EFFECT OF SALINITY STRESS ON SOME PHYSIOLOGICAL TRAITS OF BURNING BUSH

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(Received 18th Oct 2019; accepted 30th Jan 2020)

Abstract. Salinity is one of the most serious environmental stresses at the irrigated areas that causes problems for the plants and soil. *Kochia scoparia* is an annual plant of the Chenopodiaceae family, which can be a valuable source of forage growth using saline water. Salinity changes many traits and processes, including the amount of chlorophyll a and b, activity of antioxidant enzymes, protein content, and concentration of sodium and potassium in the plant. An experiment was performed with two salinity levels of 100 and 300 mM on *Kochia scoparia*. Among the measured traits, the levels of chlorophyll a, b, peroxidase and catalase specific enzyme activity, and protein content were not affected by salinity. In contrast, this plant limited sodium Influx and the amount entered accumulated in the stem. Also, the study results showed that potassium concentration in this plant significantly increased in the stem. The results of other studies indicate that potassium plays an important role in tolerance to salinity. Therefore, potassium has a function in tolerance to salinity in this plant.
Keywords: *catalase, K/Na ratio, peroxidase, potassium, sodium*

Introduction

In worldwide, the salinity is one of the main factors that cause losses of crop. The human activity including: extractions of oil, industrial waste production, salt usage for road way maintenance and intensive farming activity, are sources of soil salinization. The salinity of soil and freshwater exist in many places. The publication for this issue increased fivefold from 2004 to 2018 (Litalien and Zeeb, 2020).

Soil salinity is the most serious environmental constraint affecting the production of crops, which is estimated to affect 45 million hectares of irrigated lands and is expected to increase due to global climate changes and frequent irrigation operations (Rengasamy, 2010; Munns and Tester, 2008 quoted by Roy et al., 2014).

Kochia scoparia L. Schrad (Common name: burning bush) is an annual plant of the Chenopodiaceae family, tolerant to salinity and drought, which can be a valuable source of forage growth by saline water (Danesh Mesgaran and Stern, 2005; Riasi et al., 2008 quoted by Kafi et al., 2010).

Kochia scoparia exists in wide range of temperatures and climatic regions in the world, but this plant has adapted specifically to arid and semi-arid regions (Friesen et al., 2009 quoted by Casey, 2009). *Kochia* has shown that it is capable of rapid germination, which makes it possible to use the limited moisture content soil in the spring in arid and semi-arid regions (Eberlein and Fore, 1984 quoted by Casey, 2009). The seed germination occurs several times during the growing season, which enables the plant to use the advantage of moisture when available. The seeds of this plant are

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1802_33733385

capable to germinate under severe stress conditions, such as lack of moisture, high salt, and high pH (Friesen et al., 2009 quoted by Casey, 2009).

An experiment was done on *Kochia scoparia* at five levels of 100:00, 75:25, 50:50, 25:75, and 00:100 tap water:seawater ratio, respectively. The total dry matter (g $plant^{-1}$) of *kochia* reduces in response to this condition. A trend of decline in linear phase was observed form the highest total dry matter in control plants (100:00) to 75:25, 50:50, 25:75, and 00:100 (the lowest) respectively. Additively, the relative growth rate (RGR, g g^{-1} d^{-1}) and Specific leaf area (SLA, cm^2 g^{-1}) were measured in this study. The RGR between 100:00, 75:25, 50:50 were not significant and between 25:75 and 00:100 it was significant. The SLA trait between 100:00, 25:75, and 00:100 were significant and between of 75:25, 50:50 it was not significant (Lopez-Aguilar et al., 2013).

The salinity effect was investigated on Indian and Sabzevar genotype of *kochia* at salinity of 5, 15, and 20 dS m^{-1}. Results show that salinity reduce yield of seed Sabzevar, although at high salinity levels the yield of seeds was 890 kg ha^{-1} that approximately 80% of yield at 5 dS m^{-1}. The seed production genotype of Indian great decreased with salinity raise (Kafi et al., 2010).

Salinity stress causes many physiological changes in plants, including the followings. According to the study of Agastian et al. (2000), chlorophyll and total carotenoid is significantly reduced by salinity. In tomato leaves, total chlorophyll (Chl-a + b) chlorophyll a and beta-carotene reduced with sodium chloride stress (Khavarinejad and Mostofi, 1998 quoted by Parida and Das, 2005).

