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Research article

Stimulating antioxidant defenses, antioxidant gene expression, and salt tolerance in *Pisum sativum* seedling by pretreatment using licorice root extract (LRE) as an organic biostimulant

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ABSTRACT

Plant extracts have recently been used as exogenous adjuvants to strengthen the endogenous plant defense systems when they grow under different environmental stresses, including salinity. The study aimed at determining the effects of seed soaking using licorice root extract (LRE) on photosynthesis and antioxidant defense systems, including transcript levels of enzyme-encoding genes in pea seedling grown under 150 mM NaCl-salinity. Salt stress reduced seedling growth, photosynthesis attributes, and K^+ content, and increased oxidative stress ($O_2^{\bullet-}$ and H_2O_2, and MDA), Na^+, and Cl^-, along with an increase in antioxidative defense activities compared to control. However, LRE pretreatment enhanced seedling growth, photosynthetic attributes (chlorophylls, carotenoids, Fv/Fm, Pn, Tr, and gs), ascorbate and glutathione and their redox states, proline, soluble sugars, α-TOC, and enzyme activities compared to stressed control. LRE pretreatment also upregulated transcript levels of *CAT*-, *SOD*-, *APX*-, *GR*-, *DHAR*-, and *PrxQ*-encoding genes in salt-stressed seedlings, decreasing oxidative stress and Na^+ and Cl^- contents and increasing K^+ content and K^+/Na^+ ratio.

1. Introduction

Pea (*Pisum sativum* L.) is one of the most important leguminous crops due to its richness in proteins and carbohydrates. It is grown in many countries for dry pulses, fresh peas, and fodder. Development of salt-tolerant symbiosis is an absolute necessity to enable the cultivation of leguminous crops in salt-affected soils (Desoky et al., 2017; Rady et al., 2019a). Pea productivity is severely restricted by high salinity levels (Duzdemir et al., 2009; Osman and Rady, 2012). Approximately 50% loss of pea yield was reported with 9 dS m^{-1} using NaCl salt (Subbarao and Johansen, 1994), while in other work (Duzdemir et al., 2009), approximately 74 and 100% losses of pea yield were observed under salinity of 4 and 7 dS m^{-1}, respectively.

Salt stress is one of the major environmental problems that can directly or indirectly affect crop production. Many plants are sensitive to salt stress and are unable to tolerate even a low level of salinity. Salinity limits the ability of plants to benefit from water ("physiological drought") and cause a decrease in plant growth and yield by inhibiting the plant's metabolism through ionic and osmotic stress, which directly affects plant physiology and obstructs water relations of plant (Munns, 2002; Rady, 2011). Salt stress inhibits plant growth through excessive production of reactive oxygen species (ROS) such as free radicals like $O_2^{\bullet-}$, $OH^{\bullet}$ and non-radicals like $^1O_2$ and H_2O_2 (Mittler, 2002; Das and Roychoudhury, 2014). Excessive production of ROS causes oxidative stress, leading to cell damage and eventually cell death. ROS are produced in several ways, as a product of the mitochondrial respiratory chain, in photochemical and enzymatic reactions, as a result of exposure to salt stress. They are toxic molecules that can cause oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). Under saline conditions, the plant suffers from stress in three ways. The first is the low availability of water in the soil, causing water shortfall. Specific toxic effect of Na^+ and Cl^- ions represents the second way. The third way is the nutritional imbalance resulting from reducing the absorption of some beneficial elements (Marschner, 1995). The accumulation of salt to the toxic level in the leaf apoplasm causes loss of turgor (dehydration) and leads to death of tissues and cells. Photosynthesis is the physiological process most affected by plant exposure to salt stress (Sudhir and Murthy, 2004). The inhibition of photosynthesis caused by

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salt is associated with a reduction in chlorophyll pigment content (Rady, 2011) and the closure of stomata, thereby decreasing CO_2 pressure (Bethke and Drew, 1992) and inhibiting Rubisco enzyme and other biochemical processes, including antioxidant enzymes' activities (Khan et al., 2014; Ahanger et al., 2018).

In most harsh environmental conditions, endogenous antioxidant systems are not sufficient for plants to defend themselves. Eventually, many studies have recommended to use exogenous adjuvants such as plant-based natural extracts for plants to increase their tolerance to stress including salinity (Rady et al., 2013, 2019a; Semida and Rady, 2014; Rady and Mohamed, 2015; Desoky et al., 2018, 2019, 2019c, 2019d). It has been reported that application of organic extracts from different plant parts (e.g., *Moringa oleifera* leaf, licorice root, and maize grain extracts) increased plant tolerance to abiotic stress. They effectively restricted the production of ROS (e.g., $O_2^{\bullet-}$ and H_2O_2) and the contents of toxic ions, neutralizing the oxidative stress. They also restored nutrient contents, and increased the levels of different enzymatic and non-enzymatic antioxidants, contributing to reduction of lipid peroxidation and increase of membrane integrity and plant production in salt-stressed common bean plants (Rady et al., 2013, 2019a; Semida and Rady, 2014; Rady and Mohamed, 2015, 2019d). One of these plant extracts (organic biostimulants) reported in these studies is the licorice (*Glycyrrhiza glabra*) root extract (LRE). There are not many studies that discuss the use of LRE for stressful plants (Desoky et al., 2019; Rady et al., 2019a). LRE is rich in osmoprotectants, antioxidants, vitamins, mineral nutrients, and phytohormones (Table 1). Because it contains glycyrrhizin; calcium and potassium salts of glycyrrhizic acid and trihydroxy acid, LRE is exploited to improve plant tolerance to abiotic stresses (Newall et al., 1996) including salinity. LRE has been reported to contain other bioactive compounds including amino acids, sugars, nutrients, Se, and phytohormones (Desoky et al., 2019; Rady et al., 2019a). Therefore, these infrequent investigations have reported that application of LRE for stressed-common bean and stressed-pepper plants effectively increased their tolerance to salt and heavy metal stresses. Exogenous application of LRE limited the production of ROS (e.g., $O_2^{\bullet-}$ and H_2O_2) and the contents of toxic ions, neutralizing oxidative stress. It improved growth, productivity, physio-biochemical attributes, and anatomy features, and effectively restored nutrient contents in these stressed plants. It also elevated the activities of different enzymatic and non-enzymatic antioxidants, which contributed to maintaining the integrity of plasma membranes through controlling the level of malondialdehyde (MDA) and toxic ions (Desoky et al., 2019; Rady et al., 2019a). To our knowledge, there are no reports discussed the effect of LRE on increasing the transcript levels of genes encoding antioxidant enzymes under saline stress. In the current study, the transcript levels of genes encoding antioxidant enzymes (SOD, CAT, APX, DHAR, GR, and PrxQ), especially peroxiredoxins (PrxQ) genes that provides further insight into the mechanisms of enhanced salt tolerance by LRE pretreatment.

Table 1
Chemical analysis of the d licorice root extract (LRE) (on dry weight basis).

Component	Unit	Value
1. Antioxidants and osmoprotectants:		
Total free amino acids	g kg^{-1} DW	172
Free proline		36
Soluble sugars		148
Salicylic acid	mg kg^{-1} DW	29.5
α-Tocopherol		38.4
Glutathione (GSH)		30.2
Ascorbic acid (AsA; Vit. C)		41
Vitamin A (β-carotene)		154
Total B-group vitamins		170
Vitamin E (Tochopherol acetate)		65
Selenium (Se)		0.9
DPPH-radical scavenging activity	%	84.6
2. Phytohormones:		
Total auxins	mg kg^{-1} DW	4.2
Total gibberellins		5.2
Zeatin-type cytokinin		4.1
3. Mineral nutrients:		
Nitrogen (N)	mg kg^{-1} DW	20.2
Phosphorus (P)		21.3
Potassium (K)		47.2
Calcium (Ca)		2.2
Magnesium (Mg)		3.8
Sulfur (S)		2.4
Iron (Fe)		0.94
Manganese (Mn)		0.62
Zinc (Zn)		0.21
Cupper (Cu)		0.02

Therefore, the main objectives of this study were to evaluate the protective effect of LRE pretreatment on *Pisum sativum* growth and yield attributes, physio-biochemical attributes, and antioxidant defense system components under saline conditions, in addition to understand the tolerance mechanisms in stressed plants that improved by LRE application. It was hypothesized that the use of LRE can effectively reduce the harmful effects of salt toxicity on the performance of pea as a sensitive plant to salt stress by reducing the oxidative stress (in terms of $O_2^{\bullet-}$ and H_2O_2) and toxic ions; Na^+ and Cl^- contents and increasing the ratio of K^+/Na^+, activity of different enzymatic and non-enzymatic antioxidants and transcript levels of genes encoding antioxidant enzymes.

