

## Structural adaptations in the desert halophyte *Aeluropus lagopoides* (Linn.) Trin. ex Thw. under high salinity

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A study was conducted to evaluate structural adaptations in *Aeluropus lagopoides* (L.) Trin. ex Thw. under high salinity. Plants of this grass were collected from five different salt-affected habitats [Derawar Fort (DF); Traway Wala Toba (TT); Bai Lah Wala Dahar (BD); Ladam Sir (LS); Pati Sir (PS)] from the Lesser Cholistan Desert, Pakistan. All five ecotypes of *A. lagopoides* responded specifically to varying saline habitats in terms of root anatomical features, e.g. there was an increase in aerenchyma formation, increased sclerification in both external (hypodermis) and internal (many-layered sclerenchyma above the endodermis) cortical layers, and increased sclerification in the pith region due to the increased salinity of the habitat. At the highly salt-affected sites (LS and PS), plants had a well developed endodermis-like protective layer near the stem periphery, being a unique adaptation, not previously reported in any other grass species. Increased density of micro-hairs in *A. lagopoides* plants at both leaf surfaces was found with increase in salinity of the habitat. In conclusion, adaptive anatomical features were very conspicuous in plants growing at highly saline sites.

**Key words:** adaptation, aerenchyma, desert halophytes, endodermis, salt tolerance sclerification.

### INTRODUCTION

The Cholistan Desert in Pakistan is a unique biome with great diversity of environmental factors and multiple stresses including aridity, salinity, high temperature, and nutrient deficiency (Naz *et al.*, 2010a). A wide diversity of plant species has been recorded in the desert (Hameed *et al.*, 2011) and most of these species are believed to be adapted to one or more of the environmental stresses present (Arshad *et al.*, 2008). Of the different stressful habitats of the Cholistan Desert, the saline habitat is prevalent particularly in interdunal areas (Naz *et al.*, 2010a). Although the saline habitat possesses a variety of species, all known to be salt tolerant, *Aeluropus lagopoides* (L.) Trin. ex Thw. is an indicator species of the highly saline habitat, where no other species can easily grow (Gulzar &

Khan, 2001). This rhizomatous perennial grass, which has high protein content, can be used for forage production on highly saline wastelands (Gulzar *et al.*, 2003). It is a salt secreting grass that can also substantially accumulate toxic ions in different plant organs (Mohsenzadeh *et al.*, 2006). It dominates inundated coastal areas as well as inland saline arid flats all over Pakistan (Naz *et al.*, 2010a). All these characteristics make this perennial species a valuable candidate for saline agriculture.

Salinity causes a number of adverse effects on plants, resulting from osmotic stress (Atia *et al.*, 2011; Krasensky & Jonak, 2012). Physiological drought due to salinity limits water uptake from the soil. In addition, excess salt uptake may disrupt cellular functions and damage physiological processes such as photosynthesis and respiration due to ion toxicity and/or nutritional imbalance (Shahid *et al.*, 2011; Houmani *et al.*, 2012). Therefore it is important to keep the levels of toxic ions low in the leaves, particularly in the

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growing tissues. This can be achieved by restricting absorption of toxic ions, reducing translocation of ions to the shoot and excluding these ions from the plant body (Wang *et al.*, 2012).

Review of the literature on *A. lagopoides* reveals a number of reports on its physiological and biochemical responses to salt stress (Gulzar *et al.*, 2003; Sobhanian *et al.*, 2010), but very little information on its anatomical features, which make it thrive well in highly saline conditions. Specific anatomical adaptations in most plants related to salinity stress are generally based on two fundamental mechanisms; the first one is the efficient salt excretion through salt glands, salt hairs or micro-hairs, and trichomes (Naidoo *et al.*, 2012), or through intercellular spaces that are important for bulk salt movement in plant organs (Rana-thunge *et al.*, 2003), while the second one is water conservation to combating salinity-induced physiological drought. Structural modifications used for limiting excess water loss through the plant body include leaf rolling via bulliform cells (Bahaji *et al.*, 2002), sclerification in parenchymatous regions, thickening of cuticle, epidermis, and exodermis (Hose *et al.*, 2001; Taiz & Zeiger, 2002). Distribution, orientation and functioning of stomata is also vital for water conservation (Nilson & Assmann, 2007; Naz *et al.*, 2010a).

In view of the above reports, it was hypothesized that habitat heterogeneity has resulted in the development of different ecotypes of this grass, each of them having developed particular structural modifications during evolution to cope with their saline habitats. Thus, the present study was conducted to examine specific anatomical adaptations in various ecotypes of this species that allow them to thrive in habitats impregnated with high salt content.

## MATERIALS AND METHODS

### *Study area*

Ten plants of *A. lagopoides* were randomly collected from ten different locations at each study site in the Lesser Cholistan, Pakistan. The sites were selected on the basis of soil type, colour, texture, moisture content, and plant communities inhabiting those sites. For the collection of plant samples, 1 km<sup>2</sup> area was selected at each site.

The five collection sites were: Derawar Fort (DF), the most disturbed habitat due to anthropogenic activities, but least salt-affected (Table 1); Traway Wala Toba (TT) and Bai Lah Wala Dahar (BD): modera-

tely salt-affected; Ladam Sir (LS) and Pati Sir (PS): highly salt-affected.

