Plant growth, photosynthesis and anatomical responses of *Polygonum equisetiforme* under salt stress

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ABSTRACT: Salinity is a major environmental problem in the dry Mediterranean regions, affecting rangeland production. This study investigated the effects of salinity on *P. equisetiforme* an herbal medicine plant used in the treatment of sore throat, cold and cough and in the disinfection of wounds. The impacts of salinity on physiological and anatomical parameters were investigated with various levels of salinity (0, 100, 200, 300, and 400 mM NaCl). Biomass production, stomatal conductance (G_S), transpiration (E) and intercellular CO_2 concentration (Ci), chlorophylls (a and b) and the carotenoid decreased in parallel with salt stress degree. In contrast, net photosynthetic rate (P_N) and the leaf relative water content was declined only at high salt stress (300-400 mM NaCl). The leaf anatomy showed an increase in upper and lower epidermal thickness with salinity and the leaf lamina and mesophyll became thicker at 200-300 mM NaCl maintaining water haemostasis in the leaves. The vascular bundle area was enhanced under salinity whereas thicker-walled xylem vessels with higher density were produced at high salinity. Our results indicated also that *P. equisetiforme* is relatively high salt tolerant plant surviving up to 200 mM NaCl without any lethal effects. Nonetheless, detailed field experiments with varying salinity regimes are needed to confirm these conclusions.

Keywords: *P. equisetiforme*; salt stress; plant growth, photosynthesis; leaf anatomy.

1. Introduction

Salinity is one of the most factors, particularly in the arid regions, limiting growth and productivity of plants. The inhibitory effect of salinity has been attributed to alterations in water relations, ion toxicity, metabolic perturbations, generation of reactive oxygen species (ROS), and tissue damage [1-2]. Therefore, discovering physiological and biochemical mechanisms implicated in plant responses to salt stress are of considerable interest for sustainable plant production [2]. To cope with salt stress, plants have evolved different physiological, anatomical and biochemical mechanisms among which ion compartmentalization and leaf succulence [3]. Anatomical features like enhanced epidermal and total leaf thickness were positively correlated with salinity tolerance [3-4]. Thus leaf with higher mesophyll area can help in maintaining water content and turgor leading to osmotic and ionic adjustment of the cells [5]. The epidermal thickness not only improves the water use efficiency of plants but also provide additional space for efficient sequestration of Na+ in the leaf epidermis [6]. On the basis of the increased extent of soil salinity in Tunisia, we proposed to evaluate the effect of salinity on plant growth, photosynthesis and leaf anatomy. Therefore, the study aimed to clarify the physiological and anatomical adaptation by which *P. equisetiforme* cope with salt stress.

2. Materials and methods

2.1. Plant growth conditions

P. equisetiforme seeds were collected in 2017 from Djerba in southern Tunisia. They were naturally air-dried, purified then stocked at 25°C in the seeds bank of the laboratory. Seeds were surface sterilized for 5 min in 3 g/L calcium hypochlorite solution and then thoroughly washed with deionised water. Five seeds were sowed in each pot and watered with half strength Hoagland and Arnon's solutions (1950) to FC to facilitate germination. After two weeks of sowing, thinning was done and three healthy plants of uniform size were maintained in each pot (four pots/ treatment; 28 pots in total). Plants were grown in a growth chamber with a constant 25 ± 1 °C temperature, a relative humidity of 60 during the day and 75% at night and a 16 h light/8 h darkness regime with 250 μmol m^{-2} s^{-1} photosynthetic photon flux density (PFD) during the day period. On the 90 days after sowing (stage of tillage), pots were supplied with half strength Hoagland and Arnon's nutrient solution with different levels of salinity treatments : 0 mM (control), 100, 200, 300, 200 and 400 mM NaCl. In order to avoid a shock response, the salt concentration was increased gradually, increasing by 50 mM every 2 days until the salt levels were reached. Pots were irrigated every three days with 300 ml of their appropriate solution. This volume was enough to saturate the soil. Pots were flushed weekly with 500ml of water to prevent accumulation of salt in the

soil, and conductivities were monitored with a conductivity meter. Nutrient solutions were changed every two weeks, preventing any change in treatment conductivity levels. Harvest was applied after 45 days of salt application and all the analyses were performed in quadruplet.

