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ANATOMICAL ADAPTATIONS OF TOLERANCE TO SALT STRESS IN *CENCHRUS CILIARIS* L., A SALINE DESERT GRASS

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ABSTRACT

Cenchrus ciliaris is a potential forage grass that widely grows in saline desert environment. To examine root, stem and leaf anatomical modifications, population of *Cenchrus ciliaris* L. (Buffal grass) was collected from the naturally saline patches of the Cholistan desert, Pakistan. Four salinity treatments 0 mM of NaCl (control), 100, 200 and 300 mM of NaCl were maintained in half strength Hoagland nutrient solution by using hydroponic system. After 16 weeks of growth in nutrient solution root, stem and leaf anatomical characteristics were studied. Salt stress induced anatomical modification in root, stem and leaf that help to survive species in adverse environmental condition. At root level, salinity increased the thickness of endodermis and sclerenchyma tissues both prevent the water loss from root surface and also increased in number of parenchyma cells in pith and cortex region that improved the water storage capacity of root. At stem level, salinity contributed in epidermis and sclerenchyma thickness along with increase in number of vascular bundles and its area that might be improved conduction of water and solute, increase succulence ability and preventing water loss. In case of leaf and leaf sheath, decreased in stomatal density and its area, increased bulliform cell, and rich density of vesicular hairs and trichomes might be essential for water conservation and salt excretion. On the basis of anatomical modifications and its correlation with biomass it was concluded that *C. ciliaris* can easily be grow at salt affected areas and such anatomical modifications seemed to be critical for the better survival of species under harsh sandy and salty environment.

Keywords: salt stress; sclerification; biomass; anatomical modifications; bulliform cell.

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INTRODUCTION

Salinity is a major abiotic stress in arid and semi- arid region of the world that reduces growth and productivity of plants (Munns, 2002). In a plant, increased salinity induced specific physiological, morphological and anatomical changes at cellular, tissue and organ level (Isla *et al*., 1998; Hameed *et al*., 2013). The Cholistan desert in Pakistan is vast rangeland with rich diversity which faces many abiotic stresses such as salinity, drought, high temperature and lack of nutrients (Naz *et al*., 2013).

To survive successfully in such harsh condition, the flora of this desert might have developed many adaptive characteristics in response to multiple stresses (Hameed *et al*., 2010). *Cenchrus ciliaris, C. biflorus, Lasiurus scindicus, Ochthoclova compressa, Aeluropus lagopoides, Sporobolus ioclados* and *Cymbopogon jwarancusa* are dominated grasses of Cholistan desert. *Cenchrus ciliaris* L. (Poaceae) is a valuable and highly palatable grass (Nawazish *et al*., 2006) and grows well in sandy soil in semi-arid and arid regions (Opiyo *et al*., 2011) and has salt tolerant characteristics. High concentration of salt in root zone not only effect metabolic processes but also reduced plant growth, productivity (Athar *et al.,* 2009) along with reduction in plant biomass and photosynthesis ability (Gamma *et al*., 2007). High salinity in xero-halophytes induced a series of structural and functional modifications in morphological and anatomical characteristics which include increase in root cross section area, epidermis and cortex thickness (Naz *et al*., 2018), succulence of epidermis and cortical parenchyma (Flowers and Colmer, 2008), sclerification in root (Grigore and Toma, 2008). In stem, increased in stem cross-sectional area, epidermis, cortex and sclerenchyma thickness under salt stress, have been reported by Hameed *et al*. (2013) in *Sporobolus arabicus*, such anatomical modifications might be significantly involved in improving plant water use efficiency (Shabala *et al*., 2013). The tolerance of *C. ciliaris* to salt stress is particularly important, as it is used as fodder species in rangeland of Cholistan desert (Rafy *et al*., 2015).

The main objective of our work was to evaluate the effects of increasing salinity levels on anatomical adaptations of tolerance to salt stress in root, stem and leaf of *C. ciliaris*, a saline desert halophyte, and further correlate these anatomical adaptations with biomass of saline desert grass (*C. ciliaris*). Further research need to investigate the effect of salinity on nutritional value and palatability of such desert halophyte.

MATERIALS AND METHODS

An experiment was conducted in hydroponic system to examine the specific anatomical adaptations in *Cenchrus ciliaris* L. (Poaceae) a salt tolerant grass, under various levels of salt stress. *Cenchrus ciliaris* (Buffel grass) was collected from the saline patches in the Cholistan desert, Pakistan (pH 8.30, ECe 16.20 dSm^{-1}, Na^{+} 4531 mgL^{-1}, K^{+} 407.54 mgL^{-1}, Ca^{++} 68.77 mgL^{-1}, Cl^{-} 1592.23 mgL^{-1}) and were grown in nine inches pots filled with loam and sand for period of nine months. The plants were irrigated and kept in sunlight. Ramets of equal size were selected and shifted in hydroponic containers filled with half-strength Hoagland nutrient solution (Hoagland and Arnon, 1950) for a period of four months. Containers of twenty liter capacity were used for hydroponic system. Air pumps were used to provide aeration to the system about twelve hours daily. Ten plants of each population were used from each replicate of grass species (*C. ciliaris*) and adjusted in pore on thermopore (mineral fiber) sheet used to cover container filled with Hoagland nutrient solution. The study was planned in a 2-factor CRD (completely randomized design) with a population of *C. ciliaris* and four treatment levels, 0 (control), 100, 200 and 300 mM of NaCl in external medium. For recording anatomical characteristics and biomass, the plants were uprooted from the hydroponic after six months at the completion of experiment. To calculate dry weight, plants were oven dried at 65 °C for 72 h.

For anatomical studies a small piece (10mm length) was get from the mid-portion of lamina for leaf anatomy and from the internodal base of main tiller for sheath anatomy. Specimen for root (2cm) from the thickest root present at the junction of main stem and root and for stem (5mm) from the 3rd internode of the main tiller were taken. Tissue samples were immediately preserved in formaldehyde 5%, glycial acetic acid 5%, distilled water 35% and ethanol 70% by volume (FAA) solution for 24 hours. For the long term preservation tissue samples were transferred to solution containing 25% acetic acid and 75% ethyl alcohol by volume. Fixed samples were sectioned by free-hand sectioning technique and double staining method (safranin and fast green) was used for the preparation of permanent slides (Ruzin *et al*., 1999) by applying different grades of ethyl alcohol for dehydration. Microscopic measurement of various anatomical characteristics was recorded with an ocular micrometer, calibrated with stage micrometer. Digital photograph of preserved slides were recorded with a camera equipped stero-microscope (Nikon 104, Japan). Anatomical characteristics recorded for root, stem and leaf anatomy during the experiment were stem area, root thickness, lamina thickness and leaf thickness, exodermis thickness, endodermis and sclerenchyma thickness, cortical, pith thickness, metaxylem and phloem area, bulliform cell number and its area, hair/trichome density and stomatal area and its density (Fig. 10).

The data collected after investigation for various biomass and anatomical features were statistically analyzed by using analysis of variance (ANOVA) in complete randomized design (CRD). The results were analyzed statically by mean of Minitab software (version 18) and mean values were compared on the basis of LSD test.

RESULTS

Data after statistical analysis represented that *C. ciliaris* showed significant modification in various anatomical characteristics of root, stem and leaf with rise in salinity level of growth medium.

Root anatomy: A significant decrease in root cross sectional area was recorded ($P < 0.05$) with rise in NaCl level (Fig.1). Sclerenchyma thickness increased consistently but significantly with rise ($P < 0.05$) in NaCl contents of the growing media (Fig.2).An increase in cortical thickness and cortical cell area was recorded with increase in salinity levels (Fig.1). The maximum increase was noted in both endodermis thickness and endodermis cell area by rise in levels of salinity. Endodermis cell area on contrary, showed increase up to 200 mM NaCl and sudden fall was noticed at 300 mM of NaCl (Fig. 1). Pith thickness increased significantly ($P < 0.05$) with rise in NaCl level. However, this parameter was least affected by salinity (Fig.1). The imposition of salt stress significantly ($P < 0.05$) increased phloem and metaxylem area in *C. ciliaris*. A slight increase in pericycle thickness was observed at 300 mM NaCl salt levels (Fig. 2).An abrupt increase in aerenchyma area was observed at 100 mM NaCl but further increase in salt levels (200 and 300 mM NaCl) this character decreased (Fig. 2).

Stem anatomy: In stem anatomy of *C. ciliaris*, significant changes were noted at different salinity levels (Fig. 3). An increase in stem cross sectional area was noted at the moderate level of salinity (100 and 200 mM of NaCl) however higher NaCl level (300 mM) imparted an adverse effect on this character (Fig. 3). Epidermal thickness and epidermal cell area were significantly ($P < 0.05$) increased by the induction of salt in growth media. A significant reduction in the phloem area and vascular bundle area was observed as the level of NaCl rise in growing media. There was no definite response of vascular bundle number at 100 and 200 mM NaCl levels. However, this parameter was increased at 300 mM NaCl. Metaxylem area of *C. ciliaris* increased consistently by the induction of NaCl in the nutrient solution, however it decreased at 300 mM of NaCl level.

