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Response of some physiological and biochemical tarts of Atriplex to Saltwater stress.

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ABSTRACT

Water and soil salinity, considered one of the major abiotic stresses reducing agricultural productivity, affects in bothirrigated and non-irrigated areas of the world; the need to produce salt-tolerant crops is evident. Therefore, Atriplex plant was grown under four levels of salt water regime atdirab, south of Riyadh, faculty of agriculture, King Saud University, Saudi Arabia during the two successive seasons of 2012 and 2013. In general, the analysis of variance of data showed that, the four salt water levels had high significant effect on the all ions accumulation Ca, Na and K either in root or shoot and the ratio of shoot to root (BAF). Regarding, the pigments, the best salt water stress treatment (400 ppm), which gave the highest values 1.710, 1.181, 0.529 and 8.958mg/g dw for total chlorophyll, chlorophyll A, chlorophyll B and carotenoids, respectively. It could be concluded that, the studied Atrilex plant has varied significantly and/or highly significantly between all studied physiological and biochemical traits and each other's due to applying the salt water irrigation treatments.The maximum effect on reducing most of some ions, pigments, proline, vitamins and enzymes accumulation was obtained by the application of 1600 ppm (T_4). It could be concluded that, there is highly association between increase the concentration of salt in irrigation water and the accumulation of proline as well ascorbic acid in plant.

***Key words*:** Atriplexsp, salt stress, ions, pigments, proline, enzymes and vitamins.

Introduction

Atriplex plant *(AtriplexSpp)* is the most widely grown. The genus Atriplex is distributed nearly worldwide from subtropical to temperate and may be to subarctic regions. Many species are halophytes and are adapted to dry environments with salty soils (Kadereit*et al*., 2010). Many species are edible. However, the favored species for human consumption is Garden Orache (*A. hortensis*). Meat from sheep which have grazed on saltbush has surprisingly high levels of vitamin E, is leaner and more hydrated than regular lamb and has consumer appeal equal to grain-fed lamb. The vitamin E levels could have animal health benefits while extending the shelf-life and maintaining the fresh red colour of saltbush lamb. This effect has been demonstrated for Old Man Saltbush (*A. nummularia*) and River Saltbush (*A. ammicola*). For reasons unknown, sheep seem to prefer the more fibrous, less nutritious River Saltbush (Norman *et al*., 2004 and PearceandJacob, 2004).

In response to salinity, plants can be divided into halophytes and glycophytes. Halophytes accumulate a high amount of ions in their organisms while glycophytes exclude salt from their tissues. Halophytes have been classified as facultative and obligatory halophytes. Obligatory halophytesare not able to grow in non-saline conditions whereas facultative. Halophytes prefer non saline environment but also can grow and survive in saline areas (Flowers, 1988). Water and soil salinity directly affects plant growth and development in vegetative growth prior to reproductive stage (Allakhverdiev*et al*., 2000; Chinnusamy*et al*., 2005; Ashraf, 2009). Several studies indicated that Atriplex plant shows high response to salt water supply and stress. For example, *Atriplexvesicaria* produced high yield in 700 mMNaCl (Black, 1960), while *Atriplexnummularia* died at 600mM NaCl (Ashby and Beadle, 1957).

The magnitude of growth and metabolism reduction from salt stress in plant depends upon the growth stage at which the salt stress and water deficiency.Agricultural productivity is severely affected by water and/or soil salinity because salt levels that are harmful to plant growth affect large terrestrial areas of the world. The damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. It is estimated that 20% of the irrigated land in the world is presently affected by salinity (Yeo, 1999 and Flowers, 2004).

