**Anatomical Response of Atriplex Leaves under Different Levels of Sodium Chloride Salinity**

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**Abstract:** Salinity causes physiological, morphological, and anatomical modifications in Mediterranean saltbush *Atriplex halimus* and giant saltbush *Atriplex nummularia*. Both species, which belong to Chenopodiaceae, are true xerophytes as shown from Kranz-anatomy and salt storage trichomes. Response of both species to salinity were differed according to genetic structures. *Atriplex* species leaves characteristic by presence of salt accumulating cells on upper and lower leaf epidermis, which have ecological significance. Increasing of salinity had negative effect on anatomical measurements of *A. halimus* leaves. However, low level of salinity had positive effect in *A. nummularia* leaves, but high level of had negative effect on leaves.

**Keywords:** *Atriplex halimus* - *Atriplex nummularia* - salt stress - leaf anatomy

**INTRODUCTION**

Salinity is one of the most important topics in eco-agriculture system, which adversely affected more than 50% of crop production of the world. Salinity stress is more complicated in arid regions, caused functional, morphological, and anatomical modifications in plants (Marius-Nicušor Grigore and Toma, 2020; Keshavarzi, 2020). *Atriplex* is species-rich genus of about 300 species belong to the Chenopodiaceae, the main distribution of the family lies in the Old World. *Atriplex* species are euhalophytes, annual or perennial herbs, sub shrubs or shrubs (Kühn et al., 1993). Mediterranean saltbush *Atriplex halimus* is an evergreen fodder shrub, while giant saltbush *Atriplex nummularia* is woody perennials. Both species are true xerophyte and cultivated as salt resistant forage in ggreenhouse, and exposed to salt-water irrigation treatments, continued for six months treated with saline water every month (4 times). Soil physical and chemical properties measured according to (Cassel and Nielsen, 1986; Rhoades and Oster, 1982) as shown in Table (1).

**Table (1):** Physical and chemical properties of the soil

| Measurements               | Soil      |
|----------------------------|-----------|
| pH                         | 8.40      |
| EC (ds/m)                  | 9.1       |
| Soluble Cations meq/L      |           |
| Ca$^{2+}$                  | 35.0      |
| Mg$^{2+}$                  | 19.5      |
| Na$^+$                     | 34.3      |
| K$^+$                      | 2.0       |
| Soluble Anions meq/L       |           |
| CO$_3^{2-}$                | 0.125     |
| HCO$_3^-$                  | 3.5       |
| CI$^-$                     | 56.3      |
| SO$_4^{2-}$                | 21.9      |
| Saturation point %         | 27.3      |
| Pot capacity (at 1 bar)    | 13.6      |
| Wilting point (at 15 bars) | 6.8       |

**MATERIALS AND METHODS**

**Plant Material and Treatments:**

Seeds of both *Atriplex nummularia* and *Atriplex halimus* were cultivated under greenhouse conditions with thermos period 32:17°C fluctuation (day and night) and irrigated as normal agricultural practices. Seedlings (After occurrence of the first five true leaves) transferred to 30 cm diameter plastic pots filled with sandy loam by ratio 1:1, moved out of the
epidermis by micrometer (µ), average thickness of lower epidermis (µ), average thickness of the mesophyll (µ), average thickness of the midrib by millimeter (mm), average thickness (anticlinal diameter) of main vascular bundle MVB (mm), average width of MVB (mm), average thickness of xylem tissue per MVB (µ), average diameter of xylem vessels per MVB (µ) and average thickness of phloem tissue per MVB (µ).

**Statistical analysis:**

The data were subjected to One-way analysis of variance (ANOVA) one using CoStat Version 6.311 (CoHort soft- ware, Berkeley, CA 94701) according to (Steel, Torrie, & Dickey, 1980) with probability ≤ 0.05.

