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# ANATOMICAL ADAPTATIONS OF *CYNODON DACTYLON* (L.) PERS. FROM THE SALT RANGE (PAKISTAN) TO SALINITY STRESS. II. LEAF ANATOMY

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## Abstract

Leaf anatomical modifications were examined in a naturally adapted salt tolerant population of *Cynodon dactylon* (L.) Pers., collected from a heavily salt affected soil in the vicinity of a natural salt lake, Uchhali Lake, in the Salt Range of the Punjab province of Pakistan. An ecotype of this grass was also collected from a normal non-saline habitat from the Faisalabad region, which was considered as a control. Both populations were subjected to salt stress in hydroponics. The salt treatments used were: control (no salt), 50, 100, 150 and 200 m*M* of NaCl in Hoagland's nutrient solution. After eight weeks of growth in hydroponics leaf and leaf-sheath anatomical characteristics in the differently adapted ecotypes were studied. The ecotype from the Salt Range was much better adapted to salt stress than its counterpart from the Faisalabad region. Anatomical adaptations in the salt tolerant ecotype to saline environments were very specific, and these were not only for the excretion of toxic ions but also accumulation of ions in the parenchymatous tissue. The prominent adaptive features in the salt tolerant ecotype were increased development of vesicular hairs for the exclusion of toxic ions through leaves as well as less affected parenchymatous tissue (mesophyll, bundle sheath, and bulliform cells) due to salt stress. Additionally, some adaptations reflected were the development of xeromorphic characteristics (decreased stomatal area and size on adaxial leaf surface, less affected epidermis, increased bundle sheath, and increased bulliform cell area) essential for checking undue water loss. Highly developed dermal tissue, particularly lower epidermis and bulliform cells, and decreased stomatal density and area in the salt tolerant ecotype seemed to be crucial for checking undue water loss under critical limited moisture environments.

## Introduction

Natural populations of turfgrasses like the species of *Agrostis, Festuca, Lolium, Poa,* and even *Cynodon* had shown good tolerance to salinity (Humphreys *et al.*, 1986; Hameed & Ashraf, 2008). The evolution of salt tolerance in these grasses has occurred in a variety of habitats such as coastal marshes, saline deserts and rocky alpine habitats (Wu, 1981). Salinity stress as a natural selection pressure causes the evolution of salt tolerance in populations having considerable magnitude of genetic variation (Ahmad *et al.*, 1981).

Salt tolerant plants are capable of minimizing detrimental effects of salt stress by producing a series of anatomical, morphological, and physiological adaptations (Poljakoff-Mayber, 1988; Hamayun *et al*., 2010; Nadeem *et al.,* 2012), such as an extensive root system and salt secreting glands on the leaf surface (Marcum & Murdoch, 1990; Marcum *et al.*, 1998). The Salt Range (71˚30'-73˚30' E, 32˚23'-33˚00' N) is of unique nature in the world with the oldest series referred to as Cambrian (McKerrow *et al.*, 1992). Low precipitation is the characteristic climatic feature of the Salt Range, which is on an average 50 cm annually (Ahmad *et al.*, 2002). Soil lying between the Salt Range and River Jhelum is heavily salt affected. Water from brine springs deposits salts along its route. The run-off water particularly during the rainy season is the other source of salt deposition that is dissolved from the exposed salt rocks. A large area of foot-hill zone is salty, which is mainly due to sodium chloride (about 90%); the rest are the chlorides, carbonates and bicarbonates of sodium, potassium, magnesium and calcium (Qadir *et al.*, 2005).

Uchhali complex comprises three internationally well known wetlands including Khabeki, Uchhali and Jahlar lakes in the Salt Range, because these wetlands are the wintering sites of rare or vulnerable waterfowl species (Chaudhry *et al*., 1997). Uchhali Lake, covering an area of about 950 ha, is the largest of them, where the water is brackish to saline resulting from the seepage from adjacent agricultural lands and run-off from the surrounding hills of the Salt Range. The water and salinity levels vary with change in rainfall extent, whereas the depth varies from 0.2 to 6.0 m and the water is usually hyper-saline (Afzal *et al*., 1998).

Chaudhry *et al.* (2001) reported over 40 grass species from the Salt Range area, many of them are highly palatable and nutritious. *Cynodon dactylon*, *Sporobolus arabicus*, *Imperata cylindrica*, *Aeluropus lagopoides* and *Desmostachya bipinnata* dominate the saline and saline arid habitats. Since these grasses have been growing in the Salt Range since long, therefore, they are expected to be well adapted against very high salinity. Thus, *Cynodon dactylon* was selected for studying specific morpho-anatomical adaptations. *Cynodon dactylon* (L.) Pers. (Bermuda or Couch grass, locally called Khabbal ghas) is distributed in tropical and warm temperate regions throughout the world (Chaudhary, 1989). It is extremely variable in habit and esteemed as a lawn grass but makes excellent hay and considered as a first-class fodder in Australia, Pakistan and Southeast Asia (Cope, 1982; Chaudhary, 1989). It is generally distributed on sandy or saline soils of open sites (Gleason & Cronquist, 1991). Natural populations of Bermuda grass can have considerable genetic variation for tolerance to soil temperatures, salinity and drought (Speranza, 1995). Moderate levels of salinity may increase the grass yield, though it can tolerate relatively high salinities (Mass & Hoffman, 1977).