With increasing salinity, the leaf protein content of *Bruguiera parviflora* reduced, which indicates that the protein synthesis process may be disturbed, or more probably, proteolytic activity increase (Parida et al., 2002). The soluble protein content in the berries increased under low salinity and reduced in high salinity (Agastian et al., 2000).

Salinity stress causes water deficiency as a result of osmotic effects on a wide range of metabolic activities of plants, and this water deficiency has resulted in oxidative stress due to the formation of reactive oxygen species (ROS) such as superoxides, proxy and hydroxy radicals. The (ROS) are by-products of hyper osmotic and ionic stress that interfere with cell membrane and cell death (Bohnert and Jensen, 1996 quoted by Parida and Das, 2005). With the increase of the formation of (ROS), enzyme activities of detoxification increase in these species (Apel and Hirt, 2004; Foyer and Noctor, 2005; Logan, 2005 quoted by Munns and Tester, 2008). In halophyte *Sesuvium portulacastrum*, antioxidant enzymes such as catalase, peroxidase and polyphenol oxidase increased to 600 mM and reduced slightly at higher levels (Rajaravindran and Natarajan, 2012). Agarwal and Pandey (2004) have been shown that salt treatment increased sodium and chloride ions in *Cassia angustifolia* seedlings and increased the activity of superoxide dismutase, catalase, peroxidase and polyphenol oxidase.

Sodium concentration in rice that was treated with NaCl was significantly higher. Also, potassium concentration was higher in older stems and leaves (Morales et al., 2012). For plants ability to distinguish between sodium and potassium, the K+/Na+ ratio index usage. There are many references for this index that can be determined for plants and their organs (Flowers, 2004). The K+/Na+ ratio in leaves of rice cultivars sensitive to salinity is significantly lower than that of salinity tolerant cultivars (Moradi and Ismail, 2007 quoted by Morales et al., 2012).

Regarding tolerance of *Kochia* to salinity, this study was done to investigate physiological cases mentioned above and to find some aspects of salinity response in this plant.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Materials and methods

Sowing, sampling and experiment design

First, *Kochia* seed was sowed in sand and manure with a ratio of 3:1. Plant growth was carried out under greenhouse conditions (*Fig. 1*). About 3 to 4 weeks after planting, salt treatment at a concentration of 100 mM was performed. Two days after the treatment, samples from the roots, stems and leaves of treated and control plants were harvested in three replicates, and samples were transferred to a freezer at -70 °C. Then, the treatment was performed at a concentration of 300 mM and two days after treatment in three replications of leaves, stems and roots treatment and control plants, sampling was done with the same method as mentioned above. The experiment design which data can be analysis with two samples T-test.The controls for each treatment at 100 and 300 mM were selected separately and sampling was done. The controls for each treatment were selected separately, because environmental condition changed over time.

Figure 1. *Plants sowing in green house condition for salinity stress*

Extraction and measurement of chlorophyll

Wellburn (1994) method with partial modification was done to measure chlorophyll content. For this purpose, 50 mg of the leaf tissue well grinded with methanol in a mortar with pestle, and homogenized tissue was transferred to the microcentrifuge tubes. At the next stage, centrifugation was carried out at 5,000 rpm, the supernatant was transferred to a new tube and diluted with methanol. Using spectrophotometer (Smart Spec™ Plus, Bio-Rad), the absorbance of the resulting solution at wavelengths of 653 and 666 nm was read and chlorophyll a and b values were calculated using the following formulas:

$$Ca = 15.65\ A666 - 7.34\ A653$$

$$Cb = 27.05\ A653 - 11.21\ A666$$

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Sodium and potassium measurements

Munns et al. (2010) method with partial modification was used to measure sodium and potassium. The root, stem and leaf samples were dried in an oven at 70 °C for 48 h. The dried samples were then weighed, about 20 mg, and extracted using 1 Normal hydrochloric acid. The solution was thoroughly mixed with up and down movements and placed in a water bath for 45 min at 60 °C. During this period, the falcone is moved up and down to be mixed well with acid. Then, centrifuge was performed for 15 and 5 min at 5,000 rpm. The supernatant was transferred to the 50 ml falcon and the samples were diluted at a ratio of 1:10. Flame photometer model PFP7 (jenway) was used to measure sodium and potassium content.

