



Comparative study of the physiological performance of two species of *Atriplex* growing in the same ecological conditions

Taghried M. El-Lamey

Ecophysiology Unit, Plant Ecology and Ranges Department, Desert Research Center, Cairo, Egypt

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ABSTRACT

The naturally adapted salt - tolerant plants which are exposed to extreme environmental conditions in the arid and semi-arid regions around the Mediterranean basin possess morphological, anatomical, physiological and biochemical adaptation mechanisms to thrive on salt-affected soils. The aim of this study was to investigate the difference between anatomical structural and physiological adaptation mechanisms of *Atriplex nummularia* and *Atriplex halimus* in response to stress conditions. The results revealed that *Atriplex halimus* performed better under stress conditions than *Atriplex nummularia*. The important anatomical features of *Atriplex nummularia* and *Atriplex halimus* and other *Atriplex* spp. in relation to environmental stresses are the presence of vesiculated hairs on the leaf surface. *Atriplex halimus* had specific structural characteristics and different physiological mechanisms for adaption to environmental stresses. It showed a range of morphological and anatomical adaptive traits such as smaller leaf size, more succulent in stem and leaves, more development of vesicular hairs, more development of sclerenchymatic tissues in the hypodermis and in the cortex of the stem as well as in the vascular bundles and in-between the vascular bundles. Also, *Atriplex halimus* tended to accumulate more minerals (Na^+ , Cl^- , P and Mn^{2+}), proline and soluble sugars such as stachyose, mannitol and xylose in high concentration in dry season, which may play role in osmotic adjustment, while *Atriplex nummularia* accumulated more carbohydrates, glucose, xylose, fructose, mannitol and ribose. This study showed the most important anatomical features and physiological mechanisms of plants that are more tolerant to environmental stresses and subjected to natural selection.

Keywords: adaptation, anatomy, *Atriplex nummularia*, *Atriplex halimus*, stress.

1. Introduction

Under extreme environmental conditions in arid, semi- arid and seasonal coastal areas, a major constraint for plant growth is salinity. The naturally adapted salt - tolerant plants which are exposed to these conditions possess morphological, anatomical, physiological and biochemical adaptation mechanisms to thrive on salt-affected soils. The family of Chenopodiaceae (Amaranthaceae) contains 102 genera and 1400 species: most grow naturally in soils containing much salt (halophytes). Genera include *Beta* (6 spp.), *Chenopodium* (100–150 spp.), *Salicornia*, *Anabasis* and *Atriplex* (Evans, 2009). *Atriplex* is a large genus of the family Amaranthaceae, comprises more than 200 species (Kelley *et al.*, 1982), distributed widely in arid and semi-arid regions around the Mediterranean basin and all over the world (Le Houérou, 1992). Most of them grow in regions with annual rainfall ranging from 200 to 400mm/year (Abu-Zanat *et al.*, 2004). The xero-halophytes saltbushes (*Atriplex*) are well adapted to harsh environmental conditions and are used widely to provide forage due to their high content of protein (Le Houérou, 1986; Le Houérou, 1991).

The two species of *Atriplex*; *Atriplex nummularia* and *Atriplex halimus* are xero-halophytes perennial C_4 plants, capable of growing under saline or drought conditions (Le Houérou, 1986, 1992; Goodin and McKell, 1970; Goodin, 1979; Rogers *et al.*, 2005). They possess the C_4 photosynthetic pathway (Zarrinkamar, 2001), in which CO_2 is incorporated into the upper and lower epidermis of the leaf surface phosphoenolpyruvate (PEP) to form oxaloacetate through the action of PEP carboxylase.

Corresponding Author: Taghried M. El-Lamey, Ecophysiology Unit, Plant Ecology and Ranges Department, Desert Research Center, Cairo, Egypt. E-mail: lamytaghried_drc@yahoo.com

The activity of this carboxylating enzyme is increased by Na⁺, as it is required as an essential micronutrient (Osmond, 1970; Brownell and Crossland, 1972) for the translocation of pyruvate cross the chloroplast envelop in a number of C₄ species (Maathuis, and Amtmann, 1999).

The small number of C₄ plants, represent only about 5% of the Earth's plant biomass and 3% of known plant species (Bond *et al.*, 2005), and the agronomic and ecological importance of *Atriplex nummularia* and *Atriplex halimus* were among the important factors behind choosing these species as model species, and excellent materials for the investigation of anatomical and physiological mechanisms involved in resistance to abiotic stresses. As the identification of the adaptive mechanism of xero-halophytes species to cope with salt and /or drought is of great interest for scientists to identify the traits of stress tolerance plants. Also, the comparison of these closely related species of *Atriplex* is expected to give more information about the small differences or factors that contribute to the tolerance of these species to salt or drought stresses.

The aim of this study was to investigate the difference between anatomical structural and physiological adaptation mechanisms of *Atriplex nummularia* and *Atriplex halimus* in response to stress conditions.

2. Materials and Methods

2.1. Plant materials

The aerial parts of *Atriplex nummularia* and *Atriplex halimus* were collected from El-Hamam region, Mersa Matruh, Egypt in February and July 2019. The plant were identified and authenticated in the herbarium of Desert Research Center.

2.2. Ecological Studies:

2.2.1. Study area and climatic data

The study was carried out in El-Hamam region at Mersa Matruh (30°48'54.9"N 29°22'36.3"E). This area is classified as arid with mild winter and warm summer (UNESCO, 1977). The mean annual rainfall was 116.52mm (increasing from West to East and from South to North). During the period of study in 2019, the average annual temperature was 23.3 °C. The average annual maximum and minimum temperatures were 29.1 and 17.8 °C, respectively. While the total annual precipitation was 1.26 mm and the dry period extended from April to December. The annual average wind speed was 10.1 Km/h. The average annual humidity was 49.9%. These Meteorological data indicated that the studied habitat has an arid type of climate with high temperature especially during the dry period. Osborne *et al.* (2000) reported that the average annual temperature has increased by 0.75 °C during the past century and precipitation has shown marked variation throughout the Mediterranean basin.