2. Materials and methods

2.1. Plant materials and experiment design

With a three-time repetition, a pot experiment was conducted adopting a completely randomized design in a greenhouse. In these experiments, *Pisum sativum* (L.) seed were soaked using LRE (as an organic biostimulant) before exposing pea seedlings to short-term salt stress (150 mM NaCl for two weeks). Potential positive changes of physio-biochemical systems including gene expression were assessed under the influences of pea seed pretreatment using LRE after exposure of plants to short-term salt stress to assess the relationship between changes in antioxidative systems, gene expression and extent of plant tolerance, regarding the improvement of seedling growth.

The greenhouse conditions were 20 ± 2 °C as the mean temperature during the day (with length of 12 h) and 10 ± 1 °C as the average temperature during the night (with length of 12 h), and 62.0–65.1% as the average humidity. Certified pea (*Pisum sativum* L.) seed (cv. Master-B) were obtained from Vegetative Research Section, Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt. Each 0.5 kg of pea seed were soaked in 1 L of 0.5% licorice root extract (LRE) solution or in 1 L of distilled water for 2 h. Then, forced air was used to re-dry the soaked seeds under shade to the original weight (Sundstrom et al., 1987). For surface sterilization of the seed, they were dipped in 1% filtered $Ca(ClO)_2$ for 1 h and then washed directly using sterilize-deionized water. The seed were then planted in black-colored plastic pots of dimensions 20 cm high and 20 cm in diameter. The experimental treatments were as follows: (1) Control (seed were soaked in distilled water and seedlings were not treated with NaCl), (2) Seed were soaked in LRE and seedlings were not treated with NaCl, (3) Seed were soaked in distilled water and seedlings were treated with NaCl, and (4) Seed were soaked in LRE and seedlings were treated with NaCl. Each treatment was represented by 5 pots and each pot contained 8 seedlings. Pure sand free of anions and cations, obtained through the treatment of sand with commercial acid and re-cleaning with distilled water, was used as a growth medium. In all pots, plants were watered every 5 days (for 3 irrigations) at 100% of the field capacity using ½-strength modified Hoagland's nutritive solution (Hoagland and Arnon, 1950) free of NaCl salt (stress treatment) until full emergence (14 days after sowing; DAS). The components of Hoagland's nutritive solution (pH 5.9) were as follows: $Ca(NO_3)_2 \times 4\ H_2O$ (1.25 mM), KNO_3 (1.25 mM),

KH_2PO_4 (0.25 mM), $MgSO_4 \times 7\ H_2O$ (0.5 mM), H_3BO_3 (11.6 μmol), $MnCl_2 \times 4\ H_2O$ (2.4 μmol), $ZnSO_4 \times 7\ H_2O$ (0.24 μmol), $CuSO_4 \times 5\ H_2O$ (0.08 μmol), $Na_2MoO_4 \times 2\ H_2O$ (0.13 μmol), and Fe^{3+}-$EDTA^{+}$ (22.5 μmol). Beginning from the 15th DAS, stress treatments were started by adding NaCl in the nutritive solution at a low concentration (50 mM NaCl for the 4th irrigation). Three days later, the concentration of salt was increased to 100 mM in the following (5th) irrigation and reached 150 mM in the 6th irrigation. Every 3 days, 3 irrigations were then performed with a constant salt concentration of 150 mM NaCl. In the two stressed treatments, the concentration of NaCl was maintained in the growing medium at 150 mM and controlled by periodic evaluation using the inductively coupled plasma atomic emission spectrometry (ICP- AES, IRIS-Advan type, Thermo, USA). Pea seedlings were harvested from all treatments at 30 DAS for various measurements of this study.

2.2. Preparation and analysis of licorice root extract (LRE)

The bioactive components (biostimulants) were extracted from LRE effectively (Rady et al., 2019a). A weight of 5 g of licorice root was air-dried. This dried root was immersed in 1 L of water at 50 °C for 24 h and then filtered and the final volume was reached 1 L by distilled water. LRE (0.5%; 5 g roots per L) was applied as pea seed soaking for 2 h. Chemical components in LRE were analyzed at a specialized laboratory (the National Research Center, Egypt) and the assessments are shown in Table 1. The determinations were as follows: Total free amino acids were extracted and their content was measured according to the method of Dubey and Rani (1989). Proline content was assessed using the method of Bates et al. (1973). Total soluble sugar content was assessed according to the procedure of Irigoyen et al. (1992). Salicylic acid content was determined following the methods of Siegrist et al. (2000) and Metwally et al. (2003) using an HPLC system. α-Tocopherol was determined following the procedures of Konings et al. (1996) and Ching and Mohamed (2001). B-group vitamins were determined with HPLC system according to Wagner et al. (2006). Glutathione content was assessed by the method of Griffth (1980). The method of Kampfenkel and Van Montagu (1995) was used to assess the content of ascorbic acid. Antioxidant activity was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging activity assay using the method of Lee et al. (2003). Phytohormones were identified by GC/MS (Lavrich and Hays, 2007). For nutrients determinations, a weight of 0.2 g of dried leaves was digested with 96% H_2SO_4 in the presence of H_2O_2 (Wolf, 1982) and diluted with distilled water. N content was determined using a micro Kjeldahl method according to Chapman and Pratt (1982), P content was determined colorimetrically using ascorbic acid method of Watanabe and Olsen (1965), K content was measured using Flame photometer (Lachica et al., 1973), Se was estimated according to Advanced Microwave Digestion used for samples digestion (Krishnaiah et al., 2003), and Ca, Mg, S, Fe, Mn, Zn and Cu contents were measured using an Atomic Absorption Spectrophotometer (PerkinElmer, Model 3300) as described by the method of Chapman and Pratt (1961).

2.3. Chlorophyll content, PSII quantum yield, and CO_2 fixation rate

Total chlorophyll and total carotenoids were extracted from fresh leaf using pure acetone according to Fadeels (1962). Leaf net photosynthetic rate (Pn), rate of transpiration (Tr) and stomatal conductance (gs) were assessed for photosynthetic system by using a portable photosynthesis system (LF6400XTR, LI-COR, USA). Assessments were taken at 09:00–11:00 a.m. on the second fully expanded leaf. Calculations of maximum PS II Fv/Fm quantum yield were done using the Maxwell and Johnson (2000) formulae; $Fv/Fm = (Fm - F0)/Fm$.

2.4. Level determinations of sugars, proline, α-tocopherol (α-TOC), peroxidation of lipids, and oxidative stress (hydrogen peroxide; H_2O_2 and superoxide; $O_2^{\bullet-}$)

The method outlined by Irigoyen et al. (1992) was applied for extraction (using 96%, v/v ethanol) and determination of soluble sugars content (mg per g of leaf DW). The extract (100 μL) was reacted with 3 mL of reagent [freshly prepared; anthrone (150 mg) + H_2SO_4 (100 mL, 72% v/v)]. Then, boiling process was performed for 10 min in a water bath. After cooling, Spectronic (a Bausch and Lomb-2000) Spectrophotometer was used for absorbance reading at 625 nm. The method of Bates et al. (1973) was followed to determine proline content (μmol per g of leaf DW) using L-proline prepared as a standard. Content (μmol per g of leaf DW) of α-TOC was determined. A weight of 0.02 g of butylated hydroxytoluene (BHT) was dissolved in 0.9 L of extraction solvent consisted of n-hexane-ethyl acetate, n-hexane + 0.1 L of CH_3–COO–CH_2–CH_3. R-TOC was utilized with 0.05 g per 0.1 L n-hexane to prepare the standard solutions (0.02–0.2 mg per mL). Then, methods of Konings et al. (1996) and Ching and Mohamed (2001) were applied to determine α-TOC content using HPLC system with 94 methanol: 6 water (v/v) as the mobile phase along with 1.5 mL per min flow rate, and the UV detector, which set at 292 nm.

