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Role of Internal Antioxidant in the Adaptation of *Salsola Tetrandra* Forssk. at Different Habitats of The North Western Coast of Egypt.

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ABSTRACT

Samples of shoot system of *Salsola tetrandra* were collected from three different habitats: coastal ooletic sand dunes, salt marshes and coastal plain along the North-western coast of Egypt during wet season (EC: 1.9, 6.8 and 1.5 dSm^{-1}) and dry season (EC: 10.3, 20.7 and 3.8 dSm^{-1}). Lipid peroxidation in terms of malondialdehyde (MDA) content, carotenoid content, ascorbic acid content, proline content, glycine-betaine content, total phenols content and specific activities of peroxidase (POD), polyphenol oxidase (PPO) and ascorbate peroxidase (APX) were determined. Lipid peroxidation significantly increased during dry season to record the highest value in *S. tetrandra* inhabiting salt marshes associated with the lowest carotenoids and POD specific activity. However, the harmful effect of ROS in *S. tetrandra* inhabiting coastal ooletic sand dunes, during dry season, was achieved by stimulating specific activities of POD and PPO. Content of glycine-betaine and total phenols exhibited a significant increase in plants inhabiting coastal plain during dry season, while the proline had a reverse effect and generally decreased during dry season.

Keywords: *Salsola tetrandra*, lipid peroxidation, antioxidants.

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INTRODUCTION

Plants under natural conditions are frequently exposed to combined stresses like drought and salt (Mittler 2002). A common result of most abiotic and biotic stresses is an increased production of reactive oxygen species (ROS), which results in oxidative stress (Bowler and Fluhr 2000; Panda and Khan 2004). The production of ROS occurs in chloroplast during the light reaction of photosynthesis and from mitochondrial respiration (Mittler 2002). Reactive oxygen species may lead to the oxidations of proteins, membrane lipids or DNA injury. Malonodialdehyd (MDA) content, a product of lipid peroxidation, has been considered as an indicator of oxidative damage in the cell membrane, resulting in disruption of metabolic function and loss of cellular integrity (Scandalios 1993; Sairam and Srivastava 2002). Antioxidant systems in plant cell are known to include a wide set of enzymes (catalase, peroxidase, ascorbate peroxidase, superoxide dismutase, glutathione reductase etc.) and also accumulation of compounds, such as proline, glycine-betaine, phenols, carotenoid etc. (Blokhina *et al*. 2003; Kartashov *et al*. 2008). Harmful influence of ROS on cell macromolecules may also be alleviated by the activity of antioxidant compounds such as ascorbic acid, glutathione, carotenoids (Xiong and Zhu 2002) and polyphenolic compounds (Ksouri *et al*. 2012; Bose *et al*. 2014).

Salsola tetrandra Forssk. belonging to family Chenopodiaceae, is a succulent plant of wide ecological adaptation. In Egypt, it grows in semi-arid, i.e., -Mediterranean coastal land (Batanouny and Abol Soud 1972) areas. The ecophysiology of *S. tetrandra* has been reviewed by Abd El-Maboud (2011). New phytochemical compounds were separated from the ethyl acetate extract of *S. tetrandra* roots, which exhibited significant antioxidant effect in 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays (Beyaoui *et al*. 2012).

This study was conducted to examine antioxidant defense responses in natural plants adapted to the stressful conditions in harsh environments. *S. tetrandra* was selected to study its adaptive responses in terms of internal antioxidant enzymes POD, PPO and APX enzymes and carotenoids, ascorbic acid, proline, glycine-betaine and total phenols under different habitats, viz., coastal ooletic sand dunes, coastal salt marshes and coastal plains.

MATERIALS AND METHODS

Samples of plant shoot system and supporting soil were collected from three habitats, viz., coastal ooletic sand dunes, located at 31º 22` 32" N and 27º 11` 24" E, salt marshes, located at 31º 25` 94" N and 26º 51` 99" E and coastal plain, located at 31º 29` 05" N and 26º 35` 97" E along the North-western coast of Egypt (west Matruh) during March (wet season) and August (dry season) of 2013. The soil samples supporting plants were collected from the studied habitats at 0-30 cm depths.These soil samples were dried and then powdered gently with wooden wallet and passed through 2 mm sieve. Plant samples were divided into two parts for analyses, in the first part fresh samples were used to determine lipid peroxidation, and contents of carotenoids, ascorbic acid and specific activity of POD, PPO and APX enzymes, the second part plant samples were dried at till constant weight, then ground to fine powder to determine contents of proline, glycine-betain and total phenolics.

Soil analysis

Electrical conductivity (EC) was estimated in soil water extract (1:1) and moisture content was determined according to Rowell (1994).