Extraction of crude extract to measure enzyme activity and protein content

For this purpose, 50 mg of leaf was weighed and using sodium phosphate buffer (at a concentration of 0.1 M and pH = 6.8) based on Kar and Mishra (1976) method a homogenous solution was obtained. At the next stage, centrifugation at 10,000 (g) was carried out for 15 min at 2 °C. The supernatant was transferred to a new tube and kept at -20 °C until usage. At the extraction stage, the samples were kept on ice.

Measuring enzyme activity of peroxidase

The peroxidase enzyme activity was measured according to Mac Adam et al. (1992) method with partial modification. First 2810 µl of sodium phosphate buffer 0.1 M, 40 µl of 30 mM of hydrogen peroxide (H2O2) and 50 µl of Guaiacol solution of 200 mM were added to the 15 ml falcon. Then 100 µl of enzyme extract was added (total volume of the resulting solution was 3 ml). After moving the falcons up and down, the resulting solution was placed at room temperature for 10 min until the formation of tetraguaiacol from guaiacol (color of the solution was orange), and then absorption was read at 470 nm by spectrophotometer.

Measuring enzyme activity of catalase

Catalase activity was done according to Verma and Dubey (2003) procedure with partial modification. For this purpose, first 2840 µL of 0.1 M sodium phosphate buffer, 60 µl of hydrogen peroxide (H2O2) 1 M was added before starting the reaction and 100 µl of enzyme extract was also added. The falcon tube was moved up and down a few times to mix the reaction components and then placed at room temperature for 10 min to decompose hydrogen peroxide and the absorbance was read at 240 nm using spectrophotometer.

Measuring protein content

Protein content was measured according Bradford method (1976) with partial modification. First, a Stock 1 (mg/ml) of bovine serum albumin (BSA) was used to provide standards of protein. Then, using this we prepared series of dilutions of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 (µg/ml) in a volume of 500 µl. At the next stage, 100 µl of each of these was mixed with 900 µl of Bradford solution (total volume of 1 ml), and absorption was read at 595 nm after 2 min. The final concentration of standards was 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 (µg/ml) in 1 ml of total volume.

In order to measure the protein content in extracted samples similar to the standard method. first 100 µl of the extracted solution was mixed with 900 µl of Bradford solution and absorption was read.

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Calculation of specific enzyme activity

In order to calculate the enzyme activity, volume activity was calculated according to *Equation 1* (Bisswanger, 2011, 2014):

$$\text{Volume activity}_{(\mu mol/min/ml)} = \frac{\text{absorption/min} \cdot \text{assay volume} \cdot \text{dilution factor}}{\text{absorption coefficient} \cdot \text{path length} \cdot \text{enzyme volume}} \quad \text{(Eq.1)}$$

Absorption coefficient of tetraguaiacol and H2O2 was 0.036 and 26.6 (mM^{-1}. cm^{-1}), respectively.

Then, the obtained value was used according to *Equation 2* in order to calculate the specific enzyme activity:

$$\text{Specific enzyme activity}_{(\mu mol/min/mg)} = \frac{\text{Volume activity}}{\text{protein concentration}} \quad \text{(Eq.2)}$$

Data analysis

The data obtained from the above were evaluated using software R version 3.2.3 for the normalization of the data. Comparison between control and treatment at 100 mM was done with two samples T-test. Also Comparison between control and treatment at 300 mM was done with two samples T-test. Two samples T-test were analyzed using software SAS 9.1. The correlation coefficients between traits were calculated by Pearson method with the R software. The figures were drawing by R software.

Results

Chlorophyll content

As shown in *Figure 2*, the levels of chlorophyll a and b at 100 mM level were higher in treatment than in control, and their level reduced to less than control with an increase at 300 mM. Of course, their difference was not significant.

Sodium and potassium content

The content of potassium and sodium in the stem, root and leaf is shown in *Figure 3*, which is described in the followings.

The concentration of potassium in the stem at level of 100 and 300 mM in treatment samples is higher than in control samples. These differences are significant at a concentration of 100 mM at $\alpha = 0.05$ and at a concentration of 300 mM at $\alpha = 0.01$.

