Anatomical adaptations of four *Astragalus* L. species under salt stress

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ABSTRACT: Four *Astragalus* species have been evaluated for their tolerance and adaptability potential to salt stress. The adaptive components of salt tolerance were assessed on the basis of biomass production and anatomical changes. *A. tenuifolius* was the most tolerant among the species under study, with increased leaf water content, stable shoot dry weight, stable root thickness, thick epidermis and increased cortical cell area, increased root cortex, thick stele, stable xylem tissue. Also, observed intensive density of thick-walled vessel elements. Moreover, thicker leaves and mesophyll tissue, thick upper epidermis, increased vascular bundle area, intensive density of thick-walled vessel elements and larger stomata with unchanged density were recorded in the leaves of this species with an increase in salinity levels. *A. armatus* the second tolerant species relied on the maintenance of epidermis and stele thickness and xylem vessel diameter under salinity. Also, root and cortex thickness decreased only at 300 mM NaCl, and increased density of xylem vessels, thicker leaves and mesophyll tissue, thick upper epidermis, increased mesophyll cells size, intensive density of thick-walled vessel elements was observed. In addition, the maintenance of water content and the lower (14.2 %) reduction in biomass production. *A. mareoticus* appeared to be the most salt sensitive when the decrease in biomass production reached 56.8% with anatomical parameters severely affected. For *A. caprinus* (salt sensitive species) has shown better adaptation than *A. mareoticus* relied on increased cortical cell area as well as stable root xylem vessel diameter, also, thick upper epidermis and increased leaf vascular bundle area.

Keywords: *Astragalus armatus*, *Astragalus caprinus*, *Astragalus mareoticus*, *Astragalus tenuifolius*, anatomical changes, salt adaptations.

1. Introduction

Soil salinity is the main abiotic factor limiting plant growth and productivity of plants worldwide and thus creating a need for salt-tolerant plants [1]. This problem is more severe in arid and semi-arid regions many of which are of great intrinsic ecological values [2]. Increased salinity induces specific changes at cell, tissue and organ levels. These changes are physiological, morphological and anatomical in nature [3]. It has been revealed from many studies that salinity mostly causes specific anatomical changes. The structural changes under salinity are generally related to the growth status of the plants. Salinity affects cell division and expansion processes, and reduces the size of cortex and vascular cylinder [4]. Some interesting and intriguing features such as thick leaves, succulence in root and stem, thickening epidermis, higher stele to root cross-section area proportions and promoting stomata number are among the prominent adaptive features of salt tolerant plants [5,6].

The genus *Astragalus* L. of the family Fabaceae is the largest genus of flowering plants. Among species of *Astragalus* witch exhibit valuable economic values we are interested to four species. *Astragalus armatus* Willd. is an endemic shrub of the Northern Africa, in Tunisia it is used as tonic, stimulant and in cases of anaemia [7]. *Astragalus caprinus* L. This species spreads over different climatic zones in Tunisia. It was used in traditional medicine by its richess with flavonoids [8]. *Astragalus mareoticus* Del. annual herb growing on sandy and muddy soils in desert, semi-desert and steppe area in Northern Africa and Arabia [9]. *Astragalus tenuifolius* Desf. also called *Astragalus algerianus* E. Sheld. is distributed in Mediterranean/Sahara regional transition zone, especially in Algeria, Morocco, Tunisia and Spain [10].

The responses of these species to salinity are of particular importance, as they are among the soil fixative species that can be used as fodder in dry lands of the south of Tunisia. However, unknown information is available about the anatomical responses to salt stress in these species. In this study, we examined the effects of salinity on root and leaf anatomy, water relationship, and growth in four *Astragalus* species with increasing salinity levels. Therefore, the main objective is to evaluate the difference in the responses to different NaCl salinity levels and to identify the degree of tolerance they develop to confront salt stress. The study addressed the hypothesis that, as salinity increases, the anatomical structure may change in association with the development of salt tolerance.

2. Materials and methods

2.1. Plant growth conditions

The seeds of the four *Astragalus* species were collected in 2008-2010 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 3g/L calcium hypochlorite solution and then thoroughly washed with deionised water. They were sown in 5L plastic pots in a 2:1 mixture of sandy soil and peat in a growth chamber at 25/18°C day/night temperatures, at 65–85% relative humidity, with a photosynthetic photon flux density of 1200 μmol$m^{-2}s^{-1}$ and a 16/8 h photoperiod at the Arid Region Institute of Medenine (Tunisia). Initially 8–10 seeds were planted in each pot; 2 weeks after germination the seedlings were thinned to three per pot. The pots were arranged in a randomized complete block design with four replicates per treatment and three plants per pot for every treatment. Plants were initially grown in half-strength Hoagland and Arnon [11] solution to supply the macro- and micronutrients. Potted 90-day-old seedlings of *A.* were divided into three groups for treatments (ten plants per treatment): (1) 0 mM NaCl (control); (2) 150 mM NaCl; (3) 300 mM NaCl. The treatment was watered with 200 mL of salt solutions every two days to avoid excessive accumulation of salt due to loss of water during evaporation. The experiment was performed for a total period of 60 days. During this experiment, growth and water relations and anatomical parameters were measured.