2.2.2. Soil Analysis

Soil samples were collected from the soil supporting the investigated plants at 3 random points at two depths: the first depth from 0-10 cm and the second depth from 10-30 cm at El Hammam region. Soil texture (granulometric analysis) was determined using the international pipette method according to Gee and Bauder (1986) to determine the percentages of sand, silt and clay. Soil reaction (pH) in soil water suspension (1:2.5) was determined using pH meter instrument and the EC was measured by electrical conductivity meter. The concentrations of sodium and potassium in the soil solution were determined by using flame photometer (Jenway, PFP-7) and the concentrations of magnesium, calcium, carbonate and bicarbonate ions were determined according to the method of Rowell (1994). The concentration of chloride was determined according to the method of Jackson (1967).

2.3. Physiological studies

2.3.1. Plant water content

Plant water content, the difference between fresh weight (FW) and dry weight (DW), was calculated on a dry basis using the following formula:

$$\text{Plant water content (ml /100gDW)} = (\text{FW}-\text{DW})/\text{DW} \times 100 \dots\dots\dots(1)$$

2.3.2. Anatomical examination

Fresh samples of *Atriplex nummularia* and *Atriplex halimus* were kept in Ethyl Alcohol solution to fix and prepare them for anatomical studies. Four samples were sectioned by using microtome according to Paraffin Sectioning Method (Bani *et al.*, 2011; Mavi *et al.*, 2011). The staining slices were examined under Leica light microscope model DM-500, the images were obtained by using digital camera Leica ICC 50 HD with LAS E7 software version 2.1.0 2012.

2.3.3. Determination of photosynthesis pigments

The contents of photosynthesis pigments, chlorophyll-a (Chl a), chlorophyll-b (Chl b) and carotenoids were determined by the spectrophotometric method (Sumanta *et al.*, 2014). Half gram of fresh leaf was homogenized with 10 ml of 80% Acetone. The homogenate was centrifuged at 10,000 rpm for 15 min at 40°C. then 0.5 ml of supernatant was mixed with 4.5ml of solvent. The absorbance was read at 663, 644 and 452.5 nm using Unicam UV300 spectrophotometer. The contents of Chlorophyll-a, Chlorophyll-b and Carotenoids were calculated according to the following equations:

$$\text{Chl a} = 12.25A_{663.2} - 279A_{646.8} \dots\dots\dots(2)$$

$$\text{Chl b} = 21.5A_{646.8} - 5.1A_{663.2} \dots\dots\dots(3)$$

$$\text{C x+c} = (1000A_{470} - 1.82C_a - 85.02C_b)/198 \dots\dots\dots(4)$$

Where: A = Absorbance, Chl a = chlorophyll a, Chl. b = chlorophyll b, C x+c = carotenoids and the results were expressed as (mg/100gFW).

2.4. Plant Chemical Analysis

2.4.1. Preparation of samples

The aerial parts of *Atriplex nummularia* and *Atriplex halimus* were dried in the oven at 60 °C and ground to fine powder, then subjected to various analyses.

2.4.2. Mineral Analysis

Half gm of dried sample was digested with 10ml concentrated sulphuric acid on a hot plate at approximately 270 °C, (2-4ml) of perchloric acid was added until the digested solution become clear.

The solution was allowed to cool , then diluted to 100ml with distilled water and used for mineral analysis (Baker and Smith,1974).The concentrations of calcium, magnesium, potassium and sodium were determined according to the method of Rowell (1994), while the concentrations of manganese ,copper, zinc and iron were determined by using ICP emission spectroscopy (Jones, 1977). Whereas in the determination of the concentrations of phosphorus and chloride in the plant sample, the plant dry matter was ashed in porcelain crucibles in a muffle furnace at 550°C for 4-6 hours, part of ashed powder was dissolved in HCl to measure phosphorus contents in the plant by using phosphomolybdate methods (Rowell,1994), while the other part was dissolved in dilute nitric acid (0.01N) to extract chloride from ashed material, then titrated with standard silver nitrate (Jackson and Thomas, 1960). Nitrogen (N) content of sample was estimated by the method described by Kjeldahl (1983) and crude protein was calculated as N×6.25 (James, 1995).

2.4.3. Determination of total Carbohydrates

The total carbohydrates were extracted by dissolving 0.3gm of plant powder in 10ml of 3%HCl. The tube was sealed and heated at 100 °C for a period of 2-5 hours. The extracted sugars were estimated using the phenol–sulfuric acid assay (Buisse and Merck, 1993).

2.4.4. Determination of free proline

Free proline was determined according to Bates *et al.* (1973). Half gm of frozen plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. The homogenates were centrifuged at 6000rpm and filtrated. In a test tube, two ml of the filtrate was mixed with equal volumes of acetic acid and ninhydrin reagent (1.25 mg ninhydrin, 30ml of glacial acetic acid, 20ml 6M phosphoric acid) and incubated for 60 minutes at 100 °C. The reaction was terminated by placing the tube in an ice bath. Four ml of toluene was added with stirring. The light absorbance of the toluene was measured at 520nm. Proline concentration was determined using a standard curve of L-proline.

2.4.5. Determination of free sugars

2.4.5.1. Sample preparation

Free sugars in plant samples were determined by HPLC according to the method of (Zielinski *et al.*, 2014). The free sugars in plant samples were extracted by dissolving 1gm of plant powder in 10ml of Milli-Q water (type 1) and then filtered through a 0.22 µm filter membrane (Waters, Milford, MA, USA). An aliquot of 1.5 mL of these solutions was placed in vials for the analysis.

2.4.5.2. Equipment and operating conditions

The chromatographic system Agilent (series 1200) coupled to the refractive index detector was equipped with a quaternary pump, degasser and auto injector. The chromatographic data were acquired using the Agilent software. The samples obtained as described above were analyzed using an Aminex-carbohydrate HPX-87 column under isocratic condition with deionized water. The flow rate was 0.5 mL/ min. The column temperature was maintained at 85 °C and the detector at 50 °C. Sample detection was performed by comparing retention time's standards.

