

## ***Salicornia europaea* L. Maintains its Physiological Integrity under High Salinity Stress in Retreated Sections of the Urmia Lake, Iran**

Zare-Maivan, Hassan<sup>\*</sup>; Daviran, Marzieh; Ghanati, Faezeh; Sharifi, Mohsen

*Department of Plant Biology, Tarbiat Modares University, Tehran, IR Iran*

*Received: August 2015*

*Accepted: November 2015*

© 2015 Journal of the Persian Gulf. All rights reserved.

### **Abstract**

Arid lands and inland water bodies, such as Urmia Lake in Iran, have become vulnerable to increased salinity stress because of rising temperature as a result of global warming. High salinity enhances plants photorespiration and affects its physiology, but *Salicornia europaea* L. in Urmia Lake has adapted to grow, propagate and occupy increasingly exposed saline habitats. Plant and soil samples were collected in triplicate plots from different sites of retreated beds of Urmia Lake in fall, 2015. Salinity stress physiology of *S.europaea* L. populations were investigated in Urmia Lake through determining: (a) leaf cell membrane lipid peroxidation (Malondialdehyde production, MDA); (b) antioxidant enzymes activities (peroxidase, catalase and Phenylalanine Ammonia Lyase (PAL) and (3) changes in contents of non-enzymatic antioxidants (flavonoid and anthocyanin). Analysis of variance (ANOVA), Duncan Post-hoc tests and correlation tests were performed using the SPSS.21 statistical program when significant differences occurred at 5% level. Results showed that peroxidase activity reduced and MDA content remained constant which indicates intact integrity of leaf cell membrane and the ability of cells to scavenge Reactive Oxygen Species (ROS). Increase in catalase activity and anthocyanin and total flavonoid contents correlated strongly with increased salinity at all stations located along the exposed and drying bed of the Urmia Lake. It is concluded that *S.europaea* tolerates salinity stress and subsequent ill-effects of ROSs produced through, (1) regulating vacuolar water exchanges, (2) managing peroxisome activity and (3) developing a complex system of antioxidants. Therefore, *S.europaea* can be cultivated in retreated Urmia Lake bed for animal feed or other uses.

Keywords: *Urmia Lake, Salicornia europaea, oxidative stress, antioxidant system.*

### **1. Introduction**

Globally, temperature and precipitation patterns have changed markedly in recent decades and are predicted to change even more in the future as a result

of natural and anthropogenically driven climate change (Meehl et al., 2007). Climate change models further predict increased occurrence of extreme events (flooding, extended droughts), which will magnify the seasonal and multiannual amplitude of water level fluctuations, in turn creating hydrological stresses

<sup>\*</sup> E-mail: zare897@yahoo.com

(e.g. prolonged hydraulic retention time) in lakes (IPCC, 2014) as well as retreat of inland lakes in lower latitudes (Zare-Maivan, 2015). Decreases in precipitation and enhanced evaporation have caused retreat of many inland water bodies (for example, Urmia Lake), creating an opportunity for specific plant communities tolerant of greater aridity and salinity to grow in emerged lands or retreated lake beds. Zare-Maivan (2015) demonstrated retreating of Urmia Lake and progressing of halophyte plant communities in exposed lake bed.

*Salicornia europaea* L. (*S.persica* Akhani), is a plant species very common in Urmia Lake (Khara, et al., 2004; Akhani, et al., 1996). *S.europaea*, a succulent euhalophyte in Chenopodiaceae, is widely distributed in coastal and inland salt marshes worldwide. *S.europaea* can withstand salinity greater than 1M NaCl and is one of the most salt-tolerant plant species in the world devoid of salt glands or salt bladders (Ushakova et al., 2005; Flowers and Colmer, 2008).

Haline beds are the most obvious and predominant feature of retreated Urmia Lake. Hence, expectedly, salinity is the primary environmental factor limiting plant growth and productivity (Lv et al., 2012). Salinity effects on plants include ion toxicity, osmotic stress, mineral deficiencies, physiological and biochemical perturbations and combinations of these stresses. Tolerant plants combat salinity stress and subsequent oxidative stresses (Pérez-Lopez et al., 2009) by altering lipid composition, expression of aquaporins and controlling water permeability (Lopez-Pérez et al., 2009) through the production of antioxidants, both enzymatic and non-enzymatic antioxidants (Zare-Maivan, et al., 2015). Altering

activity of antioxidant enzymes, such as (peroxidase, catalase and Phenylalanine ammonia lyase (PAL) and membrane lipid peroxidation (Malondialdehyde production, MDA) as well as production of non-enzymatic antioxidants (flavonoids and anthocyanins) have been reported for many halophytic and arid plant species under natural or induced stresses (Zare-Maivan, et. al., 2004; Zare-Maivan, et al. 2012, Zare-Maivan, et. al., 2015).

