Celal Bayar University Journal of Science

Investigation of Salinity Tolerance Related Gene Expression in Rice (*Oryza sativa* L.)

Atilla Salman[1], Sinan Meriç[1], Tamer Gümüş[1], Çimen Atak[1], Alp Ayan[1]*

[1]Department of Molecular Biology and Genetics, Istanbul Kültür University, Istanbul, Türkiye
* a.ayan@iku.edu.tr
*Orcid No: 0000-0003-3749-0472

Received: August 7, 2024
Accepted: November 27, 2024
DOI: 10.18466/cbayarfbe.1529138

Abstract

Rice ranks second with the highest consumption rate after corn in world production. As a result of various biotic and abiotic stress factors exposed during production, plants quit normal growth. Under such conditions, plants have developed survival mechanisms at the molecular level in order to maintain their existence. Phenotypic data is widely used to evaluate plant tolerance with assistance of gene expression analysis that interprets the source of tolerance. In this study, Osmancık-97 rice variety which is extensively cultivated in Türkiye was grown under four different salt (NaCl) concentrations (60, 90, 120 mM and control) in *in vivo* conditions. The study aimed to determine the expression differences of the TPS1, NHX1, SOS1 and HKT2;1 genes under increasing salinity conditions. In the highest applied NaCl concentration (120 mM), TPS1, NHX1, SOS1 and HKT2;1 gene expression decreased 78.2, 74.0, 78.3, and 73.5% compared to the control, respectively. In the same concentration, parameters of photosynthetic pigment content, average plant length, fresh and dry weight, and root length decreased significantly. In contrast, proline accumulation and TBARS content presented significant increases. The difference in ion homeostasis and salt tolerance among species or varieties is related to the expression of regulatory genes. Rice, a moderately salt sensitive crop, has complex responses to salt stress and its sensitivity varies according to species, variety, growth and development stages and the duration of stress to which it is exposed.

Keywords: Abiotic Stress, *Oryza sativa*, OsHKT2;1, OsNHX1, OsSOS1, OsTPS1, Salinity

1. Introduction

Rice, which belongs to the Oryza genus, is a member of the Poaceae family. The genus has two cultivated species (*Oryza sativa* and *Oryza glaberrima*) and twenty-two wild-type varieties [1] . The wild-type varieties are distributed around South America, Australia, Asia and Africa, while as one of the cultivated varieties, *O. glaberrima*, is restricted to Africa. On the other hand, the second cultivated variety, *O. sativa*, is the most common variety grown in about 112 countries around the world [2]. Rice (*O. sativa* L.), which was first cultivated in China and India, is one of the earliest cultivated plants [3]. Rice has been consumed by humans for about 5000 years due to its carbohydrates, rich content of vitamins (A, E, B1 and B2), protein, minerals (calcium, phosphorus, sodium, magnesium, zinc) and low production costs [4]. Therefore, rice ranks second after maize in terms of production and share of cultivation in the world [5].

According to 2018 data from the Food and Agriculture Organization (FAO), the average annual market share of rice production in the world is 996.1 million tons. China was ranked first with 426.2 million tons, India ranked second with 172.6 million tons and Indonesia ranked third with 83 million tons. As of 2018, Türkiye produced 940 thousand tons and ranked 38th in the world [6].

Anatolia was introduced to rice 500 years ago, through Egypt and its agriculture was first practiced in Tosya, Kastamonu district [7].

Rice, as a monocot plant, is the only cereal that can grow in water as it can utilize dissolved oxygen in water. It can grow in a wide variety of geographically diverse terrains up to 2500 m altitude and below sea level in elevation [8]. It is sensitive to temperature changes and day length. Rice production requires 3000 - 5000 kg of water per kilogram yield and 80% of this water is fresh water [9].

Despite the high share of rice for feeding the growing population in our country, its cultivation area is only 0.80% of the total cereal cultivation areas. The global value for rice cultivation area is 0.11% [10]. The annual per capita rice consumption in Türkiye has increased from 3.2 kg in 1964 to 4.4 kg in 1970, 6.5 kg in 2010-2011 and 8.3 kg in 2013. In terms of production, the yield in the 1920s was 30,000-40,000 tons on less than 100,000 decares of land, while production was 225 thousand tons in the nineties. It reached 830 thousand tons in 2014 which indicates the amount of rice production in Türkiye has increased 3.9 times over the past decades [10]. However, 5-year average of rough rice production was calculated as 862 thousand tons from 2019 to 2024 [11].

Türkiye is very favorable for rice production in terms of both climate and soil structure. Regionally, East and West Marmara, Western Black Sea and Southeastern Anatolia regions and provincially Balıkesir, Çanakkale, Çorum, Edirne and Samsun have the highest production capacity [12]. In Türkiye, Marmara Region has 70%, Black Sea Region has 25% and other regions have 5% share in total rice production [13]. Of the world's total land area (13.4 billion hectares), 11% (1.5 billion hectares) can be used for agricultural activities. This corresponds to only 36% of the fertile land available for agriculture. In the world, 80% of the food needs are met by cereal crops. Rice is one of the staple crops that is the only food source of 50% of the world population. One fourth of the daily energy need per capita is met by rice, and, if the world population increases in the recent trend, a 50% increase in cultivated areas will be needed for cultivation by 2030 in order to meet the energy needs of the increasing population [10, 14].

In the last decade, factors such as increasing population, climatic and environmental problems, and intensive industrial activities have increased the negative effects of abiotic stress factors such as salinity, frost, drought and temperature fluctuation on plants and disrupted their vital functions. Therefore, increasing crop production is a very important topic for the nutritional sustainability of the world population. The first method that can be applied for this purpose is to increase the production areas. However, this method indirectly brings more negative aspects instead of being part of the solution. The second method is to increase the yield per unit area. The genotype of the cultivated plant and ecological factors are the primary properties affecting the yield per unit area [15].

Salinity is one of the most important abiotic stresses for plants. It disrupts the intracellular ion balance and prevents the water uptake at optimum levels. Hence, it leads to physiological drought. During prolonged stress, plants may suffer from decline and deterioration in energy, carbohydrate and lipid metabolism, disruptions in photosynthetic activities and protein synthesis mechanisms, and deterioration in leaf structure. In total, these effects lead the plant to yield loss. If the severity and/or duration of stress increases, it may even lead to plant death [16].

Salinity stress generally occurs in areas where the annual precipitation is below 300 mm. In these areas, excessive evaporation due to high temperatures leads to the accumulation of dissolved salts from water in the soil. Also, uncontrolled irrigation practices such as surface and flood irrigation contribute to the problem. Improper drainage properties of the fields may cause an increase in the concentration of ions such as K^+ and Na^+ in the root zone of plants and may also cause decreases in yield. In soils with high salt concentration, plants are categorized under two main groups according to their tolerance. The first group is glycophytic plants which represent the group of plants with low salt tolerance. Most plants belong to the glycophytic group, and when the concentration of salt in the soil exceeds the threshold value, their growth and development stops. Halophytes, which constitute the second group, can maintain their vital activities at high salt concentrations [17]. The amount of salinity in agricultural areas can be measured by the electrical conductivity of the soil. Salt sensitive plants can survive in soils with an electrical conductivity of 1.5 - 3 dS/m (approximately 15 - 30 mM NaCl concentration), while highly salt tolerant plants can survive in soil with an electrical conductivity of 5 - 10 dS/m (approximately 50 - 100 mM NaCl concentration) [18].

According to 2014 data of Turkish Statistical Institute (TUIK), the distribution of saline soils in Türkiye, covers a total area of 1.5 million hectares. Of this value, 599 thousand hectares are slightly saline, 508 thousand hectares are saline, 15 thousand hectares are sodic, 127 thousand hectares are slightly saline - sodic, and 268 thousand hectares are saline - sodic soils [19].

Salinity tolerance is a multigenic trait including free-radical scavenging, osmotic regulation, cellular ion homeostasis and more. Osmoprotectants such as trehalose (Tre) which is a non-reducing disaccharide of glucose, are key components of salinity tolerance in plants. As well as providing an energy source to plants, Tre has unique physicochemical properties for stabilizing dehydrated enzymes, proteins, and lipid membranes, under osmotic stress conditions. Besides Tre takes part in cellular signaling. It is also known to act as an elicitor for stress response genes [20]. TPS gene encoding trehalose -6-phosphate synthase has a crucial role in trehalose biosynthesis.

