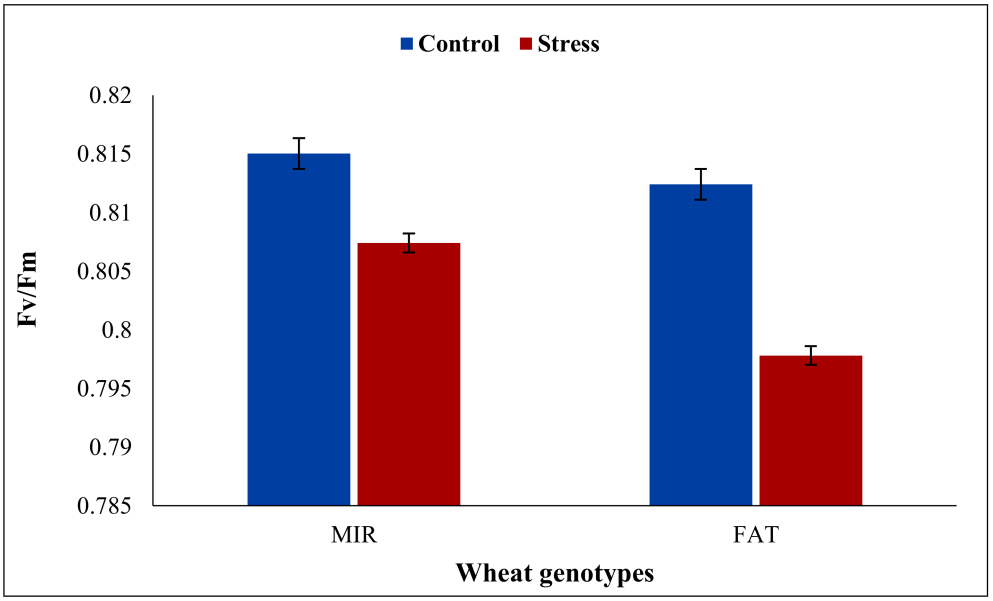

Figure 3. Effect of 150 mM NaCl on the Fv/Fm parameter (maximum quantum yield of PS II) in dark-adapted leaves of salt-tolerant Mirbashir 128 (MIR) and salt-sensitive Fatima (FAT) wheat genotypes after 10 days of treatment. The values of standard error (±SE) in each bar were calculated for each treatment from three replications.

We analyzed the content of Na^+ and K^+ in the leaves and roots of the studied genotypes (Figure 4 and Table A2). The data obtained as a result of our studies show that in both studied genotypes, the content of Na^+ in roots as well as in leaves increased under salt stress. However, these values were significantly higher for the salt-sensitive genotype FAT than for the salt-tolerant genotype MIR. The concentration of K^+ in response to salt stress, on the contrary, decreased, which was more pronounced in the salt-sensitive FAT genotype

(Figure 4A,B and Table A2). The K^+/ Na^+ ratio in the leaves was 10.5 in the MIR genotype and 9.9 in the FAT genotype under normal conditions. Under salt stress, the K^+/Na^+ ratio decreased 5.5 times in the MIR genotype and amounted to 1.9. In the FAT genotype, the decrease was 9 times.

