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PHYSIOLOGICAL, BIOCHEMICAL, AND ANTIOXIDANT PROPERTIES OF TWO GENOTYPES *OF VICIA FABA* GROWN UNDER SALINITY STRESS

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Abstract

The faba bean genotypes Hassawi-3 and ILB-4347 were evaluated under three different NaCl treatments (50 mM, 100 mM, and 150 mM) for growth, physiological parameters, and enzymatic and non-enzymatic antioxidants in leaves. Salinity stress significantly reduced the growth and biomass yield of both genotypes. Calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^+) contents were reduced, whereas sodium content was increased in both genotypes with increasing NaCl concentration. Higher levels of Ca^{2+}, Mg^{2+}, K^+, and K^+/Na^+ ratio, along with lower Na^+ accumulation were observed in ILB-4347 than those in the Hassawi-3 genotype. Chlorophyll, carotene, leaf relative water content (LRWC), proline, and protein content were reduced (by 54.61%, 51.51%, 42.33%, 105.19% and 44.80% in Hassawi-3 and 35.29%, 38.29%, 31.92%, 113.93% and 34.80% in ILB-4347) these effects were treatment and genotype dependent. Salinity stress significantly enhanced hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and electrolyte leakage (EL) in both genotypes; however, Hassawi-3 showed more accumulation compared to ILB-4347. Both genotypes subjected to salt stress showed enhancement in total antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbic acid (AsA) content. These results indicate that ILB-4347 is more tolerant than the Hassawi-3 genotype against salt stress and could be used as part of a better strategy to reclaim salt affected soils.

Key words: Salt stress; Faba bean genotypes; Growth; Pigments; Osmolytes; Reactive oxygen species; Antioxidants

Introduction

Salinity stress is one of the major abiotic stresses that affect plant growth and development and limit agricultural yield. Salinized affected areas are estimated to increase at a rate of 10% annually (Shrivastava & Kumar, 2015) and more than 20–50% of agricultural land is influenced by salt stress (Xu *et al.*, 2011). Worldwide, 800 million hectares of land and 32 million hectares of agricultural land are salt-affected (FAO, 2015). The effect of salt stress is more obvious in arid and semiarid regions, which are dominated by other environmental stresses. Drought, heat, and poor land-water management contribute to salinity problems and pose a challenge for agriculture production in these regions (de Azevedo Neto *et al.*, 2006). Limited crop production due to degradation of fertile land will affect the food demand of the increasing world population. Development of salt tolerant crops may expand the use saline-affected land for cultivation.

Most crop plants, including faba beans (*Vicia faba)*, are sensitive to ionic toxicity and osmotic stress (Munns & Tester, 2008). NaCl reduces photosynthetic activity and photoassimilate partitioning (Suwa *et al.*, 2006), which is associated with reduction in chlorophyll content and deformation of chlorophyll ultra-structures (Meng *et al.*, 2011). NaCl also suppresses the expression of genes encoding carboxylation enzymes such as Rubisco (Koch, 1996), which could be the main reason for reduced plant growth and development. Moreover, enhanced lipid peroxidation, increased production of reactive oxygen species (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and membrane leakage are also associated with NaCl toxicity (Roychoudhury *et al.*, 2008; Ahmad *et al.*, 2010a; Ahmad *et al.*, 2010b; Møller & Sweetlove, 2010; Ahmad *et al.*, 2016; Ahmad *et al.*, 2017; Ahmad *et al.*, 2018a; Ahmad *et al.*, 2018b).

Plants can accumulate osmolytes such as proline and glycine betaine to cope with salt stress, which decreases the cytoplasmic osmotic potential and facilitates water absorption (Ahmad *et al.*, 2010b; Qureshi *et al.*, 2013; Pottosin *et al.*, 2014). Plants have also developed an antioxidative system (SOD, CAT, APX, GR, and AsA) (Noctor & Foyer, 1998; Apel & Hirt, 2004) to repair the oxidative damage to macromolecules (Xiong & Zhu, 2002; Ahmad *et al.*, 2010a). Salt tolerant peas (Hernandez *et al.*, 2000), cotton (Gossett *et al.*, 1994), rice (Dionisio-Sese & Tobita, 1998), foxtail millet (Sreenivasulu *et al.*, 2000), potato (Benavídes *et al.*, 2000), sugar beet (Bor *et al.*, 2003), and wild tomato (Mittova *et al.*, 2002) show a close correlation with antioxidant capacity.

The faba bean (*Vicia faba* L.) is among the most ancient plants under cultivation. It is part of the eastern Mediterranean diet and has a relatively high content of proteins, carbohydrates, vitamins, antioxidants, and minerals. The dried seed contains proteins ranging from 26% to 41% (Picard, 1977), carbohydrate contents varying from 51% to 68% (Cerning *et al.*, 1975), and unsaturated fatty acid 88.6% (Duke, 1981). Improving tolerance to salinity stress in faba beans is necessary to increase their productivity under saline conditions. Therefore, the present study aimed to investigate the physiological and biochemical responses of two faba bean genotypes to different levels of salt stress.

Materials and Methods

Plant materials and experiments: Seeds of two faba bean (*Vicia faba*) genotypes (Hassawi-3 and ILB-4347) were procured from the legume research unit, College of Food and Agriculture – King Saud University, Riyadh. Before sowing, seeds were surface sterilized with 2.5% sodium hypochlorite for 15 min, then washed with sterile deionized water and left for germination in sterile sand at 23°C for five days. Seedlings at homogenous stages of development were selected and planted in a mixture of sterilized sand and peat (3:1 ratio). The experiment was conducted in a greenhouse with 26°C/20°C day/night temperature, 50–80% relative humidity, and a 16 h photoperiod at the Faculty of Sciences, King Saud University, Riyadh. The experimental design was arranged in a split-plot in a completely randomized manner replicated five times. The seedlings were irrigated with tap water and allowed to grow for 14 days. Hoagland nutrient solution (1/10 strength) was applied once a week. NaCl stress was initiated when seedlings were almost 21 days old, attaining 2 to 3 true leaves. The treatments comprised control, 50 mM NaCl, 100 mM NaCl, and 150 mM NaCl. Sampling for various studies was performed at 35 days after NaCl treatment.

Growth measurement: The roots were separated from the shoots and the plant height and root length was measured. The shoots and roots were weighed to determine the fresh weight and then dried at 80°C for 48 h in an oven to estimate the dry weight.

Leaf area was measured using a leaf area meter LICOR-MODEL LI-3000, Lincoln, NE, USA.

Determination of ion accumulation: Mineral elements and Na^+ content were estimated using the method described by Jackson (2005) using an atomic-absorption spectrophotometer (Perkin Elmer AA300, USA).

Determination of photosynthetic pigments: Chlorophyll a, chlorophyll b, and carotenoids were estimated according to the method of Moran and Porath (1980) and Wellburn (1994), respectively. Absorption spectra of the extracts were measured between 400 and 700 nm using the SPEKOL®1500 UV/VIS Spectrophotometer.

Determination of proline, crude protein content, and leaf relative water content (LRWC): Proline content was determined as described by Bates *et al*., (1973). Absorbance of the supernatant was spectrophotometrically recorded at 520 nm after ten minutes on the SPEKOL®1500 UV/VIS Spectrophotometer. Proline concentration was determined by a calibration curve and was expressed as µmol proline g^{-1} FW.

Crude Proteins from leaves were extracted using the micro-Kjeldahl method according to AOAC (2000). Crude protein content was calculated using the following equation:

$$V1 \times N \times 14 \times 100 \times 6.25 \text{ CP. } \% = Ws \times 1000,$$

where: CP = Crude protein, T = Titration reading, B = Blank titration reading, N = HCl normality, Ws = Sample weight, and 1000 = To convert to mg.

$$\%\ N = \frac{V_1 \times N_1 \times V_2 \times 14 \times 100}{A \times B \times 1000}$$

where, v1 = volume of HCl used, N = HCl normality 0.005, V2 = volume of diluted digested sample, 14 = molecular weight of nitrogen, A = volume of diluted sample used for titration and, B = sample weight (0.5 g), 100 = percentage conversion factor, and 1000 = to convert milligrams to grams.

