

Effects of Exogenous Linoleic Acid on Barley (*Hordeum vulgare* L.) Seedlings Under Salinity

Cüneyt UÇARLI^{1*}

ABSTRACT: Salt stress adversely affects plants and causes different levels of morphological, physiological, biochemical, and molecular changes at different growth stages. Polyunsaturated fatty acids (PUFAs), such as linoleic acid, are main components of membrane lipids and determine the fluidity and stability of the cell membrane. In addition, PUFAs have a crucial role in maintaining the structure and function of the cell membrane which is damaged by salinity. There may be a relationship between level of PUFAs in membrane lipids and salinity tolerance. The present study was carried out to examine the effects of exogenous application of 0.5 mM linoleic acid (LA) on barley seedlings (*Hordeum vulgare* L. cv. Martı) grown in hydroponic conditions under 160 mM NaCl. The treatment with LA ameliorated the stress generated by NaCl by increasing osmolyte level and decreasing ion leakage percentage and H₂O₂ content within hours. Besides, LA significantly enhanced expression of salt-responsive transcription factor *HvDRF2* and ROS scavenger gene *HvMT2* as 105- and 40-fold, respectively, in the leaves of barley seedlings under salinity conditions. While LA slightly increased the gene expression of ascorbate peroxidase (*HvAPX*), glutathione S-transferase (*HvGST6*) and copper zinc superoxide dismutase (*HvCu/ZnSOD*) in the roots of barley seedlings, the expression of these genes was not changed in the leaves under salinity compared to salt-stressed samples. This study provides novel insights for effects of LA on improvement of salinity tolerance in barley.

Keywords: NaCl, linoleic acid, ion leakage, osmolality, *HvDRF2*, *HvMT2*

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INTRODUCTION

Plants are sessile organisms and constantly subjected to a variety of deleterious environmental stresses including salinity, floods, drought, extreme temperature, fungi, bacteria and insects during their life period. Salinity is an important abiotic stress that negatively affects plant growth and development, and ultimately limits crop productivity worldwide (Chen et al., 2021). Salinity causes dehydration (osmotic stress), ionic toxicity and oxidative stress, which lead to nutrient deficiencies, membrane disorganization, reduced cell division and disruption of key physiological and metabolic processes in plants including biosynthesis of proteins, enzymatic activities, cellular homeostasis, photosynthetic activity, and phytohormone regulation, as well as plant death in extreme cases (Gupta and Huang, 2014; Wani et al., 2020). To cope with salt stress, plants have developed a variety of complex mechanisms including ion transport, compartmentalization of toxic ions, biosynthesis of osmoprotectants, accumulation of antioxidant enzyme, synthesis of antioxidant compounds, and regulation of hormones (Parida and Das, 2005; Zhang et al., 2012). A large number of genes are involved in the response to salt stress, which are generally grouped into two major categories; functional genes such as Na^+/H^+ antiporter (*HvNHX1*), lipoxygenase (*HvLOX1*), dehydrin (*HvDHN3*) and metallothionein (*HvMT2*), and regulatory genes including dehydration-responsive factor (*HvDRF2*) and *WRKY12*.

Salt stress promotes a high accumulation of reactive oxygen species (ROS) leading cause lipid peroxidation, protein oxidation, enzyme inactivation, and DNA damage in the cell (Sharma et al., 2012). In plants, efficient scavenging or detoxification of excess ROS from the cell is accomplished by an efficient ROS defense mechanism antioxidant system consists of enzymatic components and non-enzymatic antioxidants, such as glutathione and ascorbate (Singhal et al., 2021). Catalases (CAT), ascorbate peroxidases (APX), superoxide dismutases (SOD), and glutathione-S-transferases (GST) are important members of enzymatic antioxidants in plants (Gill and Tuteja, 2010; Singhal et al., 2021).

Barley (*Hordeum vulgare* L.) is one of the major cereal crops with annual production over 157 million tons in the world (FAO, 2020). Barley is mainly used for animal feed and malt production, with a smaller quantity being directly consumed by humans. Cultivated barley is grown in a range of diverse environments. Barley has a relatively short life cycle and is a self-pollinated diploid as well as the most salt-tolerant crop among cereals (Munns and Tester, 2008) As a result, it is an ideal model plant in genetic and physiological studies to understand salinity tolerance in cereal crops with its morphological, physiological, and genetic characteristics. (Wu et al., 2013).