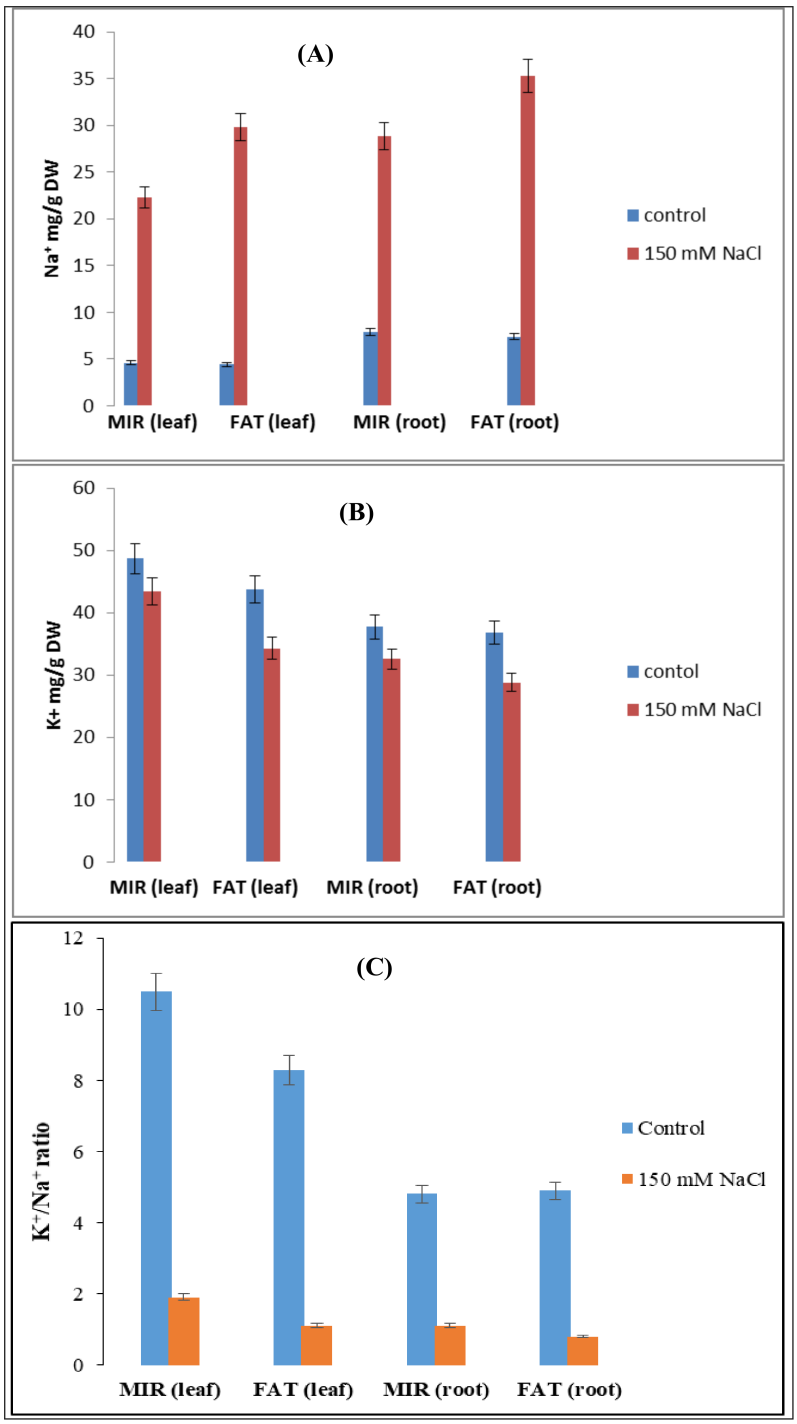

Figure 4. Effect of 150 mM NaCl on Na^+ (**A**) content, K^+ (**B**) content, K^+/Na^+ ratio (**C**) in roots and leaves of salt-tolerant Mirbashir 128 (MIR) and salt-sensitive Fatima (FAT) wheat genotypes after 10 days of salt treatment. The values of standard error (±SE) in each bar were calculated for each treatment from three replications.

The expression activity of the *HKT 1;5* gene in various tissues of wheat genotypes selected for the study after 10 days of cultivation in a high salt medium (150 mM) was assessed with RT-PCR at the level of the total amount of individual mRNAs. Total RNA was extracted from the root and leaf tissues of control and stressed plant variants, and the cDNAs synthesized from them were amplified by PCR with a specific primer to the *TaHKT1;5* gene. The activity of these genes in plants grown on a standard nutrient medium was chosen as a control. RT-PCR analysis showed that *TaHKT1;5* transcript was not synthesized in the leaves of control and stressed variants of wheat seedlings. Cultivation of wheat in the presence of 150 mM NaCl led to a decrease in the level of expression of the *HKT1;5* gene in the roots of the salt-tolerant MIR genotype compared with the control variants. Expression of the *TaHKT1;5* gene in the sensitive FAT genotype revealed some differences. Thus, the expression of this gene in the roots increased in the presence of sodium chloride. Generally, the higher expression of *TaHKT1;5* was observed in the salt-sensitive FAT genotype compared with salt-tolerant MIR (Figure 5).