It is well known that plant communities in retreated sections of the Urmia Lake might be distributed based on groundwater levels, however, due to extensive evaporation in the region and lack of data on the fluctuation of groundwater levels, it is hypothesized that the salinity tolerance and adaptive capabilities of plants are the major contributors to the succession of plant species and distribution of plant communities in retreated lake beds and saline soils. Since *S.europaea* dominates arid and saline soils of retreated Urmia Lake, its cell membrane integrity and antioxidant potential to acclimate to such conditions were investigated in 2015. *S.europaea* tolerates well in arid and saline soils and maintains its succulence and cell membrane integrity, so the purpose of this research is to investigate of resistancy *S.europaea* (enzymatic and non-enzymatic) under natural field conditions.

## 2. Materials and Methods

### 2.1. Sampling

Plant and soil samples were collected in triplicate plots from different sites of retreated beds of Urmia Lake in fall, 2015, Iran (Table 1).

Table 1: Geographical coordinates of sampling stations in retreated beds of Urmia Lake in fall, 2015, Iran

| Station | Name           | Longitude  | Latitude   | Elevation (m) |
|---------|----------------|------------|------------|---------------|
| 1       | Km60           | 37° 07.346 | 45° 27.145 | 1269          |
| 2       | Km50           | 37° 11.396 | 45° 22.354 | 1254          |
| 3       | Km40           | 37° 15.923 | 45° 19.244 | 1265          |
| 4       | Rashakan       | 37° 20.574 | 45° 17.714 | 1269          |
| 5       | Jazireh Eslami | 37° 44.559 | 45° 15.498 | 1264          |

## 2.2. Determining Soil Characteristics

Saturated soil extracts were prepared and used For pH, Electrical conductivity (EC), total dissolved solids (TDS) and salinity by (Conductivity meter PT.20 Sartorius Germany) device.

## 2.3. Determining Lipid Peroxidation (MDA content)

0.2 g fresh leaves was crushed in 3ml Trichloroacetic acid 10%, then centrifuged at 12,000 rpm for 10 min. To 1 ml of supernatant in test tube 1 ml Thiobarbituric acid was added and placed in 100°C water bath for 30 minutes until color change occurred. Test tubes were immediately placed in ice to stop reaction and their absorption was read using a spectrophotometer model UV/Visible (Analytickagena, Germany Spekol 2000) at wavelengths of 532 and 600 nm. MDA content was calculated by using  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  extinction coefficient based on mM/fresh weight (Des Vos et al., 1991).

## 2.4. Total Protein Content

100 ml of the extract (extraction method are explained below) was added 1 ml Bradford solution and vortexed. After 5 minutes, sample absorbance was measured at 595 nm. According to the protein standard curve, protein concentration of the samples was calculated (Bradford, 1979).

## 2.5. Determining Enzyme Activity

### 2.5.1. Catalase

0.2 g fresh leaves were crushed in 3ml potassium phosphate buffer 25mM, pH= 6.1 on ice and centrifuged at 4°C and 12,000 rpm for 20 min. 100  $\mu\text{l}$  of supernatant was mixed with 500  $\mu\text{l}$   $\text{H}_2\text{O}_2$  (10 mM) in potassium phosphate buffer (25 mM), pH=6.8 before reading absorbance at wavelength of 240 nm for 0 and 60 sec. The enzyme activity was

measured as absorbance changes in minute per mg protein (Cakmak and Horst, 1991).

### 2.5.2. Peroxidase

0.2 g fresh leaves were crushed in 3ml potassium phosphate buffer 60mM, pH= 6.1 on ice and centrifuged at 4°C and 12,000 rpm for 20 min. 500  $\mu\text{l}$  of supernatant was mixed with 500  $\mu\text{l}$   $\text{H}_2\text{O}_2$  (5 mM) and 500  $\mu\text{l}$  Guaiacol (28 mM) in potassium phosphate buffer (60 mM), pH=6.1 before reading absorbance at wavelength of 470 nm for 0 and 60 sec. The enzyme activity was measured as absorbance changes in minute per mg protein (Ghanati et al., 2002).

