Seasonal changes of antioxidant enzymes activities, water and osmolyte in four halophyte species

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Abstract

Seasonal changes in antioxidant enzymes activities, amount of water, ash, proline and soluble sugars were surveyed during this research in four halophyte species of Varamin County, Tehran, Iran: *Salsola turcomanica* Litwin, *Suaeda arcuata* Bunge, *Tamarix leptopetala* Bunge and *Cressa cretica* L. Plants were collected randomly from their natural habitats in spring and summer. Activity of these enzymes in response to environmental stresses such as salinity increased in shoots of most species from spring (May) to summer (Aug). In *T. leptopetala* catalase and ascorbate peroxidase activities were high while peroxidase activity was low in comparison to other species. Peroxidase activity in the other three species and catalase activity in all species were high. Thus, high activity of one antioxidant enzyme in stress condition may be accompanied by the low activity of other enzymes. According to this research, water amount in *S. arcuata* and *S. turcomanica* shoots were high and by the increase of salinity water potential reduced. The amount of ash in shoots and roots of all species increased from spring to summer but it was the most in *S. arcuata* and *S. turcomanica*. This could be the result of ion accumulation (especially Na^+ and Cl^-) in salinity condition in these two species. The amount of proline and especially soluble carbohydrates increased in shoots and roots of most species from May to Aug and it was the most in Tamarix and Suaeda.

Keywords: antioxidant enzyme Cressa cretica; Salsola turcomanica; Suaeda arcuata; Tamarix leptopetala; proline; soluble carbohydrates

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Introduction

Abiotic stresses such as salt causes ROS accumulation which in high concentrations is harmful for cells since they cause oxidative danger for lipids, membrane proteins, and nucleic acids (Smirnoff, 1993; Gomez et al., 1999 and Hernandez et al., 2001). Plants by the use of antioxidants (ascorbate, glutathione, α-tocopherol and carotenoids) and by enzymic detoxification using superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and

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ascorbate-glutathione cycle enzymes cope with oxidative stress. Activity and expression of genes coding detoxification enzymes are probably increased by ROS during abiotic stresses. Extreme expression of ROS-scavenging enzymes such as superoxide dismutase (Alscher et al., 2002), ascorbate peroxidase (Wang et al., 1999) and glutathione S-transferase/ glutathione peroxidase (Roxas et al., 1997, 2000) in transgenic plants are signs of tolerance to osmotic, heat, and oxidative stresses. Thus, detoxification by ROS is important in salt stress tolerance. Salt stress causes increase in H_2O_2 production which in concentrations less than lethal can act as a secondary peak to regulate expression of antioxidant genes in response to abiotic stress (Guan et al., 2000; Hernandez et al., 2001) and abscisic acid stress (Guan et al., 2000; Pei et al., 2000). Plants with higher levels of antioxidant (either because of their structure or induction by different factors), have a higher tolerance to environmental stress condition (Ghorbanli et al., 2004).

At present, interest in salinity and its relation with antioxidant enzymes in mangrove and other plants has increased (Jithesh et al., 2006). According to precise analyses on RNA expression records, genes of antioxidant enzymes responsible for response to decrease of salinity have become specialized in mangrove halophytes (Jithesh et al., 2006). There are some disagreements in different articles on the response of antioxidants in roots and leaves which are contradictory and need deep analysis (Ben Amor et al., 2005; Cavalcanti et al. 2007).

Most plants accumulate organic solutions in order to tolerate osmotic stress. These solutions consist of proline, betaines, polyols and soluble carbohydrate. Glycine, betaines and trehalose are solutions responsible for stabilizing 3D structure of protein and the fine organization of membranes. Mannitol acts as free radicals scavenger and stabilizes intercellular structures (membranes and proteins). These organic solutions are in fact redox potential cellular buffers during salinity stress and thus are known as osmoprotectants (Bohnert and Jesen, 1996; Chen and Murata, 2000). Complex genes act in the biosynthesis of osmoprotectants. They are extremely regulated in salinity stress condition and their concentration depends on osmotic stress tolerance (Zhu, 2002). It is seen that effect of compatible solutes are mostly on salt tolerance than on osmotic regulation.

