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Anatomical and physiological responses of three species of Suaeda Forssk. ex Scop. under different habitat conditions

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ABSTRACT

Three species of Suaeda were collected from different saline sites of Egypt. S. vera and S. pruinosa collected from coastal sand dunes (site-1) and salt marshes (site-2) at the Northwestern coast. While S. vermiculata collected from dry upper zones of salt marshes (site-3) and salt marshes (site-4) at Northeastern coast to assess their mechanism of adaptation to the different saline habitats. Anatomical modifications in leaves in relation to succulence, photosynthetic pigments and ABA contents were studied. Results showed that leaf cross section area, leaf thickness, epidermal tissue area, photosynthetic carbon assimilative tissue area (mesophyll), water storage tissue area, and chl. a were significantly decreased in the three species collected from the high saline habitats. While, cuticle thickness, number of xylem rows/median bundle and percentage of epidermis tissue were increased. On the other hand, the water storage tissue did not affected by the different saline habitats. High percentage of the vascular bundles and ABA content were detected in S. vera leaves. While it decreased in photosynthetic carbon reductive tissue area (bundle sheath) and succulence under salt marshes. S. pruinosa kept stability in photosynthetic carbon reductive tissue area and its percentage, succulence, chl. b and carotenoids, while the leaves increased in vascular bundles area and decreased in inner diameter of xylem vessels and ABA content under salt marshes. S. vermiculata uneffected significantly in percentage of vascular bundle tissues while the leaves decreased in photosynthetic carbon reductive tissue area, vascular bundle area, inner diameter of xylem vessels, degree of succulence and photosynthetic pigments and increased in percentage of photosynthetic carbon reductive (kranz type) and ABA content.

Key words: Suaeda, Leaf anatomy, Anatomical changes, Succulence, Photosynthetic pigments, ABA.

Introduction

Studies on the halophilic vegetation of arid and humid areas have been attracted great attention (Pyankov et al., 2001; Khan and Weber, 2006). The plants inhabiting saline environments have developed certain features which help them to thrive under adverse conditions. Such features are often displayed in the morpho-anatomical and physiological changes of plants (Grigore and Toma, 2007; Hameed et al., 2009; Ashraf and Harris, 2013).

Suaeda (Chenopodiaceae) includes annual and perennial herbs or rarely small trees; leaves alternate and succulent, contains about 100 species worldwide, distributed in coasts and salt steppe. In Egypt it is represented by 9 species according to Boulos (1999). A number of species have been found to be valuable feed for livestock in arid area like S. vera and S. vermiculata (El-Shaer and El-Morsy, 2008), while others have been utilized to desalinate irrigated farmlands like Suaeda maritima (Choi et al., 2012).

Fisher et al. (1997) stated that Suaeda has two pathways of CO₂ fixation, namely C₃ and C₄. Different studies proved that C₄ show higher tolerances to drought, high irradiance and salinity (Akhani et al., 1997; Wang, 2007). Most of C₄ plants require metabolic cooperation of two types of chlorochymatous tissue; the outer is the mesophyll or photosynthetic carbon assimilative tissue (PCA) and the inner is the bundle sheath or photosynthetic carbon reductive tissue (PCR), and this bundle sheath structure is known as Kranz type (Sage, 2004).

Anatomical structures of plant organs, especially of leaves change thus enabling plant adaptation to its environment. Many leaf features have been recognized to provide a protection against various environmental conditions and stresses including drought, high air temperature and high concentration of salt in soil. Most morphological and anatomical adaptations of plants from desert- saline habitats are smaller leaves, fewer stomata per unit leaf area, inceased succulence, thickness of leaf cuticle and depositon of wax (Mass and Nieman, 1978). Furthermore, the leaf histological components seem to be an ideal model to study of the relations between halomorphic and xeromorphic structures of plants and their habitat (Polić et al., 2009).
Abd El-Maboud (2011) found that *Salsola tetrandra* (Chenopodiaceae) growing at barley fields represented the best metabolism where a palisade cells composed of regular compact palisade filled with chloroplast and arranged typically like a row of stakes associated with the highest content of photosynthetic pigments. Meanwhile, leaves of the same plant inhabiting salt marshes had irregular shape of palisade cells associated with the lowest photosynthetic pigments. Flexas *et al.* (2004) and Chaves *et al.* (2009) reported that, the decrease in photosynthetic rate under stressful conditions is normally attributed to a suppression in the mesophyll conductance and stomata closure. The decrease in chlorophyll a, chlorophyll b and carotenoid contents in leaves under salinity has been reported by (Youssef, 2009; Samiullah and Bano, 2011). The salinity induced osmotic effect on plants causes a substantial accumulation of abscisic acid, particularly in the guard cells of stomata, which leads to a partial stomata closer (Zhao *et al.*, 2009).