The content of potassium in the root at treatment level of 100 and 300 mM in treatment samples was higher than in control samples. But these differences were not significant.

The content of potassium in the leaf samples at 100 mM in control was higher than in treatment samples. But at 300 mM, the content of potassium in the leaves in treatment was more than in control. The two concentrations of 100 and 300 mM were significant at $\alpha = 0.05$.

Sodium concentration in stem at both levels of 100 and 300 mM in treatment was higher than in control. As shown in *Figure 3*, this difference is only significant at $\alpha = 0.01$ at 100 mM.

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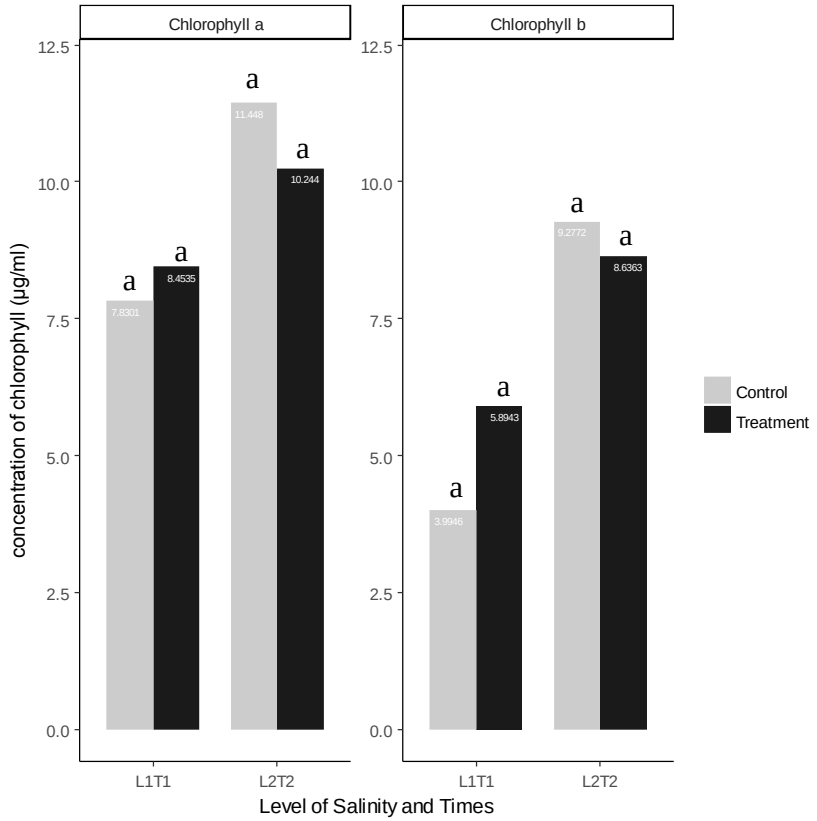

Figure 2. *Chlorophyll a and b content [L1T1: L1 = 100 mM level, T1 = two days after treatment] [L2T2: L2 = 300 mM level, T2 = two days after treatment]. Columns with a-a above are not significant*

Sodium content in the root at concentration of 100 mM is approximately not different in control and treatment samples, but in control samples it is more than in treatment samples at 300 mM. At both levels, the difference is not statistically significant.

Sodium concentration at leaf samples at 100 mM in control samples was more than in treatment samples. It is equal at 300 mM. Only at 100 mM level, a statistically significant difference was found at $\alpha = 0.05$.

K/Na ratio in stems, roots and leaves

As shown in *Figure 4*, the K/Na ratio in stem at 100 mM level in control sample was higher than in treatment samples, but at 300 mM at treatment it was higher than control. Neither of the two levels of this ratio was significant.

The K/Na ratio in root at both levels of 100 and 300 mM in treatment was higher than control and neither was statistically significant.

The K/Na ratio in leaf in treatment was higher than in control at both level and neither was statistically significant.

Protein content

As shown in *Figure 5*, protein content at the salinity level of 100 mM in control samples is higher than in treatment samples although it is reversed by increasing salinity to 300 mM. but neither was statistically significant.

