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Physiological and antioxidant responses of cultivated and wild barley under salt stress

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Abstract: Saline soil is a critical environmental problem affecting crop yield worldwide. Tibetan wild barley is distinguished for its vast genetic diversity and high degree of tolerance to abiotic stress, including salinity. The present study compared the response of antioxidant defense system in the XZ16 wild and CM72 cultivated barleys to salt stress. Wild barley was relatively more tolerant than cultivated CM72, salt-tolerant cultivar, with less Na^+ uptake and more K^+, Ca^{2+}, and Mg^{2+} retention in plant tissues. The results of diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) staining showed that XZ16 had significantly lower H_2O_2 and O_2^- concentrations than a salt-sensitive cultivar Gairdner, suggesting that the salt-tolerant genotype suffer from less oxidative damage. Moreover, XZ16 and Gairdner had the highest and lowest anti-oxidative enzyme activities and proline content in plant tissues. In addition, the microscopic examination revealed that DNA damage in cv. Gairdner was closely correlated to oxidative stress, representing that more reactive oxygen species accumulation in plants tissues leads to subsequent DNA damage. The present results show that higher salt tolerance of wild barley XZ16 is attributed to less Na^+ accumulation and stronger anti-oxidative capacity.

Keywords: *Hordeum vulgare* L.; superoxide radical; hydrogen peroxide; toxic effect; antioxidant enzyme

In normal conditions, reactive oxygen species (ROS), including hydroxyl radical, superoxide radical and hydrogen peroxide play a significant role in plant growth, development and different metabolic activities (Apel and Hirt 2004, Bartoli et al. 2004, Pandolfi et al. 2017, Abdallah et al. 2018). While under abiotic stress conditions, excessive reactive oxygen species are produced in plants (Zhu 2001), resulting in oxidative stress. Salinity is the major abiotic stress for plants growth and its toxic effect on plants is also caused by oxidative stress due to the excessive production and accumulation of reactive oxygen species in different plant tissues (Gorai and Neffati 2007, Shavrukov 2012).

Glycophyte plants, specifically large proportion of crops, cannot survive at 50 mmol or higher Na^+ concentrations in soil (Munns and Tester 2008). Mostly, yield loss of glycophytes crops occurred when the soil solution had the electrical conductivity (ECs) of 4 dS/m, approximately 40–50 mmol NaCl and the osmotic pressure reached to 0.2 MPa (Munns and Tester 2008, Tang et al. 2015). The excess salt can inhibit enzyme activity and lead to the death of leaves. Salt may also exert a toxic effect on photosynthetic processes and photosynthetic components directly by affecting the chloroplast (Munns and Tester 2008). Moreover, several genes have been reported to regulate the salt tolerant mechanism in plants. For instance, the MPK6 could play a pivotal role in sodium sequestration and detoxification through phosphorylating the Na^+/H^+ antiporters and sodium efflux (Tang et al. 2015). However, the

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halophytes acclimatise to the environmental stresses through different biochemical mechanisms such as ion regulation, sequestration and compartmentalisation, biosynthesis of compatible solutes, adjustment of osmotic potential, regulation of antioxidant enzymes and synthesis of plant hormones (Yan et al. 2012, Mbarki et al. 2020), whereas molecular mechanisms involve salt overly sensitive (SOS) pathway for ion homeostasis, protein synthesis, phytohormone signalling, regulation and expression of genes for encoding proteins, photosynthetic components, radical scavenging and vacuolar-sequestering enzymes (Tang et al. 2015, Gu et al. 2016).

On the other hand, plants have evolved multiple antioxidant defense mechanisms that are involved in enzymatic and non-enzymatic components to cope with different oxidative stress. These antioxidants include ascorbate, peroxidase, superoxide dismutase, catalase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione peroxidase and glutathione-S-transferase (Singh et al. 2008); non-enzymatic antioxidants consist of ascorbic acid, glutathione, different phenolic compounds, tocopherols and so on. These different types of antioxidants (enzymatic and non-enzymatic) form a complex network to protect plant tissues from oxidative injury (Mittler et al. 2004) mainly through ROS (Hussain et al. 2013, Abdallah et al. 2018).

Mitigation of oxidative damage by scavenging ROS is a significant approach for crops to stand under harsh environment (Zhu 2002, Miller et al. 2010). Barley is widely used in studies of salt tolerance mechanisms, because it is characterised by high salt tolerance in comparison with all other cereal crops, including rice, wheat and maize. Moreover, barley is a major cereal crop with multiple uses. However, due to the narrow genetic variation cultivated barley is more sensitive to salt stress (Zhu 2001). On the other hand, wild barley has rich genetic diversity and is more salt tolerant because of its wider genetic variation compared to different barley cultivars (Shavrukov et al. 2010). In fact, our previous studies identified some Tibetan barley accessions with salt tolerance higher than the well-known tolerant cultivar CM72 (Zahra et al. 2014, Wu et al. 2014). Hence, it is quite imperative to explore mechanisms of salt tolerance underlying the wild barley accessions. The current study was carried out to compare the differences among three barley genotypes in the physiological traits associated with salt tolerance to understand the major salt tolerance mechanism in the wild and cultivated barley.

MATERIAL AND METHODS

Plant material and experimental design. Cultivated barley CM72 (salt tolerant), Gairdner (salt sensitive) and Tibetan wild barley accession XZ16 (salt tolerant) seeds were surface sterilised with 3% H_2O_2 solution for 25 m, washed with double distilled water and then spread on moist filter papers. Germination boxes were placed into a growth chamber having 22 °C/18 °C, day/night temperature. After ten days of germination, barley seedlings were moved into 5 L plastic pots containing hydroponic solution following Wu et al. (2014). The pH of the nutrient solution was maintained up to 5.5 to 6.0 using 1 mol/L HCl or NaOH. Salt was mixed to the nutrient solution to form two levels after 10 days of germination: (1) control (0 mmol); (2) salt stress (300 mmol NaCl). The experiment was designed as a completely randomised design (CRD) with three replications. The nutrient solution in the pots was renewed on weekly basis.

Measurement of ion content. After two weeks of salt stress, plants were randomly harvested and washed 3 times with deionised water, separated into shoots and roots and oven dried at 70 °C for 72 h. The dried shoots and roots were weighted and ground for ion analysis. The plant tissue of 100 mg was dry-ashed and then mixed with 10 mL HNO_3:H_2O (1:1). Na^+, K^+, Ca^{2+} and Mg^{2+} ion contents in plant tissues were measured using a flame atomic absorption spectrometry.