Water amount control in salinity condition is part of tolerance process. Since the amounts of water and salts together determine the amounts of pressure and turgor and these amounts exactly conform to salinity degree. Water consumption in plants reduces under salinity. This could be related to the reduction of water potential around the roots and plant ability to absorb water (Levit, 1980; Chaudhury and Chaudhury, 1997). Reduction in water consumption can be due to increase of resistance in water flow paths in the plant through decrease in the number and thickness of vessels (Gadallah and Ramadan, 1997) or stomatal resistance increase and respiration decrease (Ashraf and Oleary, 1997).

Relative water content (RWC) and plant total water potential are some of the most important parameters that show plant water status. Relative water content measurement reflects the status of stomata and respiration. The potential to maintain RWC in any water potential reflects cell wall strength and its tolerance against stress (Khan and Rizvi, 1994). Khan et al. (2000a) reported that *Suaeda fruticosa* water potential reduces by the increase of salinity through which concentration water potential becomes more negative. They also reported that water potential of *Atriplex griffithii* var. stocksii leaves reduced linearly by the increase of salinity. Sairam et al. (2002) reported that RWC reduced in different wheat genotypes during different salinity condition.

Proline accumulation is one of the most dominant changes reported so far which is increased by salinity and drought stresses in plants. It seems that it is important in stress tolerance mechanisms. Although, it's exact role still remains controversial (Lutts et al., 1999). Proline is very soluble in water and it is without electrical charge in neutral pH. It acts as one of the most intensive accumulated osmolytes in stress condition. Proline is an osmoprotectant; moreover, it acts as an energy sink for redox potential regulation, hydroxyl radical scavenger, and a solution which protects macromolecules against denaturation and as a factor which reduces cellular acidity. In fact, proline acts as a main sink of energy and nitrogen during salinity

(Kavikishor et al., 1995). As seen in *Thellungiella halophyta* in comparison to *Arabidopsis thaliana*, proline accumulation increases and improves salinity tolerance (Kishor et al., 1995; Kant et al., 2006). Proline increase in stress condition was reported in *Amaranthus tricolor* (Bajji et al., 1998), *Suaeda nudiflora* (Joshi and Lyengar, 1987) and *Salicornia braciata* (Joshi, 1986). Proline accumulation occurs when leaf water potential reaches under the necessary threshold limit. Above this limit proline changes are small (Levitt, 1980). It was reported that proline accumulation usually starts when water stress is so severe that inhibits growth and causes stomatal closure (Marzouk et al., 1990).

Polyols and soluble carbohydrate are another group of osmoprotectants. Their accumulation in response to environmental stresses is related to osmotic regulation or cell membranes protection. Carbohydrates accumulate in a vast group of plants which grow in low levels of moisture and salinity. The amount of soluble carbohydrate in very sensitive genotypes is related to salinity and drought tolerance improvement. The amount of soluble carbohydrate may be a suitable mean in selection of salinity and drought tolerable species. These monosaccharides play the main role in primary response to salinity and drought. Accumulation of soluble carbohydrates (especially sucrose) in plants are usually reported as a response to salinity or drought stress; although, photosynthetic CO_2 fixation reduces (Kerepesi, 1998). There are many examples of soluble carbohydrates accumulation during salinity stress. Example are glucose and mannitol increase in olive leaves (Tattini et al., 1996) and *Atriplex halimus* (Bajji et al., 1998). In all these cases carbohydrates had an active role in osmotic adaptation process. Increase of sugars during salinity stress condition in plants was also reported by Strogonov (1964), Nieman and Clark (1973) and Doddema et al. (1986). Sugar alcohols like pinitol play an important role in intercellular osmoregulation and gathering free radicals. In *Mesembryanthemum crystallinum*, pinitol is accumulated in response to salinity stress. It was suggested that in *M. crystallinum* and a group of halophytes, main metabolic pathways containing osmolytes and ROS-scavenging enzymes may in fact be a common strategy for coping with oxidative stress which is caused by salt stress and other abiotic factors (Borland et al., 2006).

