Comparison of expression patterns of salt tolerance-related gene *HKT1;4* in bread wheat genotypes

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Received: October 30, 2023; Reviewed: November 29, 2023; Accepted: December 08, 2023

The *HKT* gene family, which encodes K^+/Na^+ transport proteins of cell membranes, plays a unique role in controlling plant responses to salt stress. The transcriptional activity of the *HKT1;4* gene was assessed in root and leaf tissues of bread wheat genotypes (tolerant Murov 2 and sensitive Aran) that differed in salt tolerance. The *HKT1;4* transcript was not found in plant leaves. A genotype-specific change in the root-specific expression of the *HKT1;4* gene was observed in response to salt stress: expression of a given gene was weakened in the salt-tolerant genotype and increased in the sensitive genotype. The data could be used to improve the salt tolerance of the wheat plant.

Keywords: *Wheat, salinity tolerance, TaHKT1;4 gene*

INTRODUCTION

Saline soils are one of the most important abiotic stressors affecting agricultural crop productivity worldwide. Salinity threatens about 6% of the world's total land area, including 20% of cultivated land and 33% of irrigated land (Kuang et al., 2019; Safdar et al., 2019). Bread wheat (*Triticum aestivum* L.) is one of the most cultivated and consumed crops worldwide. Wheat is only moderately salt tolerant. Salinity reduces grain yield by more than 60%, posing a great challenge to food security (Khan 2017). One of the methods for reducing the negative effects of salinity on crop production is to increase plant tolerance to salt stress. A comprehensive understanding of salt tolerance mechanisms and the selection of reliable screening indices are crucial for breeding salt-tolerant wheat varieties. Traditional breeding programs had little success in improving plant salt tolerance due to the genetic and physiological complexity of the traits. Although various promising morphological and physiological marker strategies for determining stress-tolerant genotypes have been developed, the molecular marker strategy is currently one of the fastest detection methods. Many tolerant wheat genotypes have been identified thanks to molecular marker technology and modern genomic research methods.

According to classical ideas, the negative effect of salinity on plants is mainly related to the violation of the ionic and osmotic balance of the cell. Excessive Na^+ ion accumulation in plant cells is particularly dangerous to their metabolic and structural-functional systems. For most glycophytes, which include cultivated plants, tolerance to salinity depends on their ability to prevent the accumulation of sodium ions in the plant (Assaha et al., 2017). Therefore, identification of the genetic basis and regulation mechanisms of the Na^+ ion transport is of great interest for the development of targeted strategies to improve plant salt tolerance.

The plant tolerance to soil salinity is provided by different mechanisms, both at the level of individual cells and at the level of the whole plant. One of such mechanisms is preventing accumulation in the stem by limiting the transport of Na^+ ions from the roots to the stem, especially leaves, with the participation of cell membrane transport proteins, re-transporting Na^+ ions from the stem to the roots and storing them in the vacuoles of the stem or leaf cells (Almeida, 2017).

The HKT (high-affinity potassium transporters) family of transport proteins has a special place among the ion transport systems that prevent the toxic accumulation of Na^+ ions in the cytoplasm of plant cells under salt-stress conditions. The activity of HKT transporters under salt stress conditions has been characterized in detail in plant species such as Arabidopsis, wheat, barley, rice, sorghum, and tomato, as well as in *Eutrema parvula* and *Eutrema salsuginea* extremophiles. Disruption of the expression of HKT family genes leads to hypersensitivity to Na^+ ions, resulting in excessive accumulation of Na^+ ions in stems and leaves.

 https://doi.org/10.62088/timbb/7.2.4

Improving the salinity tolerance of wheat is one of the main goals of breeding programs. A major genetic mechanism that plays an important role in salinity tolerance of most cereals, including rice (*Oryza sativa*) and wheat (*Triticum aestivum* and *Triticum monococcum*), is foliar Na^+ removal. The HKT protein family was reported to play a major role in this trait (Campbell et al. 2017; Xu et al. 2018). Several HKT genes, including *HKT1;1*-like, *HKT1;3*-like, *HKT1;4*-like, and *HKT1;5*-like have been identified and mapped to wheat homoeologous chromosome groups 2, 6, 2, and 4, respectively (Huang et al. 2008). Studying the involvement of stress-sensitive genes in various metabolic processes caused by high sodium chloride concentrations may be critical for increasing plant tolerance to abiotic stress.

