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ANATOMICAL ADAPTATIONS OF *CYNODON DACTYLON* (L.) PERS., FROM THE SALT RANGE PAKISTAN, TO SALINITY STRESS. I. ROOT AND STEM ANATOMY

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Abstract

A naturally adapted salt tolerant population of *Cynodon dactylon* (L.) Pers., from highly saline soils of Uchhali Lake, the Salt Range, Pakistan was evaluated for root and stem anatomical modifications. A population from the normal (non-saline) soils of the Faisalabad region was also collected for comparison. Both populations were subjected to salt stress hydroponically. The salt treatments used were: control (0 mM salt), 50, 100, 150 and 200 mM NaCl in 0.5 strength Hoagland's nutrient solution. The Salt Range population showed specific root and stem anatomical adaptations for its better survival under harsh saline environments. Increased exodermis and sclerenchyma, endodermis, cortex and pith parenchyma in roots were critical for checking water loss and enhancing water storage capability. In stem, increased stem area (succulence), increased epidermis and sclerenchyma thicknesses (preventing water loss), increased cortex thickness (increasing water storage), and increased number and area of vascular tissue (increased water conduction) seemed to be crucial for its better survival under harsh saline environments.

Introduction

Increased salt tolerance is an urgent need for crops grown in the areas that are medium to high salt affected, or at a risk of salinization. This requires new genetic traits for the identification and selection of salt-tolerant germplasm. This material can be directly used for revegetation of such affected areas and also the introduction of new salt tolerant genes to increase tolerance of crop cultivars (Munns *et al*., 2002).

Natural populations, particularly grass species like *Agrostis, Festuca, Lolium, Poa* and *Cynodon* had shown good tolerance to salinity (Humphreys *et al*., 1986; Hameed & Ashraf, 2008). A variety of salt affected habitats like coastal marshes and saline deserts may result in the evolution of specific salt tolerant traits in these grasses (Wu, 1981).

Morphological and anatomical modifications in plant body are capable of minimizing detrimental effects of salt stress (Poljakoff-Mayber, 1988). Salt tolerant species shows a range of anatomical adaptive features like increased succulence (both in root and stem), thick cuticle and deposition of wax, salt-secretory trichomes and glands, thick and many layered epidermis and well developed water storing tissues in the cortex, widening of casparian band and enhanced development of root endodermis (Akram *et al*., 2002, Wahid, 2003).

The Salt Range, Pakistan is unique in nature and ideal site for the study of natural salt tolerant population for their adaptive mechanisms. It is located between 71˚30'-73˚30' E and 32˚23'-33˚00' N, which is probably evolved in the Cambrian era (McKerrow *et al*., 1992). It is characterized by low precipitation, which is on an average

50 cm annually (Ahmad *et al*., 2002). Brine water springs deposit salts along their routes is the major source, which heavily infest the soils towards the southern side of the Salt Range. Run-off water from exposed salt depositions during rainy seasons is the other source, which resulted in salinizing large area of foot-hill zone. This salinity is mainly due to sodium chloride (about 90%); along with some salts of K, Ca and Mg (Qadir *et al*., 2005).

Cynodon dactylon (L.) Pers. (Bermuda grass, locally known as Khabbal ghas) is a species distributed abundantly in tropical and warm temperate areas (Chaudhary, 1989). Natural populations of Bermuda grass can have considerable genetic variation for tolerance to soil temperatures, salinity and drought (Speranza, 1995). Its growth is stimulated by moderate salinities, however, it can tolerate relatively high salinities (Mass & Hoffman, 1977).

This species was collected from Uchhali lake, which is a part of Uchhali complex that comprises three internationally well known wetlands (Chaudhry *et al*., 1997). Water of the Uchhali Lake is hyper-saline (Afzal *et al*., 1998) due to the seepage from adjacent agricultural lands and run-off from the surrounding hills of the Salt Range. *Cynoodn dactylon* collected from this habitat was in direct contact with hyper saline water and, hence, it is expected that this population is well adapted to high salinities.