Measurement of enzymatic activities. For determination of anti-oxidative enzyme activity, 0.5 g of fresh leaves and roots were ground in 5 mL of sodium phosphate buffer solution (PBS, pH 7.8). After grinding, they were centrifuged at 10 000 *g* for 25 min at 4 °C. The subsequent supernatants were collected for enzyme assays. Antioxidant activities were measured according to Wu et al. (2012).

Proline content. 0.5 g of fresh root and leaf proline was measured by the ninhydrin test at A520 nm according to the method defined by Bates et al. (1973). 5 mL of ninhydrin acid reaction mixture, glacial acetic acid and proline solution were added to the samples (1:1:1) and they were incubated in water bath at 100 °C for 10 min. After incubation, the reaction mixture was placed in an ice box for cooling. After cooling, 2.5 mL pure toluene was added, which allowed the samples to settle down for few minutes, then supernatant was used for measurement at A520 nm. The spectrophotometer was calibrated to zero with

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pure toluene. Proline content was calculated from a standard curve in µg/FW of the sample using L-proline as standard.

Histochemical detection of O_2^- and H_2O_2. Leaf and root H_2O_2 and O_2^- content were determined according to Velikova et al. (2000) and Jiang and Zhang (2002). For histochemical measurement of H_2O_2 and O_2^-, the plant tissues were first vacuumed with 3,3-diaminobenzidine (DAB) and nitroblue tetrazolium (NBT), respectively, as reported previously (Hernández et al. 2001). Barley leaves and roots (approx. 1 cm long) were quickly immersed into 20 mmol MES buffer (pH 6.1) and 2.0 mmol NBT solution for 15 min at room temperature, and chemical reaction was stopped by dipping the tissues into double distilled water. Hydrogen peroxide was measured with 3,3'-diaminobenzidine tetrachloride reagent. Roots and leaves were immersed in 0.05% DAB and PBS buffer solution with pH 7.4 for 2 h and the reaction was stopped by dipping roots and leaves into distilled water. Then, the leaves were bleached out in boiling ethanol (96%) for 10–15 m. This chemical treatment destained the leaves and roots with the exception of the brown patches produced by the reaction of DAB with H_2O_2. After staining, roots were photographed directly using a LEICA MZ95 stereomicroscope (Langham Creek, Suite 235 Houston, USA).

Comet assay. Comet assay was conducted on leaves of three barley genotypes following the method of Wang et al. (2013). The image of prepared samples of comets was observed under a fluorescent microscope.

Statistical analysis. Data were analysed by using the SAS statistical software 9.2 (SAS, Institute, Cary, USA). The significance level was accepted at $P \leq 0.01$ and/or $P \leq 0.05$ as per analysis of covariance. Least significant difference (*LSD*) test was used for multiple comparisons of the mean data. Means ± standard error (SE) were also calculated.

RESULTS AND ANALYSIS

Ion accumulation in plant tissues. Excessive Na^+ is harmful for plants, whereas K^+ is an adversary against Na^+ under NaCl stress. It was noted that Na^+ content elevated in roots and shoots of barley genotypes under salt stress as compared to control. However, the increased extent differed greatly among the genotypes, with Gairdner having significantly higher Na^+ concentration (91.64 mg/g dry weight (DW)) in leaf than other two genotypes, CM72 (68.38 mg/g DW) and XZ16 (45.12 mg/g DW), as compared to their respective control (Figure 1A). In roots, the Na^+ content in Gairdner was also remarkably increased (27.75 mg/g DW) than CM72 (20.59 mg/g DW) and XZ16 (16.26 mg/g DW) as compared to their respective control plants (Figure 1B). Contrary to Na^+ concentration, K^+, Ca^{2+}, and Mg^{2+} contents in both leaves and roots of all three genotypes showed dramatic reduction under salt stress in comparison with the control plants (Figure 1). In case of Gairdner, K^+ content in leaves was 17.57 mg/g DW, followed by CM72 (32.23 mg/g DW) and XZ16 (45.17 mg/g DW) as compared to their respective control plants (Figure 1C). Moreover, a significant reduction of root K^+ content in Gairdner (6.62 mg/g DW), CM72 (10 mg/g DW) and XZ16 (10.18 mg/g DW) was recorded when compared to their respective control plants (Figure 1D). Hence, the range of reduction in K^+ content varied among the genotypes, with Gairdner > CM72 > XZ16, accordingly. Notably, Ca^{2+} content in leaves and roots of three barley genotypes was also reduced; in Gairdner, it was 2.30 mg/g DW, in CM72 2.77 mg/g DW and in XZ16 2.88 mg/g DW, relative to their respective control plants (Figure 1E). By contrast, in roots the lowest Ca^{2+} content was observed in Gairdner (1.37 mg/g DW) followed by CM72 (1.65 mg/g DW) and XZ16 (1.70 mg/g DW), relative to their respective control plants (Figure 1F). Furthermore, Mg^{2+} content was also reduced in leaves and roots of three barley genotypes under salt stress. The greatest reduction was observed in Gairdner leaves (2.30 mg/g DW), followed by CM72 (2.77 mg/g DW) and XZ16 (2.88 mg/g DW) as compared to their control plants. Furthermore, the same reduction pattern was observed in case of root Mg^{2+} content in Gairdner (2.70 mg/g DW), CM72 (1.70 mg/g DW) and XZ16 (1.04 mg/g DW) relative to their control plants (Figure 1G,H). It is noteworthy that higher Na^+ concentration and reduced K^+, Mg^{2+} and Ca^{2+} concentration in Gairdner was markedly larger than those in XZ16 and CM72.

Antioxidant enzymatic activity. No significant differences were observed among barley genotypes in the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) under normal conditions without NaCl addition in the growth medium (Figure 2). Salt treatment (300 mmol NaCl) caused a significant increase of all the examined antioxidant enzyme activities both in leaves and roots. However, the increased level differed significantly among the three barley genotypes. XZ16 (368 mg/g