### 2.5.3. Phenylalanine Ammonia Lyase

0.1 g fresh leaves was crushed in 1ml potassium phosphate buffer 0.1mM, pH=7.5 on ice, and centrifuged at 4°C and 12,000 rpm for 20 min. 200  $\mu\text{l}$  Phenylalanine 0.1 M/potassium phosphate buffer 0.1M, pH=7.5 and 650  $\mu\text{l}$  potassium phosphate buffer and 150  $\mu\text{l}$  supernatant were mixed in micro tubes. They were rested at 37°C for 1 hour and after adding 50  $\mu\text{l}$  HCl 6M vortexed for 10 sec. Next, 500  $\mu\text{l}$  ethyl acetate was added and centrifuged 13000 rpm at 20°C for 10 min. Approximately 1500  $\mu\text{l}$  supernatant was collected after three times of centrifugation, dried under the hood and mixed with 1 ml NaOH 0.05 M using vortex. Absorbance of the mixture was read at 290nm using Spectrophotometer model UV/Visible (Analytickagena, Germany Spekol, 2000). PAL activity was calculated on the basis of Cinnamic acid production/mg protein. The enzyme activity was measured as absorbance changes in minute per mg protein (Wang et al., 2006).

### 2.5.4. Super Oxide Dismutase

0.2 g leaves were crushed with 3 mL potassium phosphate 50 mM, 6.8 on the ice, and centrifuged for

20 min at 4°C and 12,000 rpm. 1500 ml HEPES-KOH 50 mM with EDTA 0.1 mM (PH: 7.8), 300 ml of sodium carbonate 50 mM, 300 ml L-methionine 12 mM, 300 ml nitro blue tetrazolium (NBT) 75 µM, 300 ml Riboflavin 1 mM and 300 ml enzymatic extraction was poured into the test tubes and their absorption was measured at 560 nm using Spectrophotometer. Then, the test tubes were exposed to light for 10 minutes and their absorbance was again read at same wavelength. The enzyme activity was measured as absorbance changes per mg protein (Giannopolitis and Rice, 1977).

## 2.6. Total Flavonoid Content

0.1 g fresh leaf was crushed in 3ml acidic ethanol and centrifuged at 12,000 rpm for 15 min. One ml of supernatant was mixed with 9 ml acidic ethanol in test tube and placed in 80°C water bath for 10 minutes. The absorbance was read with spectrophotometer at 270, 300 and 330 nm. Total flavonoid content (mM/g leaf fresh weight) was calculated using 33000  $\text{mM}^{-1} \text{cm}^{-1}$  extinction coefficient (Krizek et al., 1997).

## 2.7. Leaf Pigments Contents

0.1 g fresh leaves was crushed in 3 ml acetone 80%. The extract filtered with conventional filter paper in Falcon was clear and its volume was reached to 10 ml. Then samples absorption was measured at 480, 645 and 663 nm by spectrophotometer. Chlorophyll a, Chlorophyll b and total chlorophyll was calculated from the following formula (Arnon, 1949).

$12.7(D663)-2.69(D645)) * V/1000W$  (= Chlorophyll a)  
 $22.9(D645)-4.69(D663)) * V/1000W$  (= Chlorophyll b)

Total chlorophyll=  $(20.2 (D645) + 8.02 (D663)) * V/1000W$

D: the absorbance; V: volume of extract in mili liter;  
 W: sample weight in gram

## 2.8. Anthocyanin Content

Supernatant was prepared as described for total flavonoid and let rest for 24 h in dark. Absorbance of supernatant was read at 550 nm using spectrophotometer. Anthocyanin content (mM/ leaf fresh weight) was calculated using 155  $\text{M}^{-1} \text{cm}^{-1}$  extinction coefficient The enzyme activity was measured as absorbance changes in minute per mg protein (Hara, et. al. 2003).

## 2.9. Statistical Analysis

Mean values and correlation tests were compared using the SPSS.21 statistical program. Analysis of variance (ANOVA) and Duncan Post-hoc tests was performed when significant differences occurred at 5% level.

## 3. Results

Soil primarily consisted of sand and clay. Results of average salinity, total dissolved solutes (TDS), electric conductivity (EC) and pH soil samples are shown in Table 2. Trends of changes in the salinity, EC and TDS were similar and differed from pH as the latter showed the least variation. The salinity of the soil samples was greater than average soil characteristics required for irrigated agriculture (Table 2).

Table 2: Soil chemical characteristics at different locations of the retreated section of the Urmia Lake harboring *Salicornia europaea*, 2015

| Station No | Station Name   | Soil Type       | Salinity (ppt) | TDS (g/l) | EC (ds/m) | pH   |
|------------|----------------|-----------------|----------------|-----------|-----------|------|
| 1          | Km 60          | Clay-loam       | 3              | 2.84      | 5.67      | 7.98 |
| 2          | Km 50          | Sandy-clay loam | 5              | 4.81      | 9.55      | 7.93 |
| 3          | Km 40          | Clay-loam       | 4              | 3.75      | 7.52      | 8.23 |
| 4          | Rashakan       | Clay-loam       | 4.5            | 4.58      | 9.15      | 6.95 |
| 5          | Jazireh Eslami | Sandy-clay-loam | 3.5            | 3.31      | 6.64      | 7.88 |

### 3.1. MDA Content

Mean analysis of MDA content in leaves of *S. europaea* from different stations showed no significant differences among stations which ranged between  $4 \times 10^{-3}$  and  $9 \times 10^{-3}$  mMgFW<sup>-1</sup> (Table 3).