Physiological attributes have already been studied in this population (Hameed & Ashraf, 2008) that contribute towards the adaptive mechanism for salt tolerance. Although physiological adaptations are crucial in identifying selection criteria against salt stress (Ashraf, 2004), but anatomical modification under high salinities are also important (Mass & Nieman, 1978; Fahn, 1990). Such structural changes certainly play a crucial role in combination with physiological attributes in tolerating the stress and also might have played a significant role in the survival of the salt tolerant population on highly salt affected soil.

Materials and Methods

A salt tolerant grass *Cynodon dactylon* L., from the Salt Range, Pakistan was investigated to study the root and stem anatomical adaptations against salt stress in the Botanic Garden, University of Agriculture, Faisalabad during the year 2005-6. A population was collected from the salt affected soils of the Uchhali Lake (coordinates 32º36’34.59” N, 72º13’53.72” E, soil ECe 19.92 dS m^{-1}, Na^+ 4034.86 mg kg^{-1}, Cl^- 2021.30 mg kg^{-1}). Another *C. dactylon* population was collected from the normal non-saline soils of the University campus (coordinates 31° 25’ 42.87” N, 73° 04’ 11.46” E, ECe 1.82 dS m^{-1}, Na^+ 69.24 mg kg^{-1}, Cl^- 422.46 mg kg^{-1}).

Soil samples from the root zone of each grass population from both habitats were taken for soil physico-chemical characteristics. The saturation soil extract was used to appraise the ECe using a digital EC meter. Analysis of soils was carried out following USDA Laboratory Staff (1954).

Both populations were grown in the Botanic Garden for six months in 24 cm earthen pots, which were filled with a mixture of loam and sand. The ramets uniform size were separated from each plants of each population and grown in a hydroponic system containing Hoagland’s nutrient solution (0.5 strength). The aeration of containers was made by air pumps for 12 h daily and placed under plastic covering to protect from rainfall. Twelve replicates were planted on thermopol sheets by making holes of suitable size with the help of foam. Five salinity levels, control (0 mM), 50, 100, 150 and 200 mM of NaCl were established. The experiment was designed in a two-factor (populations and salinity levels) factorial completely randomized design (CRD) with population four replications.

The material was fixed in FAA solution (formalin 10%, acetic acid 5%, ethyl alcohol 50% and distilled water 35%) for 48 h and then transferred to acetic-alcohol solution (1:3 ratio) for root and stem anatomical studies. Double staining dehydration procedure (safranin and fast green) was used for the preparation of permanent slides (Ruzin, 1999) to study various cells and tissues of root and stem. Measurements and micrographs were made with a digital camera (Nikon FDX-35) equipped on a Nikon stereo-microscope (Nikon 104, Japan).

Anatomical characteristics recorded for root were epidermis thickness and cell area, sclerenchyma thickness and cell area, parenchymatous cell area, aerenchyma area, endodermis thickness and cell area, vascular tissue (xylem, phloem and vessel areas), pith thickness and cell area. The anatomical characteristics recorded for stem were epidermis thickness and cell area, sclerenchyma thickness and cell area, parenchymatous cell area, endodermis thickness and cell area, vascular tissue (xylem, phloem and vessel areas),

The data were subjected to statistical analysis (ANOVA) using the Computer Program, MSTAT (MSTAT Development Team, 1989). Least significant difference (LSD) values ($p< 5\%$) to compare the mean values (Steel *et al*., 1997).

Results

Root anatomy: Root area in both populations decreased consistently but significantly with rise in NaCl level, but the Faisalabad population was more affected than the Salt Range (Figs. 1 and 2).

Exodermis cell area in the *C. dactylon* from Faisalabad population was generally decreased with rise in salt content of the growing media (Figs.1 and 2). The Salt Range population, on the other hand, showed stability in this character and was not much affected by increasing salt levels.

Sclerenchyma thickness and its cell area showed a slight decrease in the population from Faisalabad (Figs. 1 and 2); however, 50 mM NaCl promoted this character. However, sclerenchyma thickness and its cell area in the Salt Range population showed an increase in both the characters with increase in salt levels.