Linoleic acid (LA) is one of the most common polyunsaturated fatty acids (PUFAs), the others oleic (18:1), and α -linolenic (18:3) acids, required for normal plant growth. LA (18:2) contains 2 cis double bonds (cis-9, 12) with 18 carbon chains (He and Ding, 2020). LA plays an important role as a membrane ingredient and precursor of distinct bioactive molecules such as jasmonic acid (JA), and is involved in plant defense system against various biotic and abiotic stresses (Sumayo et al., 2014; He et al., 2020). LA has been demonstrated to have a role in the regulation of salt stress in barley, rice and peanut (Zhao and Qin, 2005; Aziz et al., 2015; Sui et al., 2018).

The current study was focused on to evaluate the short period effects of exogenous application of linoleic acid (0.5 mM) in barley seedlings under salinity (160 mM NaCl). The physiological changes on barley (*Hordeum vulgare* L.) seedlings exposed to salts stress with or without LA was determined measuring the osmolyte accumulation, ion leakage percentage and H_2O_2 level in the roots and leaves of barley. Besides, differential gene expression profiles of selected salt-responsive genes including *HvDRF2*, *HvWRKY12*, *HvLOX1*, *HvMT2*, *HvNHX1*, *HvDHN3*, and antioxidant enzyme coding genes (*HvGST6*, *HvCu-Zn/SOD*, *HvAPX*, *HvCAT2*) were determined by qPCR and RT-PCR, respectively.

MATERIALS AND METHODS

Plant material and growth conditions

In the study, barley (*Hordeum vulgare* L. cv. Martı) seeds provided by Trakya Agricultural Research Institute (Edirne, Turkey) were used. First, the barley seeds were germinated on water-moistened filter papers in the dark, then a week-old barley seedlings were transferred into plastic viols (6 cm × 6 cm × 8 cm) including perlite and watered daily with half-strength Hoagland nutrient solution at 25 °C with a 16-h light / 8-h dark photoperiod in a growth chamber until third leaf started to emerge.

Salt and linoleic acid treatments

Three-leaf-stage barley seedlings were transplanted into hydroponic systems including half-strength Hoagland nutrient solution. The salt (160 mM NaCl) and 0.5 mM linoleic acid (Sigma-Aldrich, L5900) were added in Hoagland nutrient solution and barley seedlings were incubated in this solution for 0 (control), 2 and 26 h. The samples of leaves and roots were collected and quickly frozen with liquid nitrogen, then stored at -80 °C until further analyses.

Determination of osmolality and electrolyte leakage

The osmolality of barley leaves and roots were determined according to Uçarlı and Gürel (2020) using semi-micro osmometer (K-7400, Germany). For measurement of electrolyte leakage (EL), 30 mg leaf and root were cut and the samples were washed three times with deionized water (dH₂O) to remove surface-adhered electrolytes. The samples were placed in glass tubes including 5 ml dH₂O and incubated at 25 °C in the dark for 24 h. Immediately afterwards, initial electrical conductivity of the solution (E1) was measured using a conductivity meter (HORIBA Scientific, NJ, USA). The samples were autoclaved at 121 °C for 15 min, then cooled down to room temperature. Immediately, the final electrical conductivity of the solution (E2) was measured. EL was calculated using following formula; $(E1/E2) \times 100$.

Determination of hydrogen peroxide (H₂O₂) content

The aqueous H₂O₂ extracted with PeroxiDetect kit (Sigma, PD1) from leaves and roots of barley seedlings and experiments were conducted according to the manufacturer's protocol. H₂O₂ concentration in the samples was calculated according to the following formula.

$$\text{H}_2\text{O}_2 (\mu\text{M}) = [(A_{560}(\text{sample}) - A_{560}(\text{blank})) \times \text{dilution factor}] / [A_{560}(1 \text{ nmole peroxide}) \times \text{sample}]$$

Total RNA isolation and cDNA synthesis

Total RNA was isolated from 100 mg roots and leaves of barley seedlings using TRIzol® (Invitrogen, 15596-026) according to manufacturer's manual. RNA integrity was confirmed with running of samples on agarose gel stained with ethidium bromide (EtBr). After DNase I treatment (Thermo Scientific, EN0525) to remove any DNA contamination, first strand cDNA was generated from 4 mg of total RNA using SuperScript™ kit (Invitrogen, 11904-018) with oligo(dT)₁₂₋₁₈ primers according to the manufacturer's manual.