Leaf relative water content (LRWC) was determined according to the method of Gulen & Eris (2003) and calculated by the following formula:

$$\text{LRWC} = \frac{\text{(Fresh weight - Dry weight)} \times 100}{\text{(Turgid weight - Dry weight)}}$$

Estimation of H_2O_2, MDA content, and electrolyte leakage (EL): The H_2O_2 level was measured colorimetrically as described by Aebi (1984). Absorbance of the reaction mixture containing the enzyme extract, 100 mM phosphate buffer (pH 7.0), the chromogen aminoantipyrine, and H_2O_2 was read at 510 nm.

The extent of lipid peroxidation was estimated by quantifying the malondialdehyde (MDA) content of leaves according to the method of Ohkawa *et al*., (1979). The absorbance of the supernatant was recorded at 532 nm.

Electrolyte leakage (EL) was determined as described by Dionisio-Sese & Tobita (1998). Twenty leaf discs were incubated with deionized water in a test tube and their electrical conductivity was measured (ECa). The tubes were then heated at 50°C for 25 min and electrical conductivity was measured (ECb). The same tubes were then heated at 100°C for 10 min and electrical conductivity was again measured (ECc). EL was calculated by the formula:

$$\text{EL}\% = \frac{ECb - ECa}{ECc} \times 100$$

Estimation of antioxidant enzymes assays: Leaf samples (500 mg) were ground in liquid nitrogen; the frozen powder was added to 10 mL of 100 mM phosphate buffer (KH_2PO_4/K_2HPO_4 pH 7.0, 0.1 mM Na_2EDTA, and 0.1 g of polyvinylpyrrolidone PVP). The homogenate was filtered through cheese cloth, centrifuged at 15000 × *g* for 10 min at 4°C, and the resulting supernatant was used for determination of antioxidant enzymes activity (Mukherjee & Choudhuri, 1983).

The antioxidants, superoxide dismutase SOD (EC1.15.1.1), CAT (EC1.11.1.6), Glutathione reductase GR (EC1.6.4.2), and ascorbic acid were assayed following the protocol provided by Biodiagnostic, Egypt. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm (Nishikimi *et al.*, 1972). One unit of CAT activity was estimated as the amount of enzyme that decomposes 1 µmol H_2O_2 at 510 nm sec^{-1} in 1 mg fresh tissue protein (Aebi, 1984). GR was estimated in a reaction mixture containing potassium phosphate buffer (0.1 M, pH 7.5, 1 mM EDTA, 2 mM Nicotinamide adenine dinucleotide phosphate, and 50 mM oxidized glutathione GSSG, with enzymes added to initiate the reaction. Changes in OD

were observed for 3 min at 340 nm. (Goldberg & Spooner, 1983). Ascorbic acid (AsA) was measured by the method of Huang *et al*., (2005).

Statistical analysis

The data were subjected to analysis of variance using SAS 9.2 software. SAS Institute Inc, USA and treatment means were compared using Fisher LSD at $p<0.05$.

Results

Growth and biomass yield: NaCl stress reduced the leaf area in both cultivars, and maximum reduction by 62.39% and 51.63% was reported in Hassawi-3 and ILB-4347, respectively, under 150 mM NaCl stress compared to the controls (Fig. 1A).

High NaCl concentration (150 mM) reduced the shoot and root length by 60.39% and 53.92%, respectively, for Hassawi-3; however, the ILB-4347 genotype showed 45.64% and 38.41% reduction in shoot root length, respectively, relative to the controls (Fig. 1B).

Shoot fresh weight was also significantly reduced by NaCl stress and the maximum reduction by 68.87% and 49.67% was observed in Hassawi-3 and ILB-4347, respectively, under 150 mM NaCl stress compared to the controls (Fig. 1C). Shoot dry weight was also decreased by 61.29% in Hassawi-3 and 55.29% in ILB-4347 at 150 mM NaCl, compared to the controls (Fig. 1D). NaCl (150 mM) also reduced the root fresh weight and dry weight by 59.92% and 47.36%, respectively, in Hassawi-3, whereas in ILB-4347, the root fresh weight and dry weight decreased by 51.89% and 40.47%, respectively, compared to their controls (Fig. 1C-D).

Mineral content: Calcium content reduced by 25.14% and 16.07% in Hassawi-3 and ILB-4347, respectively, under 150 mM NaCl stress compared to the control. Mg^{2+} and K^{+} also decreased with increasing NaCl concentration a maximum reduction by 47.95% and 18.84%, respectively, was observed in Hassawi-3, whereas ILB-4347 showed a reduction of 26.81% in Mg^{2+} and 19.03% in K^{+} content at 150 mM NaCl compared to that in the control (Fig. 2A-C).

The NaCl stress (50 mM) enhanced the Na^{+} content of Hassawi-3 by 2.91-fold and that of ILB-4347 by 2.38-fold (Fig. 2D). At high NaCl concentration (150 mM), the Na^{+} content was enhanced by 11.71-fold and 8.40-fold in Hassawi-3 and ILB-4347, respectively, compared to the control. The Na^{+}/K^{+} ratio was also enhanced by NaCl stress and a maximum increase by 26.70-fold and 10.32-fold was recorded in Hassawi-3 and ILB-4347, respectively, under 150 mM NaCl stress compared to the control (Fig. 2E).