2.2. Growth activity and plant water status

The dry mass (DM) is measured after the fresh material (FM) is dried at 70°C for 48 h. The fresh mass at full turgor (TM), is determined by immersing the leaf petioles in demineralised water for 48 h in darkness at 4°C. Midday leaf water potential (Ψ_W) is measured using 3rd to 4th fully expanded leaf counting from the terminal shoot apex, using a Sholander pressure chamber (Skye Instruments, Powys, UK). Leaf relative water content (RWC) is calculated as follows:

RWC = (FM - DM)/ (TM - DM) ×100

2.3. Analysis of photosynthetic gas exchange and photosynthetic pigment estimation

About 1g of fresh leaves tissue is used for each extraction. Tissue is homogenized in liquid nitrogen and total pigments extracted in 80% acetone. The absorbance of the extracts is measured on a spectrophotometer (UV-2500, Shimadzu Corp, Japan) at two wavelengths (470 and 645 nm), and chlorophylls (*a*, *b*) concentrations are calculated according to Arnon (1949).

Net CO_2 assimilation rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration (E) of the seedlings are measured on leaves of four plants per treatment at deferent NaCl doses using a Portable Photosynthesis System (Li-6200, Lincoln, NE-USA).

2.4. Anatomical study

Mature leaves of plants subjected or not to 100, 200, 300 and 400 NaCl concentrations are used. Small pieces of leaf tissue (approx. 5×5 mm), from the midportion of laminate leaves, are excised. Cut tissues are fixed in freshly prepared FAA (formaldehyde: glacial acetic acid: 70% ethanol 5:5:90 by volume) overnight at room temperature. After washing with 0.1 M phosphate buffer (pH 7.4), they are dehydrated by passage through a tertiary butyl alcohol series (15–100%), and embedded with warm (56–58 °C) paraffin. The resulting blocks are then cut in 10 µm sections with rotary microtome and stained with 2 % safranine O and fastgreen 0.2 %. Observations are performed under a light microscope (Leitz, Germany), and photographed with a digital camera (Cannon, USA). Measurements of various cells and tissues are taken with an ocular micrometer, a simply disc of glass upon which equally spaced divisions are etched. To use the ocular micrometer, calibrate it against a fixed and known ruler, the stage micrometer. The exact values are calculated with a factor derived by comparing ocular with stage micrometers.

2.5. Statistic analysis

Data are analyzed using SPSS, version 16.0. Requirements of ANOVA are checked by normality plots and by testing the homogeneity of variance of residual means. Parametric one-way ANOVA and post-hoc comparisons (Duncan's test) are conducted to determine the significant differences in the studied parameters among the different treatment samples.

3. Results

3.1. Growth and leaf water status

The biomass production (shoot and root dry weight) decreased by increasing salinity. A substantial decrease in the shoot dry weight of *P. equisetiforme* plants was observed at 100, 200, 300, and 400 mM NaCl by 16.0%, 33.6%, 51.2%, and 62.4%, respectively, compared with the control. Consistently, the root dry weight was reduced by 8.0 to 47.4 % at 100-400 mM NaCl as compared with the control, respectively. The shoot/root ratio was nearly unchanged in 100 mM NaCl treatment, but significantly decreased at 200 to 400 mM NaCl compared to the control, but no significant change was observed among the treatments (Fig. 1a). Leaf water potential (Ψ_W) of salt-treated plants decreased with increasing NaCl concentration, and it is about 4.4-fold lower at 400 mM NaCl compared to control (Fig. 1c). However, the RWC content was steady at 100 mM NaCl and decreased significantly beyond 200 mM (Fig. 1b).