### *Soil samples*

The soil adhering to the roots (about 25 cm deep) was collected to analyze physico-chemical characteristics. To measure the saturation percentage, pH and electrical conductivity (ECe) of soil collected from the root zones, 200 g of dried soil was used for the preparation of saturation paste. Saturation percentage was determined by subtracting dry weight of soil from that of the saturated paste. The soil extract was used to determine the pH and ECe using a combined pH and EC meter (WTW series InoLab pH/Cond 720, USA), respectively. Analysis was carried out according to the methods described by Handbook No. 60 (USDA Laboratory Staff, 1954).

The cations Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> in the soil extracts were estimated with a flame photometer (Jenway, PFP-7, UK). Cl<sup>-</sup> content was determined with a digital chloride meter (Model 926, Sherwood Scientific Ltd, Cambridge, UK).

### *Morphological and anatomical parameters*

The following morphological characteristics were measured: root length, shoot length, leaf area, and fresh and dry weight of root and shoot of the plants growing on each site. For anatomical studies, a portion from the thickest adventitious root at the root-shoot junction, the uppermost internode of the thickest tiller, and the flag leaf of the thickest tiller were selected. Firstly, the material was fixed in formalin-acetic-alcohol (FAA) solution for 36 hrs, which contained (v/v) formalin 5%, acetic acid 10%, ethanol 50%, and distilled water 35%. It was then subsequently transferred to acetic-alcohol solution (25% acetic acid and 50% ethanol, v/v) for long-term storage. Permanent slides of free hand-cut sections were prepared by a series of dehydrations in ethanol following Ruzin (1999). The double staining procedure was adopted to study different cells and tissues. Safranin-stained sclerified/lignified tissues, sclerenchyma and xylem vessels, and fast green thin-walled parenchymatous, mesophyll, and phloem tissues were studied. Data for different cells and tissues in the cross-sections were recorded using an ocular micrometer, which was calibrated with a stage micrometer. Micrographs of stained sections were taken with a camera equipped microscope (Nikon 104, Tokyo, Japan).

TABLE 1. Coordinates and soil physico-chemical characteristics of different habitats in the Cholistan Desert

Characteristics	Derawar Fort	Traway Wala Toba	Bai Lah Wala Dahar	Ladam Sir	Pati Sir
Coordinates	29° 24' 32.67" N 71° 27' 32.34" E	29° 10' 27.65" N 71° 09' 21.57" E	29° 38' 19.43" N 70° 93' 23.37" E	30° 53' 26.53" N 72° 64' 24.62" E	30° 35' 17.58" N 72° 63' 22.45" E
Moisture content (%)	25.17 <sup>a</sup>	27.52 <sup>b</sup>	29.65 <sup>c</sup>	25.41 <sup>a</sup>	25.40 <sup>a</sup>
Organic matter (%)	0.85 <sup>c</sup>	0.78 <sup>b</sup>	0.76 <sup>b</sup>	0.73 <sup>a</sup>	0.72 <sup>a</sup>
pH	8.40 <sup>a</sup>	8.34 <sup>a</sup>	8.42 <sup>a</sup>	8.28 <sup>a</sup>	8.31 <sup>a</sup>
ECe (dS m <sup>-1</sup> )	17.60 <sup>a</sup>	24.81 <sup>b</sup>	28.53 <sup>c</sup>	36.36 <sup>d</sup>	46.59 <sup>e</sup>
Na <sup>+</sup> (mg l <sup>-1</sup> )	3801.50 <sup>a</sup>	4381.51 <sup>b</sup>	4501.53 <sup>c</sup>	4920.85 <sup>d</sup>	5221.32 <sup>e</sup>
K <sup>+</sup> (mg l <sup>-1</sup> )	403.64 <sup>d</sup>	388.18 <sup>c</sup>	351.46 <sup>b</sup>	282.17 <sup>a</sup>	286.05 <sup>a</sup>
Ca <sup>+</sup> (mg l <sup>-1</sup> )	65.91 <sup>b</sup>	64.97 <sup>b</sup>	63.16 <sup>b</sup>	57.62 <sup>a</sup>	58.88 <sup>a</sup>
Cl <sup>-</sup> (mg l <sup>-1</sup> )	1580.28 <sup>a</sup>	2230.54 <sup>b</sup>	2403.37 <sup>c</sup>	2510.28 <sup>d</sup>	2680.33 <sup>e</sup>
NO <sub>3</sub> <sup>-</sup> (mg l <sup>-1</sup> )	2.16 <sup>a</sup>	3.21 <sup>d</sup>	2.93 <sup>c</sup>	2.91 <sup>c</sup>	2.61 <sup>b</sup>
PO <sub>4</sub> <sup>3-</sup> (mg l <sup>-1</sup> )	2.15 <sup>a</sup>	2.55 <sup>b</sup>	2.56 <sup>b</sup>	3.48 <sup>c</sup>	3.57 <sup>c</sup>
Plant community	Si-Cj	Si-Al	Si-Cj-Hs	Si-Cj-Oc	Si-Cj-Sv
Importance value	23.27 <sup>b</sup>	33.96 <sup>c</sup>	20.94 <sup>a</sup>	24.05 <sup>b</sup>	20.87 <sup>a</sup>
Species association	Cj <sup>***</sup> , Si <sup>*</sup> , Sv <sup>***</sup>	Cj <sup>***</sup> , Si <sup>*</sup> , Sim <sup>***</sup> , Sv <sup>***</sup>	Cj <sup>***</sup> , Hs <sup>*</sup> , Si <sup>*</sup> , Sim <sup>*</sup> , Sv <sup>***</sup>	Cj <sup>***</sup> , Hs <sup>*</sup> , Oc <sup>*</sup> , Si <sup>*</sup> , Sv <sup>***</sup>	Si <sup>*</sup> , Sv <sup>***</sup>