Reverse transcription polymerase chain reaction (RT-PCR)

Semi-quantitative analysis of mRNA level of antioxidant genes was performed by RT-PCR with AccessQuick™ kit (Promega, A1702). RT-PCR were carried out 25 µl reaction volume including 0.5 µg of total RNA, 1 µM forward and reverse primers of antioxidant genes (Table 1), 1X master mix and 2.5 U AMV-RT. The thermal cycle profile was 45 °C for 45 min for reverse transcription reaction, 5 min at 95 °C for initial denaturation, 30-35 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 1

min, followed by 72 °C for 5 min extension. The RT-PCR products were run on gel electrophoresis and visualized with EtBr staining.

Quantitative polymerase chain reaction (qPCR)

qPCR was performed according to Uçarlı and Gürel (2020) in a total volume of 10 µL including 100 ng of cDNA, 1 U DNA polymerase (Promega, M830), 0.2 mM dNTPs, 0.2 µM of specific primers of salt-responsive genes (Table 1), and 0.5 µL Eva Green® dye (Biotium, 31000). The thermal cycle profile was 5 min at 95 °C for 5 min, followed by 40 cycles of 10 s at 95 °C, 10 s at 60 °C and 10 s at 72 °C. Melting curve analysis was conducted at the end of cycling with a temperature gradient of 0.11 °C s⁻¹ from 65 to 95 °C. The amplification of barley Actin gene was used to normalize the mRNA levels of the salt-responsive genes.

Table 1. Designed primers for RT-PCR and qPCR expression analysis.

Gene	Accession No	Forward and Reverse Primers	Amplicon (bp)
qPCR			
<i>HvACTIN*</i>	AY145451.1	F-5' CGTGTTGGATTCTGGTGATG 3' R-5' AGCCACATATGCGAGCTTCT 3'	208
<i>HvDRF2</i>	AF521302	F-5' TGAGACGATCAAGCAATGGA 3' R-5' CGAATTTTCAGCAACCCACTT 3'	195
<i>HvWRKY12</i>	DQ840411.1	F-5' CTACCGGTGCACACATCAAG 3' R-5' GACCTGCATCTGGGTGAGTA 3'	157
<i>HvNHX1</i>	AY461511.1	F-5' CCCGCTTTCATTCTTATCCA 3' R-5' GAACGACAGTGATGGTGCTG 3'	200
<i>HvDHN3</i>	AF043089.1	F-5' GTGATCAGCAGCAGACCGG 3' R-5' CATGATGCCCTTCTTCTCGC 3'	176
<i>HvMT2</i>	BM816564	F-5' TCAGTCGAATCAACACATGGA 3' R-5' CACGAGGACGGAATAAAGC 3'	266
<i>HvLOX1</i>	U83904.1	F-5' AGCAGTGAAAGCGAGGAGAG 3' R-5' AAGTCGTTGAGGTCCAGCAC 3'	142
RT-PCR			
<i>HvAPX</i>	AJ006358	F-5' CCTACGCCGATCTCTACCAG 3' R-5' GCCTCAAGCCAACAAACATT 3'	608
<i>HvCAT2</i>	U20778	F-5' GGCGTCAACACCTACACCTT 3' R-5' TCGTCTTCTCCCTCTTCCA 3'	652
<i>HvGST6</i>	AF430069	F-5' ATCTCGTCAGAAACCCGTTT 3' R-5' CTTTCCACGACCACACATTG 3'	676
<i>HvCu/ZnSOD</i>	AK252295	F-5' CCCCTCACCAAGTCAGTCAT 3' R-5' ATTGCAAGTCGGTGTCTTTC 3'	640