2.2. Growth activity and water status

The dry mass (DM) was measured after the fresh material was dried at 70°C for 48 h. Midday leaf water potential (Ψ_W) was measured using 3rd to 4th fully expanded leaf counting from the terminal shoot apex, using a Sholander pressure chamber (Skye Instruments, Powys, UK). Each replicate was the average of three measures corresponding to three plants per pot.

2.3. Anatomy study

This study was carried out on mature leaves and roots of plants subjected or not to salinity. At the end of the experiment, small pieces of leaf tissue (approx. 5×5 mm) were excised from the midportion of laminate leaves. For roots tissue pieces, (approx. 5 mm, segments of mature roots), were sampled from control and salt-stressed cultures. Cut tissues were fixed in freshly prepared FAA (formaldehyde: glacial acetic acid: 70% ethanol 5:5:90 by volume) overnight at room temperature. After washing with a 0.1 M phosphate buffer (pH 7.4), they were dehydrated by passage through a tertiary butyl alcohol series (15–100%), and embedded with warm (56–58 °C) paraffin. The resulting blocks were then cut in 10 μm sections with rotary microtome and stained with 2 % safranine O and fastgreen 0.2 %. Observations were performed under a light microscope (Leitz, Germany), and photographed with a digital camera (Cannon, USA). Measurements of various cells and tissues were taken with an ocular micrometer and exact values were calculated with a factor derived by comparing ocular with stage micrometers. The stomatal density (number per unit leaf area) was measured in the same day of collection in the surface of ten mature leaves per treatment.

2.4. Statical analysis

The data were subjected to analysis of variance (ANOVA), and comparisons between the mean values of treatments were made by the least significant difference (Duncan post hoc) test ($P < 0.05$). Statistical analyses were performed using the SPSS statistical package (SPSS 13).

3. Results

3.1. Growth and water status

The changes in the biomass accumulation and water plant status with increasing salinity are presented in Fig. 1. In the present study, the shoot biomass accumulation was unaffected in *A. tenuifolius* by the salt levels used in this study. In contrast, the dray matter was reduced significantly as salinity rises in the three other species. The depressive effect of salt stress was more pronounced in *A. mareoticus*. Thus, in the plants submitted to 300 mM NaCl, the dry matter production was 43.2, 61.7 and 81.7% of the controls in *A. mareoticus*, *A. caprinus* and *A. armatus*, respectively. The Ψw was significantly lower in plants subjected to salt stress than in controls. At 300 mM NaCl, Ψw reached the most negative values (-3.57 MPa). Relative water content of *A. tenuifolius* was significantly higher in the presence of 150-300 mM NaCl (ca. 117% and 122% of the control) than in the absence of salt (Table 1,). However, this parameter decreased significantly ($P< 0.05$) in the three other species with salinity levels and *A. mareoticus* (ca. 80.5 to 60.7% of the control) was the most affected one.

3.2. Root anatomy

The root cross sectional thickness reduced gradually and significantly with increasing salinity levels in *A. mareoticus* (Table 2). In *A. armatus* and *A. caprinus*, a significantly decreased root diameter was observed only at 300 mM NaCl. In contrast, this parameter was unchanged in *A. tenuifolius.* The epidermis thickness was decreased significantly in *A. caprinus* at 300 mM NaCl, while increased in *A. tenuifolius* at the same salt level, but the difference was not significant in the two other species. The cortex thickness was reduced in A. *mareoticus* with increasing salinity levels. Decreased cortex thickness was also observed in *A. armatus*, but only

at 300 mM NaCl. In contrast, a significant and consistent increase in cortex thickness was observed in *A. tenuifolius* with increasing salinity levels. In *A. caprinus*, cortex thickness was increased significantly at 150 mM NaCl, and thereafter a decrease in this parameter was recorded at 300 mM. In addition the cortical cell area, increased significantly in *A. tenuifolius* and *A. caprinus* with increasing salinity, and the greater variation in this parameter was observed in *A. tenuifolius*, thus, as compared with the control, cortical cell area at 300 mM NaCl increased by 68.8% and 22.4% in *A. tenuifolius* and *A. caprinus*, respectively. In contrast, cortical cell area was unchanged in the two other species. Stele diameter decreased significantly in *A. mareoticus* with increase in salinity levels. In *A. caprinus* stele diameter was not affected at 150 mM NaCl, but thereafter significantly decreased with further increase in salinity level. The root's xylem vessel diameter was unaffected by salinity in *A. armatus*, *A. caprinus* and *A. tenuifolius*. In *A. mareoticus,* this parameter was unchanged at 150 mM NaCl, but significantly decreased at the higher salt level (300 mM NaCl). A significant increase in xylem wall thickness was observed in *A. armatus* under salt stress, while this parameter was unchanged significantly in the three other species. On the other hand, a gradual increase in xylem vessel density was recorded in *A. tenuifolius* with increasing salinity levels. Increased vessel density was also observed in *A. caprinus* and *A. mareoticus*, but only at 300 mM NaCl.