Although physiological attributes contribute towards the adaptive mechanism for salt tolerance in *Cynodon dactylon* populations (Hameed & Ashraf, 2008; Zahoor *et al.*, 2012), both halophytes and non-halophytes exhibit remarkable anatomical adaptations when exposed to elevated levels of salinity (Mass & Nieman, 1978; Hamayun *et al*., 2010a; Hameed *et al.*, 2012). However, most conspicuous adaptive changes are notable in leaf. Under salt stress conditions, increased mesophyll (Longstreth & Nobel, 1979), greater density of salt glands and hairs (Marcum *et al.*, 1998), and increased succulence (Waisel, 1972) are key adaptive features which impart greater degree of salt tolerance to plants. Increased salt tolerance is an urgent need for crops grown in the areas that are either salinity affected or at a risk of salinization. This requires new genetic sources of salt tolerance, and more efficient techniques for identifying salt-tolerant germplasm or natural populations, so that new genes for tolerance can be introduced into crop cultivars (Munns *et al.*, 2002).

Although physiological adaptations are crucial in identifying selection criteria against salt stress (Ashraf, 2004), both halophytes and non-halophytes exhibit remarkable structural modifications when exposed to high salinities, particularly in leaf anatomy (Mass & Nieman, 1978; Fahn, 1990; Dickison, 2000). Such structural changes in response to salinity stress are not very much clear, but they certainly play a crucial role in combination with physiological attributes in tolerating the stress. The present study, was therefore, carried out to investigate specific anatomical modifications in the naturally adapted populations of *C. dactylon,* which might have played a significant role in the survival of the salt tolerant population on highly salt affected soil.

## Materials and Methods

The present investigation was carried out to study the adaptability of a potential salt tolerant grass *Cynodon dactylon* L. from the Salt Range, Pakistan against salt stress. The experiment was carried out at the Botanic Garden, Department of Botany, University of Agriculture, Faisalabad during the year 2005-6.

*Cynodon dactylon* was collected from the heavily salt affected soils in the vicinity of natural salt lake, the Uchhali Lake (coordinates 32º36'34.59" N, 72º13'53.72" E, soil pH 6.62, ECe 19.92 dS $m^{-1}$, $Na^+$ 4034.86 mg $kg^{-1}$, $Cl^-$ 2021.30 mg $kg^{-1}$). This species seems to be highly salt tolerant as it grows in the habitat which is in direct contact of highly saline water of the lake. An ecotype of this grass was also collected from the normal non-saline habitats within the Faisalabad region (coordinates 31° 25' 42.87" N, 73° 04' 11.46" E, pH 9.20, ECe 1.82 dS $m^{-1}$, $Na^+$ 69.24 mg $kg^{-1}$, $Cl^-$ 422.46 mg $kg^{-1}$). This population was considered as control. For soil physico-chemical characteristics, soil samples were taken from the root zone of each grass population from both habitats. The soil extracts were used to determine the pH and ECe using pH and electrical conductivity meters, respectively. Analysis of soils was carried out according to the methods described in Handbook No. 60 (USDA Laboratory Staff, 1954).

Populations of this grass species were grown in normal non-saline soil for a period of six months. Nine-inch earthen pots filled with loam and sand in equal quantities were used for the plants. The plants were kept under full sun and irrigated daily till their establishment in Faisalabad environments (average day and night temperature 32±3 and 20±3 ºC, respectively, photoperiod 11-12 h, relative humidity 41 to 59%). The ramets, each with 3 tillers of uniform size, were detached from each plant and grown in half-strength Hoagland's nutrient solution till their establishment in hydroponics. Containers made up of fibre-glass of 25 L capacity were used for hydroponics. Each container contained twenty liters of Hoagland's nutrient solution. The containers were aerated with the help of air pumps about 12 h daily. The containers were 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 were maintained after the establishment of grass species for 8 weeks, viz., control (no salinity treatment), 50, 100, 150 and 200 m*M* of NaCl salinity in Hoagland's nutrient solution. The experiment was planned in a two-factor factorial completely randomized design (CRD) with 2 factors (ecotypes and salinity levels) and 4 replicates. Plants were carefully uprooted from the hydroponics after the completion of the project and washed with tap-water for morpho-anatomical characteristics.

For the anatomical studies, a 2cm piece was taken from the leaf base for leaf anatomy and from the base of top internode of the main tiller for leaf-sheath anatomy. The material was fixed in formalin:acetic:alcohol (FAA, formalin 10%, acetic acid 5%, ethyl alcohol 50% and distilled water 35%) solution for 48 h and subsequently transferred to acetic alcohol (acetic acid and ethyl alcohol in 1:3 ratio) solution for long-term storage. Permanent free-hand sectioning slides were prepared by a series of serial dehydrations in ethanol using standard double-stained technique of safranine and fast green stains. Measurements were taken with a light microscope (Nikon SE, Anti-Mould, Japan), using an ocular micrometer, which was calibrated with a stage micrometer.

For the calculation of area of various cells and tissues, the following formula was used:

$$\text{Area} = \frac{\text{Maximum length} \times \text{Maximum width}}{4} \text{ x } \pi$$

where $\pi$ = Constant (22/7)
This formula was derived from the area of circle, which is $\pi r^2$
That is $\pi$ x d (diameter)/2 x d/2

Considering d/2 the maximum length/2 and the maximum width/2, the formula was derived as the area of different cells was not a complete circle. Micrographs of stained sections were taken with a digital camera (Nikon FDX-35) equipped on a stereo-microscope (Nikon 104, Japan). Anatomical characteristics recorded for leaf and leaf-sheath anatomy were leaf thickness, epidermis thickness and cell area, sclerenchyma thickness and cell area, parenchymatous cell area, aerenchyma area, mesophyll cell area, bundle sheath cell area, vascular tissue (xylem, phloem and vessel areas), bulliform area, hairs/trichomes number, and stomatal density and area.