Cells respond to salt stress by osmolyte synthesis and by increase of K^+ uptake and Na^+ efflux at the plasma membrane and Na^+ accumulation at the vacuole. By increasing salinity the amount of solute in the growth or soil medium will increase and cause water deficit, because of a decrease in water potential in the growth medium. When excess Na^+ and Cl^- have been taken up an ion-specific stress develops resulting from low K^+/Na^+ ratios. The sensitivity of cytosolic enzymes to salt is similar in both glycophytes and halophytes, indicating that the maintenance of a high cytosolic K^+ /Na+ concentration ratio is a key requirement for plant growth in soils with a high concentration of salt (Glenn, 1999) Persistent salinity raises the amount of Na^+ and Cl^- in the plant to

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concentrations that inhibit plant growth (Yamaguchi and Blumwald, 2005). High salt concentrations (greater than 0.4 M NaCl) change the hydrophobic and electrostatic balance of brotein molecules and inhibit proper activity of most enzymes (Wyn and Pollard, 1983). However, toxic effects on cells occur at much lower concentrations (about 0.1 M) pointing to specific targets of salt toxicity (Serrano, 1996).

The aim of the present investigation was to share in solving the problem of the low productivity of Atriplex under salt stress conditions by testing the content and quality of the some widely of Atriplex plant and determining the proper salt stress levels to obtain maximum growth. In addition, this investigation may help plant breeder to get information on the important plant accumulation characters influencing Atriplex quality.

Materials and Methods

The present investigation was carried out during the two successive seasons of 2012 and 2013 at the experimental and agricultural research station, where the soil is sandy loam by ratio 1:1, dirab (South of Riyadh with 24 42º north, 44 46º east longitude and 600 m. up from the earth's surface), faculty of agriculture, King Saud University, Saudi Arabia. The soil mechanical and chemical analyses in addition the chemical irrigation water characters were determined according to Richards *et al*., (1964),Rhoades (1982)Cassel and Nielsen. (1986)and Gee and Bauder (1986),the data presented in Table 1.

Table 1: physic-chemical properties of the irrigation water and experimental soil.

Item		Irrigation water	Experimental soil
PH		7.10	8.40
Ec(dS/m)		0.01	0.1
Dissolved Cations mill iequivalent\ l	Ca2+	3.6	35.0
	Mg2+	0,5	19.5
	Na+	3.2	34.3
	K+	0.114	2
Dissolved Anions mill iequivalent\ l	CO_3^{2-}	0	0.125
	HCO_3^-	1	3.5
	Cl^-	4.7	56.3
	SO_4^{2-}	1.71	21.9
Saturation point %		--	27.3
Field capacity % (at 1 bar)		--	13.6
Milting point (at 15 bar)		--	6.8

Soil texture: sandy loam 1:1.

Seeds were cultivated in greenhouse to produce plantlets then, after 3 weeks. When the plantlets became 15 cm length and have three leaflets distributed to 30 cm plastic pots and moved out of the greenhouse and salt water irrigation treatments started on the first of January, 2013 for the duration of plant life till harvest date (the first of June 2013).

To investigate the effect of salt stress and evaluate the ions, green and organic pigments, proline, vitamins and enzymes accumulation of local varieties of Atrilex (*Atriplexhalimus*). The experiment included four treatments which were the combination of four levels of salt stress and Atriplex plant as follows:

A- Salt stress regime:

1- 50 ppm (T_1) 2- 400 ppm (T_2)
3- 800 ppm (T_3) 4- 1600 ppm (T_4)

B- Plant materal:

Atriplex plant (*Atriplexhalimus)* that,belong to Chenopodiaceae Family.

Studied characters:

Samples of ten plants randomly taken to determine the following physiological and biochemical attributes:

A. Ions accumulation characters:

1- Ca in shoot (mg/g).	2- Ca in root (mg/g).	3- BAF of Ca	4- Ca in soil (mg/g).
5- Na in shoot (mg/g).	6- Na in root (mg/g).	7- BAF of Na	8- Na in soil (mg/g).
9- K in shoot (mg/g).	10- K in root (mg/g).	11- BAF of K	12- K in soil (mg/g).

Bioaccumulation factor (BAF) was calculated using the formula outlined by Shin *et al*., 2002

$$BAF = \frac{ion\ accumulation\ in\ shoot}{ion\ accumulation\ in\ root}$$

B- Green and organic pigments accumulation.