3.3. Leaf anatomy

Leaf anatomy lamina thickness showed an increment in lamina and mesophyll thicknesses with increasing salt level in the four plants species (Table 4). *A. tenuifolius* and *A. armatus* had considerably thicker leaves than those recorded in the two other species. As compared with the control, lamina thickness was increased by 119%, 101%, 24% and 23% at 300 mM NaCl in *A. armatus*, *A. tenuifolius, A. caprinus* and *A. mareoticus* respectively. Epidermis thickness showed variable response to salt stress. In *A. caprinus* the upper and lower epidermis was increased at 150 mM NaCl, and thereafter decreased but it was significantly thicker than the control (Table 4). Increased epidermal thickness was also observed in the abaxial leaf surface of *A. armatus* plants submitted to 150-300 mM NaCl and in adaxial leaf surface of *A. tenuifolius* plants at 300 mM NaCl. In contrast, this parameter was unchanged in *A. mareoticus.* The mesophyll cell area decreased gradually with salinity in *A. tenuifolius* and *A. mareoticus*. In contrast, this parameter was increased significantly in *A. armatus* plants submitted to 150-300 mM NaCl. In *A. caprinus* mesophyll cell area increased at 150 mM NaCl, and thereafter decreased at 300 mM. An increase in the vascular bundle area and the bundle sheath distance was found in *A. tenuifolius* and *A. caprinus* with incrasing salinity. In *A. mareoticus,* bundle sheath distance was increased only under highest salt levels. The xylem vessel diameter showed no significant change by salinity levels in all studied species, while the vessel density was increased gradually as salinity rises in *A. armatus* and *A. mareoticus* and only at 300 mM in the two other species. As compared with the control, xylem vessel density was increased by 183.7%, 163.9%, 155.6% and 132.1% at 300 mM NaCl in *A. armatus*, *A. tenuifolius, A. mareoticus* and *A. caprinus* respectively. In addition, the xylem wall thickness was increased by salinity in all studied species especially in *A. tenuifolius* and *A. caprinus* when the wall thickness reached 1.62 to 1.69 fold than the control ones, respectively. The stomatal density was unchanged in *A. tenuifolius*, in contrast a steady increase in this parameter with increased salt stress was observed in the three other species (Table 4). The stomata size was increased in *A. tenuifolius* plants submitted to 150-300 mM but decreased in *A. mareoticus* and *A. caprinus* at the same salts levels and only at the highest salt level in *A. armatus*.

4. Discussion

Salt stress is one of the most important environmental factors limiting plant growth and development. Results from this study indicate that biomass accumulation of *A. tenuifolius* was unaffected by salinity with improvement of water content under increasing NaCl levels. In contrast, biomass and the relative water content was reduced significantly in the three other species with an increase in salinity of the culture medium, and the worst affected was *A. mareoticus*. The leaf water potential (Ψ_W) decline significantly in all studied species. This slowdown in the growth is a function of adaptation for the survival of plants under stress, reorienting redirect cells resources (energy and metabolic precursors) in the direction of defensive reactions against stress [12].

Knowledge of anatomical root modifications is essential for the explication of plants growth and hydraulic changes induced by salt stress and therefore to understand the mechanisms used to confront salinity. Our results showed that root cross-sectional and the cortex thickness decreased significantly under salinity in *A. mareoticus* and at higher salt level (300 mM NaCl) in *A. armatus* and *A. caprinus.* In contrast, root thickness was unchanged in *A. tenuifolius* with increased cortex area suggesting better adaptation to salt stress. In addition the cortical cell size increased significantly by salinity in *A. tenuifolius* and *A. caprinus* while, it was unchanged in both other *Astragalus* species. The stele diameter showed significant decrease in *A. mareoticus* and at lesser degree in *A. caprinus.* The root epidermis is directly exposed to environmental changes and therefore important to control the water movement in the roots [13, 14]. In this study it was found that salt stress significantly reduced the root epidermis of *A. caprinus* and increased in *A. tenuifolius* at 300 mM NaCl, however it was unchanged in the two other species. Increasing the thickness of the root epidermis under salt stress can play a key role in plant salt tolerance. The root and cortex thickness are generally smaller in stressed plants. Reducing

root diameter is due to the decrease of the cortex diameter and the vascular tissues thickness resulting from inhibiting the activity of the cambium cells and/or reducing the content of DNA resulting from the decrease in cell division and expansion [15]. In addition, Reinoso et al. [16] shown that the reduction in the size of root structural parameters was due to salt affected vascular cambium activity by diminishing secondary phloem and secondary xylem production. This may be the reason for the notable growth inhibition observed in *A. mareoticus* salt-treated plants. Indeed, water and ions uptake as well as the flow of photoassimilates had less chance to circulate up and down through the whole plant.

Other anatomical changes are reported in the roots such as increasing the size of the cortical parenchymatic cells observed in the roots of *A. tenuifolius* and *A. caprinus* seedlings growing under salt stress. The cortical parenchyma tissues can surely enhance the storage capacity of water and nutrients, which is crucial under unfavourable moisture conditions [17]. Indeed, the large cortical cells, particularly under severe stress, may be able to dilute the excess of salts, because they have generally large vacuoles. In *A. tenuifolius* root's the maintenance of the cross-section and cortex area accompanied by increased cortex cell size under saline conditions, suggest that this species capable of storing additional water in cortical parenchyma, which is a key factor for its survival under limited moisture environments as reported by Nawaz et al. [18].