Leaf blade anatomy: A significant ($P < 0.05$) decrease in stomatal density was noted on both adaxial and abaxial leaf surface and was adversely affected at higher salt levels (Fig. 8). However, stomatal area on both adaxial and abaxial surface of leaf was least affected characteristics by the salinity stress (Fig. 8). Lamina thickness was least affected at moderate surface, however as salinity level rise up to 300 mM NaCl, a significant decreased in lamina thickness (Fig. 4). Epidermal thickness and cell area of epidermis on both the adaxial and abaxial leaf surface showed significant ($P < 0.05$) increase with increase in salinity level. Vascular bundle area was adversely affected at higher salt levels (300 mM NaCl) and significantly decreased (Fig. 4). A sudden increase in bundle sheath and sclerenchyma thickness was recorded at all treatment levels of NaCl. Metaxylem area increased at moderate salt level (100 mM NaCl). However, it showed decrease at higher salinity (200 and 300 mM NaCl) (Fig. 5). The most adversely affected character was bulliform cell area, it significantly ($P < 0.05$) decreased as level of salinity increased. However, the bulliform cell number was constantly increased with increase in salinity (Fig. 5). An increase in phloem area was recorded with increase in external salt level (Fig. 5). A gradual increase was noted in adaxial hair density but hair density was constantly increased with increase in external salt level in nutrient solution but abaxial hair density was increased at moderate salt level (100 and 200 mM NaCl) at 300 mM NaCl no further increase was noted.

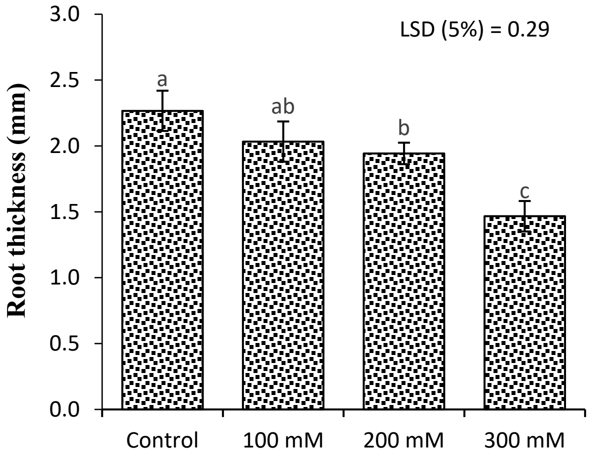
Leaf sheath anatomy: A slight decrease in leaf sheath thickness was noted by the induction of salts in growth medium (Fig. 6). A sudden increased was noted in epidermis cell area on adaxial leaf surface with increase in salinity levels its thickness decreased constantly at 100 mM NaCl and further increase in salt contents result in gradual increase. On abaxial surface of leaf epidermis thickness and its cell area was significantly ($P < 0.05$) increased along with increasing salinity gradient (Fig.6). The population of *C. ciliaris* showed a constant increase in its sclerenchyma thickness and phloem area at various levels of salinity in external medium.

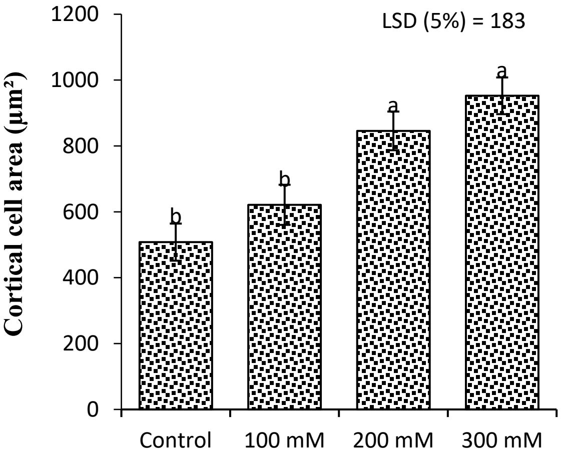
Xylem cell area was slightly decreased by increasing salinity and become constant where vascular bundle area was decreased constantly but significantly along with salinity (Fig.7).

A general increase was noted for both root and shoot dry weight at 100 mM NaCl. However, further induction of salt in growth medium up to 300 mM of NaCl, both parameters significantly decreased (Fig. 9).

Correlation: The correlation coefficient was calculated between plant biomass and anatomical parameters at 0.05 and 0.01 levels of significance. Among root anatomical characteristics root thickness, root aerenchyma cell area showed significantly positive correlation with dry weight root while phloem area showed significant positive correlation with dry weight shoot. Although aerenchyma cells showed highly significant positive correlation with dry weight shoot. However, pith thickness, metaxylem area and sclerenchyma thickness showed significant negative correlation with both dry weight shoot and root.

Among stem anatomical parameter, *C. ciliaris* generally showed significant positive correlation of dry weight root and shoot with phloem area and vascular bundle area. However, dry weight shoot showed highly significant negative correlation with epidermis cell area. In case of leaf anatomical parameters, dry weight root showed significant positive correlation with metaxylem area, bulliform cell area and vascular bundle area while dry weight shoot showed significant negative correlation with adaxial hair density, abaxial epidermis thickness and sclerenchyma thickness (Table 1).





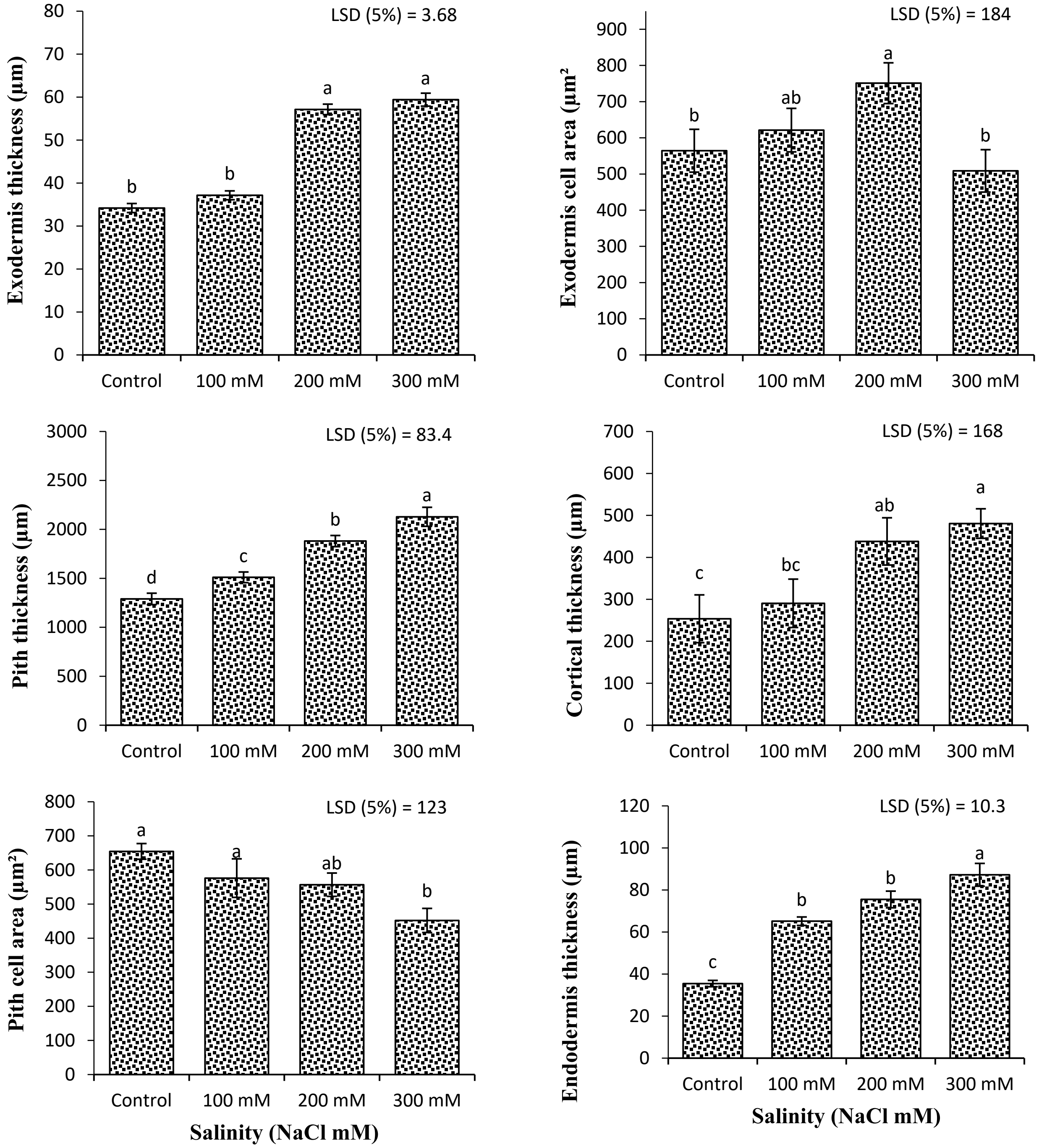


Fig. 1. Root anatomical characteristics of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.