**HvACTIN* was used as an internal reference gene for RT-PCR and qPCR

Statistical analyses

Statistical analyses were performed by SPSS21(IBM) statistical software. All data in the tables and figures are given as mean values ± SE (standard error). One-way ANOVA was used to confirm the Least Significance Difference (LSD) test for each factor of treatments at $P < 0.05$ significance level. Three biological and two technical replicates were used for each assay

RESULTS AND DISCUSSION

Physiological effects of exogenous LA treatment on barley seedlings under salt stress

Salinity adversely affects plant growth with osmotic stress, ion toxicity, and oxidative damage. Salinity causes hyperosmotic stress resulting in water deficit in plants. Plants basically counteract the negative effects of osmotic stress due to salinity by the synthesis and accumulation of osmolytes including ammonium compounds, sugars, and amino acids (Golldack et al., 2011). The osmolyte accumulation contributes to the osmotic adjustment, an adaptive mechanism in response to salt stress, and facilitates the maintenance of leaf turgor under salinity. In addition, the osmolytes serve as an osmoprotectant by protecting the structures of membranes and macromolecules (Singh et al., 2015). It

has been reported that increased osmolyte level is an important indicator of tolerance against salt stress in wheat, maize and barley (Chen et al., 2007; Carillo et al., 2008; Kaya et al., 2013). Salt stress (100 mM NaCl for 3 days) resulted in increased levels of proline (an osmolyte) in seven-week-old maize plants compared to non-stressed control plants (Kaya et al., 2013). In the present study, 160 mM NaCl induced the osmolyte accumulation in the leaves of barley seedlings and osmolyte level reached from 438 to 900.4 mosmol kg⁻¹ within 26 h (Table 2). On the other hand, it was found that salt stress did not significantly change the osmolyte content in roots within 26 hours (Table 2). The exogenous application of 0.5 mM LA resulted with increased level of osmolyte in the barley leaves under 160 mM NaCl, but It was found that this increase was not statistically significant compared to salt-stressed samples. The results show that osmolyte accumulation capacity is an important feature in the response of the barley to salt, but high accumulation of significant osmolytes does not appear to play an important role in salt stress tolerance, but rather a marker of salt sensitivity.

The electrolyte leakage (EL) demonstrates a loss of membrane integrity. An increase in electrical conductivity due to increased electrolyte leakage may be considered an indicator of membrane damage in the cell (Jiang et al., 2014). Salinity stress has found to increase the EL from 6.58 % to 36.80 %, and from 38.60 % to 89.67 % in leaves and roots, respectively, within 26 h. On the other hand, 0.5 mM exogenous LA application was found to decrease the EL in both leaf and root tissues as 33 and 44 %, respectively, within 26 h under salinity (table 2). Mahlooji et al. (2018) have reported that EL gradually increased depending on salt concentration in barley. However, EL values varied in different barley genotypes under salt conditions due to differential salinity-tolerance of barley genotypes. It seems that the ability of LA to alleviate the negative effects of salt stress on plant growth may be due to a reduction of electrolyte leakage and the increase of accumulation of osmolytes in plants.

Hydrogen peroxide (H₂O₂), non-radical group of the reactive oxygen species (ROS), can cross biological membranes through aquaporins and lead to oxidative damage far from the site of its generation (Bienert et al., 2007). The production of H₂O₂ is enhanced by abiotic and biotic stresses such as extreme temperature, salinity, and drought as well as pathogens in the plant cells due to interruption of cellular homeostasis (Sharma et al., 2012). Salinity-induced excessive generation of H₂O₂ may cause oxidative damages in a variety of cellular components including plasma membrane lipids, proteins and DNA (Mittler, 2002). Accordingly, rapid detoxification of H₂O₂ is crucial for preventing oxidative damage. In plants, H₂O₂ is produced in organelles including mitochondria, peroxisome and chloroplast. CAT and APX catalyze H₂O₂ into water and molecular oxygen. Since H₂O₂ is the end product of SOD activity, its amount is an important indicator in measuring the damage done by ROS during stress. (Shigeoka et al., 2002). Salinity stress was found not to change the H₂O₂ content in barley leaves within 26 h. On the other hand, H₂O₂ content varies in root tissues depending on exposure time of salt stress. Application of LA decreased the H₂O₂ content in leaves after 26h, but in root tissues it caused an increase in H₂O₂ level.