The study of the vascular tissue showed that the xylem vessel diameter was reduced at higher salt level in *A. mareoticus* roots however; it remained very much stable in the other species. In other hand, previous work [19, 20], shown a positive correlation between xylem density and plant salt tolerance. In our study, *A. tenuifolius* showed increased density of root xylem vessels with increasing salinity levels, and only at 300 mM NaCl in *A. caprinus* and *A. mareoticus*. Salinity influences the anatomy of cambial derivatives in such a way that xylem vessels are more numerous and narrower than those found under non-saline conditions [21]. Therefore, Sibounheuang et al. [22] reported that xylem vessel diameter is related to the maintenance of the water conductivity.

In the other hand, leaf anatomical modifications under limited moisture availability can play an important role under salt stress [23] and they are an indicator of the degree of tolerance. Similarly, we found that salt stress caused a significant augment in the leaf lamina and mesophyll thickness and the thickened leaves are of *A. tenuifolius* and *A. armatus* (Table 4, Figure 3). 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 [17], thus permitting the plant to cope with higher salt amounts.

Another remarkable leaf anatomical feature observed in *A. tenuifolius* and *A. mareoticus* plants grown under salt stress was a significant decrease of the mesophyll cells size. In contrast, this parameter was increased significantly in *A. armatus* plants. Thus, smaller size of mesophyll cells represents a major structural response to increased water stress. Indeed, small cells can resist turgor pressure better than large ones, and can contribute to turgor maintenance more effectively under drought conditions [24].

Concerning epidermis size, it is common to observe a thickening of the skin under salt stress, which can be related to salt tolerance. Also, our results showed an increased thickness for the upper epidermis of *A. armatus* and *A. caprinus* especially in *A. tenuifolius* leaves indicating its better adaptability potential to prevent undue water loss under saline environments. The presence of largely vacuolated epidermal cells, with low metabolic activity may serve as a dumping system for toxic ions that helps to protect mesophyll cells under stress [9]. In contrast, this parameter was unchanged in *A. mareoticus*.

Regarding vascular system, our results exhibited that increasing salinity increase the vascular bundle area in *A. tenuifolius* and *A. caprinus*. In contrast this parameter did not greatly modify under salt conditions in *A. armatus* and *A. mareoticus.* Better development of vascular tissue may be important for efficient transport of solutes and photosynthates under salt stress [25]. In the present study, the leaf xylem vessel diameter did not show clear trends with salinity, while the vessel density was increased as salinity raises in the four studies species especially in *A. armatus* and *A. tenuifolius.* In addition, the xylem wall thickness was increased by salinity in all studied species especially in *A. tenuifolius.* On the other hand, our results (table 4) showed that increasing salinity in the medium was associated with an improvement of leaf water content in *A. tenuifolius* and then declined slightly in *A. armatus* but decreased severely with increasing salinity in the two other species especially in *A. mareoticus*. These results led to conclude that reduction in the leaf water content can be explained by a less efficient water transport through the xylem. This suggested the leaf ability of *A. tenuifolius* and in lesser degrees for *A. armatus* to maintain comparable water transport in all treatments.

Numerous works linked the change in transpiration caused by salinity to the reduced stomatal conductance and the lower stoma density of leaves under saline conditions, as indicated by the close correlation found between these parameters [26, 27]. In the present study, leaf stomatal density was unchanged by salinity in *A. tenuifolius*, but decreased in the three other species and the more affected are *A. mareoticus* and *A.* caprinus. Our results show that leaf stomatal size was reduced with increasing salinity in the majority of studied plants and only at the highest salt level in *A. armatus,* while increased in *A. tenuifolius* under 300 mM NaCl. From these

results, it appears that *A. tenuifolius* seemed to be the better adapted as stomatal area increased decreased with the upkeep stomatal density. This may be responsible for absence of water loss through leaf surfaces.

5. Conclusion

The present study on the salt tolerance of *Astragals* species indicates that *A. tenuifolius* the better salt tolerant because of specific morphological and anatomical adaptive characteristics for its survival under saline environments. Morphological adaptation was observed mainly on increased leaf water content as well as stable shoot dry weight. Structural modifications at the root level in this specie were increased epidermis thickness, cortical cell size and increased density of xylem vessel elements as well as stable root thickness. At leaf level, the thicker leaves and mesophyll tissue, thick upper epidermis, increased vascular bundle area, intensive density of thick-walled vessel elements and larger stomata with unchanged density. For *A. armatus* the second better tolerant species relied on it's slightly decrease in leaf water content and the lowest reduction in biomass production. Moreover, root and cortex thickness decreased only at higher salt levels, epidermis thicknesses and xylem vessel diameter was unchanged; and in leaf blade, thicker leaves and mesophyll tissue, thick upper epidermis, increased mesophyll cells size, intensive density of thick-walled vessel elements was observed. In the two other species, morphological and anatomical characteristics were severely affected and the *A. mareoticus* appeared to be the least tolerant. Thus, multiples parameters measured in *A. caprinus* confirmed its better adaptation to salt stress, such as, increasing size of root cortical cell area as well as stable xylem vessel diameter, also, thick leaf upper epidermis and increased vascular bundle area. All these adaptive features may be useful for the identification of salt tolerance traits in other species. In addition, these anatomical adaptive features might be targets for incorporation into salt sensitive species through modern molecular and genetic engineering techniques.

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Table 1. Effects of NaCl concentrations on water potential (Ψw), shoot dry mass (SDM) and relative water content (RWC) in *A. tenuifolius*, *A. armatus*, *A. caprinus* and *A. mareoticus*.