The salt overly sensitive (SOS) pathway, which evolved to maintain salinity tolerance, consists of SOS1, SOS2 and SOS3. SOS1 gene encodes Na^+/H^+ antiporter protein in plasma membrane which excludes Na^+ out of cytoplasm. It takes part in cellular signaling regulation through mediating ion homeostasis under saline

conditions along with other SOS components. Particularly, SOS1 is a key component for salinity tolerance in plants. Sequesteration of Na^+ ions instead of exclusion is another strategy for maintaining cell turgor pressure under saline conditions. Na^+/H^+ antiporters (NHXs) exchange protons and Na^+ ions particularly across vacuole membranes in plants, algae, and fungi. Therefore, both SOS1 and NHX1 have been reported to improve salinity tolerance in numerous plants [21, 22]. Moreover, HKT transporters are found in plants and microorganisms. Especially, class II of HKT transporters (HKT2s) mediate Na^+/K^+ transport activity. There is substantial evidence on the physiological significance of HKTs for growth and development of plants under saline conditions. The HKT2;1 transporter has unique functions among class II HKTs which is determined to be mediating the nutritional Na^+ absorption and Na^+ uptake to compensate K^+ deficiency conditions [23].

In the present study, Osmancık-97 rice variety, which is extensively cultivated in Türkiye, was grown. Osmancık-97 comprises 80% of rice production of Türkiye along with Rocca and Baldo. It is a hybrid of Rocca x Europa and registered in 1997. Since then, it has high yield potential and adapted to various ecological condition in different regions of Türkiye. Therefore, it is a suitable candidate for abiotic stress tolerance investigations. Plants were grown under four different salt concentrations (60, 90, and 120 mM and control) in *in vivo* conditions. The study aimed to determine the expression differences of the TPS1, NHX1, SOS1 and HKT2;1 genes under increasing salinity conditions as the components of osmotic adjustment (osmopotectants and ion exclusion/sequesteration) mechanism and to evaluate growth and biochemical parameters.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Osmancık-97 rice variety was obtained from the Directorate of Trakya Agricultural Research Institute (Edirne, Türkiye) and was grown in the Plant Biotechnology Laboratory of Molecular Biology and Genetics Department of T.C. Istanbul Kültür University. Seeds of the control group and each salinity treatment group were sown in plastic plant growth containers with perlite as filler. Sowing was carried out with a total of 60 seeds per experimental group as 10 seeds per plastic container. The sown seeds were germinated in plastic containers filled with distilled water for one week. At the end of the first week, irrigation with Yoshida nutrient solution was continued. On the 28th day of germination, 60, 90 and 120 mM NaCl were added to the Yoshida solution except the control group. Salinity conditions were determined according to the preliminary test conducted. After 10 days of stress treatment, the leaves were harvested. The developmental differences of the harvested plants were evaluated based on average plant height, average root length, average fresh and dry weight (shoot and root) parameters [16].

2.2. Lipid Peroxidation Analysis

The amount of thiobarbituric acid reactive substances (TBARS) which are the products of lipid peroxidation caused by salinity stress induced oxidative stress in rice plants, was determined colorimetrically according to the method of Stewart and Bewley [24]. For each sample, 0.1 g of rice leaves was homogenized in sterile mortars with 1 mL sterile distilled water. One mL of 0.5% (v/w) thiobarbituric acid including 20% trichloroacetic acid was added on all homogenates. The samples were incubated at 95°C for 30 minutes and then the reaction was stopped by placing the tubes in an ice bath for 5 minutes. The homogenates were centrifuged at +4 °C for 30 minutes at 10.000 × g and the absorbance values were measured at 532 and 600 nm wavelengths. The results were calculated and presented as µmol TBARS/g.FW. The experiment was performed in three replicates.

2.3. Proline Accumulation

The proline accumulation caused by salt stress in rice plants was calculated according to the method of Bates et al. [25]. Rice leaf tissue (0.1 g) was homogenized with 2 mL of 3% (w/v) sulfosalicylic acid. After centrifugation at 10.000 × g for 15 minutes at +4 °C, 2 mL of each sample was transferred to a glass tube. Ninhydrin reagent was added to the samples and the reaction was carried out in an incubator at 100 °C for an hour. At the end of the duration, the samples were kept on ice to terminate the reactions. 4 mL toluene were added to each tube and vortexed. After phase separation, the supernatants of samples were measured spectrophotometrically at 520 nm wavelength. The results were calculated and presented as µmol proline/g.FW according to the standard calibration curve. The experiment was performed in three replicates.

2.4. Photosynthetic Pigment Content

Effects of salinity stress on photosynthetic pigment content of rice plants were determined according to the method of Arnon [26]. Rice leaf tissues (0.1 g) were homogenized with 80% (v/v) cold acetone. The samples were then centrifuged at 10.000 × g for 15 minutes at +4 °C. Supernatants were measured at 470, 645 and 663 nm wavelengths. The experiment was performed in three replicates. Photosynthetic pigment contents were calculated according to the equations below and presented as µg/g.FW.

$$Chlorophyll\ a = 11.24\ x\ A663 - 2.04\ x\ A645$$

$$Chlorophyll\ b = 20.13\ x\ A645 - 4.19\ x\ A663$$

$$Total\ Chlorophyll = 7.05\ x\ A663 + 13.09\ x\ A645$$

$$Carotenoid = \frac{(1000\ \text{x A470}) - (1.9\ \text{x Chll a}) - (63.14\ \text{x Chll b})}{214}$$

2.5. RNA Extraction

Plant leaves (0.3 g) were homogenized in sterile mortars with liquid nitrogen and transferred to 2 mL tubes. One mL TRIzol reagent was added. The samples were mixed gently for 20 seconds and kept at room temperature for 5 minutes. After the duration, 0.2 mL of chloroform was added, and the samples were kept at room temperature for 4 minutes after shaking for 20 seconds. Then centrifugation was performed at +4 °C, 12.000 × *g* for 15 minutes. 450 µL of supernatant was transferred to new sterile tubes and 450 µL of isopropanol was added in equal volume. After 10 minutes at room temperature, the samples were centrifuged at 12.000 × *g* for 10 minutes at 4 °C. The supernatants were discarded. One mL of 75% RNAase-free ethanol was added, and samples were centrifuged at 7500 × *g* for 5 minutes at +4 °C. The supernatant was discarded, and the tubes were kept at room temperature until the ethanol evaporated. Finally, 40 µL of RNAase-free water was added and RNA unwinding was performed. After the extraction, the concentration and purity of the obtained RNAs were measured in Nanodrop Implen NP80 device [16]. The experiment was performed in three replicates.

2.6. cDNA Synthesis

cDNA synthesis was performed using the iScript cDNA Isolation Kit. Components were used following the recommendations by the manufacturer: 4 µL 5X iScript cDNA buffer, 1 µL iScript Reverse Transcriptase, 14 µL nuclease-free water and 1 µL RNA (300 µg) sample in a total volume of 20 µl. The reaction was performed at the times and temperatures indicated in **Table 1**.

Table 1. cDNA synthesis PCR reaction protocol.

Step	Time (min.)	Temperature (°C)
Priming	5	25
Reverse Transcription	20	46
RT inactivation	1	95
Hold	-	4

2.7. Gradient PCR

The primer sequences designed for the target genes are given in **Table 2**. Thermo Scientific PCR Master Mix (2X) was used for the gradient PCR step. Mixture of 12.5 µL mix, 9.5 µL Thermo Fisher DEPC-treated water, 1 µL cDNA, 1 µL Forward Primer, 1 µL Reverse Primer were placed in a 200 µL PCR tube and vortexed. The protocol was carried out with Prima - Duo™ Hi-Media PCR device at the times and temperatures given in **Table 3**. Associated optimum binding temperatures of the primers were determined by agarose gel electrophoresis analysis of amplification products.

Table 2. Target genes, accessions, products sizes and primer sequences.