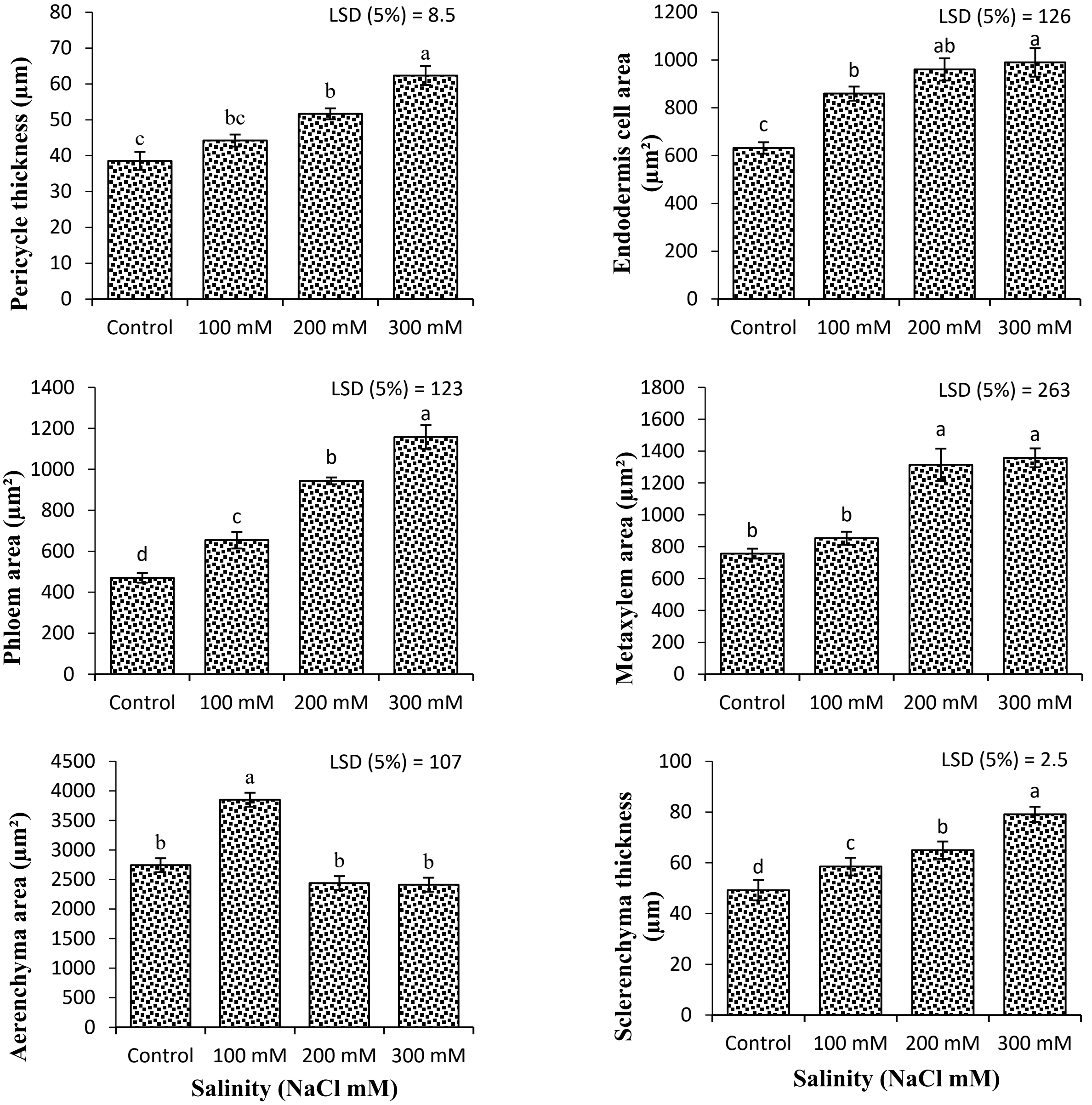
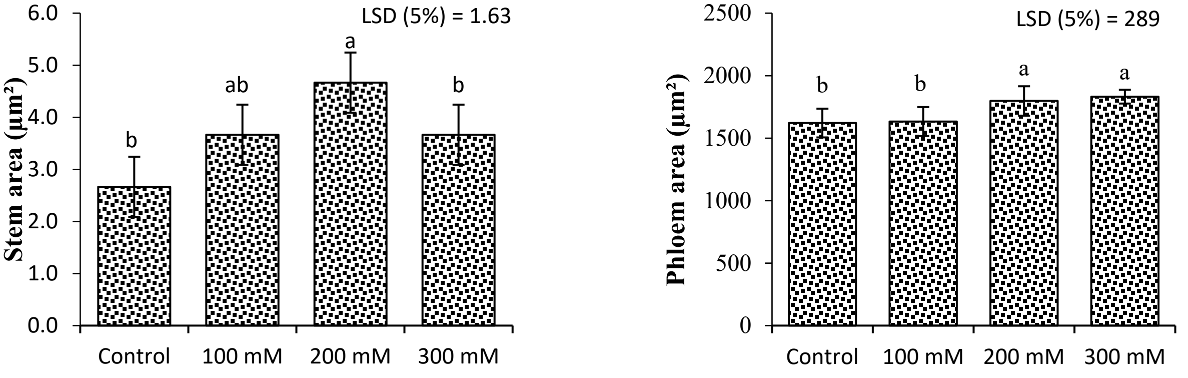


Fig. 2. Root anatomical characteristics of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.



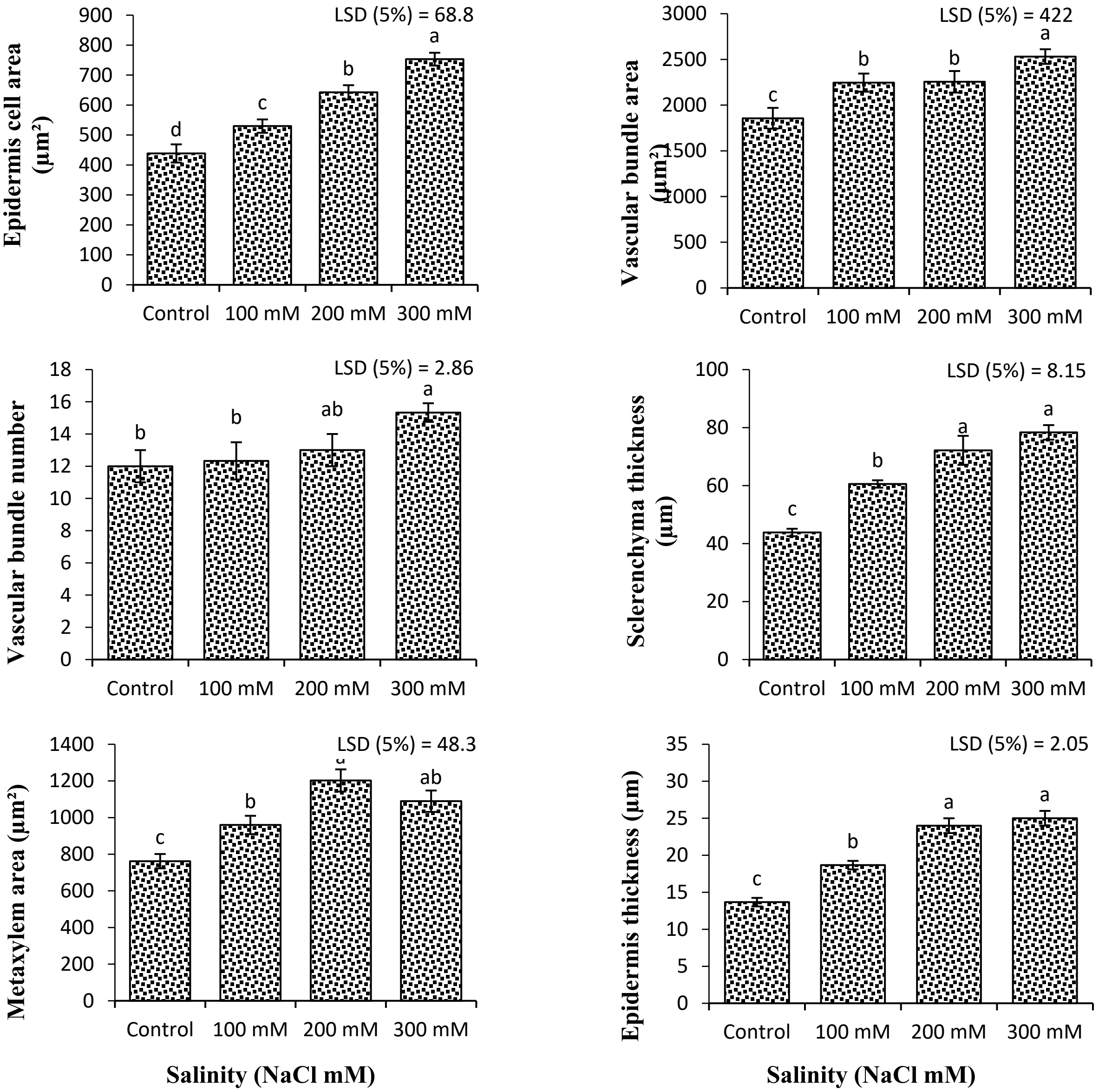
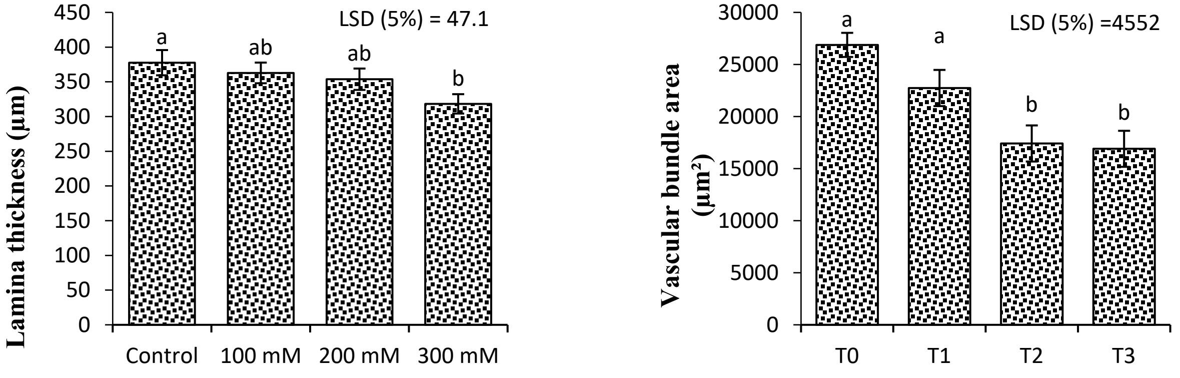


Fig. 3. Stem anatomical characteristics of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.