2.4.6. Determination of total phenolic content

Samples were prepared at a concentration of 20 mg/mL in EtOH 80%. The content of total phenolics in plant samples was determined using the Folin–Ciocalteu method as described by Attard (2013). Briefly, 10 µL of sample/standard was mixed with 100 µL of Folin-Ciocalteu reagent (Diluted 1: 10) in a 96-well microplate. Then, 80 µL of 4 N Na₂CO₃ was added and kept in dark for 20 minutes. The absorbance of blue colour was read at 630 nm. The obtained results were recorded using a microplate reader FluoStar Omega. Total phenolic content was calculated from the standard curve equation ($y = 4.1282x - 0.0894$ ($R^2 = 0.9925$)), where y is the absorbance at 630 nm and x is the concentration of gallic acid in mg/L. The results were expressed as milligrams gallic acid equivalent per gram dry weight used in extraction (mg GAE/g DW).

2.5. Statistical analysis

The data were subjected to two-way analysis of variance (ANOVA) and Duncan's multiple-range test ($P \leq 0.05$) using the statistical program, CoStat Version 6.311(CoHort soft-ware, Berkeley, CA 94701) according to Steel *et al.* (1980).

3. Results and Discussion

3.1. Plants description

Atriplex L. species are euhalophytes Herbs or shrubs. Leaves flat, generally alternate and frequently mealy, scaly or closely tomentose. Flowers polygamous, the male ones with 5-lobed perianth and 5 stamens, the female ones with the ovary included between 2 flat, leaf-like bracts, connate at base and later growing enclosing the fruit.

The morphological feature of two species of *Atriplex*; giant saltbush *Atriplex nummularia* and saltbush *Atriplex halimus* was described by Täckholm (1974) as follow:

Atriplex nummularia Lindley: robust, mostly dioecious shrub, up to 2-3 m in height with large thick rounded leaves, up to 5cm, long and often as board, frequently sharply dentate. Fruit-perianth rounded-cordate, papery, with eroded-dentate margin and a hardened base. *Atriplex nummularia* is introduced from Australia as a forage plant and naturalized.

Atriplex halimus L.: *A. halimus* is an upright perennial shrub, up to 3 m in height, branched from the base tall shrub, up to 3 m, with whitish-yellow, coriaceous leaves, the bark being grey-white in colour, and has leaves which are 10-30 mm long and 5-20 mm wide. The leaves are highly variable in form, ranging between deltoid-orbicular and lanceolate, and are attenuated at the base with a short petiole. *Atriplex halimus* is autochthonous and grows through the Mediterranean basin (Walker *et al.*, 2014).

3.2. The physical and chemical properties of the soil

The results of soil physical analysis revealed that the soil associated with *Atriplex* species at El-Hamam region was alkaline soil in nature. The soil pH with an alkaline reaction has been reported to cause nutrient deficiencies in some micronutrients such as phosphorous, nitrogen, copper, zinc,

manganese and iron, which may lead to negative impacts on the plant physiological mechanisms (Moore, 2004). As shown in Table 1, soil texture was sand, as the percentages of sand were 97.85% and 96.96% in the first (0-10cm) and second depths (10-30 cm), respectively. Others (Mojiri and Jalalian 2011; Mojiri *et al.*, 2011) reported that soil sand had a positive effect on plant physiological parameters, as it helps in penetration the roots to the deep soil.

The chemical analysis of soil indicated that the soil particles in the first depth contained the highest content of available soil nutrient (EC) mainly, Ca⁺⁺ (6.25 meql⁻¹), Na⁺ (8.88 meql⁻¹), Cl (4.85 meql⁻¹) and CaCO₃ (2.35%) compared with that in the second depth (Table 2).

Table 1: Soil physical properties

Location	Soil depth	Soil Particles Distribution			Soil Texture Class
		Sand%	Silt%	Clay%	
El -Hamam	0-10	97.85	1.68	0.47	Sand
	10-30	96.96	2.01	1.03	Sand

Table 2: Soil chemical properties

Soil depth	pH 1:2.5	EC dS/m	Cation (milliequivalent/Liter)				Anion (milliequivalent/Liter)		CaCO ₃ %
			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	Cl ⁻	HCO ₃ ⁻	
0-10	8.41	1.67	6.25	0.75	8.88	0.93	4.85	8.00	2.35
10-30	8.59	1.37	2.90	1.50	8.86	0.43	2.85	4.50	1.80

3.3. The effect of Seasonal variation on plant water content

As shown in Table 3, there was a significant difference in the content of water between seasons or species. The content of water was significantly decreased in dry to 63.0±3.68 (ml/100gDW) in *Atriplex nummularia* and to 71.10±6.60 (ml/100gDW) in *Atriplex halimus*, the decrease in the water content of plants in the dry season may attributed to the lack of rainfall and the plant exposure to prolonged drought periods of up to six months. This indicated that *Atriplex halimus* had the capacity to store water more than *Atriplex nummularia*.

Table 3: The effect of Seasonal variation in plant water content

<i>Atriplex nummularia</i> (ml/100gDW)		<i>Atriplex halimus</i> (ml/100gDW)	
Winter	Summer	Winter	Summer
138.8±2.17 ^a	63.0±3.68 ^c	101.8±17.13 ^b	71.10±6.60 ^c

Values are expressed as mean ± SD (n=3), in each row values followed different letters are significantly different at p<0.05

3.4. Anatomical characteristics

The transverse sections of the leaves of *Atriplex nummularia* and *Atriplex halimus* L. (Figs.1&2), showed that *Atriplex nummularia* and *Atriplex halimus* had the “Kranz” anatomy with a layer of bundle sheath cells surrounding each vascular bundle, which is considered as the main anatomical feature of the leaves of C₄ plants (Troughton and Card,1974; Jacobs, 2001). As the C₄ photosynthesis is associated with the compartmentation of photosynthesis between mesophyll and bundle sheath.

