한작지(Korean J. Crop Sci.), 63(4): 314~321(2018)
DOI : https://doi.org/10.7740/kjcs.2018.63.4.314

ISSN 0252-9777(Print)
ISSN 2287-8432(Online)

Original Research Article

Physiological and Genetic Responses of Salt-stressed Tunisian Durum (*Triticum turgidum* ssp. *durum*) Cultivars

Sang Heon Kim[1], Dae Yeon Kim[2], Inés Yacoubi[3], and Yong Weon Seo[4,5,†]

ABSTRACT Durum (*Triticum turgidum* L. ssp. *durum*) is a major crop species cultivated for human consumption worldwide. In Tunisia, salt stress is one of the main problems that limit crop production. 'Mahmoudi' was selected as the most salt-sensitive out of 11 Tunisian durum cultivars. Using the salt-tolerant cultivar 'Om Rabia', resistant and susceptible cultivars were evaluated to compare genetic responses under salt stress. At the fully expanded third leaf stage, salt stress was applied by submerging the pots in 500 mM NaCl for 5 min every day for saline water irrigation in the greenhouse. The treatment was applied for 1 week and salt stress tolerance was determined by changes of growth parameters to the control condition. The salt tolerance trait index and salt tolerance index were calculated and used as selection criteria. The expression levels of *TdHKT1;4*, *TdHKT1;5*, and *TdSOS1* were examined using qPCR. For further evaluation of physiological responses, salt stress (150 mM NaCl) was additionally applied for 48 h at the fully expanded third-leaf stage. Increased expression of the genes responsible for salt tolerance and proline content in tolerant durum can be used to broaden genetic diversity and provide genetic resources for the durum breeding program.

Keywords : durum, growth parameter, proline, salt tolerance, Tunisian durum

Soil salinity is one of the most serious abiotic stress which is inhibiting plant growth and organ development in the world (Himabindu *et al.*, 2016). It is our perception that the region affected by salt stress is expanding (Machado & Serralheiro, 2017). Especially, in arid and semi-arid regions, crop production can be reduced by salt stress (Munns & Gilliham, 2015). Development of salt tolerant crops in cope with salt stress is significantly important for preventing an unfavorable influence on agricultural productivity (Mian *et al.*, 2011).

Wheat (*Triticum* spp.) is a basic food crop for about 40% of people in the world (Li *et al.*, 2018). Not only hexaploid wheat (*AABBDD*, 2n=42, *T. aestivum*) but also durum (*AABB*, 2n=28, *T. turgidum* L. ssp. *durum*) is mainly cultivated and used for production of various foods in the Mediterranean Basin and North Africa (Munns *et al.*, 2012; Soriano *et al.*, 2018). Tunisia is one of the countries located on this region and has three kinds of climate such as a Mediterranean, a semi-arid to arid, and a dry dessert from northern part to southern geographic region (Kim *et al.*, 2014; Mansour & Hachicha, 2014). Soil salinity is a considerable problem in the many parts of Tunisia where durum is mainly cultivated (Brini *et al.*, 2009; Kim *et al.*, 2016). Therefore, the increasing problem of water shortage in the middle and southern parts of Tunisia resulted in soil salinity and reduced arable land area.

Salt tolerance is a complicated trait but includes usually three major mechanisms such as osmotic tolerance, Na^+ exclusion, and tissue tolerance (Wu *et al.*, 2015). Through the transpiration stream, Na^+ accumulates in leaf blade, one of the main factors that affect to grain yield. Therefore, Na^+ exclusion in shoots is a crucial factor of salt tolerance

[1)]Ph.D. Candidate, Department of Biosystems and Biotechnology, Korea University, Seoul 02841, Korea
[2)]Research Professor, Division of Biotechnology, Korea University, Seoul 02841, Korea
[3)]Associate Professor, Centre of Biotechnology of Sfax, B.P K.3038 Sfax, Tunisia
[4)]Professor, Department of Biosystems and Biotechnology, Korea University, Seoul 02841, Korea
[5)]Professor, Division of Biotechnology, Korea University, Seoul 02841, Korea