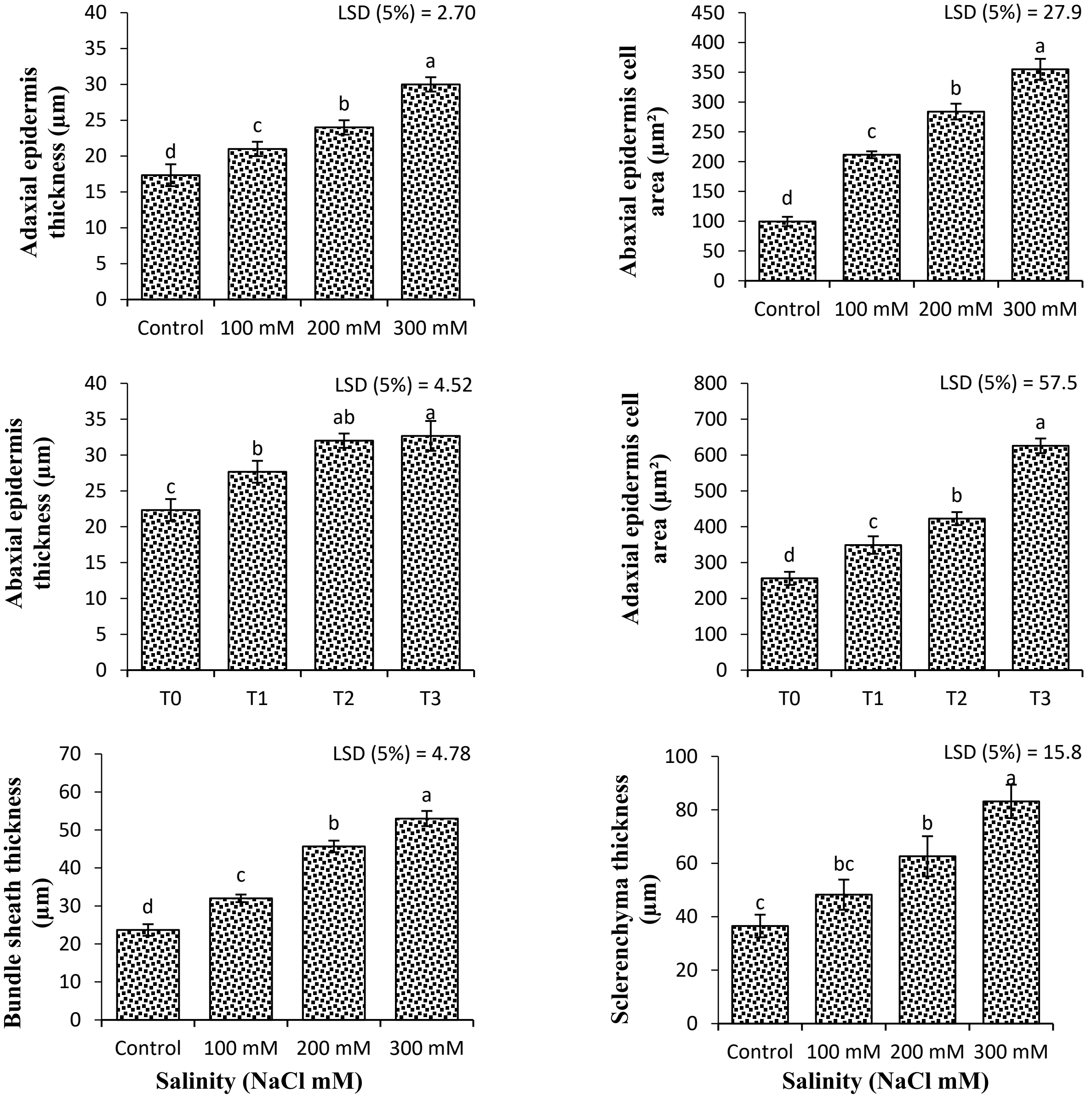
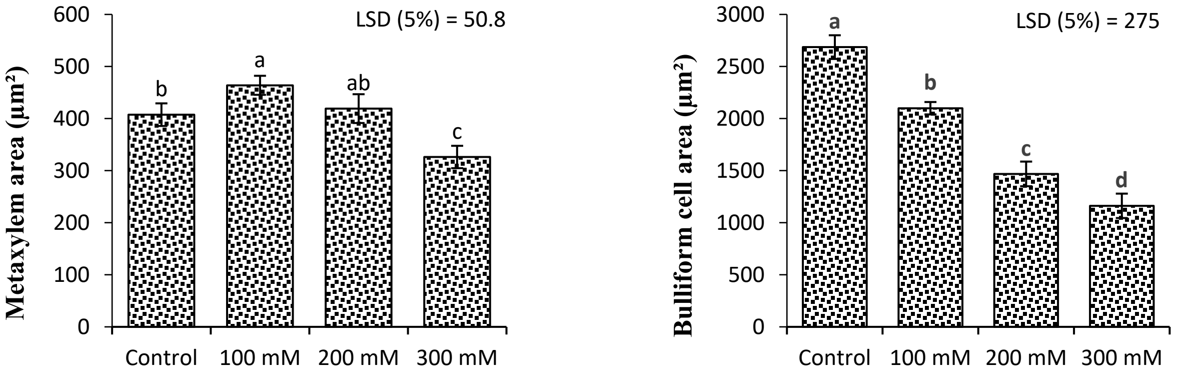


Fig. 4. Leaf blade anatomical characteristics of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.



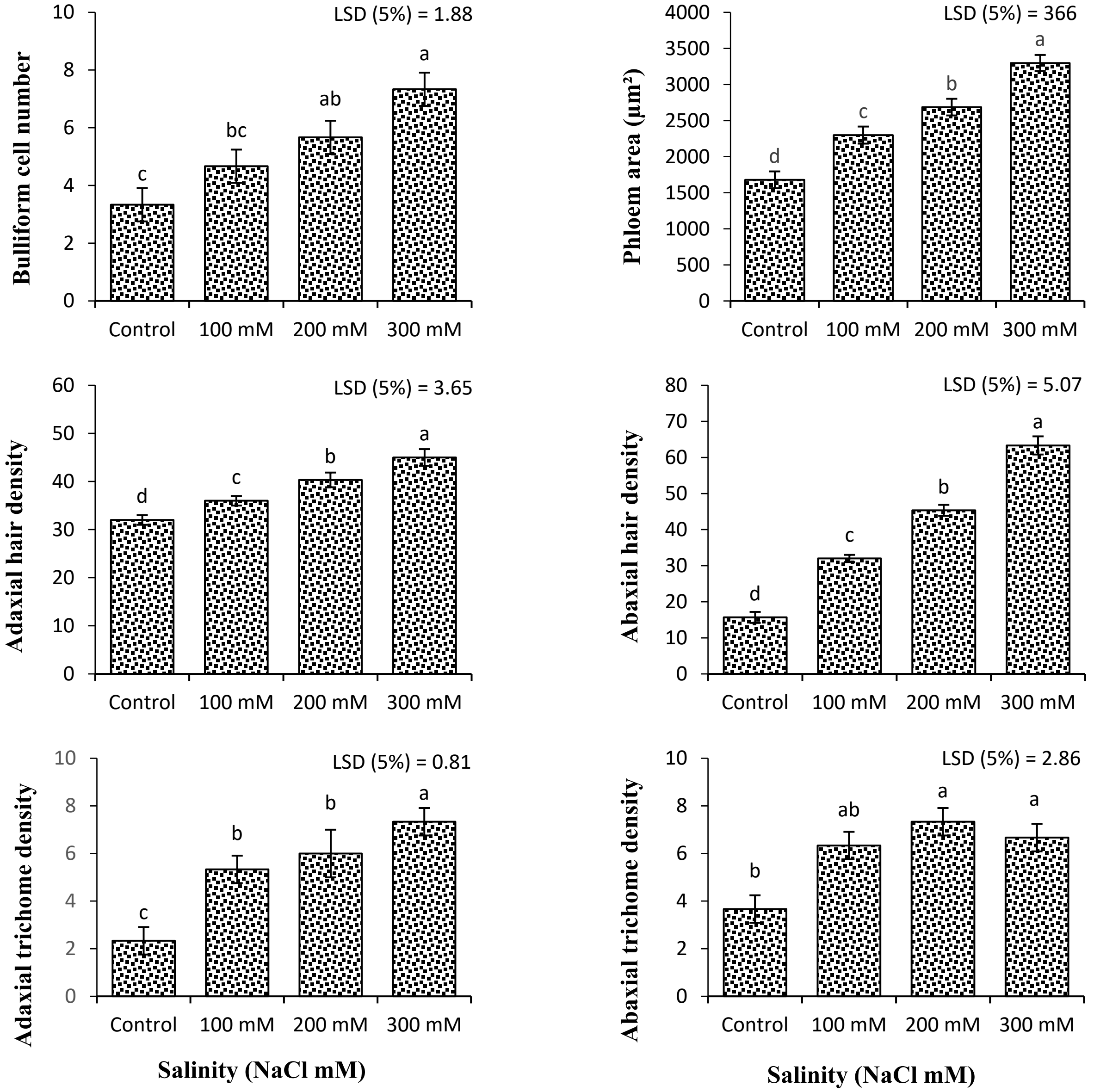
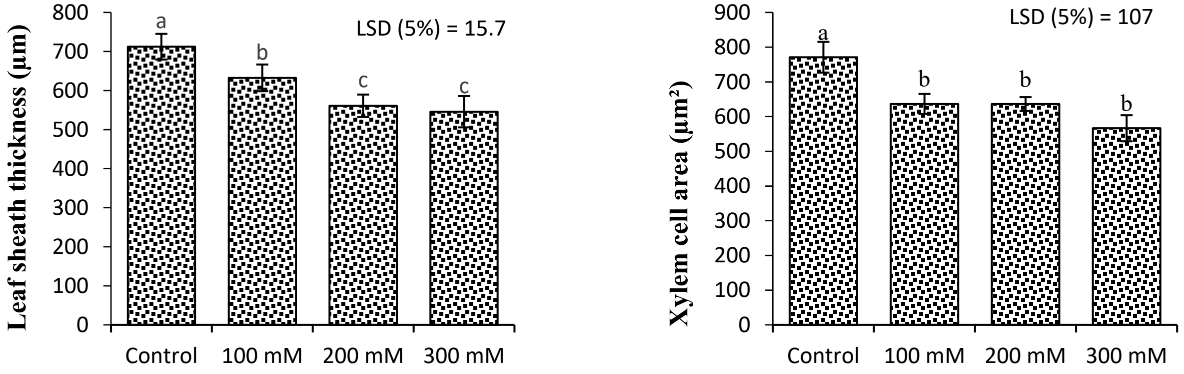


Fig. 5. Leaf blade anatomical characteristics of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.