As shown in Figs. (1) and (2), the upper and lower epidermis of the leaf surface in all the transverse sections of *Atriplex* specimens lamina were covered with vesiculated trichomes (Smaoui, *et al.*, 2011) or vesiculated hairs (Zarrinkamar,2001). The vesiculated hair consists of one bladder cell and several stalk cells, embedded in an epidermal cell. These salt bladders are considered as important anatomical feature of *Atriplex* species, which enable them to grow under salinity stress. They act as salt containers, insulators for heat and water storage tissues. More than 50% of Na⁺ and Cl⁻ are translocated to the hair (Osmond *et al.*, 1980) to protect the photosynthetic tissues and to help in maintaining the concentration of K⁺.

The average thickness of the leaf midrib, average length and average width of main vascular bundle were significantly different between species or seasons (Table 4). The average thickness of the leaf midrib of *Atriplex nummularia* was 500.16±7.90 μm in winter and significantly increased to 513.46±3.44 μm in summer, while that of *Atriplex halimus* was 358.08±7.35 μm in winter and increased to 571.37±6.76μm in dry season. succulence in the terms of leaf thickness seems to be the most adaptive

mechanism as reported in salt tolerant grass, *Imperata cylindrica* according to Hameed *et al.* (2009). The increased leaf succulence of *Atriplex halimus* was due to the enlargement of the hypodermis and palisade parenchyma (Fig.2)

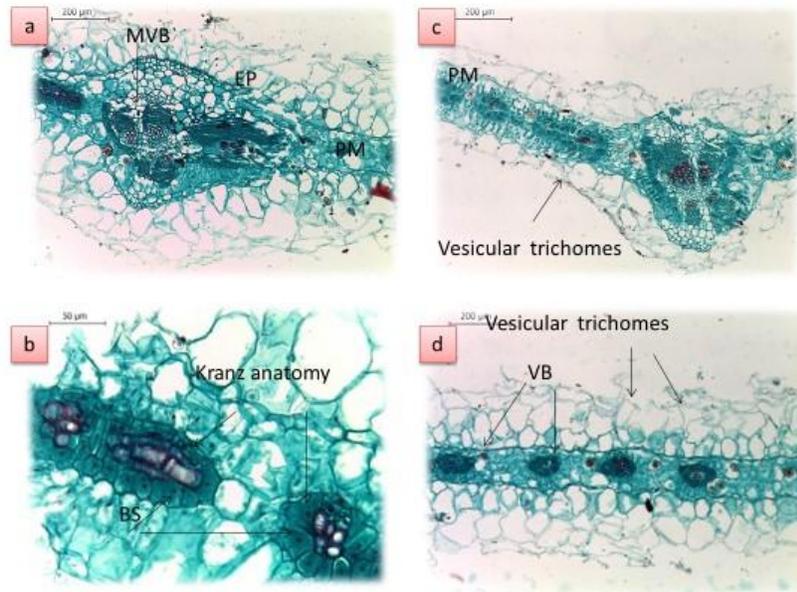


Fig 1: Transverse sections of the lamina of *Atriplex nummularia* in summer (a-b) and winter (c-d); Ep: epidermis, BS: Bundle sheath, Ca O: Calcium oxalate, PM: Palisade Mesophyll, Ph: Phloem, Scl : Sclerenchyma, VB: Vascular bundle, Xy: Xylem(Scale 50, 200μm)

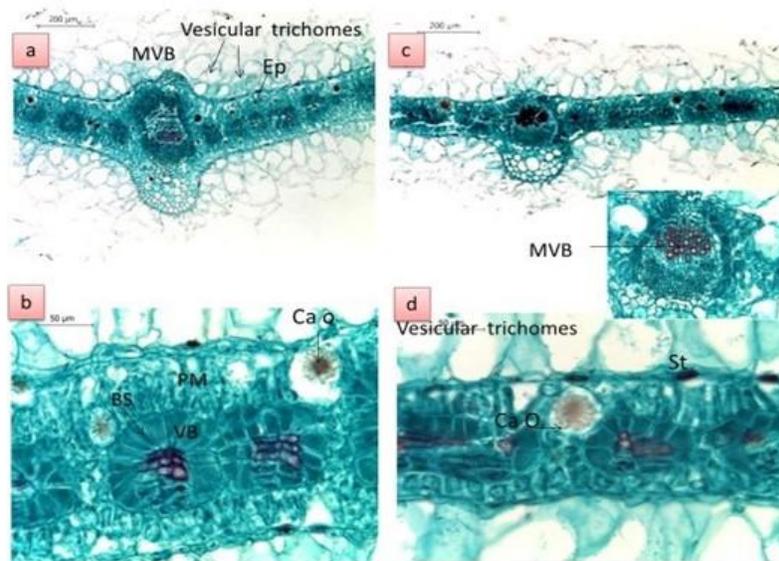


Fig. 2: Transverse sections of the lamina of *Atriplex halimus* in summer (a-b) and winter (c-d) Ep: epidermis, BS: Bundle sheath Ca O: Calcium oxalate, PM: Palisade Mesophyll, Ph: Phloem, Scl: Sclerenchyma, VB: Vascular bundle, Xy: Xylem (Scale 50,200μm)

Table 4: The effect of seasonal variations on the anatomical characters of *Atriplex nummularia* and *Atriplex halimus*

Characters	<i>Atriplex nummularia</i>		<i>Atriplex halimus</i>	
	Winter	Summer	Winter	Summer
Average Length of leaf vascular bundle (VB) (μm)	63.72 \pm 6.29 ^d	70.25 \pm 0.12 ^b	68.43 \pm 1.90 ^c	76.89 \pm 3.18 ^a
Average Width of leaf VB (μm)	111.7 \pm 5.88 ^b	118.10 \pm 2.45 ^a	53.32 \pm 1.69 ^d	58.03 \pm 3.63 ^c
Average thickness of the leaf midrib (μm)	500.16 \pm 7.90 ^c	513.46 \pm 3.44 ^b	358.08 \pm 7.35 ^d	571.37 \pm 6.76 ^a
Average diameter of stem (mm)	2.07 \pm 0.08 ^a	1.42 \pm 0.002 ^b	1.20 \pm 0.14 ^c	1.182 \pm 0.03 ^c
Average distance between the opposite VB in stem	521.1 \pm 85.9 ^b	634.08 \pm 92.3 ^a	374.57 \pm 18.1 ^c	511.36 \pm 15.1 ^b
Average Length of stem vascular bundle (VB) (μm)	256.31 \pm 17.3 ^a	192.49 \pm 2.79 ^c	238.03 \pm 8.73 ^b	199.54 \pm 3.28 ^c