### 3.2. Catalase Activity

A significant difference was observed in the activity of catalase in leaves of *S. europaea* at different stations which ranged between 1.5 and 3.5 mg protein min<sup>-1</sup> at 240 nm absorbance (Table 3). The lowest catalase activity was observed in leaves of plants collected from Rashakan station located on

the west side of the lake.

There was a strong positive correlation between catalase activity and salinity and negative correlation with shoot length (Table 4).

### 3.3. Peroxidase Activity

A significant difference was observed in the activity of peroxidase in leaves of *S. europaea* at different stations and ranged between 40 and 50 mg protein min<sup>-1</sup> at 470 nm absorbance (Table 3). The lowest catalase activity was recorded from leaves of plants collected from Jazireh Eslami. There was a negative correlation between peroxidase activity and salinity as well as shoot length (Table 4)

Table 3: The average of measured parameters characteristics at different locations of the retreated section of the Urmia Lake harboring *Salicornia europaea*, 2015.

|   | Station 1                  | Station 2                  | Station 3                  | Station 4                  | Station 5                  |
|---|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| MDA (mM/g F.W)                                      | 0.004 <sup>a</sup> ±0.0004 | 0.006 <sup>a</sup> ±0.0005 | 0.007 <sup>a</sup> ±0.0006 | 0.005 <sup>a</sup> ±0.001  | 0.009 <sup>a</sup> ±0.003  |
| Chlorophyll a<br>(mg/g F.W)                         | 0.087 <sup>c</sup> ±0.04   | 0.06 <sup>d</sup> ±0.004   | 0.13 <sup>b</sup> ±0.015   | 0.081 <sup>c</sup> ±0.007  | 0.166 <sup>a</sup> ±0.004  |
| Chlorophyll b<br>(mg/g F.W)                         | 0.114 <sup>bc</sup> ±0.006 | 0.109 <sup>c</sup> ±0.001  | 0.147 <sup>a</sup> ±0.004  | 0.118 <sup>bc</sup> ±0.031 | 0.138 <sup>ab</sup> ±0.002 |
| Total Chlorophyll<br>(mg/g F.W)                     | 0.192 <sup>c</sup> ±0.015  | 0.157 <sup>d</sup> ±0.007  | 0.273 <sup>b</sup> ±0.019  | 0.176 <sup>cd</sup> ±0.01  | 0.304 <sup>a</sup> ±0.001  |
| Anthocyanine<br>(mM/g F.W)                          | 0.049 <sup>b</sup> ±0.001  | 0.055 <sup>a</sup> ±0.003  | 0.039 <sup>c</sup> ±0.001  | 0.046 <sup>b</sup> ±0.001  | 0.046 <sup>b</sup> ±0.002  |
| Total Flavonoids<br>(mM/g F.W)                      | 0.214 <sup>c</sup> ±0.005  | 0.334 <sup>a</sup> ±0.0265 | 0.235 <sup>c</sup> ±0.001  | 0.283 <sup>b</sup> ±0.0475 | 0.254 <sup>bc</sup> ±0.002 |
| POX activity<br>(ΔAbs. 470/mg protein.min)          | 44.16 <sup>a</sup> ±0.299  | 36.257 <sup>b</sup> ±1.519 | 37.543 <sup>b</sup> ±6.758 | 44.083 <sup>a</sup> ±0.644 | 47.297 <sup>a</sup> ±1.805 |
| CAT activity<br>(ΔAbs. 240/mg protein.min)          | 1.406 <sup>ab</sup> ±0.355 | 3.388 <sup>a</sup> ±0.361  | 1.969 <sup>b</sup> ±0.349  | 1.498 <sup>ab</sup> ±0.29  | 1.082 <sup>c</sup> ±0.206  |
| SOD activity<br>(U/mg protein)                      | 1.612 <sup>a</sup> ±0.084  | 1.647 <sup>a</sup> ±0.051  | 1.388 <sup>b</sup> ±0.058  | 1.248 <sup>b</sup> ±0.03   | 1.338 <sup>b</sup> ±0.129  |
| PAL activity<br>(μM cinamic<br>acid/mg protein.min) | 0.307 <sup>a</sup> ±0.015  | 0.547 <sup>ab</sup> ±0.099 | 0.474 <sup>ab</sup> ±0.097 | 0.601 <sup>b</sup> ±0.0938 | 0.341 <sup>ab</sup> ±0.073 |

