The *HKT2;3* gene expression analysis in bread wheat genotypes under salt stress imposition

Suleymanova Zarifa Jahandar

Laboratory of Genome Structure and Expression, Institute of Molecular Biology & Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan, 11 Izzat Nabiyev Str., Baku AZ1073, Azerbaijan

For correspondence: jzarifa@yahoo.com

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The mechanisms of adaptation at the cellular level ensure reducing the Na^+ concentration in the cytoplasm of cells of photosynthetic tissues. The high-affinity potassium transporter (HKT) genes play an important role in the response to salt stress. In a controlled environment, the effect of a high concentration of NaCl (150 mM) on the accumulation of Na^+ and the expression of the *HKT2;3* gene in the roots and leaves of two *T.aestivum* L. genotypes with contrasting salt tolerance was studied. The comparative analysis of Na^+ transport in the two genotypes revealed that the salt-tolerant wheat genotype is able to limit the flow of sodium ions from the roots to the shoots. Semi-quantitative RT-PCR experiments showed a differential expression pattern of *HKT2;3* gene in the root and leaf tissues of the two wheat genotypes. A negative correlation was established between the salt tolerance of the genotype and an increase in the expression level of the *HKT2;3* gene. The results obtained suggest that the salt tolerance of bread wheat depends both on its ability to control Na^+ influx into the root and excess Na^+ out flow from photosynthetic tissues, and on the regulation of HKT gene expression in plant roots and leaves under salt stress.

Keywords: *Wheat, HKT2;3 gene, Na^+ transport, salt stress*

INTRODUCTION

Currently, one of the main ecological problems of irrigated lands is salinization. Increased content of NaCl in the soil, which is not involved in plant metabolism, leads to the inhibition of almost all vital functions and is considered a negative factor. Since each soil contains sodium ions in a certain amount, the effect of the salt factor can be considered a long-term effect leading to global restructuring of the metabolic and structural-functional systems of plants. Salinization of any type is a source of excess hydrated Na^+ ions. The main reason causing inhibition of plant growth under salt stress (except a decrease in water potential) is an excess of Na^+ ions, which are not required by glycophytes for normal growth (Assaha et al., 2017). Because physicochemical properties (ionic radius and hydration energy) of Na^+ and K^+ ions are similar, Na^+ competes with K^+ to enter the plant cell through the membrane transport system under high salt concentration. Since K^+ plays an important role in many enzymatic functions, a high Na^+/K^+ ratio slows down many enzymes (Kader et al., 2006). This, in turn, results in the disruption of cellular processes in roots and leaves. Several important plasma membrane Na^+-transporters have been identified in plants, which reduce high Na^+ concentration, among which HKT (High-Affinity K^+ Transporters) transporters play an extremely important role. The plant HKTs are divided into two distinct classes based on their transport characteristics (Almeida et al., 2017). Most members of class I transporters (HKT1;x) have been implicated in controlling Na^+ accumulation in the shoot as Na^+selective exclusion transporters. They have been shown present in all higher plant species and to play crucial roles in salinity tolerance in different plant species (Arzani and Ashraf, 2016). The class II transporters of HKT (HKT2; y) mostly function as K^+-Na^+ symporters and so far have been only identified in cereal monocots (Asins et al., 2013; Suzuki et al., 2016). All members of identified HKT2 are shown to be involved in mediating Na^+ influx in root tissues under K^+ starvation conditions. Much less information is available for transporters from HKT subfamily 2 and their possible involvement in plant adaptation to saline conditions.

Wheat is the most demanded food plant on the globe. Every year, a significant part of the wheat crop is lost as a result of exposure to various abiotic stresses such as salinity, drought, and cold. Understanding the molecular basis of mechanisms

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of tolerance to salt stress in wheat today becomes mandatory for the screening of local wheat genotypes and the creation of elite lines with increased tolerance to salt stress. In wheat, the exclusion of sodium ions from cells with the participation of transport proteins of cell membranes is an important salt tolerance strategy. Evidence has been obtained that members of the HKT family should be involved in the exclusion of Na^+ from leaves in crops. For instance, the key role that *HKT1;5* (previously *HKT8*) plays in salt tolerance through sequestering Na^+ to the non-photosynthetic reservoir, leaf sheaths and roots has been reported in wheat (Byrt et al., 2007).