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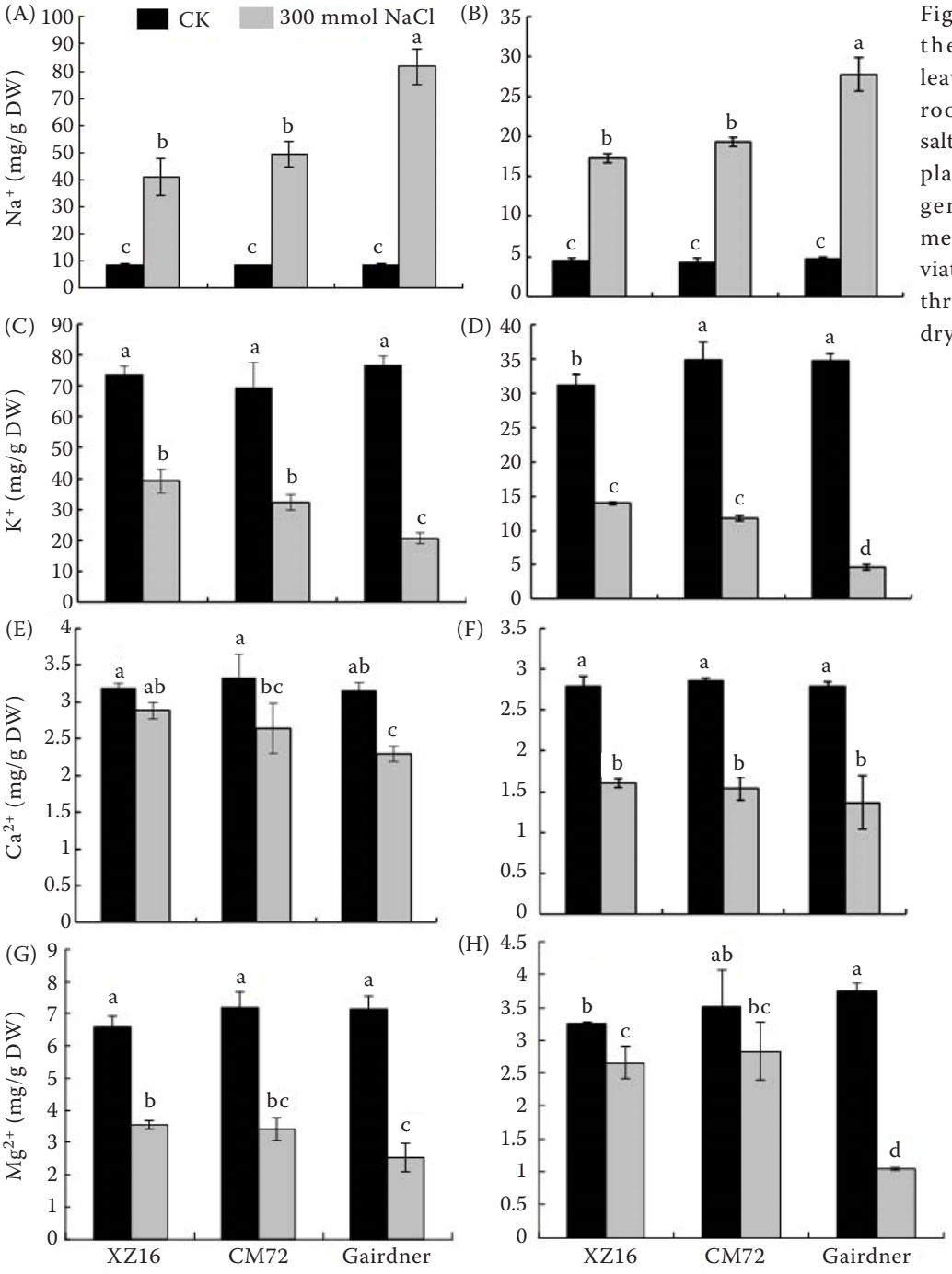

Figure 1. Analysis of the ion content in leaves (left panel) and roots (right panel) of salt-treated and control plants of three barley genotypes. Data are means ± standard deviation calculated from three replicates. DW – dry weight

DW) and CM72 (277 mg/g DW) had the highest and Gairdner (243 mg/g DW) had the lowest SOD activities in leaves (Figure 2A). Under salt stress, the respective SOD activity values were 276 mg/g DW for XZ16 and CM72 had and 145 mg/g DW for Gairdner (Figure 2B) as compared to their control plants. Similarly, the two salt-tolerant genotypes XZ16 and CM72 showed significantly higher leaves POD activity of 7.05 mg/g DW and 6.86 mg/g DW, respectively, than the salt-sensitive genotype Gairdner (5.09 mg/g DW) as compared to their respective control plants (Figure 2C). Moreover, in roots, the POD activity was remarkably high in XZ16 (6.32 mg/g DW) followed by CM72 (5.85 mg/g DW) and Gairdner (2.99 mg/g DW), respectively, as compared to their control plants (Figure 2D). Concerning the CAT activity, XZ16 and CM72 showed a significant increase in both leaves (7.60 mg/g DW, 4.87 mg/g DW) and roots (4.66 mg/g DW, 3.44 mg/g DW) under salt stress in comparison with the control. However,

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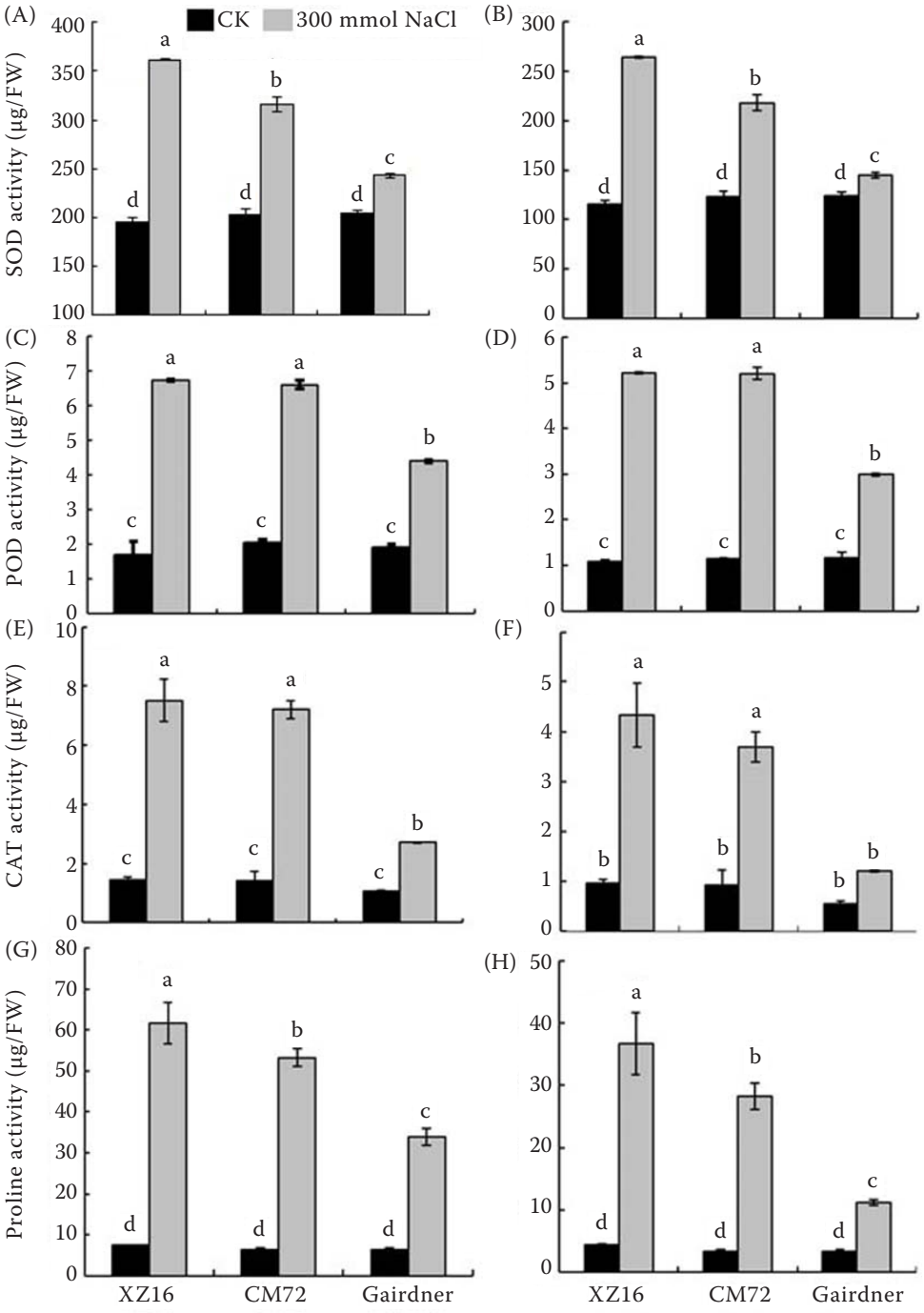