[†]Corresponding author: Yong Weon Seo; (Phone) +82-2-3290-3005; (E-mail) seoag@korea.ac.kr
<Received 8 September, 2018; Revised 1 October, 2018; Accepted 2 October, 2018>

to avoid the toxicity in leaf blade by Na^+ accumulation (Alqudah *et al.*, 2018; Arabbeigi *et al.*, 2014; Munns, 2002). The *high-affinity* K^+ *transporter* (*HKT*) and the *salt overly sensitive 1* (*SOS1*) are involved in accumulation of Na^+ in shoots process, reduction of the long distance transport of Na^+ and energy loss on Na^+ exclusion, which minimize shoot damage (Munns & Tester, 2008). Many other studies have demonstrated that those genes are involved in salt tolerance and enhance the salt tolerance of wheat (Amar *et al.*, 2014; Huang *et al.*, 2008; Sathee *et al.*, 2015; Wu *et al.*, 2015).

Proline accumulates in plant cells under salt stress and exogenous proline reduces the harmful effects of salt stress (Hoque *et al.*, 2007). Plant response to salt stress by generating antioxidants which have the ability to detoxify reactive oxygen species (ROS) that are detrimental to plant development (Ashraf, 2009). Therefore, they can be used as one of potential selection criteria of salt tolerance.

In this study, Tunisian durum cultivars were evaluated for their salt tolerance by phenotypic traits. The selected salt tolerant and susceptible cultivars were analyzed for genetic and physiological responses to salt stress. The obtained results could be applied in durum breeding for salt tolerance in arid- or semi-arid regions where soil salinity is an important problem.

MATERIALS AND METHODS

Experiment I: evaluation of durum for salt tolerance

Plant materials and experimental conditions

Eleven Tunisian durum cultivars were evaluated for salt tolerance (Table 1). The seeds were kindly provided by the National Plant Germplasm System (NPGS), United States Department of Agriculture (USDA/ARS). The basic agronomic information on each cultivar was collected from the Agricultural Research Service-Germplasm Resources Information Network (ARS-GRIN) (https://npgsweb.ars-grin.gov/).

The salt tolerance of each cultivar was evaluated in a greenhouse at Korea University's Research Farm (Namyangju-si, Gyeonggi-do, Korea) during the 2014 growing season. Seeds were germinated at 4°C for 2 weeks and each plant was transplanted to a pot (5 × 8 / 5 cm × 5 cm × 16 cm height) filled with soil (50 g dry weight; Sunshine mix #1, Sun Gro Horticulture, Canada). Five plants from each cultivar was allocated to either the non-treated control or treated group. All plants were grown with sufficient irrigation until the beginning of the salt treatment. At the fully expanded 3rd leaf stage (Zadok scale 13), plants in the treated group were subjected to salt stress by submerging the pots in 500 mM NaCl solution for 5 min every 2 days. Plants in the non-treated control received normal water every 2 days. The treatment was applied for 2 weeks. After the treatment, all plants were given sufficient water and grown until spike harvest.

Table 1. Durum cultivars used to evaluate salt tolerance.

Accession Number	Name	Species	Supplier	Improve status	STI (%)
PI 41041	Agili Pubescent	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	73.2
PI 41049	Allemand	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	83.6
PI 185195	Sbei 7	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	76.7
PI 189778	Chili	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	72.1
PI 306572	Chili 931	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	83.4
PI 306573	Mahmoudi	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	66.5
PI 324939	Inrat 69	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	83.5
PI 433749	Amal 72	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	88.3
PI 433756	Inrat 69	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	74.5
PI 433758	Maghrebi 72	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	90.6
PI 520062	Maghrebi 72	*Triticum turgidum* ssp. *durum*	NPGS, USDA/ARS	Cultivar	83.7

Electrical conductivity (EC) of the soil was measured at the start and the end of treatment using a Direct Soil Activity and Solution Conductivity Measurement Kit (HI 993310, Hanna Instruments, Woonsocket, RI, USA) with a stainless EC probe (HI 76305, Hanna Instruments, Woonsocket, RI, USA). EC of soils in both control and treatment conditions were measured as 0.35±0.09 dS/m at the start of the treatment, whereas at the end of the treatment, those in control condition and in treatment condition were measured as 0.54±0.15 dS/m and 10.24±0.85 dS/m, respectively.

The phenotypic parameters such as plant height, average leaf length, number of tillers, and leaf chlorophyll content were scored at the beginning and end of treatment.