Values are expressed as mean \pm SD (n=3), in each row values followed different letters are significantly different at $p < 0.05$

As shown in Fig. (3), the average area of the leaf surface was significantly different between species. Its values were $1 \pm 0.173 \text{ cm}^2$ in *Atriplex nummularia* and $0.26 \pm 0.057 \text{ cm}^2$ in *Atriplex halimus*. Small leaves are expected to have many advantages in regulating the leaf temperature in hot and dry environments to avoid overheating (Niinemets and Kull, 1994) and have adaptive value for plants evolved for hot environments. Furthermore, the decrease in leaf size with decreasing water availability has been reported by others (Basal *et al.*, 2005; Cramer *et al.*, 2009). Previous studies confirmed that the leaf size variation can significantly modify the integrative photosynthetic activity of the whole leaves, that is, generally higher the mass-based photosynthetic activity of smaller leaves (Poorter and Evans, 1998; Niinemets *et al.*, 2006, 2007). Thus, Leaf size may be a direct product of natural optimum selection (Kleiman and Aarssen, 2007; Tozer *et al.*, 2015).



Fig. 3: Morphology of *Atriplex nummularia* (a) and *Atriplex halimus* (b)

As Shown in Figs. (4) & (5), stress conditions in summer season induced significant alternation in stem anatomy of both species of *Atriplex*. The hypodermal thickness and pith area increased significantly with stress. Whereas the stems of most species of vascular plants form a single cylindrical vascular cambium, the stem of *Atriplex nummularia* and *A. halimus* showed an increase in diameter in wet season through the meristematic activity of several successive cambia (Fahn and Zimmermann, 1982).

The transverse sections of the stem of *Atriplex nummularia* and *Atriplex halimus* L. showed the presence of vesiculated trichomes connected with the epidermal cell of the stem surface. Average diameter of stem of *Atriplex nummularia* was $2.07 \pm 0.08 \text{ mm}$ in winter and significantly decreased to $1.42 \pm 0.002 \text{ mm}$ in summer, while in *Atriplex halimus* L., there was no significant change in stem diameter between seasons.

The vascular bundle is arranged regularly and collaterally forming a circuit around the cell of the pith (parenchymatic cells) and attached with sclerenchymatic cells. In comparing the stem sections of the two species of *Atriplex* in winter and summer seasons, it was observed that the thickness of the cell

wall and the number of sclerenchymatic cells, that are in the cortex region and in-between stem vascular bundles were increased significantly in the summer season. Which may protect against water loss (Yentür, 2003; Makbul *et al.*, 2011). The induce of sclerification by salt stress has been reported in other plants such as *Spartina alterniflora* (Walsh, 1990) and *Imperata cylindrica* (Hameed *et al.*,2009).

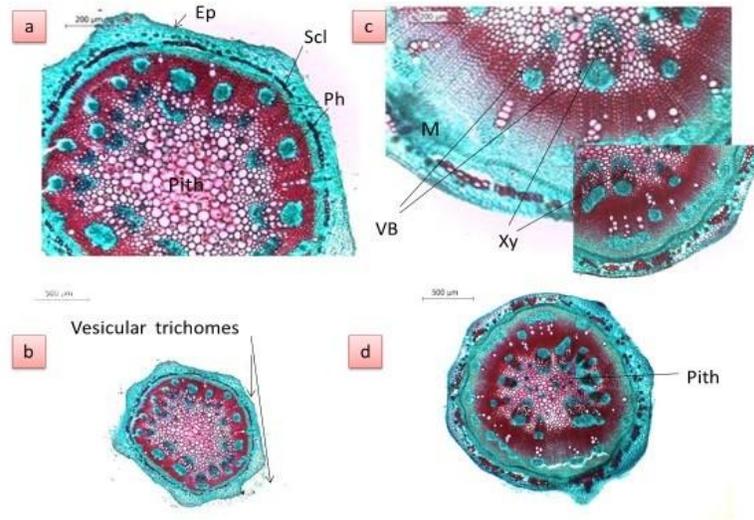


Fig. 4: Transverse sections of the stem of *Atriplex nummularia* in summer (a-b) and winter (c-d) Ep: epidermis, M: Mesophyll, Ph: Phloem, Scl: Sclerenchyma, VB: Vascular bundle, Xy: Xylem (Scale 200,500µm).

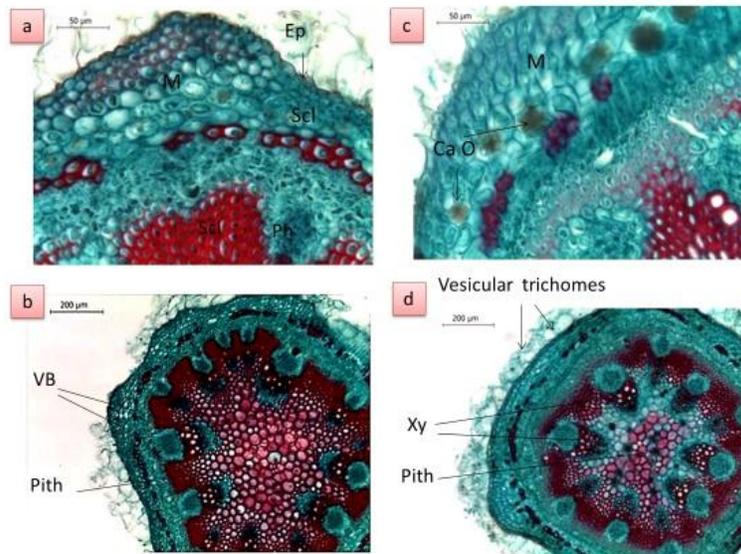


Fig. 5: Transverse sections of the stem of *Atriplex halimus* in summer (a-b) and winter (c-d); Ep: epidermis, Ca O: Calcium oxalate, M: Mesophyll, Ph: Phloem, Scl : Sclerenchyma, VB: Vascular bundle, Xy: Xylem (Scale50, 200 µm).