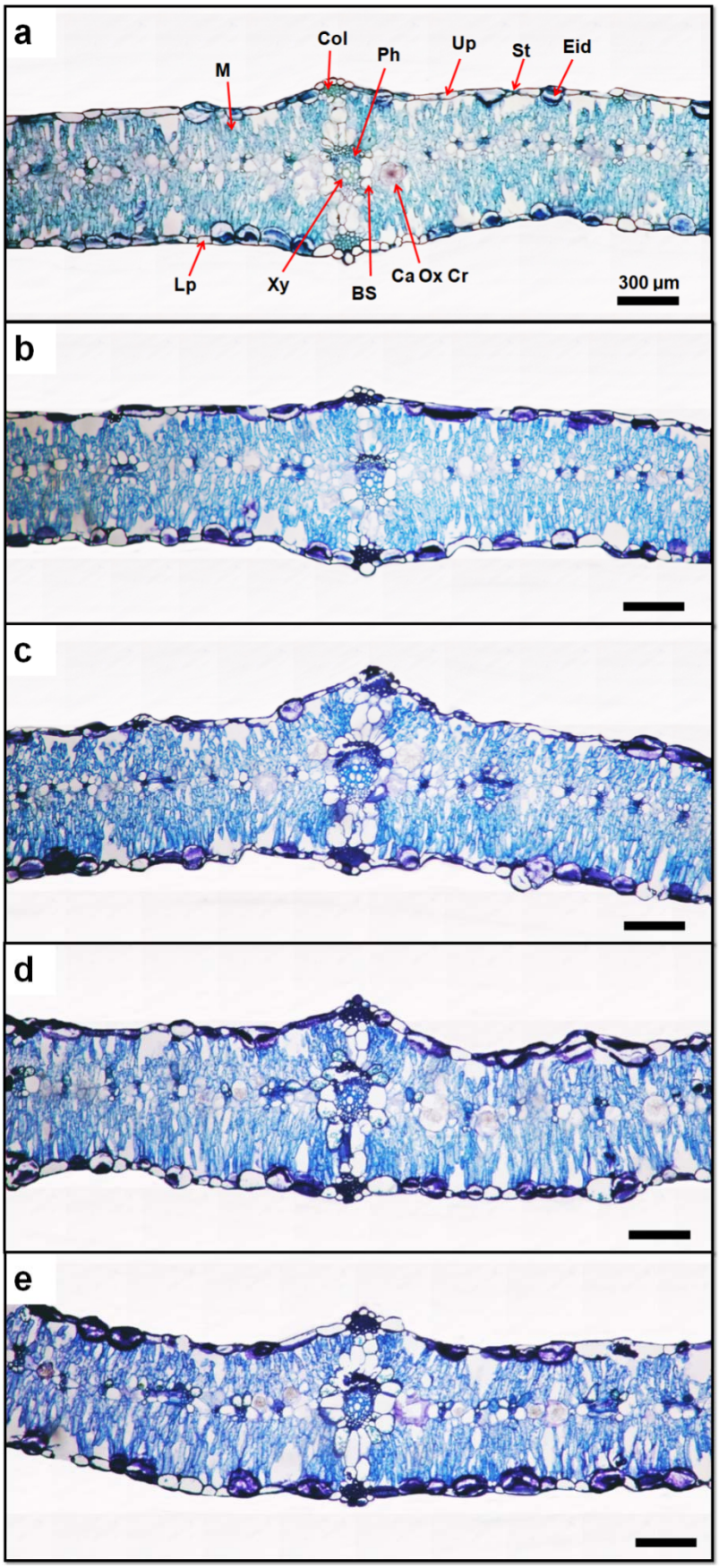

Figure 1: Leaf blade cross-sections showing leaf anatomical parameters (a, b, c, d and e) of *A. gombiformis* plants grown at 0 (a), 100 (b), 200 (c), 300 (d) and 400 mM NaCl (e). BS, bundle sheath; Ca Ox Cr, Calcium oxalate crystals; Col, Collenchyme; Eid, epidermal idioblast; Lp, lower epidermis; Up, upper epidermis; Ph, phloem; PM, mesophyll; St, Stomata; VB, vascular bundle; Xy, xylem. Scale bar: 300 µm.