The objective of this research was to study antioxidant enzymes activities, water, ash, proline, and soluble carbohydrate in four dicotyledon halophyte plants including *Tamarix leptopetala* Bunge (Tamaricaceae family), *Cressa cretica* L. (Convolvulaceae family), *Salsola turcomanica* Litwin, and *Suaeda arcuata* Bunge (Chenopodiaceae family).

Materials and Methods

Plant materials: Samples of *Salsola turcomanica* Litwin, *Suaeda arcuata* Bunge, *Tamarix leptopetala* Bunge, and *Cressa cretica* L. were randomly harvested from their natural habitats, South of Varamin county, Tehran (which has a generally saline loam soil with high pH) based on four replications in spring (May) and summer (August) and immediately transferred to the lab. Some samples were immediately used to derivate protein extract and others were frozen by liquid nitrogen and kept in the freezer and were later thawed with distilled water.

Enzyme Extractions: Enzymes were extracted from fresh leaves (based on 4 replications for each species) in 0-4 °C. 1 g of fresh tissue was completely ground in a porcelain mortar containing 5 ml of 0.05 M Tris- HCl buffer placed in an ice bath for 30 min. The homogenous mixture was transferred to centrifuge tubes and remained still for 10 min then centrifuged in 4 °C for 20 min at 13000 rpm (Sudhakar et al., 2001). The upper solution was filtered and the enzyme extracts were used in peroxidase and catalase enzymes activities surveys. Since ascorbate peroxidase enzyme is very transient in the absence of ascorbate, 0.2 ml ascorbate was added to the protein extract (Benavides et al., 2000).

Peroxidase Enzyme Activity Measurements: to determine kinetic activity of peroxidase enzyme 100 mM Tris buffer (pH 7), 5 mM H_2O_2 and 10 mM pirogalol were used as indicator. In an ice bath, 2.5 ml of this indicator was mixed with 50 μl of the enzyme extract mixture. Absorbance changes curve at 425 nm were read by Spectrophotometer (Uvmini 1240, SHIMADZU Company). Enzyme activity was

calculated according to absorbance unit changes per min mg protein (Mishra and Kar, 1976).

Catalase Enzyme Activity Measurements: kinetic activity of catalase enzyme was determined using 2.5 ml of 50 mM Tris buffer (pH 7) and 0.3 ml of 3% (v/v) H_2O_2 as indicator. In an ice bath, the indicator was mixed with 60 µl of the enzyme extract mixture. Absorbance changes at 240 nm were read by Spectrophotometer. Enzyme activity was calculated according to absorbance unit changes per min g leaves fresh weight (Mishra and Kar, 1976).

Ascorbate Peroxidase Enzyme Activity Measurements: indicators used for determining kinetic activity of ascorbate peroxidase enzyme were: 2 ml of 0.05 M phosphate buffer (pH 7), 0.2 ml of 3% (v/v) H_2O_2, and 0.2 ml of 50 µl ascorbate. 25 µl of the enzyme extract mixture was added to the indicator immediately after they were mixed in an ice bath. Absorbance changes were read at 290 nm. Enzyme activity was calculated according to absorbance unit changes per min g leaves fresh weight (Nakano and Asada, 1981).

The exact fresh weight of roots and shoots were separately determined by digital balance. The samples were then transferred to oven and kept for 24 h at 75 °C in order to determine dry weight and tissue water percentage. Dried samples were ground and transferred into an electrical furnace at 550 °C for 4-5 h to determine ash percentage. Part of samples was also used to derivate protein extract and others were frozen by liquid nitrogen and kept in the freezer.

Proline Concentration Measurment (Battes et al., 1973): 0.5 g of leaves and roots fresh substance was separately ground in 10 ml 30% sulfuric acid to produce a completely homogenous extract. The extract was filtered by Whatman filter paper No.2. Then 2 ml of this filtered extract was added to 2 ml ninhydrin indicator and 2 ml pure acetic acid and kept at 100 °C for 1 h. This solution was cooled in an ice bath. 4 ml toluene was then added to this solution and the mixture was severely stirred for 15-20 sec. The upper phase which consisted of toluene and proline was separated from the water phase and its absorbance was determined by the spectrophotometer at 520 nm; toluene was used as control. Proline concentration (mg/g . FW) in each sample was determined according to absorbance and specific concentrations in standard curve.