Increasing salinity resulted in a significant decrease in cortical thickness of *C. dactylon* from Faisalabad region but at 50 mM NaCl level slight increase was noted in this character (Figs. 1 and 2). The Salt Range population showed gradual increase in cortical thickness with increase in salt levels. Cortical cell area, on contrary, showed increase up to 100 mM NaCl in both the populations and decreased with further increase in salt levels.

Endodermis thickness increased in both populations up to moderate salt level, but the highest salt regime caused a significant decrease in this character. Pericycle cell area was one of the least affected characteristics (Figs. 1 and 2). In the Faisalabad population it was increased by higher salt levels i.e., 100 mM and above, while in the Salt Range population, only 100 mM NaCl level showed some increase.

Both populations showed similar response to increase in salt levels in external growth medium in relation to vascular region thickness (Figs. 1 and 2). There was an increase in this characteristic at lower salt levels i.e., 50 and 100 mM NaCl, but the Faisalabad population responded little more.

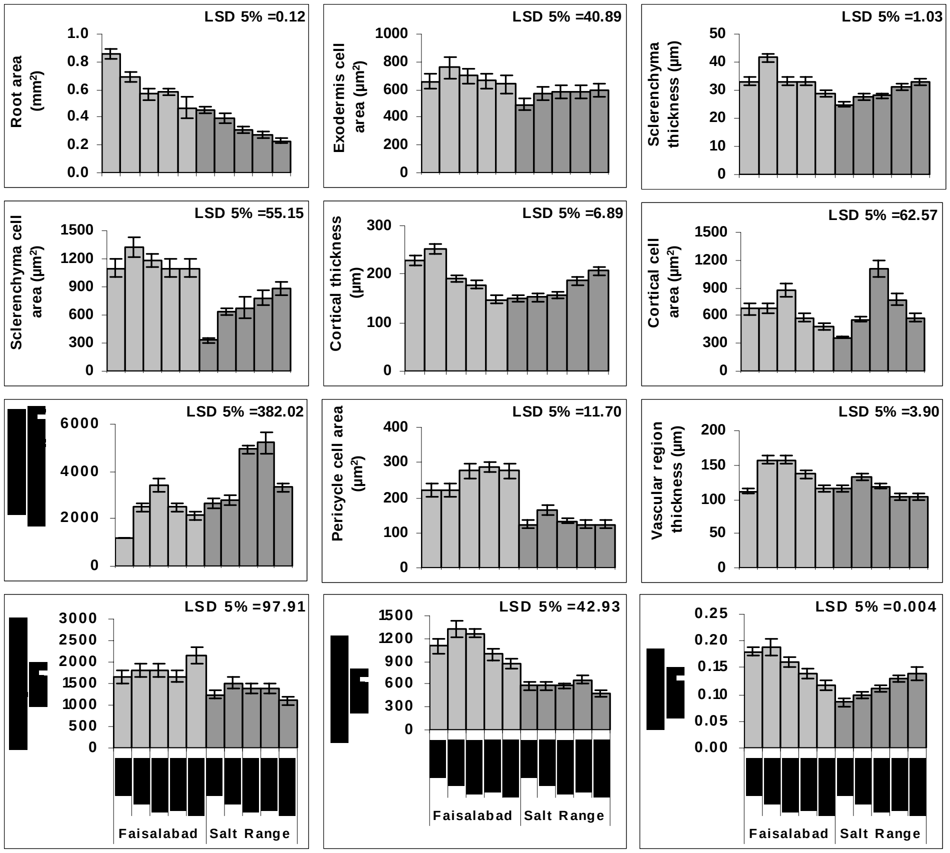

Fig. 1. Root anatomical characteristics in *Cynodon dactylon* from the Salt Range and Faisalabad grown hydroponically under different salt levels (Mean ± S.E; *n* = 12).

A slight increase in metaxylem vessel area of *C. dactylon* from Faisalabad and Salt Range populations was observed at variable salt levels. The Faisalabad population was little more responsive to increasing salt regime than its counterpart from Salt Range.