Leaf chlorophyll content was estimated by using a portable chlorophyll meter (SPAD-502, Minolta, Japan) which can determine leaf chlorophyll content quickly without damaging leaves (Yıldırım *et al.*, 2010). The salt tolerance trait index (STTI) and salt tolerance index (STI) were computed with the formula used in the previous other studies (Ali *et al.*, 2007; Kim *et al.*, 2016; Shahzad *et al.*, 2012).

$$STTI=\frac{Value\ of\ trait\ under\ treatment\ condition}{Value\ of\ trait\ under\ control\ condition}\times 100$$

$$STI=The\ mean\ of\ STTIs$$

Eleven durum cultivars were identified as either tolerant or sensitive to salt stress based on STI. One sensitive cultivar ‘Mahmoudi’ was selected and used for further analysis comparing with another Tunisian durum cultivar ‘Om Rabia’, which is known for salt-tolerant (provided by Centre of Biotechnology of Sfax, Sfax, Tunisia). ‘Om Rabia’ is considered as salt-tolerant durum cultivar (Amar *et al.*, 2014; Khoufi *et al.*, 2012).

Experiment II: comparison of genetic responses of Tunisian durum cultivars

Plant materials and experimental conditions

The second experiment using two tolerant and susceptible Tunisian durum cultivars was conducted as previous method with some minor modification. The salt-stress treatment was applied for 1 week and the phenotypic parameters such as plant height and leaf chlorophyll content were scored at the beginning and end of treatment. Shoots of each plant from both non-treated and treated groups at the beginning and the end of treatment were sampled to analyze the gene expression.

Gene expression analysis

RNA (ribonucleic acid) was extracted from shoots using the Trizol method. The first strand cDNA was synthesized with the Power cDNA Synthesis Kit (Intron Biotechnology, Seoul, Korea) following the manufacturer’s protocol. The qPCR using the genes associated with the salt tolerance was performed (Table 2).

Experiment III: comparison of physiological responses of Tunisian durum cultivars

Plant materials and experimental conditions

Two Tunisian durum cultivars (‘Om Rabia’ and ‘Mahmoudi’)

Table 2. Primers used in the qPCR analysis.

Genes	Feature	Primer Sequence (5'→3')	Annealing Temperature (ºC)
TaActin	*Internal standard*	F: ACAATGGAACCGGAATGG R: TGTGATGCCAGATTTTCTCC	60
TdHKT1;4-1	*High-affinity K^+ transporter*	F: TCGAGATGGAGGTGTTCTCC R: CTTGCTTCCTCAGCTTGGAC	60
TdHKT1;4-2	*High-affinity K^+ transporter*	F: CAAGAGCACGCTTCTGTCAC R: GGTCCTCCTTGAGCTTTTCC	60
TdHKT1;5-B1	*High-affinity K+ transporter*	F: GCACCACCAGAAAAGGGTAA R: TTGAAGTTGAGGGGGTCATC	60
TdSOS1	*Salt Overly Sensitive*	F: GCCTTGCAAGTCAGCATGTA R: GAAGGCACCTTTGGATACGA	60

were also used in the third experiment. Seeds were germinated at 4°C for 2 weeks and each plant was transplanted to the magenta boxes containing 1000-fold diluted Hyponex solution (Type: 6-10-5, Hyponex, Japan). Ten plants in each magenta box were grown with refreshing Hyponex solution every day until the beginning of the salt treatment. At the fully expanded 3rd leaf stage (Zadok scale 13), plants were subjected to salt stress by changing the solution to the saline solution (150 mM NaCl). The saline solution was renewed every day and the salt-stress treatment was applied for 48 hours. Leaf blades of each cultivar were sampled at both the beginning and the end of the treatment.

Crude protein/enzyme extraction

Crude protein/enzyme extraction was carried out according to Chen & Zhang (2016) with some modifications. Leaf blades were grinded with mortar and pestle in liquid nitrogen. The leaf powder (0.1 g) were homogenized in 1.5 ml of 100 mM sodium phosphate buffer (PBS, pH 7.8) on ice and centrifuged at 14,000 rpm for 20 min at 4°C. The supernatant was 10-fold diluted with 100mM PBS (pH 7.8) and used to measurement of proline content and Superoxide dismutase (SOD) activity.