Table 4: Correlation between salinity and shoot length with leaf pigments and antioxidants of *S.europaeae* grown on the bed of the Urmia Lake, 2015

| Character                                 | Salinity | Shoot length |
|---|----------|--------------|
| Salinity                                  | -        | -0.70**      |
| Chlorophyll a                             | -0.51    | 0.44         |
| Chlorophyll b                             | -0.23    | -0.10        |
| Chlorophyll Total                         | -0.48    | 0.30         |
| Anthocyanine                              | 0.32     | 0.14         |
| Flavonoids (Total)                        | 0.82**   | -0.33        |
| Superoxide dismutase (SOD) activity       | -0.14    | -0.04        |
| Peroxidase (POX) activity                 | -0.54*   | -0.79**      |
| Catalase (CAT) activity                   | 0.77**   | -0.74**      |
| Phenylalanin amonia layase (PAL) activity | 0.64**   | 0.48         |

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).

### 3.4. PAL Activity

There was a positive correlation between salinity and PAL activity. A significant difference was observed in the activity of PAL in leaves of *S. europaea* plants collected from Rashakan station on the west side of the Urmia Lake (the lowest activity) and the Km 60 station, the farthest eastern station (the highest enzyme activity) (Table 3). There was a mild correlation between PAL and salinity as well as shoot length (Table 4).

### 3.5. Superoxide Dismutase Activity

Significant differences were observed in superoxide dismutase activity of leaves of *S. europaea* at different stations. Maximum enzyme activity was observed at km 50 and km 60 stations. These stations were stations with the highest and lowest salinity, respectively (Table 3). There was no significant correlation between SOD activity and salinity (Table 4).

### 3.6. Total Flavonoid

Significant differences were observed in the flavonoid contents of leaves of *S. europaea* at different stations. Leaves collected from Jazireh Eslami showed

the highest total flavonoid content (3.2 mM FW g<sup>-1</sup>) (Table 3). There was a strong positive correlation between flavonoid content and salinity but not with shoot length (Table 4).

### 3.7. Contents of Leaf Pigments

Pigment contents of leaves of *S. europaea* varied between stations and showed weak or negative correlation with salinity (Table 4). There were significant differences in anthocyanin contents in different stations. The highest and the lowest contents of anthocyanin (mM FW g<sup>-1</sup>) were observed in leaves of *S.europaea* collected from Jazireh Eslami and station at Km 60 (Table 3). There was a weak positive correlation between anthocyanin content and salinity (Table 4).

## 4. Discussion

Production of Reactive Oxygen Species (ROS), which are commonly produced under normal conditions as a by-product of metabolism in all plants and halophytes, is exacerbated under physiological stresses; For example, when a plant faces increase in rhizosphere salinity levels, its cellular water potential lowers, plant gas exchange capacity alters, accessible

CO<sub>2</sub> as a results of stomata closure lowers, frequent potential redox changes in the cell followed by shifts in overall cell metabolic homeostasis occurs and ROS production enhances drastically, causing oxidative stress in the cells (Ozgur, et al., 2013; Amor,et al., 2006). Afterward, reactions leading to the production of antioxidants initiate and cell membrane peroxidation ensue.

Polyunsaturated fatty acids (PUFAs), a major component of biomembranes, are easily peroxidized in response to cellular oxidative stress. In the process, one of the most reactive aldehydes formed after the breakdown of lipid hydroperoxides is malondialdehyde (MDA), MDA can attach to proteins and nucleic acids and deteriorates their functions in the process (Yamauchi, et al., 2008). In plant biomembranes, especially in chloroplast membrane, tri-unsaturated fatty acids (C18 and C16), in mixture with di- and mono-unsaturated fatty acids (2-25%), compose a large percentage (50-90%) of membrane fatty acids (Douce, et al., 1973). Tri-unsaturated fatty acids, such as linolenic acid which exists in *S.europaeae*, is an easily peroxidizable fatty acid. Therefore, peroxidation products caused by oxidation of PUFAs in chloroplast membrane (especially thylakoid membrane), are plenty (Yamauchi, et al., 2008) and can be used as good indicators of plants under stress.

Results of this research showed no significant difference in the concentration of MDA among samples collected from different stations (Table 3), despite differences in the chemical characteristics of the soil at each station (Table 2). It is expected that greater salinity of the substrate potentially cause NaCl-induced oxidative stress and manifest itself in the form of differences in the content of MDA of leaves (Amor et al., 2006), but in *S.europaeae* because of strong ability to activate enzymatic and non-enzymatic antioxidant systems (Adam et al., 1995; Bestwick et al., 2001), differences in MDA contents of leaves at different locations remained insignificant. This is an indication of cell membrane integrity and

the minimalist peroxidation of membrane lipids. Besides, measuring antioxidants such as catalase, peroxidase and PAL as well as flavonoids has highlighted the antioxidant strategy of *S.europaeae*, as depicted by Gholizadeh and Kohnehrouz (2010), and characterized its ability in regulating water, ionic relations and other salt effects efficiently (Rout and Shaw, 2001; Saleh and Plieth, 2009).