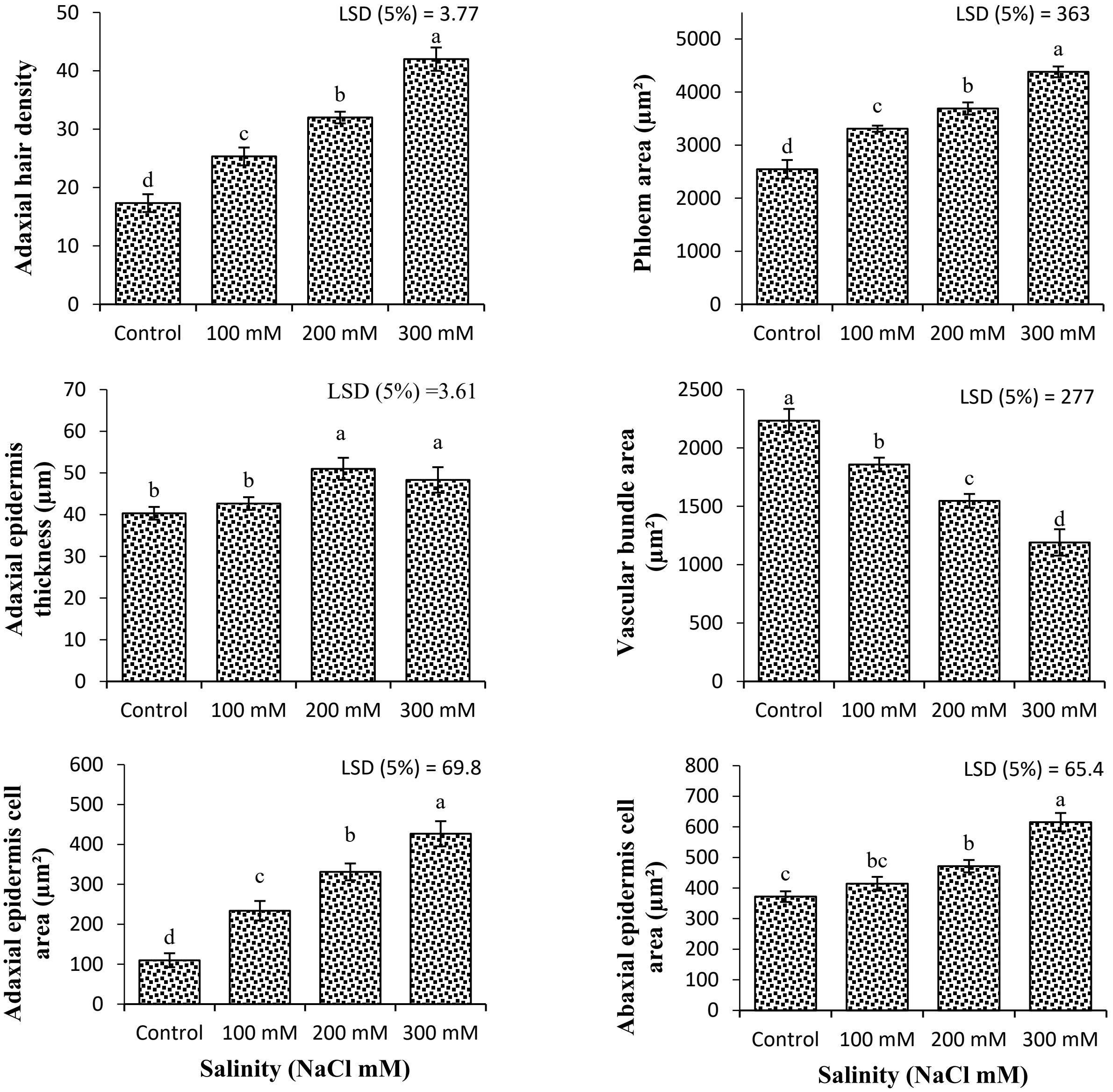


Fig. 6. Leaf Sheath anatomical characteristics of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.

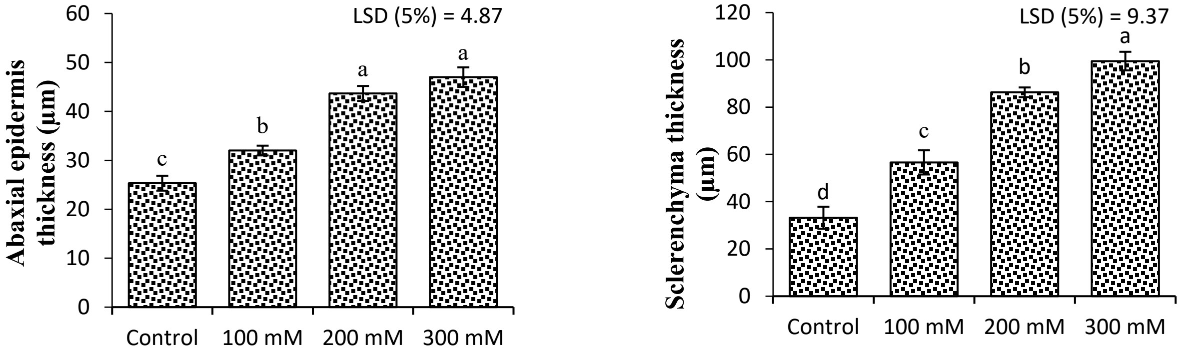


Fig. 7. Leaf sheath anatomical characteristics of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.

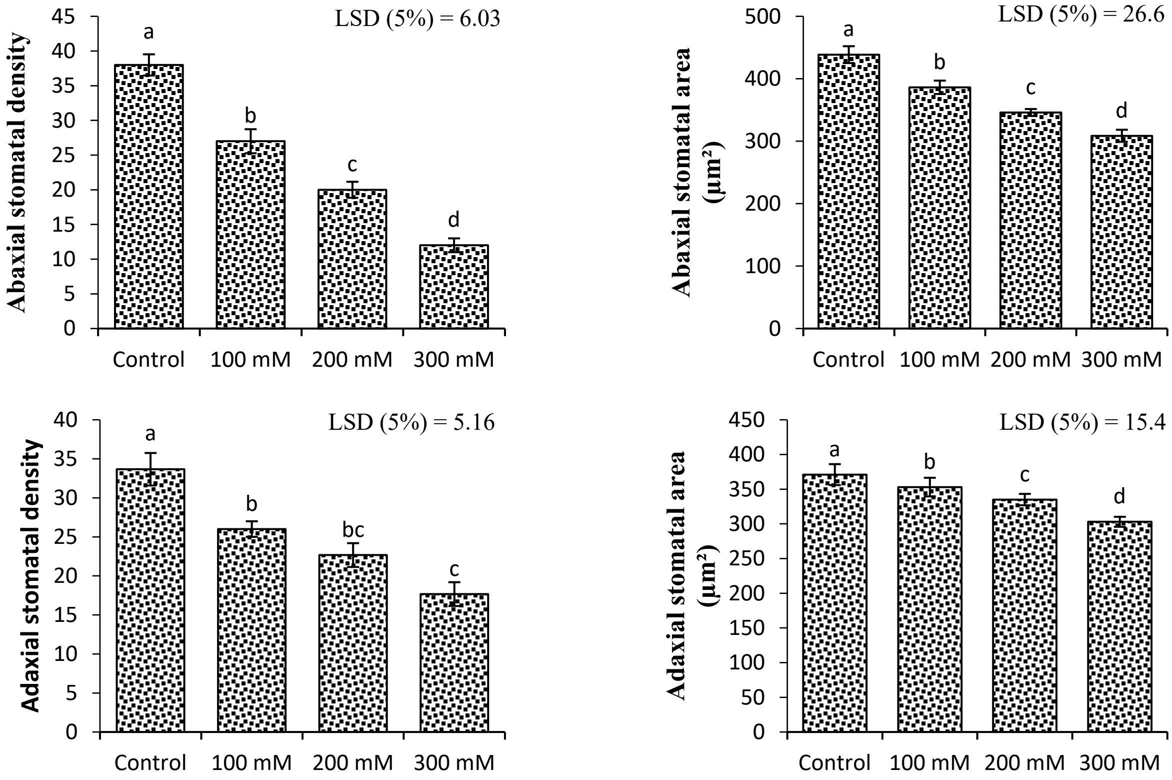


Fig. 8. Stomatal characteristics of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.