Target Genes	Accession	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Product length (bp)
OsTPS1	HM050424	CTGATGAGAGA GAAAAGCGACA T	GACTAGGGAGA TCAGGTGGAAC T	153
OsNHX1	XM_015789089	CTGGATTGCTCA GTGCATACATA	ACCACAGAAGA ATACGGTGAGA A	155
OsHKT2;1	AJ491852	GTCAACCTCTGC TCTGACACTTT	GAAAACTCTGG GTTGTGCTTA TG	168
OsSOS1	KY752550	GGCTTCCTTCTT CTGCTCTATGT	ACTGCATCACTA GCACGCTTAAC	185

OsTPS1: Trehalose 6 Phosphate Synthase 1, OsNHX1: Na^+/H^+ antiporter, OsHKT2;1: High Affinity K^+ transporters, OsSOS1: Salt Overly Sensitive 1

Table 3. Gradient PCR protocol.

Step	Time (min.)	Temperature (°C)	Cycles
Initial Denaturation	3	95	1
Denaturation	0.5	95	35
Annealing	0.5	54, 56, 58, 60, 62, 64, 66, 68	
Extension	1	72	
Final Extension	10	72	1

2.8. Quantitative Real Time PCR Analysis

Gene expression analysis was performed by following the BIO-RAD iQ™ SYBR® Green Supermix protocol. The reaction mixture containing BIO-RAD iQ™ SYBR®Green Supermix (2X) 10 µL, 1 µL forward primer, 1 µL reverse primer, 1 µL cDNA and 7 µL Thermo Fisher DEPC-treated water was used. The reactions were carried out on BIO-RAD CFX Connect Real-Time PCR device. Ubiquitin 5 primer was selected as endogenous control. Reaction times and temperatures are presented in **Table 4**.

Table 4. Quantitative real-time PCR protocol.

Step	Time (sec.)	Temperature (°C)	Cycles
Polymerase activation and DNA denaturation	180	95	1
Denaturation	15	95	39
Annealing / Extension and Plate Read at Optimum Temperature	45	60	
Melting Curve Analysis	5	65 - 95	1

2.9. Statistical Analysis

The data obtained from the evaluation of morphological damage and biochemical analysis (lipid peroxidation, proline accumulation, photosynthetic pigment), plant height, root length, fresh weight and fresh length of the control and experimental groups of rice plants treated with different concentrations of NaCl were statistically analyzed by using GraphPad Prism 8 (https://www.graphpad.com) statistical program. One-way ANOVA test was performed, and the comparison of the groups found to be statistically significant in the test were further analyzed by Student- Newman Keuls post-test. The experiments were performed in three biological and technical replicates.

3. Results and Discussion

In this study, proline accumulation levels, TBARS levels, photosynthetic pigment content, plant fresh and dry weights, root lengths, plant lengths and salt stress response gene expression profiles of Osmancık-97 rice variety were analyzed under increasing salinity stress conditions (no salt as control, 60, 90, and 120 mM NaCl for salinity stress).

In all salinity treatment groups of Osmancık-97 rice variety, with increasing NaCl concentration, root lengths, average plant weights and average plant lengths decreased, significantly (**Figure 3**). In Osmancık-97 variety, the average root length in the control group was 7.80 cm, while the root length of plants exposed to 60 mM salinity stress decreased by 1 cm to 6.80 cm. The average root length in plants exposed to 90 mM and 120 mM NaCl decreased to 6.60 cm and 6.30 cm, respectively. The root lengths of the group exposed to 120 mM salt decreased by 19% compared to the control (**Figure 1**). Similarly, it was reported that salinity application (50 mM NaCl) significantly reduced shoot and root growth morphological indices of two rice varieties, and especially in certain varieties, salinity application for 8 - 10 days significantly reduced root biomass in rice plants [2].

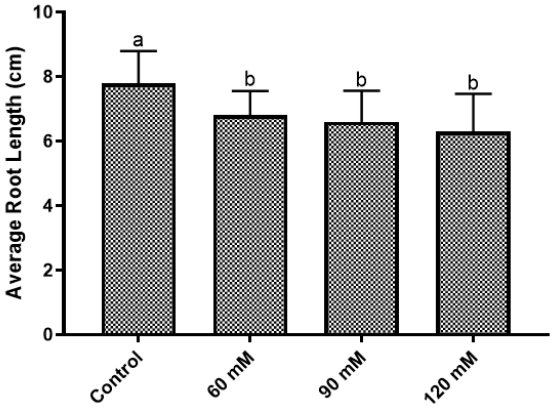

Figure 1. Effect of increasing NaCl concentrations on average root length of Osmancık-97 rice variety.

Similarly, in a study performed with salinity tolerant Pokkali and susceptible IR-28 rice varieties, it was reported that short-term salt stress negatively affected root lengths on all varieties, but IR-28 variety was much more affected by the stress factor compared to Pokkali [27]. In another study performed with tolerant Pokkali and sensitive IR-28, it was emphasized that increasing salinity stress (60 or 120 mol m^{-3} NaCl) had negative effects on IR-28 root lengths, but this change was not significant for both varieties [28]. In another study, it was reported that root length and root total volume presented a decreasing trend during the salinity treatment (50 mM) period in Huanghuazhan rice variety compared to the control, and root length and root total volume decreased by 30.08% and 40.8% on the 10th day of treatment, respectively [29]. The reduced root surface area under salt stress reduces nutrient uptake and water absorption and further inhibits the cell division process. As a result, it causes a decrease in root length and root biomass [30].

In Osmancık-97 variety, statistically significant decreases were measured gradually in the fresh and dry weights following the salinity treatment (60, 90, and 120 mM NaCl) compared to the control group. The average fresh weight of the plants in the control group was 0.45 g, while the dry weight was 0.12 g. The average fresh weight of the plants exposed to 60 mM salinity stress was measured as 0.39 g and dry weight as 0.11 g. The fresh weight and dry weight of the rice plants exposed to 90 mM salinity decreased to 0.30 and 0.09 g, respectively. Similarly, in plants exposed to 120 mM NaCl, the fresh weight was measured as 0.30 g, while the dry weight was measured as 0.09 g (**Figure 2**).

Celal Bayar University Journal of Science
Volume 21, Issue 1, 2025, p 109-120
Doi: 10.18466/cbayarfbe.1529138

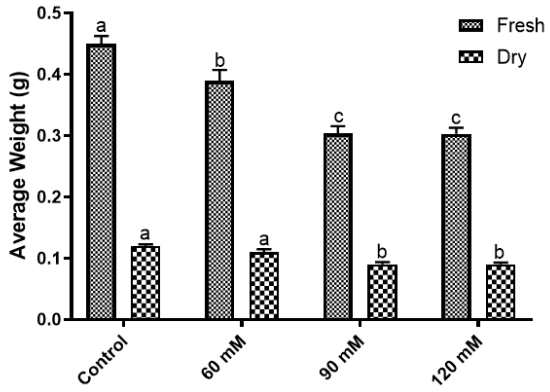

Figure 2. Effect of increasing NaCl concentrations on average fresh and dry weights of Osmancık-97 rice variety plants.

In the study performed by Chen et al. [29], in Chaoyouqianhao rice variety, shoot dry weight was decreased under salinity treatment compared to the control. Especially, stronger decrease was occured between 6th and 10th days. In the same study, it was reported that both total dry weight and root dry weight were strongly inhibited during the salinity treatment period of Huanghuazhan rice variety compared to the control (18.86% and 19.09%, respectively). When the fresh weights were evaluated, despite the 0.06 g decrease between the control group and 60 mM salinity-treated rice plants, the highest difference occurred between 60 mM and 90 mM salinity-treated rice plants by 0.09 g. In the highest applied 120 mM NaCl treated group, 33% decrease occurred compared to the control group.

In Osmancık-97 variety, the average plant length in the control group was 23.8 cm, while the length of plants exposed to 60 mM salinity stress was 1.7 cm shorter, decreasing to 22.1 cm. The average length of plants exposed to 90 mM salinity was 19.8 cm, while the average plant length in plants exposed to 120 mM NaCl stress was 19.2 cm. The decrease in the length of plants treated with 120 mM salinity compared to the control group was 19% (**Figure 3**).