Soluble carbohydrate Measurement (Kochert, 1978): 0.1 g of plant dry matter (separately ground roots and shoots) was placed in a large test tube followed by immediate addition of 10 ml 70% ethanol and kept in the refrigerator for one week until soluble sugars were free. Then 0.5 ml (shoots mixture) and 1 ml (roots mixture) of the upper solution was separated and their volume was set to 2 ml using distilled water. 1 ml of phenol 5% was added to this solution and after mixing 5 ml concentrated sulfuric acid was added by pressure. A yellow solution was produced which changed color. Since this reaction was extremely exothermic, the solution was kept at lab temperature for 30 min until it cooled down and produced its final color. The absorbance of the color at 485 nm which was related to soluble carbohydrate concentrations (mg/g.DW) was read by the spectrophotometer.

Statistical analyses

Experiments were conducted in two factorial completely randomized block design based on 4 replications. Analysis of variance (ANOVA) was done using SPSS (ver. 14) software and means were compared with Duncan's Multiple Range Test. Graphs were drawn by Excel software.

Results

Peroxidase enzyme activity in shoots of all four species increased from May to August, this increase was significant (α=5%) except in *T. leptopetala* (Table 1). *S. turcomanica* shoots had the highest enzyme activity (in Aug). The activity of this enzyme increased in roots of all species from May to Aug, this increase was significant (α=5%) in *T. leptopetala* and *S. turcomanica* (Table 1). *S. arcuata* roots had the highest enzyme activity (in Aug).

Table 1
Mean of peroxidase enzyme activity in four halophytes (OD/g.FW.min)

Species	shoot				Pr > F	root				Pr > F
	May	Aug	Decrease	Increase		May	Aug	Decrease	Increase	
T. leptopetala	3.51±0.51	4.10±0.26		+	ns	2.54±0.19	4.00±0.41		+	*
C. cretica	8.06±1.26	12.89±1.05		+	*	15.31±1.02	16.17±1.49		+	ns
S. turcomanica	10.82±1.36	15.74±0.60		+	*	16.82±0.32	18.83±0.69		+	*
S. arcuata	12.06±1.05	14.65±1.29		+	*	18.37±0.84	19.63±1.53		+	ns

^{ns} and * show not significant and significant at 0.05 levels, respectively.

Table 2
Mean of catalase enzyme activity in in four halophytes (OD/g.FW.min)

Species	shoot				Pr > F	root				**Pr > F**
	May	Aug	Decrease	Increase		May	Aug	Decrease	Increase	
T. leptopetala	3.93±0.66	5.83±0.64		+	*	6.00±0.73	6.89±0.23		+	*
C. cretica	4.39±0.84	5.88±0.67		+	*	4.03±0.36	5.06±0.37		+	*
S. turcomanica	3.31±1.12	5.07±0.37		+	*	4.05±0.41	5.26±0.46		+	*
S. arcuata	5.00±0.42	5.36±0.72		+	ns	4.77±1.15	5.18±0.61		+	ns

^{ns} and * show not significant and significant at 0.05 levels, respectively.

Catalase enzyme activity in shoots of all four species increased from May to Aug, this increase was significant at 0.05 levels except in *S. arcuata* (Table 2). The highest enzyme activity was observed in *C. cretica* shoots (in Aug). This enzyme activity increased in roots of all species from May to Aug, the increase was not significant *S. arcuata* (Table 2) and *T. leptopetala* roots had the highest enzyme activity (in Aug).

Ascorbate peroxidase enzyme activity in shoots of all four species increased from May to Aug, this increase was significant at 0.05 levels except in *S. turcomanica* (Table 3). The highest enzyme activity was in *T. leptopetala* shoots (in Aug). Enzyme activity increased in roots of all species from May to Aug although the increase was not significant in *S. turcomanica* and *S. arcuata* (Table 3). The highest enzyme activity was in *T. leptopetala* roots (in Aug).