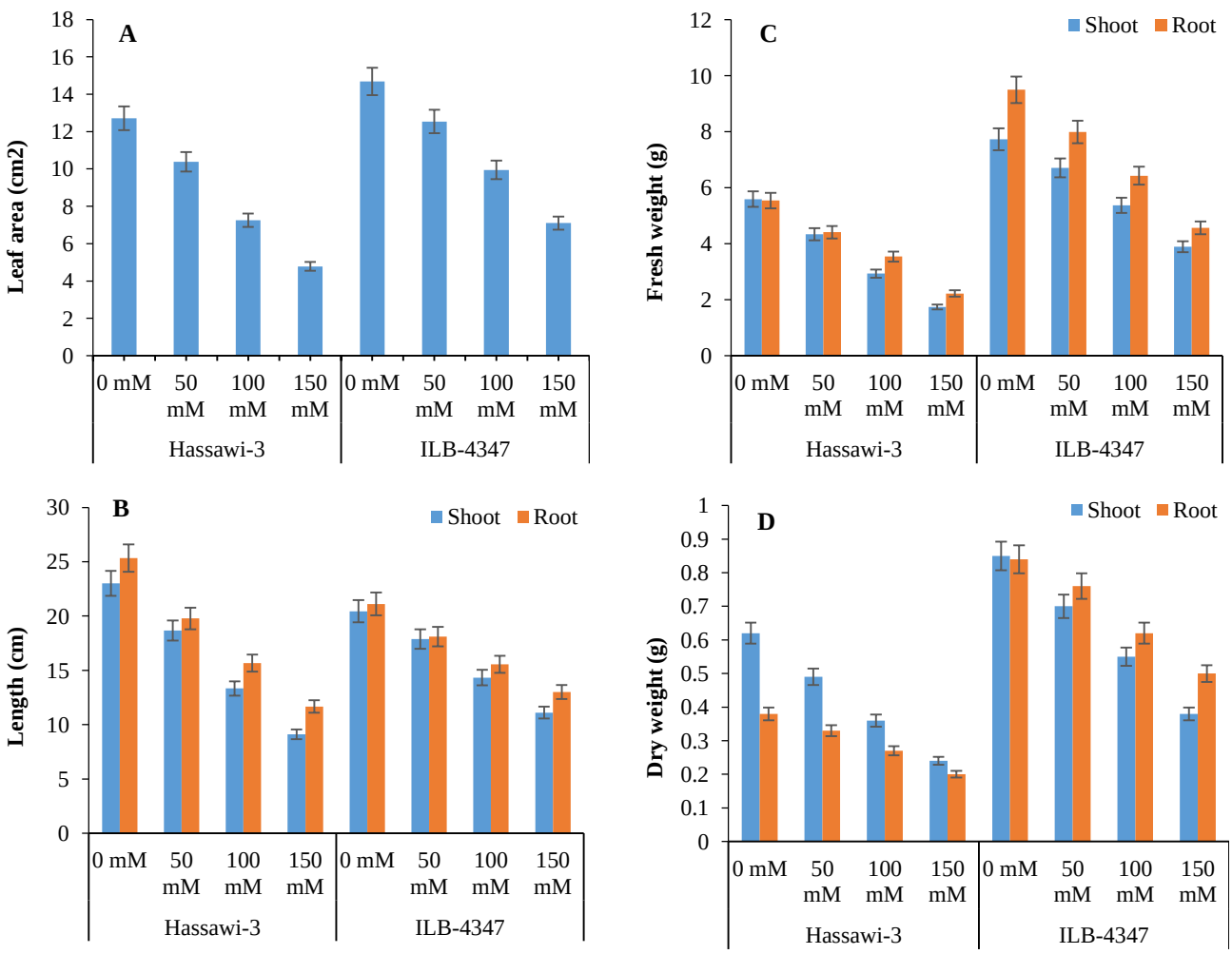

Fig. 1. Effect of salinity treatment on (A) leaf area, (B) shoot and root length, (C) shoot and root FW and (D) shoot and root DWin Hassawi-3 and ILB-4347 genotypes. Each point is the mean of three replications and the bars indicate +S.E.

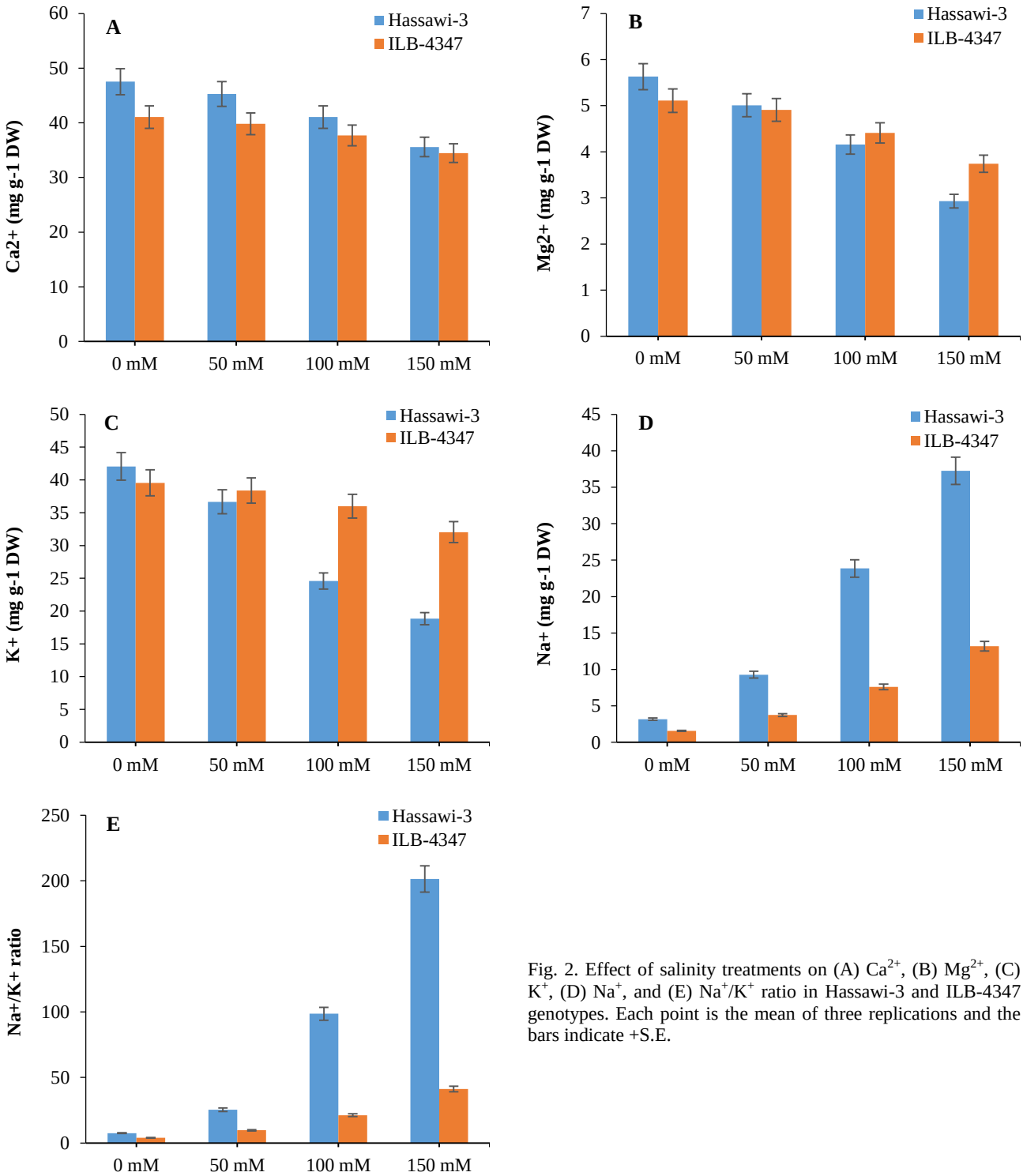

Fig. 2. Effect of salinity treatments on (A) Ca^{2+}, (B) Mg^{2+}, (C) K^{+}, (D) Na^{+}, and (E) Na^{+}/K^{+} ratio in Hassawi-3 and ILB-4347 genotypes. Each point is the mean of three replications and the bars indicate +S.E.

Physiological parameters: NaCl stress reduced the pigment content in both genotypes (Fig. 3A-D). High concentration of NaCl (150 mM) reduced the Chl 'a' by 54.61% and 35.36% in Hassawi-3 and ILB-4347, respectively. Chl 'b' was decreased by 51.85% in Hassawi-3 and by 35.36% in ILB-4347 under 150 mM NaCl stress relative to the control. Carotene also showed reduction of 51.51% and 38.29% in Hassawi-3 and ILB-4347, respectively, under 150 mM NaCl stress (Fig. 3A).

Proline concentrations were increased by 105.19% and 113.93% in Hassawi-3 and ILB-4347 genotypes, respectively, upon 150 mM NaCl treatment (Fig. 3B). The soluble protein content was decreased significantly in both genotypes, and a maximum decrease of 44.80% was recorded in Hassawi-3, whereas ILB-4347 showed a decrease of 34.80% under 150 mM NaCl treatment compared to control plants (Fig. 3C).

Plants under salinity stress showed lower leaf relative water content (LRWC) compared to the control plants. Hassawi-3 showed 42.33% LRWC reduction by 150 mM NaCl treatment, whereas the ILB-4347 genotype showed 31.92% LRWC reduction under 150 mM NaCl treatment compared to the control (Fig. 3D).