Phloem area showed a decreasing trend in the population of *Cynodon dactylon* from both Faisalabad and the Salt Range, but the Faisalabad population had larger phloem area than its counterpart from Salt Range (Figs. 1 and 2).

The imposition of salt stress generally decreased the pith area in the *C. dactylon* from Faisalabad population (Figs. 1 and 2), but in contrast, this character was enhanced in Salt Range population with increase in salt levels.

Stem anatomy: Stem area in *C. dactylon* from both Faisalabad and the Salt Range populations consistently increased with increase in salt level (Figs. 3 and 4). In general, the Salt Range population had thinner stem than its counterpart from the Faisalabad region, but it showed greater increase in its stem area with rise in NaCl level.

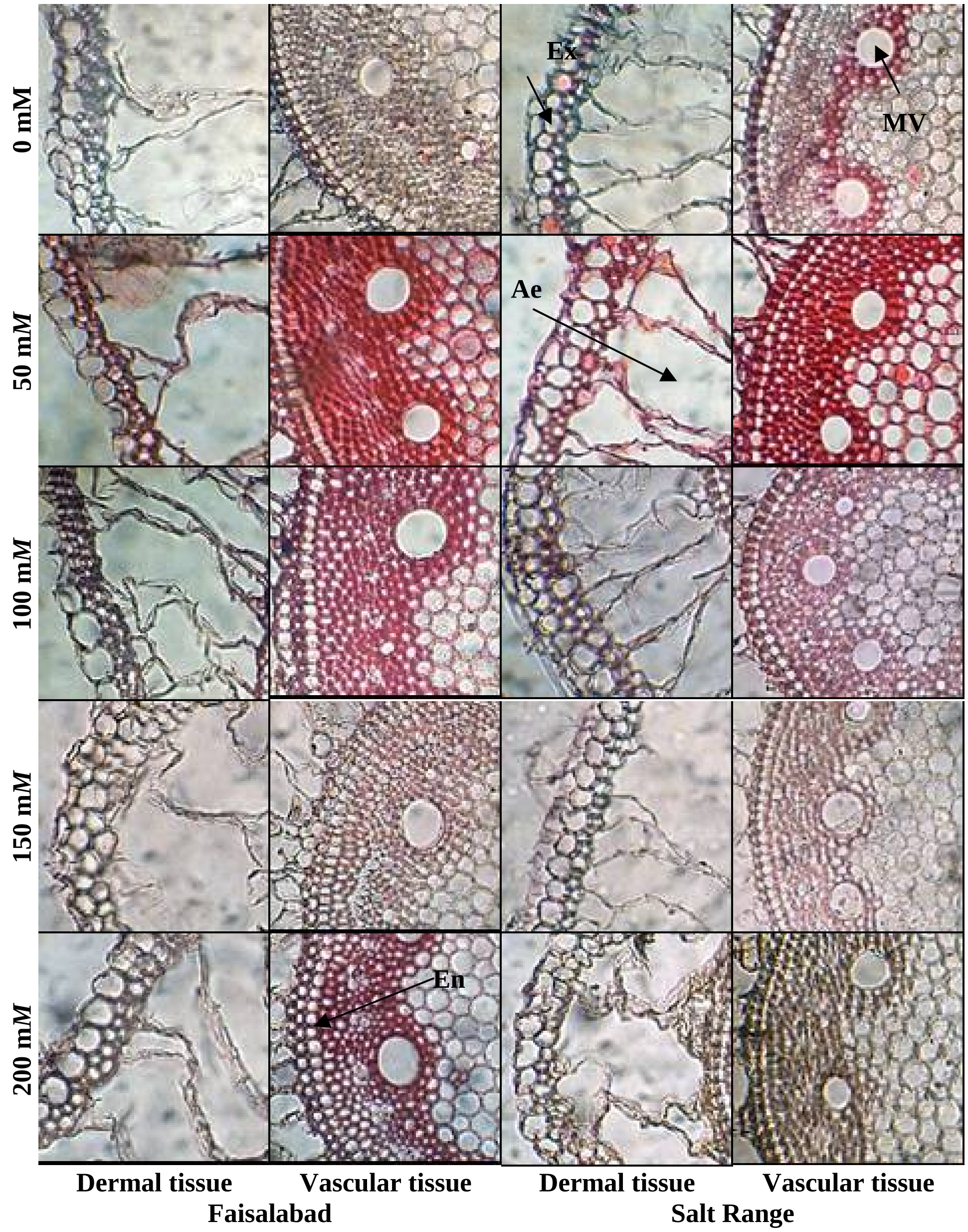

Fig. 2. Transverse section of root in *Cynodon dactylon* ecotypes from the Salt Range and Faisalabad subjected to different salt levels (Ae = Aerenchyma, En = Endodermis, Ex = Exodermis, MV = Metaxylem).

Epidermis cell area in the population of *C. dactylon* from Faisalabad population was sharply increased by the induction of salt in growing medium, but thereafter gradually decreased by further increase in salt content. In contrast, the Salt Range population showed steady increase with increase in salt level.

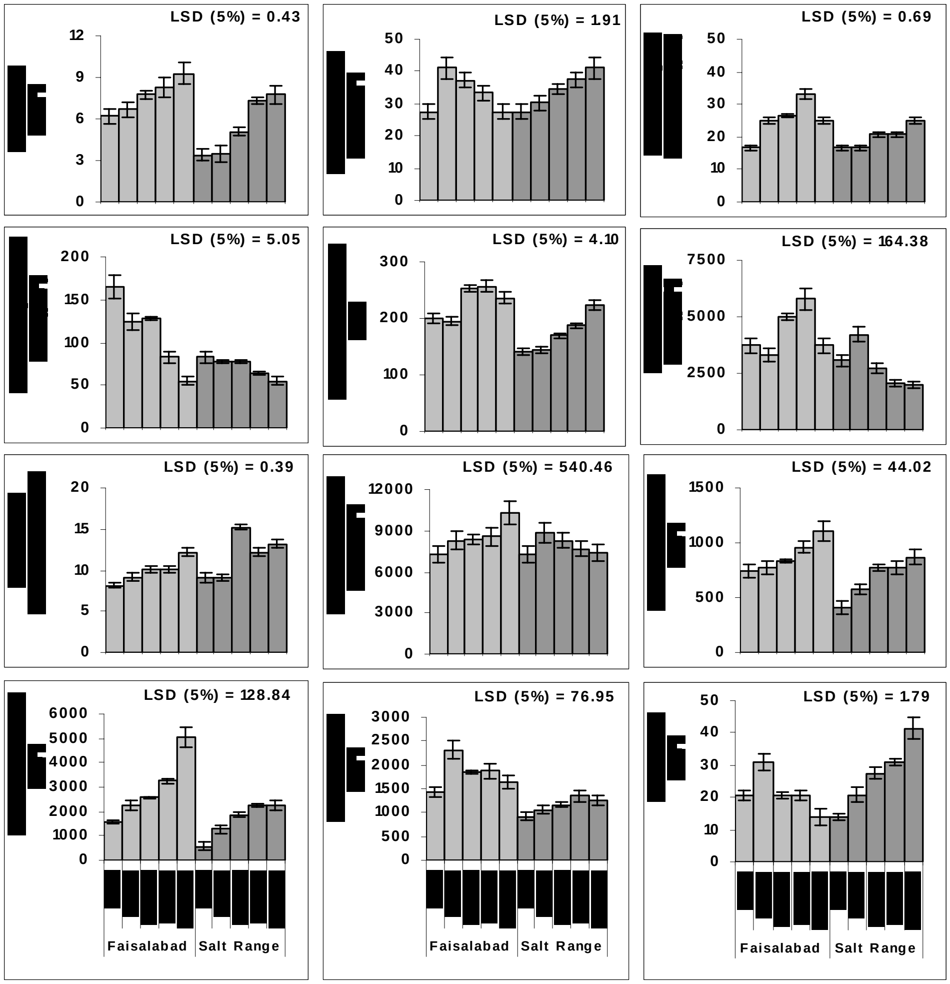

Fig. 3. Stem anatomical characteristics in *Cynodon dactylon* from the Salt Range and Faisalabad grown hydroponically under different salt levels (Mean ± S.E; n = 12).