Peroxidation of lipids was assessed by determining the level of malondialdehyde (MDA; μmol per g of leaf FW). MDA assessment was performed by using the same H_2O_2 extracts (Heath and Packer, 1968). Calculation was performed with $0.155 \times 10^{-3}\ M^{-1}\ cm^{-1}$ as a coefficient of molar extinction to record the content of MDA. For determining H_2O_2 level (μmol per g of leaf FW), 0.25 g fresh leaf was homogenized in 5 ml 5% TCA. Homogenate centrifugation (12,000 × g) was performed at 4 °C for 15 min. After collecting supernatant, it was added to a reaction medium; 10 mM of buffer (potassium phosphate, pH 7.0) + 1 M of KI. Using a spectrophotometer, absorbance reading was recorded at 390 nm using a standard prepared from H_2O_2 (Velikova et al., 2000). For assessing $O_2^{\bullet-}$, pea fresh fully-expanded leaf (100 mg) was cut into 1 mm × 1 mm fragments. The fragments were then immersed for 1 h at room temperature in 10 mM K-phosphate buffer, pH 7.8, 0.05% NBT and 10 mM NaN_3. A volume of 2 ml of immersed solution was heated at 85 °C for 15 min and cooled rapidly. Optical density was measured colorimetrically at 580 nm and the $O_2^{\bullet-}$ content was expressed as $A_{580}\ g^{-1}$ FW (Kubis, 2008).

2.5. Determination of antioxidants activities

Extraction for enzyme activity was performed according to Vitoria et al. (2001). The activity of catalase (CAT) enzyme was measured spectrophotochemically according to Chance and Maehly (1955). Peroxidase (POD) activity was estimated according to Thomas et al. (1982). Ascorbate peroxidase (APX) was determined spectrophotochemically according to Fielding and Hall (1978). Activity of superoxide dismutase (SOD) was determined by recording the drop in absorbance of superoxide-nitro blue tetrazolium complex by the enzyme (Sairam et al., 2002). Glutathione reductase (GR) activity was measured after monitoring the oxidation of NADPH for three absorbance readings taken at 340 nm (Rao et al., 1996), and the other enzyme activities were expressed as $A_{564}\ min^{-1}\ mg^{-1}$ protein.

2.6. Estimation of non-enzymatic antioxidant compounds

The Kampfenkel and Van Montagu (1995) method was applied to estimate AsA level (μmol per g of leaf FW). The mixture; 30 mM of buffer (potassium phosphate, pH 7.4) + TCA (2.5%) + phosphoric acid (8.4%) + bipyridyl (0.8%) + ferric chloride (0.3%) was received leaf extract. The reaction was conducted (40 °C, 30 min), and absorbance was read at 525 nm. Content of AsA + DHA (oxidized AsA) was assessed after the addition of extract to 500 μM of DTT to estimate total AsA reduction by reading the absorbance on 525 nm and L-AsA was

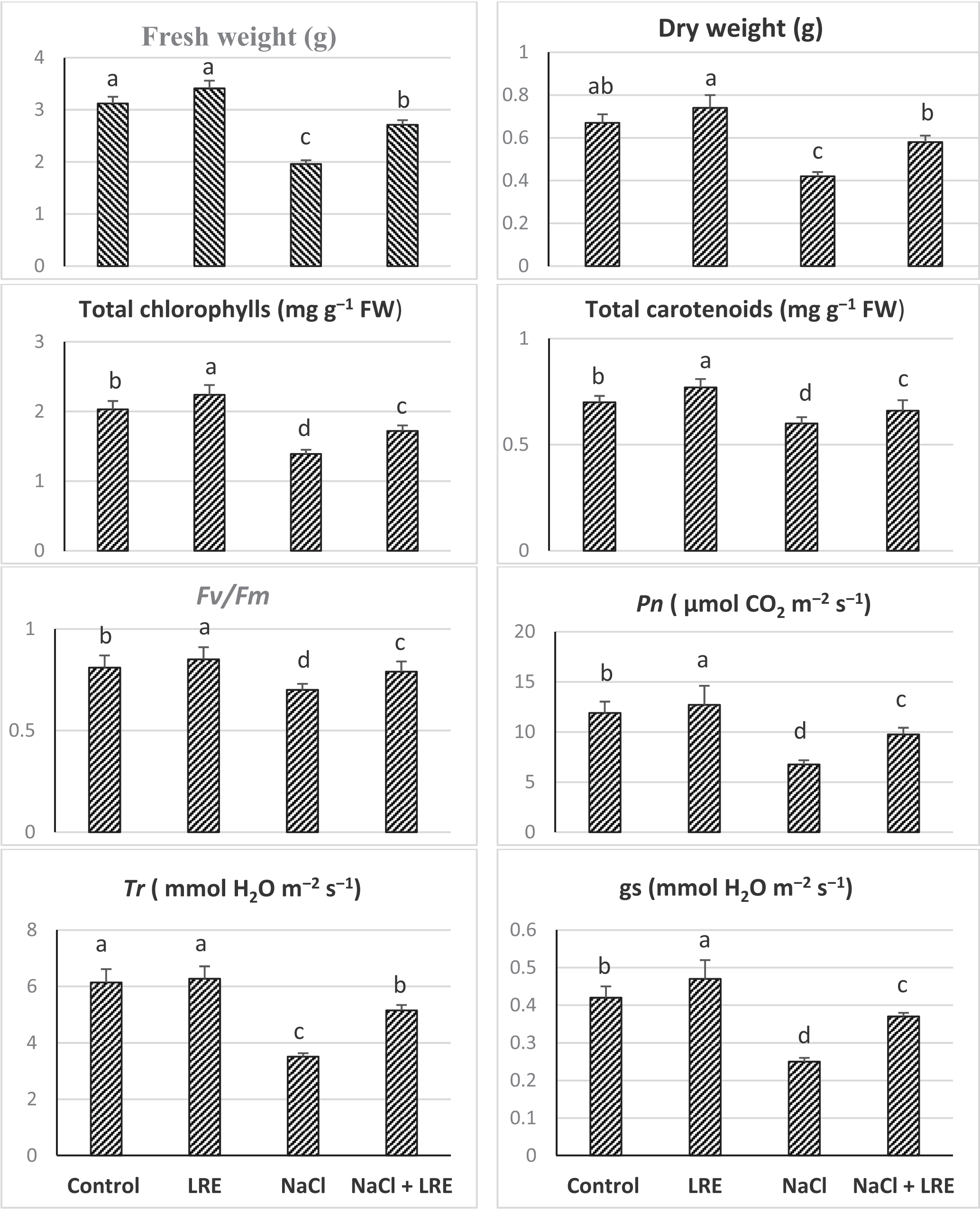


Fig. 1. Fresh and dry weights, photosynthetic pigments, Fv/Fm, net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (gs) of salt-stressed pea plant as affected by seed soaking in LRE. Different letters above bars within treatment groups are significantly different ($P < 0.05$). Fv/Fm means Maximal quantum efficiency of PSII, and PI means photosynthetic performance index.

used as a standard, and the following formula was used to record AsA redox state:

$$\text{AsA redox state \%} = \frac{\text{AsA}}{\text{AsA} + \text{DHA}} \times 100$$