Table 2: Osmolality, ion leakage and H₂O₂ concentration in the leaves and roots of cv. Martı after dH₂O (Control), 160 mM NaCl for 2 h (2h-NaCl), 160 mM NaCl and 0.5 mM LA for 2h (2h-NaCl+LA), 160 mM NaCl for 26 h (26h-NaCl), 160 mM NaCl and 0.5 mM LA for 26 h (26h-NaCl+LA) treatments.

Treatment	Osmolality (mosmol kg ⁻¹)		Electrolyte Leakage (%)		H ₂ O ₂ concentration (µM)	
	Leaf	Root	Leaf	Root	Leaf	Root
Control	438.0±26.5 c	172.8±25.6 a	6.58±0.77 d	38.60±2.92 c	44.16±0.92 a	27.59±0.10 d
2h-NaCl	483.7±21.3 c	238.0±31.5 a	15.49±1.61 bc	57.87±8.04 b	42.15±0.57 a	42.22±0.28 c
2h-NaCl+LA	693.3±80.9 b	146.7±31.8 a	13.26±0.60 cd	54.97±1.30 bc	41.25±2.24 a	53.58±0.46 a
26 h-NaCl	900.4±21.4 a	188.7±28.8 a	36.80±4.70 a	89.67±7.40 a	43.71±1.47 a	28.46±0.19 d
26 h-NaCl+LA	963.3±43.6 a	210.0±43.59 a	24.51±1.94 b	50.55±3.62 bc	28.55±0.57 b	45.25±0.30 b

Mean values in each column followed by different letters are significantly different at P < 0.05. according to LSD test

Gene expression profiles of antioxidant and salt-responsive genes

High salinity leads to an excessive production of ROS such as H_2O_2 , 1O and $O_2^{\bullet-}$. ROS cause lipid peroxidation in the membrane, resulting in changes in cell permeability, composition and structure, and impaired membrane integrity. ROS also cause the deterioration of ion homeostasis and damage to the macromolecules such as nucleic acid and protein. Antioxidant enzymes are the frontline of defense in the detoxification of ROS (Bose et al., 2014). The effectiveness of antioxidant enzymes can be done both directly by measuring enzyme activities and indirectly by transcript analysis (Hu et al., 2012). The transcript level of *HvAPX*, *HvCAT2*, *HvGST6* and *HvCu/ZnSOD* genes were determined at three time points (0, 2, and 26 h) under salinity (160 mM NaCl) with or without 0.5 mM LA in barley roots and leaves with RT-PCR. In the leaves of cv. Martı, expression of *HvAPX*, *HvGST6* and *HvCu/ZnSOD* was slightly upregulated under 160 mM NaCl with or without LA compared the control plants. On the contrary, expression of *HvCAT2* gene was not observed in salt-treated and control plants (Figure 1). In the roots, expression of *HvAPX*, *HvGST6* and *HvCu/ZnSOD* was decreased under salt stress within 2 h compared to control samples, while expression of these genes increased by the time. LA application was decreased the mRNA level of *HvAPX*, *HvGST6* and *HvCu/ZnSOD* compared to salt-stressed samples within 26 h. Shagimardanova et al. (2010) reported that after 25-day old barley seedlings were exposed to 500 mM NaCl for 2 days, transcript analysis of *GST*, *SOD*, *CAT* and *APX* genes have been shown to increase by salt stress. In another study, activities of antioxidant enzymes SOD, APX and CAT were dramatically enhanced by salinity (300 mM NaCl) in the leaves of salt-tolerant *Hordeum marinum* Huds. (sea barleygrass). However, in cultivated barley (*Hordeum vulgare*), activity of SOD decreased under salinity, while activities of CAT and APX enzymes did not change (Seckin et al., 2010). All findings have suggested that activity of antioxidant enzymes and expression of genes coding antioxidant enzymes change according to the salt concentration, stress exposure time and salt-tolerance level of plants.