Sclerenchyma thickness was increased in *C. dactylon* from the Salt Range with increasing salt concentration of the growth medium (Figs. 3 and 4). Sclerenchyma in the population from the Faisalabad region showed improvement in sclerenchyma thickness up to 150 mM NaCl, but at the highest level it was abruptly decreased. Sclerenchyma cell area, in contrast, decreased with increase in external salt concentration. It was also decreased in the Salt Range population but only slightly.

There was no definite response of cortical thickness and its cell area in the Faisalabad population, where both these characters were increased at 100 and 150 mM NaCl levels and decreased at 200 mM NaCl. In the Salt Range population cortical thickness was increased gradually with rise in salt treatment; however, cortical cell area was increased at 50 mM NaCl and thereafter it reduced with increase in external salt level (Figs. 3 and 4).

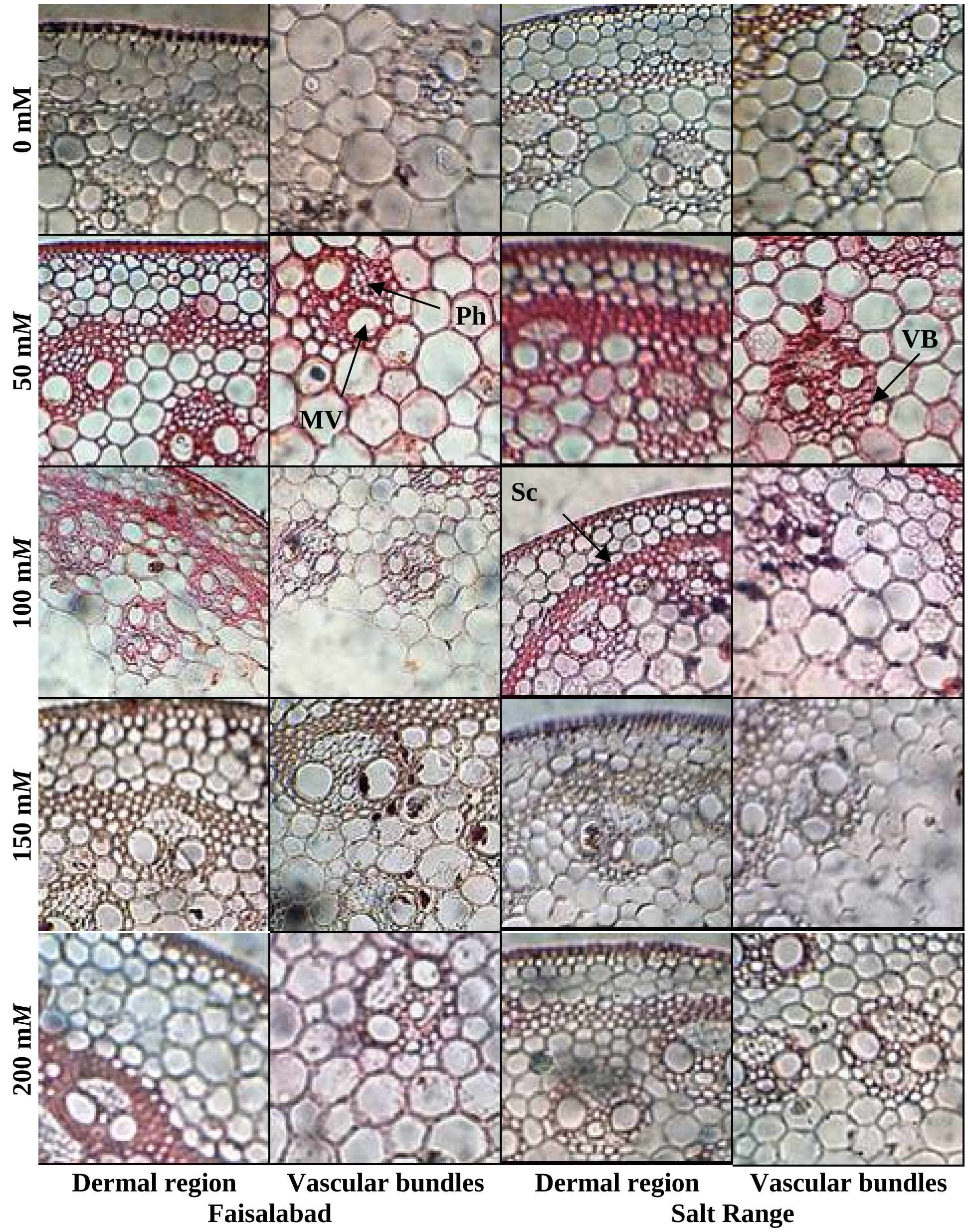

Fig. 4. Transverse section of stem in *Cynodon dactylon* ecotypes from the Salt Range and Faisalabad subjected to different salt levels (MV = Metaxylem, Ph = phloem, Sc = Sclerenchyma, VB = vascular bundle).

Number of vascular bundles in the Faisalabad population increased consistently with increase in NaCl concentration, but in the Salt Range population was improved by 100 mM NaCl and slightly decreased thereafter at higher levels. Vascular bundle area in the Faisalabad population remained stable under varying salt levels up to 150 mM NaCl, but increased at the highest regime (Figs. 3 and 4). In the Salt Range population, in contrast, this character was increased by the imposition of salt in growth medium and by further induction of salt in medium it was gradually decreased.

Increasing salt level did not affect metaxylem area in *C. dactylon* from both Faisalabad and the Salt Range. Metaxylem area in the population from the Salt Range, however, increased nearly 100% only at 150 mM NaCl level (Figs. 3 and 4). Protoxylem area increased in both populations with increase in salt levels, but the Salt Range population showed greater response to increase in salt levels than its counterpart from the Faisalabad region.