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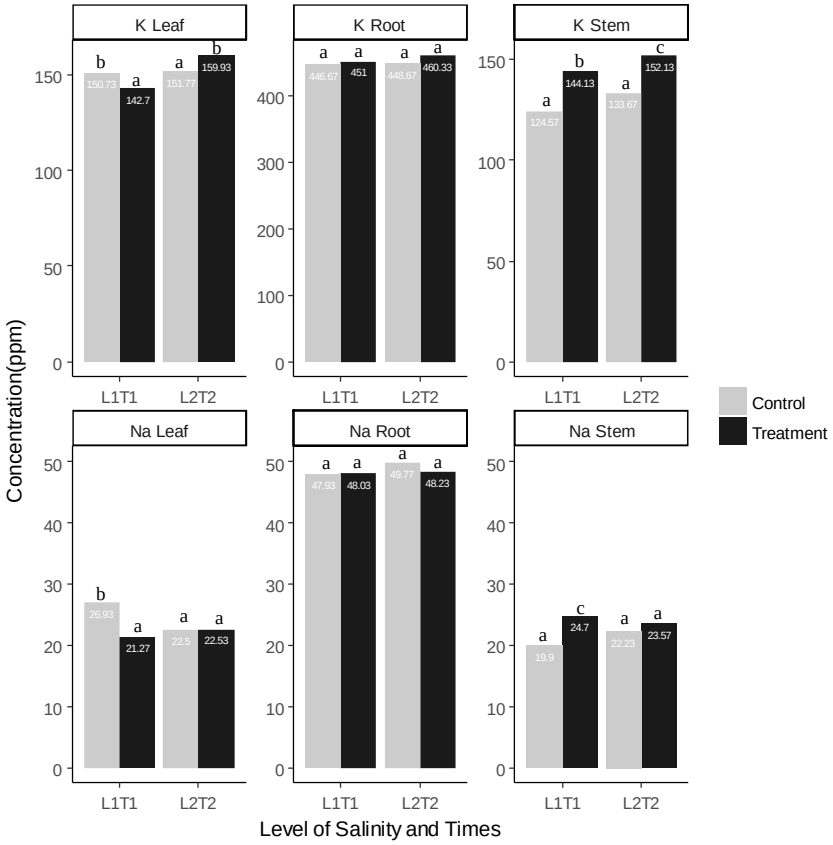

Figure 3. *Potassium and sodium content in stems, roots and leaves. columns with a-a above are not significant. Columns with a-b and a-c above are significant at α = 0.05 and α = 0.01 respectively. Legend same as Figure 2*

Peroxidase

According to *Figure 6*, the activity of this enzyme at 100 mM concentration and two days after the treatment was more than control, and it was equal at a concentration of 300 mM. Of course, there is no statistically significant difference between them.

Catalase

As showed in *Figure 6*, the specific enzyme activity of catalase at 100 mM concentration in control was higher than in treatment, but vice versa at 300 mM concentration. Although these differences were not significant.

Study of correlation coefficients

The values of correlation coefficients are given in *Figure 7*. Among all correlation coefficients, 14 cases were significant at $\alpha = 0.05$. Among correlation coefficients, chlorophyll a and protein, chlorophyll b and leaf K/Na ratio, stem Na and stem K, stem Na and leaf K/Na ratio, leaf K and protein, leaf K and stem K/Na ratio, stem K and root K, stem K and leaf K/Na ratio showed significant and positive correlation. However, between chlorophyll a and peroxidase, protein and peroxidase, leaf Na and stem Na, leaf Na and stem K, leaf Na and leaf K/Na ratio, root Na and root K/Na ratio a significant and negative correlation was found.

