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 xerophyte as shown from Kranz-anatomy and salt storage trichromes. 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-Nicuşor 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 grazing systems (Norman *et al.,* 2004). *Atriplex* is a halophyte with potential interest for saline soil reclamation and Phytoremediation (Benzarti *et al.,* 2014). The anatomy of *Atriplex* genus leaves and the marked anatomical variations of it proved important from an ecological viewpoint (Black, 1954; Evert, 2006). *Atriplex* species leaves characteristic by presence of salt bladders, fused vesicles on upper and lower leaf epidermis, which have ecological significance. Genus *Atriplex* consider halophyte salt includer, particularly adapted to arid, semi-arid and salt-affected areas (de Araújo *et al.,* 2006; de Villiers *et al.,* 1996). The present paper describes, the leaf different anatomical structure related to salt tolerance of both species *Atriplex halimus* and *A. nummularia*, to monitor the anatomical response of *Atriplex* leaves under different levels of sodium chloride salinity.

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 greenhouse, 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
	Cl^{-}	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

The plants irrigated regularly with sodium chloride solutions concentration as follows, 50 ppm (T1) as control, 400 ppm (T2), 800 ppm (T3) and 1600 ppm (T4). The final concentration of NaCl in soil was 200, 4800, 9600 and 19200 ppm.

Anatomical Structure:

For studying the anatomical response of *Atriplex* leaves, killing and fixation of the leaves in 50% F.A.A (formalin alcohol acetic acid) solution, dehydration and clearing in xylol and embedded in pure paraffin wax (melting point 56°C) were carried out as described by (Das, 1971). Using a rotary microtome, transverse sections of the lamina (10 µ) were obtained and double stained with safranin and light green. To monitor the anatomical response of *Atriplex* leaves under different levels of sodium chloride salinity, the following characters were measured, average thickness of upper

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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 Figure1. 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
***Atriplex halimus* L.**					
Average thickness of upper epidermis (µ)	15.64^a	9.64^b	9.21^b	8.99^b	5.23
Average thickness of lower epidermis (µ)	10.47^a	9.27^{ab}	6.91^{ab}	6.39^b	3.98
Average thickness of the mesophyll (µ)	161.48^a	137.95^b	88.75^c	145.42^b	13.14
Average thickness of the midrib (mm)	0.421^c	0.500^a	0.460^b	0.440^{bc}	0.029
Average thickness (anticlinal diameter) of MVB (mm)	0.169^c	0.239^a	0.207^b	0.198^b	0.018
Average width of MVB (mm)	0.175^c	0.263^a	0.194^c	0.225^b	0.021
Average thickness of xylem tissue per MVB (µ)	75.28^{ab}	86.48^a	68.91^{ab}	56.41^b	24.88
Average diameter of xylem vessels per MVB (µ)	12.77^a	6.92^b	9.09^b	8.26^b	3.17
Average thickness of phloem tissue per MVB (µ)	45.67^b	64.42^a	39.79^b	57.48^a	10.19
***Atriplex nummularia* Lindl.**					
Average thickness of upper epidermis (µ)	11.224^{bc}	13.720^{ab}	15.322^a	8.957^c	2.92
Average thickness of lower epidermis (µ)	8.758^c	12.342^b	15.566^a	9.312^c	2.299
Average thickness of the mesophyll (µ)	165.147^b	203.251^a	169.282^b	162.048^b	16.53
Average thickness of the midrib (mm)	0.280^c	0.465^a	0.398^b	0.440^a	0.030
Average thickness (anticlinal diameter) of MVB (mm)	0.137^c	0.215^a	0.195^b	0.188^b	0.012
Average width of MVB (mm)	0.106^c	0.202^a	0.176^b	0.166^b	0.016
Average thickness of xylem tissue per MVB (µ)	56.171^b	83.129^a	63.297^b	57.396^b	13.77
Average diameter of xylem vessels per MVB (µ)	5.818^c	10.873^a	10.956^a	8.127^b	1.66
Average thickness of phloem tissue per MVB (µ)	31.692^b	49.257^a	39.229^{ab}	43.196^a	10.68

Abbreviations: MVBs= main vascular bundles, mm= millimeter, µ= micrometer. T1. T2, T3 and T4 = salinity treatments