The Griffth (1980) method was applied to assess the levels (µmol per g of leaf FW) of reduced GSH and the total (GSH + GSSG). For GSH assessment, extract of leaf +0.13 M of buffer (sodium phosphate, pH 7.4) + 0.007 M of buffer (sodium phosphate, pH 6.8) + 0.006 M of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) as a reaction mixture was stayed on 30 °C for 10 min. Then, absorbance reading was taken on 412 nm. Total GSH level was determined after reducing of GSSG to GSH by adding leaf extract to 0.13 M of buffer (sodium phosphate, pH 7.4) + 1 U of GSH-reductase. The absorbance was read on 412 nm. Content of GSH, as well as GSH + GSSG, was assessed along with a standard (GSH), and calculation of GSH redox state was done:

$$\text{GSH redox state\%} = \frac{\text{GSH}}{\text{GSH} + \text{GSSG}} \times 100$$

2.7. Determination of potassium (K^+), sodium (Na^+) and chlorine (Cl^-) contents

To assess K^+, Na^+, and Cl^- contents, pea leaves were dried in an electric oven at 70 °C for 48 h and ground to powder form. Then, samples were digested using 96% H_2SO_4 in the presence of H_2O_2 (Wolf, 1982). Contents of K^+ and Na^+ were assessed according to Lachica et al. (1973) in a suspension made from 50 mg of freeze-dried leaf powder and centrifuged at 3000 × g for 10 min at 25 °C. Content of leaf Cl^- was assessed according to the method of Gaines et al. (1984).

2.8. Determination of mRNA levels

Total RNA isolated from leaf samples of pea (*Pisum sativum*) plants using a RNeasy Mini Kit (Qiagen GmbH, Germany), the subsequent cDNA synthesis were performed using a RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas GmbH, Germany). Primers sequences for semi-quantitative and quantitative RT-PCR of the stress-related genes in pea plant are as follows:

The gene	Reference Seq.	5′–3′ primer sequence	T_A
Actin	AB181991	F: CTCTGACAATTTCCCGCTCA R: ACACGCTTCCTCATGCTATCC	58 °C
DHAR	AY074784	F: TCAAGAACGTGACAAAGGTGG R: TAACGGTGGTGATGGCAAAT	
SOD	MG893090.1	F: TTCGCCATGCTGGTGATCTT R: CATGGACAACTACGGCCCTT	
CAT	GU984379	F: GGCTGCTTGAAGTTGTTCTCCT R: CTGCTAGTACCTCCTGATCCGTT	
APX	KU747079.1	F: TGGCCTGCTCTTCCTCTAGT R: CATGCCACGCTAATCGAAGC	
GR	KX828561.1	F: CAACGCGCTTTGGTAACTCC R: GGGCCCTAATGAAGTGGAGG	
PrxQ	AY789643	F: ACTTCACGCTCAAGGACCAG R: CCGCCTTCTTGTACTTCTCG	

Quantitative PCR (qRT–PCR) analysis was performed on the iCycler Thermal Cycler (*Bio-Rad*, USA) using the iQ SYBR Green Supermix (*Bio-Rad*, USA) manufacturer's instructions. Actin gene was used as a reference gene for qPCR data normalization. Efficiencies of reactions were calculated using LinRegPCR Software (Ruijter et al., 2009). Signal values were subsequently derived from the threshold cycles, with the average background subtracted, using the equation of Pfaffl (2001).

2.9. Statistical analysis

One-way ANOVA was used to evaluate the effect of treatments on all parameters. When significant differences appeared ($P \leq 0.05$), a Fisher Test was used (Sokal and Rohlf, 1980).

3. Results and discussion

3.1. Plant growth and nutrient contents

In comparison to the control, soaking pea seed in licorice root extract (LRE, 0.5%) did not affect pea seedling growth in terms of fresh and dry weights, and Na^+ and Cl^- contents, while K^+ content and K^+/Na^+ ratio were significantly increased (Fig. 1, Tables 2 and 4). Under salt stress (150 mM NaCl) for two weeks, seedling fresh and dry weights, K^+ content and K^+/Na^+ ratio were significantly reduced, while Na^+ and Cl^- contents were severely increased. However, pretreatment with LRE mitigated the deleterious effects of salt stress and significantly increased seedling fresh and dry weights, K^+ content and K^+/Na^+ ratio, while Na^+ and Cl^- contents were significantly decreased compared to the salt stress treatment.

Plant biomass production was evaluated as a result of the positive performance (e.g., growth promotion) of stressful plants pretreated with LRE. It has been concluded that LRE, which is applied as seed soaking, is considered as an effective natural biostimulant to increase salt tolerance in plants grown under salt stress (Desoky et al., 2019; Rady et al., 2019a). These reports have concluded that LRE pretreatment can provide seeds with bioactive components (i.e., Se, amino acids, sugars, vitamins, phytohormones, and mineral nutrients, Table 1) to improve seed germination and seedling growth. Semida and Rady (2014) have reported that the increase in the germination percentage of plant extract-soaked seeds may be attributed to that seeds absorbed biostimulant substances from the extract. In addition, seedlings obtained from plant extract-soaked seeds showed improved growth in terms of seedling fresh and dry weights, and also showed an increase in salt tolerance in terms of increased membrane stability index and relative water content and reduced ion leakage under salt stress. The richness of LRE in various biologically active components (Table 1), in addition to its high DPPH-radical scavenging activity (84.6%) makes it as a source of many defense mechanisms for NaCl-salt-stressed pea seedlings.

In the present study, salt stress (150 mM NaCl) significantly reduced pea shoot fresh and dry weights (Fig. 1, Table 4). This result may be due to the high content of toxic ions (Na^+ and Cl^-) and low K^+ content observed in salt-stressed pea seedlings, which were reflected in the reduction of K^+/Na^+ ratio (Tables 2 and 4). In addition, reduction in pea seedlings growth may be caused by worsening metabolic functions and high respiratory rate due to energy requirements, resulting in reduced activities in the meristems, cellular divisions, and elongations (Semida and Rady, 2014). However, salt-stressed pea seedlings pretreated with LRE showed an increase in plant biomass, which was associated with a reduction in Na^+ and Cl^- contents, high K^+ content and K^+/Na^+ ratio compared to the salt-stressed control (Fig. 1, Tables 2 and 4). Along this line, LRE pretreatment decreased the contents of Na^+ and Cl^- and increased K^+ content and K^+/Na^+ ratio in some plant species exposed to salt stress (Desoky et al., 2019; Rady et al., 2019a). The accumulation of K^+ is a common response to cultivated plants under salinity conditions due to their direct competition with Na^+ for charge dependent binding sites (Chen et al., 2007). LRE may reduce K^+ efflux in salt-stressed seedlings to compete well with Na^+ thus improving K^+/Na^+. Different bioactive constituents of LRE (effective bio-stimulants) can enhance seedling growth as reported recently (Desoky et al., 2019; Rady et al., 2019a). In the current study, the increase in fresh and dry weights of salt-stressed pea seedlings pretreated with LRE may be due to improved mobilization of germination linked metabolites and dissolved substances (e.g., mineral nutrients, sugars, vitamins, antioxidants, Se, and phytohormones) found in LRE. This has resulted in improved growth of plume and/or increased sugars content and amylase activity, participating in improved growth activity to increase seedling biomass under stress (Elham et al., 2014; Rady et al., 2019a). K^+ imbalance in cytosol occurred under salt stress was positively ameliorated by LRE pretreatment, thus maintaining a high accumulation of K^+ within the cytosol and maintaining adequate K^+/Na^+ ratio (Tables 2 and 4) as a basic mechanism to help plant to withstand salt stress. The increase in K^+ content and K^+/Na^+ ratio by LRE pretreatment may be due to the fact that LRE contains high concentration of K^+ and other bioactive ingredients (Table 1) that may act as some mechanisms against salt stress conditions. Seed priming in LRE-

Table 2
Elemental status in salt-stressed pea plant as affected by seed soaking in LRE.