The present work was aimed to study leaf anatomical structures in addition to some physiological parameters (succulence, photosynthetic pigments and ABA) of three halophilic species; *Suaeda vera*, *Suaeda pruinosa* and *Suaeda vermiculata* under different habitat conditions of the Egyptian deserts.

**Materials and Methods**

1-Plant material:

*Suaeda vera* Forssk. ex J. F. Gmel, *Suaeda pruinosa* Lange and *Suaeda vermiculata* Forssk ex J. F. Gmel were collected from their natural habitats at the Northern coast of Egypt during Marsh 2012. *Suaeda vera* and *Suaeda pruinosa* were collected together from two sites at Northwestern coast (West Matruh). Site-1; coastal sand dunes, the geographical position system reading (GPS) is: 31° 29' 19.6" N and 26° 37' 43.6"E. Site-2; salt marsh, the GPS reading is: 31° 23' 04.6" N and 27° 03' 51.7" E. While *Suaeda vermiculata* was collected from two other habitats at Northeastern coast (North Sinai). Site-3; dry upper zones of salt marshes, GPS reading is: 31° 02' 28.1" N and 33° 16' 43.6" E. Site-4; salt marshes, the GPS reading is 31° 01' 54.5" N and 32° 35' 16.3 " E. All the studied species were identified by the aid of textbooks of flora (Täckholm, 1974; Bolous, 1999). Voucher specimens were kindly identified at the Herbarium of Faculty of Science, Cairo University.

2-Soil analysis:

The soil samples of plant habitats were collected from two successive depths; upper depth (0-20 cm) and lower depth (20-40 cm). Electrical conductivity (EC) was estimated and determined in soil water extract (1:1) by using electric conductivity meter. The hydrogen ion concentration was measured by means of glass electrode pH meter according to Rowell (1994). The soil moisture content was determined using method described by Rowell (1994).

3-Leaf anatomy:

Specimens from the middle part of leaves of the three species were detached (five leaves per species) and fixed in FAA solution (formalin, acetic acid and 70% ethyl alcohol, 1:1:18 by volume) for 24h. The schedule of paraffin method described by Johansen (1940) was followed. Serial transverse sections (8µm in thickness) were made by LEICA rotary microtome model RM 2125 RTS and fixed on slides by means of Haupt’s adhesive (Sass, 1951). The sections were stained with a safranin- fastgreen combination, and then mounted in Canada balsam (Sass, 1951). Sections were examined and viewed with a LEICA light research microscope model DM 2500 supplied with a digital camera. Different histological parameters were measured including cross-section areas of (1) epidermis (sum of upper and lower epidermal layers), (2) PCA the palisade layer in C₄ or mesophyll in C₃ plants, including all chlorenchymatous ground tissues, intercellular space, and substomatal cavities, (3) water storage tissue WST, (4) PCR the kranz anatomy in C₄ or [BS] bundle sheath in C₃, and the vascular tissues. The percentages of the abovementioned parameters were calculated and expressed as percentages of total cross-section leaf area. Leaf thickness at the thickest part of the median vein sector was determined for leaves.

4-Succulence and physiological analysis:

The degree of succulence was calculated as fresh/dry weight according to Dehan and Tall (1978).

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined quantitatively as described by Metzner *et al.* (1965). Abscisic acid (ABA) was determined by HPLC with Column : 3.9×300mm M Bonda Pakc18, Data Module: Waters 746, Detector: Tumble Absorbance, Pump: HPLC 510, Volume injection: 10ml as described by Wasfy *et al.* (1975).
5-Statistical analysis:

Obtained data of leaf anatomy was done by statistical analysis system (SAS, 1999). Tukey test was used for separation between means.

Data obtained from the experiment of physiological analysis were subjected to the proper statistical analysis of variance of the complete randomized design according to the method of Snedecor and Cochran (1969). Mean values of treatments were compared at 5% level according to (Duncan, 1955).