Figure 2. Activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and proline content in leaves (left panel) and roots (right panel) of salt treated and control plants of three barley genotypes. Data are means ± standard deviation calculated from three replicates. FW – fresh weight

Gairdner had significantly lower values of CAT activities in leaves (2.71 mg/g DW) and roots (1.21 mg/g DW) than the other two genotypes (Figure 2E,F).

Proline concentration. There was no significant difference in proline concentration of both leaves and roots among the three barley genotypes under the normal conditions. Salt stress caused a dramatic increase of proline concentration in the plant tissues. However, the extent of increased proline in leaves differed among the genotypes, namely XZ16 (65.69 mg/g DW) > CM72 (55 mg/g DW) > Gairdner (55 mg/g DW), respectively, whereas in roots, proline content was observed in the order of XZ16 (38.90 mg/g DW) > CM72 (28.43 mg/g DW) > Gairdner (11.21 mg/g DW) respectively, as compared to their control plants (Figure 2G,H).

H_2O_2 and O_2^- production. Obvious effects of NaCl stress on the accumulation of H_2O_2 and O_2^- were observed in leaves and roots. Both leaves and roots of Gairdner were severely damaged by oxida-

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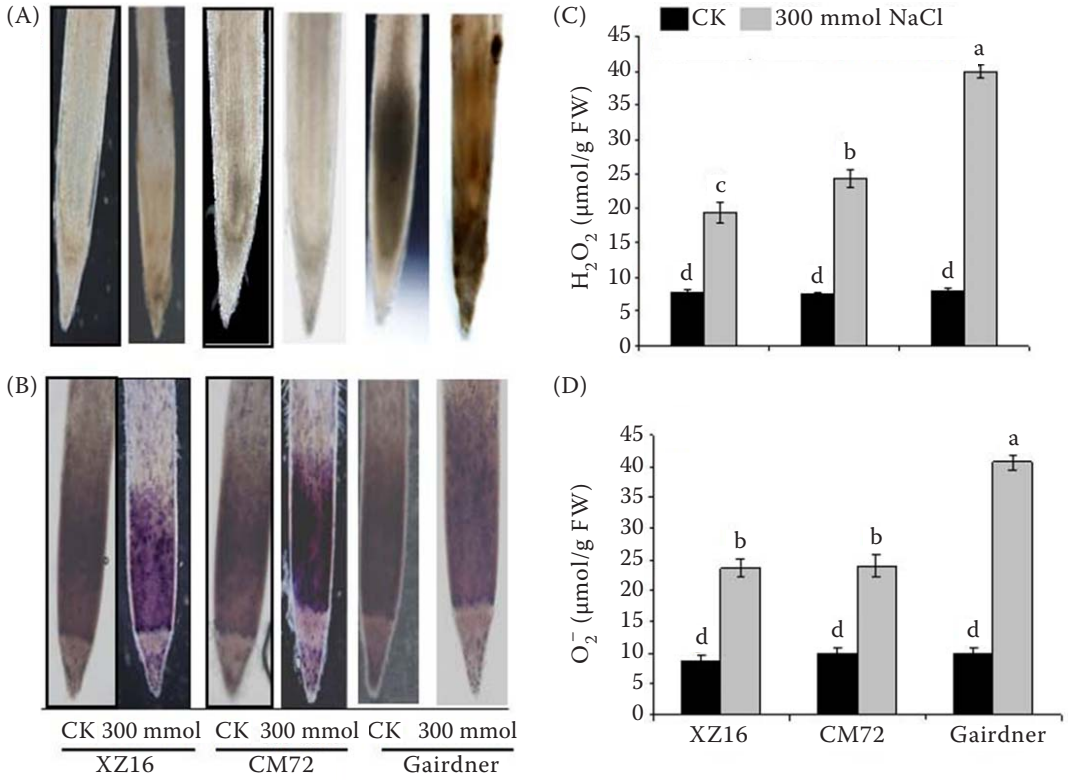

Figure 3. Accumulation of H_2O_2 and O_2^- in the roots after salt stress. (A) H_2O_2 and (B) O_2^- accumulation was detected by diaminobenzidine (DAB) (brown) and nitroblue tetrazolium (NBT) (dark blue) staining. (C) H_2O_2 and (D) O_2 content in roots under control and salt stress. FW – fresh weight