3.2. Photosynthetic pigment content and leaf gas exchange

Various photosynthetic parameters were investigated in *P. equisetiforme* grown under various salt treatments for 45 days. Under low salinity (100 mM NaCl), the chlorophyll a and b as well as total carotenoid contents showed no significant differences with control, however, higher salinity levles (200-400 mM NaCl) caused a significant reduction ($P<0.05$) in photosynthetic pigments. At 400 mM NaCl, chlorophyll a, b and carotenoid contents are 38.6%, 28.6%, and 48% lower than controls, respectively. The net photosynthesis rate (P_n) remained unchanged in 100 and 200 mM NaCl treatments, but decreased significantly at 300 and 400 mM NaCl by 26.9 and 34.5%, respectively, compared to control. The stomatal conductance (*gs*), showed a gradual decreasing pattern upon increasing concentration of NaCl, i.e., 12%, 36.3%, 52.8% and 62.6% reduction in the plants treated with 100, 200, 300 and 400 mM NaCl, respectively, with respect to the control. Similarly, a progressive decrease of the intercellular CO_2 concentration (C*i*) and the transpiration rate (*E*) is observed compared to controls. At 400 mM NaCl treatment, the smallest reduction is observed for C*i* (56.5%) while the higer (77.2 %) for the transpiration rate (*E*) in comparison to control. There is a good linear correlation ($r = 0.81$, $F = 34.718$, $P<0.0001$; Table 2) between photosynthesis and stomatal conductance. The intercellular CO_2 concentration (C*i*) is highly correlated with P*n* ($r = 0.85$, $F = 49.419$, $P<0.0001$; Table 2). A positive relationship is observed between P*n* and SDM ($r = 0.77$, $F = 26.939$, $P<0.0001$; Table 2).

Table 1. Effects of NaCl concentrations on water potential (Ψw), Relative water content (RWC), shoot dry mass (SDM), root dry mass (RDM) and shoot/root ratio in *P. equisetiforme* plants treated with different salinity levels (0, 100, 200, 300 and 400 mM NaCl).

Characters	Traitements				
	Control	100 mM	200 mM	300 mM	400 mM
Leaf water potential (Ψ_W, MPa)	-0.951± 0.053a	-1.552±.0.091b	-2.370±0.078c	-3.453±0.103d	-4.211 ±0.165e
Relative water content (RWC, %)	75.20± 0.953a	69.71± 0.925ab	66.27± 1.023ab	64.23± 1.229b	57.52± 0.836c
Shoot dry mass (SDM, g plant^{-1})	0.967± 0.023a	0.671± 0.019 b	0.433± 0.022c	0.350 ± 0.031d	0.258±0.015e
Root dry mass (RDM, g plant^{-1})	0.177 ± 0.012a	0.148± 0.018ab	0.128±0.0015b	0.090± 0.008c	0.069 ±0.007d
Shoot/root ratio	5.453± 0.158a	4.517± 0.239a	3.384±0.110b	3.821± 0.167b	3.705 ±0.198b

Data are means values ± SE of four measurements. Values in each line with the same letter are not significantly different (P = 0.05) as described by Duncan's test.

Table 2. Effects of NaCl concentrations on net CO_2 assimilation rate (P_N), stomatal conductance (G_S), transpiration (*E*), internal CO_2 concentration (*C*i), and photosynthetic pigments contents in leaves of *P. equisetiforme* and the correlations coefficients (*R*) of the stomatal conductance, internal CO_2 concentration and shoot dry weight (SDW) with net CO_2 assimilation rate.

Treatment (mM)	P_N (µmol m^{-2} s^{-1})	Ci (µmol mol^{-1})	G_S (mmol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})	**Pigment** (mg g^{-1} FM)		
					Chl*a*	**Chl*b***	**Carotenoïds**
Control	4.35 ± 0.15 a	497.1± 15.6a	166.1 ± 9.5 a	3.61 ± 0.09 a	1.06 ± 0.05 a	0.89 ± 0.06 a	0.045 ± 0.002 a
100	4.27 ± 0.23 a	418.7± 12.1b	145.9± 9.1 b	2.75 ± 0.11 b	0.98 ± 0.06 a	0.88 ± 0.05 a	0.037 ± 0.004ab
200	4.11 ± 0.19 a	390.2 ± 11.8 b	105.6 ± 7.3 c	1.81 ± 0.08 c	0.85 ± 0.05 b	0.79 ± 0.04 b	0.027 ± 0.003 bc
300	3.18 ± 0.13 b	306.3 ± 8.4 c	78.1 ± 8.1 d	0.99 ± 0.06 d	0.78 ± 0.04 b	0.70 ± 0.03 c	0.029 ± 0.003 bc
400	2.85 ± 0.16 b	216.7 ± 8.1 d	62.0 ± 8.5 e	0.82 ± 0.06 e	0.65 ± 0.03 c	0.61 ± 0.05 c	0.023 ± 0.002 c
Correlation coefficient (*R*)							
G_S	0.908***						
P_N		0.907***					
SDW	0.892***						