The average length of the stem vascular bundle of two species of *Atriplex* was decreased significantly in response to stress conditions. All the stem and leaf sections were characterized by the presence of crystals of calcium oxalate in the cortex of the stem and in the parenchymatic tissues of the pith as well as in the palisade cells of the leaf.

3.5. The effect of seasonal variations on the content of photosynthetic pigments in *Atriplex* species

As shown in Table (5), under stress conditions in summer season, the concentration of Chl a was decreased significantly ($p < 0.05$) in *Atriplex nummularia* and *Atriplex halimus* to 5.53 ± 0.70 and 2.53 ± 0.30 mg/100g FW, while the concentration of Chl b was significantly increased by stress to 9.60 ± 0.72 and 3.53 ± 0.75 mg/100g FW, respectively.

Measuring the content of essential photosynthetic pigments in plants may help in indicating the efficiency of the photosynthetic process and the rate of energy utilization, it also assists in identifying the physiological performance of the two species of *Atriplex* under stress conditions. According to Sairam *et al.*, (1997), this reduction in the content of primary photosynthetic pigment (Chl a) under water deficit have been reported for drought-tolerant plants and stress-tolerant genotypes of some crop species. Also, the ratio of Chl a to Chl b in the two species of *Atriplex* was significantly decreased in the summer season. The same attitude has been recorded in the resistant species of tomato under water stress, which may indicate that photosystem II may have a vital role in protecting the plant against heat and water stress (Ghorbanli *et al.*, 2013).

Regarding the content of carotenoids, the obtained results indicated that there was a significant decrease in the concentration of carotenoids by stress in *Atriplex nummularia* and a significant increase in *Atriplex halimus*. The ability of *Atriplex halimus* to maintain a high content of carotenoid gives the plant a competitive trait that makes it more adaptable to the desert environment than *Atriplex nummularia*. Due to the antioxidant activity of carotenoid and its capacity to quenching of 1O_2 and peroxy radicals (Demmig and Adams, 1996), which may protect cellular components from photo-oxidative damage during environmental stresses. A previous study performed by Knox and Dodge (1985) proved this important role of carotenoids and revealed that the inhibition of its biosynthesis by norflurazon led to an increase in the production of free radicals and caused oxidative damage to the plant cell.

Table 5: The effect of seasonal variations on the concentration of photosynthetic pigments in *Atriplex nummularia* and *Atriplex halimus*

Plant species	Season	Chlorophyll a (mg/100g FW)	Chlorophyll b (mg/100g FW)	Carotenoids (mg/100g FW)	Chl a/Chl b
<i>Atriplex nummularia</i>	Winter	12.5 ± 0.80^a	1.26 ± 0.23^c	4.20 ± 0.34^b	9.98 ± 2.33^a
	Summer	5.53 ± 0.70^c	9.6 ± 0.72^a	2.26 ± 0.46^c	0.58 ± 0.08^b
<i>Atriplex halimus</i>	Winter	7.76 ± 0.23^b	1.1 ± 0.34^c	0.86 ± 0.11^d	7.12 ± 2.47^a
	Summer	2.53 ± 0.30^d	3.5 ± 0.75^b	5.13 ± 1.47^a	0.71 ± 0.12^b

Values are expressed as mean \pm SD (n=3), in each column values followed different letters are significantly different at $p < 0.05$

3.6. The effect of seasonal variations on the content of minerals in *Atriplex* species

As shown in Table (6), the concentrations of Na^+ and Cl^- were significantly higher in *A. halimus* than in *A. nummularia*, under stress conditions in summer season. Their values were 4.34 ± 0.05 and 6.17 ± 0.01 g/100g in *A. halimus*, while in *A. nummularia*, their values were 2.92 ± 0.05 and 4.29 ± 0.03 g/100g, respectively. Like other halophytes, *A. halimus* accumulates the ionic osmolytes, such as Na^+ , (Nemat Alla *et al.*, 2012) Cl^- and other ions (Ben Ahmed *et al.*, 1996; Ben Hassine *et al.*, 2009) in the vacuole in response to salinity. Kader *et al.* (2011) suggested that the accumulation of Na^+ in cell vacuole improved the growth by increasing cell volume and enhancement of photosynthesis and sugar synthesis through increasing the photoassimilatory surface area of the leaves (Martinez *et al.*, 2005). Many species of *Atriplex* have been reported Na^+ and Cl^- accumulation in large amounts, such as *A. nummularia* (Uchiyama, 1987; Ramo *et al.*, 2004), *A. barclayana* (Nerd and Pasternak, 1992) and *A. hortensis* (Wilson *et al.*, 2000). An increase of ion accumulation for osmotic adjustment to decrease the plant osmotic potential is a common trait in many halophytic species such as *A. triangularis* (Karimi and Ungar, 1984), *A. semibacata* (Viliers *et al.*, 1996) and *A. griffithii* (Khan *et al.*, 2000). The accumulation of ions in the vacuole provides cheap solutes for osmotic adjustment, as it is less energy and carbon demanding compared to the adjustment by organic solutes (Flowers *et al.*, 1977; Wyn Jones, 1981; Munns, 2002).