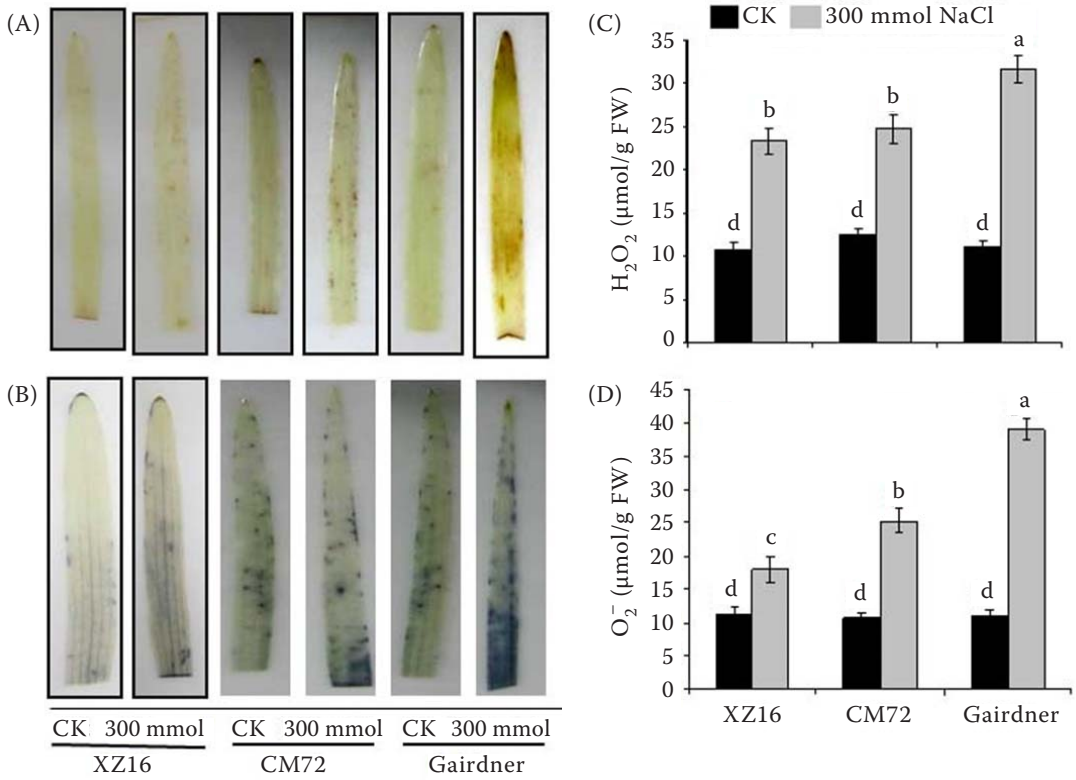

Figure 4. Detached leaves were infiltrated with diaminobenzidine (DAB) (brown) for (A) H_2O_2 and nitroblue tetrazolium (NBT) (dark blue) for (B) O_2^-, respectively, (C) H_2O_2 and (D) O_2^- content in leaves under control and salt stress. FW – fresh weight

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tive stress due to salt treatment, as shown by more staining with brown spots being an indicator of more H_2O_2 (Figures 3A, 4A) and blue spots being an indicator of more O_2^- accumulation (Figures 3B, 4B). In the normal conditions (control), there was no significant difference among the three genotypes in H_2O_2 and O_2^- concentrations of both leaves and roots. Salt stress caused a significant increase of the two ROS compounds relative to the control. Moreover, an increased extent differed greatly among the genotypes. Hence, Gairdner (23.47 mg/g DW) had the highest H_2O_2 concentration in leaves followed by CM72 (24.21 mg/g DW) and XZ16 (23.47 mg/g DW), while root H_2O_2 concentration was observed higher in Gairdner (24.67 mg/g DW) followed by CM72 (24.02 mg/g DW) and XZ16 (43.21 mg/g DW), respectively (Figures 3C, 4C). Additionally, it was observed that O_2^- concentration increased in roots and leaves among three barley genotypes. In roots, O_2^- content ranked in the following order: Gairdner (43.0 mg/g DW) > XZ16 (24.67 mg/g DW) > CM72 (24.0 mg/g DW), respectively, while in leaves the concentration of O_2^- was higher in Gairdner (41 mg/g DW) followed by CM72 (25 mg/g DW) and XZ16 (18 mg/g DW) as compared to their respective control plants (Figures 3D, 4D).

DNA damage. This assay was used to examine the DNA damage due to salt stress. No visible signs of the comet head DNA and no reduction in comet tail DNA or tail moments were observed for either of genotypes under the normal conditions (Figure 5A–C). Yet, obvious differences could be detected among three genotypes in the DNA damage (Figure 5B–F). XZ16 and CM72 had no significant change in comet head DNA and tail moment under salt stress treatment in comparison with the control (Figure 5B,D), while Gairdner showed the marked gain in comet head DNA and great increase in tail moment under salt stress (Figure 5D).

Correlation analysis. Moreover, Pearson's correlation analysis was conducted among antioxidative enzyme activities and Na^+, K^+, Ca^{2+}, Mg^{2+}, proline, H_2O_2 and O_2^- contents in roots and leaves of three barley genotypes (Figure 6A,B). The data revealed that the enzymatic activities in leaves showed a positive correlation with proline, H_2O_2, O_2 and

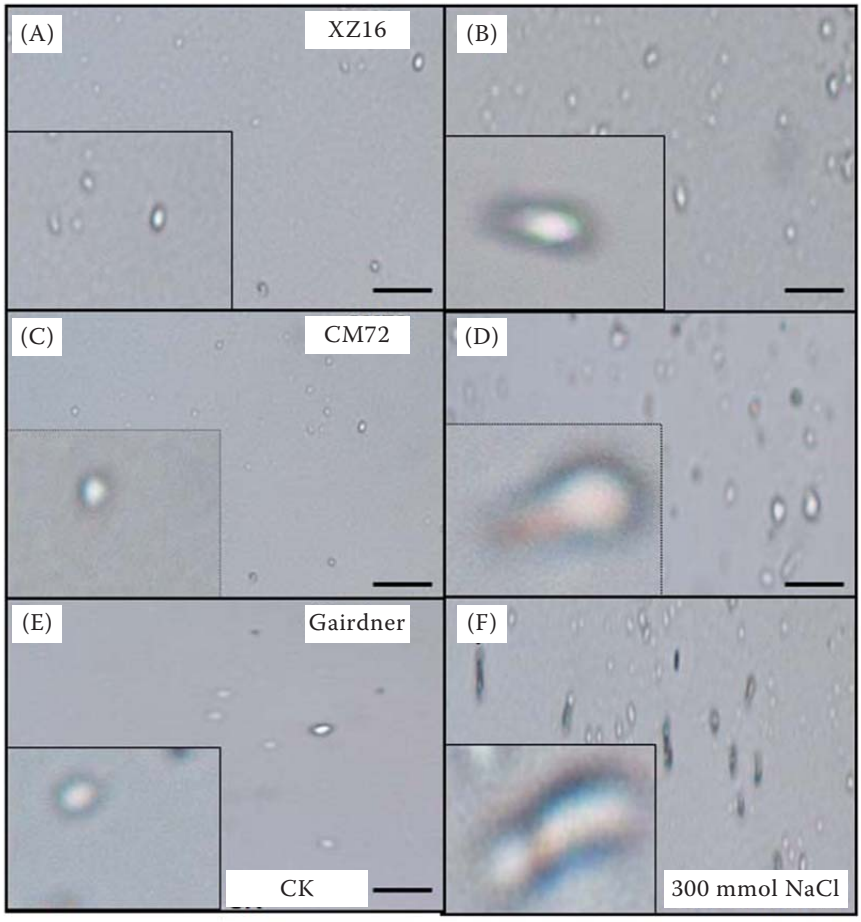

Figure 5. Exposure of barley seedling to salt stress causes DNA damage at the single cell level. A, C, E – control (undamaged DNA); B – treated XZ16 (undamaged DNA, no tail); C – CM72 (little damage); F – Gairdner (almost all DNA damage) under salt stress. Comets were stained with ethidium bromide