Analysis of variance of the data for each attribute was computed using the MSTAT Computer Program (MSTAT Development Team, 1989). Least significant difference (LSD) values at 5% level of probability were used to test the differences among mean values (Steel *et al.*, 1997).

## Results

On the basis of performance and growth attributes like fresh and dry matter production of the *Cynodon dactylon* ecotypes under different salt levels of the growth medium, the Salt Range ecotype was regarded as highly salt tolerant (Hameed & Ashraf, 2008).

**Leaf blade anatomy:** A steady increase in the stomatal density on adaxial leaf surface with increase in salt stress was observed in the Faisalabad ecotype of *C. dactylon* (Figs. 1 and 2). The Salt Range ecotype generally had a much higher stomatal density, but had a gradual reduction in stomatal density on adaxial leaf surface with increase in salt level on adaxial leaf surface. Stomatal density on the abaxial leaf surface increased slightly in the Faisalabad ecotype with increase in salt level, but in the ecotype from the Salt Range this increase was more prominent.

Stomatal area on the adaxial leaf surface of both populations was the least affected characteristic by the salt stress (Fig. 1). Stomatal area in both ecotypes of *C. dactylon* was increased at moderate salt levels (50 and 100 m*M*), but this increase was more pronounced in the Faisalabad ecotype. However, stomatal area on the abaxial surface in *C. dactylon* from both Faisalabad and the Salt Range were adversely affected at higher salt levels (150 and 200 m*M* NaCl).

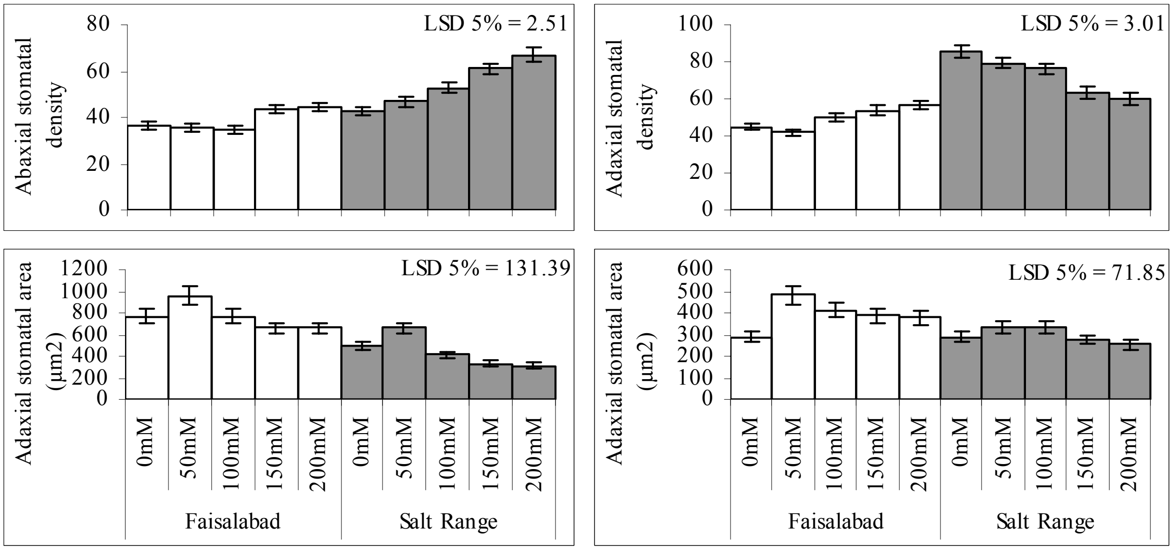

Fig. 1. Stomatal density and area in *Cynodon dactylon* from the Salt Range and Faisalabad grown hydroponically under different salt levels (Mean ± S.E; *n* = 12).

Midrib thickness in ecotypes of *C. dactylon* from Faisalabad remained very much constant under varying salt levels (Figs. 3 and 4). However, the ecotype from the Salt Range progressively decreased with an increase in salt level. Lamina thickness in the ecotype from Faisalabad gradually increased with increase in salt level up to 150 m*M* NaCl level, but again the ecotype from the Salt Range lamina thickness decreased with increase in salt level. Epidermis thickness and its cell area on the adaxial leaf surface decreased with increase in salt level in both ecotypes, however, the effect of salt stress was more severe in the Faisalabad ecotype than recorded in the Salt Range ecotype (Fig. 3). Epidermis thickness and its cell area on the abaxial leaf surface increased with increase in salt level. The population from the Salt Range showed a gradual increase in both epidermis thickness and its cell area, but in that from the Faisalabad region salt stress level up to 100 m*M* NaCl significantly increased epidermis thickness and cell area, but thereafter there was a sharp decrease in these attributes.

A sudden increase in mesophyll area was recorded in both ecotypes at 50 m*M* NaCl. A further increase in salt level resulted in a gradual decrease in mesophyll cell area in both Faisalabad and Salt Range ecotypes.

Bundle sheath cell area in the ecotype from the Faisalabad region increased with increase in salt level in the external medium, however the highest level (200 m*M* NaCl) imparted an adverse effect. On the other hand, this anatomical characteristic remained more or less stable in the Salt Range ecotype.

Bulliform area generally increased in both ecotypes with increase in salt level, and the response was more pronounced in the ecotype from the Salt Range (Figs. 3 and 4). Bulliform area in the Faisalabad ecotype increased up to moderate salt level (100 m*M* NaCl) but further increase in salt level resulted in a reduction in bulliform area.