Results of this study showed that there were significant differences between all stations for the activity of antioxidant enzymes, catalase, peroxidase, and PAL as well as non-enzymatic antioxidants of flavonoids and anthocyanin. Catalase showed the lowest activity in the leaves of *S.europaeae* in Rashakan on the west side of the lake, peroxidase activity was the lowest in plants collected from Jazireh Eslami, PAL activity was the lowest and the highest in leaves collected from Rasakan and Km 60 on the southeast side of the lake, respectively. Both total flavonoids and anthocyanin showed the highest activity in leaves collected from Jazireh Eslami. Peroxidase and SOD showed negative and PAL and catalase as well as total flavonoids and anthocyanin contents showed a positive correlation with salinity. Shoot length, as biomass, showed negative or at best moderate correlation with antioxidant enzymes and compounds (Table 4).

Peroxidase showed negative and catalase showed a positive correlation with salinity. In fact by increased level of salinity, increasing trend of catalase and decreasing trend of peroxidase were observed. Catalase and peroxidase are commonly stored in peroxisomes, cytosol, and vacuole, respectively (Prasad et al., 1995 and Mhamdi et al., 2010). Usually, salinity causes increases in H<sub>2</sub>O<sub>2</sub> content of the cell and expectedly, greater involvement of peroxisomes in H<sub>2</sub>O<sub>2</sub> break down than cytosol and vacuole; But, since *S.europaea* is able to compartmentalize excessive sodium in its vacuoles, it can better maintain its cellular homeostasis (Lv et al., 2012) and tolerate excess salinity.

These show that salinity caused H<sub>2</sub>O<sub>2</sub> production in peroxisomes more than in cytosol and vacuole. This means that salinity does not much affect cellular homeostasis negatively in this species; because *S.europaea* is able to compartmentalize excessive sodium in vacuoles. Multiple compartmentalization of sodium in vacuoles confers salt tolerance to *S.europaea* (Jiang et al 2012).

The results of SOD activity showed the highest activity of this enzyme in the station with the highest salinity (Station km 50), which has the highest catalase activity, and in the station with the lowest salinity (Station km 60), which has the highest peroxidase activity.

SOD enzyme has different isoforms that each act in particular subcellular. Mn-SOD is, generally, found in mitochondria, Fe-SOD in chloroplasts and CuZn-SOD in chloroplasts and/or in cytosol, compartment-specific responses of the antioxidative response system can be distinguished (Amor et al., 2005).

The maximum CAT activity has been in plants of the station with the highest salinity, which is due to the increase of superoxide anion detoxification by SOD into peroxisome. But, probably the stations that are less saline, cytosolic SOD activity increased due to increasing of superoxide anion in the cytosol, and then peroxidase activity increased for detoxification of H<sub>2</sub>O<sub>2</sub> in the cytosol. In fact, in the stations with the highest salinity, CAT detoxify ROS within peroxisomes, but in the stations with the lowest salinity, due to reduction of CAT activity, detoxification of ROS is performed in the cytosol, increased POX activity indicates this (Table 3).

Phenolic compounds, including simple phenols (phenolic acids and derivatives) and polyphenols (flavonoids and polymeric compounds), play an important role in the detoxification of free radicals (Ksouri et al., 2007). Phenylalanine ammonia-lyase (PAL) catalyzes the first step in the biosynthesis of phenylpropanoids, which form a wide variety of plant secondary products, such as flavonoids,

including anthocyanins and condensed tannins, via p-coumaric acid of the phenylpropanoid pathway (Bevan et al., 1989).

A common response of plants to water deficit is increased synthesis of phenolic compounds (Parida et al., 2004). High PAL activity is associated with the accumulation of anthocyanins and flavonoids in plant tissues (Table 3). However, the degree of significance or correlation between salinity and enzymatic and nonenzymatic antioxidants varies. For example, while total flavonoids contents of leaves correlated very strongly with salinity, anthocyanin content and catalase activity showed relatively strong and PAL activity moderate correlations in response to salinity. In contrast, peroxidase showed a negative correlation to salinity.