Proline content measurement

The proline content was measured according to Chen & Zhang (2016) with some modifications. 50 µl of 10-fold diluted crude protein/enzyme extract was mixed with 1 ml of the reaction solution which contains 0.25 ml of 3% sulphosalicylic acid, 0.25 ml of glacial acetic acid, and 0.5 ml of 2.5% acid-ninhydrin. The reaction mixture was boiled for 15 min and cool down on ice for 5 min. The absorbance was recorded at 520 nm. A standard curve of known concentrations of L-proline was applied to determine the proline content in samples. The results were calculated as μg/g FW (Fresh weight).

Superoxide dismutase (SOD) activity analysis

SOD activity was analyzed according to Chen & Zhang (2016) with some modifications. 180 µl of 10-fold diluted crude protein/enzyme extract was mixed with 20 µl of the reaction solution which contains 2 µl of 1 mM EDTA-2Na, 6 µl of 130 mM methionine, 6 µl of 750 µM nitroblue tetrazolium (NBT), and 6 µl of 20 µM riboflavin. The activity of SOD was analyzed by evaluating its ability to inhibit photochemical reduction of NBT at 560 nm.

RESULTS

Experiment I: evaluation of durum for salt tolerance

The magnitudes of plant growth parameters of plants in both control and treatment conditions were evaluated at the initiation and end of salt stress. After 14 days of salt treatment, plants in treatment condition showed decreased plant height, average leaf length, number of tillers, and leaf chlorophyll contents compared to those in control condition (Supplementary Table 1). 'Mahmoudi (PI306573)' showed the lowest STI (66.5%) whereas 'Magrhrebi 72' (PI 433758) showed the highest STI (90.6%) (Table 1).

Experiment II: comparison of genetic responses of two Tunisian durum cultivars

Both 'Om Rabia' and 'Mahmoudi' under salt stress condition showed reduced plant height compared to those in control condition after 7 days of salt treatment (Fig. 1). However, the difference of plant heights of 'Mahmoudi' between control and treatment conditions was larger than that of 'Om Rabia'. Leaf chlorophyll contents in both

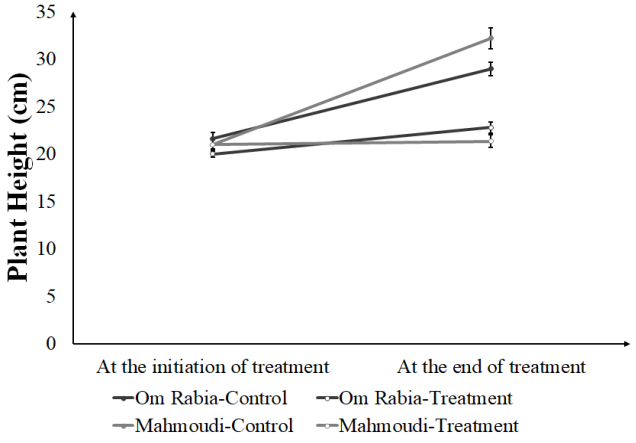

Fig. 1. Average plant heights of 'Om Rabia' and 'Mahmoudi' after 7 days of salt treatment. The values are means (±SE) of five biological replicates. Closed symbols indicate plants grown under control conditions and open symbols indicate plants grown under salt stress (treatment) conditions.

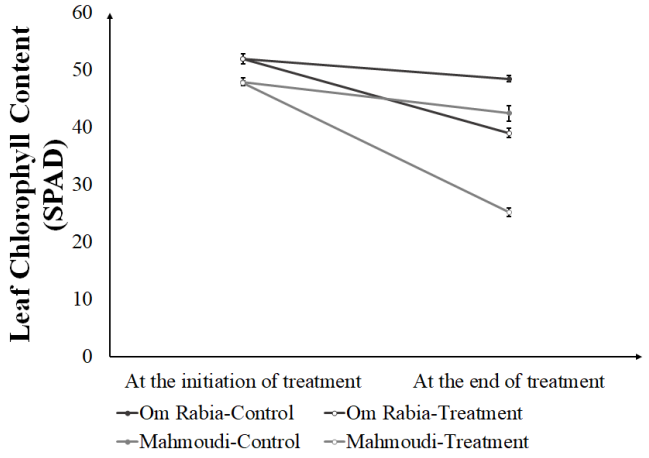

Fig. 2. Average leaf chlorophyll contents of 'Om Rabia' and 'Mahmoudi' after 7 days of salt treatment. The values are means (±SE) of five biological replicates. Closed symbols indicate plants grown under control conditions and open symbols indicate plants grown under salt stress (treatment) conditions.