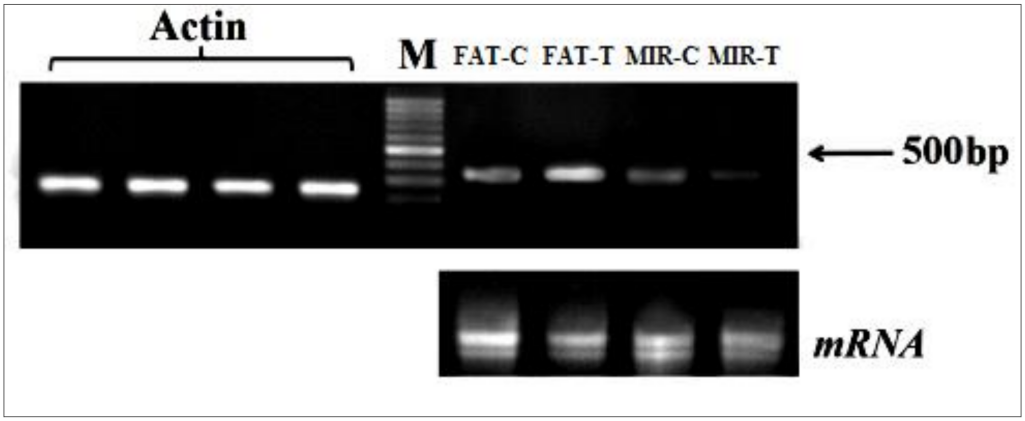

Figure 5. RT-PCR analysis of the expression level of *TaHKT1;5* gene in roots of salt-sensitive Fatima (FAT) and salt-tolerant Mirbashir 128 (MIR) genotypes under control (C, control) and 150 mM NaCl stress (T, salt treatment) conditions using specific gene primers. A 250 pb actin gene fragment was amplified by RT-PCR as the internal control. M, Marker, 100 bp DNA ladder. The arrow shows a 500 bp band of DNA ladder. Total RNA samples extracted from control and stressed plant variants are also shown.

4. Discussion

One of the mechanisms that ensures the tolerance of plants to stress factors at the cellular level, including salt stress, is the change in the activity of antioxidant enzymes involved in the scavenging of reactive oxygen species. Due to the reduced stomatal conductance in the leaves under salt stress, CO_2 cannot be well absorbed by the plant. As a result, disruptions occur in the electron transport in chloroplasts, leading to the formation of ROS [25,26]. ROS have high activity and disrupt the normal metabolism of the cell, adversely affecting proteins, lipids, and amino acids. The lifespan of ROS depends directly on the antioxidant system that protects the cell against oxidative stress [27]. This system includes non-enzymatic antioxidants such as ascorbate, glutathione, tocopherol, and various phenolic compounds, as well as main antioxidant enzymes such as catalase, superoxide dismutase, and peroxidases [28,29].

Recent research has shown the role of the catalase and ascorbate peroxidase enzymes in the protection of higher plants, including wheat against hydrogen peroxide and reactive oxygen species [30,31]. In this regard, the study of the activity of antioxidant system enzymes in wheat genotypes exposed to stress is of particular importance. Peroxidases use H_2O_2 as a substrate in oxidation reactions in the synthesis of organic substances. In our study, the activity of CAT and APX increased by the effect of NaCl in the leaves of both genotypes studied, and the highest activity was recorded in the MIR genotype. The

enzyme catalase is involved in the conversion of hydrogen peroxide to water, which is formed by the β-oxidation of fatty acids in peroxisomes. The high activity of the enzymes catalase and peroxidase leads to a decrease in the concentration of H_2O_2 in the cell, and as a result, the integrity of the cell membrane is preserved [32]. Similarly, Sofo et al. [33] reported that CAT and APX are key enzymes for the detoxification of ROS in the cell. These enzymes, especially APX, perform the conversion of H_2O_2 to water in the cell cytosol, mitochondria, and peroxisomes, as well as in the apoplastic space.