Results:

Table 1: Physical properties of soil supporting Suaeda vera, Suaeda pruinosa and Suaeda vermiculata at the studied sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Species</th>
<th>Depth cm</th>
<th>EC dS m⁻¹</th>
<th>TDS mg/L</th>
<th>pH</th>
<th>Moisture content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. vera</td>
<td>0-20</td>
<td>3.31</td>
<td>2118.4</td>
<td>7.34</td>
<td>4.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-40</td>
<td>7.7</td>
<td>4928</td>
<td>7.68</td>
<td>6.56</td>
</tr>
<tr>
<td></td>
<td>S. pruinosa</td>
<td>0-20</td>
<td>3.70</td>
<td>2368</td>
<td>7.30</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-40</td>
<td>8.2</td>
<td>5248</td>
<td>7.63</td>
<td>6.66</td>
</tr>
<tr>
<td>2</td>
<td>S. vera</td>
<td>0-20</td>
<td>9.8</td>
<td>6272</td>
<td>7.43</td>
<td>5.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-40</td>
<td>15.6</td>
<td>9964</td>
<td>7.68</td>
<td>12.25</td>
</tr>
<tr>
<td></td>
<td>S. pruinosa</td>
<td>0-20</td>
<td>10.52</td>
<td>6732.8</td>
<td>7.71</td>
<td>5.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-40</td>
<td>16.27</td>
<td>10412.8</td>
<td>7.74</td>
<td>12.69</td>
</tr>
<tr>
<td>3</td>
<td>S. vermiculata</td>
<td>0-20</td>
<td>4.85</td>
<td>3104</td>
<td>7.20</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-40</td>
<td>5.29</td>
<td>3385.6</td>
<td>7.06</td>
<td>11.66</td>
</tr>
<tr>
<td>4</td>
<td>S. vermiculata</td>
<td>0-20</td>
<td>11.92</td>
<td>7628.8</td>
<td>7.17</td>
<td>15.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-40</td>
<td>20.1</td>
<td>12864</td>
<td>7.03</td>
<td>25.35</td>
</tr>
</tbody>
</table>

Electrical conductivity of soil supporting S. vera and S. pruinosa was higher in site-2 than in site-1 and increased from first to second layer as shown in Table 1. EC of soil supporting S. vermiculata varied from (4.85 to 5.29 dS m⁻¹) at site-3 and (11.92 to 20.1 dS m⁻¹) at site-4 from first to second layer. Soil pH was slightly alkaline ranged from (17.03 up to 7.74) in soil supporting the three Suaeda species. Moisture content increased from first to second layer in all studied sites, varied from 4.65 to 6.66% in Suaeda inhabiting site-1 and from 5.11 to 12.69% in Suaeda inhabiting site-2. Moisture content of soil supporting S. vermiculata recorded 2.91%, 11.66% at site-3 and 15.45%, 25.35% at site-4 for first and second layer repsectively.

Leaf anatomy:

**Suaeda vera** has relatively oblong alternate leaves nearly 5 to 20 mm in length and 2 to 4 mm in width. The leaf consists of the common epidermal layers enclosing in between the mesophyll. The latter is intervened by small vascular bundles. The abaxial and adaxial epidermis are uniseriate or simple with thin cuticle (Figs. 1&2). The mesophyll consists of two distinct layers. The first is recognized underneat the epidermis and differentiated into one or two rows of nearly isodiametric or slightly elongated cells. Their long axes are perpendicular to the epidermis. These cells have abundance of plastids and small intercellular spaces it define as the photosynthetic carbon assilimative PCA (Fig. 3). The second and innermost layer of the mesophyll is developed into thin walled parenchyma cells with few scattered chloroplasts. Because of the large size of these cells, it may participate in water storage in addition to photosynthesis (Fig. 2). The same distribution of the photosynthetic tissue forms a bifacial leaf. The vascular bundles were arranged into a crescent shape. The concave side occurred toward the upper side. Each bundle is bordered with a thin layer of compact parenchymatous layer. Its cells are plastids free (Fig. 4) it define as the bundle sheath BS or the photosynthetic carbon reductive PCR. The largest bundle located in the center, and then their size gradually decreased toward the leaf margin. Small interveinal zones, of only one parenchyma cell separating the lateral or small bundles were observed.
**Suaeda vermiculata** has alternate, succulent, semi cylindrical leaves nearly 5 to 7mm in length and 3 to 4 mm in width. The abaxial and adaxial epidermal layers consist of a single large layer with succulent cells. The plant has isodiametral leaves (Fig. 6). The mesophyll consists of three types of cell layers. The outermost layer consists of palisade cells with dense, small chloroplasts and small intercellular spaces or "the photosynthetic carbon assimilative PCA". The second chlorophyllumalous layer is generally one cell thick. It consists of small isodiametral cells containing many large chloroplasts occurred on the inner tangential and the radial walls. The outer tangential wall is almost plastide free. This layer appears as a sheath comprising the inner layers or "the photosynthetic carbon reductive PCR" these tissues in C₄ leaves, a structural pattern is known as Kranz type (Figs. 7&8). The third type of the mesophyll located in the center of the leaf and was developed of several layers of thin walled parenchymatous water storage tissue embedding the vascular bundles. This specialized anatomy is typical for most C₄ species of Suaeda. The cells of the water storage tissue contained few scattered chloroplasts which were smaller than those of the sheath layer, but resembled those of the outer chlorophyllumalous palisade layer. The midvein has a small single vascular bundle formed of a patch of phloem elements outwards and few narrow xylem elements inwards.