Plant analysis

The level of lipid peroxidation in *S. tetrandra* samples was determined in term of malondialdehyde (MDA) content according to the protocols of Heath and Packer (1968). Carotenoids were determined quantitatively as described by Metzner *et al*. (1965). Ascorbic acid was estimated by titrating with 2,6-dichlorophenolindophenol sodium solution as described by Okeri and Alonge (2006). Free proline was measured by the sulfosalicylic acid- ninhydrin method according to Bates *et al*. (1973). Glycine-betaine content was estimated colorimetrically as described by (Grieve and Grattan 1983). Total phenolics was determined using Folin-Denis reagent as described by Shahidi and Nacz (1995). A known weight of plant sample was extracted by 80% ethanol, 1 ml of the extract, 0.5 ml of Folin reagent were mixed well, 1 ml of saturated

Na_2CO_3 and mixed well then 3 ml of dist. water were added. After 1 hour read the developed blue color at 725 nm by spectrophotometer using catechol as a standard.

Enzymes specific activity

Leaf tissues (0.5 g) was ground in 4 ml of 50 mM potassium phosphate buffer (pH 7.0), 1 mM ethylenediamine tetraacetic acid (EDTA), 0.2 mM ascorbic acid (AsA), 1% (w/v) polyvinylpyrrolidone (PVP) and 0.05% (w/v) Triton X-100 using a chilled pestle and mortar. The homogenate was centrifuged at 10,000 g for 20 min at 4°C and the supernatants thus collected was used for the assays of peroxidase (POD), polyphenol oxidase (PPO) and ascorbate peroxidase (APX). Protein concentrations in the enzyme extract were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

The activity of POD was assayed according to Hammerschmidt *et al*. (1982) with some modifications. The reaction mixture (3 ml) consisted of 2.9 ml 0.25% guaiacol in 10 mM sodium phosphate buffer, pH 6.0 containing 10mM H_2O_2 followed by addition of 100 µl enzyme extract to initiate the reaction. The changes of optical density at 470nm were recorded in a spectrophotometer. Unit of enzyme (U) equal 0.01 ΔA470. min^{-1}. The specific activity expressed as (units. mg^{-1} protein). PPO activity was determined according to (Galeazzi *et al*. 1981). The reaction mixture consisted of 3.0 ml of 0.6 M catechol, freshly prepared in 0.05 M sod. Phosphate buffer at pH 6.5 and a predetermined quantity of enzyme extract. The reference cuvette contained only the substrate solution and the change in O.D was recorded at 30 s interval up to 3 min at 420 nm. Unit of enzyme (U) equal 0.001 ΔA420. min^{-1}. The specific activity expressed as (units. mg^{-1} protein). APX was assayed by the method as described by Nakano and Asada (1981**)**. The reaction mixture (3 ml) consisted of 2.9 ml 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 0.5 mM ascorbic acid and 0.1 mM H_2O_2 and 100 µl enzyme extract. The decrease in absorbance at 290 nm for 1 min was recorded and the specific activity was recorded as µMol ascorbate oxi. min^{-1} mg^{-1} protein with the extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Statistical analysis

The experiment included three locations, which were arranged in a randomized complete block design with three replications. Data obtained were analyzed according to MSTATC software program (1991). Means values were differentiated using Duncan at 5% level as mentioned by Duncan (1955).

RESULTS AND DISCUSSION

Data in Table, 1 indicated that moisture content was higher in wet season than in dry in all the three habitats. Highest value was observed at salt marshes (10.25%) in wet season. EC ranged from 1.47 dSm^{-1} at coastal plain during wet season to 20.72 dSm^{-1} at salt marshes during dry season.

Table 1: Some chemical properties of soil supporting *Salsola tetrandra*.

Locations	Moisture content (%)		EC (dSm^{-1})	
	Wet season	Dry season	Wet season	Dry season
Ooletic sand dunes (L1)	5.15	4.04	1.89	10.3
Salt marshes (L2)	10.25	7.35	6.75	20.72
Coastal plain (L3)	4.09	3.35	1.47	3.8