Previous studies showed a correlation between peroxidase activity and plant tolerance of salt or water deficiency stresses [30,34]. Marvi and colleagues studied the response of eight different wheat genotypes to salt stress. In addition to several physiological parameters, the current research also studied the biochemical responses of the plant to different salt concentrations (0, 100, 200, and 300 mM NaCl) and concluded that the effect of salt increased the activity of APO and CAT enzymes in the leaves of the plant [35]. Sen and Alikmanoglu showed that the activity of CAT and APO enzymes increased in wheat with rising salt concentration [36].

Saturated fatty acids, some of the main components of membrane lipids, undergo peroxidation by free radicals [37]. Peroxidation of lipids leads to disruption of membrane integrity due to increased permeability [38]. MDA is a product of lipid peroxidation and is considered an indicator of oxidative damage. Therefore, MDA is widely studied in stressed plants and is considered the best salt-tolerance marker for them. NaCl-induced lipid peroxidation was observed in various plants: mustard [39], bean [40], wheat, and barley [31,41,42]. Lipid peroxidation occurs more in salt-sensitive species than in salt-tolerant ones [43,44]. Our results are consistent with the results of previous studies [27,45,46]. Thus, lipid peroxidation was higher in the salt-sensitive FAT genotype. As shown in Figure 4, the difference between two genotypes affected by 150 mM NaCl was obvious. In the salt-sensitive FAT genotype, MDA increased 2.8 times compared with the control plants, whereas the MIR genotype showed 1.9-fold increase.

Osmotic regulation is one of the important mechanisms for plant adaptation to conditions with low water potential, and it ensures the maintenance of cell turgor. Osmotic regulation leads to a decrease in the osmotic potential due to the accumulation of osmolytes such as proline, soluble sugars, polyols, and glycine betaine in the cell [47]. In the presented study, the water status of the plant was assessed based on the osmotic potential. The lower the osmotic potential, the lower the water potential of plants. According to some authors, the osmotic potential is an important parameter for the selection of plants for drought and salt tolerance [31,48,49]. The accumulation of proline and sugars under salt stress regulates the osmotic balance between the cytosol and vacuoles [18]. In the presented study, the accumulation of sugars and proline was observed in the leaves of salt-exposed plants, and the increase was higher in the salt-tolerant genotype MIR. The increase in the amount of proline and sugars in plants is considered to be the result of osmotic regulation. The result of the present study was in agreement with previous studies [31,50]. Similarly, Yassin et al. [31] reported that under different levels of salinity (50, 100, 150 mM NaCl) the sensitive genotype "Sakha 95" exhibited a more negative potential compared with tolerant "Misr 2". We also found that the salinity-tolerant genotype MIR displayed a higher osmotic adjustment than the sensitive genotype FAT under salinity stress conditions.

Chlorophyll fluorescence was measured to determine plant performance under control and salinity conditions. The value of the Fv/Fm parameter is in the range of about 0.80–0.83 in plants grown under normal conditions [51]. When the Fv/Fm ratio is lower than the indicated value, the plant is thought to be stressed. Therefore, Fv/Fm is considered one of the important indicators in the selection of plants for salt tolerance [52]. The results of our research show that photo-protection of the MIR genotype was stronger than in FAT. These results are consistent with those presented by Sun et al. [53], Ibrahim et al. [54], and Kalaji et al. [23].