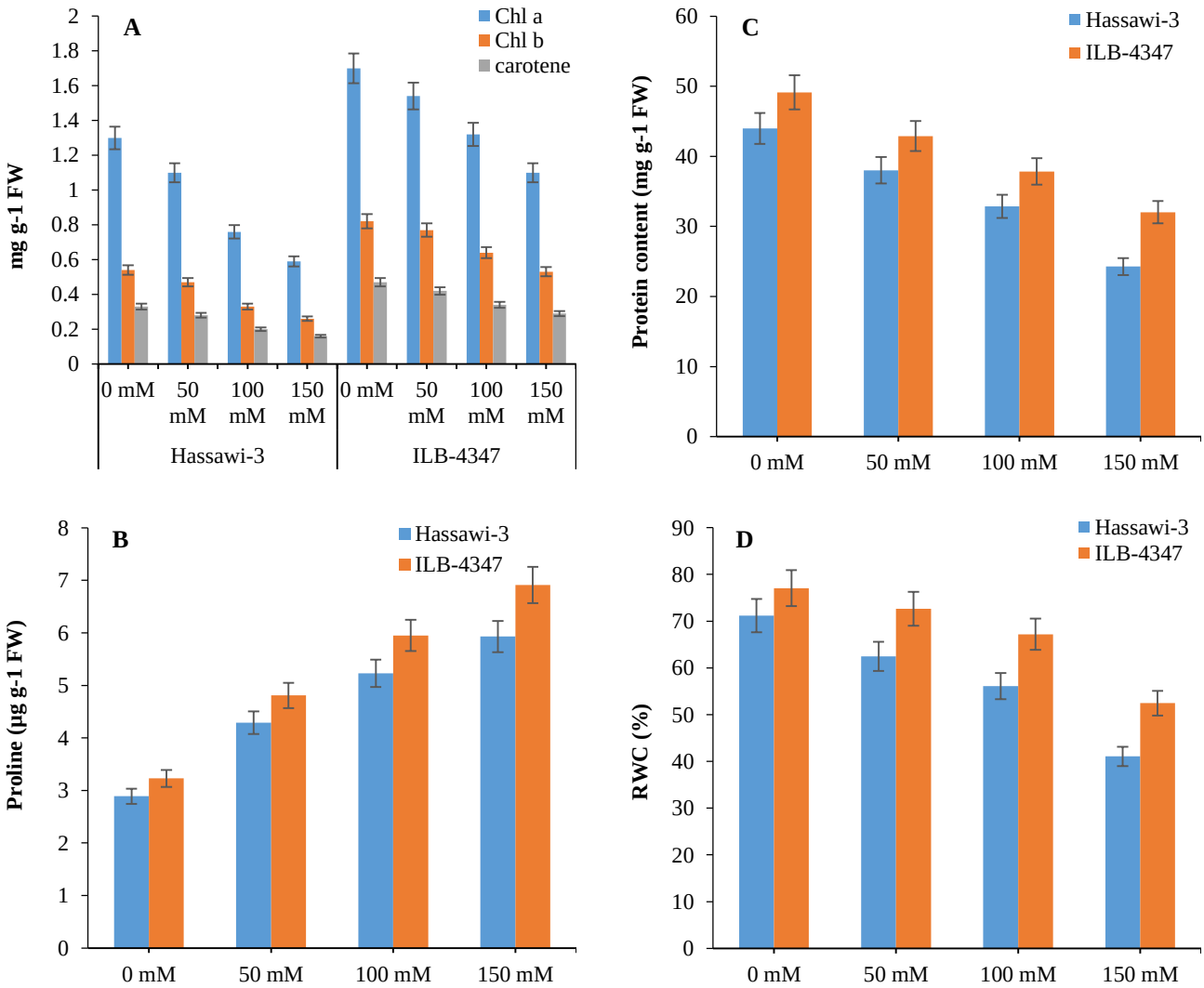

Fig. 3. Effect of salinity treatments on (A) pigment content, (B) proline content, (C) protein content and (D) LRWC in Hassawi-3 and ILB-4347 genotypes. Each point is the mean of three replications and the bars indicate +S.E.

H_2O_2, MDA, and EL: H_2O_2, MDA, and EL increased under NaCl stress in both genotypes (Fig. 4A-C). However, ILB-4347 showed an increase of 90% in H_2O_2, 66.45% in MDA content, and 84.27% in EL under 150 mM NaCl stress relative to the control. Under the same NaCl concentration, the Hassawi-3 genotype showed an increase of 128%, 91.72%, and 96.26% in H_2O_2, MDA, and EL, respectively, compared to the control.

Enzymatic and non-enzymatic antioxidants: Salinity stress significantly enhanced, SOD, CAT, GR, and AsA in both Hassawi-3 and ILB-4347 genotypes compared to their respective controls (Fig. 5A-D).

SOD activity increased with the increase in salinity. It increased by 48.23% in Hassawi-3 and 86.56% in ILB-4347 under 150 mM NaCl treatment compared to the control. CAT and GR activity increased by 24.08% and 63.31%, respectively, in Hassawi-3 under 150 mM NaCl treatment. Under the same NaCl concentration, ILB-4347 showed enhanced CAT activity by 34.01% and GR activity by 63.75%, compared to the control. The AsA content was enhanced by 42.30% and 50.00% in Hassawi-3 and ILB-4347 genotypes, respectively, under 150 mM NaCl stress compared to the control.

Discussion

Legume plants such as the faba bean are highly sensitive to salinity (Maas *et al.*, 1986; Hashem *et al.*, 2014; Ahmad *et al.*, 2018b). Tavakkoli *et al.*, (2010) found that salinity treatments reduced both biomass production and water uptake in the faba bean. Helal & Mengel (1981) showed the deleterious effects of NaCl on plant metabolism that retard growth in salt sensitive species. Reduced growth and biomass yield in faba beans by NaCl is also reported by Dawood & EL-Awadi (2015), Hashem *et al.*, (2014) and Ahmad *et al.*, (2018b). The reduction in growth and biomass yield due to salinity is attributed to inhibition of cell division and cell elongation (Ahmad *et al.*, 2015; Ahmad *et al.*, 2016). Reduced growth and biomass yield by NaCl also occurs due to reduced mineral uptake, generation of reactive oxygen species, enzyme activity inhibition, and hormonal imbalance (Munns, 1993; Sumer, 2004; Ashraf *et al.*, 2010). Sensitive cultivars are more susceptible to salinity stress compared to tolerant ones and have been reported by Ahmad *et al.*, (2016) in pea plants and by Ahmad *et al.*, (2010b) in mulberry seedlings.