The goal of this study was to compare the expression of the *HKT1;4* gene, which is involved in wheat salt tolerance under conditions of excessive NaCl, in two Azerbaijani wheat genotypes with contrasting salt tolerance.

MATERIALS AND METHODS

Plant material, germination assay, and stress conditions: The objects of study were two genotypes of bread wheat, Murov 2 and Aran. Sensitivity to chloride salinity was assessed using the roll culture method. In the experiments, 30 seeds were taken, in 3 replicates. After sterilization, wheat seeds were laid out on 20 cm strips of filter paper. The rolls were placed in beakers with 150 ml of water (control) or 150 mM NaCl solution. Cultivation was carried out in a growth chamber for 7 days at 22 ± 2°C with 16-h light and 8-h dark photoperiod. After completion of the experiment, biometric indicators, linear dimensions, and weight of wheat seedlings were measured. Each variant was represented by three biological replicates of 20–25 seedlings. For gene expression experiments, root and shoot (200 mg) samples from salt-treated and control plants were quickly frozen in liquid nitrogen and stored at -80ºC until further use.

RNA isolation and cDNA synthesis: Total RNA was isolated from root and shoot tissue samples (200 mg) of wheat genotypes under control and salt treatment (150 mM NaCl) conditions using the Monarch total RNA miniprep kit (BioLabs) as directed by the manufacturer. By treating RNA with Monarch DNase I (BioLabs), the remaining genomic DNA was removed. The quality of the isolated RNA samples was evaluated using 1% agarose gel electrophoresis (Fig. 1), and the concentration of total RNA was determined using Nanodrop2000c (Thermo Scientific, USA).

RNA isolation and cDNA synthesis: in batches. Total RNA from the root and shoot tissues of tolerant and sensitive wheat genotypes under control and salt treatment (150 mM NaCl) conditions was isolated with Monarch total RNA miniprep kit (BioLabs) following the manufacturer's instructions. The remaining genomic DNA was removed by treating RNA with Monarch DNase I (BioLabs). The quality of the isolated RNA samples was tested by electrophoresis on 1% agarose gels (Fig.1) and the concentration of total RNA was determined using Nanodrop2000c (Thermo Scientific, United States).

Only RNA samples with a 260/280 ratio of 1.9-2.2 and a 260/230 ratio greater than 2.0 were used for cDNA synthesis. The first strand cDNA was synthesized from 0.5 μg of total RNA, using the LunaScript® RT SuperMix Kit (E3010), following the manufacturer's protocol. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed on first-strand cDNA generated from total RNA, including salt-treated and untreated samples from either the Aran or Murov-2 genotypes. To examine the expression of *HKT1;4*, semi-quantitative RT-PCR was carried out using the gene-specific primers: (Forward: 5′-ATTCAGGCAACACCTAATCATGC-3′, Reverse: 5′-GCATCACAAGAATGAGGATGAGC -3′). The constitutively expressed actin gene (UniGene cluster Ta54825; forward primer: 5'-TGGGATGCCACCAAAGAC-3'; reverse primer: 5'-TGATACGCAAATGTTGAGC-3') was used as a reference gene in the semi-quantitative RT-PCR analyses. The RT-PCR experiments were performed at least three times.

RESULTS AND DISCUSSION

Biomass: Murov 2 and Aran bread wheat genotypes were used in this study to determine the relationship between the *TaHKT1;4* gene, which encodes the Na^+ transporter, and salt tolerance. The initial assessment of salt tolerance of wheat varieties was carried out according to the degree of inhibition of growth by sodium chloride. Under laboratory conditions, the salt tolerance of plants was evaluated by the roll method. One of the most sensitive processes that reflect the physiological state of stressed plants is their growth. Weakening of cell growth and division in an unfavorable environment slows down the development of the plant as a whole, which can be considered one of the main defense mechanisms.