The formation of increased plant tolerance in response to salinity is associated with a wide range of protective and adaptive mechanisms, including changes in the expression of

a large number of genes and proteins involved in various metabolic processes. The HKT transporter gene families play the most important role in the development of salt tolerance and regulation of the ionic status in the cells of the root system and shoots. Eliminating sodium ions from cells is one of the strategies used by many plants, including wheat. Ability to survive and salt tolerance of the plants depends on the efficiency of HKT proteins. *TaHKT1;5* was found to be a critical determinant of salt tolerance in wheat. *TaHKT1;5-D* plays a significant role in salt tolerance via Na^+ exclusion from leaves and controlling xylem loading in roots [54]. In the current study, these two contrasting genotypes were further characterized for root and shoot Na^+ and K^+ concentrations as well as for the expression of gene *TaHKT1;5* related to ion homeostasis under 0 mM (control) and 150 mM NaCl treatments for 10 days.

The absorption of large amounts of salt by plant cells in saline environments is known to change the ionic balance. The change in the membrane transport of ions under stress is one of the most important reactions plant cells use to maintain and restore homeostasis. In the salt-tolerant genotypes, the entry of Na^+ ions into the roots and removal of their excessive amounts from the photosynthetic tissues are controlled by exclusion mechanisms (regulation of HKT gene expression) [55]. Our results show that in contrast with the salt-sensitive genotype, the access of Na^+ to roots of the salt-tolerant genotype is limited and their efflux occurs to ensure a tolerable level in the leaves. The accumulation of Na^+ ions in plant cells is undoubtedly important, but it is not the only indicator of their sensitivity/tolerance to the effects of salt stress. It is known that the existing systems of active transport under normal conditions maintain constant osmotic pressure in the cell and the optimal concentration of K^+ and pump out Na^+. The toxic effect of salinity is directly related to the fact that Na^+ replaces K^+ in the cytosol. The ability of plant cells to maintain a stable level of cytosolic K^+ in an environment with a high Na^+ concentration is a key factor determining salt tolerance since potassium is an activator of many plant cell enzymes [4].

In addition to the increase in the content of sodium ions, a tendency to a decrease in the level of potassium ions in the leaves and roots of the studied genotypes was revealed (Figure 4A,B and Table A2). Moreover, the K^+ content was higher in the leaves and roots of the tolerant genotype MIR compared with the sensitive genotype under stress conditions. This means that, unlike the FAT genotype, the MIR genotype had a pronounced ability to maintain a significant concentration of K^+ ions under salt stress, thereby maintaining the excess of K^+ over Na^+, which obviously contributed to the maintenance of intracellular ion homeostasis in this genotype under salt stress. In the MIR genotype, due to the more efficient selective transport of K^+ from roots to leaves, the K^+/Na^+ ratio was higher in the leaves compared with the FAT genotype (Figure 4C and Table A2).

In the current study, the expression of *HKT1;5* was observed in the roots of genotypes rather than in their leaves. Failure to detect the *HKT1;5* gene transcript in the leaves can be attributed to complete silencing of this gene in the leaves or very low, undetectable levels of expression. These results are in line with previous reports confirming that the *TaHKT1;5-D* gene expression occurs in the root rather than in the shoot [18,56]. Microchip-based expression analysis of the *HKT1;5* gene performed under 22 different conditions showed its activation in the roots and weakening in the leaves of rice varieties with contrasting tolerance. There was a significant difference in the level of expression of this gene in the root tissues of wheat genotypes. The expression of *TaHKT1;5* increased in the salt-sensitive FAT genotype under salt stress.

This effect may lead to an increase in the Na^+ concentration in plant cells, especially in the xylem. At the cellular level, one of the most dangerous consequences of salt stress is the intake of Na^+ and a decrease in K^+ levels. Most likely, this effect along with the increased expression activity of *TaHKT1;5* in the roots is connected to the sensitivity of the FAT genotype to salt stress. In the case of MIR, expression level of *TaHKT1;5* decreased under salt stress, possibly leading to restriction of Na^+ entry. An excess of Na^+ is known to negatively affect the physiological processes in plants; consequently, decreasing the

expression of genes that control the synthesis of transport proteins can be one of the mechanisms preventing the entry of excess amounts of ions into the cell. It should be noted that the detected expression profile is similar to the expression profile of the *TaHKT1;4* gene in contrasting Indian wheat genotypes [57,58].