Means with same letters in each row are statistically non-significant (n = 10)

\* : significant at  $p < 0.05$ , \*\* : significant at  $p < 0.01$

ECe: electrical conductivity of soil extract, Importance value: Relative Density, Relative Frequency, Relative Cover

Al: *Aeluropus lagopoides*, Cj: *Cymbopogon jwarancusa* (Jones) Schult., Hs: *Haloxylon stocksii* (Boiss.) Benth. & Hook., Oc: *Ochthochloa compressa* (Forssk.) Hilu, Si: *Sporobolus ioclados* (Trin.) Nees, Sim: *Salsola imbricata* Forssk., Sv: *Suaeda vera* Forssk. ex J. F. Gmel.

Statistical analysis

The data for both morphological and anatomical parameters, derived from a completely randomized design (CRD) with 10 replications, were subjected to analysis of variance (ANOVA). Mean values were compared by the Duncan’s Multiple Range (DMR) test. Standard error (SE) was calculated for each mean value.

RESULTS

Morphological parameters

Root length showed no significant increase with increasing salinity of the site up to the moderately saline BD. However, it increased significantly with a further increase in salinity of the habitat ( $p < 0.05$ , Fig. 1).

Shoot length of the *A. lagopoides* ecotypes growing on the least saline DF and moderately saline TT and BD did not differ significantly ( $p > 0.05$ ). However, the ecotypes from the highly saline LS and PS showed a significant reduction in shoot length, although they did not differ significantly from each other (Fig. 1).

Number of leaves per plant decreased progressively with an increase in salinity of the habitats (Fig. 1). This decrease in leaf number was consistent up to the moderately saline BD, but thereafter a sharp decrease was recorded in the ecotype from the highly saline LS and PS. Both flag leaf area and total leaf area per plant showed a significant decrease with increasing soil salinity of the habitats ( $p < 0.05$ ), but no significant difference was recorded between highly