The content of chlorophyll a, b and total chlorophyll showed negative correlation with soil salinity, it means that with increasing salinity, chlorophyll content is reduced. There are two reasons for reduction in chlorophyll content under salt stress. Under water shortage or salinity, proline act as osmolyte for supporting cell osmotic challenges. (Hare and Cress, 1996). Proline and chlorophyll have a common precursor for their construction, perhaps with increasing of salinity, and consequently increasing of proline production, the common precursor is consumed for proline production and thus, reduce the amount of chlorophyll synthesis. The effects of salinity, such as increasing production of ROS, increasing light inhibition, increasing enzyme activity of chlorophyllase and changes in ratio of lipid to protein in protein-pigment complex, enhances the degradation of chlorophyll and thus, reduces its content. (Iyengar and Reddy, 1996; Kato and Shimizu 1985)

These findings reinforce the notion that catalase in peroxisomes plays the primary role and removes the H<sub>2</sub>O<sub>2</sub> and facilitates ROS breakdown and non-enzymatic antioxidants play a secondary role in the physiological operation of the cell. It is concluded that

*S.europaea* tolerates salinity stress and subsequent ill-effects of ROSs produced through: (1) regulating vacuolar water exchanges, (2) managing peroxisome activity and (3) developing a complex system of antioxidants. Therefore, *S. europaea* can be cultivated in retreated lake bed for animal feed or other uses.

## Acknowledgements

Authors acknowledge assistance and cooperation of Regional Department of Environment of West Azarbayjan, Research Deputy of Ministry of Science and Technology and Tarbiat Modares University, Iran.

## References

- Akhani, H. 1996. A new species and a synonym in Chenopodiaceae from Iran. *Sendtnera*, 3: 5-10.
- Amor, N. B., Jiménez, A., Megdiche, W., Lundqvist, M., Sevilla, F. and Abdelly, C., 2006. Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritima*. *Physiologia Plantarum*, 126(3): 446-457.
- Amor, N. B., Hamed, K. B., Debez, A., Grignon, C. and Abdelly, C., 2005. Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. *Plant Science*, 168(4): 889-899.
- Adam, A. L., Bestwick, C. S., Barna, B. and Mansfield, J. W., 1995. Enzymes regulating the accumulation of active oxygen species during the hypersensitive reaction of bean to *Pseudomonas syringae* pv. phaseolicola. *Planta*, 197(2), 240-249.
- Bestwick, C. S., Adam, A. L., Puri, N. and Mansfield, J. W., 2001. Characterisation of and changes to pro and anti-oxidant enzyme activities during the hypersensitive reaction in lettuce (*Lactuca sativa* L.). *Plant Science*, 161(3), 497-506.
- Bevan, M., Shufflebottom, D., Edwards, K., Jefferson, R. and Schuch, W., 1989. Tissue-and cell-specific activity of a phenylalanine ammonia-lyase promoter in transgenic plants. *The EMBO Journal*, 8(7).
- Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2): 248-254.
- Cakmak, I. and Horst, W. J., 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum*, 83(3): 463-468.
- De Vos, C., Schat, H., De Waal, M., Vooijs, R. and Ernst, W., 1991. Increased copper-induced damage of the root plasma membrane in copper tolerant silene cucubalus. *Plant Physiology*. 82: 523-528.
- Douce, R., Holtz, R. B. and Benson, A. A., 1973. Isolation and properties of the envelope of spinach chloroplasts. *Journal of Biological Chemistry*, 248(20), 7215-7222.
- Flowers, T. J. and Colmer, T. D., 2008. Salinity tolerance in halophytes. *New Phytologist*, 179(4): 945-963.
- Ghanati, F., 2002. Studies on boron tolerance mechanisms of suspension-cultured tobacco cells. Ph D Thesis, Shizouka University.
- Gholizadeh, A. and Kohnehrouz, B. B., 2010. Activation of phenylalanine ammonia lyase as a key component of the antioxidative system of salt-challenged maize leaves. *Brazilian Journal of Plant Physiology*, 22(4): 217-223.
- Giannopolitis, C.N. and Ries, S.K., 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant physiology*. 59: 309-314.
- Hare, P. D. and Cress, W. A., 1996. Tissue-specific accumulation of transcript encoding  $\Delta$  1-pyrroline-5-carboxylate reductase in *Arabidopsis thaliana*. *Plant growth regulation*, 19(3): 249-256.
- Hara, M., Oki, K., Hoshino, K. and Kuboi, T., 2003. Enhancement of anthocyanin biosynthesis by sugar in radish (*Raphanus sativus*) hypocotyl. *Plant Science*, 164(2), 259-265.
- IPCC. 2014. Summary for policymakers. Barros, D.J.,