The results obtained indicate that the salt tolerance of wheat depends both on its ability to prevent Na^+ from entering the cells of the root and above-ground parts, and on the levels of regulation of the *TaHKT1;5* gene expression in roots under salt stress.

5. Conclusions

The tolerance of plants to salinity is a complex physiological mechanism. Along with the regulation of gene expression and metabolism, regulation of ion transport in cell membranes is necessary for maintaining the optimal ionic composition of the cytosol and the water potential of plant cells under salt stress. The studies revealed a pronounced genotypic specificity of the response to salinization in seedlings of the *Triticum aestivum* L. genotypes. It was found that under salinity conditions, the salt-tolerant genotype was characterized by a low MDA level, a low Na^+ content in the above-ground parts and roots, high activity of antioxidant enzymes such as APX and CAT, high amounts of proline and soluble sugars, high water potential, and constant Fv/Fm values. It was also shown that, in response to salt stress, the *TaHKT1;5* gene activity changed differently in the roots of two genotypes with contrasting tolerance. Summarizing the above, we can conclude that the greatest difference between the studied bread wheat varieties was manifested in the greater ability of the salt-tolerant MIR variety to constrain the input of Na^+ into photosynthetic tissues as a result of the down-regulation of the *TaHKT1;5* gene expression in the roots and that it would be useful to develop a salinity-tolerant wheat variety in future breeding programs.

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Appendix A

Table A1. APX: CAT, osmotic potential, Fv/Fm, and MDA of wheat varieties under both control and salt stress condition.

Treatments	APX (µmol/mg Protein)				CAT (µmol/mg Protein)				Osmotic Potential			
	Control	Salinity	Mean	CV (%)	Control	Salinity	Mean	CV (%)	Control	Salinity	Mean	CV (%)
MIR	1.03a	2.22a	1.63A	7.18	0.63a	1.10a	0.87	19.25	−0.58	−3.68	−2.13	−36.27
FAT	0.99a	1.63b	1.31B		0.61a	0.83b	0.72		−1.57	−5.67	−3.62	
Mean	1.01B	1.93A			0.62B	0.97A			−1.08A	−4.67B		
CV (%)	5.47				6.77				−31.11			
	Fv/Fm				MDA							
MIR	0.82	0.81	0.81	0.54	0.0005a	0.0008b	0.00065A	16.09				
FAT	0.81	0.79	0.80		0.0006a	0.0015a	0.00105B					
Mean	0.81A	0.80B			0.00055A	0.00115B						
CV (%)	0.54				17.16							

Within each trait, means followed by the same lowercase letter(s) in a column or in a row followed by the same capital letter(s) indicate significant differences ($p \leq 0.05$) according to DMRT.

Table A2. K, Na, and K/Na concentration in leaves and roots of both wheat varieties under both control and salt stress condition.

Treatments	Leaves											
	K^+				Na^+				K^+/Na^+			
	Control	Salinity	Mean	CV (%)	Control	Salinity	Mean	CV (%)	Control	Salinity	Mean	CV (%)
MIR	48.7	43.4	46.05A	5.40	4.6a	22.3b	13.45B	7.41	10.63	1.93	6.28	11.70
FAT	43.8	34.3	39.05B		4.4a	29.8a	17.10A		9.93	1.10	5.52	
Mean	46.25A	38.85B			4.5A	26.05B			10.28A	1.52B		
CV (%)	7.08				5.52				12.72			
	Roots											
MIR	37.57	32.60	35.09	11.06	7.90a	28.80b	18.35B	4.08	4.73	1.13	2.93	6.82
FAT	36.80	28.80	32.8		7.40a	35.30a	21.35A		4.93	0.80	2.88	
Mean	37.18A	30.70B			7.65A	32.05			4.83A	0.97B		
CV (%)	8.31				5.97				9.50			

Within each trait, means followed by the same lowercase letter(s) in a column or in a row followed by the same capital letter(s) indicate significant differences ($p \leq 0.05$) according to DMRT.

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