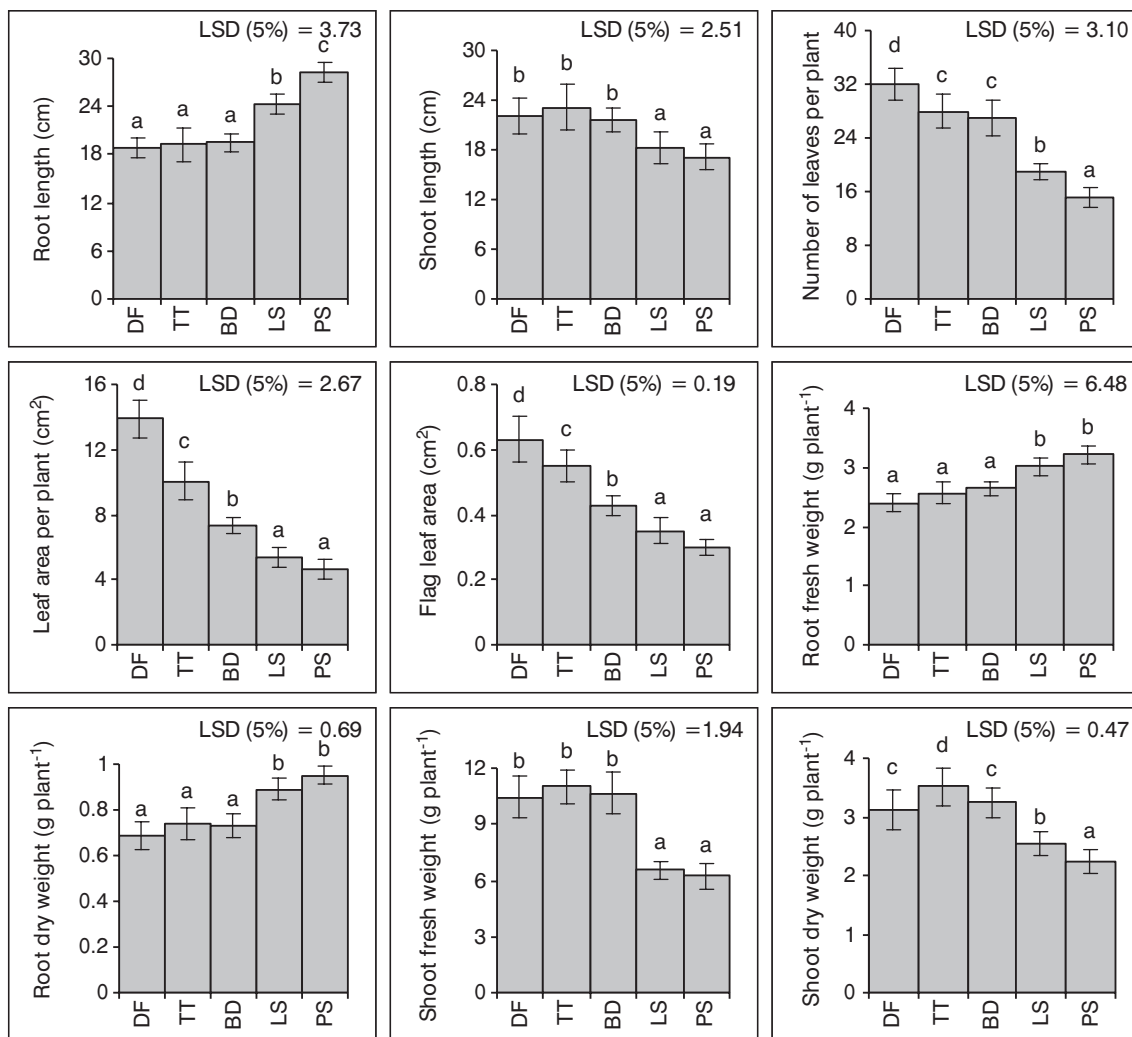


FIG. 1. Growth attributes of *Aeluropus lagopoides* from different habitats in the Cholistan Desert (n = 10, mean values ± SE). Means with same letters are statistically non-significant. DF: Derawar Fort, TT: Traway Wala Toba, BD: Bai Lah Wala Dahar, LS: Ladam Sir, PS: Pati Sir.

saline LS and PS with respect to these characters ( $p > 0.05$ ).

Fresh and dry root weight of *A. lagopoides* plants showed a gradual increase with increase in salinity of habitat, and this increase was more prominent in the ecotype from highly saline soil which had root weight that differed significantly from those of plants from lower or moderate salinities (Fig. 1). Compared with least saline affected sites, shoot fresh weight was found to be slightly increased in plants from the moderate salinity sites but significantly decreased at sites LS and PS with higher levels of salinity (Fig. 1). Shoot dry weight showed a pattern of decrease similar to that recorded for shoot fresh weight. However, in the

ecotype from the moderately saline site TT, shoot dry weight increased significantly ( $p < 0.05$ ).

### Anatomical parameters

#### Root anatomy

The root cross-section area of *A. lagopoides* showed a gradual and significant increase ( $p < 0.05$ ) with increase in salinity of the habitat, being greater in the population from the highly salinity affected site, PS. However, no significant difference was recorded between the highly saline LS and PS with respect to root area ( $p > 0.05$ , Fig. 2). Root hair length did not differ significantly between populations from the moderately saline TT with respect to that from the least saline