The level of K^+ of both species of *Atriplex* was significantly reduced in dry season, its value was decreased significantly from 3.05 ± 0.04 g/100g in winter to 1.85 ± 0.04 g/100g in summer in *A.*

nummularia, and from 2.04±0.02 to 1.82±0.05 g/100g in *A. halimus*. The results indicated that the ratio of Na⁺ to K⁺ in the two species of *Atriplex* was significantly different between species or seasons, where its value in the summer season in *A. halimus* (2.37±0.08) was much higher than that of *A. nummularia* (1.57±0.05). Due to the similarity of hydrated ionic radii (Tester and Davenport, 2003; Munns, 2005), Na⁺ competes with K⁺, which cause the uptake of K⁺ to decrease (Nakamura *et al.*, 1990; Marschner,1995). This ratio is mainly influenced by changes in Na⁺ concentration, which are usually relatively much larger than changes in K⁺, as reported by Negrão *et al.* (2017). The maintenance of a low ratio of Na⁺ to K⁺ helps plants grow well under saline conditions (Ashraf, 2004).

The increased content of Na⁺ in the aerial parts in most plants is generally associated with relatively low content of K⁺, Ca²⁺ (Munns and Tester ,2008) and /or Mg²⁺ (Bajji *et al.*,1998). Thus, the concentrations of Ca²⁺ and Mg²⁺ were significantly decreased in the two species of *Atriplex* in response to stress conditions. This physiological disturbance stimulates the antioxidant system. The ratio of Na⁺ to Ca²⁺ was significantly higher in *A. halimus* (6.57±0.35 g %) than in *A. nummularia* (4.00±0.25g%), under stress condition in summer season. The concentrations of micronutrient Fe²⁺, Mn²⁺ and Cu²⁺ were increased significantly in dry season.

Table 6: The effect of seasonal variations on the concentrations of minerals in *Atriplex nummularia* and *Atriplex halimus*

Minerals	<i>Atriplex nummularia</i>		<i>Atriplex halimus</i>	
	Winter	Summer	Winter	Summer
Nitrogen (N) g%	1.83±0.14 ^a	1.01±0.04 ^b	1.30±0.03 ^{ab}	1.54±0.01 ^a
Sodium (Na) g%	4.58±0.05 ^a	2.92±0.05 ^d	3.50±0.03 ^c	4.34±0.05 ^b
Potassium(K) g%	3.05±0.04 ^a	1.85±0.04 ^c	2.04±0.02 ^b	1.82±0.05 ^c
Na/K	1.50±0.02 ^d	1.57±0.05 ^c	1.71±0.03 ^b	2.37±0.08 ^a
Calcium (Ca) g%	0.92±0.04 ^a	0.73±0.03 ^b	0.77±0.02 ^c	0.66±0.04 ^d
Na/Ca	4.98±0.23 ^b	4.00±0.25 ^d	4.41±0.15 ^c	6.57±0.35 ^a
Magnesium (Mg) g%	1.02±0.01 ^a	0.41±0.03 ^d	0.90±0.03 ^a	0.62±0.04 ^c
Phosphorus (P) g%	0.08±0.01 ^b	0.06±0.01 ^c	0.09±0.03 ^b	0.13±0.02 ^a
Chloride (Cl) g%	5.53±0.02 ^b	4.29±0.03 ^c	3.72±0.05 ^d	6.17±0.01 ^a
Iron (Fe) g%	0.24±0.004 ^c	0.42±0.002 ^a	0.22±0.001 ^d	0.28±0.001 ^b
Manganese (Mn) mg/ 100g	16.5±0.12 ^d	24.0±0.59 ^b	19.6±0.18 ^c	47.5±0.04 ^a
Zinc (Zn) mg/100g	25.70±0.28 ^a	18.11±0.25 ^c	23.02±0.08 ^b	15.73±0.08 ^d
Copper (Cu) mg/100g	18.6±0.11 ^b	23.1±0.70 ^a	16.18±0.02 ^c	19.2±0.15 ^b

Values are expressed as mean ± SD (n=3), in each row values followed different letters are significantly different at p<0.05

3.7. The effect of seasonal variations on the concentrations of total carbohydrates and soluble sugars in *Atriplex* species

Regarding the concentrations of organic osmolytes (Table7), there was a significant difference between species or seasons in the content of total carbohydrates, its values were increased significantly in *A. nummularia* from 4.19±0.10 in winter to 4.68±0.06 g/100g in summer. Whereas in *A. halimus*, its values were decreased significantly by stress from 4.98±0.07 in winter to 4.86±0.04 g/100g in summer season. *Atriplex nummularia* respond to stress condition in summer season by accumulation of glucose, xylose, fructose, mannitol and ribose. Whereas in *A. halimus*, the soluble sugars that increased in concentration in summer season were stachyose, mannitol and xylose. The accumulation of compatible osmolytes assists in maintaining tissue turgor and generates low values of plant water potential (more negative than the external medium) to insure the water uptake (Kan *et al.*, 2000; Kamel, 2007).

3.8. The effect of seasonal variations on the contents of proline and protein in *Atriplex* species

As shown in Table 8, the content of proline was significantly different between species or seasons. Where, the value of proline was significantly reduced in the dry season in *A. nummularia*, while in *A. halimus*, its value was significantly increased in response to stress conditions. The higher proline accumulation in *A. halimus* in response to salinity or drought could have been one of the important factors in adaption of *A. halimus* to adverse environmental conditions, this result agrees with Bajji *et al.*, (1998). Various studies have been reported the accumulation of proline as a compatible organic osmotic in the cytoplasm in plants under stress condition to maintain an osmotic equilibrium across the

tonoplast (Matinez *et al.*, 2003, 2004; Mansou *et al.*, 2005; Desingh and kanagaraj, 2007). In addition to the role of proline as an osmoticum, it acts as an osmo-protectant and involved in stabilizing cellular membranes, protecting proteins and enzymes (Rudolph *et al.*, 1986; Gadallah, 1999; Vinocur and Altman, 2005). It also has the capacity to scavenge free-radical (Lin *et al.*, 2002; Szabados and Savoure, 2010)

Table 7: The effect of seasonal variations on the concentrations of total carbohydrates and soluble sugars in *Atriplex nummularia* and *Atriplex halimus*