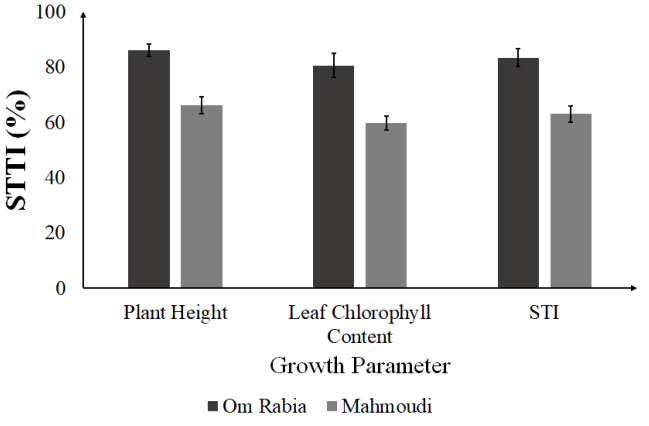

Fig. 3. Salt tolerance trait index (STTI) and salt tolerance index (STI) of 'Om Rabia' and 'Mahmoudi' after 7 days of salt treatment. STI was calculated on the basis of plant height and leaf chlorophyll content, which are the traits affected by salt stress. The values are means (±SE) of five biological replicates.

cultivars were decreased after salt treatment (Fig. 2). 'Mahmoudi' showed much more reduction of leaf chlorophyll content than 'Om Rabia', which is in accordance with the result of plant height. After calculating STTI and STI, 'Om Rabia' showed higher STTI and STI than 'Mahmoudi' (Fig. 3). These results indicated that 'Om Rabia' is more tolerant to salt stress than 'Mahmoudi'

The transcription levels of the genes associated with salt tolerance were analyzed by qPCR (Fig. 4). Both cultivars

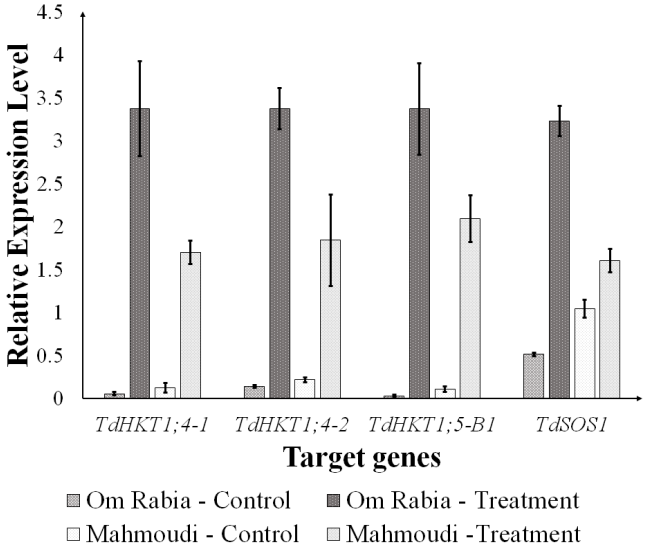

Fig. 4. Relative expression levels of the genes associated with salt tolerance on the basis of the qPCR analysis. *TdHKT1;4*, *TdHKT1;5*, and *TdSOS1* are the genes associated with salt tolerance in durum. The values are means (±SE) of three replicates.

showed low expression levels in control condition but high expression levels in treatment condition. In the treatment condition, 'Om Rabia' showed significantly higher expression levels than 'Mahmoudi', which resulted in greater expression differences in 'Om Rabia' than 'Mahmoudi' between control and treatment condition.

Experiment III: comparison of physiological responses of two Tunisian durum cultivars

After 48 hours of salt stress treatment, proline contents in leaf blade were increased in both 'Om Rabia' and 'Mahmoudi' (Fig. 5). Furthermore, the proline content of 'Om Rabia' was increased more compared to that of 'Mahmoudi'. There was no significant difference in SOD activities in leaf blades between initiation and end of salt treatment (Fig. 6).