1. Total Chlorophyll (mg/g fw).
2. Chlorophyll A (mg/g fw).
3. Chlorophyll B (mg/g fw)
4. Carotenoids (mg/gfw).

The pervious green and organic pigments concentration had been determined in mg/g fresh weight (FW) of sample as follow. The extraction of leaf pigments was performed with 80% acetone, and the absorbance at 663 and 645 nm was measured with an Ultrospec 2100 pro spectrophotometer (Amersham Biosciences). Total chlorophyll content, chlorophyll a, chlorophyll b, and total carotenoid quantities were calculated according to the method of Arnon (1949) using the following formula:

Total Chl (a and b) (mg/l) = 20.2 (A_{645}) + 8.02 (A_{663})
Chl a (mg/l) = 12.7 (A_{663}) - 2.69 (A_{645})
Chl b (mg/l) = 22.9 (A_{645}) - 4.68 (A_{663})
Carotenoids (mg/l) = A_{480} x 200

C- Proline, vitamins and enzymes accumulation.

1. Proline (µmol g-1 fw)
2. Ascorbate (unit)
3. Peroxidase (U g-1fw)
4. Catalase (unit)
5. Superoxide dismutase (Ug−1 fw)

Determination of free proline:

Extraction and estimation of free proline were conducted according to the procedures described by Bates *et al*., (1973). Plant tissues (0.5 g) were homogenized with 5 ml of 3% sulfosalicylic acid and the homogenates were centrifuged at 3000×*g* for 20 min. In a test tube, 1 ml of the supernatant was mixed with 1 ml acid ninhydrin reagent and1 ml of glacial acetic acid and incubated in 100°C in water bath for 1 h, and then the absorbance at 520 nm was determined. Free proline content insampleis estimated by referring to a standard curve made from known concentrations of prolineand the results were expressed as µmolproline g^{-1} FW.

Determination of Ascorbate activity:

Ascorbate peroxidase (APX EC 1.11.1.11) activity was measured according to the methods of Wang *et al*.(1991)The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM hydrogen peroxide, and 20 µl of enzymeextract in a total volume of 1 ml. The concentrationof oxidized ascorbate was calculated by the decreasein absorbance at 290 nm. The absorption coefficientwas 2.8 mM^{-1} cm^{-1}. One unit of APX was defined as 1mmol ml^{-1}ascorbate oxidized min^{-1}.

Extraction and analysis of antioxidant enzymes:

Leaf samples (0.5 g fresh weight, FW) were homogenized in 3 ml extraction buffer (0.1 M potassium phosphate buffer (pH 7.0), 1mMEDTA, 0.05% Triton X-100) in a pre-chilled pestle and mortar, centrifuged at 15,000 g for 20 min at 4°C. The supernatant was used for the estimation antioxidant enzyme activities.

Peroxidase activity:

Peroxidase (POX) activity was estimated by the method of Sakharov andArdilla(1999). Peroxidase enzyme activity was determinedby the oxidation of guaiacol in the presence of H_2O_2. Theincrease in absorbance was recorded at 470nm for 1min witha spectrophotometer. The reaction mixture contained 50 µlof 28 mMguaiacol, 900 µl of 50 mM potassiumphosphate buffer (pH 6.0), 50 µl of 5mM H_2O_2, and 10 µlof crude extract. POX activity of the extract was expressed as the POX Ug−1 FW

Catalase activity:

Catalase (CAT EC 1.11.1.6) activity was measuredspectrophotometricallyaccording to the method of Aebi(1983). The disappearanceof H_2O_2 at 240 nm in a reaction mixture containing 50 m M sodium phosphate

buffer (pH 7.0),20 mM H_2O_2 and 5 µl of supernatant.The decrease in the absorption was followed for 1min at 240 nm, and 1 m mol H_2O_2oxidized ml^{-1} min^{-1} was defined as 1 U of CAT activity.