Levels of lipid peroxidation were highest in *S. tetrandra* growing at salt marshes, followed by those growing at coastal plain while those growing at ooletic sand dunes recorded the lowest value, indicating least oxidative stress in plant growing in ooletic sand dunes as shown in Table, 2. Carotenoid content, ascorbic acid and APX specific activity recorded the highest values in *S. tetrandra* growing at salt marshes, while, POD and PPO specific activity were the highest in those growing in ooletic sand dunes. The increase of APX under salt marshes agreed with Freipica and Ievinsh (2010) who found that APX increased in explants of *Glaux maritima* and *Dianthus arenarius* by 100 mM NaCl treatment. Contents of proline, glycine-betaine and total phenolics were highest in those inhabiting coastal plain. In response to salinity, the PPO specific activity increased in plants inhabiting salt marshes and ooletic sand dunes comparable to those inhabiting coastal plain (the lowest in salinity) increased by 3.96 and 4.78 fold respectively. On the other hand, total phenolics increased as PPO

decreased recording the highest accumulation in *S. tetrandra* inhabiting coastal plain comparable to the others, increased about 3.03 and 4.33 fold from those inhabiting salt marshes and ooletic sand dunes respectively. We can deduce that PPO decreases under moderate conditions in *S. tetrandra* to induce the accumulation of total phenols. Lower MDA content in *S. tetrandra* growing in ooletic sand dunes might be associated with the minimum oxidative damage to membrane as a result of lower production of free radicals therefore suggest the presence of strong antioxidant defense represented by POD and PPO specific activity.

Regarding seasons effect, values of lipid peroxidation, glycine-betaine, total phenolics and specific activity of POD and PPO were higher in the dry season, while carotenoids, ascorbic acid, proline and APX specific activity decreased in dry season. The higher accumulation of carotenoid content, ascorbic acid, proline and APX specific activity of *S. tetrandra* in wet season may be due to its lower lipid peroxidation (minimum oxidative damage). The increase of MDA, POD and PPO during dry season are in agreement with those obtained by Poodeetip *et al*. (2013) who investigated biochemical substances and their relationship into two groups of plants; halophytes and salt tolerance species during the rainy and dry seasons. They found that in the dry season all the studied plants produced more contents of POD activity and MDA than in the rainy season. In addition, Sanaeirad (2014) found that the activity of PPO in shoots and roots of *Salsola crassa* was higher in summer season than winter. Kamiński *et al*. (2012) found a negative relation between MDA and APX activity in the roots of *Salicornia europaea* (Chenopodiaceae).

The levels of lipid peroxidation were highest in *S. tetrandra* growing at salt marshes in dry season, followed by those inhabiting coastal plain in dry season, ooletic sand dunes in dry and wet seasons, coastal plain in wet season and salt marshes in wet season. Carotenoid recorded the highest concentration in plants inhabiting salt marshes during wet season followed by those inhabiting ooletic sand dunes in wet season, coastal plain in wet season, ooletic sand dunes in dry season, while the lowest concentration was recorded in those inhabiting salt marshes during dry season. Values of ascorbic acid were highest in plants inhabiting coastal plain during wet season, followed by plants inhabiting salt marshes in dry season and the lowest value in both growing at ooletic sand dunes and coastal plain during dry season. Carotenoid and ascorbic acid are resistance to salinity but sensitive to drought stress in *S. tetrandra*. In this trend, Abd El-Maboud and Khalil (2013) reported that ascorbic acid tended to increase in *Suaeda vera* under salt marshes to dilute deleterious effect caused by free radicals under salinity stress. Sai *et al*. (2012) found that water deficit decreased the content of carotenoid and anthocyanin pigments in leaves of *Atriplix hortensis* var*. rubra*. They reported that plant can cope with carotenoid damage; under water stress carotenoids are induced in an attempt to protect the cell against this insult. Carotenoids have essential functions in photosynthesis and photoprotection. Besides their structural roles, they are well known for their antioxidant activity by quenching ^{3}Chl and $^{1}O_2$, inhibiting lipid peroxidation, and stabilizing membranes (Demmig- Adams and Adams 1992; Frank and Cogdell 1996 and Niyogi 1999).

Proline was highest accumulation in plants inhabiting coastal plain (the lowest salinity) during wet season, followed by those inhabiting ooletic sand dunes during wet season and the lowest accumulation was observed in those inhabiting ooletic sand dunes during dry season. In certain halophytes proline has been advocated as the major cytoplasmic osmoticum and reliable biochemical marker for salt stress (Kishor *et al*. 2005; Jia *et al*. 2011 and Grigore *et al*. 2011). In these Chenopod species, proline levels were relatively low over the range of plants inhabiting salt marshes and/ or those exposed to drought stresses. The results of present study were coincided with the previous work on *Suaeda vera* (Chenopodiaceae) which increased in proline accumulation under moderate salinity at sand dunes and decreased at salt marshes (Abd El-Maboud and Khalil 2013). Also, Kong-ngern *et al*. (2012) observed a negative relationship between the amount of proline accumulation and the level of salt tolerance did not support the widely advocated role of proline as an osmoprotectant under salt stress. Arbona *et al*. (2008) found that the protective role of proline has to be considered minimal as its accumulation was inversely correlated with tolerance to the stress. Generally, glycine-betaine content increased during dry season, recorded the highest accumulation in plants inhabiting coastal plain indicated that betaine is more effective as a defense against free radicals under drought than salinity stress.