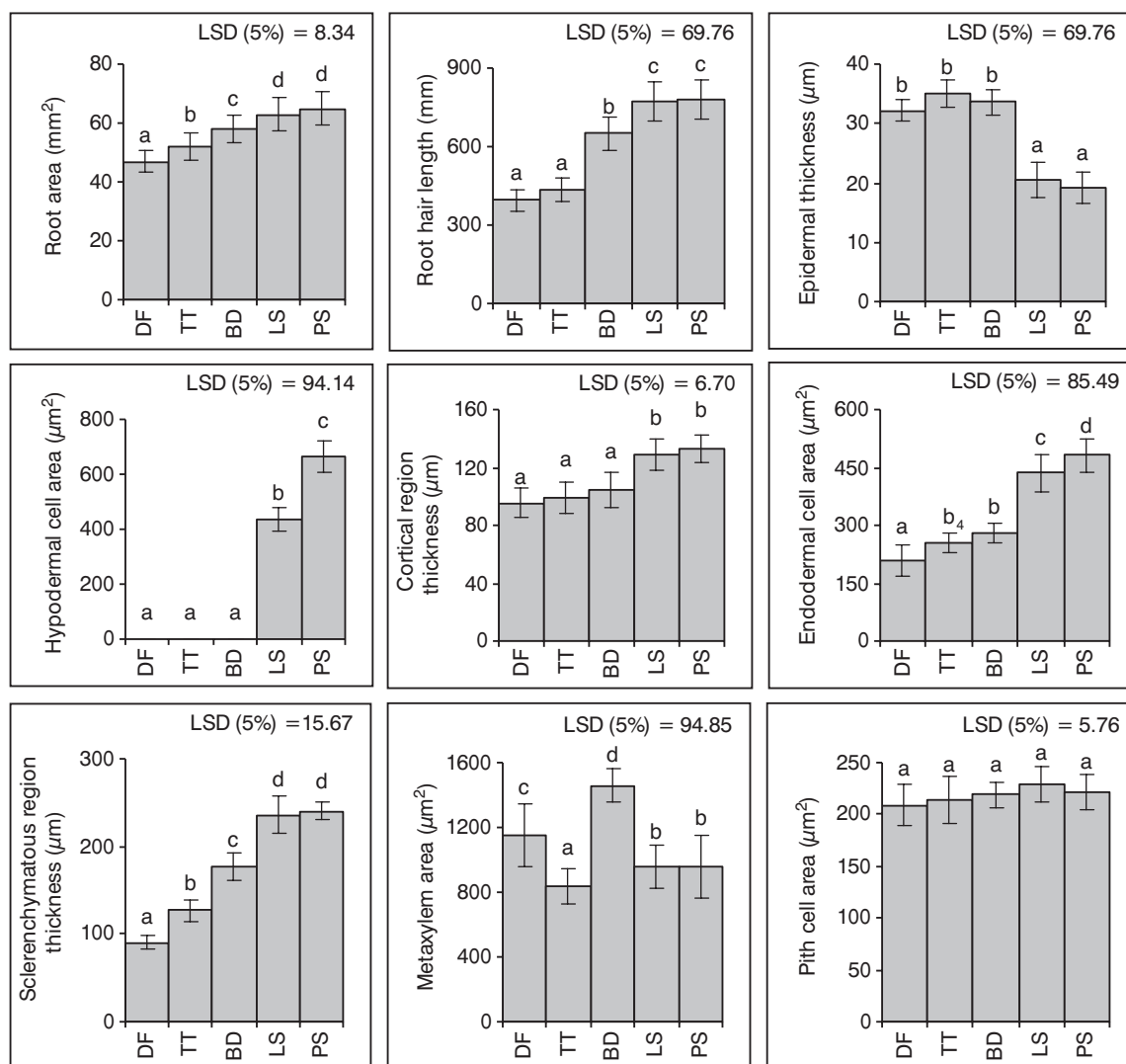


FIG. 2. Root anatomical characteristics of *Aeluropus lagopoides* from different habitats in the Cholistan Desert ( $n = 10$ , mean values  $\pm$  SE). Means with same letters are statistically non-significant. DF: Derawar Fort, TT: Traway Wala Toba, BD: Bai Lah Wala Dahar, LS: Ladam Sir, PS: Pati Sir.

DF ( $p > 0.05$ ), but was significantly higher in the ecotypes inhabiting the moderately saline BD and highly saline LS and PS sites ( $p < 0.05$ ). Epidermal layer thickness was not affected in the ecotypes from lower and moderately saline soils, but in those from highly sal-

ine LS and PS, this parameter significantly decreased. Sclerified hypodermis in the outer cortical region was not found in the plants from the least saline DF and moderately saline TT and BD. A distinct hypodermis was recorded in the ecotype from LS and was marke-