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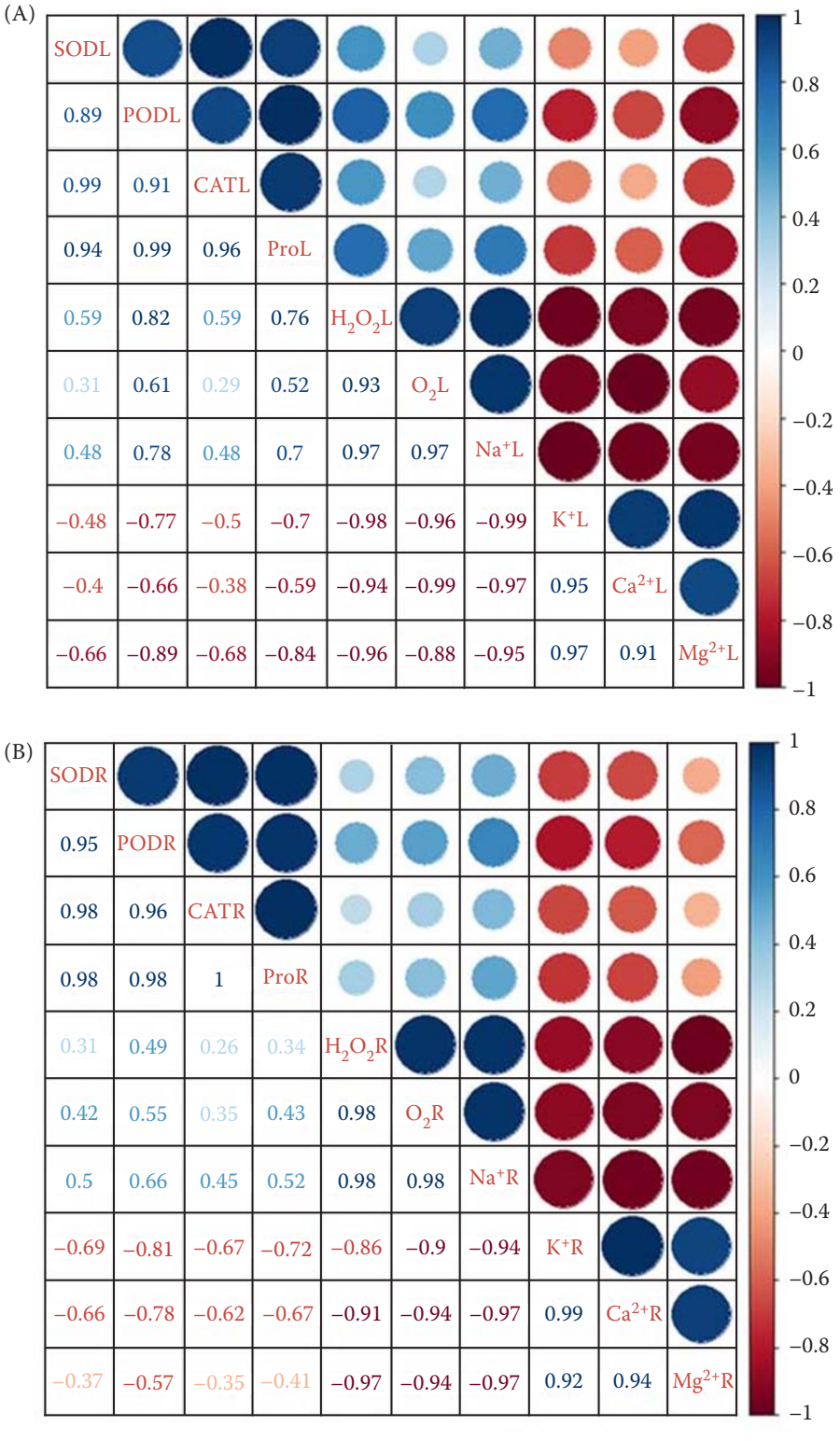

Figure 6. Correlation analysis of (A) leave and (B) root of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), Na^+, K^+, Ca^{2+} and Mg^{2+} concentration, proline (Pro), H_2O_2 and O_2 content of salt treated and control plants of three barley genotypes. Pearson's correlation coefficient among parameters was analysed using the R package. L – for leave; R – for root

Na^+, and a negative correlation with K^+, Ca^{2+} and Mg^{2+} contents of three barley genotypes (Figure 6A). Moreover, enzymatic activities in roots showed a positive correlation with proline, H_2O_2, O_2 and Na^+, and a negative correlation with K^+, Ca^{2+} and Mg^{2+} (Figure 6B).

DISCUSSION

A comprehensively harmful effect of salt stress is the inhibition of plant growth, which could be attributed to specific osmotic stress, ion toxicity, disturbance in ion homeostasis, decrease in chlorophyll content,

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stomatal closure, DNA damage and higher production of reactive oxygen species (Chen et al. 2005, Gunes et al. 2007, Daneshmand et al. 2010, Tian et al. 2015). In this research, salt stress increased the sodium ions accumulation in the leaf and root parts of all the genotypes relative to their respective control. However, the three barley genotypes showed a dramatic difference in tissue Na^+ concentration, with salt-sensitive Gairdner having significantly higher Na^+ ions than other two genotypes. In contrast, K^+, Ca^{2+} and Mg^{2+} ions concentration in plant tissues were significantly reduced under salt stress for all three genotypes, with Gairdner showing the greatest reduction. Results of the present study showed that lower Na^+ uptake and lower inhibition of K^+ and other nutrient elements uptake are important traits closely attributed to salt stress tolerance in barley.