Treatments	K^+ (mg g^{-1} DW)	Na^+ (mg g^{-1} DW)	Cl^- (mg g^{-1} DW)	K^+/Na^+ ratio
Control	14.8 ± 0.33 b	2.36 ± 0.04c	1.86 ± 0.02 b	6.29 ± 0.13b
LRE	18.9 ± 0.39a	2.24 ± 0.05c	1.79 ± 0.03b	8.45 ± 0.22a
NaCl	9.58 ± 0.16 c	13.4 ± 0.11a	3.19 ± 0.09 a	0.71 ± 0.02d
NaCl + LRE	14.7 ± 0.23b	4.18 ± 0.09b	1.99 ± 0.04b	3.55 ± 0.09c

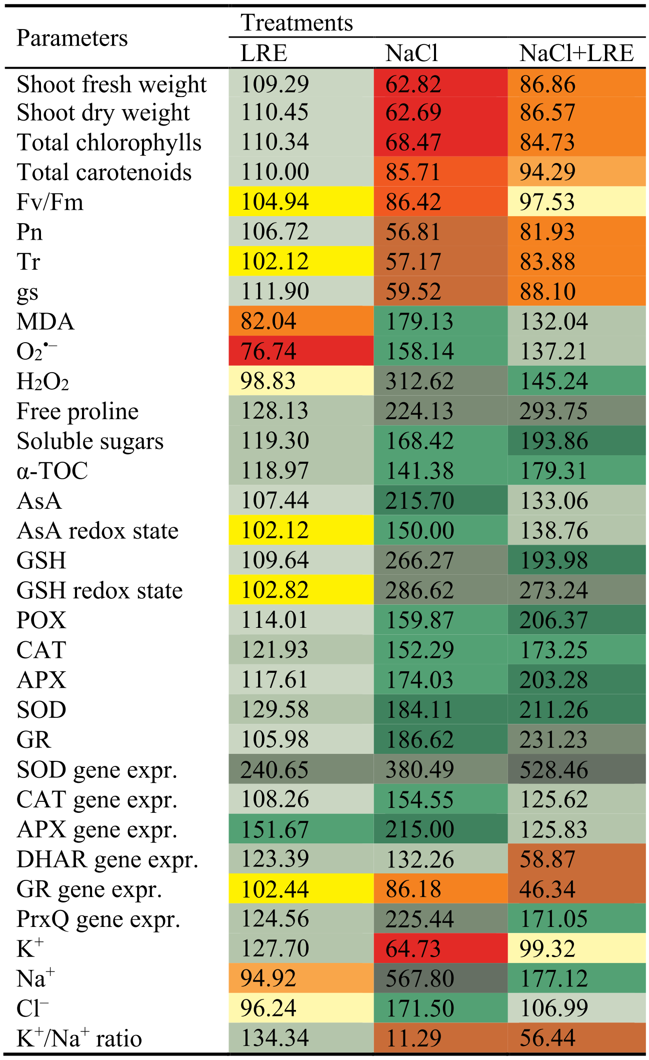
Different letters after means ± SE in each column indicate significant differences according to the LSD test ($P \leq 0.05$).

Table 3
Enzymatic activities in salt-stressed pea plant as affected by seed soaking in LRE.

Treatments	POX	CAT	APX	SOD	GR
A_{564} min^{-1} mg^{-1} protein					
Control	1.57 ± 0.04c	41.5 ± 1.21d	33.5 ± 0.98d	4.53 ± 0.34d	26.9 ± 1.21d
LRE	1.79 ± 0.06c	50.6 ± 1.35c	39.4 ± 0.88c	5.87 ± 0.41c	28.5 ± 1.42c
NaCl	2.51 ± 0.09b	63.2 ± 1.64b	58.3 ± 1.73b	8.34 ± 0.79b	50.2 ± 2.45b
NaCl + LRE	3.24 ± 0.12a	71.9 ± 1.55a	68.1 ± 1.68a	9.57 ± 0.95a	62.2 ± 2.44a

Different letters after means ± SE in each column indicate significant differences according to the LSD test ($P \le 0.05$).

Table 4
Relative to control values, changes (%) observed in growth and other physio-biochemical parameters and antioxidant systems in *Pisum sativum* under NaCl-salinity stress and pretreatment using LRE. Three color scale heatmap; red color for change % < 100, yellow color as a midpoint for 100% change (equal to control values), and green color for change % > 100.



Parameters	Treatments		
	LRE	NaCl	NaCl+LRE
Shoot fresh weight	109.29	62.82	86.86
Shoot dry weight	110.45	62.69	86.57
Total chlorophylls	110.34	68.47	84.73
Total carotenoids	110.00	85.71	94.29
Fv/Fm	104.94	86.42	97.53
Pn	106.72	56.81	81.93
Tr	102.12	57.17	83.88
gs	111.90	59.52	88.10
MDA	82.04	179.13	132.04
$O_2^{\bullet-}$	76.74	158.14	137.21
H_2O_2	98.83	312.62	145.24
Free proline	128.13	224.13	293.75
Soluble sugars	119.30	168.42	193.86
α-TOC	118.97	141.38	179.31
AsA	107.44	215.70	133.06
AsA redox state	102.12	150.00	138.76
GSH	109.64	266.27	193.98
GSH redox state	102.82	286.62	273.24
POX	114.01	159.87	206.37
CAT	121.93	152.29	173.25
APX	117.61	174.03	203.28
SOD	129.58	184.11	211.26
GR	105.98	186.62	231.23
SOD gene expr.	240.65	380.49	528.46
CAT gene expr.	108.26	154.55	125.62
APX gene expr.	151.67	215.00	125.83
DHAR gene expr.	123.39	132.26	58.87
GR gene expr.	102.44	86.18	46.34
PrxQ gene expr.	124.56	225.44	171.05
K^+	127.70	64.73	99.32
Na^+	94.92	567.80	177.12
Cl^-	96.24	171.50	106.99
K^+/Na^+ ratio	134.34	11.29	56.44

containing gibberellins (GAs) may increase GAs seed content, which may increase the activity of GAs biosynthesis enzymes such as GA20ox and GA3ox (Li et al., 2016). This leads to the promotion of seed germination, producing seedlings that can overcome salt stress conditions. Among GAs, GA_4 is unequivocally linked to the GA receptor to legitimize GA signals and maintain the appropriate dimension of endogenous GAs to enhance seed germination (Sun, 2008) under salt stress despite GAs catalytic effects at various stages of plant development. Gibberellins activate cellular division and elongation, resulting in expanded leaf area and enhanced photosynthesis rate (Elham et al., 2014).

3.2. Physio-biochemical attributes and oxidative stress (e.g., H_2O_2 and $O_2^{\bullet-}$)

Soaking pea seed in LRE did not affect transpiration rate (Tr) and hydrogen peroxide (H_2O_2), and significantly increased total chlorophylls and total carotenoids contents, Fv/Fm, net photosynthetic rate (Pn), stomatal conductance (gs), and soluble sugars content, while malondialdehyde (MDA) and superoxide ($O_2^{\bullet-}$) contents were significantly reduced in pea seedlings compared to the control (Figs. 1 and 2). Under salt stress, total chlorophylls and total carotenoids contents, Fv/Fm, Pn, Tr, and gs were significantly reduced, while MDA, H_2O_2, $O_2^{\bullet-}$, and soluble sugars contents were significantly increased. However, pretreatment with LRE significantly reduced the adverse effects of salt stress and significantly increased total chlorophylls, total carotenoids and soluble sugars contents, Fv/Fm, Pn, Tr, and gs, while MDA, H_2O_2, and $O_2^{\bullet-}$ contents were significantly decreased compared to the salt stress treatment.