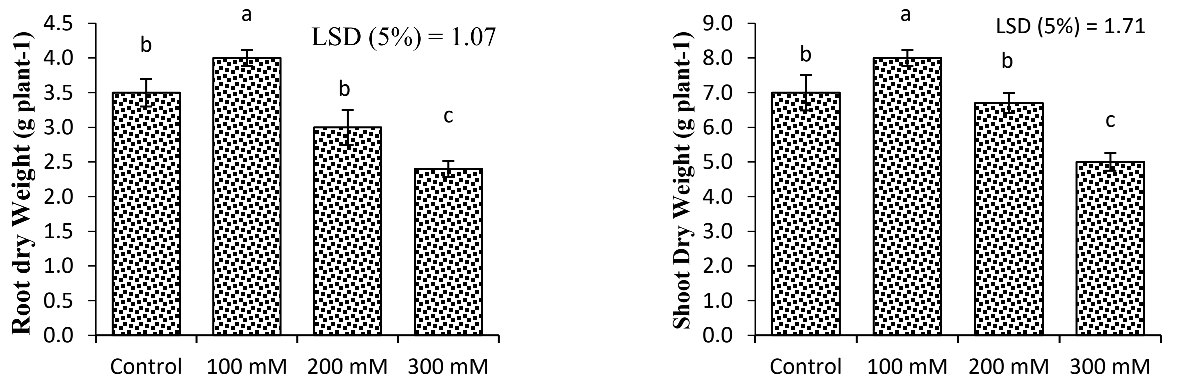


Fig. 9. Root and shoot dry weight of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.

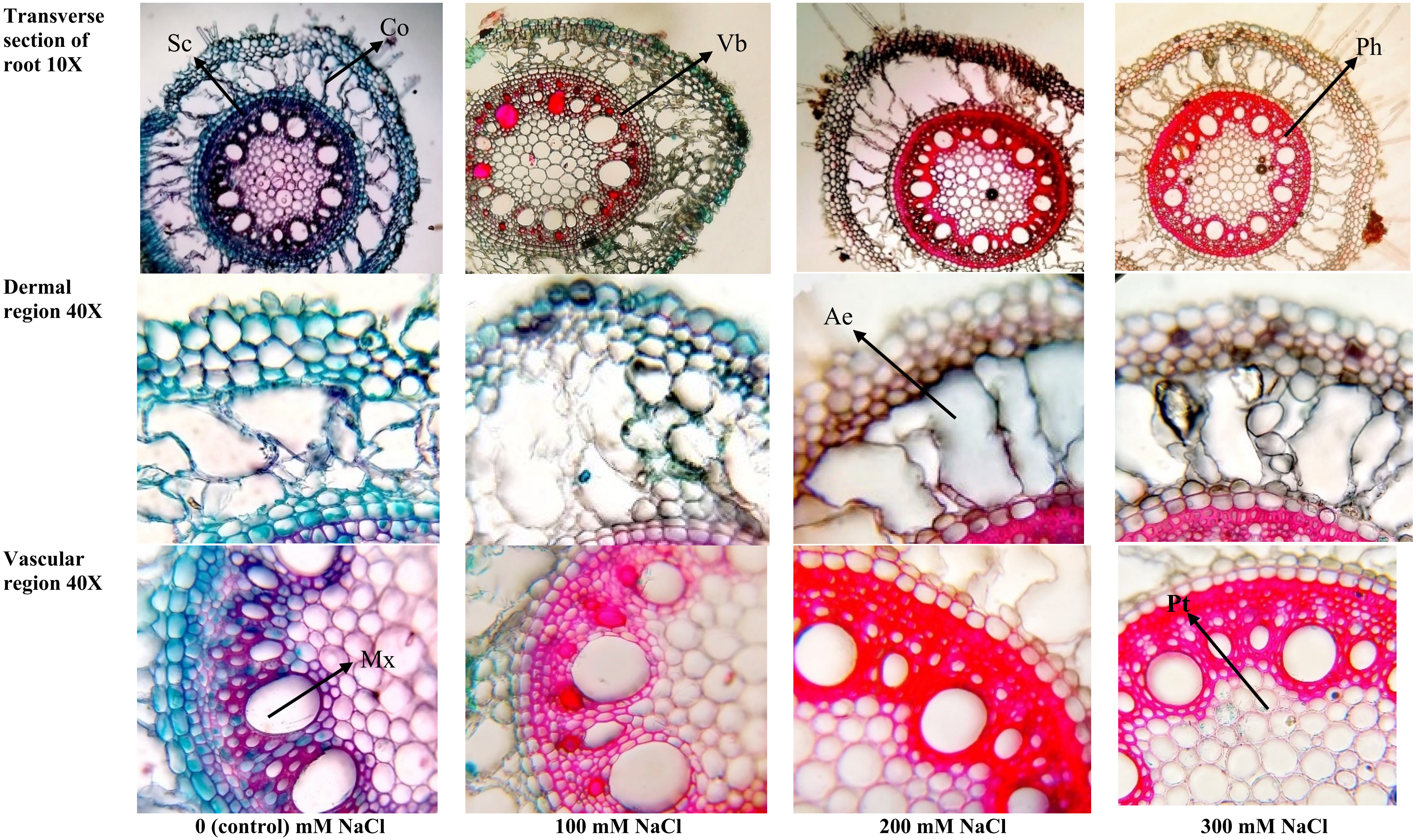


Fig.10. Root anatomy of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.
(Mx = Metaxylem, Ph = Phloem, Sc = Sclerenchyma, Vb = Vascular bundle, Pt = Pith, Ae = Aerenchyma, Co = Cortex).