The higher levels of NaCl (150 and 200 m*M*) resulted in increased area of vascular bundles in the ecotype from Faisalabad, whereas in the ecotype from the Salt Range this anatomical feature progressively decreased with increase in the salt level of the growth medium.

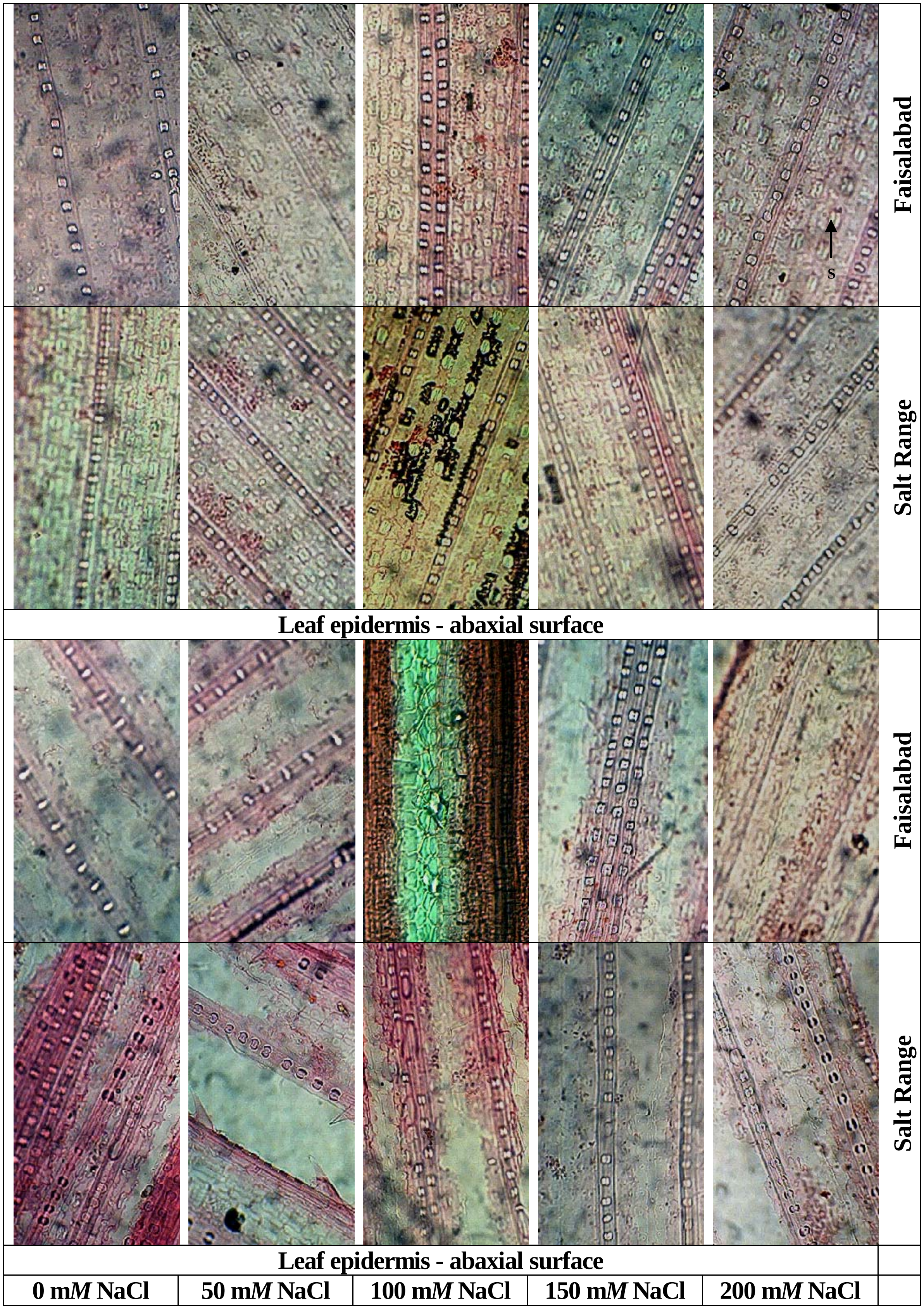

Fig. 2. Leaf surface view in *Cynodon dactylon* from the Salt Range and Faisalabad subjected to different salt levels (S = stomata).