Salt stress (150 mM NaCl) severely reduced leaf photosynthetic pigments and photosynthetic efficiency, but pretreatment using LRE greatly improved these photosynthetic attributes (Fig. 1, Table 4). Due to the reduction or inhibition of nutrient uptake under salt stress (Tables 2 and 4), chlorophyll content is strongly affected, which might be attributed to the synthesis of certain inhibited chlorophyll forms, in parallel with an increase in chlorophyll-degrading enzymes (Rezende et al., 2018). In this study, oxidative stress resulting from excess ROS (e.g., H_2O_2 and $O_2^{\bullet-}$) production under salinity stress (Fig. 2, Table 4) contributes to damage to plasma membranes (in terms of increased malondialdehyde; MDA content) and chlorophyll levels and loss of pigment, which are seen as indicators of oxidative damage (Rady et al., 2018). This is a result of increased Na^+ accumulation in seedling leaves, which causes degradation in chlorophyll or reduced uptake of Mg, which may affect the synthesis of chlorophyll molecule. However, pretreatment using LRE increased photosynthetic parameters because of LRE biostimulants acted to reduce oxidative stress (e.g., excessive H_2O_2 and $O_2^{\bullet-}$ contents) and MDA content, reflecting in plasma membranes reform (Figs. 1 and 2, Table 4).

LRE contains some nutrients and phytohormones, especially cytokinins that suppress leaf senescence and maintain higher leaf area and expand leaf photosynthetic pigments. Further, micronutrients, especially Fe in LRE, can be accessed to support the main content in pretreated seedlings to stimulate many enzymes associated with the pathway of chlorophyll biosynthesis and some antioxidant enzymes, for example, APX and GR that scavenge ROS and protect chlorophyll from degradation (Zayed et al., 2011). The *Fv/Fm* ratio reflects the primary photochemistry of PSII capacity, which is highly sensitive to different environmental stressors (Sheng et al., 2008), including salinity. It is undoubtedly highly sensitive to salt stress and is usually an indicator of photoinhibition and/or other deterioration as a result of PSII complexes (Ranjbarfordoei et al., 2006). The *Fv/Fm* ratio was significantly increased in this study by pretreatment using LRE under salt stress (Fig. 1, Table 4). The activity of cell physiology, especially photosynthesis, has been reported to decrease under salt stress due to osmotic stress, nutritional imbalance, and oxidative stress (Rios et al., 2017). As

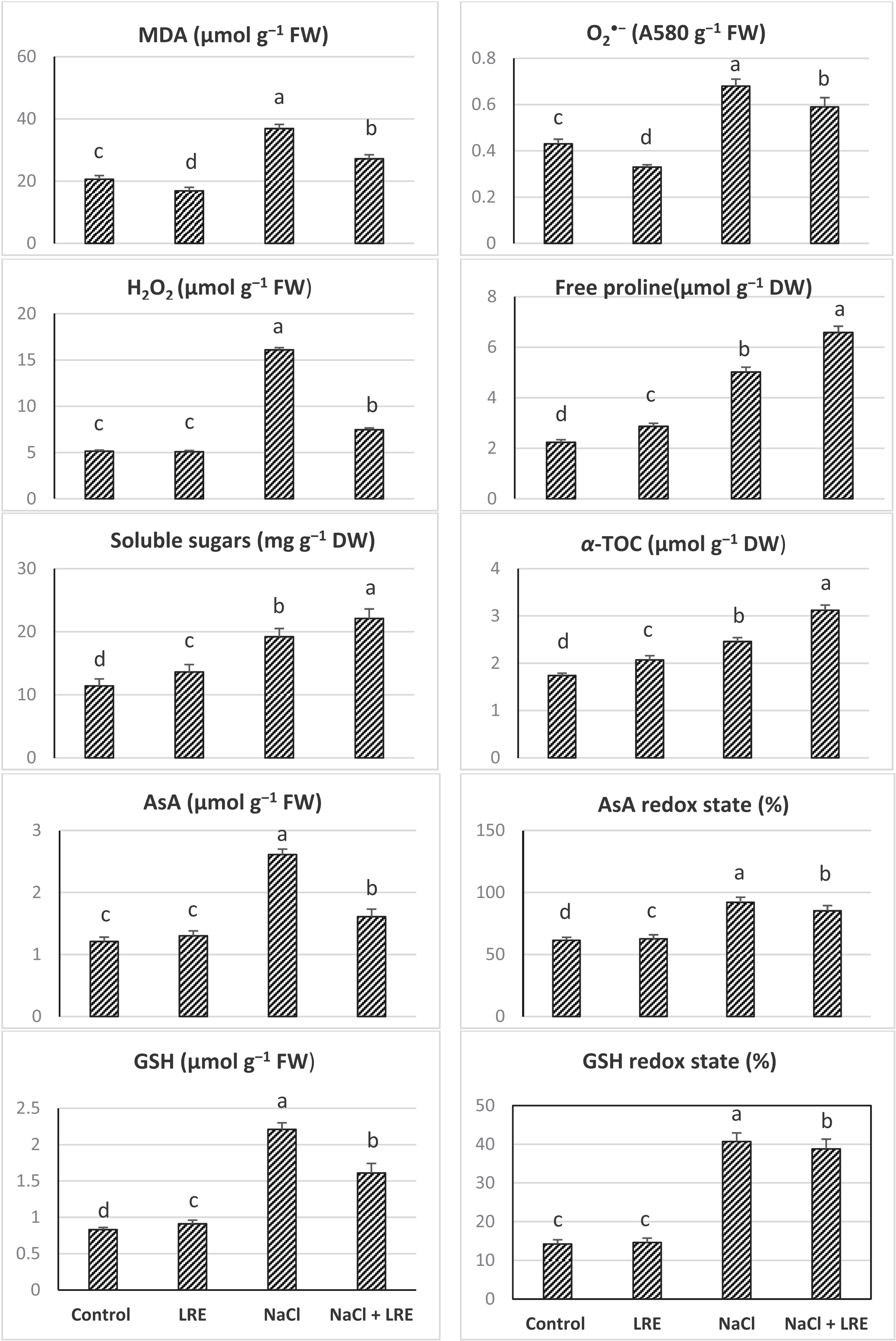


Fig. 2. Oxidative stress (MDA, $O_2^{\bullet -}$, and H_2O_2), defensive compounds (free proline, soluble sugars, α-TOC), ASA and GSH and their redox states of salt-stressed pea plant as influenced by seed soaking in LRE. Different letters above bars within treatment groups are significantly different ($P < 0.05$).

necessary bases for photosynthesis, salt-stressed leaf gas exchange, stomatal conductance (gs), net photosynthesis rate (Pn), transpiration rate (Tr) were effectively retrieved by pretreatment using LRE that highly increased photosynthetic effectiveness in this study (Fig. 1, Table 4). As one of the main components of LRE (Table 1), K^+ that significantly increased in pea seedlings under salt stress with LRE pretreatment (Tables 2 and 4) is considered to be one of the major osmoprotectants to maintain water content in plant tissues and manage stomatal opening/closing that control photosynthesis rate of stressful plants (Dubey, 2005).

In this study, lipid peroxidation was determined as malondialdehyde (MDA) content, which appears to be a good biochemical indicator of tolerance/sensitivity to salt stress (Hernández and Almansa, 2002). The MDA content was associated with oxidative stress ($O_2^{\bullet-}$ and H_2O_2) and increased in pea seedlings under salt stress (Fig. 2). Increased MDA and oxidative stress are associated with increased electrolyte leakage (EL) and decreased membrane integrity and cellular water content (Rady et al., 2019a). These negative properties adversely affect metabolic functions and consequently plant biomass production (Fig. 1). The rate of photosynthesis in salt-stressed seedlings is reduced by closing the stomata, leading to a decrease in CO_2 fixation, while electrons exchange and light response usually continue. In addition, the acceptance electrons by NADP will be decreased and oxygen can go as a receptor for electrons, prompting the production of more ROS such as $^1O_2$, $O_2^{\bullet-}$, H_2O_2, and $OH^{\bullet}$ radicals that lead to cell membrane peroxidation and increase EL (Alireza et al., 2014). However, salt-stressed pea seedling pretreated with LRE showed significant decreases in MDA, $O_2^{\bullet-}$, and H_2O_2 contents. These traits have been repaired by antioxidants and phytohormones present in LRE along with the induction of endogenous antioxidants in pea seedling to minimize damage caused by oxidative stress.