Data are means values ± SE of four measurements. Values in each column with the same letter are not significantly different (p = 0.05) as described by Duncan's test. The probabilities are shown as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and not significant (ns).

3.3. Leaf anatomy

The leaf anatomical features of *P. equisetiforme* showed significant alterations imposed by salt stress. The lower epidermis thickness was increased gradually by 2.1, 5.5, 49.5 and 51.8% in 100, 200, 300 and 400 mM NaCl treatements as compared to control. However, the upper epidermis thickness showed no significant change up to 200 mM NaCl, and increased by 37.8 to 47.2% respectively at higher salt levels (300-400 mM NaCl) compared to control. Likewise, the entire lamina and mid-vein leaf thickness remained unaffected at 100 mM NaCl, whereas they increased by about 8.2- 8.8 % and 12.2- 12.6% in 200 and 300 mM NaCl treated plants as compared with control, respectively. At high salinity (400 mM NaCl), the entire lamina and mid-vein thickness was increased only by 5.8 and 5.6% with respect to control, respectively while remaining significantly higher than the untreated plants. On the other hand, thickness of mesophyll tissue remained unchanged at low salinity (100 mM NaCl) and increased significantly by 9.4% at moderate salinity (200 mM NaCl) whereas at 300 mM NaCl it increased by 5.3%. At 400 mM NaCl treatment the mesophyll thickness remains significantly higher than control but increased only by 3.2%. The mesophyll cell and collenchym layers showed no significant changes at 100 mM NaCl, whereas, theses anatomical parameters was increased by 26.8 to 28.5% and by 32.9 to 37.7% at higher salt levels (200 to 400 mM NaCl) respectively as compared to control. In addition, the average vascular bundle diameter was unchanged by salt stress. The distance between vascular bundle was enhanced by 4.8, 5.2 and 11.4% in 100, 300 and 400 mM NaCl treatements in comparison to control. But at 200 mM NaCl treated plants it was unchanged with respect to control.

In the leaf mid-vein, the vascular bundle length was unaffected by salt stress, while the vascular bundle width was increased by 15.6, 19.4 and 25.9% in 200, 300 and 400 NaCl treatement compared to control. The phloem tissue area was unchanged under 100 and 400 400 NaCl treated plants, but increased marginally by 6.5 to 9.1% at 200 and 300 mM NaCl treatement compared to control. The xylem tissue area was enhanced by salt stress but no significant changes between the salt tratements (100-400 mM NaCl). The xylem vessel diameter remained unchanged under low salt stress (100 mM NaCl) and declined by 5.5 to 21.4% under higher salinities (200-400 mM NaCl). However, the xylem vessel dencity was enhanced by about 9, 31.2 and 38% in 200, 300 and 400 mM NaCl treated plants compared to control. The xylem wall thickness is significantly thinner than that of controls when the salinity levels exceed 200 mM NaCl. There was no significant change observed in the calcium oxalate crystal diameter of *P. equisetiforme* leaves induced by salt stress as compared to control. However, the crystal dencity was increased progressively with increasing salinity. As compared to control, the dencity of calcium oxalate crystal dencity remained unchanged at low to moderate salinity (100-200 mM NaCl) but was enhanced by 96.1 to 157.7% at higher salt levels (300-400 mM NaCl) compared to untreated plants, respectively.

Table 3. Anatomical variables and properties of leaves from *P. equisetiforme* plants treated with different salinity levels (0, 100, 200, 300 and 400 mM NaCl).