The main contributing factor to salt stress is Na^+, and ion transporters, in particular Na^+ transporters, are considered important candidate genes controlling traits that promote salt tolerance in crops (Fan et al., 2016; Hazzouri et al., 2018). In this study, a comparative analysis of the expression of the *HKT2;3* gene involved in the regulation of Na^+ transport from the roots to shoots was carried out under salt stress in the two contrasting wheat genotypes, Mirbashir-128 and Fatima. Responses to salt stress of these genotypes were evaluated based on multivariable biochemical and physiological analyses (Ibrahimova et al., 2021).

MATERIALS AND METHODS

Plant material, germination assay, and stress conditions: The objects of the present study were two Azerbaijani local wheat (*Triticum aestivum* L.) genotypes, Mirbashir 128 (salt-tolerant) and Fatima (salt-sensitive). Seeds were surface sterilized with 10% NaClO 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, seedlings were transferred into the half-strength Hoagland solution containing 150 mM NaCl for 14 days. Leaf and root samples from treated and control plants were quick-frozen in liquid nitrogen and stored at -80ºC until further use.

Measurement of sodium ions: The content of Na^+ was determined using a PFP7 (Jenway-2007, England) flame photometer. First, plant samples were combusted according to the method (Allen et al., 1986). A mixture of sulphuric and perchloric acids (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 ions. Standard solutions of NaCl salts were used to construct the calibration curve.

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 7 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 RNA from root and shoot tissues of tolerant and sensitive wheat genotypes under control and salt treatment (150 mM NaCl) 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 a Nanodrop2000c (Thermo Scientific, United States).

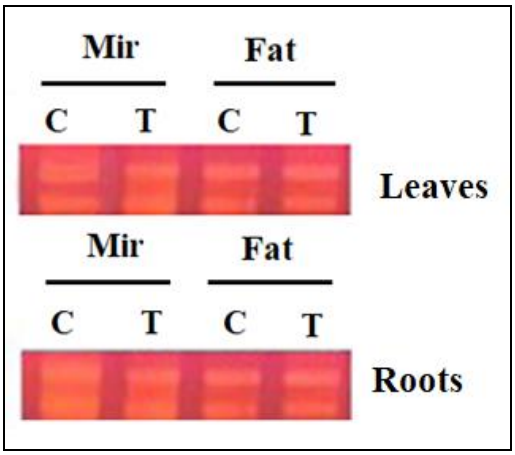

Fig. 1. Total RNA from leaf and root tissues of salt-tolerant Mirbashir 128 (Mir) and salt-sensitive Fatima (Fat) genotypes under control (C) and salt (150 mM NaCl) stress (T) conditions.

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 from 0.5 µg of total RNA, using the LunaScript® RT SuperMix Kit (E3010), according to the manufacturer's protocol. The first-strand cDNA generated from total RNA including salt-treated and untreated samples from either the Mirbashir-128 or Fatima genotype was subjected to semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis. To examine the expression of *HKT2;3*, semi-quantitative RT-PCR was carried out using the gene-specific primers: Forward: 5′-TCTTAGTTCGGCAAGGCATATCA -3′; Reverse: 5′-TGCACGGTAACCGATGTAACTCT-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′; Reverse: 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 30s, for a total of 40 cycles. Reactions were carried out under the following conditions: the first cycle for 4 minutes at 94°C, followed by 35 cycles at 94°C for 45s, 58°C for 45s, 72°C for 45s, 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.

RESULTS AND DISCUSSION

One of the vital mechanisms of plant salt tolerance is the ability to maintain a low concentration of Na^+ ions in the cytoplasm under stress, wherein the plasma membrane is the most likely site for selective regulation of ion transport (Kader et al., 2006). Therefore, the accumulation of Na^+ by the roots and leaves of seedlings of two wheat genotypes was analyzed. According to the data obtained (Fig. 2), under conditions of chloride salinity, plants accumulate significantly higher concentrations of Na^+ ions in the roots than in the leaves.