Data in Table (2) showed that some anatomical leaf characters (leaf cross section area, leaf thickness, epidermis tissue area, PCA tissue area and water storage tissue area) in the three species of *Suaeda* were significantly decreased in leaves collected from site-2 and site-4 as compared to site-1 and site-3. The same trend observed in PCR tissue area for *S. vera* and *S. vermiculata*, the vascular bundles area in *S. vermiculata* as well as the average of inner diameter of xylem elements of *S. purinosa* and *S. vermiculata*. Whereas the cuticle thickness, number of xylem rows in the median bundle increased in the three species of *Suaeda* collected from site-2 and site-4. The same trend for areas of the vascular bundles of *S. purinosa* which gave the highest significant values in leaves collected from site-2 comparing with those from site-1. On the other hand, no significant differences were noticed in the PCR area in *S. pruinosa* and the vascular bundles area in leaves of *S. vera* and its inner diameter of xylem elements.

Regarding to the percentages of the leaf tissues, the percentage of epidermis tissue area was significantly increased while the percentage of PCA was decreased under the high saline habitats in site-2 and site-4 in all *Suaeda* sp. The percentage of PCR (kranz type) was significantly increased in *S. vermiculata* collected from site-4 as compared to site-3. Also, the percentage of vascular bundles in *S. vera* and *S. pruinosa* recorded highest values in site-2 than site-1. Whereas, no significant differences were noticed in the percentage of WST in the three species in all sites.