Characters	**Traitements**				
	Control	100 mM	200 mM	300 mM	400 mM
Total leaf thickness (µm)	646.7± 11.5**c**	642.8 ±.12.9**c**	699.8 ±15.7a	704.2±16.3a	684.6 ±12.3**b**
Upper epidermis thickness (µm)	32.3± 0.6**c**	31.8± 0.9 **c**	32.7 ± 0.8**c**	44.6 ± 1.2**b**	47.6 ±1.4**a**
Lower epidermis thickness (µm)	36.8 ± 0.9**e**	37.6± 1.2**d**	38.8±1.7**c**	55.0 ± 1.9**b**	55.8 ±2.6**a**
Mesophyll thickness (µm)	539.1 ± 8.3**d**	546.2± 8.1cd	590.2±9.4**a**	567.6 ± 7.6**b**	556.8 ±9.8**bc**
Mesophyll cell area (µm^2)	2061.1 ± 32.4**b**	2077.6 ± 36.1**b**	2649.7 ± 29.6**a**	2615.3 ± 35.9**a**	2636.4 ±41.5**a**
Collenchyma tissue (µm)	63.7± 1.6**b**	68.2± 1.1**b**	84.7± 1.8**a**	87.8 ± 1.9**a**	86.6 ±2.3**a**
Distance between VB (µm)	166.2± 3.7**c**	174.2± 4.1**b**	164.7.7±4.2**c**	174.8±4.5**b**	184.2± 5.2**a**
Vascular bundle diameter (µm)	106.8± 2.8**a**	109.3± 2.5**a**	110.5± 1.8**a**	105.5± 2.4**a**	107.6± 1.9**a**
Stomata size (µm)	73.1 ± 1.8**a**	71.2 ± 1.9**a**	70.9 ± 2.2**a**	72.3± 1.9**a**	67.2 ±1.7**b**
Stomatal density (nu mm^{-2})	29.1± 1.3**a**	30.8± 1.4**a**	27.1 ± 1.1**b**	25.4± 1.1**c**	20.3 ±0.8**d**
Calcium oxalate crystal diameter (µm)	79.7± 1.5**a**	80.2± 1.5**a**	78.8± 1.9**a**	84.3±1.6**a**	84.5± 1.5**a**
Calcium oxalate crystal density (nu mm^{-2})	8.7± 0.15**c**	9.3± 0.15**c**	10.1± 0.12**c**	17.2± 0.23**b**	22.3± 0.24**a**
Leaf main-vein					
Total leaf thickness (µm)	877.5± 16.1**c**	865.6± 18.7**c**	984.7± 21.5**a**	988.7± 25.1**a**	927.3± 22.9**b**
Vascular bundle length (µm)	495.1± 12.5**a**	499.4± 11.9**a**	502.2± 11.8**a**	490.1± 14.8**a**	524.3± 11.4**a**
Vascular bundle width (µm)	262.1± 5.2**c**	249.1± 5.8**c**	303.4± 7.1**b**	313.5± 6.4**b**	330.2± 8.6**a**
Xylem tissue thickness (µm)	117.2± 1.9**b**	128.2± 2.4**a**	134.1± 2.3**a**	131.9± 2.1**a**	130.4± 2.6**a**
Phloem tissue thickness (µm)	42.6± 0.85**c**	43.5± 0.91**bc**	45.5± 0.99**ab**	46.5± 1.1**a**	44.5± 0.81**abc**
Xylem vessel diameter (µm)	16.1± 0.27**a**	16.0± 0.29 **a**	15.2± 0.21**b**	12.9± 0.18**c**	12.7± 0.22**c**
Xylem vessel density (nu mm^{-2})	655.6± 17.1**d**	634.7± 18.5**d**	714.2± 22.8**c**	860.3± 18.1**b**	904.6±2 5.3**a**
Xylem vessel wall (µm)	3.39± 0.08**b**	3.47± 0.09**b**	3.45± 0.07**b**	3.85± 0.13**a**	3.82± 0.15**a**

Data are means values ± SE of four measurements. Values in each line with the same letter are not significantly different (P = 0.05) as described by Duncan's test.