High Na^+ accumulation and low concentrations of K^+, Ca^{2+} and Mg^{2+} in plant tissues would result in the enhanced ROS accumulation (Chen et al. 2005, Munns 2005, Wu et al. 2014). The transportation, distribution and sequestration of ions in different plant parts are important mechanisms of salt tolerance (Gu et al. 2016, Tahjib-Ul-Arif et al. 2019). In addition to ionic and osmotic stress, salinity also induces reactive oxygen species, which leads to oxidative stress (Tang et al. 2015). To cope with the excessive ROS, plants have evolved diverse mechanisms to produce enzymatic and non-enzymatic antioxidants (Blum et al. 1996, Munns and Tester 2008). In the current study, the XZ16 and CM72 showed significantly higher enzymatic activities in both leaves and roots than the sensitive genotype Gairdner. In tolerant plants, SOD and CAT enzymes help in neutralisation, removal of surplus H_2O_2 and play important role in the protection against oxidative stress in plants (Birben et al. 2012, Tahjib-Ul-Arif et al. 2019). Obviously, the activities of these anti-oxidative enzymes could be enhanced by salt stress, but Na^+ or K^+ concentrations in plant tissues are not parallel to the enzymatic activity. It may be assumed that the enhancement of anti-oxidative enzyme activity under salt stress is an inheritable trait closely associated with high salt stress tolerance.

Proline accumulation is enhanced when plants are under abiotic stress. Proline may protect plants against abiotic stresses by possibly mitigating and scavenging the production of free radicals (Fedina and Benderliev 2000, Sperdouli and Moustakas 2012). Generally, plants accumulate more proline content to cope with osmotic stress. Higher concentration of proline in plants is also considered as a marker of osmotic stress (Tahjib-Ul-Arif et al. 2019 et al. 2019). Compatible solutes play an important role in maintaining ion homeostasis and osmotic adjustment by mitigating the toxic effects of ion, lowering the water potential, helping membrane stability and proper regulation, increasing biosynthesis and enzymatic activities and decreasing degradation in plants (Gu et al. 2016, Mbarki et al. 2020).

In the current study, the increase of proline concentration by salt stress differed significantly among all the genotypes, with XZ16 salt tolerant genotype and Gairdner salt sensitive genotype showing the most and least increase. Clearly, the enhancement of proline accumulation in plant tissues is beneficial for development of salt stress tolerance.

Examination of ROS level in cell could be useful for detecting the oxidative damage caused by abiotic factors (Miller et al. 2010), while H_2O_2 and O_2^- are the most prominent ROS accumulated in plant cells under abiotic stress. Detection of H_2O_2 with DAB and O_2^- with NBT at the cellular level was used to determine ROS accumulation and oxidative stress (Hernández et al. 2001, Fukao et al. 2011). Superoxide dismutase reacts with the superoxide radical to produce H_2O_2, which detoxifies and converts it into H_2O and O_2 by the CAT and/or ascorbate-glutathione cycle. Hence, high activities of both CAT and APX may reduce H_2O_2 level in cell under abiotic stress, increasing membrane stability as well as CO_2 fixation, because numerous enzymes of the Calvin cycle within chloroplasts membrane are very sensitive to H_2O_2 (Yamazaki et al. 2003).

In the present work, the result of DAB and NBT staining demonstrated that H_2O_2 and O_2^- concentrations were higher in Gairdner than other two genotypes (CM72 and XZ16), proving that the two salt-tolerant genotypes suffered from less oxidative damage than Gairdner. Such less oxidative damage in XZ16 and CM72 seedlings is closely related with lower Na^+ and higher K^+ concentrations and anti-oxidative enzyme activities in plant tissues.

In addition, ROS may cause DNA disintegration. In this research, a comet assay was used to evaluate the DNA damage of each genotype. The results showed that the enhancement of DNA damages is greatly correlated to oxidative stress, indicating that it is the ROS accumulation in plants tissues that causes subsequent DNA damage.

Our results suggest that Tibetan wild barley XZ16 is relatively more tolerant than CM72 and Gairdner.

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The salt tolerance mechanisms of XZ16 could be elucidated in terms of: (1) less Na^+ uptake and more K^+, Ca^{2+} and Mg^{2+} retention in roots and leaves; (2) the enhancement of proline accumulation in tolerant plant tissues is beneficial for development of stress tolerance; (3) lesser oxidative stress *via* stimulating detoxifications of ROS by keeping up redox homeostasis, and (4) increased activity of antioxidant enzymes, such as CAT, APX and POX activity, which detoxifies excess of H_2O_2 and O_2^-. These are important traits, closely attributed to salt stress tolerance in XZ16 wild barley. Moreover, it is highly recommended to explore in depth molecular mechanism(s) of Tibetan wild barley XZ16 to unravel the novel genes involved in salinity tolerance.

REFERENCES

Abdallah M.B., Trupiano D., Polzella A., De Zio E., Sassi M., Scaloni A., Zarrouk M., Youssef N.B., Scippa G.S. (2018): Unraveling physiological, biochemical and molecular mechanisms involved in olive (*Olea europaea* L. cv. Chétoui) tolerance to drought and salt stresses. Journal of Plant Physiology, 220: 83–95.

Apel K., Hirt H. (2004): Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology, 55: 373–399.

Bartoli C.G., Gómez F., Martínez D.E., Guiamet J.J. (2004): Mitochondria are the main target for oxidative damage in leaves of wheat (*Triticum aestivum* L.). Journal of Experimental Botany, 55: 1663–1669.

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

Birben E., Sahiner U.M., Sackesen C., Erzurum S., Kalayci O. (2012): Oxidative stress and antioxidant defense. World Allergy Organ Journal, 5: 9–19.

Blum A., Munns R., Passieoura J.B., Turner N.C., Sharp R.E., Boyer J.S., Nguyen H.T., Hsiao T.C., Verma D.P.S. (1996): Genetically engineered plants resistant to soil drying and salt stress: how to interpret osmotic relations? Plant Physiology, 110: 1051–1053.

Chen Z., Newman I., Zhou M., Mendham N., Zhang G., Shabala S. (2005): Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. Plant, Cell and Environment, 28: 1230–1246.

Daneshmand F., Arvin M.J., Kalantari K.M. (2010): Physiological responses to NaCl stress in three wild species of potato *in vitro*. Acta Physiologiae Plantarum, 32: 91.

Fedina I.S., Benderliev K.M. (2000): Response of *Scenedesmus incrassatulus* to salt stress as affected by methyl jasmonate. Biologia Plantarum, 43: 625–627.

Fukao T., Yeung E., Bailey-Serres J. (2011): The submergence tolerance regulator SUB_1A mediates crosstalk between submergence and drought tolerance in rice. The Plant Cell, 23: 412–427.