Superoxide dismutase activity:

Superoxide dismutase activity (SOD EC 1.15.1.1) activity was estimated by the ability of this enzyme to inhibit the photochemical reduction of nitrobluetetrazolium salt (NBT). The SOD activity was measured at 560 nm according to the method of Beyer and Fridovich (1987) and the assays were carried out at 25°C. The final assay volume of 3 ml contained 50 mMTris-HCl buffer (pH 7.8), 33 µM NBT, 10 mM L-methionine, 0.66 mM disodium EDTA, 0.0033 mM riboflavin and 50 µl of supernatant. Reaction was started under fluorescent light for 15 minutes by adding 10 µl of riboflavin solution. One unit of SOD was defined as the amount of enzyme activity that inhibited the photo reduction of NBT to blue formazan by 50%.The SOD activity of each extract was expressed as Ug−1 FW.

Statistical procedures:

A Randomize Complete Block Design (RCBD) with three replications was used in this investigation. The obtained data were subjected to regular statistical analysis of variance as well as differences between means of salt water irrigation were tested for significant against LSD values at 5 and 1% levels of probability according to (Snedecore and Cochran 1990). The MSTAT computerized package program was subjected to the regular statistical analysis of variance (Nissen*et al*., 1985).

Results and Discussion

Effect of salt stress levels on:

I- Ions accumulation characters:

Regarding to some of elements accumulation characters in shoot, root, soil and bioaccumulation factor (BAF) under four levels of salt stress, the analysis of variance of data showed that, the four salt water levels had high significant effect on the all ions accumulation Ca, Na and K either in root or shoot and the ratio of shoot to root (BAF) in addition to the ion accumulation in the soil (Tables 2, 3 and 4).

Table 2: Effect of salt stress treatments on Ca(mg/g) element accumulation.

Character Treatment	Shoot	Root	BAF	Soil
T_1	14.083	36.417	0.590	85.323
T_2	13.500	8.400	0.280	77.417
T_3	11.623	40.717	0.160	331.183
T_4	6.733	43.700	0.317	158.943
F test	**	**	**	**
LSD at 5%	0.120	0.367	0.101	0.320
at 1%	0.187	0.571	0.142	0.498

T1:50 ppm, T2:400 ppm, T3:800 ppm, T4:1600 ppm, and **: significant at 1% levels of probability

Regarding to the four salt water treatments mean, the highest Ca accumulation in shoot (14.083 mg/**g**) was obtained from the first salt water irrigation (50 ppm), while the lowest Ca accumulation in shoot (6.733 mg/**g**) was recorded by the fourth of water salt irrigation.The highest Ca accumulation in root (43.700 mg/**g**) was obtained from the fourth salt water irrigation (1600 ppm) with highly significant with the other treatments, while the lowest Ca accumulation in root (8.400 mg/**g**) was recorded by the second of water salt irrigation. The Bioaccumulation factor (BAF) is an indicator for the ratio of ion accumulation in shoot/root which exhibited the third treatment gave the highest ratio and followed by the fourth one, in the main while the second water salt treatment ranked the lowest ratio. On the other side the Ca element accumulation in the soil differ from a salt water regime to another one; anywhere the third treatment give the highest ion accumulation (331.183 mg/**g**) at the same time as the second one gave the lowest accumulation of Ca (77.417 mg/**g**) in the soil. We can notice that the treatment which gave the highest ratio of shoot/root accumulation is that gave the highest Ca accumulation in the soil.

As mention before that, the analysis of variance of data showed highly significant effect of the four salt water regimes on Na accumulation either in root or shoot and the ratio of shoot/root (BAF) in addition to the ion accumulation in the soil (Table 3).

Salt water treatment number two gave the highest value (82.167mg/**g**) of Na accumulation in the shoot parts of Atrilexplant; as well the same previous salt water treatment gave the highest value (14.327 mg/**g**) of Na

element accumulation in the root. The highest Bioaccumulation factor (BAF) of Na (162.42 mg/**g**) obtained from the first salt water regime while the lowest value (0.157 mg/**g**) obtained from the last treatment (1600 ppm) that reflect obviously the equally effect of salt water stress number four on Na accumulation in both shoot and root. Regarding Na accumulation in the soil, data shown that the treatment four which gave the lowest BAF that as well gave the highest value (128.107 mg/**g**) of Na accumulation in the soil.