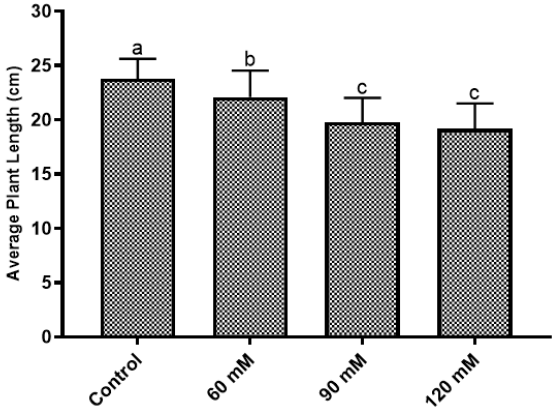

Figure 3. Effect of increasing NaCl concentrations on average plant length of Osmancık-97 rice variety.

In a study performed with Mekongga, Banyuasin, Madura, Ciherang, Inpara-3 and Inpari 13 rice varieties, 50, 100, 150, and 200 mM levels of NaCl were applied to the varieties together with the control group for four weeks. As a result, it was determined that there was a decrease in plant lenght in all rice varieties [31]. Similarly, plant length of Chaoyouqianhao and Huanghuazhan rice varieties gradually decreased during the 10-days salinity treatment, and presented a decrease of 17.7% and 11.5% on the 10th day of the treatment, respectively [29].

In Osmancık-97 variety, the effects of increasing salinity stress (60, 90, and 120 mM NaCl) on the photosynthetic pigment contents were measured spectrophotometrically. Following the increase in salinity concentrations, decreases in the amounts of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were detected (**Figure 4**).

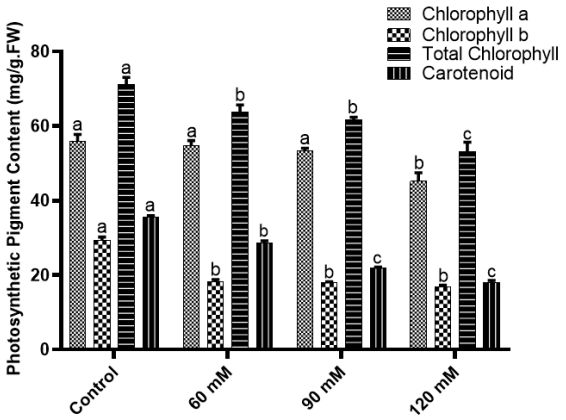

Figure 4. Effect of increasing NaCl concentrations on photosynthetic pigment content of Osmancık-97 rice variety.

Since salt stress largely inhibits biomass accumulation, it reduces leaf area and photosynthetic parameters [32]. Numerous salinity stress studies have reported significant decreases in chlorophyll concentration as the intensity of salinity stress increases [33, 34] In our study, there was no significant difference between control and 60 mM, 90 mM salinity applied plants for chlorophyll a pigment in Osmancık-97 variety similar to the results of the study conducted by Chen et al. in which they applied 50 mM salt to rice plants [29]. In some salt applications (80 mM NaCl), it was reported that chlorophyll a, b and total chlorophyll contents increased in rice seedlings [35]. Chlorophyll content may temporary increase during the growing period under 100 Mm NaCl salinity treatment, which was indicated to be mainly due to the stimulating effect of stress conditions [36].

The highest alteration of chlorophyll a concentration was observed in 120 mM salinity applied group. When we compared the chlorophyll a concentrations between control and 120 mM salinity stress, an 18% decrease was

measured with increasing salinity. For chlorophyll b, 45% initial concentration loss was measured. When the difference in carotenoid concentration between control and 120 mM was evaluated, 48.5% concentration loss was measured. When the total chlorophyll amount was evaluated, a decrease was observed in Osmancık-97 rice plants exposed to 60 and 90 mM salinity compared to the control, while the greatest change occurred between control plants and rice plants applied with 120 mM salinity. The change in total chlorophyll concentration resulted in a loss of 25% between control and 120 mM. Similarly, it was reported that chlorophyll a (11.34% and 15.84%), chlorophyll b (12.71% and 19.48%), carotenoid (10.86% and 17.73%) and total chlorophyll contents (11.71% and 16.78%) of two rice varieties decreased significantly under salinity treatment compared to the control [29]. In another study, it was reported that chlorophyll a, chlorophyll b and total chlorophyll concentrations of Gala and Edirne rice varieties decreased under 300 mM salinity stress [37].

The most important energy source for plants is photosynthesis. Although the tolerance of plants to salt stress varies, photosynthetic pigment determination is used as a biochemical marker in abiotic stress factor tolerance [38]. There is sufficient evidence to report that exposure of plants to stressful environments such as salinity reduces chlorophyll content, which leads to general growth retardation. Short-term exposure to salt induces osmotic stress in plants, which results in reduced water absorption, redox imbalance, stomatal closure, and inhibition of new leaf growth and root system development. However, long-term exposure to salt causes ionic toxicity due to Na^+ accumulation in mature leaves. This reduces the rate of photosynthesis and nutrient accumulation, resulting in premature senescence [39]. Excessive salinity significantly inhibits plant growth and biomass accumulation, and causes a decrease in photosynthetic parameters [40]. In addition, the decrease in photosynthetic pigments can be explained by the increase in chlorophyll-ase activity caused by salinity and the degradation of chlorophyll [41].

The change in proline concentration in Osmancık-97 variety under 60, 90 and 120 mM NaCl treatment compared to the control is represented in **Figure 5**. High salt concentration limits water uptake for plants and thus osmotic stress occurs. Plants store compatible solutes such as proline in order to protect themselves from osmotic stress [42]. A statistically significant increase was observed between the control group (0.64 µmol / g.FW) and the 90, 120 mM NaCl treated rice plants (0.86 µmol / g.FW and 1.07 µmol / g.FW, respectively). Especially, when the proline amounts in the control group and the 120 mM salinity treated rice plants were examined, an increase of 40% was determined.

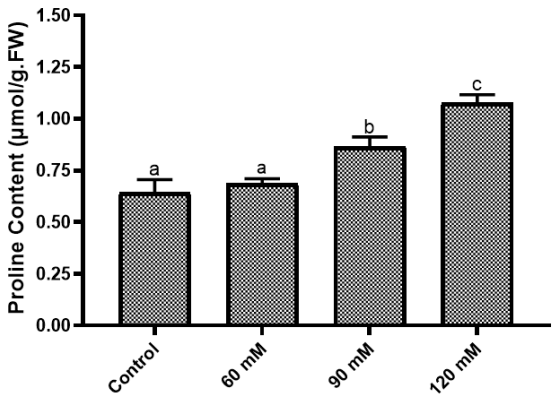

Figure 5. Effect of increasing NaCl concentrations on proline accumulation of Osmancık-97 rice variety.

In a study performed with salt sensitive IR-29, tolerant Pokkali and 12 other rice varieties, an increase in proline accumulation was observed in all groups compared to the control at all salinity concentrations [43]. In another study, it was reported that proline demonstrated a gradual accumulation in accordance with increasing NaCl concentrations. In the same study, plants exposed to 150 and 210 mM NaCl had the highest proline content with 4.47 and 4.81 fold increase over the control, respectively[16].

In Osmancık-97 rice variety, an increase in TBARS levels was measured and found to be directly proportional to the increasing salinity (**Figure 6**). Salt stress leads to membrane damage and stomatal closure. This process leads to a decrease in carbon dioxide fixation and hydrolase activity, and an increase in lipid peroxidation levels. The formation of reactive oxygen species such as O_2^-, H_2O_2 and OH^- can be stimulated. ROS can cause oxidative damage to cell membranes, proteins, DNA and lipids, and can also cause TBARS accumulation, disrupting various biochemical and metabolic processes in plants [44]. In particular, unsaturated fatty acids in the plant cell membrane are subjected to lipid peroxidation by reactive oxygen species formed as a result of abiotic and biotic stresses. Thus, ion transport systems and proteins in the membrane and cell membrane structure are damaged. TBARS, which is the product of this damage, has been used as an indicator of oxidative damage in membranes [45]. While the value obtained as a result of colorimetric measurement of TBARS amounts in the Osmancık-97 rice control group was 0.004 µmol / g.FW, this value increased to 0.0063 µmol / g.FW in proportion to stress in the 60 mM group. The amount of TBARS in rice plants exposed to 90 mM salt was very close to the 60 mM salt treated group and was determined as 0.0064 µmol / g.FW, while this value presented the highest increase in the 120 mM salinity group and was measured as 0.011 µmol / g.FW. In our study, TBARS concentrations increased by 60% in plants exposed to 120 mM NaCl compared to the control group.