Species	Traitements (mM NaCl)	Parameters		
		Water Potential (Ψw)	**Shoot dry mass (SDM)**	**Relative water content (RWC)**
A. tenuifolius				
	0	-0.74 ± 26.4**a**	6.278 ±37.5**a**	81.2 ±29.6**c**
	150	-2.15 ± 3.6**b**	6.213 ± 4.1**a**	82.4 ± 6.8**a**
	300	-2.96 ± 10.3**c**	6.105 ± 19.5**a**	84.1 ±24.1**b**
A. armatus				
	0	-0.73 ± 17.3**a**	4.351 ±16.7**a**	78.1 ±15.9**a**
	150	-2.31 ± 3.4b	3.852 ± 3.1**b**	77.5 ± 5.7**ab**
	300	-3.57 ± 8.5**c**	3.557± 9.2**c**	76.2±7.6**b**
A. caprinus				
	0	-0.86 ± 25.3**a**	5.143±19.8**a**	75.1±20.1**a**
	150	-2.51 ± 4.5**b**	4.249 ± 6.4**b**	69.7 ± 4.9**b**
	300	-3.05 ± 12.5**c**	3.219 ± 15.8**c**	61.1 ± 10.7**c**
A. mareoticus				
	0	-0.68± 41.1**a**	4.122± 28.3**a**	76.3 ± 18.2**a**
	150	-1.95 ± 3.5**b**	2.935 ± 3.3**b**	61.4 ± 4.1**b**
	300	-2.56± 11.5**c**	1.781 ±10.2**c**	46.3± 6.5**c**

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 root anatomical parameters of *A. tenuifolius*, *A. armatus*, *A. caprinus* and *A. mareoticus*.

Species / Characters	Traitements		
	Control	**150 mM**	**300 mM**
A. tenuifolius			
Total root thickness (µm)	1297.8 ± 26.4**a**	1316,2 ±37.5**a**	1311,3 ±29.6**a**
Epidermis thickness (µm)	75.1 ± 3.6**b**	76.6 ± 4.1**b**	94.5 ± 6.8**a**
Cortex thickness (µm)	427.5 ± 10.3**c**	490.1 ± 19.5**b**	512.5 ±24.1**a**
Cortical cell area (µm^2)	617.0± 11.6**c**	938.2± 21.3**b**	1042.3± 32.7**a**
Stele diameter (µm)	282.5 ± 8.3**a**	267.4 ± 18.4a**b**	272,6 ± 17.3a**b**
Xylem vessel diameter (µm)	21.5 ± 1.9**a**	20.0 ± 1.7**a**	20.1 ± 2.1**a**
Xylem vessel density (nu mm^{-2})	1832.5 ± 28.1**c**	2189.2±19.1**b**	2454.0 ±20.5**a**
Vessel wall thickness (µm)	0.99 ± 0.06**a**	0.98 ± 0.05**a**	0.97 ± 0.07**a**
A. armatus			
Total root thickness (µm)	1335.1 ± 17.3**a**	1362.5 ±16.7**a**	1205.2 ±15.9**b**
Epidermis thickness (µm)	66.1 ± 3.4a	68.5 ± 3.1a	64.2 ± 5.7a
Cortex thickness (µm)	297.5 ± 8.5**a**	293.3± 9.2**a**	252.5±7.6**b**
Cortical cell area (µm^2)	1509.5± 23.5**ab**	1558.7± 27.1**a**	1471.4± 41.8**b**
Stele diameter (µm)	635.0 ± 15.4**a**	616.2 ± 15.8**a**	642.5±21.4**a**
Xylem vessel diameter (µm)	27.8 ± 1.3a**b**	29.2 ± 0.9**a**	28.5 ± 1.4**a**
Xylem vessel density (nu mm^{-2})	808.7 ± 18.2**b**	823.2±12.3**a**	753.7±17.2**c**
Vessel wall thickness (µm)	1.01 ± 0.08**c**	1.23 ± 0.09**b**	1.28 ± 0.12**a**
A. caprinus			
Total root thickness (µm)	1687.5 ± 25.3**a**	1661.2±19.8**a**	1216.2±20.1**b**
Epidermis thickness (µm)	96.9 ± 4.5**a**	92.5 ± 6.4**a**	73.6 ± 4.9**b**
Cortex thickness (µm)	543.7 ± 12.5**b**	663.8 ± 15.8**a**	415.0 ± 10.7**c**
Cortical cell area (µm^2)	1228.4 ± 20.5**c**	1331.5 ± 19.7**b**	1504.2 ± 22.5**a**
Stele diameter (µm)	512.5 ± 13.2**a**	505.1 ± 10.8**a**	225.8±8.3**b**
Xylem vessel diameter (µm)	23.5± 0,8**a**	23.6 ± 1.1**a**	24.7 ± 0.9**a**