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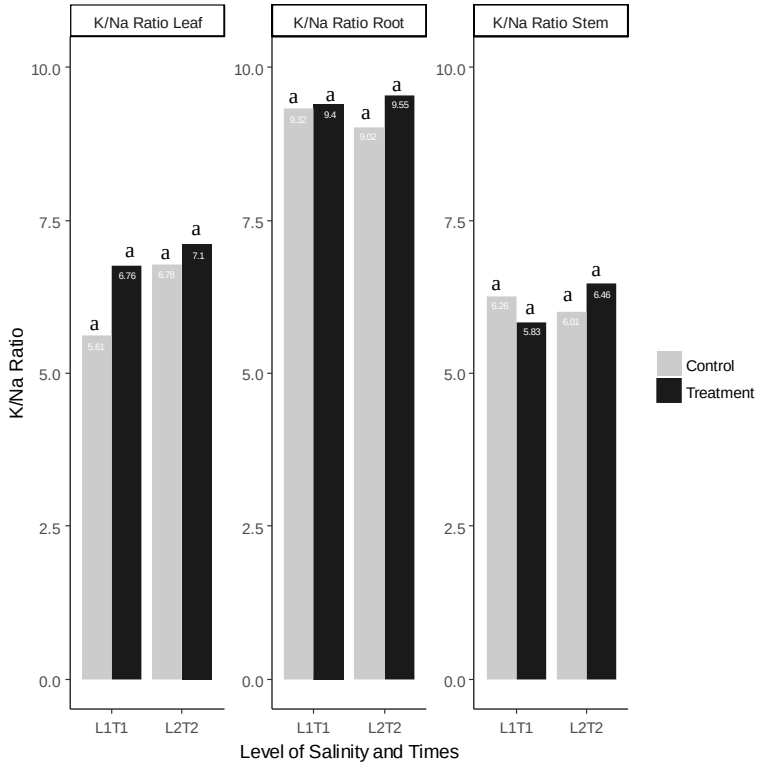

Figure 4. *The K/Na ratio in stems roots and leaves. Columns with a-a above are not significant. Legend same as Figure 2*

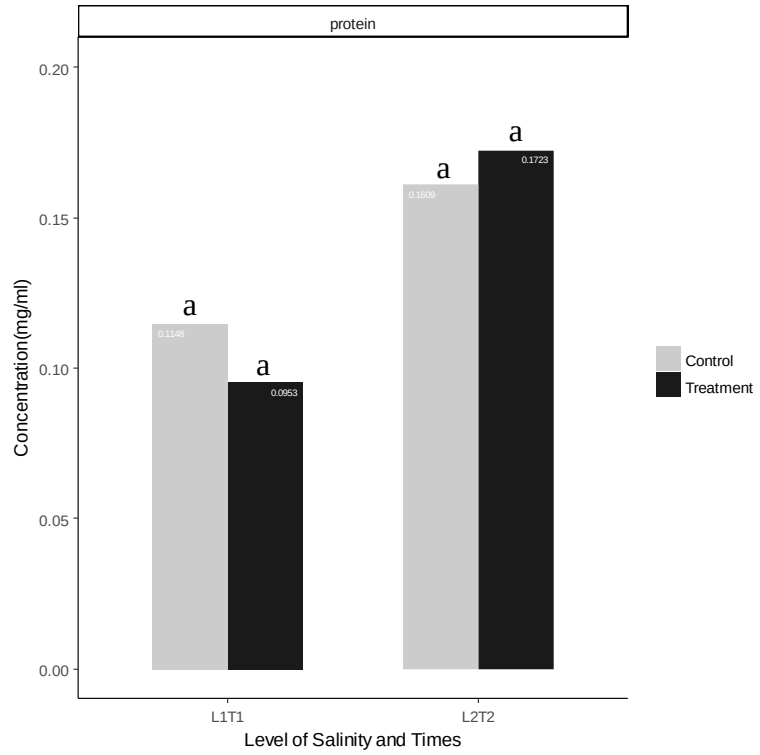

Figure 5. *Protein content in leaves. Columns with a-a above are not significant. Legend same as Figure 2*

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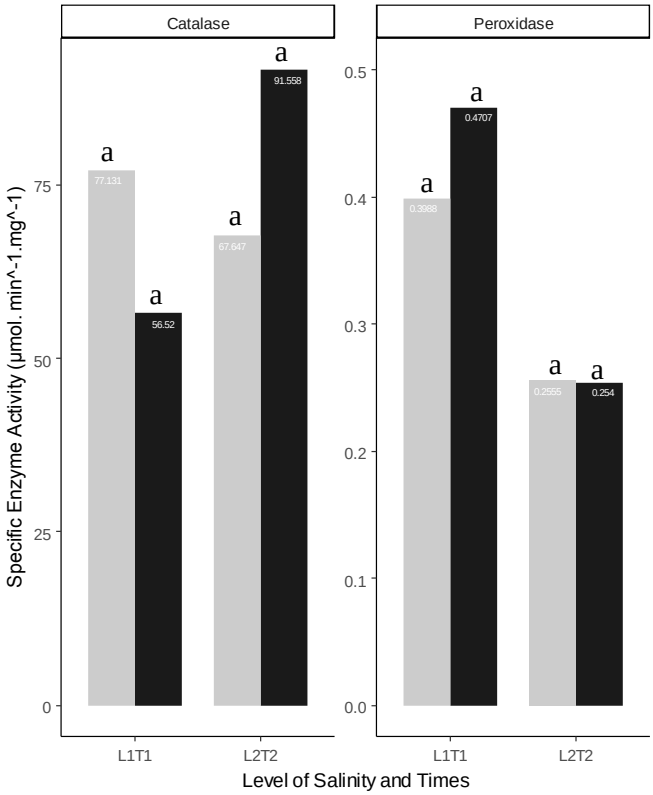