As an important one of the major osmoprotectants necessary for stressed-plants, accumulation of soluble sugars (Fig. 2, Table 4) is required for cell membrane integrity and relieving toxic ions (Alzahrani and Rady, 2019). Osmoregulation by soluble sugars may be one of the mechanisms stimulated by priming LRE in this study. Growing stressed-plants create and accumulate various osmoprotectants as compatible solutes a mechanism to protect themselves against adverse effects of stress. This effective mechanism maintains water in cells for normal physio-biochemical processes (Rady et al., 2019b). Under stress conditions in this study, accumulation of soluble sugars along with proline (Fig. 2, Table 4) safeguards plant cells by the balance between cytosol and vacuole osmotic strengths and osmotic strength of outer saline environment.

3.3. Antioxidative defense system; enzymatic and non-enzymatic components

In comparison to the control, soaking pea seed in LRE did not affect ascorbic acid (AsA) content, glutathione (GSH) redox state, and peroxidase (POX) activity, while free proline, α-tocopherol (α-TOC) and GSH contents, AsA redox state, and activities of CAT, APX, SOD, and GR were significantly increased in pea seedlings compared to the control (Fig. 2, Tables 3 and 4). Under salt stress, all the enzymatic and non-enzymatic attributes mentioned above were further increased. However, pretreatment with LRE significantly ameliorated the adverse effects of salt stress and significantly increased free proline and α-TOC contents, and activities of all enzymes (e.g., POX, CAT, APX, SOD, and GR), AsA and GSH contents, and AsA and GSH redox states were significantly decreased compared to the salt stress treatment.

Proline is a non-enzymatic antioxidant that changes positively through the application of LRE (Desoky et al., 2019; Rady et al., 2019a). It is one of the main components of the LRE (Table 1). It plays an important role in osmotic adjustment of plant cells. Plants accumulate more content of proline under saline conditions (Zhu, 2001; Rady et al., 2019a). By LRE application, proline content was further accumulated (Fig. 2, Table 4). This accumulation of proline in salt-stressed plant tissues contributed to the increased tolerance in pea seedling to salt stress and to a significant decrease in damage caused due to oxidative stress generated under salt stress. Increased proline content is associated with improved antioxidant systems in pea seedlings to counter salt stress damage. In addition, other antioxidants and biologically active ingredients present in LRE are integrated with proline to improve antioxidative systems in pea seedlings to counter salt stress (Rady et al., 2019a). Typically, salt stress reactions in plants involves a reduction in cellular water potential through improvements in osmotic adjustments or net groups of solute, an important use for the continuation of both cellular water content and turgor (Farhangi-Abriz and Torabian, 2017). Like proline, the accumulation of soluble sugars gives the cell a guarantee by maintaining harmony between the osmotic quality of the cytosol and that of the vacuole and external condition (Sairam et al., 2002). Where LRE contains in its composition on proline, soluble sugars and other important biologically active compounds at high concentrations, LRE is considered as an important tool involved in osmotic adjustment and may help modify the gene expression that plays a pivotal role in storage functions, metabolic processes and tolerance (Desoky et al., 2019; Rady et al., 2019a). Among the important non-enzymatic antioxidants, tocopherols are capable of scavenging ROS and reducing oxidative stress under salt stress (Bano et al., 2014). Results of this study indicated that *α*-TOC content was significantly increased in pea seedlings under salt stress and further increased through LRE pretreatment (Fig. 2, Table 4). The increase in *α*-TOC content was associated with a decrease in MDA (membrane lipid peroxidation, Fig. 2, Table 4) content due to its pivotal role as an antioxidant, forming an important part of defense systems to counter oxidative stress (Valero et al., 2016).

Results of this study showed that the activity of all enzymes (e.g., POX, CAT, APX, SOD, and GR) were significantly increased in pea seedlings under salt stress and further increased through LRE pretreatment (Tables 2 and 4). Consistently, APX activity must be strictly adjusted along with a balance of the AsA and GSH pool, which improves antioxidant capacity in plant cells and decreased oxidative damage (Foyer and Noctor, 2011). Ascorbate (AsA) has the ability to donate electron in many enzymatic and non-enzymatic reactions so that it's a highly powerful ROS scavenger. It is able to protect plasma membranes by scavenging $O_2^{\bullet-}$ and $OH^{\bullet}$ directly (Semida and Rady, 2014). Glutathione (GSH) and AsA are the main antioxidants of AsA-GSH cycle that decrease the concentration of H_2O_2 in plant cells. Primarily, glutathione reductase (GR), MDHAR and DHAR are responsible for providing substrates for APX through the formation of GSH and AsA (Zhou et al., 2017). In addition, the conversion of GSSG to GSH is catalyzed by GR. Salt stress causes an increase in the level of H_2O_2 so that the content of AsA and the activity of AsA redox state are elevated as a response to stress (Hasanuzzaman et al., 2017). However, LRE application significantly reduced AsA content and AsA redox state in salt-stressed pea seedlings (Fig. 2, Table 4). Like AsA content and AsA redox state, GSH content and GSH redox state were behaved the same trend (Fig. 2, Table 4). Rady et al. (2019a) have reported that under salt stress, LRE pretreatment overcame the accumulation of $O_2^{\bullet-}$ and H_2O_2 because LRE contains AsA and GSH at high concentrations to eliminate oxidative stress. The reduction in AsA and GSH contents and their redox states by pretreatment with LRE may be due to that other bioactive component present in LRE have been effectively acted upon to overcome the adverse effects of salt stress in pea seedlings (Fig. 1–4, Table 1–4). Plants create many antioxidant defense systems including non-enzymatic (e.g. proline, AsA, GSH, tocopherols, carotenoids, etc.) and enzymatic (e.g., GR, SOD, CAT, POX, APX, etc.) antioxidants to avoid damage occurred in salt-stressed pea seedlings due to oxidative stress (Fig. 1–4, Table 1–4). Pretreatment using LRE highly activated these antioxidant defense systems in salt-stressed pea seedlings, which showed less oxidative damage (Fig. 2, Tables 2 and 4). The first line of defense against ROS is the SOD which converts $O_2^{\bullet-}$ to H_2O_2 (Abogadallah, 2010). The activity of SOD was also increased in pea seedlings under salt stress and further increased by pretreatment with LRE (Tables 2 and 4) to add more defensive functions for biological systems. CAT is the main scavenger of ROS in leaves, playing an important role in eliminating the H_2O_2. It is found exclusively in glyoxysomes and peroxisomes and may inhibit the formation of hydroxyl radicals, which is responsible for lipid peroxidation of cell membranes and has many detrimental effects on plant growth (Abogadallah, 2010). In the present study, CAT was strongly activated in salt-stressed pea seedlings pretreated with LRE (Tables 2 and 4). It was played a pivotal role in reducing oxidative damage in seedling tissues, as evidenced by fewer contents of H_2O_2 and MDA (Fig. 2, Table 4). Like CAT, APX removes H_2O_2, and the activity of