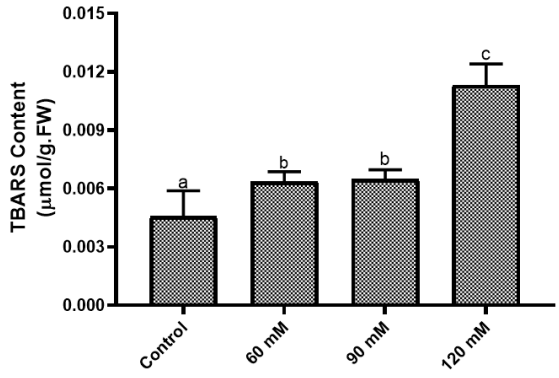

Figure 6. Effect of increasing NaCl concentrations on TBARS content of Osmancık-97 rice variety.

Similarly, it was indicated that TBARS concentration in plants increases in direct proportion to salinity stress [46–48]. Hossen et al. [48] indicated that the MDA content in 150 mM NaCl-treated rice seedlings increased by 98%. In another study, TBARS amount was found to be particularly high in a salt-sensitive variety, while it was detected at low levels in a tolerant variety [45]. In tolerant rice genotypes, the positive relationship between the Na^+/K^+ ratio and hydrogen peroxide (H_2O_2), TBARS and the activity of antioxidant enzymes indicates that they are important in improving salinity tolerance. Salt tolerant rice varieties demonstrated lower Na^+/K^+ ratio, higher proline accumulation, lower H_2O_2 accumulation and TBARS content, and higher catalase and ascorbate peroxidase activities [45]. Wu et al. demonstrated that overexpression of the MADS-box gene OsMADS57 plays an important role in response to salinity stress and improves salt tolerance via antioxidant mechanism in rice. In this process, TBARS content was also significantly decreased in OsMADS57 overexpressing lines compared to controls [49].

Plants should develop different strategies to reduce the harmful effects of salt stress and protect themselves from the harmful effects of ROS. Salt stress is among the environmental stress factors that lead to the accumulation of ROS at toxic levels. Antioxidant mechanisms are one of the most important mechanisms that protect cells from the harmful effects of reactive oxygen species (ROS). In previous studies, the responses of 14-day-old seedlings of Osmancık-97 rice (*Oryza sativa* L.) variety to NaCl stress were shown by transcriptional analyses of genes responsible for antioxidant enzymes (Cyt-APX, CAT A, Cyt-GR1 and proline metabolism-related genes). It was reported that these genes were expressed differently between the two rice varieties under different salt concentrations [50]. Salt stress responsive genes including TPS1, NHX1, SOS1 and HKT2 genes are another important part of this effective strategy. In the present study, we analyzed the expression of NHX1, HKT2 and SOS1 genes, which are members of the three main Na^+ transporter gene families that play an important role in salinity tolerance, under different salt concentrations. We also examined the expression profile of the TPS1 gene, which belongs to trehalose metabolism and is also associated with salt tolerance. The gene expression levels of target genes were determined by qRT-PCR.

In Osmancık-97 variety, expression of OsTPS1 gene was examined in 60, 90, and 120 mM salinity conditions (**Figure 7**). OsTPS1 gene expression in 60 mM salinity treated rice demonstrated 63.3% decrease compared to the control. OsTPS1 gene expression decreased by 85% in 90 mM salinity treated rice, while a 78.2% decrease was detected in 120 mM salinity group. Trehalose is a non-reducing disaccharide that protects cellular proteins and membranes against abiotic stress factors. Trehalose production in plants occurs through trehalose-6-phosphate synthase (TPS) / trehalose-6-phosphate phosphatase (TPP). In Arabidopsis seedlings grown in medium containing trehalose and without sucrose, increased expression of the starch synthesis gene ApL3, excessive starch accumulation in cotyledons and inhibition of root growth were observed [51].

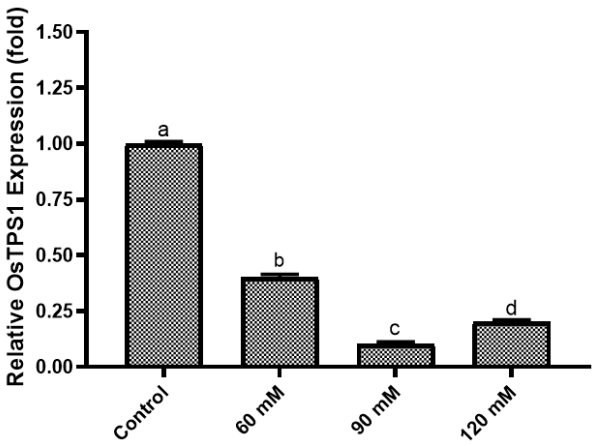

Figure 7. Effect of increasing NaCl concentrations on OsTPS1 gene expression of Osmancık-97 rice variety.

In our study, as a result of statistically significant decreases in the expression of OsTPS1 gene at increasing salinity concentrations in Osmancık-97 rice variety, it was hypothesized that our variety may be tolerant to salinity. There are two different cases in the literature regarding the TSP1 gene expression under stress conditions. It has been stated that both increase and decrease in TSP1 gene expression in different plant species or varieties are associated with stress tolerance. In a previous study, bioinformatics analyses including co-expression network, gene expression, chromosomal map analysis of cis element and phylogenetic relationships of 11 OsTPS gene sequences downloaded from NCBI were performed in *O. sativa*. Similar to our study, it was reported that the expression of OsTPS1 gene was downregulated in abiotic stresses of dehydration, cold and drought and in biotic stresses of *X. oryzea* [52]. Also, it was shown that the expression levels of genes related to trehalose biosynthesis (TPS1, TPS2, TPP1 and TPP2) in tomato seedlings were reduced under NaCl

application [53]. In the same study, molecular evidence has revealed that the up regulation of TPS1, TPS2, TPP1 and TPP2 genes in tomato plants is only realized through plant hormone strigolactones and then supports tomato seedling growth. In another study, it was indicated that increased expression of OsTPS1 gene in rice plants has an important role in developing tolerance to salinity, drought and low temperature [54]. Similarly, in study performed with *Arabidopsis thaliana* plant, high expression of AtTPS1 gene was associated with drought stress [55].

In our study, OsNHX1 gene expression in 60 mM salinity treated rice plants decreased by 51.9% compared to the control. OsNHX1 gene expression decreased by 79.1% in 90 mM salinity treated rice, while a 74% decrease was detected in 120 mM salinity group (**Figure 8**). NHX antiporters regulate salt and pH levels by providing cation/H^+ balance in the cell. In a study performed with transgenic rice plants, it was reported that salt tolerance improved as a result of increased expression of Na^+, K^+/H^+ antiporter gene (OsNHX1) [56]. In another study, it was shown that OsNHX gene provided intracellular Na^+ balance in transgenic corn plants and improved salt tolerance by providing Na^+ ion transport to vacuoles [57]. K^+ is the basic macronutrient found in the cell cytosol. The presence of Na^+ ion in high concentrations inside the cell disrupts the cell water balance and changes the cell ion charge balance by competing with K^+ ion. Studies have reported that NHX1 type transporters generate tolerance to salt stress by removing Na^+ ion in wheat, rice and Arabidopsis plants [22, 58, 59]. It has been reported that the NHX1 gene was statistically down-regulated in salt-tolerant plant shoots under NaCl treatment (75 mM) for 14 days compared to the control, and statistically significantly up-regulated in salt-sensitive plant shoot. On the contrary, the NHX1 gene was statistically significantly up-regulated in the salt-tolerant plant roots under salinity stress compared to the control, while it was statistically significantly down-regulated in the salt-sensitive plant roots [60].

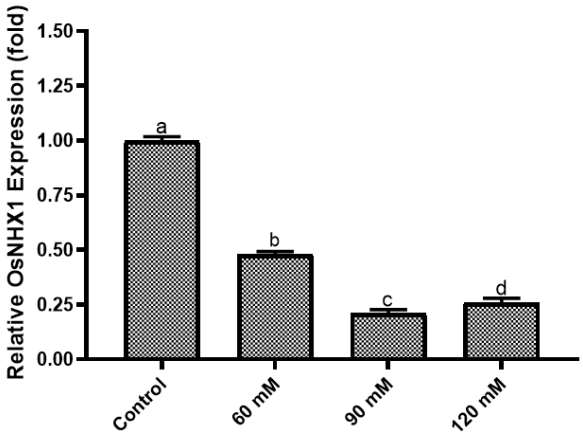

Figure 8. Effect of increasing NaCl concentrations on OsNHX1 gene expression of Osmancık-97 rice variety.