### Table 2: Leaf anatomical characteristics in three species of *Suaeda* collected from different saline habitats

<table>
<thead>
<tr>
<th>Species</th>
<th>LCS mm²</th>
<th>L th μm</th>
<th>C th μm</th>
<th>Ep μm²</th>
<th>PCA μm²</th>
<th>PCR or BS%</th>
<th>WST μm²</th>
<th>V Bs μm²</th>
<th>N X rows</th>
<th>X diameter μm</th>
<th>Ep %</th>
<th>PCA %</th>
<th>PCR or BS%</th>
<th>WST%</th>
<th>V Bs%</th>
</tr>
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<tbody>
<tr>
<td>Site-1</td>
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</tr>
<tr>
<td>Sp1×site-1</td>
<td>3.09 a</td>
<td>1206 a</td>
<td>2.51 b</td>
<td>203500</td>
<td>1902199</td>
<td>35173 a</td>
<td>900636 a</td>
<td>49734 u</td>
<td>6-7</td>
<td>4.59 a</td>
<td>6.62 b</td>
<td>61.34 a</td>
<td>1.15 a</td>
<td>29.28 a</td>
<td>1.62 b</td>
</tr>
<tr>
<td>Site-2</td>
<td>1.65 b</td>
<td>1080 b</td>
<td>6.7 a</td>
<td>171912 b</td>
<td>935275 b</td>
<td>34122 b</td>
<td>4485096</td>
<td>45247 a</td>
<td>8-12</td>
<td>4.18 a</td>
<td>10.4 a</td>
<td>37.78 b</td>
<td>3.07 a</td>
<td>27.13 a</td>
<td>2.61 a</td>
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<td>Site-3</td>
<td>2.11 a</td>
<td>1447 a</td>
<td>3.7 b</td>
<td>228229 a</td>
<td>1075954 a</td>
<td>11325 a</td>
<td>787201 a</td>
<td>12871 b</td>
<td>3-4</td>
<td>5.49 a</td>
<td>10.79 b</td>
<td>50.85 a</td>
<td>0.55 a</td>
<td>37.22 a</td>
<td>0.61 a</td>
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<td>Site-4</td>
<td>2.72 b</td>
<td>1230 b</td>
<td>5.8 a</td>
<td>187326 b</td>
<td>522312 b</td>
<td>13241 a</td>
<td>553767 a</td>
<td>19189 a</td>
<td>4-5</td>
<td>3.26 b</td>
<td>44.68 a</td>
<td>49.94 b</td>
<td>1.04 b</td>
<td>41.83 a</td>
<td>1.50 a</td>
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<td>Site-5</td>
<td>3.85 a</td>
<td>1455 a</td>
<td>5.67 b</td>
<td>717165 a</td>
<td>671231 a</td>
<td>16949 a</td>
<td>253205 a</td>
<td>15087 a</td>
<td>5-6</td>
<td>4.90 a</td>
<td>13.30 b</td>
<td>22.30 a</td>
<td>4.43 b</td>
<td>65.85 a</td>
<td>3.93 a</td>
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<td>Site-6</td>
<td>2.47 b</td>
<td>957 b</td>
<td>5.2 a</td>
<td>248964 b</td>
<td>313931 b</td>
<td>60992 b</td>
<td>809522 a</td>
<td>67194 a</td>
<td>4-6</td>
<td>3.52 b</td>
<td>16.87 b</td>
<td>26.88 b</td>
<td>3.48 b</td>
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<td>Sp2×site-1</td>
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<td>75.37 a</td>
<td>19.65 a</td>
<td>451694</td>
<td>139957 a</td>
<td>21169 a</td>
<td>101776 a</td>
<td>15720 a</td>
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<td>65.07 b</td>
<td>17.81 a</td>
<td>20.77 a</td>
<td>337.3 a</td>
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<td>6.81 b</td>
<td>8.78 b</td>
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<tr>
<td>Sp2×site-4</td>
<td>4.22 a</td>
<td>17.46 d</td>
<td>5.26 b</td>
<td>7.40 b</td>
<td>73.9 d</td>
<td></td>
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<td></td>
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</tbody>
</table>

### Table 3: Effect of sites and species on succulence, chlorophyll (a, b), carotenoid and ABA in *Suaeda vera* and *S. pruinosa*.

<table>
<thead>
<tr>
<th>Species</th>
<th>succulence</th>
<th>Chl. a (mg/100g f. wt.)</th>
<th>Chl. b (mg/100g f. wt.)</th>
<th>Carotenoid (mg/100g f. wt.)</th>
<th>ABA (µg/100g F. wt.)</th>
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<tr>
<td>Sp1×site-1</td>
<td>3.81 b</td>
<td>74.3 a</td>
<td>19.65 a</td>
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<td>Sp1×site-2</td>
<td>3.26 c</td>
<td>65.07 b</td>
<td>20.77 a</td>
<td>337.3 a</td>
<td></td>
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<tr>
<td>Sp2×site-1</td>
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<td>8.78 b</td>
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<td>4.22 a</td>
<td>17.46 d</td>
<td>7.40 b</td>
<td>73.9 d</td>
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### Table 4: Effect of sites on succulence, chlorophyll (a, b), carotenoid and ABA in *Suaeda vermiculata*.

<table>
<thead>
<tr>
<th>sites</th>
<th>succulence</th>
<th>Chl. a (mg/100g f. wt.)</th>
<th>Chl. b (mg/100g f. wt.)</th>
<th>Carotenoid (mg/100g f. wt.)</th>
<th>ABA (µg/100g F. wt.)</th>
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<tr>
<td>Sp3×site-3</td>
<td>4.98 a</td>
<td>45.2 a</td>
<td>13.44 a</td>
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<td>646.9 b</td>
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</table>
Light micrographs of transverse sections of *Suaeda* species leaves, **Figs. 1-4** *Suaeda vera*. Fig. 1: General view of the laminate isolateral leaf. Fig. 2: The structure of internal tissues, note the vascular bundles embedded in the water storage tissue. Fig. 3: Magnified part of Fig. 1 (the rectangle) showed the PCA tissue consists of two layers of elongate cells having abundance of plastids. Fig. 4: Magnified medium vascular bundle showed the bundle sheath is free of chloroplasts. **Fig. 5: Suaeda pruinosa**, part of the cylindrical leaf showing the structure of internal tissues, note the bundle sheath is free of chloroplasts. **Figs. 6-8** *Suaeda vermiculata*. Fig. 6: General view of the semi cylindrical isolateral leaf. Fig. 7: The structure of internal tissues. Fig. 8: Magnified part of Fig. 6 (the rectangle) showed the mesophyll is differentiated into 3 distinct layers; a layer of palisade (PCA), an inner chloroncymatous sheath (PCR) and central water storage tissue.