DISCUSSION

Some of the physiological and phenotypic parameters were reduced in their magnitudes under the salt stress. Shafi *et al.* (2009) indicated that chlorophyll content of leaf and plant height were reduced under salt stress compared with non-stressed condition. Also, both tiller

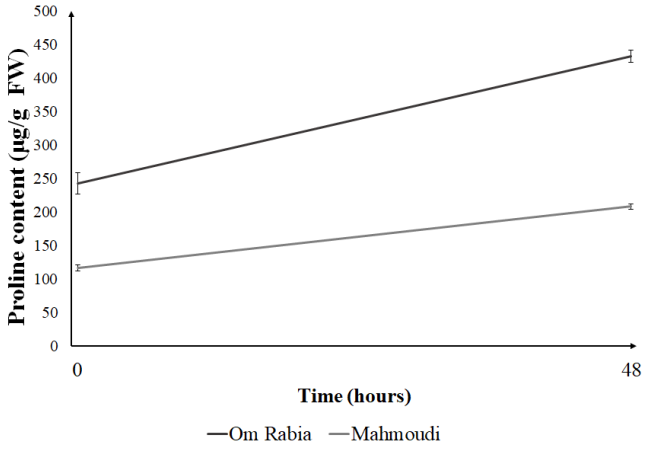

Fig. 5. Proline content in the leaf blades of 'Om Rabia' and 'Mahmoudi' after 48 h of salt treatment. Proline content was increased in both cultivars after 48 h of salt stress treatment. The values are means (±SE) of five technical replicates.

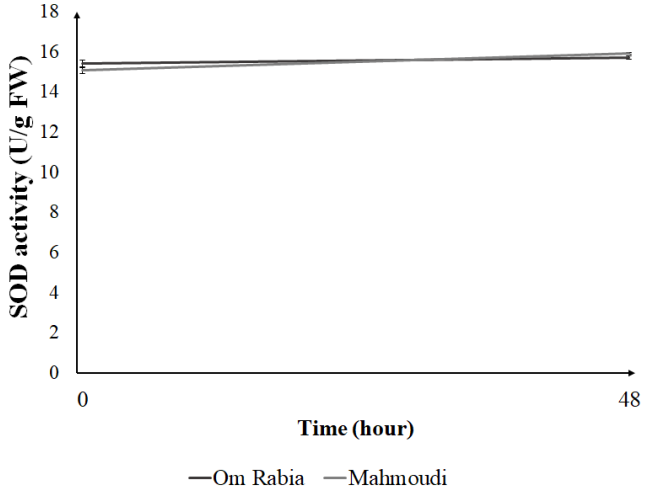

Fig. 6. SOD activity in leaf blades of 'Om Rabia' and 'Mahmoudi' after 48 h of salt treatment. No significant differences in SOD activities were observed between initiation and end of the salt treatment. The values are means (±SE) of five technical replicates.

numbers and leaf length were decreased under salt stress (El-Hendawy *et al.*, 2005; Saqib *et al.*, 2012). Within these parameters, chlorophyll content of leaf is considered as more critical indicator to salt stress susceptibility/ tolerance than other parameters (Kim *et al.*, 2016). Therefore, evaluation of salt tolerance based on the STI of these parameters is a valid method that it can be utilized in breeding programs

The transcription levels of the genes, *TdHKT1;4*, *TdHKT1;5*, and *TdSOS1* were analyzed by qPCR (Fig. 4). Those genes are associated with accumulation process of Na^+ in shoots and lessen the impairment in shoot (Munns & Tester, 2008). James *et al.* (2011) identified that the salt tolerance of common wheat was improved by the introgression of *HKT1;4* and *HKT1;5* genes from *T. monococcum* into common wheat. Cotsaftis *et al.* (2012) reported that higher expressions of *HKT1;4*, involved in sheath to blade transfer of Na^+, and *HKT1;5*, involved in root to shoot transfer of Na^+, can protect the photosynthetic part of shoot in rice. Overexpression of *SOS1* in *Arabidopsis* showed enhanced salt tolerance (Shi *et al.*, 2003). Thus, the accessions that have higher expression levels of those genes can have more tolerance to salt stress.

