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Research article

# Growth, physiological adaptation, and gene expression analysis of two Egyptian rice cultivars under salt stress

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ABSTRACT

Abiotic stressors, such as high salinity, greatly affect plant growth. In an attempt to explore the mechanisms underlying salinity tolerance, physiological parameters of two local Egyptian rice (*Oryza sativa* L.) cultivars, Sakha 102 and Egyptian Yasmine, were examined under 50 mM NaCl stress for 14 days. The results indicate that Egyptian Yasmine is relatively salt tolerant compared to Sakha 102, and this was evident in its higher dry mass production, lower leaf $Na^+$ levels, and enhanced water conservation under salt stress conditions. Moreover, Egyptian Yasmine exhibited lower $Na^+/K^+$ ratios in all tissues examined under salinity stress. The ability to maintain such traits seemed to differ in the leaves and roots of Egyptian Yasmine, and the root $K^+$ content was much higher in Egyptian Yasmine than in Sakha 102. In order to understand the basis for these differences, we studied transcript levels of genes encoding $Na^+$ and $K^+$ transport proteins in different tissues. In response to salinity stress, Egyptian Yasmine showed induction of expression of some membrane transporter/channel genes that may contribute to $Na^+$ exclusion from the shoots (*OsHKT1;5*), limiting excess $Na^+$ entry into the roots (*OsLti6b*), $K^+$ uptake (*OsAKT1*), and reduced expression of a $Na^+$ transporter gene (*OsHKT2;1*). Therefore, the active regulation of genes related to $Na^+$ transport at the transcription level may be involved in salt tolerance mechanisms of Egyptian Yasmine, and these mechanisms offer the promise of improved salinity stress tolerance in local Egyptian rice genotypes.



## 1. Introduction

Soil salinity is a major environmental problem that has deleterious effects on world agriculture, especially in irrigated lands (Flowers, 1999). Salinity stress causes serious damage to many cellular and physiological processes including photosynthesis, nutrient uptake, water absorption, root growth, and cellular metabolism, which all lead to yield reduction (Pardo, 2010). Furthermore, excess $Na^+$ causes an imbalance in cellular ion homeostasis, resulting in ion toxicity (Mandhania et al., 2006; Assaha et al., 2013). Cellular $Na^+$ and $K^+$ homeostasis play a fundamental role in the growth and development of higher plants. As one of the most important macronutrients in plants, $K^+$ is necessary for the maintenance of membrane potential and turgor pressure, activation of enzymes, regulation of osmotic pressure, stomatal movement, and tropisms (Golldack et al., 2003). In contrast to animal cells, $Na^+$ is not an essential element for plant cells; therefore, high $K^+/Na^+$ ratios maintain osmotic balance in plant cells. Genes from many functional classes including those encoding transcription factors, signal transduction, cell wall components, and membrane transporters were found to be differentially regulated in response to salt stress (Walia et al., 2007; Ueda et al., 2002, 2004, 2006).

The transmembrane movement of $Na^+$ and $K^+$ in plants is mediated by several types of transporters and/or channels (Yao et al., 2010), and a number of transporters have been implicated in leaf $Na^+$ exclusion. These include members of the high-affinity $K^+$ transporters (HKTs), including *Arabidopsis thaliana* HKT (AtHKT1;1) and its ortholog in rice (OsHKT1;5), which retrieves $Na^+$ from the xylem to the surrounding parenchyma cells (Ren et al., 2005; Horie et al., 2009). Plasma membrane protein 3 (PMP3) is a small hydrophobic peptide that plays a role in shoot $Na^+$ exclusion by preventing excess $Na^+$ entry into the plant roots (Nylander et al., 2001). In addition to $Na^+$ exclusion, plants may

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avoid toxic $Na^+$ accumulation in the cytosol by sequestering excess $Na^+$ into vacuoles, which is a process mediated by the $Na^+/H^+$ antiporter (NHX1) localized in vacuolar membranes (Venema et al., 2002). However, these transporters only function to counteract the activities of other transporters that are known to induce $Na^+$ influx into roots. This may occur through cyclic nucleotide-gated channels (CNGCs), which are considered the dominant pathways of $Na^+$ influx in many plants (Roberts and Tester, 1997). Some members of the HKT family are also thought to mediate a substantial $Na^+$ influx in some species, including rice (Golldack et al., 2003).

$K^+$ is the most abundant cation in plants, and it is an essential nutrient for plant growth and development. Under salinity stress conditions, plants suffer from $K^+$ deficiencies stemming from the competitive inhibition of its uptake by $Na^+$, and this often leads to high $Na^+/K^+$ ratios that disrupt cellular homeostasis (Tester and Davenport, 2003). However, potassium inward rectifying channels (KIRC) (e.g., AKT1) have high $K^+/Na^+$ selectivity and are known to activate $K^+$ influx upon plasma membrane hyperpolarization (Sentenac et al., 1992). The activity of the AKT1 channel is important in maintaining elevated $K^+/Na^+$ ratios in plants. Thus, the activities of the transporters/channels involved in $Na^+$ exclusion and $K^+$ uptake are important to the improvement of salt stress tolerance in plants.

Rice is an important cereal that is very sensitive to salt stress. Although the degree of sensitivity varies with cultivars (Munns and Tester, 2008), some cultivars are capable of growing in very high salt concentrations. Sakha 102 and Egyptian Yasmine, are important local rice cultivars in Egypt, where soil salinization is becoming increasingly challenging for agriculture. Whereas Sakha 102 has been established as a salt-sensitive cultivar (Darwish et al., 2009), the response of Egyptian Yasmine has not been clearly established. Therefore, the objective of this study was to elucidate differences in the mechanisms of salinity tolerance between Egyptian Yasmine and Sakha 102 by comparing the physiological parameters and expression profiles of the genes that encode $Na^+$ and $K^+$ transport proteins.