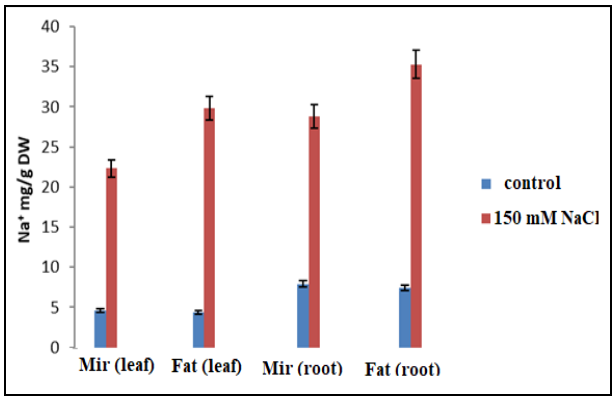

Fig. 2. Effect of 150 mM NaCl on Na^+ content in roots and leaves of salt-tolerant Mirbashir 128 (Mir) and salt-sensitive Fatima (Fat) genotypes.

The concentration of Na^+ in plant roots in both genotypes increased under the action of a high concentration of NaCl, however, the accumulation of Na^+ was higher in the Fatima genotype than in the Mirbashir 128 genotype. The greatest difference between the genotypes was manifested in the accumulation of Na^+ in the aboveground part of the seedlings. The accumulation of Na^+ in the leaves of the genotypes is almost the same under control conditions. However, Na^+ accumulation in leaves of the Fatima genotype increased 2-fold compared to the Mirbashir- 128 genotype under salt stress. In seedlings of the Mirbashir-128 genotype, approximately 44% of the sodium accumulated in the root passed into the shoots, while in Fatima, it amounted to approximately 60%. A comparison of the values of Na^+ accumulation in the roots and shoots shows that the lower accumulation of Na^+ in the aboveground part of the seedlings of the Mirbashir-128 genotype is probably due to its ability to limit the flow of Na^+ into the xylem. The data obtained in recent years indicate that the salt tolerance of cereal crops depends both on their ability to limit the flow of Na^+ ions from the roots to shoots, and on the regulation of the function of HKT transporters, which are localized on the plasma membrane of root cells surrounding xylem vessels. Under salt stress conditions, the role of the class I *HKT* transporter in the roots is to remove Na^+ ions from the xylem in the roots in order to reduce their content in the shoots. According to the literature data, Na^+ ions enter the root through nonselective ion channels and the HKT1 symporter. Na^+ from the shoots can move back along the phloem to the roots through the HKT1 symporter and from it to the environment (Hauser and Horie, 2010). Violation of the expression of HKT genes leads to hypersensitivity to Na^+ ions and excessive accumulation of sodium in the shoots.

Based on the above, a comparative study of the effect of high NaCl concentration on the expression level of the *HKT2;3* gene in the roots and leaves of the studied wheat genotypes was carried out. This gene encodes a transporter involved in Na^+ homeostasis by extruding Na^+ from root epidermal cells at the root-soil interface. Using the RT-PCR method, the expression activity of this gene was assessed at the level of the total content of individual mRNAs after 14 days of growing plants in media with a high NaCl concentration. The activity of these genes in plants grown in a standard nutrient medium was chosen as a control. Semi-quantitative RT-PCR analysis showed that the *HKT2;3* gene was differentially expressed in these two wheat genotypes (Fig.2). The relative expression level of the *HKT 2;3* gene in plants of both wheat genotypes revealed a slight increase in the expression of this gene in roots compared to leaves when grown under control conditions. The level of gene expression in the leaves and roots of the salt-tolerant Mirbashir-128 genotype grown in the medium with high salt concentration was practically unchanged. A completely different picture is observed in the roots and leaves of the

salt-sensitive wheat genotype: the expression level of the *HKT2;3* gene in the leaves and roots of the Fatima genotype increases in the presence of NaCl. A possible physiological explanation is that increased expression of *HKT2;3* could lead to a higher influx of Na^+ into the cytosol of plant cells in the sensitive genotype, which makes it more salt sensitive. Under salinity stress conditions, the expression of the *HKT 2;3* gene in leaves and root tissues was lower in the salt-tolerant genotype, compared to the salt-sensitive genotype. Similarly, expression of *HKT2;1* and *HKT2;3* genes in roots and shoots were differentially regulated in contrasting bread wheat genotypes under salt stress (Kumar et al., 2017). A similar observation on differential expression of TaSOS1 associated with Na^+ flux roots to shoots has been reported in contrasting durum wheat varieties (Brini et al., 2009).