Xylem vessel density (nu mm^{-2})	1121.7 ± 14.7**b**	1137.5±13.9**b**	1475.1±18.8**a**
Vessel wall thickness (µm)	0.94 ± 0.08**a**	0.97 ± 0.09**a**	0.95 ± 0.08**a**
A. mareoticus			
Total root thickness (µm)	3056.2± 41.1**a**	2463.7± 28.3**b**	1267.5 ± 18.2**c**
Epidermis thickness (µm)	59.0 ± 3.5**a**	55.9 ± 3.3**b**	58.2 ± 4.1**a**
Cortex thickness (µm)	1033.7± 11.5**a**	873.6 ±10.2**b**	427.4± 6.5**c**
Cortical cell area (nu mm^{-2})	3588.4± 56.1**a**	3624.5 ± 61.2**a**	3425.2 ± 45.8**a**
Stele diameter (µm)	817.5 ± 7.8**a**	621.2 ± 6.3**b**	247.5 ± 3.4**c**
Xylem vessel diameter (µm)	24.0± 1.0**a**	23.1± 0.8**a**	16.2 ± 0.7**b**
Xylem vessel density (nu mm^{-2})	2485.6± 34.5**b**	2460.7 ± 39.1**b**	3231.5± 42.9**a**
Vessel wall thickness (µm)	1.02 ± 0.07**a**	0.95 ± 0.06**a**	0.82 ± 0.05**b**

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 3. Anatomical variables and properties of leaves from *A. tenuifolius*, *A. armatus*, *A. caprinus* and *A. mareoticus* plants treated with different salinity levels (0, 150 and 300 mM NaCl).

Species	**Characters**	**Traitements**		
		Control	**150 mM**	**300 mM**
A. tenuifolius				
Total leaf thickness (µm)		438.3± 10.3**c**	470.8 ±9.6**b**	883.3 ±23.7**a**
Upper epidermis thickness (µm)		38.3± 1.9**b**	38.2± 2.1**b**	45.7 ± 3.2**a**
Lower epidermis thickness (µm)		20.1 ± 1.3**b**	22.5± 1.4**b**	44.6±3.7**a**
Mesophyll thickness (µm)		268.3 ± 6.9**c**	317.5± 8.2**b**	603.4± 21.4**a**
Mesophyll cell area (µm^2)		1122.1 ± 52.6**a**	967.7 ± 23.4**b**	891.6 ± 24.5**c**
Vascular bundle diameter (µm)		36.5± 2.6**b**	55.3 ± 4.6**a**	54.1 ± 5.0**a**
Distance between VB (µm)		178.6± 6.1**c**	204.1± 7.2**b**	327.5± 8.6**a**
Xylem vessel diameter (µm)		22.8± 0.3**a**	23.2± 0.2**a**	23.0± 0.2**a**
Xylem vessel density (nu mm^{-2})		914.2± 8.3**b**	903.5 ± 7.8**b**	1498.2 ± 12.9**a**
Vessel wall thickness (µm)		1.89± 0.07**b**	3.04±0.12**a**	3.08±0.11**a**
Stomata size (µm)		31.2 ± 2.0**b**	32.4 ± 1.8**b**	41.0 ± 2.3**a**

Stomatal density (nu mm^{-2})	53.4± 3.2**a**	54.1± 2.9**a**	52.9 ± 5.1**a**
A. armatus			
Total leaf thickness (µm)	327.5± 9.8**c**	588.3 ±19.1**b**	720.1 ±25.4**a**
Upper epidermis thickness (µm)	16.7± 1.5**a**	16.8 ± 1.5**a**	16.6 ± 1.6**a**
Lower epidermis thickness (µm)	154.3± 6.7**a**	152.2± 7.1**a**	130.1±5.7**b**
Mesophyll thickness (µm)	274.1± 9.1**c**	467.5± 12.3**b**	613.3± 19.7**a**
Mesophyll cell area (µm^2)	1635.7 ± 33.9**b**	1753.0 ± 35.1**a**	1785.3±37.4**a**
Vascular bundle diameter (µm)	50.1± 2.8**a**	42.2± 1.9**b**	41.0 ± 2.0**b**
Distance between VB (µm)	263.4± 7.9**a**	202.5± 6.6**b**	276.6± 9.2**a**
Xylem vessel diameter (µm)	13.8± 0.1**a**	14.2± 0.2**a**	14.6± 0.2**a**
Xylem vessel density (nu mm^{-2})	1801.2± 22.7**c**	2548.3± 31.8**b**	3310.7± 42.5**a**
Vessel wall thickness (µm)	1.63± 0.10**c**	1.89±0.12**b**	2.03±0.21**a**
Stomata size (µm)	29.5± 2.1**a**	28.9 ± 2.2**a**	25.4± 2.2**b**
Stomatal density (nu mm^{-2})	44.5± 2.8**a**	42.5 ± 2.9**b**	37.7± 2.0**c**
A. caprinus			
Total leaf thickness (µm)	314.1 ± 9.8**c**	353.4±10.8**b**	388.5±12.3**a**
Upper epidermis thickness (µm)	16.5± 1.7**c**	28.4 ± 2.5**a**	26.5± 3.1**b**
Lower epidermis thickness (µm)	16.4 ± 1.9**c**	25.6± 2.7**a**	21.4± 2.5**b**
Mesophyll thickness (µm)	233.4± 6.1**c**	249.6 ± 5.9**b**	292.1± 7.2**a**
Mesophyll cell area (µm^2)	1518.5± 25.3**b**	1580.2± 23.9**a**	1400.1±26.4**c**
Vascular bundle diameter (µm)	60.6 ± 3.2**b**	71.3± 3.8**a**	72.9 ± 3.3**a**
Xylem vessel diameter (µm)	19.4± 1.6**b**	20.2 ± 1.8**ab**	20.6 ± 1.9**ab**
Xylem vessel density (nu mm^{-2})	758.7± 23.1**b**	712.7 ± 33.6**c**	1002.5 ± 39.8**a**
Vessel wall thickness (µm)	2.04± 0.11**b**	3.55±0.23**a**	3.46±0.27**a**
Stomata size (µm)	34.3± 2.1**a**	31.0 ± 1.7**b**	26.4 ± 1.2**c**
Stomatal density (nu mm^{-2})	50.1± 2.9**a**	45.5± 3.1**b**	38.6± 2.8**c**
A. mareoticus			
Total leaf thickness (µm)	322.5± 5.8**b**	319.1± 5.2**b**	397.5± 7.2**a**