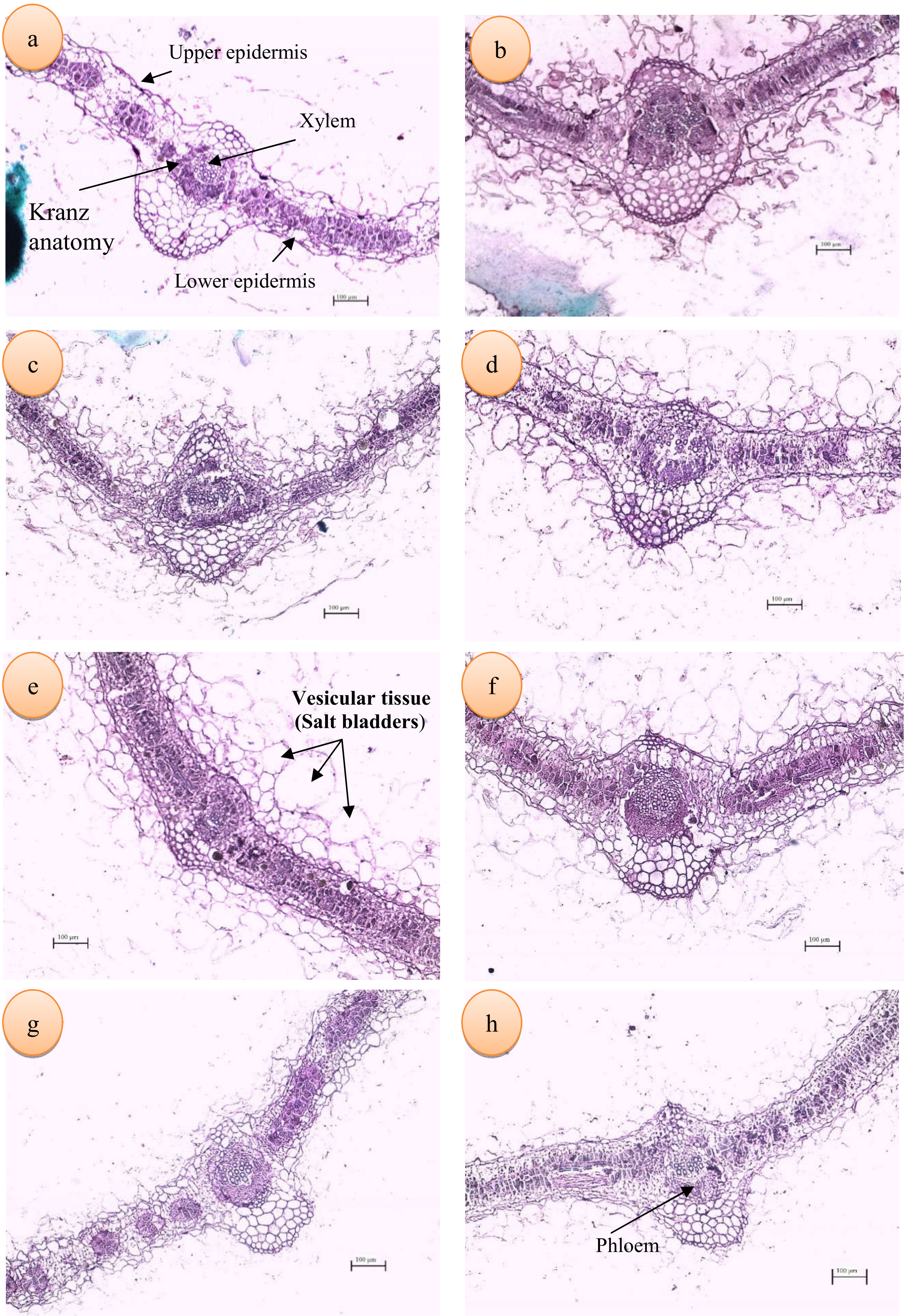

Figure (1): Transverse sections of the lamina of *Atriplex* (a-b) *Atriplex halimus* and (e-g) *Atriplex nummularia* under T1, T2, T3 and T4 respectively

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 (anticlinal 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. halimus* lamina. 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; Boughalleb *et al.,* 2009; Marius-Nicusor Grigore *et al.,* 2014; Marius-Nicușor Grigore and Toma, 2017; Kelley *et al.,* 1982; Martìnez *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 differed between two species of *Atriplex* under salinity stress. Halophytes such as *Atriplex* showed same trend of response to high level of salinity as glycophytes.

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الاستجابة التشريحية في أوراق نبات القطف *Atriplex* تحت مستويات مختلفة من ملوحة كلوريد الصوديوم

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تسبب الملوحة تغييرات فسيولوجية ومورفولوجية وتشريحية في نبات القطف *Atriplex* بنوعيه *Atriplex halimus* و *Atriplex nummularia* حيث ينتمي النوعان للعائلة الرمرامية Chenopodiaceae وكلاهما من النباتات الجفافية الحقيقية المتحملة للملوحة والتي تزرع كنبات اعلاف ومراعي. يعد نبات القطف *Atriplex* نبات ملحي وقد يفيد في استصلاح الأراضي الملحية وتنظيف التربة. وضح تشريح الأوراق في جنس *Atriplex* أن هناك اختلافات ملحوظة ذات أهمية بيئية. تتميز أنواع *Atriplex* بوجود شعيرات حويصلية مخزنة للملح في أكثر من طبقة على البشرة العليا والسفلى للأوراق، وذلك عند مستويات مختلفة من كلوريد الصوديوم من ٤٠٠ وحتى ١٩٢٠٠ جزء في المليون في نهاية التجربة. أدت زيادة الملوحة إلى حدوث تأثير سلبي على قياسات أنسجة الورقة المختلفة للنوع *Atriplex halimus*. بينما زيادة الملوحة في البداية كان لها تأثير إيجابي وتنشيط النمو في أوراق النوع *Atriplex nummularia* ، ولكن المرتفعة منها كان لها تأثير سلبي على القياسات التشريحية المختلفة لأنسجة الورقة.