Figure 6. *Specific enzyme activity of peroxidase and catalase. Columns with a-a above are not significant. Legend same as Figure 2*

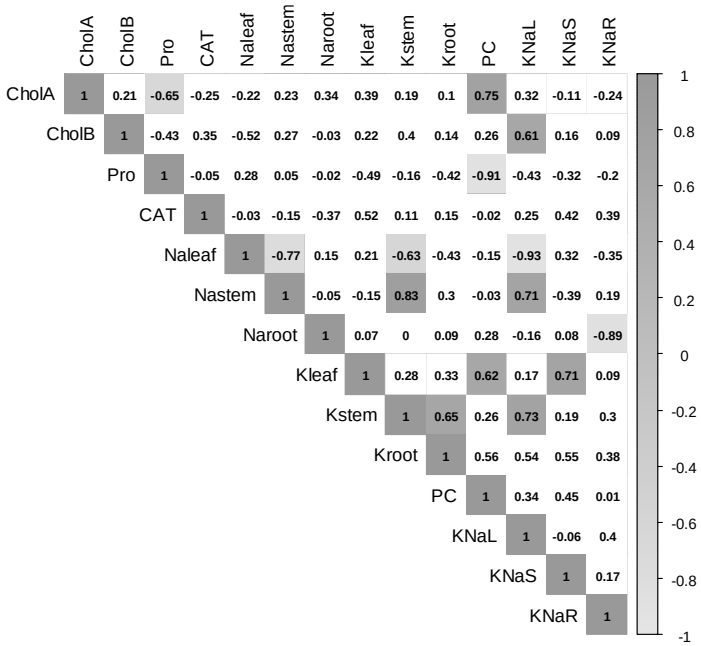

Figure 7. *Pearson correlation coefficients between the measured traits. Colour boxes are significant at α = 0.05, right bar shows the direction (positive and negative) and degree (colour intensity, from 1 to – 1) of correlation). CholA: chlorophyll a, CholB: Chlorophyll b, Pro: Peroxidase, CAT: Catalase, PC: Protein content, KNaL: leaf K/Na ratio, KNaS: Stem K/Na ratio, KNaR: Root K/Na ratio*

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Discussion

Chlorophyll a and b and total carotenoid in *Morus alba* L were affected differently by salinity, but their total amount reduced with salinity (Agastian et al., 2000).

The leaf chlorophyll content of *Bruguiera parviflora* increased by 6% in plants treated with 100 mM of NaCl compared to the control plants, and then reduced at 200 and 400 mM (Parida et al., 2004).

Leaf total chlorophyll and carotenoid reduced under salt stress (Parida and Das, 2005). But Wang and Nil (2000) quoted by Parida and Das (2005) reported that chlorophyll content increased under salinity conditions in Amaranthus.

But results of this study showed that Chlorophyll a and b at 100 mM level in treatment was higher than in control, while at 300 mM level this relationship was reversed, however, neither was significant. The study results showed that salinity had no significant effect on the amount of chlorophyll a and b.

The most important trait among salinity specific traits is the plant's ability to limit the high accumulation of sodium entering into xylems. This trait is described by the term "Na^+ exclusion". Within a species or even within a genus, sodium concentration in leaves can be considered as a sign of relative ability to "exclude Na^+", especially if well-defined tissues are sampled (Colmer et al., 2006). According to *Figure 3*, it seems that the plant allows low sodium entering the root, but the low amount entered accumulated in the stem. In leaves at L1T1, sodium is higher in control sample than in treatment sample with unknown reason, but in L2T2 it is reduced and equal in both samples.