Upper epidermis thickness (µm)	23.9± 1.4**a**	23.5 ± 1.3**a**	23.4 ± 1.7**a**
Lower epidermis thickness (µm)	23.8± 1.2**a**	23.4 ±1.3**a**	23.5± 1.4**a**
Mesophyll thickness (µm)	216.1± 6.5**c**	281.7 ± 7.2**b**	340.4 ± 9.3**a**
Mesophyll cell area (µm^2)	1206.2± 19.6**a**	1070.6± 15.1**b**	1057.2 ± 16.9**b**
Vascular bundle diameter (µm)	62.8± 3.4**b**	72.5± 3.9**a**	66.8± 4.2**ab**
Distance between VB (µm)	198.6± 5.9**b**	201.9 ± 3.8**b**	231.6± 6.2**a**
Xylem vessel diameter (µm)	13.6± 0.9**ab**	13.1± 1.1**b**	14.0± 1.0**a**
Xylem vessel density (nu mm^{-2})	801.2± 18.3**c**	1194.5± 23.1**b**	1247.1± 21.3**a**
Vessel wall thickness (µm)	1.55± 0.09**c**	1.76 ± 0.12**b**	2.04 ± 0.15**a**
Stomata size (µm)	43.7± 1.8**a**	39.6± 1.1**b**	31.7± 1.2**c**
Stomatal density (nu mm^{-2})	52.1± 1.7**a**	41.8± 1.5**b**	33.9± 1.3**c**

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.