From May to Aug water loss was seen in shoots of all four species (Table 4). In *S. turcomanica* and *S. arcuata* (having the highest water percentage) shoots the reduction was not significant at 0.05 levels. Water percentage in *T. leptopetala* and *C. cretica* shoots reduced significantly (α=5%) and this reduction was higher

Table 2
Mean of ascorbate peroxidase enzyme activity in four halophytes (OD/g.FW.min)

Species	shoot					root				
	May	Aug	Decrease	Increase	Pr > F	May	Aug	Decrease	Increase	Pr > F
T. leptopetala	9.23±0.82	10.07±0.15		+	*	5.16± 0.64	6.21± 0.52		+	*
C. cretica	1.67±0.24	2.54±0.33		+	*	3.92 ±0.51	4.96± 0.31		+	*
S. turcomanica	5.4±0.59	5.77±0.67		+	ns	5.34± 0.27	5.55± 0.16		+	ns
S. arcuata	1.87±0.48	3.19±0.25		+	*	4.29± 0.41	4.34± 0.39		+	ns

ns and * show not significant and significant at 0.05 levels, respectively.

Table 4
Mean of water percentage in shoots and roots

Species	shoot					root				
	May	Aug	Decrease	Increase	Pr > F	May	Aug	Decrease	Increase	Pr > F
T. leptopetala	71.12±1.93	51.18±3.58	+		*	60.94±1.2	49.17±2.38	+		*
C. cretica	66.12±0.63	58.16±5.16	+		*	59.64±5.59	64.42±0.38		+	*
S. turcomanica	82.62±1.44	79.3±0.87	+		ns	54.58±1.59	41.25±1.2	+		*
S. arcuata	88.16±0.58	85.11±0.42	+		ns	62.91±1.6	56.57±4.12	+		*

in *T. leptopetala* (Table 4).Water percentage was significantly reduced during this time in roots of all species (α=5%) except for *C. cretica* which had a significant increase at 0.05 levels (Table 4).

Ash percentage in shoots of all four species had a significant increase (α=5%) from May to Aug. Ash percentage in *S. turcomanica* and *S. arcuata* was much higher in comparison to *T. leptopetala* and *C. cretica* which could be due to ion accumulation (Table 5). Ash percentage in *S. turcomanica*, *T.* leptopetala, and *C. cretica* roots increased from May to Aug. This increase was significant in *T. leptopetala* and *C. cretica* (α=5%). A slight and not significant increase was also seen in *S. arcuata* (Table 5).

Proline amount increased in shoots of all species from May to Aug. This increase was significant at 0.05 levels in *S. arcuata* and *T. leptopetala* while it was not significant in *C. cretica* and *S. turcomanica* (Table 6). Although it should be mentioned that the annual precipitation during the year when this study was being conducted, amount of rain was high in Varamin County causing reduction in soil salinity which could affect

Table 5
Mean of ash percentage in shoots and roots

Species	shoot					root				
	May	Aug	Decrease	Increase	Pr > F	May	Aug	Decrease	Increase	Pr > F
T. leptopetala	17.53±0.63	23.34±1.08		+	*	10.17±1.99	15.37±0.64	+		*
C. cretica	24.30±4.75	45.55±0.66		+	*	11.24±0.84	13.25±0.96	+		*
S. turcomanica	40.66±1.49	48.24±1.44		+	*	7.91±0.38	8.68±0.93	+		ns
S. arcuata	40.34±1.55	48.98±1.05		+	*	8.95±0.33	8.66±0.81		+	ns

Table 6
Mean of proline content in shoots and roots (mg/g.Fw)

Species	shoot					root				
	May	Aug	Decrease	Increase	Pr > F	May	Aug	Decrease	Increase	Pr > F
T. leptopetala	29.82±9.67	45.74±2.78		+	*	31.07±12.73	39.74±9.66		+	ns
C. cretica	0.69±0.37	1.08±0.26		+	ns	0.72±0.31	0.91±0.22		+	ns
S. turcomanica	1.79±1.59	2.80±0.83		+	ns	4.93±4.62	2.96±0.85	+		ns
S. arcuata	26.73±9.63	37.94±13.98		+	*	40.1±5.31	42.27±10.9		+	ns

proline content. Proline amount increased slightly but not significantly from May to Aug in *S. arcuata*, *T. leptopetala*, and *C. cretica* (Table 6).