Table 3: Effect of salt stress treatments on Na (mg/g) element accumulation.

Character Treatment	Shoot	Root	BAF	Soil
T_1	38.123	9.537	162.42	0.297
T_2	82.167	14.327	73.96	1.307
T_3	14.96	1.667	10.27	1.62
T_4	10.63	9.727	0.157	128.107
F test	**	**	**	**
LSD at 5%	0.295	0.097	61.311	0.025
at 1%	0.459	0.151	95.460	0.039

T1:50 ppm, T2:400 ppm, T3:800 ppm, T4:1600 ppm and **: significant at 1% levels of probability

Concerning the K element accumulation, the statistical analysis of data showed highly significant effect of the four salt water treatments on K accumulation for all studied characters showed in Table 4.The K element accumulation in shoot parts of Atriplex differs from salt water treatment to another between 18.570 mg/**g** (second treatment) to 6.407 mg/**g** (fourth treatment). In the same time, the first salt water regime highly surpassed the all other treatments (which was convergent for) and gave 6.197 mg/**g** of K accumulation in root. The values of bioaccumulation factor (BAF) varied for the all salt water treatment, where the highest ratio (43.053 mg/**g**) were obtained from the second treatment of salt stress, and the lowest ratio (27.307 mg/**g**) of shoot/root K accumulation were obtained from the third treatment. The all values of K accumulation in the soil by the all four salt water treatments were convergent (from 0.543 to 0.337 mg/**g**) but with highly significant differences.

Table 4: Effect of salt stress treatments on K (mg/g) element accumulation.

Character Treatment	Shoot	Root	BAF	Soil
T_1	14.003	6.197	39.367	0.513
T_2	18.570	4.837	43.053	0.543
T_3	8.543	4.003	27.307	0.460
T_4	6.407	4.007	30.937	0.337
F test	**	**	**	**
LSD at 5%	0.113	0.055	0.630	0.001
at 1%	0.176	0.085	0.981	0.002

T1:50 ppm, T2:400 ppm, T3:800 ppm, T4:1600 ppm and **: significant at 1% levels of probability

The distribution of homeostasis occurs at both the cellular and the whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death. To achieve salt tolerance, three interconnected aspects of plant activities are important. First, damage must be prevented or alleviated. Second, homeostatic conditions must be re-established in the new, stressful environment. Third, growth must resume, although at a reduced rate (Borsani 2003 and Zhu *et al*., 2005). Hamdia (2005) show that salinity stress affected nitrogen content and some minerals (Na+, K+, and Ca++) in plants. De Pascale *et al*., (2005) examine the influence of residual soil salinity on mineral composition of cauliflower and broccoli. The same result of salinity experimental on water relations and solute composition of crop halophyte had been done by Koyro (2006).

2- Green and organic pigments accumulation:

Data in Table (5) show the effect of the four salt water levels on green pigments *i.e*., total chlorophyll, chlorophyll A, chlorophyll B and organic pigments *i.e*., carotenoids accumulation. The Pigment degradation appears obviously when the concentration of salt in irrigation water increased. The analysis of variance of data showed that, the four salt water levels had highly significant effect on the all previous studied characters. Focusing in the data, we can give us a sharp idea about the best salt water stress treatment (second one) which gave the highest values 1.710, 1.181, 0.529 and 8.958 mg/**g**fwfor total chlorophyll, chlorophyll A, chlorophyll B and carotenoids, respectively. On the other hand the lowest values (0.489 and 0.275 mg/**g**fw) of total chlorophyll, chlorophyll A accumulation, respectively were obtained by the fourth salt water stress, while the third treatment gave the lowest values (0.149 and 5.940 mg/**g**fw) for chlorophyll B and carotenoids, respectively. Therefore, it could be concluded that, there highly correlation between these green and organic

pigments.Hamdia (2010) show that salinity stress affected the chlorophylls contents, in plants. These results are in general agreement with these obtained by the experimental of salinity on photosynthesis, crop halophyte. Koyro (2006).Parida*et al*., 2002 stated that the total Chlorophyllcontent increased for 14 d after treatment with 100 mMNaCI, then gradually stabilized. At 400 mM, the total Chlorophyllcontent slowly decreased over the 45-d test period.Çiçek and Çakırlar, 2008came to the same result when studied the effects of salt stress on some physiological and photosynthetic traits on soya bean plant.