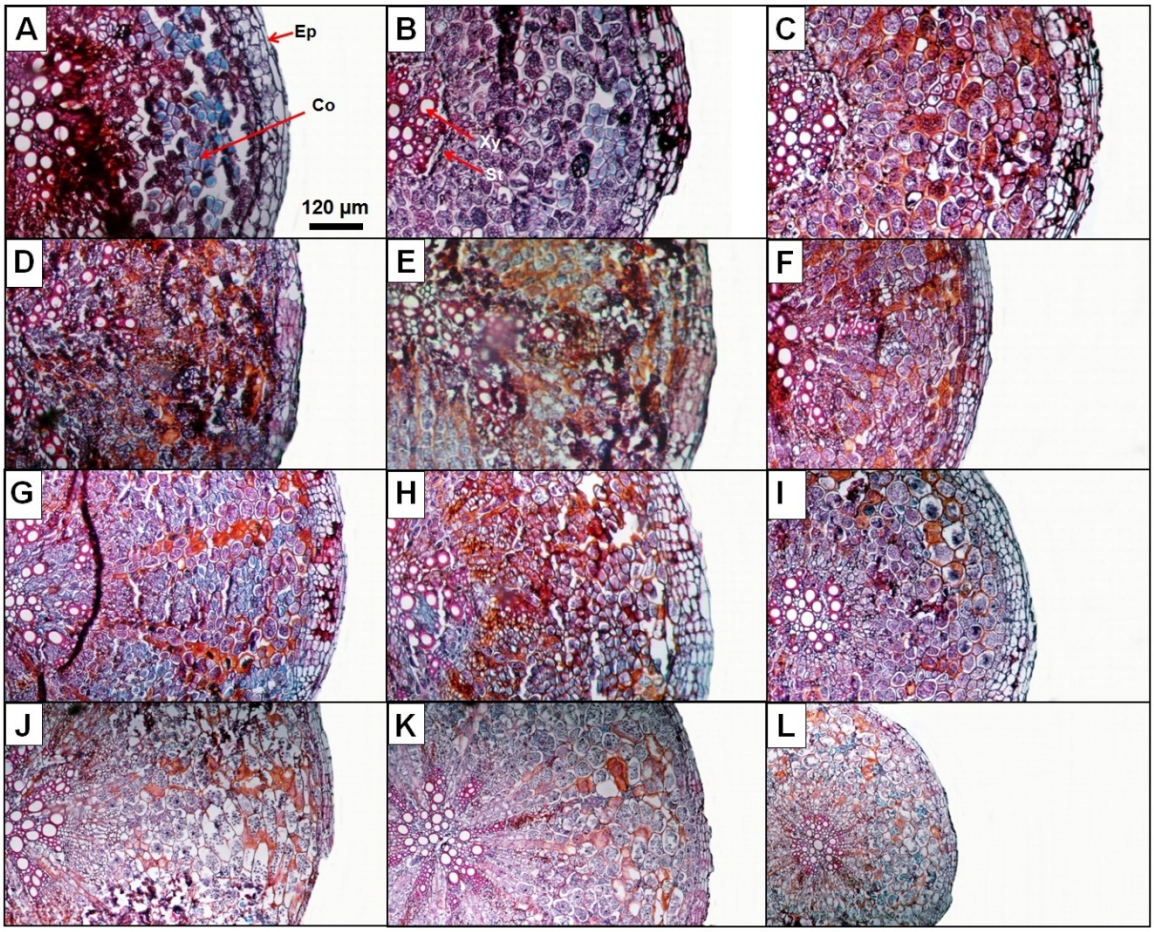

Figure 1: Root cross-sections showing the xylem tissue details in *A. tenuifolius* (a, b and c), *A. armatus* (d, e and f), *A. caprinus* (g, h and i) and *A. mareoticus* (j, k and l) plants grown at 0 (a, d, g and j), 150 (b, e, h and k) and 300 mM NaCl (c, f, i and l), respectively. Bars = 120 µm. Co, cortex, Ep, epidermis; St, stele; Xy, xylem.

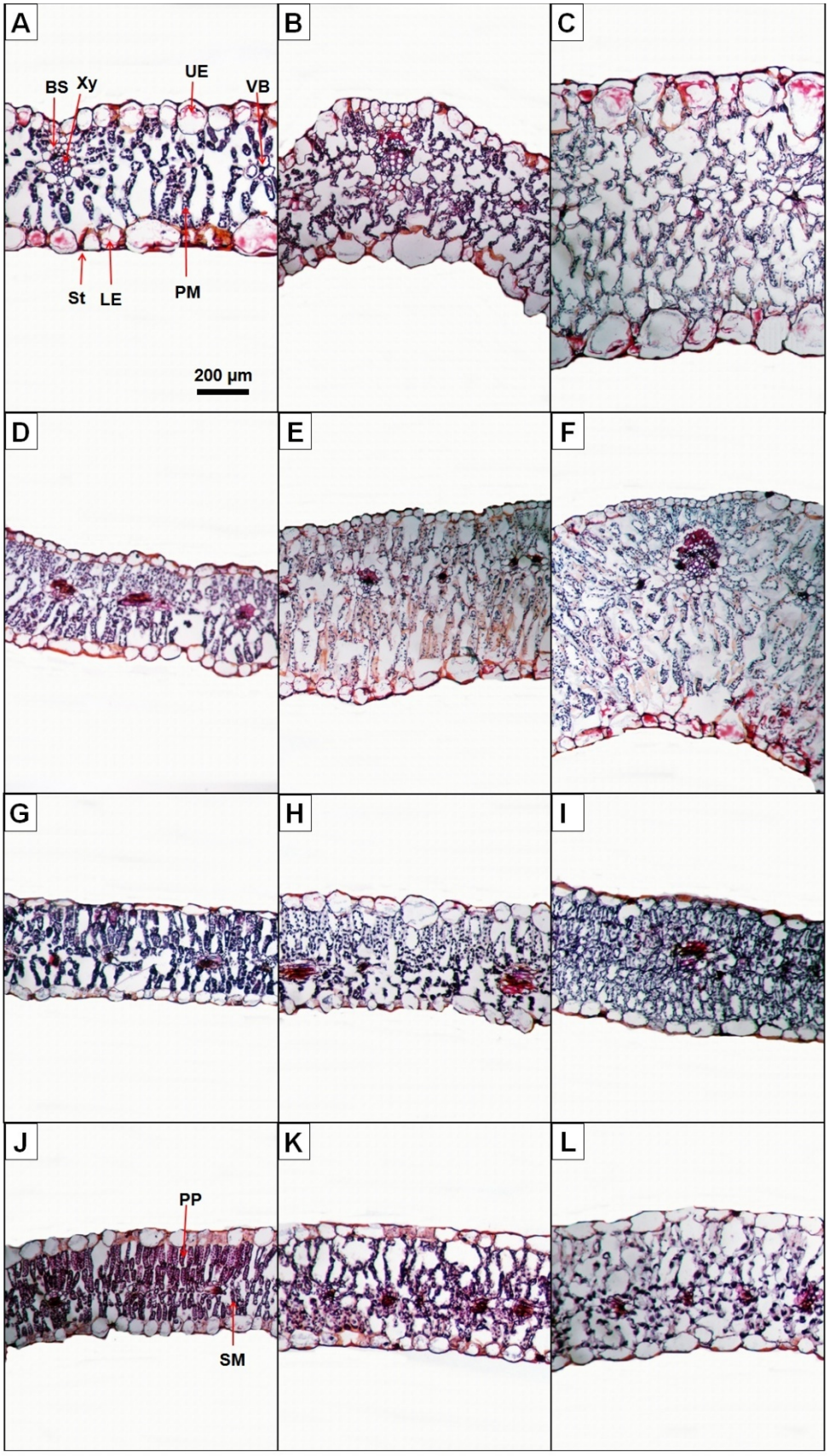

Figure 2: Leaf blade cross-sections showing leaf anatomical changes in *A. tenuifolius* (a, b and c), *A. armatus* (d, e and f), *A. caprinus* (g, h and i) and *A. mareoticus* (j, k and l) plants grown at 0 (a, d, g and j), 150 (b, e, h and k) and 300 mM NaCl (c, f, i and l), respectively. Bars = 200. BS, bundle sheath; LE, lower epidermis; UP, upper epidermis; M, mesophyll cells; PM, palisade mesophyll; SM, spongy mesophyll; St, Stomata, VB, vascular bundle.