Gorai M., Neffati M. (2007): Germination responses of *Reaumuria vermiculata* to salinity and temperature. Annals of Applied Biology, 151: 53–59.

Gunes A., Inal A., Alpaslan M., Eraslan F., Bagci E.G., Cicek N. (2007): Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. Journal of Plant Physiology, 164: 728–736.

Gu M.F., Li N., Shao T.Y., Long X.H., Brestič M., Shao H.B., Li J.B., Mbarki S. (2016): Accumulation capacity of ions in cabbage (*Brassica oleracea* L.) supplied with sea water. Plant, Soil and Environment, 62: 314–320.

Hernández J.A., Ferrer M.A., Jiménez A., Barceló A.R., Sevilla F. (2001): Antioxidant systems and O_2^-/H_2O_2 production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiology, 127: 817–831.

Hussain N., Irshad F., Jabeen Z., Shamsi I.H., Jiang L.X. (2013): Biosynthesis, structural, and functional attributes of tocopherols in planta; past, present, and future perspectives. Journal of Agricultural and Food Chemistry, 61: 6137–6149.

Jiang M.Y., Zhang J.H. (2002): Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. Journal of Experimental Botany, 53: 2401–2410.

Mbarki S., Skalický M., Vachová P., Hajihashemi S., Jouini L., Živčák M., Tlustoš P., Brestič M., Hejnák V., Khelil A.Z. (2020): Comparing salt tolerance at seedling and germination stages in local populations of *Medicago ciliaris* L. to *Medicago intertexta* L. and *Medicago scutellata* L. Plants, 9: 526.

Miller G., Suzuki N., Ciftci-Yilmaz S., Mittler R. (2010): Reactive oxygen species homeostasis and signaling during drought and salinity stresses. Plant, Cell and Environment, 33: 453–467.

Mittler R., Vanderauwera S., Gollery M., Van Breusegem F. (2004): Reactive oxygen gene network of plants. Trends in Plant Science, 9: 490–498.

Munns R. (2005): Genes and salt tolerance: bringing them together. New Phytologist, 167: 645–663.

Munns R., Tester M. (2008): Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59: 651–681.

Pandolfi C., Bazihizina N., Giordano C., Mancuso S., Azzarello E. (2017): Salt acclimation process: a comparison between a sensitive and a tolerant *Olea europaea* cultivar. Tree Physiology, 37: 380–388.

SAS Institute Inc. (2008): SAS/STAT 9. 2 User's Guide. Cary, SAS Institute.

Shavrukov Y. (2012): Salt stress or salt shock: which genes are we studying? Journal of Experimental Botany, 64: 119–127.

Shavrukov Y., Gupta N.K., Miyazaki J., Baho M.N., Chalmers K.J., Tester M., Langridge P., Collins N.C. (2010): HvNax3 – a locus controlling shoot sodium exclusion derived from wild barley (*Hordeum vulgare* ssp. *spontaneum*). Function of Integrative Genomics, 10: 277–291.

https://doi.org/10.17221/169/2020-PSE

Singh S., Anjum N.A., Khan N.A., Nazar R. (2008): Metal-binding peptides and antibinding peptides and antioxidant defence system in plants: significance in cadmium tolerance. In: Khan N.A., Singh S.G. (eds.): Abiotic Stress and Plant Responses. New Delhi, IK International, 159–189. ISBN: 978-81-89866-95-2

Sperdouli I., Moustakas M. (2012): Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of *Arabidopsis thaliana* to drought stress. Journal of Plant Physiology, 169: 577–585.

Tahjib-Ul-Arif Md., Sohag A.A.M., Afrin S., Bashar K.K., Afrin T., Mahamud A.G.M.S.U., Polash M.A.S., Hossain Md.T., Sohel Md.A.T., Brestič M., Murata Y. (2019): Differential response of sugar beet to long-term mild to severe salinity in a soil-pot culture. Agriculture, 9: 223.

Tang X.L., Mu X., Shao H.B., Wang H.Y., Brestič M. (2015): Global plant-responding mechanisms to salt stress: physiological and molecular levels and implications in biotechnology. Critical Reviews in Biotechnology, 35: 425–434.

Tian X.Y., He M.R., Wang Z.L., Zhang J.W., Song Y.L., He Z.L., Dong Y.J. (2015): Application of nitric oxide and calcium nitrate enhances tolerance of wheat seedlings to salt stress. Plant Growth Regulation, 77: 343–356.

Velikova V., Yordanov I., Edreva A. (2000): Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science, 151: 59–66.

Wang Y., Xu C., Du L.Q., Cao J., Liu J.X., Su X., Zhao H., Fan F.-Y., Wang B., Katsube T., Fan S.J., Liu Q. (2013): Evaluation of the comet assay for assessing the dose-response relationship of DNA damage induced by ionizing radiation. International Journal of Molecular Science, 14: 22449–22461.

Wu D.Z., Shen Q.F., Qiu L., Ye L.Z., Han Y., Zahra J., Shu Q.Y., Zhang G.P. (2014): Proteomics analysis reveals mechanism of ion homeostasis and salt tolerance in wild and cultivated barley. Proteomics, 14: 1303–1444.

Wu G.Q., Zhang L.N., Wang Y.Y. (2012): Response of growth and antioxidant enzymes to osmotic stress in two different wheat (*Triticum aestivum* L.) cultivars seedlings. Plant, Soil and Environment, 58: 534–539.

Yamazaki J.-Y., Ohashi A., Hashimoto Y., Negishi E., Kumagai S., Kubo T., Oikawa T., Maruta E., Kamimura Y. (2003): Effects of high light and low temperature during harsh winter on needle photodamage of *Abies mariesii* growing at the forest limit on Mt. Norikura in Central Japan. Plant Science, 165: 257–264.

Yan C., Fansheng K., Xingling O. (2012): Antioxidant and antiglycated activities of polysaccharides *in vitro* isolated from *Hedyotis diffusa* Willd. Journal of Medicinal Plants Research, 6: 2895–2900.

Zahra J., Nazim H., Han Y., Syed M.J., Zeng F.R., Zeng J.B., Zhang G.P. (2014): The differences in physiological responses, ultrastructure changes, and Na^+ subcellular distribution under salt stress among the barley genotypes differing in salt tolerance. Acta Physiologiae Plantarum, 36: 2397–2407.

Zhu J.K. (2001): Plant salt tolerance. Trends in Plant Science, 6: 66–71.

Zhu J.K. (2002): Salt and drought stress signal transduction in plants. Annual Review of Plant Biology, 53: 247–273.

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