- Dokken, KJ., Mach, MD., Mastrandrea, TE., Bilir, M., Chatterjee, KL., Ebi, YO., Estrada, RC., Genova, B., Girma, ES., Kissel, AN., Levy, S., MacCracken, PR., Mastrandrea and White LL., (eds.). *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge: 1–32.
- Iyengar, E. R. R. and Reddy, M. P., 1996. Photosynthesis in highly salt tolerant plants. *Handbook of photosynthesis.* Marshal Dekar, Baten Rose, USA, 909.
- Kato, M. and Shimizu, S., 1985. Chlorophyll metabolism in higher plants VI. Involvement of peroxidase in chlorophyll degradation. *Plant and Cell Physiology*, 26(7): 1291-1301.
- Khara, J. and Zare-Maivan, H., 2004. Comparative evaluation of halophyte and glycophyte mycorrhizae in protected islands of Urmia Lake. *Journal of Science.* Tehran University. 30(3):425- 440.
- Ksouri, R., Megdiche, W., Debez, A., Falleh, H., Grignon, C. and Abdelly, C., 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiology and Biochemistry*, 45(3): 244-249.
- Krizek, D. T., Mirecki, R. M. and Britz, S. J., 1997. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cucumber. *Physiologia Plantarum*. 103(1), 1-7.
- López-Pérez, L., del Carmen Martínez-Ballesta, M., Maurel, C. and Carvajal, M., 2009. Changes in plasma membrane lipids, aquaporins and proton pump of broccoli roots, as an adaptation mechanism to salinity. *Phytochemistry*, 70(4): 492-500.
- Lv, S., Jiang, P., Chen, X., Fan, P., Wang, X. and Li, Y., 2012. Multiple compartmentalization of sodium conferred salt tolerance in *Salicornia europaea*. *Plant Physiology and Biochemistry*, 51, 47-52.
- Meehl, G. A., Stocker, T. F., Collins, W. D., Friedlingstein, P., Gaye, A. T., Gregory, J. M. and Raper, S. C. B., 2007. Global Climate Projections. In ‘Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change’. (Eds S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor, HL Miller) pp. 747–845.
- Ozgun, R., Uzilday, B., Sekmen, A. H. and Turkan, I., 2013. Reactive oxygen species regulation and antioxidant defence in halophytes. *Functional Plant Biology*, 40(9), 832-847.
- Prasad, T. K., Anderson, M. D. and Stewart, C. R., 1995. Localization and characterization of peroxidases in the mitochondria of chilling-acclimated maize seedlings. *Plant Physiology*, 108(4), 1597-1605.
- Parida, A. K., Das, A. B., Sanada, Y. and Mohanty, P., 2004. Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*. *Aquatic Botany*, 80(2): 77-87.
- Pérez-López, U., Robredo, A., Lacuesta, M., Sgherri, C., Muñoz Rueda, A., Navari Izzo, F. and Mena-Petite, A., 2009. The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO<sub>2</sub>. *Physiologia Plantarum*, 135(1): 29-42.
- Rout, N. P. and Shaw, B. P., 2001. Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. *Plant Science*, 160(3), 415-423.
- Saleh, L. and Plieth, C. 2009. Fingerprinting antioxidative activities in plants. *Plant Methods*. 5: 2.
- Ushakova, S. A., Kovaleva, N. P., Gribovskaya, I. V., Dolgushev, V. A. and Tikhomirova, N. A., 2005. Effect of NaCl concentration on productivity and mineral composition of *Salicornia europaea* as a potential crop for utilization NaCl in LSS. *Advances in Space Research*, 36(7), 1349-1353.
- Wang, J. W., Zheng, L. P., Wu, J. Y. and Tan, R. X., 2006. Involvement of nitric oxide in oxidative burst, phenylalanine ammonia-lyase activation and

- Taxol production induced by low-energy ultrasound in *Taxus yunnanensis* cell suspension cultures. *Nitric Oxide*, 15(4): 351-358.
- Yamauchi, Y., Furutera, A., Seki, K., Toyoda, Y., Tanaka, K. and Sugimoto, Y., 2008. Malondialdehyde generated from peroxidized linolenic acid causes protein modification in heat-stressed plants. *Plant Physiology and Biochemistry*, 46(8): 786-793.
- Zare-Maivan, H., 2013. A conceptual analysis of retrogression of Urmia Lake and progression of plant species. *Journal of the Persian Gulf*, 4(13), 59-64.
- Zare-Maivan, H., Ebrahimzadeh, H. and F. Karimi., 2004. Arbuscular vesicular fungi in roots of *Artemisia sieberi* of Khartouran protected area, Semnan in relation to rhizosphere soil and biota characteristics. *Iranian Journal Biology*. 17(1): 70-79.
- Zare-Maivan, H., Noori, A. S. and Alaie, E., 2012. Changes in total phenol and flavonoid contents in *Chrysanthemum leucanthemum* under crude oil contamination. *Advances in Environmental Biology*. 6 (12):3057-3064.
- Mhamdi, A., Queval, G., Chaouch, S., Vanderauwera, S., Van Breusegem, F. and Noctor, G., 2010. Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. *Journal of Experimental Botany*. 15: 4197-4220.