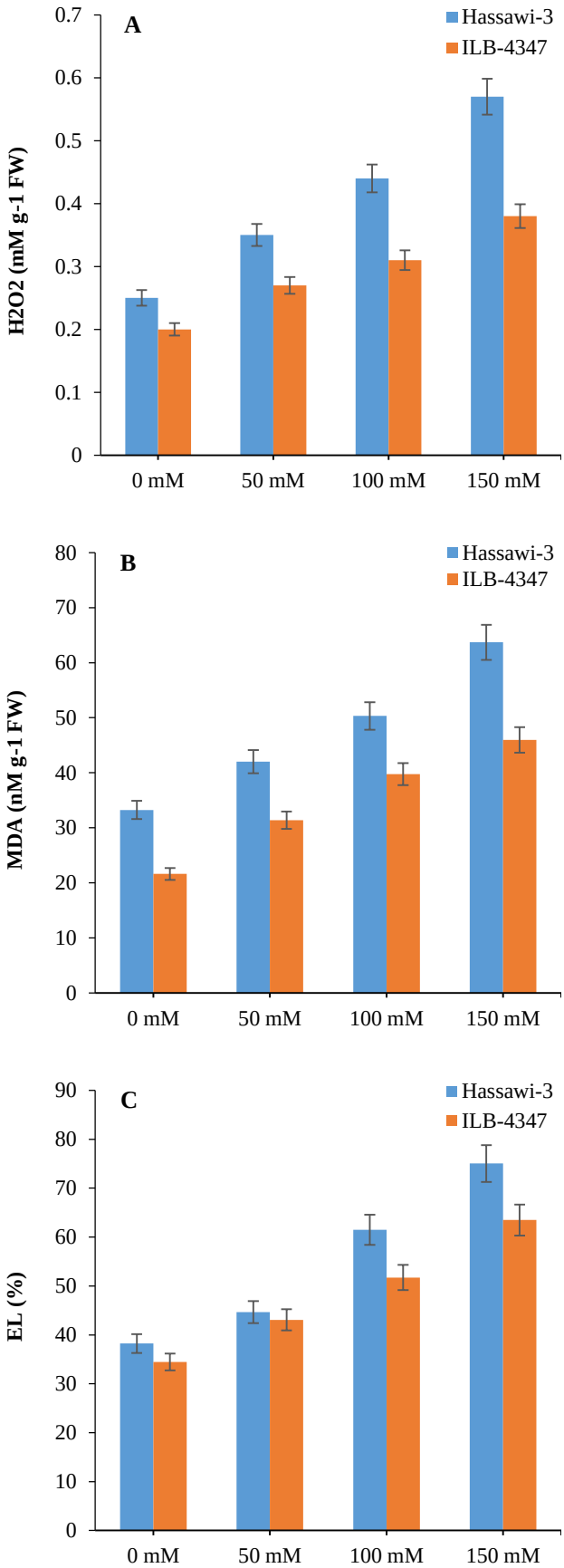

Fig. 4. Effect of salinity treatments on (A) H_2O_2 content, (B) MDA content, and (C) electrolyte leakage in Hassawi-3 and ILB-4347 genotypes. Each point is the mean of three replications and the bars indicate +S.E.

Results related to the decreased uptake of Ca^{2+}, Mg^{2+}, K^+ and enhanced accumulation of Na^+ and Na^+/K^+ ratio under NaCl stress corroborate the findings of Hashem *et al*., (2015) in *Solanum lycopersicum* and those of Ahmad *et al*., (2018b) in *Vicia faba*. According to Ahanger *et al*., (2017), the Na^+ and K^+ ions compete for uptake as both ions share a similar physicochemical structure. Increased Na^+ accumulation by NaCl accompanied with decreased K^+ and Ca^+ uptake have been reported in other plant species, including mustard (Ahmad *et al*., 2015) and strawberry (Karlidag *et al*., 2009). Restricted uptake of mineral elements is the main reason for reduced growth and development (Ahmad *et al*., 2015; Ahanger *et al*., 2017). Salinity stress tolerance in plants is reportedly conferred by K^+ retention and Na^+ exclusion from the shoots (Shabala, 2017). Positive correlations between K^+ retention in plant tissues and salinity tolerance have been reported in wheat (Wu *et al*., 2015), alfalfa (Guo *et al*., 2016), cotton (Wang *et al*., 2016), mustard (Chakraborty *et al*., 2016), and cucumber (Redwan *et al*., 2016). Salt tolerant genotypes maintain high K^+/Na^+ ratios under salinity stress; this has been reported by Wu *et al*., (2015) in barley and by Xiong *et al*., (2018) in melon. In the present study, higher K^+ content and K^+/Na^+ ratio was recorded in the ILB-4347 genotype, which thus showed more tolerance than Hassawi-3 to salt stress.

The effect of salinity on photosynthetic activity and chlorophyll pigment damage was dependent on concentration, duration of stress, and genotype. The results of the present study indicate that increasing NaCl concentration leads to reducing chlorophyll pigments and carotene contents, and corroborate the findings of Ahmad *et al*., (2018b). Decreased pigments content by NaCl stress has also been reported in *Phaseolus vulgaris* (Turan *et al*., 2007; Taïbi *et al*., 2016) and *Vigna subterranean* (Taffouo *et al*., 2010). Chlorophyll is used as an indicator for evaluating PSII integrity under salt stress (Smethurst & Shabala, 2003) and reduction in chlorophyll content indicates oxidative stress symptoms (Smirnoff, 1996). NaCl stress-mediated decrease in pigments is attributed to distortion in chlorophyll ultra-structure (Meng *et al*., 2011; Rady, 2011), inhibition of Rubisco (Soussi, 1998), and stomata closure leading to decreased leaf intercellular CO_2 pressure (Bethke & Drew, 1992), thus resulting in poor photosynthesis, plant growth, and productivity. Decreased chlorophyll content is also attributed to retarded synthesis of chlorophyll, activation of chlorophyllase enzymes (Santos, 2004), pigment protein complex abnormalities, and restricted uptake of minerals such as Mg^{2+} (Kusaba *et al*., 1998; Ashraf *et al*., 2010; Ahmad *et al*., 2018b). Carotene plays a role in light collection for photosynthesis, quenches triplet chlorophyll and O_2, dissipates excess energy via the xanthophyll cycle, stabilizes the chloroplast membrane, and reduces membrane fluidity and susceptibility to lipid peroxidation. The importance of carotenoids in detoxifying plants from ROS is also well known (Verma & Mishra, 2005).

The NaCl treatments induced a significant decrease in plant soluble proteins, whereas the plants accumulated extensive proline compared to the controls in both genotypes. Reduced protein contents and increased proline due to salinity have been reported in different plant species (Gadallah & El-Enany, 1999; Abdul Qados, 2011; Ahmad *et al.*, 2015). Ahmad (2012) also reported that salt tolerant cultivars of mustard accumulated more proline than the susceptible cultivar.Besides osmoregulation, antioxidative activity of proline could protect the plants against stress (Ahmad, 2012; Ahmad *et al.*, 2015; Ahanger *et al.*, 2017; Kibria *et al.*, 2017). Proline is reported to protect macromolecules such as enzymes, stabilizes their structures, and serves as a hydroxyl radical scavenger (Smirnoff & Cumbes, 1989; Zhu, 2002; Ashraf & Harris, 2004; Pottosin *et al.*, 2014). This renders proline accumulation as one of the most important physiological responses of plants against salt stress. The results related to the decline in LRWC under salt stress corroborate the findings of Ahmad (2012) in mustard and Karlidag *et al.*, (2009) in strawberry plants. The decline in LRWC due to salinity stress may be attributed to the decreased water and mineral uptake (Ahmad, 2012; Ahmad *et al.*, 2016). Enhanced H_2O_2, MDA content, and EL by NaCl in the present study is in agreement with the results of Ahmad (2012) in mustard genotypes. ROS reacts with polyunsaturated fatty acids (PUFA) to form lipid hydroperoxides and decreases membrane permeability. This results in increased electrolyte leakage and has been reported by Karlidag *et al.*, (2009) in strawberry and Ahmad (2012) in mustard. Lipid peroxidation is used as an indicator of oxidative stress damage and has been used in many plant species as a selection tool for salinity stress injuries. Khan and Panda (2008) found that salt tolerant rice cultivars maintained low levels of lipid peroxidation compared to sensitive cultivars, and they attributed this tolerance to higher free radical scavenging capacity and more efficient protection mechanisms of tolerant cultivars compared to sensitive ones against salt stress. Salt tolerant genotypes showed less accumulation of H_2O_2, MDA content, and EL compared to susceptible genotypes and this has been reported by Ahmad (2012) in mustard. In the present study, ILB-4347 maintained low MDA content, indicating salt-tolerance compared to Hassawi-3.