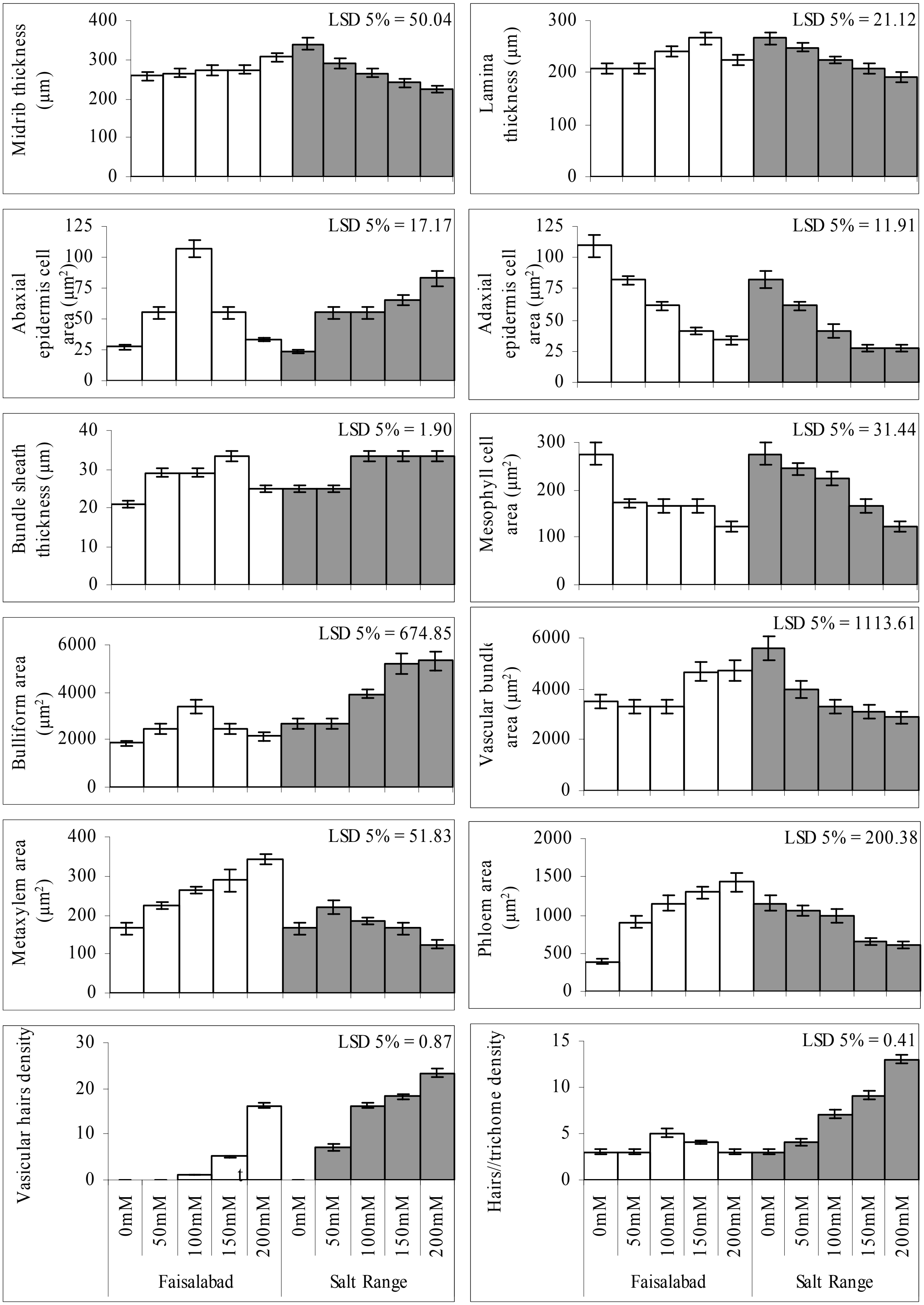

Fig. 3. Leaf blade anatomical characteristics in *Cynodon dactylon* from the Salt Range and Faisalabad grown hydroponically under different salt levels (Mean ± S.E; $n$ = 12).

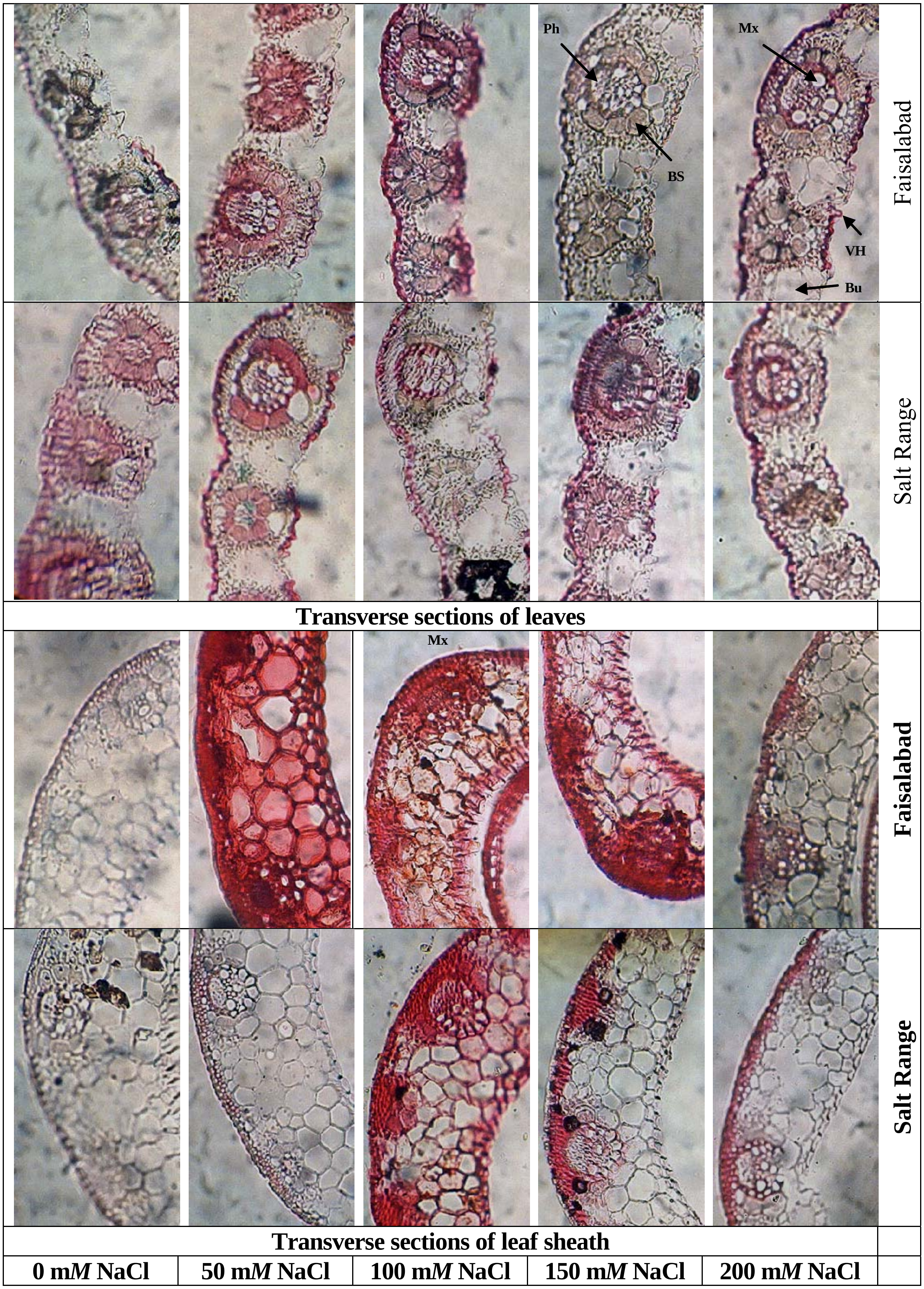
Ph
BS
Mx
VH
Bu
Faisalabad
Salt Range
Transverse sections of leaves
Mx
Faisalabad
Salt Range
Transverse sections of leaf sheath
0 mM NaCl
50 mM NaCl
100 mM NaCl
150 mM NaCl
200 mM NaCl