**Figure abbreviations:** BS, bundle sheath; Ep, Epidermis; PCA, photosynthetic carbon assimilative tissue; PCR, photosynthetic carbon reductive tissue or kranz type; VB, vascular bundle; WST, water storage tissue.
Generally, the interaction between sites and species had a significant effect on degree of succulence, chlorophyll (a, b), carotenoids and ABA content as shown in Table 3. Succulence was higher in S. pruinosa than in S. vera, recorded the highest value in S. pruinosa at site-2 (4.22) followed by S. pruinosa at site-1 (4.11), S. vera at site-1 (3.81) and the lowest value in S. vera at sit-2 (3.26). However succulence had increased in S. pruinosa inhabiting site-2 compared to that inhabiting site-1, but this increase did not reach to 5% of significant. Concerning photosynthetic pigments chl. a was the highest concentration followed by carotenoids while chl. b was the lowest. Chl. a tended to decrease from plants inhabiting site-1 to those inhabiting site-2, recorded the highest value in S. vera at site-1 (74.73 mg/100g f. wt.). The significant effect of chl. b and carotenoids turned to species only without sites. With regard to ABA the highest value (337.3 μg/100g f. wt.) was recorded in S. vera at sit-2 while the lowest (73.9 μg/100g f. wt.) recorded in S. pruinosa at site-2.

An inverse proportion was found between ABA content from one side and both succulence and photosynthetic pigments of S. vermiculata from other sides Table (4). This means that increasing in ABA contents under high salinity led to decrease in both succulence and photosynthetic pigments and vice versa is true.

Discussion:

Observations of cross section shape of S. vera leaves showed laminate with an isolateral leaf anatomy, whereas S. pruinosa have cylindrical leaves and showed varying degrees of bifaciality, due to the differentiation of the leaf mesophyll into densely packed palisade parenchyma on the adaxial side and more loosely packed tissue, somewhat resembling spongy parenchyma on the abaxial side. While S. vermiculata have an isolateral leaf anatomy with a cylindrical shape. Isolateral (unifacial) leaf anatomy is common in plants that grow under high light conditions (Napp-Zinn, 1984/1988). This feature is a common characteristic found in plants from hot and arid environments because it tends to minimize heating of the leaves and thus reduces transpirational demands (Ehleringer and Forseth, 1980). This is true in desert environments and on shorelines, the habitat for many species of Suaeda.

All species S. vera, S. pruinosa and S. vermiculata grow in high saline habitats site-2 and site-4 having small values in leaf cross section area Table (2). The reduction in leaf area is the principal strategy that makes it possible to attenuate the effects of the reduced availability of water under saline stress (Alem et al., 2002). The reduction in cross-sectional area may be due to reduction in DNA content resulted in reduced cell expansion (Wignarajah et al., 1975). In addition, Abd Elbar et al. (2012) observed reduction in cross-sectional area of Leptochloa fusca leaves treated with NaCl due to a decrease in the size of the midrib and large veins and decrease the thickness of mesophyll layers.

Thickness of laminate or cylindrical leaves is significantly decreased by increasing saline soil and undergoes to become thinner leaves Table (2). The reduction of leaf thickness might be attributed to that salinity reduces the ability of plants to absorb or take up water and this quickly causes reduction in photosynthetic capacity and the growth rate of the plant (Munns, 2002).

Drought-adapted plants generally had thick cuticles (Esau 1977; Boom et al., 2005). Therefor, all species which collected from high saline habitats site-2 and site-4 have thick cuticles Table (2). These findings seems to be fully justified the better adaptation for S. vera, S. pruinosa from the site-2 and S. vermiculata from site-4 to adverse moisture limited conditions. The plants with waxy cuticle in the leaves minimize the loss of water under salinity stress conditions.