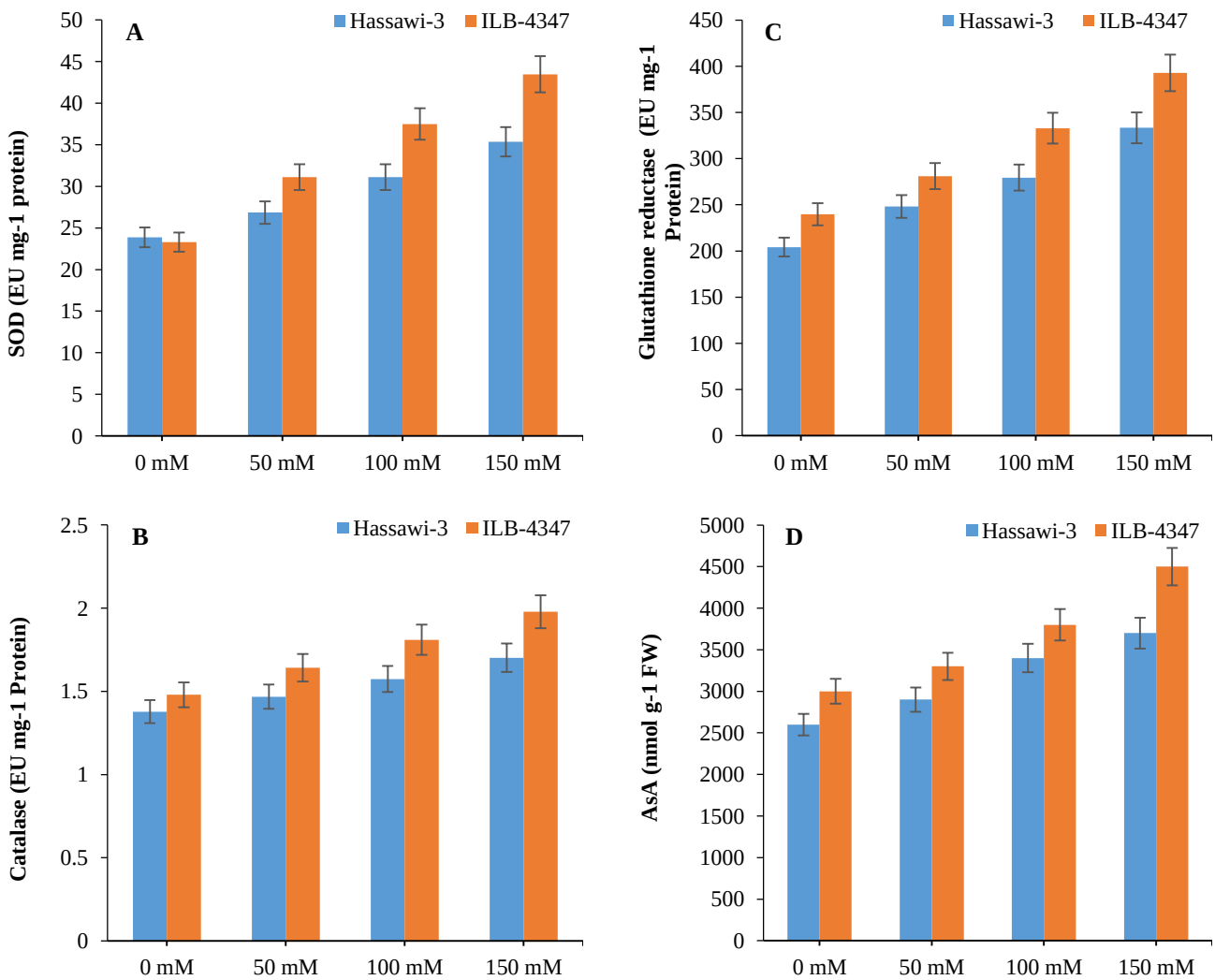

Fig. 5. Effect of salinity treatments on (A) SOD, (B) CAT, (C) GR and (D) AsA in Hassawi-3 and ILB-4347 genotypes. Each point is the mean of three replications and the bars indicate +S.E.

NaCl enhanced the activity of antioxidant enzymes SOD, CAT, and GR and the results are analogous with earlier reports on canola (Ashraf & Ali, 2008), sunflower (Noreen & Ashraf, 2009), proso millet (Sabir *et al.*, 2011), wheat (Ashraf *et al.*, 2010), safflower (Siddiqi, 2010), mustard (Ahmad, 2012), and chickpea (Ahmad *et al.*, 2016). SOD has been reported as the first line of defense against oxidative stress and converts $O2^{-\bullet}$ to H_2O_2 (Mittler, 2002; Ashraf, 2009; Ahmad *et al.*, 2010b). This accumulated H_2O_2 is also dangerous for the cell and is dismutated by CAT to H_2O and O_2 (Van Breusegem *et al.*, 2001; Ashraf, 2009; Ahmad *et al.*, 2010a). Enhanced CAT activity under saline conditions decreases the H_2O_2 from cells and has also been reported previously (Sekmen *et al.*, 2007; Ashraf, 2009; Noreen *et al.*, 2010; Ahmad, 2012). Another versatile antioxidant enzyme is GR, which catalyzes oxidized glutathione (GSSG) to its reduced form, GSH (Mannervik, 1987; Meister, 1988). $NADP^+$ accepts electrons from the photosynthetic electron transport chain, thus minimizing the flow of electrons to O_2 and $O_2^{\bullet-}$ formation. The ratio of $NADP^+$/NADPH is enhanced by GR activity (Bishop, 1971). Many studies have reported that the salt-tolerant genotypes tend to enhance or have higher antioxidant enzyme activity under salt stress compared to salt-sensitive genotypes (Ashraf, 2009; Noreen & Ashraf, 2009; Sabir *et al.*, 2011; Ahmad, 2012). In the present study, total antioxidants, SOD, CAT, and GR were enhanced in both Hassawi-3 and ILB-4347 genotypes under NaCl stress, and the enhancements were more pronounced in ILB-4347.

In conclusion, NaCl stress decreases the growth and biomass yield, pigment content, and LRWC, but increases H_2O_2, MDA content, EL, and antioxidant enzymes such as SOD, CAT, and GR in both faba bean genotypes (Hasawe-3 and ILB-4347). The ILB-4347 genotype showed a reduced decrease in growth and biomass yield along with other physiological and biochemical attributes compared to Hassawi-3. The proline content, SOD, CAT, and GR activity was also higher in ILB-4347 than that in Hassawi-3. H_2O_2, MDA, and EL was higher in Hassawi-3 than that in ILB-4347 genotype. Thus, the ILB-4347 genotype (tolerant) shows better protection against salt stress through modification of biochemical properties and activities of enzymatic and non-enzymatic antioxidants under salt stress compared to those in Hassawi-3.

Acknowledgments

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References

Abdul Qados, A.M.S. 2011. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (l.). *J. Saudi. Soc. Agric. Sci.* 10(1): 7-15.

Aebi, H. 1984. Catalase *In vitro*. In: (Eds.): Colowick, S. and N. Kaplan, *Methods in Enzymology*. Elsevier, Florida, pp: 121-126.

Ahanger, M.A., N.S. Tomar, M. Tittal, S. Argal and R.M. Agarwal. 2017. Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. *Physiol. Mol. Biol. Plants*, 23(4): 731-744.

Ahmad, P. 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). *Afr. J. Biotechnol.*, 11(11): 2694-2703.

Ahmad, P., A. Hashem, E.F. Abd-Allah, A.A. Alqarawi, R. John, D. Egamberdieva and S. Gucel. 2015. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system. *Front. Plant Sci.*, 6:868.

Ahmad, P., A.A. Abdel Latef, A. Hashem, E.F. Abd_Allah, S. Gucel and L.-S.P. Tran. 2016. Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. *Front. Plant Sci.*, 7: 347.

Ahmad, P., C.A. Jaleel and S. Sharma. 2010b. Antioxidant defense system, lipid peroxidation, proline-metabolizing enzymes, and biochemical activities in two *Morus alba* genotypes subjected to NaCl stress. *Russ. J. Plant Physiol.*, 57(4): 509-517.