Phloem area increased significantly in the Faisalabad population with the imposition of salt in growth medium; however, a further increase in salt level resulted in a consistent decrease in this character. The Salt Range population, in contrast, phloem area gradually increased with increase in salt levels. Sieve area in the Salt Range population significantly increased with increase in salt levels, whereas, in the Faisalabad population it was motivated by 50 mM NaCl, and with a further increase in external salt concentration this parameter was significantly decreased (Figs. 3 and 4).

Discussion

The Salt Range population was considered as the tolerant on the basis of fresh and dry masses of root and shoot (Hameed & Ashraf, 2008). The Salt Range formed most probably during the late Cambrian. Multiple stresses like drought and salinity are operating simultaneously in the salt Range, therefore, plants inhabiting there should have developed specific stem and root structural adaptations against high salinity.

As salt level increased, decrease was observed in root diameter in both the Faisalabad and the Salt Range populations, as reported by De Villiers *et al*., (1995). Degano (1999) related succulence in root with the mechanisms of adaptation to saline conditions. The less affected root area in the Salt Range population may be the better adaptation.

Salinity is known to stimulate suberization of the root hypodermis and endodermis (Kozlowski, 1997). Saline tolerant species are often characterized by thick inner tangential walls of endodermis and lignified walls of cortical parenchyma (Baumeister & Merten, 1981; Walsh, 1990; Hwang & Chen, 1995; Baloch *et al.*, 1998; YuJing & Yong, 2000; YuJing *et al.*, 2000). These reports are in agreement with those of present study where lignified sclerenchyma was prominent in the salt tolerant population.

Parenchymatous cell, both cortical and pith, increased in the Salt Range population. These tissues can surely enhance the storage capacity, which is crucial under unfavourable moisture conditions (Baloch *et al.*, 1998). Akram *et al.*, (2002) reported decreased cortical and pith region in wheat under salinity, though well developed parenchyma is a characteristic feature of salt tolerant species such as saltgrass (Alshammary *et al.*, 2004).