4. Discussion

To evaluate the salt stress tolerance of *P. equisetiforme* numerous parameters were developed. In this study, it was found that salt stress had a negative effect on plant growth. This reduction in growth may be due to the osmotic effect induced by salinity leading to a decrease in turgor pressure, as well as several metabolic changes [2-7]. The increased SDM/ SDM ratio of *P. equisetiforme* under moderate to higher salt levels (200-400 mM NaCl), suggesting that roots are more tolerant to salinity stress than shoots [2]. This decline in shoot/ root ratio could be as an adaptive mechanism improving the water absorption capacity through the increased roots surface area and lowering water loss by reducing the transpiration area [8]. Salinity also has a negative effect on RWC, showing a gradual reduced values when plant exposes to salinity exceeding 100 mM NaCl, which leads to water deficit and loss of turgor [9]. In addition, the leaf water potential of *P. equisetiforme* was significantly decreased with increasing salinity. In the cell cytoplasm, the reduced water potential can be interpreted as adaptive mechanisms to improve the ability of cells to maintain turgor pressure at low water potentials [10].

Generally salt stress has a negative effect on plant photosynthesis that can be caused by stomatal and non-stomatal factors [11-12]. In the present study, the stomatal conductance (G_S), transpiration and intercellular CO_2 concentration were declined by NaCl salt treatments, however, the net photosynthetic rate (P_N) was inhibeted only at higher (300-400 mM NaCl) salinities. The reduction in intercellular CO_2 concentration during the experimental period may be the result of the declined in stomatal conductance that led to the decrease in photosynthesis [13]. In addition, a highly significant correlation (r = 0.908, Table 2) between net photosynthetic rate (P_N) and stomatal conductance (G_S), suggests that stomatal conductance as the primary factor limiting photosynthesis under salt stress. In *P. equisetiforme* plants imposed to salt stress, the stomatal closure may cause the decline in stomatal conductance thereby preventing water loss as evidenced from the relative maintenance of RWC in the plant imposed to 300-400 mM NaCl. Thus, the stomatal closure can act as an an adaptive measures to protect photosynthesis organs against rapid dehydration by reducing transpiration under salt stress. Maggio et al. [14] showed that the higher resistance of plants to salinity is linked to lower transpiration rates. Based on our findings the highly positive correlation between SDM and P_N (data not shown) suggesting that plant growth reduction could be explained by the decrease in photosynthetic ability.

The chlorophylls and carotenoids are vital components involved in the synthesis of metabolites that promote the plant growth [15]. The alterations of the content of photosynthetic pigments might influence significantly the plant metabolism affecting the function of the photosynthetic apparatus, which can lead to inhibition of photosynthesis [16]. In our study, the chlorophyll a and b and the carotenoid contents showed a significant decline under moderate to higher salt levels (200-400 mM NaCl), whereas theses pigments remained unchanged with 100 mM NaCl treatment. The chlorophyll loss is used as an indicator of cellular stress [17], evenly this decrease of pigments could also be an adaptive strategy to prevent oxidative stress by reducing the amount of light intercepted and therefore reducing the amount of ROS generated by chloroplasts [18].The reduction in chlorophyll content may be due to the perturbation of chloroplast structure and chlorophyll synthesis and/or activation of the chlorophyllase enzyme, involved in chlorophyll degradation [19]. The carotenoids are reported to stabilize the light harvesting complex proteins and the phospholipids of thylakoid protecting membranes from salt stress [20]. Furthermore, carotenoids has multiple functions in plants, in addition to their direct role in photosynthesis, theses pigments possesses important antioxidant properties which act as scavengers of singlet oxygen or protecting chlorophylls from the deleterious effects of photooxidation reactions [21].