Fig. 4. Transverse section of leaf midrib in *Cynodon dactylon* ecotypes from the Salt Range and Faisalabad subjected to different salt levels (BS = bundle sheath, Bu = Bulliform cells, MX = metaxylem, Ph = phloem, VH = vesicular hairs).

Metaxylem area was generally greater in the Faisalabad ecotype, which showed a further increase with increase in salt level of the growth medium. In the Salt Range ecotype, it was slightly increased due to salt stress, but thereafter it gradually decreased at higher salt levels. Phloem area was consistently increased in response to increasing salt levels in the Faisalabad ecotype (Fig. 3). However, this attribute was adversely affected in the Salt Range ecotype, where a gradual decrease was recorded in this characteristic with increase in the external salt level.

Number of trichomes per unit area increased in both ecotypes at moderate salt levels, i.e., 50 and 100 m*M* NaCl (Fig. 3). However, higher salt levels resulted in a decline in the trichome number in the Faisalabad ecotype, but a marked increase in number of trichomes was recorded in the ecotype from the Salt Range. Vesicular (bladder) hairs were drastically increased in both populations of *C. dactylon* with increased in external salt level (Fig. 3). However, the ecotype from the Salt Range generally had much higher number than that from the Faisalabad region.

**Leaf sheath anatomy:** Leaf sheath thickness was reduced in both ecotypes of *C. dactylon* with increase in external salt level (Fig. 5), however, this decrease was slightly more prominent in the Salt Range ecotype. On adaxial surface, epidermis thickness and its cell area was increased up to 100 m*M* salt in the ecotype from the Salt Range, whereas a further increase in the external salt level resulted in a gradual decrease (Fig. 5). In contrast, in the Faisalabad ecotype, these parameters were increased by the lowest salt level (50 m*M* NaCl) and thereafter, they decreased considerably with a further increase in salt level. Epidermis thickness and its cell area on abaxial side were similarly affected as the adaxial side, but in this case the effect of salt level was relatively more severe in the Faisalabad ecotype.

Parenchyma cell area generally decreased with increase in salt levels, however, lowest salt level (50 m*M* NaCl) promoted parenchyma cell area in the Faisalabad ecotype (Fig. 5). In the Salt Range ecotype, it was consistently decreased with increase in salt level of the growth medium. A slight variation in number of vascular bundles was recorded in both populations of *C. dactylon*, however, higher salt levels (150 and 200 m*M* NaCl) resulted in a slight decrease in this character.

Vascular bundle area in both ecotypes was increased up to 100 m*M* salt level, but at subsequent salt levels there was a progressive reduction in vascular bundle area. However, the Faisalabad ecotype showed much higher increase in this parameter than that recorded in the Salt Range ecotype, and sharp decrease in vascular bundle area was recorded at higher external salt levels. Metaxylem area in the Faisalabad ecotype decreased gradually with increase in salt level of the growth medium, but higher salt levels (150 and 200 m*M* NaCl) resulted in a significant increase in vessel area in the Salt Range ecotype (Fig. 5).

The Faisalabad ecotype showed a gradual increase in its phloem area up to 150 m*M* salt (Fig. 5) and thereafter at the highest salt level it showed an abrupt decrease in this character. In contrast, the Salt Range ecotype showed a slight increase in phloem area at 100 m*M* NaCl level.

## Discussion

The evolution of a particular character depends primarily on two principal determinants occurring together for a considerable length of time, i.e., the existence of a great magnitude of appropriate genetic variation and the occurrence of appropriate natural selection (Ashraf *et al.,* 1986; Ashraf, 1994). Historical records suggest that the Salt Range originated during the late Cambrian era. Plants inhabiting there should have developed very specific adaptations against a combination of several environmental stresses, mainly salinity and drought, in view of a sufficient length of time they have been growing there.

On the basis of various growth attributes measured in the previous study (Hameed & Ashraf, 2008), the ecotype of *Cynodon dactylon* from the Salt Range was categorized highly tolerant to salt stress. However, *C. dactylon* was collected from near the Ucchali Lake where it had a direct contact with hyper-saline water of the lake. The soil of its site was highly saline (ECe 19.92 dS $m^{-1}$, $Na^+$ content 4134.86 mg $kg^{-1}$, and $Cl^-$ 2021.32 mg $kg^{-1}$). This population may have adapted specific morpho-anatomical and physiological mechanisms to withstand such harsh climatic conditions for its survival and normal growth.