## 2. Materials and methods

### 2.1. Plant material, growth conditions, and salt treatment

*Oryza sativa* rice seeds of varying genotypes were obtained from The Field Crops Research Institute, Giza, Egypt. These genotypes were chosen from the germplasm collections based on their reputation of salt tolerance in terms of agronomic performance. The subset of the germplasm samples included the subspecies *O. sativa* ssp. *indica* and *O. sativa* ssp. *japonica*.

Rice seeds were surface sterilized via immersion in a 5% sodium hypochlorite solution for 30 min, and were then thoroughly rinsed with distilled water. Seeds were subsequently soaked in tap water for 24 h at 28 °C. After germination, the seeds were transferred to a nylon mesh floating on 20 L of tap water for two days. Water was then replaced with half-strength Kimura B solution. Twenty-eight-day old seedlings (4–5 leaf stage) were transferred to either Kimura B nutrient solution (control) or nutrient solution supplemented with 50 mM NaCl (salinity) for two weeks. The solutions were replaced every two days and the pH was adjusted to 5.0–5.5 each day. Seedlings were grown in a growth chamber under the following controlled environmental conditions: 70% relative humidity, 24 ± 2 °C, and a 16 h photoperiod at a photosynthetic photon flux density of 250–350 μmol $m^{-2}$ $s^{-1}$.

### 2.2. Measurement of fresh and dry weight, relative water content, and electrolyte leakage ratio

The fresh weight (FW) was measured following the separation of leaves, stems, and roots. For dry weight (DW) determination, leaves, stems, and roots were dried at 70 °C for three days prior to being weighed. To determine the relative water content (RWC), four plants from each treatment were randomly selected and the method described by Weatherly (1950) was implemented. Briefly, leaf samples were weighed to determine the FW. The leaf samples were then soaked in fresh deionized water for 24 h under light, and were placed on tissue paper to remove excess water. The samples were then weighed to determine the fully turgid weight (TW). Samples were next oven-dried at 70 °C for three days, and the DW was obtained. The RWC was determined using the following formula: $RWC = (FW - DW)/(TW - DW) \times 100$.

To determine the electrolyte leakage ratio (ELR), the third leaf from the top of each plant was dissected and soaked in a bottle containing 30 mL of deionized water, and the bottles were gently shaken overnight. Conductivity of the solution was measured with an EC meter (EC1). The bottles were then autoclaved and cooled, and the conductivity of the solution was again measured (EC2). The ELR was calculated as the ratio of the conductivity before autoclaving to the conductivity after autoclaving using the following formula: $ELR = (EC1/EC2) \times 100$.

### 2.3. Determination of $Na^+$ and $K^+$ content

The $Na^+$ and $K^+$ content in leaves, sheaths, and roots was measured using a flame photometer (ANA-135; Tokyo Photoelectric, Tokyo, Japan) according to the method of Kushizaki (1968). Dried samples were gently agitated in 1 N HCl overnight, and the content of $Na^+$ and $K^+$ ions was estimated from the $Na^+$ and $K^+$ standard curves.

### 2.4. Expression analysis of genes encoding $Na^+/K^+$ transport proteins

Total RNA was extracted from the leaves, sheaths, and roots of the control and stressed plants using TRIzol reagent (Invitrogen, Carlsbad, CA). After digestion with DNaseI, total RNA (1 μg) was reverse-transcribed to cDNA using a ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan). Quantitative polymerase chain reaction (qPCR) was conducted as previously described (Ueda et al., 2013), using a Thunderbird SYBR qPCR Mix (Toyobo) and an ABI Step One Plus system (Applied Biosystems). Quantitative RT-PCR was performed using the following profile: an initial incubation at 95 °C for 1 min, followed by 40 cycles of denaturation at 95 °C for 15 s and extension at 60 °C for 60 s. Relative expression level of the gene transcripts was calculated with the comparative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) using the *OsUBQ-5* gene as an internal

**Table 1**
Primers used for quantitative real-time RT-PCR.

| Genes | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|---|---|---|
| *OsHKT1;5* | CCCATCAACTACAGCGTCCT | AGCTGTACCCCGTGCTGA |
| *OsLti6a* | CCTTCCAAGGTGATGGTGAA | CCGTCCAAAGAACCAGAAAA |
| *OsLti6b* | GCTCCAAACCGCTTCATCTA | CAAGAATTGGAGCACTCAGGA |
| *OsHKT2;1* | TGCATTCATCACTGAGAGGAG | GGTGCAGTTTCTGCAACCTC |
| *OsNHX1* | AATGATCACCAGCACCATCA | AAGGCTCAGAGGTGACAGGA |
| *OsSOS1* | ATACTGAGTGGGGTTGTTATTGC | AAAGGTAAATTTCAAAAGGTACATGG |
| *OsAKT1* | GAAACGAGCAATGCGTCAG | CTTCTCACACAGCGCTTCC |
| *OsHAK7* | TGCTGTGACACTTGGTTTCC | AAATAACAAGGCGAGCAGGA |
| *OsCNGC1* | TGCAATAGCAAAGCGATACTTG | TTTTGGCTTTTGCAACCTCT |
| *OsUBQ5* | ACCACTTCGACCGCCACTACT | ACGCCTAAGCCTGCTGGTT |