Proline contents in leaf blade were increased in both cultivars but the salt-tolerant cultivar 'Om Rabia' showed more increased content compared to that of susceptible 'Mahmoudi'. Hoque *et al.* (2007) reported that proline accumulates in plant cells under salt stress and exogenous proline reduces the detrimental effects of salt stress. Therefore, the accessions, which can produce much more proline under salt stress, could be used as crossing materials to develop the salt tolerant cultivars.

Sairam *et al.* (2005) reported that salt-tolerant cultivars displayed higher increment in SOD. However, in this study, there was no significant difference of SOD in leaf blade between both cultivars after 48 hours of salt-stress treatment. The severity and duration of stress as well as cultivar difference should be associated with SOD activity.

In this study, Tunisian durum cultivars were evaluated for their salt tolerance. Also, genetic and physiological responses of either tolerance or susceptible cultivars to salt stress were analyzed. Most of obtained results were in accordance with other previous studies, which suggest the future direction to develop salt-tolerant durum cultivars. The results acquired in this study might enhance breeding programs for salt tolerance in durum.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT) (No. NRF-2017

K1A3A1A09085568).

REFERENCES

Ali, Z., A. Salam, F. M. Azhar, and I. A. Khan. 2007. Genotypic variation in salinity tolerance among spring and winter wheat (*Triticum aestivum* L.) accessions. South African Journal of Botany 73 : 70-75.

Alqudah, A. M., H. M. Youssef, A. Graner, and T. Schnurbusch. 2018. Natural variation and genetic make-up of leaf blade area in spring barley. Theoretical and Applied Genetics 131: 873-886.

Amar, S. B., F. Brini, H. Sentenac, K. Masmoudi, and A. -A. Véry. 2014. Functional characterization in *Xenopus* oocytes of Na^+ transport systems from durum wheat reveals diversity among two *HKT1;4* transporters. Journal of Experimental Botany 65 : 213-222.

Arabbeigi, M., A. Arzani, M. M. Majidi, R. Kiani, B. E. S. Tabatabaei, and F. Habibi. 2014. Salinity tolerance of *Aegilops cylindrica* genotypes collected from hyper-saline shores of Uremia Salt Lake using physiological traits and SSR markers. Acta Physiologiae Plantarum 36 : 2243-2251.

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnology Advances 27 : 84-93.

Brini, F., I. Amara, K. Feki, M. Hanin, H. Khoudi, and K. Masmoudi. 2009. Physiological and molecular analyses of seedlings of two Tunisian durum wheat (*Triticum turgidum* L. subsp *Durum* [Desf.]) varieties showing contrasting tolerance to salt stress. Acta Physiologiae Plantarum 31 : 145-154.

Cotsaftis, O., D. Plett, N. Shirley, M. Tester, and M. Hrmova. 2012. A two-staged model of Na^+ exclusion in rice explained by 3D modeling of HKT transporters and alternative splicing. PLoS One 7 : e39865.

El-Hendawy, S. E., Y. C. Hu, G. M. Yakout, A. M. Awad, S. E. Hafiz, and U. Schmidhalter. 2005. Evaluating salt tolerance of wheat genotypes using multiple parameters. European Journal of Agronomy 22 : 243-253.

Himabindu, Y., T. Chakradhar, M. C. Reddy, A. Kanygin, K. E. Redding, and T. Chandrasekhar. 2016. Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes. Environmental and Experimental Botany 124 : 39-63.

Hoque, M. A., E. Okuma, M. N. A. Banu, Y. Nakamura, Y. Shimoishi, and Y. Murata. 2007. Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. Journal of Plant Physiology 164 : 553-561.

Huang, S., W. Spielmeyer, E. S. Lagudah, and R. Munns. 2008. Comparative mapping of *HKT* genes in wheat, barley, and rice, key determinants of Na^+ transport, and salt tolerance. J. Exp. Bot. 59 : 927-937.

James, R. A., C. Blake, C. S. Byrt, and R. Munns. 2011. Major genes for Na^+ exclusion, *Nax1* and *Nax2* (wheat *HKT1;4* and *HKT1;5*), decrease Na^+ accumulation in bread wheat leaves under saline and waterlogged conditions. Journal of Experimental Botany 62 : 2939-2947.

Khoufi, S., K. Khamassi, J. A. T. da Silva, R. Chaabane, and M. B. B. Naceur. 2012. Morphological and molecular characterization of six of the most frequently cultivated hard wheat varieties in Tunisia. Journal of Plant Breeding and Crop Science 4 : 106-114.