**RESULTS AND DISCUSSION**

The leaves of all treatments (control and different concentration of NaCl) of *Atriplex* specimens of the two species, *Atriplex halimus* and *A. nummularia*, transverse sections showed the presence of Kranz-type (assimilated sheath) leaf anatomy as seen in Figure 1. The vascular surrounded by two layers of bundle-sheath cells, rich in chloroplasts, with bundle sheath cells seems densely stained (Fig. 1a). (Frankton and Bassett, 1970; Jacobs, 2001; Troughton and Card, 1974) reported that C4 plants had Kranz cells or bundle sheath such as *Atriplex halimus* and *A. nummularia*. All transverse sections of *Atriplex* specimens’ lamina characteristic by presence of a firmly fused vesicular tissue as vesicles on both upper and lower epidermis. Those salt bladders had ecological significance such as; a heat insulator and reflector prevent excessive transpiration, a water-storage tissue and a medium to absorb atmospheric moisture into the mesophyll of the leaf. Such adaptation is worth under desert condition where intense solar heat and extreme drought (Black, 1954). Also, (Freitas and Breckle, 1992; Mozafar and Goodin, 1970; Osmond et al., 2012; Yuan et al., 2016) suggested that bladders accumulate Na+ and Cl ions, and these bladders are associated with the *Atriplex* species tolerance to salinity.

Table (2) and Figure (1) summarizes the anatomical response of *Atriplex* as affected by different salinity concentrations compared to normal level of NaCl (such as halophytes). Upper and lower epidermis thickness (µ) of *A. halimus* significantly decrease with the increasing of salinity from 15.64 µ in control to 8.99 µ in T4, whereas, the same thickness of *A. nummularia* vary with increasing salinity as shown in (Table 2).

**Table (2):** Effect of NaCl levels on *Atriplex* lamina anatomy in transverse sections of *Atriplex halimus* and *A. nummularia* under T1, T2, T3 and T4 treatments respectively

| Variables                                                  | T1             | T2             | T3             | T4             | L.S.D ≤0.05 |
|------------------------------------------------------------|----------------|----------------|----------------|----------------|-------------|
| Average thickness of upper epidermis (µ)                   | 15.64a         | 9.64b          | 9.21b          | 8.99b          | 5.23        |
| Average thickness of lower epidermis (µ)                   | 10.47a         | 9.27b          | 6.91b          | 6.39b          | 3.98        |
| Average thickness of the mesophyll (µ)                    | 161.48b        | 137.95c        | 88.75c         | 145.42bc       | 13.14       |
| Average thickness of the midrib (mm)                       | 0.421c         | 0.500a         | 0.460b         | 0.440bc        | 0.029       |
| Average thickness (anticlinal diameter) of MVB (mm)        | 0.169c         | 0.239a         | 0.207b         | 0.198b         | 0.018       |
| Average width of MVB (mm)                                 | 0.175c         | 0.263a         | 0.194c         | 0.225b         | 0.021       |
| Average thickness of xylem tissue per MVB (µ)              | 75.28ab        | 86.48a         | 68.91ab        | 56.41b         | 24.88       |
| Average diameter of xylem vessels per MVB (µ)              | 12.77a         | 6.92b          | 9.09b          | 8.26b          | 3.17        |
| Average thickness of phloem tissue per MVB (µ)             | 45.67b         | 64.42a         | 39.79b         | 57.48a         | 10.19       |

| Atriplex nummularia Lindl.                                  |                |                |                |                |             |
|------------------------------------------------------------|----------------|----------------|----------------|----------------|-------------|
| Average thickness of upper epidermis (µ)                   | 11.224bc       | 13.720ab       | 15.322a        | 8.957c         | 2.92        |
| Average thickness of lower epidermis (µ)                   | 8.758c         | 12.342b        | 15.566a        | 9.313c         | 2.299       |
| Average thickness of the mesophyll (µ)                    | 165.147b       | 203.251a       | 169.282b       | 162.048b       | 16.53       |
| Average thickness of the midrib (mm)                       | 0.280c         | 0.465a         | 0.398b         | 0.440a         | 0.030       |
| Average thickness (anticlinal diameter) of MVB (mm)        | 0.137c         | 0.215a         | 0.195b         | 0.188b         | 0.012       |
| Average width of MVB (mm)                                 | 0.106c         | 0.202a         | 0.176b         | 0.166b         | 0.016       |
| Average thickness of xylem tissue per MVB (µ)              | 56.171b        | 83.129a        | 63.297a        | 57.396b        | 13.77       |
| Average diameter of xylem vessels per MVB (µ)              | 5.818c         | 10.873a        | 10.956a        | 8.127b         | 1.66        |
| Average thickness of phloem tissue per MVB (µ)             | 31.692b        | 49.257a        | 39.229ab       | 43.196c        | 10.68       |