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Table 2: Lipid peroxidation, carotenoids, ascorbic acid, proline, betain, total phenolics and specific activity of peroxidase, polyphenol oxidase and ascorbate peroxidase in *S. tetrandra*.

Locations									
Habitats	**Lipid peroxidation (µmol MDA g^{-1} fr. wt.)**	**Carotenoid content [mg (100 g)$^{-1}$ fr. wt.]**	**Ascorbic acid content [mg (100 g)$^{-1}$ fr. wt.]**	**Proline content (µg g^{-1} dry wt.)**	**Glycine betaine content (µmol g^{-1} dry wt.)**	**Total phenolics content (mg g^{-1} dry wt.)**	**Peroxidase activity (U. mg^{-1} protein)**	**Polyphenol oxidase activity (U. mg^{-1} protein)**	**Ascorbate peroxidase activity (µMol AsA oxi. Min^{-1} mg^{-1} protein)**
Ooletic sand dunes (L1)	0.182^{c}	3.090^{a}	15.275^{c}	54.757^{b}	145.593^{c}	1.325^{c}	6991^{a}	1746^{a}	1.394^{c}
Salt marshes (L2)	0.229^{a}	3.220^{a}	29.700^{a}	46.317^{c}	163.197^{b}	1.895^{b}	4022^{c}	1569^{b}	2.385^{a}
Coastal plain (L3)	0.213^{b}	2.770^{b}	25.983^{b}	92.848^{a}	235.618^{a}	5.733^{a}	4395^{b}	396^{c}	2.195^{b}
Seasons									
Wet season (W)	0.146^{b}	4.736^{a}	32.344^{a}	92.842^{a}	156.341b	2.933^{b}	4824^{b}	1149^{b}	2.239^{a}
Dry season (D)	0.270^{a}	1.484^{b}	14.961^{b}	36.439^{b}	206.598^{a}	3.036^{a}	5449^{a}	1325^{a}	1.744^{b}
Interaction between locations and seasons									
L1*W	0.165^{d}	4.760^{b}	26.000^{c}	93.390^{b}	118.753^{f}	1.507^{e}	5286^{b}	1463^{c}	1.409^{d}
L1*D	0.198^{c}	1.920^{d}	4.550^{d}	16.123^{e}	172.433^{d}	1.143^{f}	8696^{a}	2029^{a}	1.380^{d}
L2*W	0.133^{e}	5.427^{a}	23.400^{c}	66.483^{c}	141.677^{e}	2.100^{c}	4292^{d}	1529^{bc}	2.124^{c}
L2*D	0.326^{a}	1.013^{e}	36.000^{b}	26.150^{d}	184.717^{c}	1.690^{d}	3754^{e}	1609^{b}	2.646^{b}
L3*W	0.139^{e}	4.020^{c}	47.633^{a}	118.653^{a}	208.593^{b}	5.193^{b}	4894^{c}	456^{d}	3.185^{a}
L3*D	0.287^{b}	1.520^{d}	4.333^{d}	67.043^{c}	262.643^{a}	6.273^{a}	3897^{e}	336^{e}	1.205^{e}

Peroxidase specific activity was highest in plants inhabiting ooletic sand dunes during dry season while it decreased in those inhabiting salt marshes and coastal plain during dry season. Polyphenol oxidase specific activity was highest in *S. tetrandra* inhabiting ooletic sand dunes during dry season, followed by those inhabiting salt marshes during dry and wet seasons, and ooletic sand dunes during wet season, lowest in those inhabiting coastal plain during dry season. The highest specific activity of POD and PPO in *S. tetrandra* inhabiting ooletic sand dunes are similar to those obtained by (Rajaravindran and Natarajan 2012) who found that antioxidant enzymes such as catalase, POD and PPO in *Suaeda maritima* increased up to the optimum level of 300 mM NaCl concentration and beyond these levels the contents decreased marginally.

APX specific activity was highest in plants inhabiting coastal plain during wet season followed by those inhabiting salt marshes in dry and wet seasons, respectively, while the lowest activity was in plants inhabiting coastal plain during dry season. APX plays an important role in the metabolism of hydrogen peroxide in higher plants (Liu *et al*. 2013).

In conclusion, the relationship between lipid peroxidation and antioxidant enzymes is not always stable among the studied habitats. However, we found a reversible relation between them in *S. tetrandra* inhabiting coastal plain. Proline has a reverse effect under drought and /or salinity stress, while glycine-betaine has an obvious effect against free radicals under drought stress in *S. tetrandra*. Total phenolics content increase as PPO specific activity decrease and vice verca.

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