The high salt uptake competes with other nutrient ions uptake, especially potassium, that cause deficiency of this element. An increase in sodium chloride treatment induces an increase in sodium and chloride and leads to a reduction in calcium, potassium and magnesium in a number of plants (Khan et al., 1999, 2000; Khan and Aziz, 2001 quoted by Parida and Das, 2005). As shown in *Figure 3*, in stems, roots and leaves at two levels and times, which for each element is six cases in total, in three cases potassium is higher in the treatment compared to the control, and in one case control is more than the treatment and is statistically significant. But sodium is significant in two cases, that one is more in control and one more in treatment. According to these results, it seems that in the competition between potassium and sodium, more potassium enters the plant, which is contrary to the above-mentioned works. With low sodium uptake, this substance accumulates less in the plant and prevents the toxicity it can cause.

The plant cells require high levels of potassium to maintain normal metabolic reactions. Potassium also plays an important role in maintaining turgor pressure (Sairam and Tyagi, 2004). It is possible that the plant at the early stages that is exposed to water deficiency stress also absorbs more potassium to maintain turgor pressure. It is also necessary to maintain the uptake of potassium and transfer to growing tissues for salinity tolerance (Greenway and Munns, 1980 quoted by Colmer et al., 2006). These reasons can be explanations to the increased K uptake of this plant.

Analysis of ions had shown that tolerant genotypes had lower sodium concentration in the shoot, and more potassium in the shoot that sensitive genotypes. Salt-tolerant crops are characterized by absorbing more potassium than sodium (Teakle and Tyerman, 2010; Kausar et al., 2014 quoted by Oyiga et al., 2016). The ratio of K/Na was significantly higher in the shoot compared to sensitive ones, which is due to high concentration of potassium in the shoot and low concentration of sodium in the shoot. The optimum ratio of K/Na plays an important role in maintaining the optimum osmotic

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1802_33733385

potential and membrane potential for regulating cell volume in the plant under salinity stress and participate in increasing the tolerance of salinity in wheat (El-Hendawy et al., 2009 quoted by Oyiga et al., 2016). According to *Figure 4*, in most cases, K/Na ratio is higher in the treatment plants, which indicates the *kochia* tolerance to salinity and as mentioned above has a role in salinity tolerance of this plant.

As mentioned in Introduction, with the increase of hyperosmotic stresses, the activity of antioxidant enzymes increases. According to *Figure 6*, the activity of peroxidase in L1T1 in stressed plants is more than in the control, but catalase at this level and time in the control samples is more than in the treatment. In L2T2, the activity of catalase in treatment is more than in control, and peroxidase at this level and time is almost equal in the control and in the treatment. It can be said that the function of both enzymes is the opposite of each other except of L2T2 peroxidase that was different. Although these differences are not significant, they are remarkable. Probably a factor or process makes these two enzymes at any time and level one is higher for lower energy consumption.

As shown in *Figure 7*, a significant and negative correlation (r = -93) was found between the leaf Na and leaf K/Na ratio. Also, a significant and negative correlation was found between root Na and root K/Na ratio (r = -89). This result is consistent with the study results of Karimi et al. (2014) in safflower, which concluded that a negative and significant correlation was found between the leaf Na and leaf K/Na ratio, and the root Na and root K/Na ratio.

Furthermore, a significant and negative correlation was observed between chlorophyll a and peroxidase activity (r = -65). Moreover, a significant and positive correlation was found between chlorophyll a and protein content (r = 75). Anaya et al. (2017) reported a significant and positive correlation between chlorophyll a and peroxidase activity. They also observed a significant and negative correlation between chlorophyll a and protein. As mentioned, the study results were inconsistent with the study results of Anaya et al. (2017).

The results showed that salinity had no significant effect on chlorophyll a, b, peroxidase, catalase, and protein content. But this plant was limited sodium entering to the plant, and prevented its toxic effects. It also has been shown that potassium plays an important role in tolerance to salinity.

Acknowledgements. We thank to Masood Fakherfeshani, Ahmad Javai, Ghasem Parmoon, Jamshid Ardan, Ekhtiear Aghazade and others who helped in this work. This work was financed by a PhD fellowship from the University of Mohaghegh Ardabili.

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