One of the major anatomical modifications in the ecotype of *C. dactylon* from the Salt Range was increased hairiness (trichomes), which had been considered a characteristic feature in grass leaves at highly saline habitat (Cheng & Chou, 1997). In addition to simple trichomes, significantly increased salt secreting vesicular (bladder) hairs on this ecotype provide a clear indication of its excellent adaptability to high saline environments. These results can be supported by some earlier findings in different species, e.g., *Cynodon* and *Distichlis* (Oross & Thomson, 1982), in salt tolerant species (Hagemeyer, 1997), subfamily Chloridoideae (Marcum, 1999), and *Odyssea paucinervis* (Somaru *et al.,* 2002) who reported salt glands on leaf surface for salt excretion.

Plant anatomy is often altered by salinity stress. Leaves of most plants become thicker and more succulent under saline conditions (Kozlowski, 1997). Increased thickness has been reported by several authors, but more reports are on relatively salt sensitive dicot species, e.g., *Distichlis spicata* (Kemp & Cunningham, 1981), *Kandelia candel* (Hwang & Chen, 1995), and kidney bean (El-Araby & Hegazi, 1999). However, in contrast, Omer and Schlesinger (1980) reported no relation of leaf thickness with increasing NaCl concentration in *Jaumea carnosa*, a salt marsh species. The salt tolerant ecotype of *C. dactylon* from the Salt Range in our study showed similar response but that from the Faisalabad region mainly relied on leaf succulence, which might have helped in dumping off toxic ions inside the plant body (Zheng *et al.,* 2002).

Halophytic or salt tolerant species are generally equipped with thick cuticles and epidermis and this is perhaps the most effective mechanism against water loss during limited moisture availability (Yu Jing *et al.,* 2000). However, Bahaji *et al*., (2002) related large size epidermal cells to salt tolerance and regarded it a selection tool for *in-vitro* identification of tolerant lines. Increased thickness by increasing salinity was also reported in different plant species, e.g., in *Ziziphus* cultivars (Awasthi & Pathak, 1999) and *Ziziphus mauritiana* (Awasthi *et al.,* 1999). The Salt Range ecotype with relatively less affected epidermis thickness on the adaxial leaf surface and enlarged thickness and cell size on abaxial surface at the highest salt level makes it a better suitable to withstand physiological droughts than that recorded in the Faisalabad ecotype.