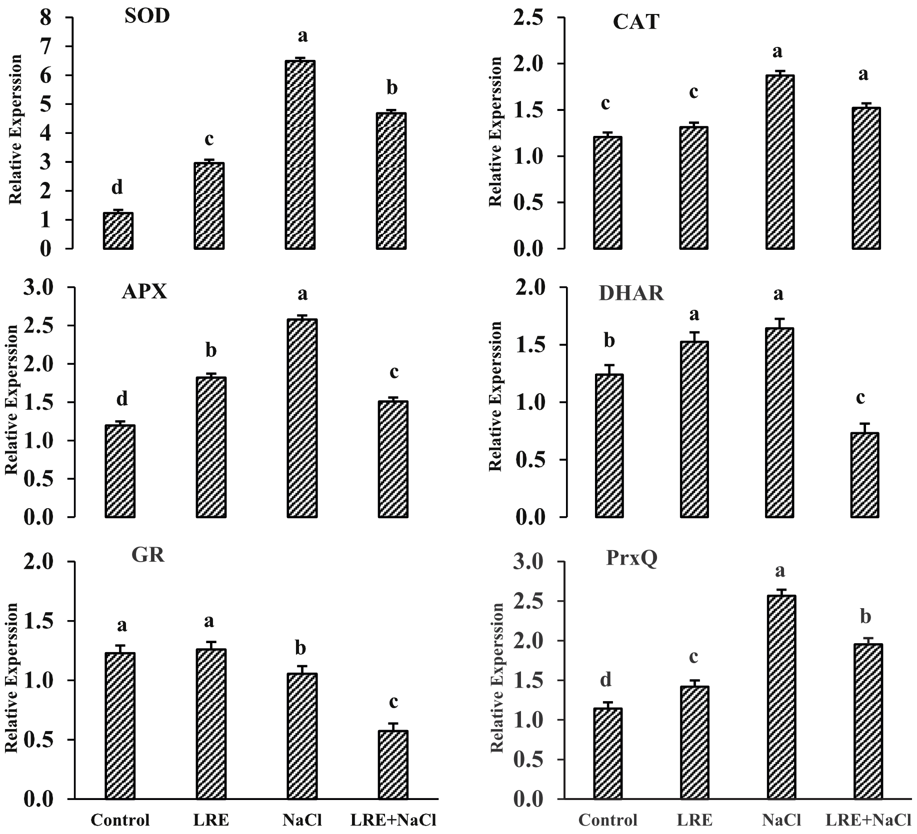


Fig. 3. Transcript levels of antioxidant enzymes encoding genes in salt-stressed pea seedling as affected by seed soaking in LRE. Transcript amounts were quantified by qPCR and relative to actin transcript levels. qPCR data represent the average from three independent experiments with two technical replicates each. Different letters above bars within treatment groups are significantly different ($P < 0.05$).

both enzymes increase in several plant species under salt stress (Rady et al., 2013; Zaki and Rady, 2015; Desoky et al., 2018).

3.4. Transcript levels of genes encoding antioxidant enzymes

Quantitative RT–PCR and conventional RT-PCR were used to compare the expression of different genes encoding antioxidant enzymes in normal seedlings, NaCl-salt-stressed seedlings, and LRE pretreated + salt-stressed seedlings (Figs. 3 and 4, Table 4). The Cu–Zn/SOD transcript level was highly increased in salt-stressed pea seedlings followed by LRE pretreated + salt-stressed seedlings compared to the control. However, the expression of CAT-encoding gene showed significant increase under LRE pretreatment + salt-stress compared with the other treatments. No significant difference was observed between the control seedlings and LRE pretreated seedlings. The transcript level of CAT gene was increased under salt stress and LRE pretreatment compared to the other treatments. The transcript level of APX gene was significantly increased in LRE pretreated + salt-stressed seedlings compared with the control. Also, pretreated plants using LRE showed only increased expression of the APX gene compared with salt-stressed and control seedlings. The transcript level of GR gene showed a significant drop under salt stress. However, the amount of GR gene transcript level was increased in pea seedlings when pretreated with LRE. Similarly, the level of gene transcript of DHAR was significantly decreased under salt stress only compared to the other treatments. However, there was a significant increase in the DHAR gene transcript level in pea seedlings pretreated with LRE and stressed with 150 mM NaCl compared to salt-treated seedlings. The highest transcript level of PrxQ gene was observed in LRE pretreated + salt-stressed seedlings. In contrast, with LRE pretreatment without NaCl stress resulted in much lower PrxQ gene transcript level compared with the other NaCl-salt treatments.

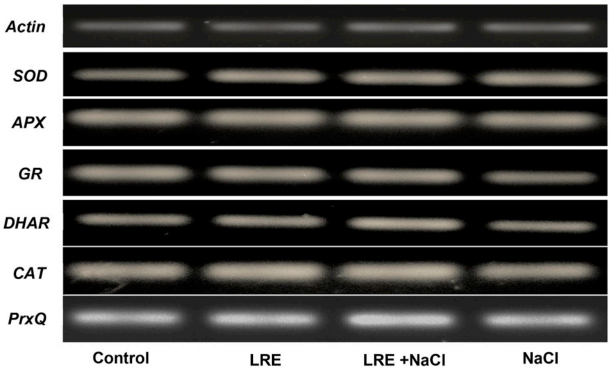


Fig. 4. Semi-quantitative RT-PCR of antioxidant enzymes genes in pea seedling from cDNA of under salt stress and control conditions, compared with exogenous treatments of licorice root extract (LRE). Samples standardized to Actin. RT-PCR products were separated on agarose gels (1.5%) and visualized by ethidium bromide stain.

Overall, results of qRT–PCR showed that pretreatment with LRE upregulated gene expression of *SOD*, *CAT*, *APX*, *DAHR*, *GR* and *PrxQ* in salt-stressed pea seedlings. The changes in transcript levels of antioxidant enzyme-encoding genes in pea seedlings were somewhat consistent with the observed trend of the corresponding antioxidant enzyme activities. To gain further insight into the mechanisms of salt tolerance in pea seedlings pretreated with LRE, the gene expression of peroxiredoxins (*PrxQ*) was studied. The functions of the different plant peroxiredoxins and their isoforms have not been properly characterized, but all enzymes appear to play a role in detoxification of alkyl hydroperoxide in different plant compartments (Rouhier and Jacquot, 2002). The expression of *PrxQ* gene was enhanced and upregulated in salt-stressed pea seedlings pretreated using LRE. Hossain et al. (2017) found that *PrxQ* transcript and protein levels were slightly increased during long-term salinity stress. The *PrxQ* is assumed to be chloroplastic and detoxifies alkyl hydroperoxides (Rouhier and Jacquot, 2002). The high expression of *PrxQ* transcript in salt-stressed pea seedlings pretreated with LRE suggests that this gene is involved in seedling antioxidant defense and redox signaling. Like *PrxQ* gene, all the other genes (*SOD*, *CAT*, *APX*, *DAHR*, and *GR*) were behaved the same trend and transcript levels to contribute to increase in the tolerance to salt stress in pea seedlings.

4. Conclusions

To increase salt tolerance in pea seedlings to withstand salt stress, pretreatment can be applied using LRE as an effective source of natural biostimulants. Pea seed soaking with LRE can provide seed and then seedling with more bioactive components (i.e., Se, GAs, amino acids, sugars, vitamins, phytohormones, and mineral nutrients) to improve germination growth, increasing seedling tolerance to salt stress. The validity of LRE in overcoming salt stress in pea seedlings that reflect better performance is found to be due to improved antioxidant systems; non-enzymatic and enzymatic antioxidants (i.e., free proline, soluble sugars, carotenoids, CAT, POX, SOD, APX, and GR) with low oxidative stress (H_2O_2 and $O_2^{\bullet-}$ radicals) and toxic ions (Na^+ and Cl^-) through the application of LRE applied as seed soaking. Many mechanisms have been developed and adopted by pea seedlings to enhance their tolerance to salt stress through pretreatment using LRE. From these mechanisms, the increased contents of soluble sugars, proline, and K^+ as effective osmoprotectants, increased ratio of K^+/Na^+, increased levels of non-enzymatic antioxidants and their redox states, increased activities of antioxidant enzymes, and increased transcript levels of genes encoding antioxidant enzymes, especially peroxiredoxins (*PrxQ*) to gain further insight into the mechanisms of salt tolerance in pea seedlings pretreated with LRE. This suggests that this gene is involved in seedling antioxidant defense and redox signaling. The validity of LRE has also been reported in our study as a “stay green effect” due to the LRE bioactive components (i.e., mineral nutrients, phytohormones, sugars, vitamins, and amino acids), which support the antioxidative defense systems in pea seedlings to withstand salt stress.

Author contributions

Conceived and designed the experiments: EMD and AIE. Performed the experiments: EMD, AIE, and AMAM. Analyzed the data: EMD and AIE. Contributed reagents/materials/analysis tools: EMD, AIE, and AMAM. Wrote the paper: EMD, AIE, AMAM, and MMR. Revised the paper: MMR. All authors read and approved the final manuscript.

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