Generally, saline conditions can reduce plant stem area as was earlier observed by Datta & Som (1973) and Reinoso *et al*., (2004) in *Prosopis strombulifera*. However, in the salt tolerant population of *C. dactylon* from the Salt Range the stem area was markedly increased under saline regime. This increased succulence in stem may aid to store additional water and hence better survival under harsh environments. Furthermore, increased sclerenchyma thickness but reduced size of sclerenchma cells was observed in the stem of this population from the Salt Range. This characteristic may offer some resistance to water loss through stem and again may play a crucial role in adaptation to unfavourable conditions. Earlier reports on *Spartina alterniflora* (Walsh, 1990) and *Puccinellia tenuiflora* (YuJing *et al.,* 2000) support the present findings, but those in rice

(Chandramony & George, 1975) and *Deschampsia antarctica* (Gielwanowska *et al.*, 2005) are in disagreement.

Increased metaxylem area and phloem area in the Salt Range population perhaps plays important role in the conduction of water and photosynthates, particularly under adverse saline conditions. This has been supported by previous reports in different plant species, e.g. in rice (Datta & Som, 1973), *Kandelia candel* (Hwang & Chen, 1995), *Ziziphus* cultivars (Awasthi & Pathak, 1999), and *Arabidopsis thaliana* (Baloch *et al.*, 1998).

Halophytic or salt tolerant species generally possess thick epidermis and this serves as an effective mechanism against water loss during limited moisture availability (Botti *et al.*, 1998; YuJing *et al.*, 2000). In the present studies epidermal cell area increased in the Salt Range population, and this will surely ensure preventing of water loss through the stems.

The Faisalabad population had thicker metaxylem area but greater increase in the Salt Range indicates its better adaptation to high salinities, as water conduction might be easier and this is critical under harsh climates (Awasthi & Pathak, 1999). These studies are in contrary to the findings of Akram *et al.*, (2002) and Hu *et al.*, (2005) who reported decreased metaxylem area.

Cortical area decreased at higher salinities in the Faisalabad population, as reported by Akram *et al*., (2002), but increased cortical area under salt stress in the Salt Range population may be critical under physiological drought for its better storage capacity (Baloch *et al.*, 1998).

Salinity generally reduces epidermal cell area in stem (Akram *et al.*, 2002), but in the present studies, this character was increased by increasing salt levels in growth medium in both populations. In the Salt Range population, epidermis thickness greatly increased, which showed its better adaptability because thick epidermis is a characteristic feature of salt tolerant species (Awasthi & Pathak, 1999). This characteristic is critical under limited moisture availability as thick epidermis is capable of checking water loss through stems (Mansoor *et al*., 2002; Hameed *et al*., 2002; Nawazish *et al*., 2006).

Awasthi *et al*., (1999) reported increased vascular bundle area in relatively less tolerant plants (*Zizyphus mauritiana*) and similar finding was recorded in the less tolerant population from the Faisalabad region. The Salt Range population, however, showed slight decrease in this character along with increased in its number may be better adaptation for efficient water uptake as reported by YuJing *et al.*, (2000) in highly salt tolerant *Puccinellia tenuiflora.*

Xylem vessels generally reduced under salt stress, as reported by several authors such as Gadallah & Ramadan (1997) and LingAn *et al*., (2002). This characteristic, however, remained very much stable in both the Salt Range and the Faisalabad populations.

The Faisalabad population reduces both its phloem area and sieve area at higher salinities, and such findings were correlated to less tolerant species by Goncharova & Dobren'kova (1981). In view of this, the Salt Range population can be safely concluded as the better adapted population in areas hit by high salinities, where both phloem and sieve area considerably increased.

In conclusion, the salt tolerant ecotype from the Salt Range adapted very specific root and stem adaptive characteristics for its better survival under harsh saline environments. Increased exodermis and sclerenchyma in roots for preventing water loss through root surface, endodermis for preventing redial flow of water and nutrients, and increased cortex and pith parenchyma for better water storage. In stem it had increased stem area by increasing succulence, increased epidermis and sclerenchyma thicknesses for checking water loss, increased cortex thickness for enhanced water storage, increased number of vascular bundles, and metaxylem, protoxylem, phloem and sieve area for better conduction of water, nutrients and photosynthates.

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