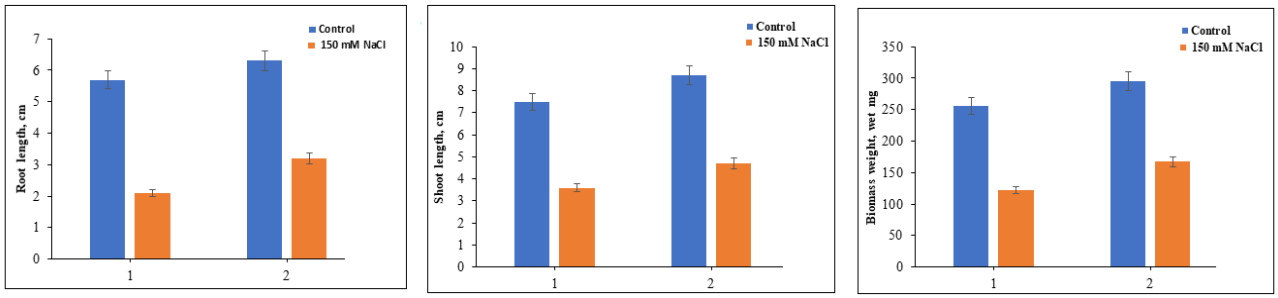

Fig. 1. Biometric parameters of wheat seedlings grown in normal and 150 mM NaCl solutions; 1. Aran, 2. Murov 2.

Observations show that tolerant forms adapt to the effects of stress faster and restore the growth process. As seen in Fig.1, the length of both the stem and the roots depends on the environment in which the plants grow, and under the influence of salinity, these indicators decrease sharply. It should be noted that the degree of retardation of growth processes of plant organs is also determined by the genotypic characteristics of wheat.

Thus, the length of Aran genotype seedlings exposed to 150 mM NaCl for 10 days decreased by 52%, while this indicator was 46% for Murov 2 seedlings. Salts are known to inhibit the growth of roots more strongly than above-ground organs, because roots, unlike above-ground organs, are constantly in contact with saline soil. Salts damage the zone of cell elongation and the zones of salt absorption and water penetration. In our research, we observed a similar picture. Salt stress reduced the roots of the Aran and Murov 2 genotypes by 63% and 49%, respectively. According to these results, there was also a decrease in the biomass of wheat genotypes, which indicates that salt stress suppresses the general metabolism of plant tissues. The negative effect of salt stress on plant biomass was less noticeable in Murov 2 than in Aran (Fig. 1). Based on the comparison of morphometric parameters of wheat genotypes exposed to salt stress, it can be concluded that the Murov-2 genotype is more tolerant to salinity than the Aran genotype at the early stage of development.

Expression analysis of *HKT1;4*. Molecular-genetic assessment of the expression level of genes controlling the synthesis of proteins responsible for plant stress tolerance is considered a priority research area. In the presented work, the activity of the *HKT1;4* gene in salt stress responses of Murov-2 and Aran genotypes was studied. After the wheat genotypes chosen for the study were grown in the medium containing high NaCl concentration (150 mM) for 10 days, the expression activity of the *HKT1;4* gene in leaf and root tissues was assessed by semi-quantitative expression analysis (RT-PCR) at the level of the total amount of individual mRNAs. To carry out gene expression experiments, cDNA preparations synthesized on the basis of total RNA extracted from root and leaf tissues of control and stress variants of plants were used for PCR. RT-PCR analysis using the *HKT 1;4* gene-specific primer did not detect the *HKT1;4* transcript in leaves of control and stress variant plants. This may be due to the complete silencing of the given gene in leaves or a very low expression level that cannot be detected by RT-PCR. Microchip-based expression analysis of rice varieties with contrasting tolerance under different conditions showed activation of the *HKT1;4* gene in roots and attenuation in leaves (Shohan et al., 2019). A different picture was observed in the relative expression level of the *HKT 1;4* gene in root tissues of wheat genotypes grown in the presence of high concentrations of salt. As seen in Fig. 2, salt stress caused an increase in the *HKT1;4* transcript in the roots of the Aran genotype, and a decrease in the Murov 2 genotype. The change in the *HKT1;4* gene expression level indicates the development of stress protection mechanisms in plants.

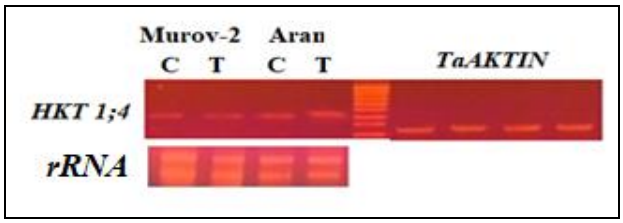

Fig. 2. Semi-quantitative expression analysis of the *HKT1;4* gene in roots of salt-tolerant Murov 2 and salt-sensitive Aran genotypes under control (C) and salt stress (T) conditions. M, Marker, 100bp DNA ladder.