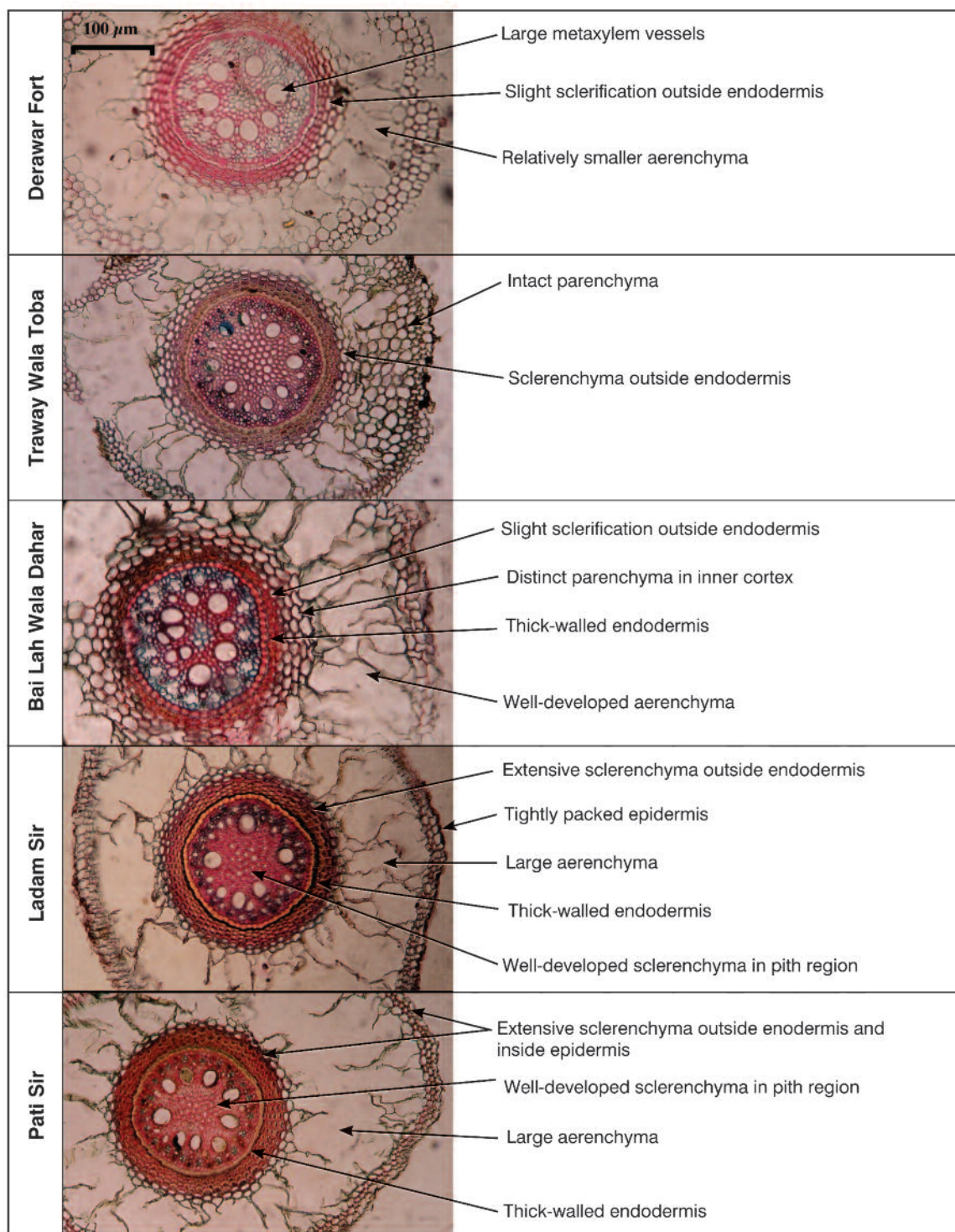


FIG. 3. Cross sections of *Aeluropus lagopoides* roots from different habitats in the Cholistan Desert.

dly developed in the plants from highly saline PS. There was an overall increase in the cortical region thickness in the root transverse section with increase in salinity of the habitat, but no significant differences were observed between least and moderately saline sites (Fig. 2).

Endodermal cell cross-section area increased considerably with increase in salinity level of the habitats, but this increase was more pronounced in the plants from highly saline sites, LS and PS (Fig. 2). Sclerenchymatous region thickness increased consistently and significantly with increase in salinity of the habitats ( $p < 0.05$ ), but at the highly saline LS and PS sites this anatomical feature did not differ significantly ( $p > 0.05$ ). No clear-cut response of metaxylem vessel cross-section area was observed in response to different salinity level of the habitats, and the largest vessels were recorded in plants from the moderately saline BD; followed by the plants from least saline area, while significantly smaller vessels were recorded in the plants from moderately saline TT (Fig. 2). Pith cell cross-section area, in contrast, was very much stable in all the populations of this grass.

The impact of increasing salinity on root anatomy was very conspicuous (Fig. 3). Epidermal thickness significantly decreased with increase in salinity of the habitats. The formation of aerenchyma by disintegration of the cortical region increased and sclerification outside the endodermis and inside the epidermis increased significantly with increasing salinity. Endodermis became more prominent, and sclerification in the pith region increased with increase in habitat salinity.

#### Stem anatomy

Stem cross-section area decreased consistently with increase in salinity and in plants from DF and TT, this parameter was significantly higher than in those from the other habitats (Fig. 4). Sclerenchymatous cell cross-section area was significantly greater in the ecotypes from the habitats with higher salt content than in those from the low DF and moderately saline TT sites. Nevertheless, sclerification in the outermost region of stem was much more prominent in the plants from highly saline PS.

Salinity resulted in a development of a new endodermis-like layer near the stem periphery, which further increased in size and compactness as salinity level of the habitat increased, being greatest in plants from the highly saline PS (Figs 4 and 5). Cortical cell cross-section area was the greatest in plants from modera-

tely saline sites (TT and BD), but thereafter this parameter decreased consistently with a further increase in salinity of the habitats.

Vascular bundle area was significantly greatest in plants collected from the highly salt affected site, PS. Metaxylem vessel cross-section area was significantly higher in plants from moderately and highly saline TT, BD and LS than in those from DF with low salinity, which showed the narrowest metaxylem vessels (Fig. 4). A similar pattern was recorded for protoxylem cross-section area, which was greatest in plants from BD and least in those from DF.

Phloem cross-section area was significantly greater in plants from moderately saline TT and BD than in those from other habitats. The smallest value for this parameter was recorded in plants from the least saline DF. Sieve cross-section area decreased consistently and significantly with increase in salinity of the habitats. Plants from both highly saline LS and PS sites showed significantly narrower sieves in the phloem than those recorded from the other habitats (Fig. 4).

The outer cortex and epidermis of *A. lagopoides* were disintegrated in the plants from all habitats, and they were protected by increased sclerification and an endodermis-like layer. A stem cross-section of this grass from the Faisalabad region with relatively high rainfall showed distinct cortical parenchyma and epidermis (Fig. 5). Another significant change with increasing salinity of the habitats was the orientation of vascular tissues, which became closer to the stem periphery as the salinity level of the habitats increased (Fig. 5).

#### Leaf anatomy

Leaf thickness decreased gradually and significantly with increase in the habitat salinity up to the moderately saline BD, but thereafter, a significant increase in leaf thickness was observed at the highly saline LS and PS sites (Fig. 6). Epidermal thickness increased gradually and significantly with increasing salinity and was the highest in plants from PS ( $p < 0.05$ ). Bulliform cell area was the smallest in plants from DF site with low salinity, but increased consistently and significantly with increase in salinity of the habitat. In the plants from the highly saline, LS and PS, bulliform cells were highly developed (Fig. 6).

Mesophyll thickness, in contrast, was not affected by increasing salinity up to the moderately saline BD ( $p > 0.05$ ), but decreased significantly at higher salinity ( $p < 0.05$ ). Bundle sheath thickness showed a grad-

ual and significant decrease with increase in salinity of the habitats, being lowest in plants from the highly saline habitats (Fig. 6).