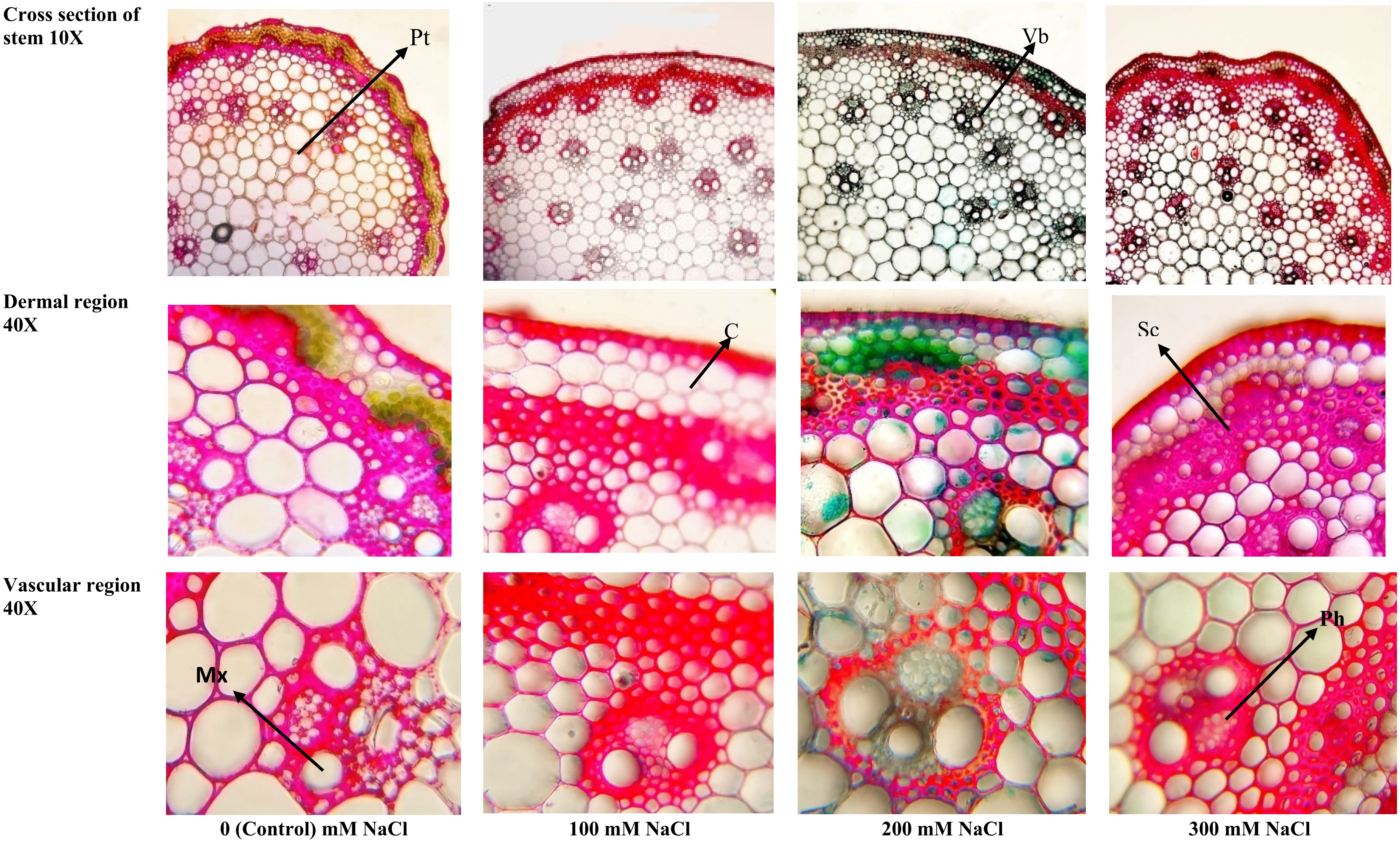
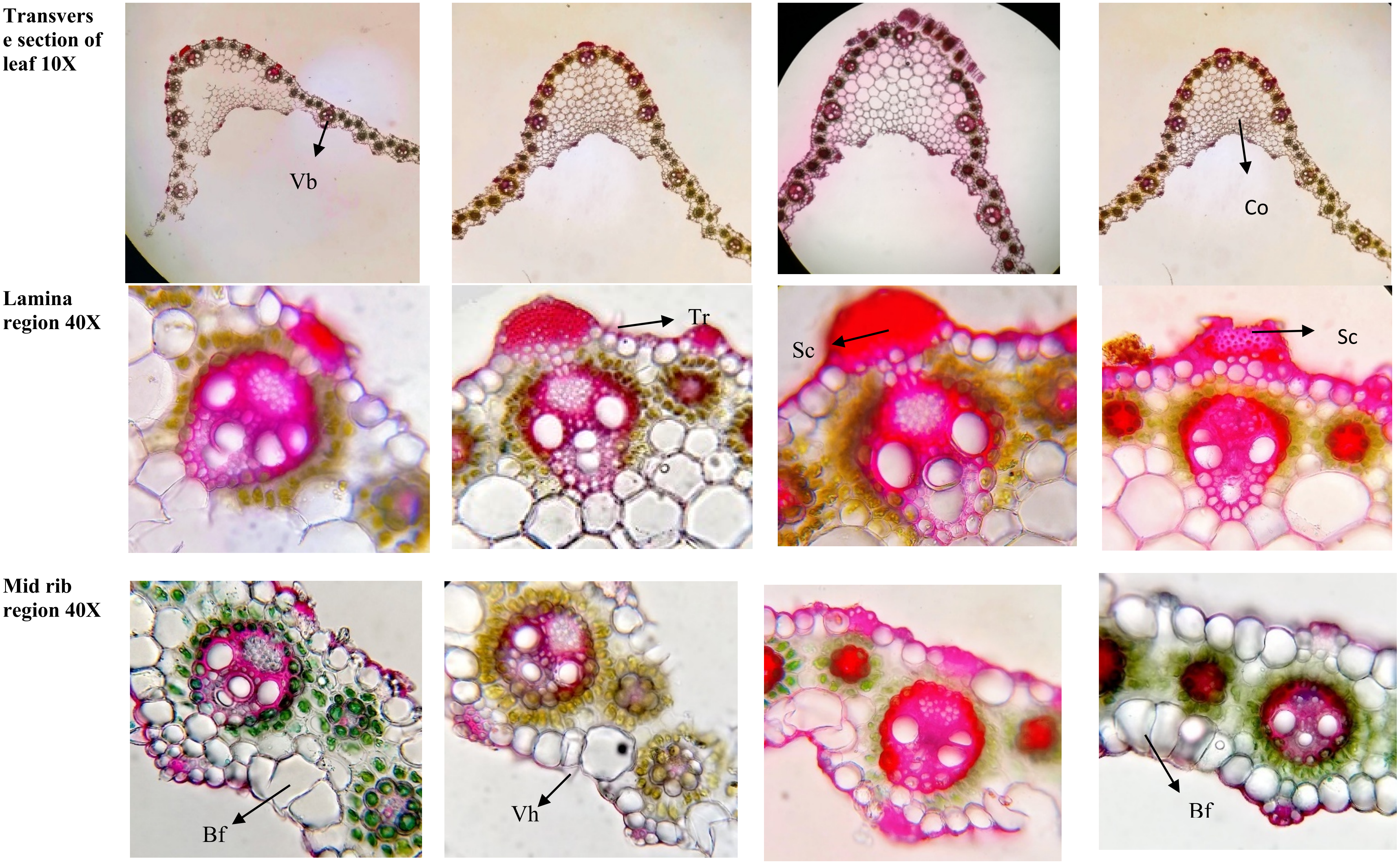
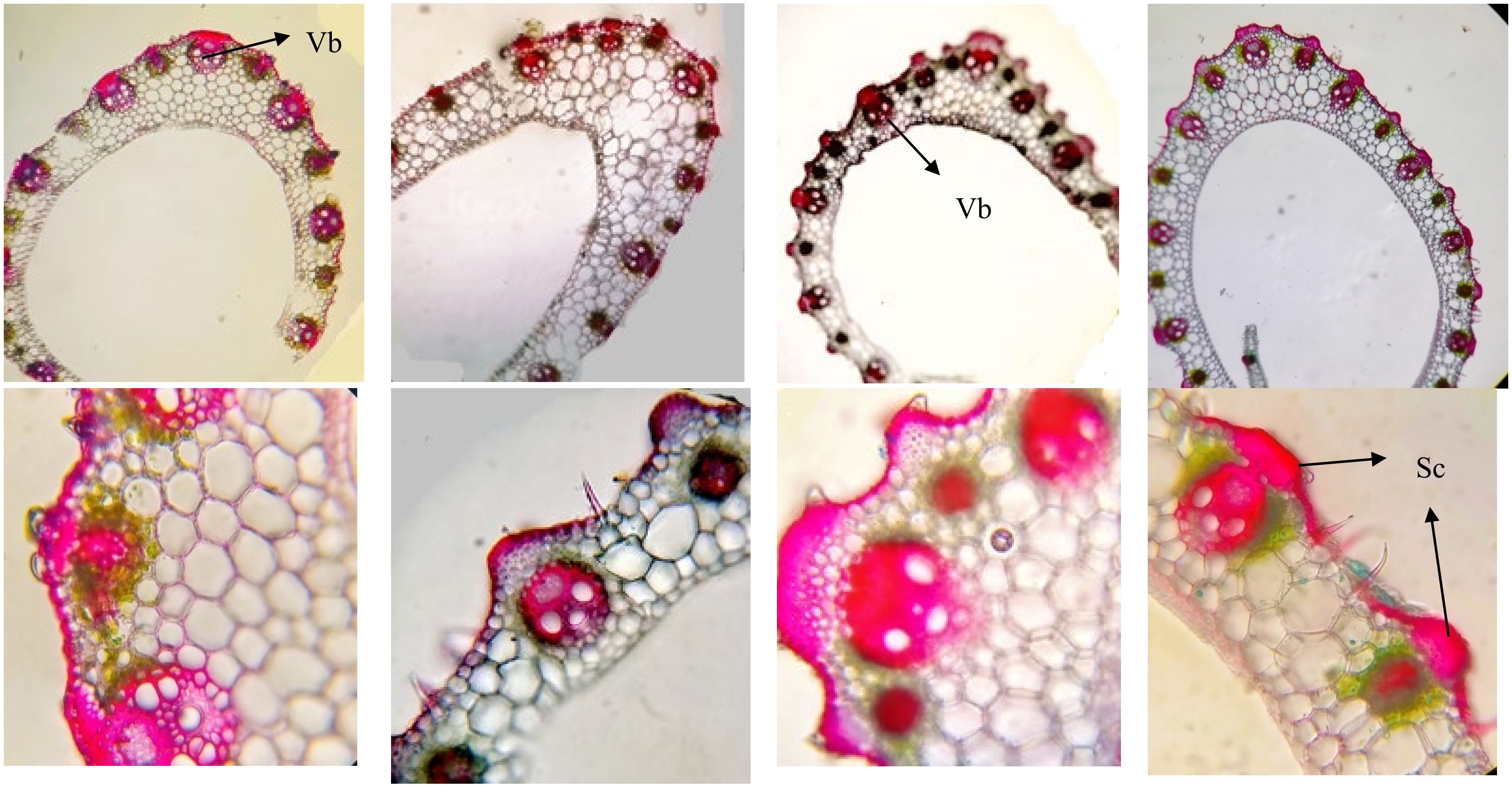


Fig.11. Stem anatomy of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.
(Mx = Metaxylem, Ph = Phloem, Sc = Sclerenchyma, Vb = Vascular bundle, Pt = Pith, Co = Cortex).



0 (Control) mM NaCl 100 mM NaCl 200 mM NaCl 300 mM NaCl

Fig.12. Leaf anatomy of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.
(Vh = Vesicular hair, Sc = Sclerenchyma, Vb = Vascular bundle, Bf = Bulliform, Co = Cortex, Tr = Trichome).



Transverse section of leaf sheath 10X

Lamina region 40X

0 (Control) mM NaCl **100 mM NaCl** **200 mM NaCl** **300 mM NaCl**

Fig.13. Leaf Sheath anatomy of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.
(Sc = Sclerenchyma, Vb = Vascular bundle).