Soluble carbohydrate levels in shoots had a dramatic increase from May to Aug which was significant at 0.05 levels. In *T. leptopetala* and *S. arcuata*. *T. leptopetala* shoots the highest amounts of soluble carbohydrates were observed in Aug (Table 7). The amount of soluble carbohydrates in roots increased dramatically in all species from May to Aug (α=5%). *T. leptopetala* roots had the highest soluble carbohydrate levels (in Aug) while *S. arcuata* roots contained the lowest level of soluble carbohydrate (in May) (Table 7).

Discussion

In this research, results showed that with the increase of salinity stress from spring to summer, activities of peroxidase, catalase and ascorbate peroxidase in shoots and roots of all four species had a dramatic increase in most cases.

Table 3
Mean of soluble sugar content in shoots and roots (mg/g.DW)

Species	shoot					root				
	May	Aug	Decrease	Increase	Pr > F	May	Aug	Decrease	Increase	Pr > F
T. leptopetala	204.01±17.90	248.18±13.50		+	*	259.54±9.22	322.00±7.17		+	*
C. cretica	166.67±8.79	178.21±23.27		+	ns	254.93±10.65	312.32±4.77		+	*
S. turcomanica	88.44±6.03	111.33±16.22		+	ns	239.25±7.46	268.42±33.05		+	*
S. arcuata	97.35±22.34	199.46±29.51		+	*	136.85±23.25	182.84±14.75		+	*

Catalase had a higher activity in all species especially, *T. leptopetala* and *C. cretica*; while, peroxidase activity in *T. leptopetala* was much lower than other species. Ascorbate peroxidase activity in shoots of *T. leptopetala* was much higher than other species although it was almost active in roots of all species. Thus, the lower activity of one enzyme in a plant is accompanied by the higher activity of other enzymes. When plants are under environmental stresses such as salinity, drought, high temperature, air pollution, heavy metals, and varied soil pH the balance between ROS production and antioxidant systems is disrupted. Accumulation of ROS in cells causes oxidative damages in plants. Plants which have higher amounts of antioxidants (whether because of their structure or induction of different factors), show higher tolerance against environmental stresses (Ghorbanli et al., 2004). In order to minimize or prevent damages caused by active oxygens, plants produce a variety of antioxidant enzymes, antioxidant molecules with low molecular weight, and specific secondary metabolites which can eliminate active oxygen species. In other words, they chemically decrease and neutralize these destructive oxidants (Srivastava, 1999; Noctor and Foyer, 1998). Quan et al. (2008) believe that in *Thllungiella salsuginea*, TsMT3 gene probably has a complex role in tolerance phenomenon and metal elements homeostasis and affect active oxygen species content.

Increase of peroxidase enzyme activity was seen in *Suaeda nudiflora* callus under high concentrations of salt (Cherian and Reddy, 2003). Peroxidase enzyme activity increase in shoots and roots of *Avicennia marina* was also seen under controlled salt condition (Cherian et al., 1999). Temporary increase of peroxidase activity in halophytes such as *Crithmum maritimum* in different concentrations of NaCl was also reported (Ben Amor et al., 2005).

Catalase enzyme along with superoxide dismutase and peroxidase form a strong defensive system against superoxide and hydrogen peroxide radicals (especially fatty acids β-oxidation) (Savoure et al., 1999). Ascorbate peroxidase is one of the key compounds in cytosolic and chloroplastic H_2O_2 elimination system in higher plants. The presence of ascorbate peroxidase has been known in chloroplasts (lacking catalase) and cytosol. Recently, its presence was also reported in glyoxysomes and peroxysomes which show the importance of this enzyme in H_2O_2 elimination even in such organelles that are rich in catalase. The high tendency of this enzyme to H_2O_2 and the ability to increase its activity in response to stresses are some of the important reasons that ascorbate peroxidase is a key enzyme in active oxygen species detoxification processes (May et al., 1998). Ascorbate peroxidase enzyme activity increase after salt stress was reported in cotton (Gosset et al., 1994), pea (Hernandez et al., 2000) and rice (Lee et al., 2001). In pepper increase of

peroxidase activity was related to increase of other physiological parameters such as increase of ethylene production, increase of color changes in fruits from green to red, decrease of respiration, and skin thickness (Bilesc et al., 1993).