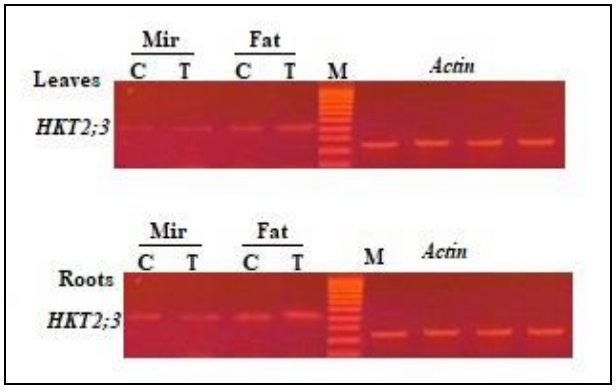

Fig. 3. Semi-quantitative expression analysis of *HKT2;3* gene in leaves and roots of salt-tolerant Mirbashir 128 (Mir) and salt-sensitive Fatima (Fat) genotypes under control (C) and salt (150 mM NaCl) stress (T) conditions.

A lower gene expression level in leaves and roots of the Mirbashir 128 genotype compared to the Fatima genotype under salt stress conditions is attributed to the activation of the Na^+ exclusion mechanisms. Sodium exclusion does not allow the accumulation of Na^+ to toxic levels within leaves, leading to high K^+/Na^+ discrimination, which has frequently been described as an important mechanism involved in salt tolerance in cereal crops such as bread wheat, durum wheat, barley, and rice (Arabbeigi et al., 2018). The exclusion of Na^+ can also be achieved due to the removal of Na^+ ions from the xylem in the roots, which can correlate with the lowest accumulation of Na^+ in the shoots of Mirbashir- 128 under stress conditions.

Summarizing the above, it can be concluded that the greatest difference between wheat genotypes (Mirbashir- 128 and Fatima) with contrasting salt tolerance manifested itself in the greater ability of the salt-tolerant genotype to limit the Na^+ flow into photosynthetic tissues, as well as in the different expression levels of the *HKT2;3* gene in the seedlings of these two genotypes.

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Duz stresinin təsiri altında buğda genotiplərində *HKT2;3* genin ekspressiya analizi

Süleymanova Zərifə Cahandar qızı

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

Hüceyrə səviyyəsində adaptasiya mexanizmləri fotosintetik toxumaların hüceyrələrinin sitoplazmasında Na^+ qatılığını azaltmaqdan ibarətdir. Yüksək affin kalium daşıyıcıları (HKT) ailəsinin nümayəndələrini kodlaşdıran genlər bitkilərin duz stresinə cavab reaksiyalarında əhəmiyyətli rol oynayırlar. Nəzarət olunan mühitdə yüksək qatılıqlı (150 mM) NaCl-un duza davamlılığına görə fərqlənən iki *T.aestivum* L genotiplərinin kök və yarpaqlarında Na^+ ionlarının toplanmasına və *HKT2;3* genin ekspressiyasına təsiri öyrənilmişdir. Iki genotipdə Na^+ nəqlinin müqayisəli təhlili göstərdi ki, duza dözümlü buğda genotipi natrium ionlarının köklərdən gövdəyə axınını məhdudlaşdırmağa qadirdir. RT-PCR təcrübələri ilə iki buğda genotipinin kök və yarpaq toxumalarında *HKT2;3* genin fərqli ekspressiyası aşkar edilmişdir. Əldə edilən nəticələr göstərmişdir ki, çörək buğdasının duza davamlı olması onun köklərə Na^+ axınına və fotosintetik toxumalardan Na^+ ionlarının artıq miqdarının xaricə axınına nəzarət etmək qabiliyyətindən, həm də duz stresinin təsiri altında bitkilərin kök və yarpaqlarında HKT genlərinin ekspressiyasının tənzimlənməsindən asılıdır.

Açar sözlər: *Buğda, HKT2;3 geni, Na^+ transport, duz stresi*