Ahmad, P., C.A. Jaleel, M.A. Salem, G. Nabi and S. Sharma. 2010a. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Crit. Rev. Biotechnol.*, 30(3): 161-175.

Ahmad, P., M.A. Ahanger, M.N. Alyemeni, L. Wijaya, D. Egamberdieva, R. Bhardwaj and M. Ashraf. 2017. Zinc application mitigates the adverse effects of NaCl stress on mustard [*Brassica juncea* (L.) czern & coss] through modulating compatible organic solutes, antioxidant enzymes, and flavonoid content. *J. Plant Interact.*, 12(1): 429-437.

Ahmad, P., M.A. Ahanger, P. Alam, M.N. Alyemeni, L. Wijaya, S. Ali and M. Ashraf. 2018a. Silicon (si) supplementation alleviates NaCl toxicity in mung bean [*Vigna radiata* (L.) wilczek] through the modifications of physio-biochemical attributes and key antioxidant enzymes. *J. Plant Growth Regul*.doi.org/10.1007/s00344-018-9810-2.

Ahmad, P., M.N. Alyemeni, M.A. Ahanger, D. Egamberdieva, L. Wijaya and P. Alam. 2018b. Salicylic acid (sa) induced alterations in growth, biochemical attributes and antioxidant enzyme activity in faba bean (*Vicia faba* L.) seedlings under NaCl toxicity. *Russ. J. Plant Physiol.*, 65(1): 104-114.

Apel, K. and H. Hirt. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, 55(1): 373-399.

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv.*, 27(1): 84-93.

Ashraf, M. and P.J.C. Harris, 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, 166(1): 3-16.

Ashraf, M. and Q. Ali. 2008. Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). *Environ. Exp. Bot.*, 63(1-3): 266-273.

Ashraf, M.A., M. Ashraf and Q. Ali. 2010. Response of two genetically diverse wheat cultivars to salt stress at different growth stages: Leaf lipid peroxidation and phenolic contents. *Pak. J. Bot.*, 42(1): 559-565.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39(1): 205-207.

Benavídes, M.P., P.L. Marconi, S.M. Gallego, M.E. Comba and M.L. Tomaro. 2000. Relationship between antioxidant defence systems and salt tolerance in *Solanum tuberosum*. *Funct. Plant Biol.*, 27(3): 273-278.

Bethke, P.C. and M.C. Drew. 1992. Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. *Plant Physiol.*, 99(1): 219-226.

Bishop, N.I. 1971. Photosynthesis: The electron transport system of green plants. *Annu. Rev. Biochem.*, 40(1): 197-226.

Bor, M., F. Özdemir and I. Türkan. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. *Plant Sci.*, 164(1): 77-84.

Cerning, J., A. Saposnik and A. Guilbot. 1975. Carbohydrate composition of horse beans (*Vicia faba*) of different origins. *Cereal Chem*., 52: 125-137.

Chakraborty, K., J. Bose, L. Shabala, A. Eyles and S. Shabala. 2016. Evaluating relative contribution of osmotolerance and tissue tolerance mechanisms toward salinity stress tolerance in three Brassica species. *Physiol. Plant.,* 158: 135-151.

Dawood, M.G. and M.E. EL-Awadi. 2015. Alleviation of salinity stress on *Vicia faba* L. plants via seed priming with melatonin. *Acta Biológica Colombiana*, 20(2): 223-235.

de Azevedo Neto, A.D., J.T. Prisco, J. Enéas-Filho, C.E.B.d. Abreu and E. Gomes-Filho. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ. Exp. Bot.*, 56(1): 87-94.

Dionisio-Sese, M.L. and S. Tobita. 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.*, 135(1): 1-9.

Duke, J.A. (Ed.) *Handbook of legumes of world economic importance.* New York: Plenum press, Cambridge University Press (CUP), pp. 345.

FAO. 2015. Faostat agricultural database. In: *Choice Reviews Online*.

Gadallah, M. and A. El-Enany. 1999. Role of kinetin in alleviation of copper and zinc toxicity in *Lupinus termis* plants. *Plant Growth Regul.*, 29(3): 151-160.

Goldberg, D.M. and R.J. Spooner. 1983. Assay of glutathione reductase In: *Methods of Enzymatic Analysis*, (Eds.): Bergmeyen, H. Verlag Chemie, Deerfield Beach, pp: 258-265.

Gossett, D.R., E.P. Millhollon and M.C. Lucas. 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Sci.*, 34(3): 706.

Gulen, H. and A. Eris. 2003. Some physiological changes in strawberry (Fragaria × Ananassa cv. 'Camarosa') plants under heat stress. *J. Hort. Sci & Biotech*., 78:894-898.

Guo, P., H.X. Wei., W.J. Zhang and Y.J. Bao. 2016. Physiological responses of alfalfa to high-level salt stress: root ion flux and stomatal characteristics. *Int. J. Agri. Biol.*, 18: 125-133.

Hashem, A., E. Abd_Allah, A. Alqarawi, G. El-Didamony, M. Alwhibi, D. Egamberdieva and P. Ahmad. 2014. Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. *Pak. J. Bot.*, 46(6): 2003-2013.

Hashem, A., E.F. Abd_Allah, A.A. Alqarawi, A.A. Al Huqail, D. Egamberdieva and S. Wirth. 2015. Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. *Saudi J. Biol. Sci.*, 23(2): 272-281.

Helal, H.M. and K. Mengel. 1981. Interaction between light intensity and NaCl salinity and their effects on growth, CO_2 assimilation, and photosynthate conversion in young broad beans. *Plant Physiol.*, 67(5): 999-1002.

Hernandez, J.A., A. Jimenez, P. Mullineaux and F. Sevilia. 2000. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. *Plant Cell Environ.*, 23(8): 853-862.

Huang, C. 2005. Increased sensitivity to salt stress in an ascorbate deficient arabidopsis mutant. *J. Exp. Bot.,* 56: 3041-3049.

Jackson, M.L. 2005. *Soil chemical analysis: Advanced course.* UW-Madison Libraries Parallel Press.

Karlidag, H., E. Yildirim and M. Turan. 2009. Salicylic acid ameliorates the adverse effect of salt stress on strawberry. *Sci. Agric.*, 66(2): 180-187.

Khan, M.H. and S.K. Panda. 2008. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiol. Plant.*, 30(1): 81-89.

Kibria, M.G., M. Hossain, Y. Murata and M.A. Hoque. 2017. Antioxidant defense mechanisms of salinity tolerance in rice genotypes. *Rice Sci.*, 24(3): 155-162.

Koch, K.E. 1996. Carbohydrate-modulated gene expression in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 47(1): 509-540.

Kusaba, S., Y. Kano-Murakami, M. Matsuoka, M. Tamaoki, T. Sakamoto, I. Yamaguchi and M. Fukumoto. 1998. Alteration of hormone levels in transgenic tobacco plants overexpressing the rice homeobox gene Osh1. *Plant Physiol.*, 116(2): 471-476.

Maas, E.V., J.A. Poss and G.J. Hoffman. 1986. Salinity sensitivity of sorghum at three growth stages. *Irrigation Sci.*, 7(1): 1-11.

Mannervik, B. 1987. The enzymes of glutathione metabolism: An overview. *Biochem. Soc. Trans.*, 15(4): 717-718.

Meister, A. 1988. Glutathione metabolism and its selective modification. *J. Biol. Chem.*, 263(33): 17205-17208.

Meng, H.B., S.S. Jiang, S.J. Hua, X.Y. Lin, Y.L. Li, W.L. Guo and L.X. Jiang. 2011. Comparison between a tetraploid turnip and its diploid progenitor (*Brassica rapa* L.): The adaptation to salinity stress. *Agri. Sci. China*, 10(3): 363-375.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, 7(9): 405-410.

Mittova, V., M. Tal, M. Volokita and M. Guy. 2002. Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. *Physiol. Plant.*, 115(3): 393-400.