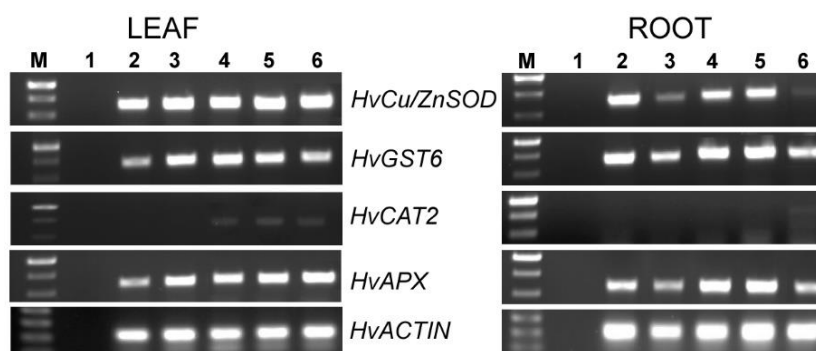


Figure 1. The accumulation of antioxidant genes after NaCl and Linoleic acid (LA) treatments. M: Marker, 1: Non template control, 2: Control (0 h), 3: 160 mM NaCl for 2 h, 4: 160 mM NaCl and 0.5 mM LA for 2h, 5: 160 mM NaCl for 26 h, 6: 160 mM NaCl and 0.5 mM LA for 26 h. *HvACTIN* was used as a reference gene.

qPCR experiments were conducted to investigate expression patterns of salt stress-related genes and transcription factors including *DRF2*, *WRKY12*, *NHX1*, *DHN3*, *MT2*, and *LOX1* in roots and leaves of 3-leaf-stage barley seedlings after 160 mM NaCl with or without 0.5 mM LA treatments for short time (0, 2 and 26 h). qPCR data were visualized as graph in Figure 2. In the leaves, the transcript level of *HvWRKY12* was not changed in all conditions after 26 h. Addition of LA into the root medium did not change the expression of *HvWRKY12* compared with salt-stressed seedlings. While the *WRKY* gene was induced in root tissues by salt stress, no significant change was observed in leaf tissues. Salt stress significantly induced the expression of transcription factor *HvDRF2* as 25 and 29 fold in barley leaves and roots, respectively, compared to controls. Exogenous application of LA dramatically

increased *HvDRF2* level by 105-fold and 45-fold in the leaves and roots, respectively, at 26h of treatments compared the non-stressed plants. *HvDRF2* is homologue of *DREB2A* and expression of *DREB2s* is upregulated by dehydration and high salinity (Nayak et al., 2009, Uçarlı and Gürel 2020). Yin et al. (2018) have reported that the transcript level of *AmDREB2C* was increased by salt and dehydration treatment in *Ammopiptanthus mongolicus*. Besides, the constitutive expression of *AmDREB2C*, a third AP2/ERF member classified into subgroup 2 of the DREB TF family, in *Arabidopsis* substantially increased the level of poly unsaturated fatty acids such C18:2 (linoleic acid) and C18:3 (linolenic acid) and induced the ω -3 FAD (fatty acid desaturase) genes *FAD3* and *FAD8*, which are responsible formation of linolenic acid from linoleic acid (Yin et al., 2018). In the current study, LA treatment significantly induce *HvDRF2* gene in barley leaves and roots. All of these findings have contributed to a certain association between polyunsaturated fatty acids (PUFAs) and DREB transcription factors.