Leaf anatomical modifications were observed in *P. equisetiforme* plants imposed by various NaCl salt treatments. The thickness of lower epidermis was increased in all salt levels as compared to control, while the upper epidermis was thicker only at 300-400 mM NaCl. The presence of thick epidermis largely vacuolated provides additional space for efficient sequestration of Na^+ which helps to protect mesophyll cells under stress and improves the water use efficiency (WUE) of plants [6-22]. Also we found that moderate to high salt stress caused a significant augment in the leaf lamina and mesophyll thickness of *P. equisetiforme* leaves with higher values occurred at 200-300 mM NaCl. The necessity to conserve water renders the leaves succulent thus, increase leaf thickness. These anatomical features may help in storing ions inside the plant body due to increased vacuolar volume [3], thus permitting the plant to cope with higher salt amounts. On the other hand, the mesophyll cells size increased under moderate to high salt stress. Generally, small cells can resist turgor pressure better than large ones, and can contribute to turgor maintenance more effectively under drought conditions [23].

Regarding vascular system, our results exhibited that moderate to high (200-400 mM) salinity increase the vascular bundle area in *P. equisetiforme* leaves. Likewise, the xylem tissue area was enhanced under all salt levels while the phloem tissue area was increased only at 200-300 mM NaCl. Better development of vascular tissue, may be important for efficient transport of solutes and photosynthates under salt stress [24]. The leaf xylem vessel diameter was unchanged under low salinity while decreased significantly at higher salt levels, in parallel the xylem vessel density increased and the xylem wall become thicker than that of controls at high salt stress (300-400 mM NaCl). Stiller et al. [25] reported that cavitation occurs when the flow of water in the xylem vessels cannot keep the sweat rate. Thus, selection for narrow vessels in response to improved leaf water use efficiency would reduce

the risk of xylem embolisms in saline habitats. In addition, cavitation resistance is also highly correlated to vessel wall thickness [26]. Xylem vessel wall reinforcement is required to prevent wall implosion and cavitation, when xylem pressure is highly negative [27].

Numerous works linked the change in transpiration caused by salinity to the reduced stomatal conductance and the lower stomatal density of leaves under saline conditions, as indicated by the close correlation found between these parameters [28]. In the present study, leaf stomatal density was decreased by salinity. In addition, the stomatal size was reduced only at 400 mM NaCl.The reduction in stomatal density can be considered as adaptation by which a plant may optimize WUE under salt stress [6]. A positive correlation between G_S and stomatal density might imply that photosynthesis inhibition induced by salinity can be explained by stomatal factors. However, the relationships of stomatal density and size with gas exchange may be complex, suggesting that some compromises can occur during plant adaptation to varying degrees of stress [3].

Druse and prismatic crystals are well known as general occurrence in *Polygonaceae.* Other observations have indicated vast distribution of oxalate calcium crystals in *Polygonum* species like *P. acuminatum*, *P. ferrugineum*, *P. ferrugineum* and *P. hydropiperoides* [29]. In *P. equisetiforme* leaves, the calcium oxalate crystal diameter was unchanged by salinity while the crystal dencity was significantly higher at high salt levels (300-400 mM NaCl). Similarly, Brown et al. [30] have recorded that the number of the CaOx crystals in the phyllodes of the *Acacia* species increases with aridity. The formation of CaOx crystals was found to be related with changes in calcium levels within the plant [31]. These deposits confer some metabolic advantages, considering that they are metabolically and osmotically inactive [32].

5. Conclusion

In order to study the physiological and anatomical processes of *P. equisetiforme* under different levels of salt stress, various physiological parameters were evaluated. Leaf water potential, RWC, leaf Chl a and b contents, carotenoid content, net photosynthetic rate (P_N), stomatal conductance (G_S), transpiration (E), intercellular CO_2 concentration (Ci) and anatomical parameters. Results showed that *P. equisetiforme* could be considered a glycophyte salt-tolerant species because it had a less affected chlorophyll content and net photosynthetic rate (P_N). Leaf anatomical assessment showed increased leaf epidermis and mesophyll thickness, characteristic features of salt-adapted plants. In addition, The vascular bundle area was enhanced under salinity whereas thicker-walled xylem vessels with higher density were produced at high salinity. In addition, developed vascular area, and thicker-walled xylem vessels with higher density were produced at high salinity under higher salinity provide the anatomical basis for salt resistance in *P. equisetiforme* plants.

Acknowledgements

We gratefully acknowledge all the technical staff of the Pastoral Ecology Lab of the Arid Lands Institute-Medenine (IRA) for their help to conducting these experiments.

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