Epidemis tissue area was decreased in all species under high salinity habitat. But the percentage of the epidermis area compared to other tissues in leaf was increased. This may be due to the reduction of leaf area under high saline habitats (site 2 &4) and this tissue seems to be more succulent. The percentage of the epidermis tissue in C4 S. vermiculata recorded the highest value 16%. While the C3 species S.vera recorded 10%, and S. pruinosa reached14%. Enhancing epidermal volume may simply maintain leaf thickness required for physical strength (when Mesophyll tissue is reduced) or provide succulent water storage tissue. Developing a large volume of epidermis tissue enhances internal light levels in C4 may thus be an anatomical adaptation to enhance photon flow to PCR and PCA tissues (Muhaidat et al., 2007).

The observed reduction in PCA tissue area and the percentage of PCA in all species collected from the high saline habitats in site-2 and site-4 may suggest that salinity reduced the capacity for conductance. Therefore, the reduction in PCA tissue volume is associated partly with a reduction in leaf thickness and/or number of mesophyll layers (McKown and Dengler, 2007; Abd Elbar et al., 2012). This observation accompanied with decrease in Chl. a in plants collected from site-2 and site-4 as shown in Tables (3&4). Photosynthetic pigments present in the photosystems are believed to be damaged by salinity resulting in a reduced light-absorbing efficiency of both photosystems (PSI and PSII) by impairment in electron transport and hence a reduced photosynthetic capacity (Zhang et al., 2011). Light energy absorbed by Chl. is transformed into Chl. fluorescence (Maxwell and Johnson 2000). It gives a valuable insight into exploitation of the excitation energy by PSII, and indirectly by the other protein complexes of the thylakoid membranes (Roháček, 2002),
particularly in plants exposed to stressful conditions. Carotenoids (xanthophylls) are necessary for protection of photosynthesis and they play an important role as a precursor in signaling during the plant development under abiotic/biotic stress (Misra et al., 2006). Zeaxanthin (one of xanthophylls) plays a key role in minimizing the overexcitation in higher plants (Kuczyńska et al. 2012). The dissipation of excitation energy is determined as nonphotorunchemical quenching (NPQ) of Chl fluorescence during photosynthetic electron transport, which significantly correlates with contents of zeaxanthin and antheraxanthin produced during the xanthophyll cycle (Niyogi et al. 1997). This means that decreasing in PCA area (or mesophyll area) and photosynthetic pigments by plants collected from high saline habitats is necessary and adaptive feature for impairment light absorbed.

Despite the observed relative stability in PCA area in S. pruinosa leaves collected from the high saline habitat in site-2, there is reduction in PCR area in leaves of S. vera or Kranz type area in S. vermiculata collected from site-2&4, which is high saline habitats compared to site-1&3.

The percentage of PCR (or bundle sheath BS) tissue area was significantly higher in C₄ eudicots S. vermiculata than those of C₃ S. vera or in S. pruinosa. Kanani and Edwards, 1999 found that the enhancing of PCR tissue volume of C₄ species reflects the physiological requirement for accommodating the numerous and large organelles that are involved in the C₄ cycle and are required for generating high levels of CO₂ around Rubisco. This finding explains the observed increase in the percentage of PCR tissue in S. vermiculata under the high saline at site-4 compared to low saline at site-3. Unexpectedly, the percentage of PCR tissue (bundle sheath BS) in S. vera and S. pruinosa which characterized by free of plastids, was increased but this increasing does not reach the significant values. The authors suggest an extensive study of all types of enzymes in the PCR or bundle sheath cells in leaves of S. vera and S. pruinosa to clarify the reasons for the observed increase in adverse conditions in site-2.

A well developed WST was recorded in the leaves of C₄ species (S. vermiculata) in comparison with S. vera and S. pruinosa. Although the reduction in WST tissue area in all studied species collected from high saline habitats, the percentage of WST showed no significant difference between the tested saline habitats in S. pruinosa and S. vermiculata. Succulence causes a dilution effect upon the salts accumulated in plants, upon the toxic ions from the cells, thus permitting the plant to cope with higher salt amounts (Abd El- Maboud, 2011).