Vascular bundle area was greatest in plants from the least saline DF, significantly higher than in plants from any other habitat. Both moderately saline habitats showed the lowest value for this parameter (Fig. 6). No significant variation was recorded in the metaxylem vessel area, but the plants from moderately saline TT had greatest cross-section area of metaxylem vessels. In contrast, protoxylem area increased considerably and significantly with increase in salinity level of the habitats, being greatest in plants from the

highly saline PS (Fig. 6). No clear-cut pattern in response to increasing salinity was recorded with respect to phloem area. Phloem area in plants from the least saline DF, and highly saline LS and PS sites did not differ significantly, whereas it was significantly lower in those from the moderately saline TT and BD (Fig. 6).

The density of micro-hairs and trichomes at both leaf surfaces increased with increase in salinity of the habitats. However, the density of micro-hairs was relatively greater on the abaxial leaf surface in plants from highly saline LS and PS sites (Fig. 6).

The most prominent modification of leaf trans-

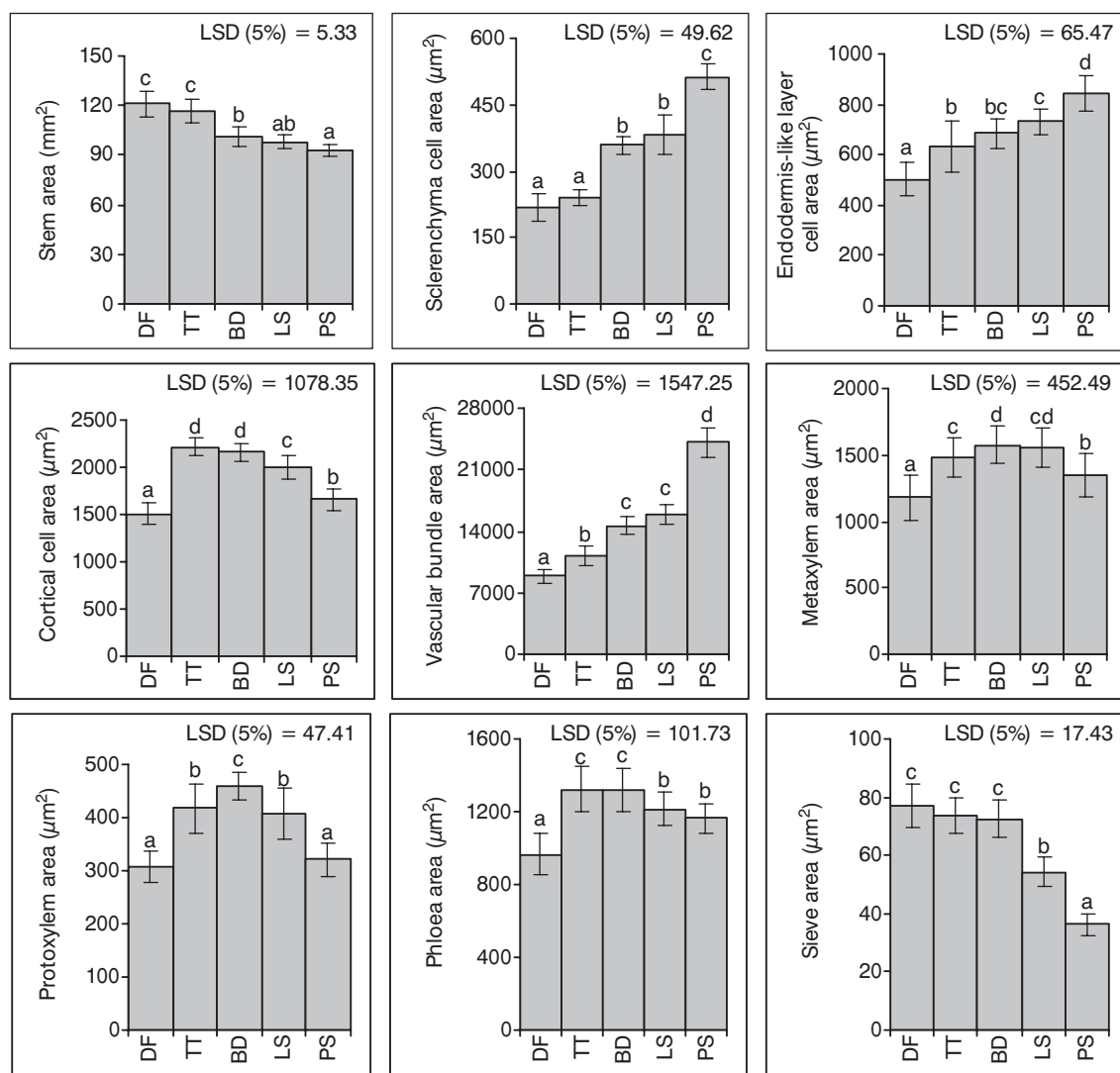


FIG. 4. Stem anatomical characteristics of *Aeluropus lagopoides* from different habitats in the Cholistan Desert (n = 10, mean values ± SE). Means with same letters are statistically non-significant. DF: Derawar Fort, TT: Traway Wala Toba, BD: Bai Lah Wala Dahar, LS: Ladam Sir, PS: Pati Sir.



verse sections in response to increasing salinity was the increased sclerification above and below the vascular bundles. Hairiness and depression occurred prominently on the adaxial leaf surface due to bulliform cells (Fig. 7).