3- Proline, vitamins and enzymes accumulation accumulation:

The influence of four studied salt water levels on proline and some vitamins and enzymes (*i.e.*,ascorbate, peroxidase, catalase and superoxide dismutase) accumulation of Atriplex, presented in Table (6).

Table 5: Effect of salt stress treatments on green and organic pigments accumulation (mg/gfw) of Atriplex.

Character Treatment	Total chlorophyll	Chlorophyll A	Chlorophyll B	Carotenoids
T_1	0.837	0.604	0.233	6.031
T_2	1.710	1.181	0.529	8.958
T_3	0.556	0.410	0.149	5.940
T_4	0.489	0.275	0.215	7.034
F test	**	**	**	**
LSD at 5%	0.024	0.017	0.017	0.083
at 1%	0.037	0.026	0.026	0.129

T1:50 ppm, T2:400 ppm, T3:800 ppm, T4:1600 ppm and **: significant at 1% levels of probability

Table 6: Effect of salt stress treatments on proline(µmol g-1 FW) vitamins and enzymes (U g-1 FW)accumulation of Atriplex.

Character Treatment	Proline	Ascorbate	Peroxidase	Catalase	Superoxide dismutase
T_1	0.394	0.210	9.683	221.147	12.350
T_2	0.963	0.753	8.720	147.050	15.040
T_3	1.213	0.933	6.083	106.923	14.723
T_4	1.370	3.563	7.697	217.837	17.520
F test	**	ns	**	**	**
LSD at 5%	0.068	--	0.254	0.186	0.267
at 1%	0.105	--	0.394	0.289	0.415

T1:50 ppm, T2:400 ppm, T3:800 ppm, T4:1600 ppm, **: significant at 1% levels of probability and ns: not significant

The data analysis of variance showed that, the four salt water levels had highly significant effect on the all previous studied items accumulation except the stock of ascorbate vitamin. It could be concluded that, there is highly association between increase the concentration of salt in irrigation water and the accumulation of proline in plant. Which increase from 0.394 µmol g-1 FW to 1.370 µmol g-1 FW by increasing salt water concentration from 500 ppm to 1600 ppm. Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell (Misra and Gupta, 2005 and Veeranagamallaiah*et al*., 2007). The accumulation of ascoebate takes the same trend of proline. The store of ascorbate in plant increased by application salt water regime with differed from treatment to another without any significant, that may be reflecting the role of ascorbic acid as a biological regulator in plant (Oertli, 1987, Beltagi, 2008 and El-Saidy*et al*., 2011). The highly values (9.683 and 221.147) of peroxidase and catalase enzymes were obtained by first salt water treatment (50 ppm), respectively, in the same time, the lowest value (12.350) of superoxide dismutase obtained by the same treatment (first one), that may be due to the roll of each one in cell division and metabolism system in plant, plus the partial inhibition co-with vitamins of a few interaction in reactive oxygen species production (Gadalla, 2009 and Ratnakar and Rai 2013).

In general, from the previous results it could be concluded that,the studied traits of Atrilex plant has varied significantly and/or highly significantly among them due to applying the salt water irrigation treatments.The maximum effect on reducing most of some ions, pigments, proline, vitamins and enzymes accumulation was obtained by the application of 1600 ppm (T4). The effects of salt water deficiency on different Atreilex characters were also reported by nemours researchers. It could be concluded that, there is highly association between increase the concentration of salt in irrigation water and the accumulation of proline as well ascorbic acid in plant.

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