Similarly, the SOS1 gene was statistically significantly down-regulated in the salt-tolerant plant shoots under NaCl treatment compared to the control, while no significant difference was obtained in the salt-sensitive plant shoots. The SOS1 gene was statistically significantly up regulated in the roots of all tolerant or sensitive plant groups [60]. Thus, decreased expressions of SOS1 and NHX1 in shoots may be related to salinity tolerance of Osmancık-97 rice plant. Osmancık-97 rice variety was stated to be more salt tolerant than other local rice varieties in a previous study [16]. Transport of Na^+ and K^+ between different tissues and subcellular locations may help plants adapt to saline environment [61]. According to previous reports, the relative expression of OsNHX1 was detected maximum in the roots and greater than shoot tissues cvs. 9311 and JYGY-1 exposed to 150 mM NaCl for 24 h. After long-term NaCl exposure (72 h), OsNHX1 expression level in seedlings under salt stress decreased [62].

In our study, OsSOS1 gene expression decreased significantly with increasing stress amount in rice plants exposed to 60, 90, and 120 mM NaCl (**Figure 9**). In Osmancık-97 variety, OsSOS1 gene expression in rice exposed to 60 mM salinity decreased by 63.3% compared to the control. While OsSOS1 gene expression decreased by 85.1% in rice exposed to 90 mM salinity, a decrease of 78.3% was reported in the 120 mM salinity group. The uptake of Na^+ ion into the cell occurs instantly. This disrupts ion homeostasis in the cell and damages the plants. SOS1 antiporters ensure the intracellular Na^+ /H^+ balance and provide the removal of Na^+ out of the cell. Studies indicated that in mutant Arabidopsis plants lacking SOS1, there are irregularities in the excretion of Na^+ ions, and that tolerance to salt is significantly reduced, and defects occur in ion transport from roots to shoots [63].

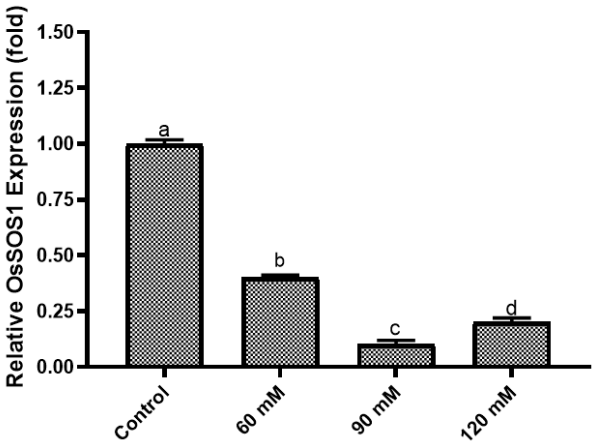

Figure 9. Effect of increasing NaCl concentrations on OsSOS1 gene expression of Osmancık-97 rice variety.

When SOS1 mutants and wild type plants were treated with 25 mM salinity, wild type plants accumulated Na^+ more than mutants. This indicated that SOS1 is involved in controlling the sodium ion transfer to the xylem and its

transfer to the shoots [64]. However, in contrast, in control and mutant plants treated with 100 mM salinity, more sodium ion accumulation was observed in mutants compared to controls. It was thought that this could be due to the inability to export Na^+ ions from the root epidermis and the disruption of the Na^+ electrochemical gradient along the xylem-symplast boundary [64]. It was also suggested that under high salinity stress, the Na^+ ion concentration difference between the xylem parenchyma and sap could be higher than the pH gradient, and as a result, SOS1 activity could reverse, resulting in sodium ion uptake from the xylem [64]. In previous studies, it was observed that SOS1 and NHX1 exhibited a different expression pattern related to leaf age in pomegranate (*Punica granatum* L.) plants. In mature leaves, low transcript levels of these genes were detected in the early period of treatment (10 h). In young leaves, increased expression of SOS1 and NHX1 was reported (except NHX1 at 24 h) [65].

In our study, OsHKT2;1 gene expression decreased significantly with increasing salt concentration in rice plants exposed to 60, 90 and 120 mM NaCl (**Figure 10**). In Osmancık-97 variety, OsHKT2;1 gene expression in rice exposed to 60 mM salinity decreased by 51.4% compared to the control. While OsHKT2;1 gene expression decreased by 78.8% in plants exposed to 90 mM salinity, a decrease of 73.5% was detected in the 120 mM salinity group. OsHKT2 proteins are located in the plasma membrane and control the Na^+ entry from the medium into plant cells [66].

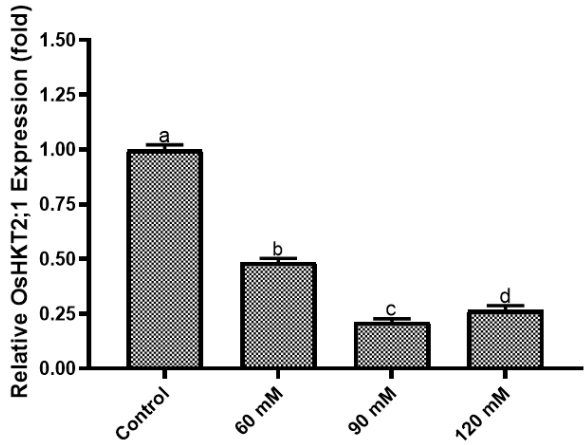

Figure 10. Effect of increasing NaCl concentrations on OsHKT2;1 gene expression of Osmancık-97 rice variety

It was reported that the expression of HKT2;1 gene decreased in the leaves of both salt-tolerant Pokkali rice variety and salt-sensitive IR29 rice variety at early hours (1-6 h) of 200 mM salt stress. In root tissues, the expression of HKT2;1 gene was decreased in the salt tolerant Pokkali variety while it decreased in the salt sensitive IR29 variety at early hours (1-6 h). Zhang et al. reported that OsHKT2;1 was down-regulated in four rice varieties (Nipponbare, 9311, JYGY-1 and JYFN-4) exposed to 125 mM NaCl for 24 and 72 h by under salt stress [62]. Another study reported that the relative expression level of OsHKT2;1 in leaves and roots of two rice cultivars, Tampha and MSE9, exposed to 120 mM NaCl for 96 h was very low [67]. In addition, downregulation of OsHKT2;1 transporter in both root and leaf tissues limits Na^+ influx and Na^+ efflux from xylem to leaf organ. When plants are exposed to high NaCl stress, K^+ deficiency plays an important role in reducing the expression level of OsHKT2;1, which is largely regulated by the availability of K^+ ions [68].

4. Conclusion

The difference in ion homeostasis and salt tolerance among species or varieties is related to the regulation of the expression of NHX, HKT and SOS family genes [65]. However, it is worth noting that the levels of these genes do not represent a clear trend that directly explains the dynamics of Na^+ and K^+ content in different organs from different species or varieties. Soils with EC exceeding 4 dS/m (~ 40 mM) and 8 dS/m (~ 80 mM) are considered as moderate and high salt stress, respectively. Salinity levels greater than 5 dS/m (~ 50) cause a significant decrease in yield for rice. Rice, a moderately salt sensitive crop, has complex responses to salt stress and its sensitivity varies according to species, variety, growth and development stages and the duration of stress to which it is exposed. Further research is required to better understand the role of Na^+ $(K^+)/H^+$ antiporters in intracellular compartmentalization under lower and higher salt stress conditions in different rice varieties.

Acknowledgement

Authors acknowledge the support of Ministry of Agriculture and Forestry, Directorate of Trakya Agricultural Research Institute (Edirne, Türkiye) in providing source of seed materials

Author's Contributions

Atilla Salman: Experimentation

Sinan Meriç: Experimentation, drafted and wrote the manuscript, result interpretation.

Tamer Gümüş: Experimentation

Çimen Atak: Supervised the experiment's progress, result interpretation and helped in manuscript preparation.

Alp Ayan: Supervised the experiment's progress, drafted and wrote the manuscript, experimentation, result interpretation.