Plant species	Season	Total carbohydrates g/100g	Glucose g/100g	Xylose g/100g	Fructose g/100g	Mannitol g/100g	Ribose g/100g	Stachyose g/100g	Sucrose g/100g
<i>Atriplex nummularia</i>	Winter	4.19±0.10 ^d	0.041	0.058	0.166	0.265	0.003	6.670	0.749
	Summer	4.68±0.06 ^c	0.540	0.124	0.792	3.187	0.037	5.356	-
<i>Atriplex halimus</i>	Winter	4.98±0.07 ^a	0.057	0.093	0.329	0.152	0.027	6.960	0.957
	Summer	4.86±0.04 ^b	0.026	0.287	0.197	0.651	0.004	10.670	0.932

Values are expressed as mean ± SD (n=3), in each column values followed different letters are significantly different at p<0.05

Table 8: The effect of seasonal variations on the contents of proline and protein in *Atriplex nummularia* and *Atriplex halimus*

Species	Season	Proline mg/g	Protein (mg/g)
<i>Atriplex nummularia</i>	Winter	9.03±0.08 ^b	114.3±8.70 ^a
	Summer	4.18±0.23 ^d	62.8±2.88 ^d
<i>Atriplex halimus</i>	Winter	7.59±0.23 ^c	81.4±1.94 ^c
	Summer	10.33±0.26 ^a	96.6±0.69 ^b

Values are expressed as mean ± SD (n=3), in each column values followed different letters are significantly different at p<0.0

Regarding the concentrations of protein, there was a significant difference between species or seasons. The value of protein was decreased significantly in *A. nummularia* from 114.3±8.70 mg/g in winter to 62.8±2.88 mg/g in summer. Whereas in *A. halimus*, its values were increased significantly in response to stress conditions from 81.4±1.94 mg/g in winter to 96.6±0.6 mg/g in summer season.

3.9. The effect of seasonal variations on the content of total phenolics in *Atriplex* species

As shown in Table (9), the content of total phenolics was significantly different between species or seasons. The highest values of total phenolic content were detected in *A. nummularia* (4.88±0.25mg g⁻¹) and in *A. halimus* (4.19±0.18 mg g⁻¹) in the dry season. The ability of *Atriplex* spp. to accumulate a high content of total phenolics under stress conditions gives the plants a competitive trait that makes them more adaptable to the desert environment and oxidative stress. Due to the ability of these compounds to scavenge free radicals and reactive oxygen species and form complexes with the metals that inhibit the activity of oxidizing enzymes and catalyze oxygenation reaction (Sokół-Łętowska, 1997).

Table 9: The effect of seasonal variations on the concentration of total phenolics in *Atriplex nummularia* and *Atriplex halimus*

Plant species	Season	Total Phenolics (mg/g)
<i>Atriplex nummularia</i>	Winter	3.71±0.11 ^c
	Summer	4.88±0.25 ^a
<i>Atriplex halimus</i>	Winter	3.21±0.21 ^d
	Summer	4.19±0.18 ^b

Values are expressed as mean ± SD (n=3), in each column values followed different letters are significantly different at p<0.05

4. General Conclusion

The two species of *Atriplex* showed a wide range of anatomical features that aid the plant to be widely distributed in arid and semi-arid regions around the Mediterranean basin, these anatomical

features included thick epidermis, development of vesiculated trichomes on the leaf and stem surfaces, development of sclerenchymatic tissues in vascular bundles and in-between the vascular bundles, in the hypodermis as well as in the cortex of the stem and the leaf mesophyll tissues. Vesiculated trichomes consists of bladder cells, where the salts are accumulated. When the concentration of salt reached the optimum concentration within these cells, they burst and eliminate the salts.

The anatomical examination of the leaf and stem sections by light microscope indicated the presence of anatomical variations between the two species of *Atriplex*. These variations were related to the size of leaf, average diameter of stem, average thickness of the leaf midrib, development of sclerenchymatic cells and development of vesiculated trichomes.

The results indicated that each species of *Atriplex* exhibited a different adaptive mechanism according to genetic structures.

The results showed the anatomical differences between *Atriplex* spp. that can be associated with their degree of adaptation to external conditions and revealed that *Atriplex halimus* performed better under stress conditions than *Atriplex nummularia*. The wide distribution of *Atriplex halimus* and field observation supported this conclusion. Similarly, Ayyad and El- Ghareeb (1982) demonstrated that *Atriplex halimus* dominant on elevated, well-drained, saline soils and considered as one of the dominant species in the salt marshes of the western Mediterranean desert of Egypt, in the transitional zones between areas of shallow and deep-water tables. Also, Cañadas *et al.* (2010) found that *A. halimus* was the dominant species on the soils of higher salinity. Furthermore, the germination of *A. halimus* seems to be more resistant to salinity than that of *A. canescens* or *Atriplex nummularia* Lindl as reported by Máalen and Rahmoune, (2009).

The results revealed that *Atriplex halimus* has specific structural characteristics and different physiological mechanisms for adaption to environmental stresses. It showed a range of morphological and anatomical adaptive traits such as smaller leaf size, more succulent in stem and leaves, more development of vesicular hairs, more development of sclerenchymatic tissues in vascular bundles and in-between the vascular bundles, in the hypodermis as well as in the cortex of the stem.

The adaptation of *Atriplex halimus* to the arid environment in term of osmotic adjustment was the most important adaptation mechanism to maintain the water potential more negative than the external medium to ensure the continuous uptake of water. The results revealed that *Atriplex halimus* tended to accumulate more minerals (Na^+ , Cl^- , P and Mn^{2+}), proline and soluble sugars such as stachyose, mannitol and xylose in high concentrations in dry season, which may play role in osmotic adjustment, while *Atriplex nummularia* accumulated more carbohydrates, glucose, xylose, fructose, mannitol and ribose.

The ability of *Atriplex* spp. to accumulate a high content of total phenolics under stress conditions gives the plants a competitive trait that makes them more adaptable to the desert environment and oxidative stress.

The study showed the most important anatomical features and physiological mechanisms of plants that are more tolerant to environmental stresses and subjected to natural selection.

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