According to a review of the literature, all studies on the role of ion transporters focused on the short-term effects of NaCl on plants. Under salt stress, the function of class I HKT (HKT1) transporters in roots is to remove Na^+ ions from the root xylem. Due to their work, the flow of Na^+ ions to the above-ground tissues of plants is prevented, reducing cell damage from the toxic effect of Na^+ ions. As a result, the high expression level of HKT1 class genes leads to the reduction of harmful ions in cells. "Silencing" the *TaHKT1;4* gene in transgenic bread wheat lines caused an increase in the amount

of Na^+ ions in the leaves (Byrt et al., 2014). Overexpression of the *AtHKT1* gene in the roots (root stele) of transgenic arabidopsis plants led to their salt tolerance, but high expression in the whole plant resulted in plant hypersensitivity (Møller et al., 2009). These facts indicate that the *TaHKT1;4* transporter plays an important role in limiting the Na^+ transport from roots to leaves. RNAi-mediated knockdown of *OsHKT1;4* expression in roots of a Japanese rice variety did not impair Na^+ and K^+ accumulation in any plant tissue during the vegetative growth stage (Campbell et al., 2017). However, a recent study showed the involvement of *OsHKT1;4* in transgenic rice lines in the release of Na^+ from the xylem in roots under the influence of different concentrations of Na^+ ions (Khan et al., 2020). However, we observed an inverse relationship between the salt tolerance of plants and the HKT1;4 gene expression level under long-term stress conditions. The expression of *HKT1;4* increased in the roots of the Murov 2 genotype and strong morphological changes occurred in the plant. The increase in the amount of the *HKT1;4* transcript in the salt-sensitive genotype compared to the salt-tolerant genotype may physiologically lead to more Na^+ flow into the cytoplasm of plant tissues, and the plant salt sensitivity may be related to this effect. On the other hand, the activation of the *HKT1;4* gene can also induce other Na^+ transporters, which can result in an increase in the amount of Na^+ in plant cells, especially in the xylem. It is known that the excess amount of Na^+ ions in cells has a negative effect on physiological processes in plants. Weakening of the expression of genes that control the synthesis of transport proteins can be one of the mechanisms that prevent the entry of excessive amounts of ions into plant cells.

Similar results were obtained in the regulation of *HKT1;4* gene expression during long-term exposure to salt stress in contrasting wheat genotypes of India. Comparison of nucleotide sequences of *HKT1;4* genes of salt-tolerant and sensitive wheat genotypes showed 2 deletions, 6 transitions, and 12 inversions in *HKT1;4* in salt-sensitive wheat. Point mutations resulting in the loss of the "-Arg motif" and the "selectivity filter" can cause structural/functional changes observed in *TaHKT1;4*. Ion channels generally bind to the membrane through a highly conserved Gly-Arg motif. Considering the importance of these motifs, it is assumed that due to their absence, *TaHKT1;4* cannot perform its function efficiently in the salt-sensitive genotype (Kumar et al., 2017).

Thus, elucidating the tissue-specific expression of HKTs and their control mechanisms is important for improving wheat salt stress tolerance.

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Yumşaq buğda genotiplərində duzadavamlılıqla əlaqəli *HKT1;4* geninin ekspressiya nümunələrinin müqayisəsi

Zərifə Süleymanova, Ələmdar Məmmədov

Azərbaycan Respublikası Elm və Təhsil Nazirliyi Molekulyar Biologiya və Biotexnologiyalar İnstitutunun Genomun quruluşu və ekspressiyası laboratoriyası, Bakı, Azərbaycan

Bitkilərin duz stresinə qarşı reaksiyalarının idarə edilməsində iştirak edən genlər arasında hüceyrə membranlarının K^+/Na^+ daşıyıcı zülallarını kodlaşdıran HKT gen ailəsinin xüsusi rolu vardır. Verilmiş tədqiqatda duzadavamlılığına görə fərqlənən yumşaq buğda genotiplərinin (davamlı Murov-2 and həssa Aran) kök və yarpaq toxumalarında *HKT1;4* genin ekspressiya aktivliyi transkripsiya səviyyəsində qiymətləndirilmişdir. Bitkilərin yarpaqlarında *HKT1;4* transkripti aşkar olunmamışdır. Duz stresinə cavab olaraq, *HKT1;4* genin kök-spesifik ekspressiyasında genotip-spesifik dəyişiklik müşahidə olunmuşdur: Duzadavamlı genotipdə verilmiş genin ekspressiyası zəifləmiş, həssas genotpdə artmışdır. Əldə olunan nəticələr buğdanın duzadavamlılığının yüksəldilməsi üçün faydalı ola bilər.

Açar sözlər: *Buğda, duzadavamlılıq, TaHKT1;4 geni*

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