Kim, S. H., D. Y. Kim, I. Yacoubi, and Y. W. Seo. 2014. Phenotypic and Genotypic Analyses of Drought Tolerance in Korean and Tunisian Wheat Cultivars. Plant Breeding and Biotechnology 2 : 139-150.

Kim, S. H., J. Y. Kim, D. Y. Kim, J. S. Yoon, W. J. Jung, I. Yacoubi, and Y. W. Seo. 2016. Development of a SCAR marker associated with salt tolerance in durum wheat (*Triticum turgidum* ssp. *durum*) from a semi-arid region. Genes & Genomics 38(10) : 1-10.

Li, C. X., W. G. Xu, R. Guo, J. Z. Zhang, X. L. Qi, L. Hu, and M. Z. Zhao. 2018. Molecular marker assisted breeding and genome composition analysis of Zhengmai 7698, an elite winter wheat cultivar. Scientific Reports 8 : 322.

Machado, R. M. A. and R. P. Serralheiro. 2017. Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. Horticulturae 3: 30.

Mansour, M. and M. Hachicha. 2014. The Vulnerability of Tunisian Agriculture to Climate Change. Emerging Technologies and Management of Crop Stress Tolerance : 485-500.

Mian, A., R. J. Oomen, S. Isayenkov, H. Sentenac, F. J. Maathuis, and A. A. Very. 2011. Over-expression of an Na^+-and K^+-permeable HKT transporter in barley improves salt tolerance. Plant J. 68 : 468-479.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant, Cell & Environment 25 : 239-250.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant. Biol. 59 : 651-681.

Munns, R. and M. Gilliham. 2015. Salinity tolerance of crops - what is the cost? New Phytol. 208 : 668-673.

Munns, R., R. A. James, B. Xu, A. Athman, S. J. Conn, C. Jordans, C. S. Byrt, R. A. Hare, S. D. Tyerman, M. Tester, D. Plett, and M. Gilliham. 2012. Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. Nature Biotechnology 30 : 360-U173.

Sairam, R., G. Srivastava, S. Agarwal, and R. Meena. 2005. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. Biologia Plantarum 49 : 85.

Saqib, Z. A., J. Akhtar, M. A. Ul-Haq, and I. Ahmad. 2012. Salt induced changes in leaf phenology of wheat plants are

regulated by accumulation and distribution pattern of Na^+ Ion. Pak. J. Agr. Sci. 49 : 141-148.

Sathee, L., R. K. Sairam, V. Chinnusamy, and S. K. Jha. 2015. Differential transcript abundance of salt overly sensitive (SOS) pathway genes is a determinant of salinity stress tolerance of wheat. Acta Physiologiae Plantarum 37 : 169.

Shafi, M., J. Bakht, M. J. Hassan, M. Raziuddin, and G. Zhang. 2009. Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (*Triticum aestivum* L.). Bulletin of Environmental Contamination and Toxicology 82 : 772-776.

Shahzad, A., M. Ahmad, M. Iqbal, I. Ahmed, and G. M. Ali. 2012. Evaluation of wheat landrace genotypes for salinity tolerance at vegetative stage by using morphological and molecular markers. Genetics and Molecular Research 11 : 679-692.

Shi, H., B. H. Lee, S.J. Wu, and J. K. Zhu. 2003. Over-expression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in Arabidopsis thaliana. Nat. Biotechnol. 21 : 81-85.

Soriano, J. M., D. Villegas, M. E. Sorrells, and C. Royo. 2018. Durum Wheat Landraces from East and West Regions of the Mediterranean Basin Are Genetically Distinct for Yield Components and Phenology. Frontiers in Plant Science 9 : 80.

Wu, H., L. Shabala, M. Zhou, G. Stefano, C. Pandolfi, S. Mancuso, and S. Shabala. 2015. Developing and validating a high-throughput assay for salinity tissue tolerance in wheat and barley. Planta 242 : 847-857.

Yıldırım, M., H. Kılıç, E. Kendal, and T. Karahan. 2010. Applicability of Chlorophyll Meter Readings as Yield Predictor in Durum Wheat. Journal of Plant Nutrition 34 : 151-164.