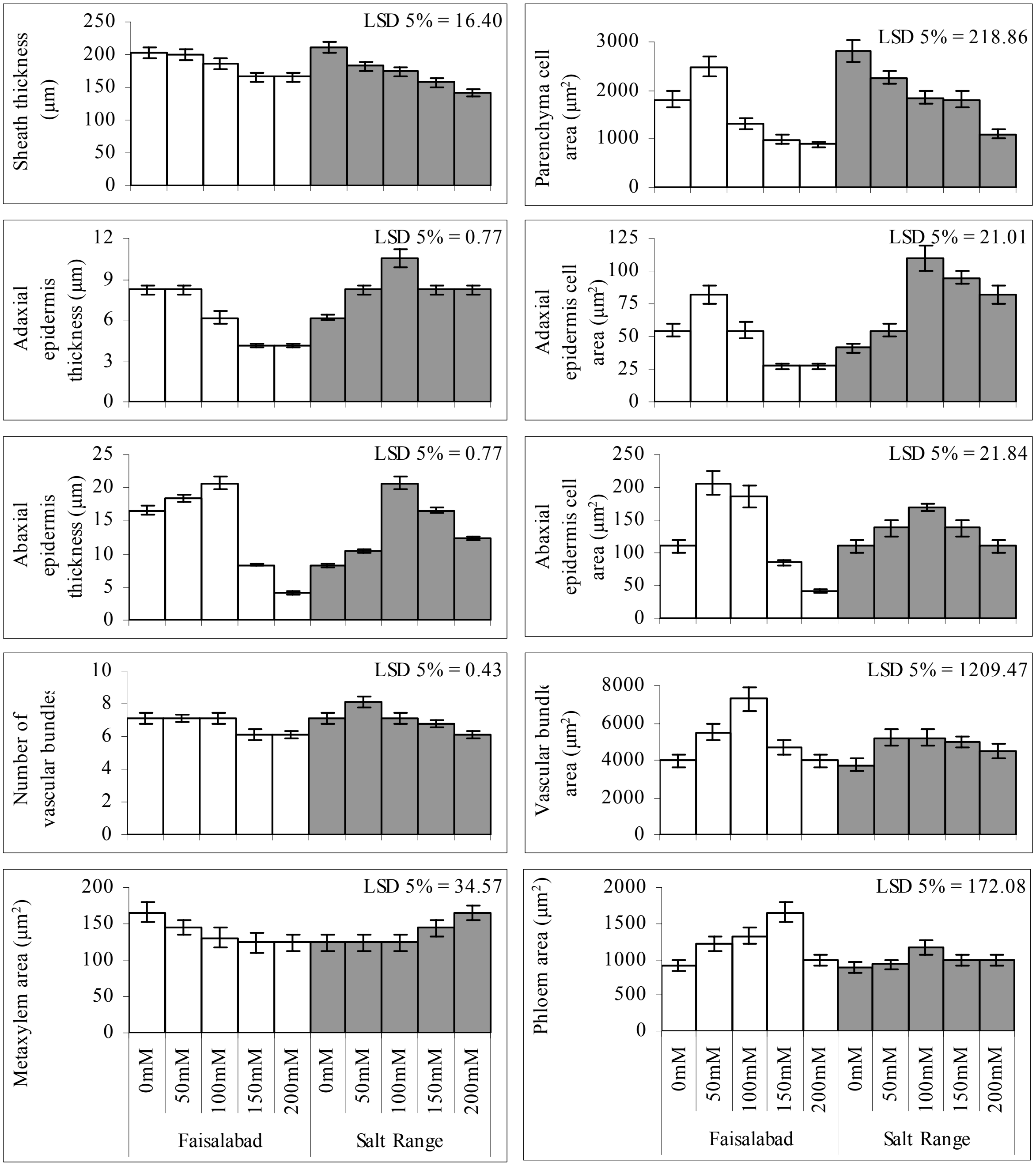

Fig. 5. Leaf-sheath anatomical characteristics in *Cynodon dactylon* from the Salt Range and Faisalabad grown hydroponically under different salt levels (Mean ± S.E; *n* = 12).

The Salt Range ecotype showed a gradual decrease in mesophyll cell size with increase in salinity level, but in the Faisalabad ecotype there was a sharp decrease in mesophyll area by the induction of salt stress. However, both ecotypes were affected by the addition of salt to the growth medium, but relatively less affected mesophyll cells and a gradual decrease in mesophyll cell area in the Salt Range ecotype may be an indication of better survival under harsh climates as it is adapted to tolerate high salinities. Parida *et al*., (2004) reported a significant decrease in mesophyll cell thickness in mangroves, which is in agreement with the present findings. Additionally, Kemp & Cunningham (1981) and Vigo *et al*., (2005) reported less affected mesophyll cells in salt tolerant *Distichlis spicata* and olives. This again supports our findings for the Salt Range ecotype.

Another prominent feature in the Salt Range ecotype was the increased bundle sheath thickness, particularly at the highest salt level. Since *C. dactylon* is a $C_4$ grass and bundle sheath cells play an important role in $CO_2$ conductance, the larger bundle sheath cells may help increase the rate of photosynthesis thereby resulting into enhanced salt tolerance as in the Salt Range ecotype. Such adaptations were also found to be of considerable importance in *Spartina alterniflora,* a dominant species of salt marshes (Walsh, 1990).

The larger size of bulliform cells are distinctively related to saline stress and these can provide tools for the *in vitro* identification of tolerant phenotypes (Bahaji *et al.,* 2002). The grass population from the Salt Range had significantly increased bulliform cells and, therefore, it can safely be concluded that these may substantially contribute to salinity tolerance by efficient leaf rolling, as they are helpful in checking undue water loss during physiological droughts. Zheng *et al.* (2002) reported that under sandy or less salt affected habitats, $Na^+$ and $Cl^-$ are accumulated in vascular bundle sheath or mesophyll cells, but at highly salt-affected habitats bulliform cells act as the main site for toxic ion accumulation. These anatomical features make the Salt Range ecotype to grow vigorously on saline habitats.

One of the most prominent anatomical features in the Salt Range ecotype was considerable development of salt hairs (bladder or vesicle hairs) under high saline environments. The stalk cells of such hairs are capable of transporting toxic $Na^+$ and $Cl^-$ ions into the vacuoles of bladder cells, which eventually burst or die releasing salts outside the plant body (Hagemeyer, 1997). Such type of hairs had been reported by several authors in saline tolerant and halophytic grasses (Marcum, 1999; Somaru *et al.,* 2002; Alshammary *et al.,* 2004). Such increased development of vesicular or bladder hairs in the Salt Range ecotype is probably the most efficient mechanism for the perfect adaptation of this ecotype to highly salt-affected soils.

Reduction in stomatal density and size might also be an efficient feature of checking undue water loss via transpiration during limited water availability under high salinities as reported by different researchers (Walsh, 1990; Bray & Reid, 2002). The Salt Range ecotype showed decreased stomatal area and density under saline conditions on the adaxial leaf surface, so due to this it can be regarded as the best adapted ecotype against highly saline environments.

While assessing the degree of salt tolerance of both ecotypes of *C. dactylon* by examining various anatomical characteristics, it was found that the ecotype from the Salt Range was much better adapted to salt stress than its counterpart from the Faisalabad region. The ecotype from the Salt Range had developed very specific adaptive mechanisms for its survival under harsh climates of the Salt Range. Mechanism of adaptation to saline environments is very specific, and it is not only for the excretion of toxic ions through vesicular hairs but also for the accumulation of these ions in large vacuoles of parenchymatous tissue. Additionally, development of some xeromorphic characteristics (decreased stomatal area and density at adaxial side, stability in epidermis thickness and its cell area, increased bundle sheath that prevents radial flow of water, increased bulliform cell area that is crucial for leaf rolling and greatly increased density of hairs/trichomes) seems to be essential for checking undue water loss.

The most prominent adaptive feature of the salt tolerant ecotype from the Salt Range is the increased development of vesicular hairs, which generally tends to exclude toxic ions through leaves and this salt exclusion mechanism was much more developed under the highest salt level (200 m*M* NaCl). Relatively well developed and less affected parenchyma in the salt tolerant population under high salinities (mesophyll, bundle sheath, and bulliform cells) surely has a great potential of accumulating toxic ions in vacuolar sap. Highly developed dermal tissue, particularly lower epidermis and bulliform cells, and decreased stomatal density and area in the salt tolerant population seem to be crucial for checking undue water loss under low availability of water, particularly under osmotic stress caused by salinity stress. All these characteristics must have played a significant role for the successful predominance of the salt tolerant ecotype on highly salt affected soils.

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