Results from water content measurement showed that shoots of *S. turcomanica* and *S. arcuata* had a higher amount of water than other species. This could be due to succulence and salt accumulation. Water amount in roots did not differ among species. On the other hand, ash amount in respect to dry weight was higher in these two species which could be the result of ion accumulation in their vacuoles, unlike salt excretor species such as *T. leptopetala* and *C. cretica*. In salt excretor plants, a considerable amount of absorbed ions are excreted from salt glands or bladders by secretory pumps or antiporters (Hill and Hill, 1973; Shimony et al., 1973; Weiche and Breckle, 1990). Thus, ion accumulation and ash amount is usually low in these plants. Ash measurement in roots also showed that ash amount in roots of perennial plants such as *T. leptopetala* is more than other species. Osmoregulation is done in some halophytes by ion accumulation in their cells; this mechanism is shown by ash measurement (Elzam and Epstein, 1969; Eshel and Waisel, 1979; Naidoo and Rughunanan, 1990).

In this research, increase of salinity from spring to summer resulted in ash amount increase according to dry weight in leaves and roots of all four species which conforms to Khan et al. (2000 a,b) results in *Suaeda fruticosa* and *Atriplex griffithi*. It is possible that high amount of ash in Chenopodiaseae species could be the result of salt accumulation in them.

From spring (May) to summer (Aug) when salinity increased, proline amount increased in shoots and roots of all four species and this increase was significant in most of the samples. It seems that plants use proline for adaptation against salinity. Results gained in this research conform to results of Joshi (1986) on *Salicornia braciata*, Joshi and Lyengar (1987) on *Suaeda nudiflora*, Bajji et al. (1998) on *Amaranthus tricolor*, and Sairam et al. (2002) on wheat genotypes. As shown in a comparison between *Thellungiella halophyta* and *Arabidopsis thaliana* (Kishor et al., 1995; Kant et al., 2006), increase in proline accumulation improves salinity tolerance. Proline increase is probably due to the ability of some plants in organic and inorganic compounds accumulation in cytoplasm in order to reduce water potential and change osmotic gradient to absorb water (Rosa and Maiti, 1995). There is a threshold for proline accumulation in plants. In other words, proline accumulation does not occur unless salt concentration and consequently monovalent cations (especially Na^+) concentrations reaches an appropriate limit (Morgan, 1984).

The results showed that carbohydrate amount increased by increase of salinity from spring to summer, as also seen in alfalfa (Irigoyen et al., 1992), olive (Tattini et al., 1996), *Atriplex halimus* (Bajji et al., 1998), and wheat (Sairam et al., 2002). Increase of soluble carbohydrates in plants during salinity stress was also reported by Strogonov (1964), Nieman and Clark (1973), and Doddema et al. (1986). Soluble carbohydrate increase in shoots during stress condition is in fact an important response to water deficiency and probably a result of starch hydrolysis during water deficiency stress in tissues and soil water potential decrease (Jones and Qualset, 1984). Increase of soluble carbohydrate in roots could be due to starch conversion to soluble sugars, reduction in their consumption or reduction in their transmission throughout the phloem (Irigoyen et al., 1992). Plants use part of their carbon resources to produce such osmolytes used for adaptation instead of using them for growth and production, which could affect their growth. Thus, in these plants part of osmoregulation during salinity is done by compatible solutes. Although, since this kind of adaptation uses nutrients such as Na^+ and Cl^- , it is less costly than osmoregulation. For instance, in order to use 1 M of NaCl as an compatible factor, 4 M ATP is consumed in roots and 7 M in leaf cells while this is much higher for organic compounds such as mannitol (34), raline (41), glycine betaine (50) and sucrose (52) (Greenway and Munns, 1980).

Conclusion

According to this research, *S. arcuata* and *T. leptopetala* had better tolerance mechanisms under salt desert condition. These species could be used for revitalizing environment in saline

regions. Although, in order to find species with more tolerance to stress condition more research needs to be conducted on various species, other compatible solutes, and different ways of tolerance to salinity.

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