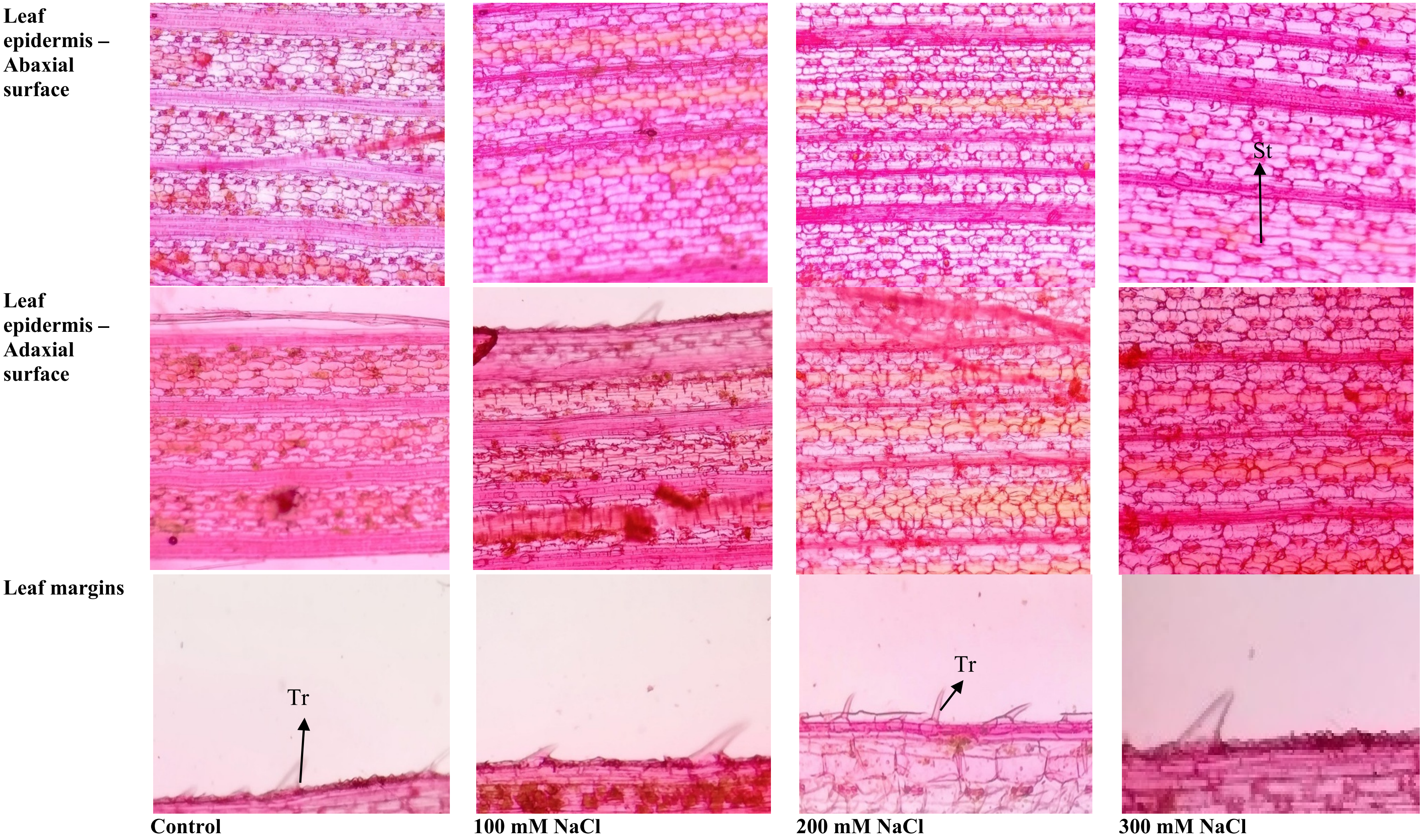


Fig.14. Leaf surface and leaf margin anatomy of *Cenchrus ciliaris* subjected to different levels of salt stress for 60 days.
(Tr = Trichome, St = Stomata)

Table.1. Pearson's Correlation Coefficient (r) between plant biomass and anatomical characteristics of *Cenchrus ciliaris.*

Root anatomy	DW root	DW shoot	**Stem anatomy**	DW root	DW shoot	**Leaf sheath anatomy**	DW root	DW shoot	**Leaf blade anatomy**	DW root	DW shoot
Root thickness	▣	▣	Stem area			Leaf sheath thickness		▣	Lamina thickness	□	▣
Exodermis thickness	○	○	Epidermis cell area	◉	●	Adaxial hair density	◉	●	Adaxial epidermis thickness	◉	●
Cortex thickness	□	□	Vascular bundle number	◉	●	Adaxial epidermis thickness	○	○	Abaxial epidermis thickness		○
Pith thickness	◉	●	Metaxylem area			Adaxial epidermis cell area	◉	◉	Bundle sheath thickness	◉	●
Pith cell area	□	▣	Phloem area	■	■	Xylem cell area			Vascular bundle area	□	▣
Cortical cell area	◉	●	Vascular bundle area	▣	▣	Phloem area	◉	◉	Abaxial epidermis cell area	◉	◉
Exodermis cell area			Sclerenchyma thickness	○	◉	Vascular bundle area	▣	▣	Adaxial epidermis cell area	◉	●
Cortical thickness	◉	●	Epidermis thickness	○	◉	Abaxial epidermis cell area	●	●	Sclerenchyma thickness	●	●
Endodermis thickness	○	○				Abaxial epidermis thickness	◉	●	Metaxylem area	■	■
Pericycle thickness	◉	●				Sclerenchyma thickness	◉	●	Bulliform cell number	◉	●
Phloem area	◉	●							Adaxial hair density	◉	●
Aerenchyma area	▣	■							Adaxial trichome density		
Metaxylem area	◉	●							Phloem area	◉	◉
Endodermis cell area									Bulliform cell area	▣	■
Sclerenchyma thickness	◉	●							Abaxial hair density	◉	●
									Abaxial trichome density		

0.01	0.05	0.1	Significance level
■	▣	□	Positive correlation
●	◉	○	Negative correlation

DISCUSSION

In desert, plants exposed to severe conditions with multiple stresses including aridity, drought, high temperature and soil salinity, which promote the evolution of salt tolerant plants (Breckle, 2004). In *C. ciliaris* development of structural features in response to high salt stress, indicating an adaptive potential of this species to salinity.

According to the results of the study, in *C. ciliaris* a decrease in root thickness was observed (Fig. 10) as salinity increased also reported by De Villers *et al.* (1995) and Hameed *et al*. (2010). The less affected root diameter in *C. ciliaris* at Cholistan desert may be the better adaptation against salty environment. *C. ciliaris* showed increased value for many root anatomical parameters like exodermis thickness and its cell area, cortical cell area and its thickness and endodermis cell area and its thickness at increasing salinity in nutrient solution. However, a decrease in exodermis cell area was seen at 300 mM of NaCl. All these modified anatomical characteristics might be enhance water and solute storage capacity and check radial flow of water from root surface. Increased in cortical thickness might be accompanied by an increase in number of parenchyma tissue, which store additional water under osmotic stress during salinity. Therefore such modifications considered as crucial for survival under extreme saline sandy environment. Such structural adaptations have also been recorded in many desert halophytes by Naz *et al.* (2018) and Qurat *et al.* (2019).

Sclerification, outside the epidermis and in the outer cortex is one of the important anatomical modifications of root under rising salinity level. Sclerification provide mechanical strength to root tissues (Lo *et al*., 2008), check the loss of water and prevent from desiccation (Balsamo *et al*., 2006; Voltolini *et al*., 2009). At root level, *C. ciliaris* showed sclerification in pith and cortical region, a crucial anatomical adaptation to high salinity, which protect the root from damage and give its mechanical strength. Increased sclerification and endodermal thickness increased the survival of species under harsh environmental conditions and check the loss of water through root (Naz *et al*., 2018). Similar finding have also been reported in many grass species by several researches like Hameed *et al.* (2012) in *Sporobolus arabicus; Cynodon dactylon,* Naz *et al.* (2016) in *Sporobolus ioclados* and Qurat *et al.* (2019) in *Leptochloa fusca*. Larger vascular tissues are important for the better transport of water and nutrients under limited supply of water (Awasthi *et al*., 1999), and therefore, increased thickness in vascular area in *C. ciliaris* with increasing NaCl levels is an indication of enhanced salt tolerance.

Moreover, increase in vascular tissues, mainly metaxylem area in *C. ciliaris* at high salinities may enhance conduction of water and nutrients and minimize resistant in conduction (Horie *et al*., 2012). According to Colmer and Flowers (2008) aerenchyma tissues appeared in salt tolerant plants under wetland conditions. *C. ciliaris* exhibited a significant rise in aerenchyma formation at moderate salinity (100 mM NaCl) but at 300 mM of NaCl aerenchyma might be transferred into parenchyma. These parenchyma tissues may act as storage tissues (succulence) for toxic ions; hence provide an important strategy against high salinity (Akhtar *et al*., 1998; Hameed *et al*., 2010).

Generally salinity has a negative impact on stem cross-sectional area as was earlier noted by Reinose *et al*. (2004). However in the present studies, stem area was markedly increase under moderately salinity levels, same was reported by Wu *et al*. (2010), Hameed *et al*. (2010) and Naz *et al*. (2016). This adaptation may increase stem succulence and help to conserve water for better survival under harsh environment. Increased sclerification in the present studies (Fig. 11) perhaps plays significant role in preventing water loss from stem, under adverse saline conditions. Same has been reported by previous reports in various grass species e.g. *Sporobolus arabicus* (Hameed *et al.*, 2012) and *Aeluropus lagopoides* (Naz *et al.*, 2018).

At leaf level, the major impact of salinity was increased in epidermis thickness with thick cuticle, which prevents the water loss from leaf surface in desert condition, and considered as significant adaptation against physiological drought in saline environment (Barhoami *et al.*, 2007) and a prominent feature of most drought and salt tolerant species in desert environment (Jian-jing *et al.*, 2012). Number of bulliform cell increased significantly (Fig. 12) in leaf blade indicating its roll in leaf rolling, which may reduce water loss (transpiration) through the leaf surface and considered as an important adaptive defensive feature against salinity in *C. ciliaris*.

One of the crucial modification in leaf of *C. ciliaris* under salinity was considerable development of salt hair (vesicular hair) and trichomes on both adaxial an abaxial leaf surface. In *C. ciliaris* salt hair (vesicular hair) might be important for exclusion of toxic salts and leaf trichomes for checking water loss and maintained leaf temperature (Khokhar *et al.*, 2012; Naz *et al.*, 2018). In saline desert environment, reduction in stomatal density and area (Fig. 13) on leaf surface might be critically important for checking unnecessary water loss has been reported in *Triticum aestivum* (Akram *et al.*, 2002), *Cynodon dactylon* (Hameed *et al.*, 2012). In *C. ciliaris*, stomatal density and area decrease at adaxial leaf surface, this might be positively lower rate of transpiration in saline desert environment and considered as a survival strategy when water scarcity is a limiting factor. In halophyte, Xu and Zhou (2008), Naz *et al.* (2016) and Qurat *et al.* (2019) recorded similar results. In the present study, root and shoot biomass generally stimulated at lower salinity, this increase in biomass indicated the high tolerance of this grass against salinity. Similar results have been reported by several researchers in desert halophytes for example Hameed *et al.* (2013), Naz *et al.* (2018) and Qurat *et al.* (2019).

Conclusion: It is concluded that *C. ciliaris* has the ability to survive at moderate salinity and developed very crucial anatomical adaptations in salty desert environment, which not only check water loss in desert condition but also conserve water under physiological drought in saline environment. In root, increased sclerification for preventing water loss and increased parenchyma tissue in cortex for water storage. In stem, increased sclerification preventing water loss and increased vascular tissue for water conduction. In leaf and leaf sheath, decreased in stomatal density and area, increased bulliform cell, and rich density of vesicular hairs and trichome might be essential for water conservation and salt excretion.

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