Data in Table (2) revealed that the area of vascular bundles tissues for S. vera was nonsignificant difference while it was increased in leaves of S. pruinosa under the high saline habitats in site-2 compared to site-1. These results were combined with increasing in number in xylem strands in the median bundle. This feature enhanced the water conduction and seemed to be estimated for its better survival under harsh saline environments. This observation is also recorded in the proportion of the vascular bundles area of S. vera and S. pruinosa. Although, the vascular bundles area decreased in leaves of S. vermiculata (Table 2) collected from site-4 compared to those from site-3, the proportion of these tissues keep stability under adverse moisture limited condition.

Metaxylem inner diameter decreased in S. pruinosa and S. vermiculata under high salinity stress at site-2 and site-4 Table (2), this decrease does not reach the significant value in S. vera. Decrease metaxylem can be immensely important under harsh climates like drought as was reported by Vavilatti et al. (2001) reported decreasing metaxylem vessels diameter, thus lowering the risk of embolisms and increasing water-flow resistance. These plastic responses may confer an ability of S. vera, S. pruinosa and S. vermiculata from the high saline habitats site-2 & 4 to withstand sudden events drought.

Data of the present study indicated that, degree of succulence in S. pruinosa was stable under saline habitats while it decreased by high saline habitats (site-2 &4) in S. vera and S. vermiculata. In this regard Story and Jones (1979) found that succulence of Saeda monoica increase at low salinity reflecting an increase in the water content of the tissue (leaf and root) and reduce at high salinity. Regarding the decrease in chl. a in both S. vera and S. pruinosa associated with reduction in the percentage of PCA tissues under salt marshes habitat Flexas et al. (2004) and Chaves et al. (2009) reported that, the decrease in photosynthetic rate under stressful conditions (salinity, drought and temperature) is normally attributed to a suppression in the mesophyll conductance and stomata closer at moderate and sever stress. As respect to S. vermiculata, data in Table (5) showed decrease in photosynthetic pigments at site-4 associate with the decrease in succulence. Reduction in photosynthetic pigments, such as chl. a, chl. b and carotenoids by salinity has been reported by many authors (Youssef, 2009; Samiullah and Bano, 2011). Moreover Cao et al. (2005) reported that carotenoids concentration of Suaeda salsa decreased by salinity and drought stress indicating that carotenoid cleavage dioxygenase enzyme may be involved in the carotenoid catabolism.

ABA content was found to be increase in both of S. vera and S. vermiculata at site-2 and site-4 respectively. While it decreases in S. pruinosa at site-2 under saline conditions. ABA can be considered as a physiological marker to salinity stress in S. vera and S. vermiculata. Taiz and Zeiger (2002) reported that, high concentration of salts can stimulate ABA production and movement of ABA to leaves. ABA is well known to be involved in physiological processes elicited or enhanced by stress causing dehydration (Pons et al., 2013). A reversible relation was observed between ABA and carotenoids in S. vermiculata. ABA is synthesized from b-carotene through several enzymatic steps. The salinity stress-induced activation of many ABA biosynthetic genes, such
as zeaxanthin oxidase, 9-cis-epoxycarotenoid dioxygenase, and ABA-aldehyde oxidase, appear to be regulated through a calcium-dependent phosphorylation pathway (Zhu, 2002; Chinnusamy et al., 2004). Also, a reversible relation was observed between succulence and ABA in the three studied species of *Suaeda*. High ABA level has been found under salinity stress to cause an increase in cytosolic Ca\(^{2+}\) and activation of plasma membrane-localized anion channels (Kohler and Blatt 2002). This, in turn, causes potassium efflux, guard cell depolarization, loss of guard cell volume and turgor, high \(\text{H}_2\text{O}_2\) production, and finally the stomata closure (Zhang et al. 2006).

**Conclusions:**

The *Suaeda* plants of each site exhibited halomorphic characteristics, specially from site-2 and site-4 (salt marshes) showed specific adaptation of leaf anatomical characteristics. These can be summarized as decreased leaf cross sectional area is a characteristic feature of salinity-adapted plants. Thick cuticle deposition accompanied with increased in the percentage of epidermal tissue and ABA is crucial for preventing water loss through leaf surfaces and acquired as succulent epidermis. The reduced in PCA area is adaptive feature for impairment light absorbed. Highly developed of vascular tissues but reduced in metaxylem cross-sectional area can easily be correlated to efficient water uptake and moisture column maintenance, and therefore, essential under harsh moisture climates. The amount of salt and ions in the soil, is important factor that enhance the adaptive potential of *Suaeda* species.

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**References**