Salt stress induced the expression of *NHX1*, *DHN3*, *MT2* and *LOX1* in barley leaves and roots. Among these genes, LA application dramatically increased the mRNA level of *NHX1* in roots and enhanced *MT2* and *LOX1* in leaves within 26 h under salinity (Figure 2). On the other hand, expression of *DHN3* was decreased in leaf tissues by LA application compared to salt-stressed plants (Figure 2). Lipoxygenases (LOXs) play crucial role in the synthesis of fatty acid metabolites in plants by the hydroperoxidation of polyunsaturated fatty acids (Rosahl 1996). Linoleic (LA) and linolenic acids in plants (LeA) are the most abundant substrates for LOX enzymes. The LOXs are important enzymes involved in diverse physiological functions in seed germination, growth, and development in plants (Viswanath et al., 2020). LOX-1 catalyzes the formation of 9-HPOD (9-hydroperoxide) (Guo et al., 2014). The studies have shown that expression of the *HvLOX1* gene is increased by salt stress in barley (Ueda et al., 2004; Walia et al., 2006). Gogna and Bhatla, (2020) reported that increased levels of lipoxygenase (LOX) activity and its further upregulation by salinity are the uncommon characters of salt-sensitive sunflower seedlings. On the other hand, salt-tolerance is associated with lower LOX activity in sunflower seedlings. Gogna and Bhatla, (2020) also have shown that linoleic acid content significantly increased in response to salt stress in all salt-tolerant and -sensitive seedlings of sunflower. Lim et al. 2015 reported that the CaLOX1 have an important role in high salinity response via reduced H₂O₂ content and lipid peroxidation. Distinct LOX enzymes are shown to play a crucial role in ROS scavenging through reducing H₂O₂ accumulation and influencing antioxidative enzymes. Besides, LOXs induced stress responsive genes such as *DREB2A*, *RD22*, *NCED3* (Viswanath et al. 2020). LOX expression upregulated by LA in response to salt stress may have a correlation with altered fatty acids composition, which could possibly serve as a potential substrate for the LOX enzyme.

Metallothioneins (MTs) are small metal-binding proteins and characterized with a relatively high cysteine residue content, almost 30% of their amino acid content (Joshi et al. 2016). MTs have a crucial role in heavy metal detoxification, stress response and ROS scavenging as well as some important developmental processes including root development and fruit ripening in plants (Hassinen et al. 2011). In the present study, transcript levels of *HvMT2* were significantly increased with 12- and 4-fold in the leaves and roots of seedlings, respectively, under 160 mM NaCl within 26 h. Similarly, Xue et al. (2009) reported that salinity increased the expression of a type 3 MT gene (*GhMT3a*) in cotton (*Gossypium hirsutum*) seedlings, and overexpression of *GhMT3a* conferred salinity tolerance to transgenic tobacco plants compared with wild-type plants by scavenging ROS. LA application significantly enhanced the expression of *HvMT2* in the leaves under salinity, whereas no changes were observed in the roots (Fig 2).