Ethics

There are no ethical issues after the publication of this manuscript.

References

[1]. Jena KK. The Species of the Genus Oryza and Transfer of Useful Genes From Wild Species Into Cultivated Rice, *O. sativa*. *Breeding Science* 2010; 60: 518–523.

[2]. Chen T., Shabala S., Niu Y., et al. Molecular Mechanisms of Salinity Tolerance in Rice. *The Crop Journal* 2021; 9: 506–520.

[3]. Civan P., Ali S., Batista-Navarro R., et al. Origin of the Aromatic Group of Cultivated Rice (*Oryza sativa* L.) Traced to the Indian Subcontinent. *Genome Biology and Evolution* 2019; 11: 832.

[4]. Fukagawa NK., Ziska LH. Rice: Importance for Global Nutrition. *Journal of Nutritional Science and Vitaminology*2019; 65: S2–S3.

[5]. Erenstein O., Jaleta M., Sonder K., et al. Global Maize Production, Consumption and Trade: Trends and R&D Implications. *Food Security* 2022; 145 2022; 14: 1295–1319.

[6]. Öztürk D., Akçay Y. Güney Marmara Bölgesinde Çeltik Üretiminin Genel Bir Değerlendirmesi. *Journal of Agricultural Faculty of Gaziosmanpaşa University* 2010; 2010: 61–71.

[7] Taşligil N., Şahin G. Türkiye'de Çeltik (*Oryza sativa* L.) Yetiştiriciliği ve Coğrafi Dağılımı. *Adıyaman Üniversitesi Sosyal Bilimler Enstitüsü Dergisi* 2011; 182–203.

[8]. Paudel MN. Rice (*Oryza sativa* L.) Cultivation in the Highest Elevation of the World. *Agronomy Journal of Nepal* 2011; 2: 31–41.

[9]. Bouman BAM., Hengsdijk H., Hardy B., et al. Water-wise Rice Production. *IRRI*, 2002.

[10] Samal P., Babu SC., Mondal B., et al. The Global Rice Agriculture Towards 2050: An Inter-continental Perspective. *Outlook on Agriculture* 2022; 51: 164–172.

[11]. Turkey Rice Area, Yield and Production, https://ipad.fas.usda.gov/countrysummary/Default.aspx?id=TU&crop=Rice (accessed 11 November 2024).

[12]. Beşer N., Rice Food Security and Production in Turkey. https://ageconsearch.umn.edu/record/164415/files/AdvancesIn.pdf#page=97 (accessed 1 August 2024).

[13]. H.Sürek. Rice Production and Research Activities in Turkey. https://om.ciheam.org/om/pdf/c24-2/CI011101.pdf(accessed 1 August 2024).

[14]. van Dijk M., Morley T., Rau ML., et al. A Meta-analysis of Projected Global Food Demand and Population at Risk of Hunger For the Period 2010–2050. *Nature Food* 2021; 27 2021; 2: 494–501.

[15]. Gümüş T., Meriç S., Ayan A., et al. Plant Abiotic Stress Factors: Current Challenges of Last Decades and Future Threats. *Plant Abiotic Stress Responses and Tolerance Mechanisms* 2023; DOI: 10.5772/intechopen.110367.

[16]. Çelik Ö., Meriç S., Ayan A., et al. Epigenetic Analysis of WRKY Transcription Factor Genes in Salt Stressed Rice (*Oryza sativa* L.) plants. *Environmental and Experimental Botany*; 2019; 159 DOI: 10.1016/j.envexpbot.2018.12.015.

[17]. Sairam R., Physiology and Molecular Biology of Salinity Stress Tolerance in Plants. *Current Science* 2004 86:3, 407-421.

[18]. Zhu JK. Plant Salt Tolerance. *Trends Plant Science* 2001; 6: 66–71.

[19]. Tunç E., Tekin MS., Demir M., et al. Halophytic Species in Natural Areas Close to Agricultural Areas of Araban (Gaziantep, Turkey). *Journal of Agricultural Chemistry and Environment* 2020; 9: 48–58.

[20]. Sadak MS. Physiological Role of Trehalose on Enhancing Salinity Tolerance of Wheat Plant. *Bulletin of the National Research Centre*. 2019 431 2019; 43: 1–10.

[21]. Liang L., Guo L., Zhai Y., et al. Genome-wide Characterization of SOS1 Gene Family in Potato (*Solanum tuberosum*) and Expression Analyses Under Salt and Hormone Stress. *Frontiers in Plant Science*. 2023; 14: 1201730.

[22]. Solis CA, Yong MT, Zhou M, et al. Evolutionary Significance of NHX Family and NHX1 in Salinity Stress Adaptation in the Genus Oryza. *Int J Mol Sci*; 23. Epub ahead of print 1 February 2022. DOI: 10.3390/IJMS23042092.

[23]. Hamamoto S., Horie T., Hauser F., et al. HKT Transporters Mediate Salt Stress Resistance in Plants: From Structure and Function to the Field. *Current Opinion in Biotechnology* 2015; 32: 113–120.

[24]. Stewart RRC., Bewley JD. Lipid-Peroxidation Associated with Accelerated Aging of Soybean Axes. *Plant Physiology*. 1980; 65: 245–248.

[25]. Bates LS., Waldren RP., Teare ID. Rapid Determination of Free Proline for Water-Stress Studies. *Plant and Soil*. 1973; 39: 205–207.

[26]. Hoagland DR., Arnon DI. The Water-Culture Method for Growing Plants Without Soil. *Circular. California Agricultural Experiment Station*; 347:39.

[27]. Vaidyanathan H., Sivakumar P., Chakrabarty R., et al. Scavenging of Reactive Oxygen Species in NaCl-stressed Rice (*Oryza sativa* L.) - Differential Response in Salt-Tolerant and Sensitive Varieties. *Plant Science*. 2003; 165: 1411–1418.

[28]. Demiral T., Türkan İ. Comparative Lipid Peroxidation, Antioxidant Defense Systems and Proline Content in Roots of Two Rice Cultivars Differing in Salt Tolerance. *Environmental and Experimental Botany*. 2005; 53: 247–257.

[29]. Chen G., Zheng D., Feng N., et al. Physiological Mechanisms of ABA-induced Salinity Tolerance in Leaves and Roots of Rice. *Scientific Reports*. 2022 121; 12: 1–26.

[30]. Atta K., Mondal S., Gorai S., et al. Impacts of Salinity Stress on Crop Plants: Improving Salt Tolerance Through Genetic and Molecular Dissection. *Frontiers in Plant Science*. 2023; 14: 1241736.

[31]. Hariadi YC., Nurhayati AY., Soeparjono S., et al. Screening Six Varieties of Rice (*Oryza sativa*) for Salinity Tolerance. *Procedia Environmental Sciences*. 2015; 28: 78–87.

[32]. Harizanova A., Koleva-Valkova L. Effect of Silicon on Photosynthetic Rate and the Chlorophyll Fluorescence Parameters at Hydroponically Grown Cucumber Plants Under Salinity Stress. *Journal of Central European Agriculture*. 2019; 20: 953–960.

[33]. Jamil M., Bashir S., Anwar S., et al. Effect of Salinity on Physiological and Biochemical Characteristics of Different Varieties of Rice. *Pakistan Journal of Botany*. 2012; 44: 7–13.

[34]. Saddiq MS., Iqbal S., Hafeez MB., et al. Effect of Salinity Stress on Physiological Changes in Winter and Spring Wheat. *Agronomy*. 2021; 11: 1193.

[35]. Gadelha CG., Coutinho ÍAC., Pinheiro SK de P., et al. Sodium Uptake and Transport Regulation, and Photosynthetic Efficiency Maintenance as the Basis of Differential Salt Tolerance in Rice Cultivars. *Environmental and Experimental Botany*. 2021; 192: 104654.

[36]. Chen F., Chen S., Guo W., et al. Salt Tolerance Identification of Three Species of *Chrysanthemums*. *Acta Horticulturae* 2003; 618: 299–305.

[37]. Orcan P. Tuz Stresine Maruz Bırakılan Çeltik (*Oryza sativa* L.) Çeşitlerinde Radikal Söndürme ve Antioksidan Enzim Aktiviteleri, *Dicle Üniversitesi Fen Bilimleri Enstitüsü* http://acikerisim.dicle.edu.tr/xmlui/handle/11468/3582 (2017, accessed 1 August 2024).