**Abbreviations:** MVBs= main vascular bundles, mm= millimeter, µ= micrometer. T1, T2, T3 and T4 = salinity treatments
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Figure (1): Transverse sections of the lamina of *Atriplex* (a-b) *Atriplex halimus* and (e-g) *Atriplex nummularia* under T1, T2, T3 and T4 respectively.
In addition, the average thickness of the mesophyll decreased significantly under high level of salinity from 161.48 µ in control to 88.75 µ in T3, on contrast A. nummularia average thickness of the mesophyll increased from 165.147 µ in control (T1) to 203.251 µ in T2 and this is normal because of A. nummularia is euhalophyte (de Souza et al., 2012).

Average thickness of the midrib (mm), average thickness (anticalcinal diameter) of main vascular bundle (mm) and average width of MVB (mm) were not significantly different among all treatments. Average thickness of xylem tissue per MVB (µ), average diameter of xylem vessels per MVB (µ) were highly indicated-character for salinity in A. halimus as shown in Table (2) and Fig. (1); but vice versa as shown in A. nummularia. Average thickness of phloem tissue per MVB was vary among treatments from 45.67 µ in control (T1) of A. halimus to 57.48 µ in T4; whereas, in A. nummularia were 31.69 µ in T1 and 43.19 µ in T4. These findings indicated that salt accumulation from 400 ppm to 19200 ppm NaCl (0.30 to 30 dS/m), respectively, had negative effect on growth and anatomical, even in halophytes as shown at structure of A. nummularia. However, increasing salinity levels had positive effect in A. nummularia. It mean that salinity treatment of species significantly different according to genetic structure. These findings agreed with, (Benzarti et al., 2014; Boughalleh et al., 2009; Marius-Nicusor Grigore et al., 2014; Marius-Nicusor Grigore and Toma, 2017; Kelley et al., 1982; Martinez et al., 2004; Ounaissia et al., 2019; Troughton and Card, 1974; Walker et al., 2014).

CONCLUSION

Results of the present study indicate that, anatomical characters of lamina were differered between two species of Atriplex under salinity stress. Halophytes such as Atriplex showed same trend of response to high level of salinity as glycohytes.

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الاستجابة التشريحية في أوراق نبات القطف الصوديوم

الاستجابة التشريحية في أوراق نبات القطف الصوديوم

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تسبب الملوحة تغييرات فسيولوجية ومرورولوجية وتشريحة في نبات القطف الصوديوم Atriplex النموه Atriplex halimus. يتسم نبات القطف الصوديوم Atriplex halimus ببروز سطح نبات ملحي وقد يزيد في استصلاح الأراضي الملحية وتظيف التربة. وضح تشريح Atriplex halimus أن هناك اختلافات ملحوطة ذات أهمية بينية. تتميز أوراق Atriplex halimus الأوراق في جنس نبات القطف الصوديوم Atriplex halimus بأنها تتميز ببروز شعيرات حويضية مخزنة للملح في أكثر من طبقة على البشرة العليا والسفلية للأوراق، وذلك عند مستويات مختلفة من كلويريد الصوديوم من 0.01 وحتى 1200 جزء في المليون. بينما أوراق نبات القطف الصوديوم Atriplex halimus في نهاية التحريج. أدت زيادة الملوحة إلى حدوث تأثير ملحي على قياسات نسبية الورقة المختلفة لنوع Atriplex halimus. ولكن المرتبطة منها كان لها تأثير ملحي للورقة، ولكن المرتبطة منها كان لها تأثير ملحي للورقة.