Møller, I.M. and L.J. Sweetlove. 2010. ROS signalling – specificity is required. *Trends Plant Sci.*, 15(7): 370-374.

Moran, R. and D. Porath. 1980. Chlorophyll determination in intact tissues using n, n-dimethylformamide. *Plant Physiol.*, 65(3): 478-479.

Mukherjee, S. and M. Choudhuri. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant.*, 58(2): 166-170.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. *Plant Cell Environ.*, 16(1): 15-24.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59(1): 651-681.

Nishikimi, M., N. Appaji Rao and K. Yagi. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.*, 46(2): 849-854.

Noctor, G. and C.H. Foyer. 1998. Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant Biol.*, 49(1): 249-279.

Noreen, S. and M. Ashraf. 2009. Modulation of salt (NaCl)-induced effects on oil composition and fatty acid profile of sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid. *J. Sci. Food Agric.*, 90(15): 2608-2616.

Noreen, Z., M. Ashraf and N.A. Akram. 2010. Salt-induced regulation of some key antioxidant enzymes and physio-biochemical phenomena in five diverse cultivars of turnip (*Brassica rapa* L.). *J. Agron. Crop. Sci*., 196: 273-285.

Ohkawa, H., N. Ohishi and K. Yagi. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95(2): 351-358.

Picard, J. 1977. Some results dealing with breeding for protein content in *Vicia faba* L. In: *Protein quality from leguminous crops*. EVR 5686 EN, Commission of European Communities, Coordination of Agricultural Research, pp. 339.

Pottosin, I., A.M. Velarde-Buendia, J. Bose, I. Zepeda-Jazo, S. Shabala and O. Dobrovinskaya. 2014. Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: Implications for plant adaptive responses. *J. Exp. Bot.*, 65(5): 1271-1283.

Qureshi, M.I., M.Z. Abdin, J. Ahmad and M. Iqbal. 2013. Effect of long-term salinity on cellular antioxidants, compatible solute and fatty acid profile of Sweet Annie (*Artemisia annua* L.). *Phytochemistry*, 95: 215-223.

Rady, M.M. 2011. Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. *Sci. Hort.*, 129(2): 232-237.

Redwan, M., F. Spinelli, L. Marti, M. Weiland, E. Palm, E. Azzarello and S. Mancuso. 2016. Potassium fluxes and reactive oxygen species production as potential indicators of salt tolerance in *Cucumis sativus*. *Funct. Plant Biol.,* 43: 1016-1027.

Roychoudhury, A., S. Basu, S.N. Sarkar and D.N. Sengupta. 2008. Comparative physiological and molecular responses of a common aromatic indica rice cultivar to high salinity with non-aromatic indica rice cultivars. *Plant Cell Rep.*, 27(8): 1395-1410.

Sabir, P., M. Ashraf and N.A. Akram. 2011. Accession variation for salt tolerance in proso millet (*Panicum miliaceum* L.) using leaf proline content and activities of some key antioxidant enzymes. *J. Agron. Crop. Sci.*, 197(5): 340-347.

Santos, C.V. 2004. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Sci. Hort.,* 103: 93-99.

Sekmen, A.H., İ. Türkan and S. Takio. 2007. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media*. *Physiol. Plant.*, 131(3): 399-411.

Shabala, S. 2017. Signalling by potassium: Another second messenger to add to the list? *J. Exp. Bot.*, 68(15): 4003-4007.

Shrivastava, P. and R. Kumar. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.*, 22(2): 123-131.

Siddiqi, E. 2010. Influence of salt stress on some physiological and biochemical attributes and oil composition of a potential oilseed crop safflower (*Carthamus tinctorius* L.). In: Department of Botany. University of Agriculture, Faisalabad.

Smethurst, C.F. and S. Shabala. 2003. Screening methods for waterlogging tolerance in Lucerne: Comparative analysis of waterlogging effects on chlorophyll fluorescence, photosynthesis, biomass and chlorophyll content. *Funct. Plant Biol.*, 30(3): 335-343.

Smirnoff, N. 1996. Botanical briefing: The function and metabolism of ascorbic acid in plants. *Ann. Bot.*, 78(6): 661-669.

Smirnoff, N. and Q.J. Cumbes. 1989. Hydroxyl radical scavenging activity of compatible solutes. *Phytochem.*, 28(4): 1057-1060.

Soussi, M. 1998. Effects of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (*Cicer arietinum* L.). *J. Exp. Bot.*, 49(325): 1329-1337.

Sreenivasulu, N., B. Grimm, U. Wobus and W. Weschke. 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). *Physiol. Plant.*, 109(4): 435-442.

Sumer, A. 2004. Evidence of sodium toxicity for the vegetative growth of maize during the first phase of salt stress. *J. App. Bot.*, 78: 135-139.

Suwa, R., N.T. Nguyen, H. Saneoka, R. Moghaieb and K. Fujita. 2006. Effect of salinity stress on photosynthesis and vegetative sink in tobacco plants. *Soil Sci. Plant Nutr.*, 52(2): 243-250.

Taffouo, V.D., O.F. Wamba, E. Youmbi, G.V. Nono and A. Akoa. 2010. Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranea* (L.) verdc.) landraces grown under saline conditions. *Int. J. Bot.*, 6(1): 53-58.

Taïbi, K., F. Taïbi, L. Ait Abderrahim, A. Ennajah, M. Belkhodja and J.M. Mulet. 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. *S. Afr. J. Bot.*, 105: 306-312.

Tavakkoli, E., P. Rengasamy and G.K. McDonald. 2010. High concentrations of Na+ and Cl– ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *J. Exp. Bot.*, 61(15): 4449-4459.

Turan, M.A., V. Katkat and S. Taban. 2007. Salinity-induced stomatal resistance, proline, chlorophyll and ion concentrations of bean. *Int. J. Agr. Res.*, 2(5): 483-488.

Van Breusegem, F., E. Vranová, J.F. Dat and D. Inzé. 2001. The role of active oxygen species in plant signal transduction. *Plant Sci.*, 161(3): 405-414.

Verma, S. and S.N. Mishra. 2005. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. *J. Plant Physiol.*, 162(6): 669-677.

Wang, N., H.K. Qi, G.L. Su, J. Yang, H. Zhou, Q.H. Xu, Q. Huang and G.T. Yan. 2016. Genotypic variations in ion homeostasis, photochemical efficiency and antioxidant capacity adjustment to salinity in cotton (*Gossypium hirsutum* L.). *Soil Sci. Plant Nut.*, 62: 240-246.

Wellburn, A.R. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.*, 144(3): 307-313.

Wu, H., M. Zhu, L. Shabala, M. Zhou and S. Shabala. 2015. K^+ retention in leaf mesophyll, an overlooked component of salinity tolerance mechanism: A case study for barley. *J. Integr. Plant Biol.*, 57(2): 171-185.

Xiong, L. and J.K. Zhu. 2002. Molecular and genetic aspects of plant responses to osmotic stress. *Plant Cell Environ.*, 25(2): 131-139.

Xiong, M., X. Zhang, S. Shabala, L. Shabala, Y. Chen, C. Xiang, M.A. Nawaz, Z. Bie, H. Wu, H. Yi, M. Wu and Y. Huang. 2018. Evaluation of salt tolerance and contributing ionic mechanism in nine Hami melon landraces in Xinjiang, China. *Sci, Hort.*, 237: 277-286.

Xu, F.J., C.W. Jin, W.J. Liu, Y.S. Zhang and X.Y. Lin. 2011. Pretreatment with h_2o_2 alleviates aluminum-induced oxidative stress in wheat seedlings. *J. Integr. Plant Biol.*, 53(1): 44-53.

Zhu, J.-K. 2002. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.*, 53(1): 247-273.

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