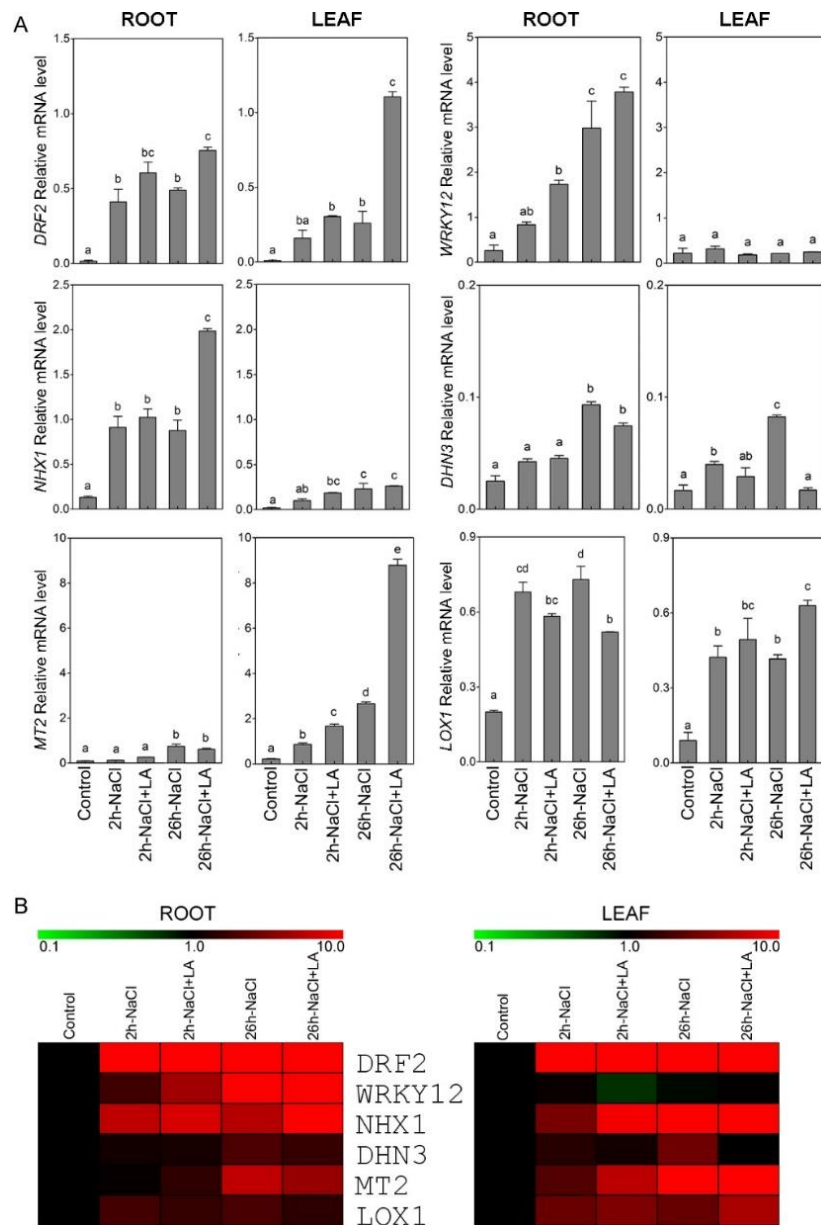


Figure 2. A) Expression profiles of the salt-responsive genes in the leaves and roots of barley seedlings treated with 160 mM NaCl for 0 h (Control), 160 mM NaCl for 2 h (2h-NaCl), 160 mM NaCl and 0.5 mM LA for 2h (2h-NaCl+LA), 160 mM NaCl for 26 h (26h-NaCl), 160 mM NaCl and 0.5 mM LA for 26 h (26h-NaCl+LA). Different letters are significantly different at $P < 0.05$. B) Heat map showing the expression pattern of salt-responsive genes after NaCl with or without LA treatments. Red and green boxes represent induction and repression of genes, respectively

Intracellular Na^+/H^+ antiporters (NHXs) play important roles in cellular osmotic adjustment and Na^+/K^+ homeostasis (Uçarlı and Gürel, 2020). Salt stress significantly increased the expression of *NHX1* by 7- and 12-fold in the roots and leaves of barley seedlings, respectively. In the roots, LA treatment dramatically upregulated *HvNHX1* transcripts by 128 % compared to only NaCl-treated seedlings within 26 h. However, the increase of *HvNHX1* transcripts was limited to 14 % in leaves (Figure 2). Dehydrins, group 2 late embryogenesis abundant proteins (LEAs), are promoted by salinity, drought, and cold (Uçarlı et al. 2016). *DHN3* gene was induced by 160 mM NaCl, whereas transcript level of *DHN3* was decreased by LA treatment under salinity in the leaves and roots of barley seedlings by 80 % and 20 %, respectively, compared to only NaCl-treated samples within 26 h.

CONCLUSION

This study characterized the short term effects of exogenous application of linoleic acid (LA) on barley seedlings under salt stress (160 mM NaCl). LA has been found to cause a series of physiological and molecular changes, including osmolality, ion leakage, H₂O₂ and differentially gene expression of salt-responsive genes, leading to an increased tolerance to salt stress. Polyunsaturated fatty acids (PUFAs) such as linoleic acid, as main components of membrane lipids determine the fluidity and stability of the cell membrane. In addition, PUFAs have a crucial role in maintaining the structure and function of the cell membrane. Generally, in salt-sensitive plants, fatty acids are more saturated, whereas fatty acids are more unsaturated in salt-tolerant plants. In the present study, exogenous application of LA decreased the electrolyte leakage and H₂O₂ while increasing osmolality under salt stress within hours in the leaves of barley seedlings. Besides, LA dramatically increased the expression of *HvDRF2* TF and ROS associated gene *HvMT2*. The results of the current study and literatures demonstrate that there is an association between unsaturation level of membrane fatty acids and salt-tolerance of plants. However, more detailed researches are still needed to understand the mechanisms of the improving effects of the LA on plants in response to salinity.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Yazar Katkısı

The authors declare that they have contributed equally to the article.

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