[38]. Alharby HF., Al-Zahrani HS., Hakeem KR., et al. Identification of Physiological and Biochemical Markers for Salt (NaCl) Stress in the Seedlings of Mungbean [*Vigna radiata* (L.) Wilczek] genotypes. *Saudi Journal of Biological Science*. 2019; 26: 1053–1060.

[39]. Munns R., Tester M. Mechanisms of Salinity Tolerance. *Annual Review of Plant Biology* 2008; 59: 651–681.

[40]. Hu C hong, Zheng Y., Tong C ling, et al. Effects of Exogenous Melatonin on Plant Growth, Root Hormones and Photosynthetic Characteristics of Trifoliate Orange Subjected to Salt Stress. *Plant Growth Regulation*. 2022; 97: 551–558.

[41]. Mohamed HI., Latif HH. Improvement of Drought Tolerance of Soybean Plants by Using Methyl Jasmonate. *Physiology and Molecular Biology of Plants.* 2017; 23: 545–556.

[42]. Meriç S., Ayan A., Atak Ç. Molecular Abiotic Stress Tolerans Strategies: From Genetic Engineering to Genome Editing Era. *Abiotic Stress Plants*. 2020. DOI: 10.5772/Intechopen.94505.

[43]. Chunthaburee S., Dongsansuk A., Sanitchon J., et al. Physiological and Biochemical Parameters for Evaluation and Clustering of Rice Cultivars Differing in Salt Tolerance at Seedling Stage. *Saudi Journal of Biological Sciences*. 2016; 23: 467–477.

[44]. Suzuki N., Koussevitzky S., Mittler R., et al. ROS and Redox Signalling in the Response of Plants to Abiotic Stress. *Plant Cell & Environment.* 2012; 35: 259–270.

[45]. Rasel M., Tahjib-Ul-Arif M., Hossain MA., et al. Discerning of Rice Landraces (*Oryza sativa* L.) for Morpho-physiological, Antioxidant Enzyme Activity, and Molecular Markers' Responses to Induced Salt Stress at the Seedling Stage. *Journal of Plant Growth Regulation.* 2020; 39: 41–59.

[46]. Khan MH., Panda SK. Alterations in Root Lipid Peroxidation and Antioxidative Responses in Two Rice Cultivars Under NaCl-salinity Stress. *Acta Physiologiae Plantarum.* 2008; 30: 81–89.

[47]. Singh A., Sengar RS., Shahi UP., et al. Prominent Effects of Zinc Oxide Nanoparticles on Roots of Rice (*Oryza sativa* L.) Grown under Salinity Stress. *Stresses.* 2023; 3: 33–46.

[48]. Hossen MS., Karim MF., Fujita M., et al. Comparative Physiology of Indica and Japonica Rice under Salinity and Drought Stress: An Intrinsic Study on Osmotic Adjustment, Oxidative Stress, Antioxidant Defense and Methylglyoxal Detoxification. *Stresses* 2022; 2: 156–178.

[49]. Wu J., Yu C., Huang L., et al. A Rice Transcription Factor, OsMADS57, Positively Regulates High Salinity Tolerance in Transgenic *Arabidopsis thaliana* and *Oryza sativa* plants. *Physiologia Plantarum* 2021; 173: 1120–1135.

[50]. Çelik Ö., Çakır BC., Atak Ç. Identification of the Antioxidant Defense Genes Which May Provide Enhanced Salt Tolerance in *Oryza sativa* L. *Physiology and Molecular Biology of Plants.* 2019; 25: 85–99.

[51]. Ramon M., Rolland F., Thevelein JM., et al. ABI4 Mediates the Effects of Exogenous Trehalose on Arabidopsis Growth and Starch Breakdown. *Plant Molecular Biology* 2007. 63: 195–206.

[52]. Saidi A., Hajibarat Z. Computatıonal Analysis and Expression Study of Trehalose 6-Phosphate Synthase (TPS) in Rice (*Oryza sativa*), http://bar.utoronto.ca/welcome.htm (2021, accessed 5 August 2024).

[53]. Lu X., Liu X., Xu J., et al. Strigolactone-Mediated Trehalose Enhances Salt Resistance in Tomato Seedlings. *Horticulturae.* 2023; 9: 770.

[54]. Li HW., Zang BS., Deng XW., et al. Overexpression of the Trehalose-6-Phosphate Synthase Gene OsTPS1 Enhances Abiotic Stress Tolerance in Rice. *Planta.* 2011; 234: 1007–1018.

[55]. Avonce N., Leyman B., Mascorro-Gallardo JO., et al. The Arabidopsis Trehalose-6-P Synthase AtTPS1 Gene is a Regulator of Glucose, Abscisic Acid, and Stress Signaling. *Plant Physiol* 2004; 136: 3649–3659.

[56]. Fukuda A., Nakamura A., Tagiri A., et al. Function, Intracellular Localization and the Importance in Salt Tolerance of a Vacuolar Na^+/H^+ Antiporter from Rice. *Plant and Cell Physiology.* 2004; 45: 146–159.

[57]. Chen M., Chen QJ., Niu XG., et al. Expression of OsNHX1 Gene in Maize Confers Salt Tolerance and Promotes Plant Growth in the Field. *Plant, Soil and Environment.* 2007; 53: 490–498.

[58]. Yarra R. The Wheat NHX Gene Family: Potential Role in Improving Salinity Stress Tolerance of Plants. *Plant Gene* 2019; 18: 100178.

[59]. Pabuayon ICM., Jiang J., Qian H., et al. Gain-of-function Mutations of AtNHX1 Suppress SOS1 Salt Sensitivity and Improve Salt Tolerance in Arabidopsis. *Stress Biology.* 2021; 1: 1–19.

[60]. Liu M., Song X., Jiang Y. Growth, Ionic Response, and Gene Expression of Shoots and Roots of Perennial Ryegrass Under Salinity Stress. *Acta Physiologiae Plantarum.* 2018; 40: 1–8.

[61]. Silva EN., Silveira JAG., Rodrigues CRF., et al. Physiological Adjustment to Salt Stress in *Jatropha curcas* is Associated With Accumulation of Salt Ions, Transport and Selectivity of K^+, Osmotic Adjustment and K^+/Na^+ Homeostasis. *Plant Biology*. 2015; 17: 1023–1029.

[62]. Zhang Y., Fang J., Wu X., et al. Na+/K+ Balance and Transport Regulatory Mechanisms in Weedy and Cultivated Rice (*Oryza sativa* L.) Under Salt Stress. *BMC Plant Biology.* 2018; 18: 1–14.

[63]. Qiu QS., Guo Y., Dietrich MA., et al. Regulation of SOS1, A Plasma Membrane Na^+/H^+ Exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. *Proceedings of the National Academy of Sciences.* 2002; 99: 8436–8441.

[64]. Shi H., Quintero FJ., Pardo JM., et al. The Putative Plasma Membrane Na^+/H^+ Antiporter SOS1 Controls Long-Distance Na^+ Transport in Plants. *Plant Cell.* 2002; 14: 465–477.

[65]. Calzone A., Cotrozzi L., Pellegrini E., et al. Can the Transcriptional Regulation of NHX1, SOS1 and HKT1 Genes Handle the Response of Two Pomegranate Cultivars to Moderate Salt Stress?: Salt-tolerance of Two Pomegranate Cultivars. *Scientia Horticulturae.* 2021; 288: 110309.

[66]. Wu H. Plant Salt Tolerance and Na^+ Sensing and Transport. *Crop Journal.* 2018; 6: 215–225.

[67]. Omisun T., Sahoo S., Saha B., et al. Relative Salinity Tolerance of Rice Cultivars Native to North East India: A Physiological, Biochemical and Molecular Perspective. *Protoplasma.* 2018; 255: 193–202.

[68]. Theerawitaya C., Tisarum R., Samphumphuang T., et al. Expression Levels of the Na^+/K^+ Transporter OsHKT2;1 and Vacuolar Na^+/H^+ Exchanger OsNHX1, Na Enrichment, Maintaining the Photosynthetic Abilities and Growth Performances of Indica Rice Seedlings Under Salt Stress. *Physiology and Molecular Biology of Plants.* 2020; 26: 513–523.