## DISCUSSION

The study sites inhabited by *A. lagopoides* differed significantly in terms of various physico-chemical characteristics measured (Table 1). These factors determine the community structure and species association

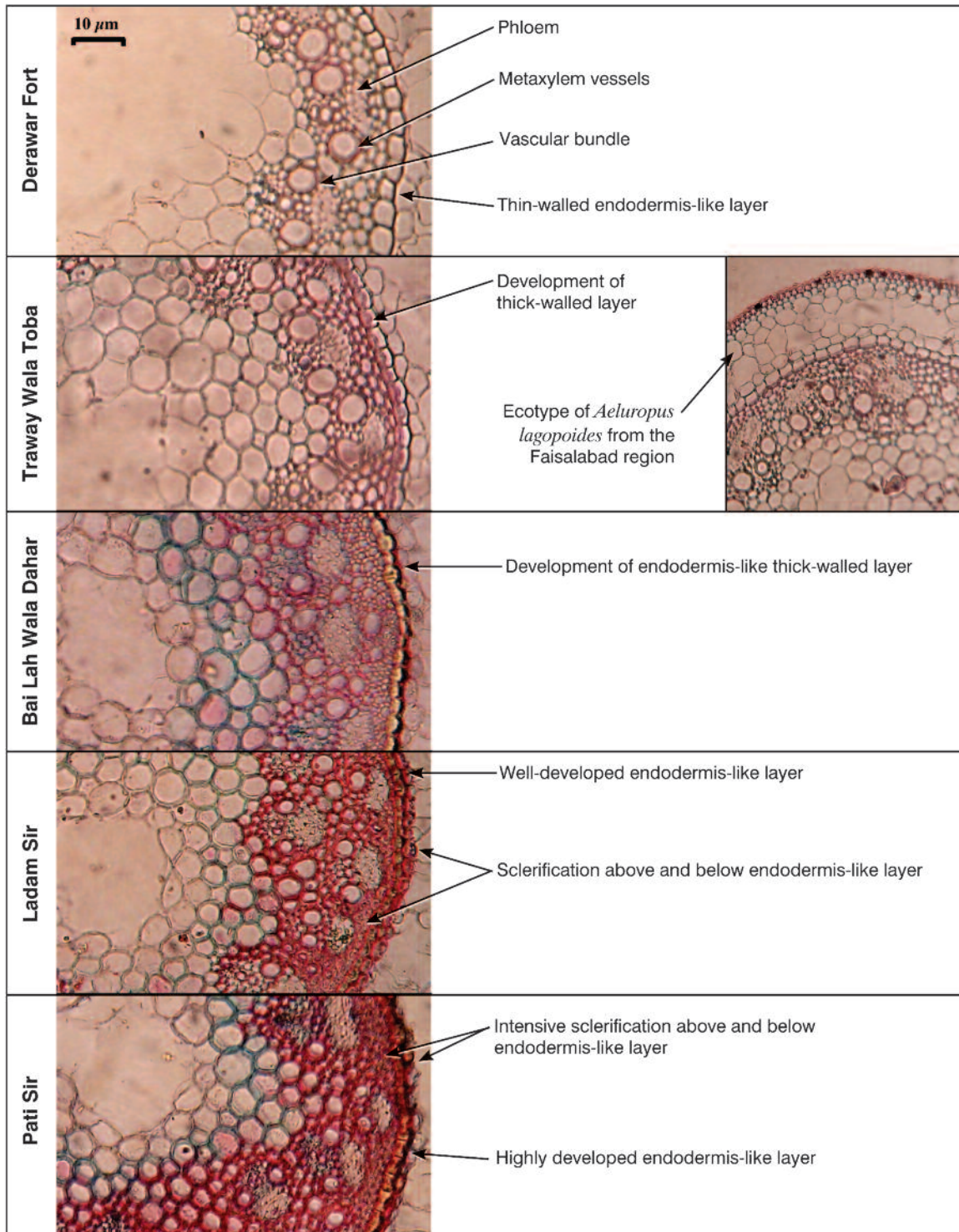


FIG. 5. Cross sections of *Aeluropus lagopoides* stems from different habitats in the Cholistan Desert.

of the study sites (Naz et al., 2010a,b). The differences in the biotic and abiotic factors, and the considerable separation distance between habitats may be sufficient for certain characteristics to have become fixed in this grass over a long period. Such variation in differently adapted ecotypes has earlier been reported in plant species such as *Phragmites australis* (Zheng et

al., 2002), *Sporobolus virginicus* (Bell & O’Leary, 2003), *Panicum antidotale* (Ahmad et al., 2010), *Imperata cylindrica* (Hameed et al., 2009), and *Paspalum vaginatum* (Lee et al., 2005).

Increasing salinity of the habitats may have selected for specific morphological and anatomical changes, indicating the adaptive potential of this grass to

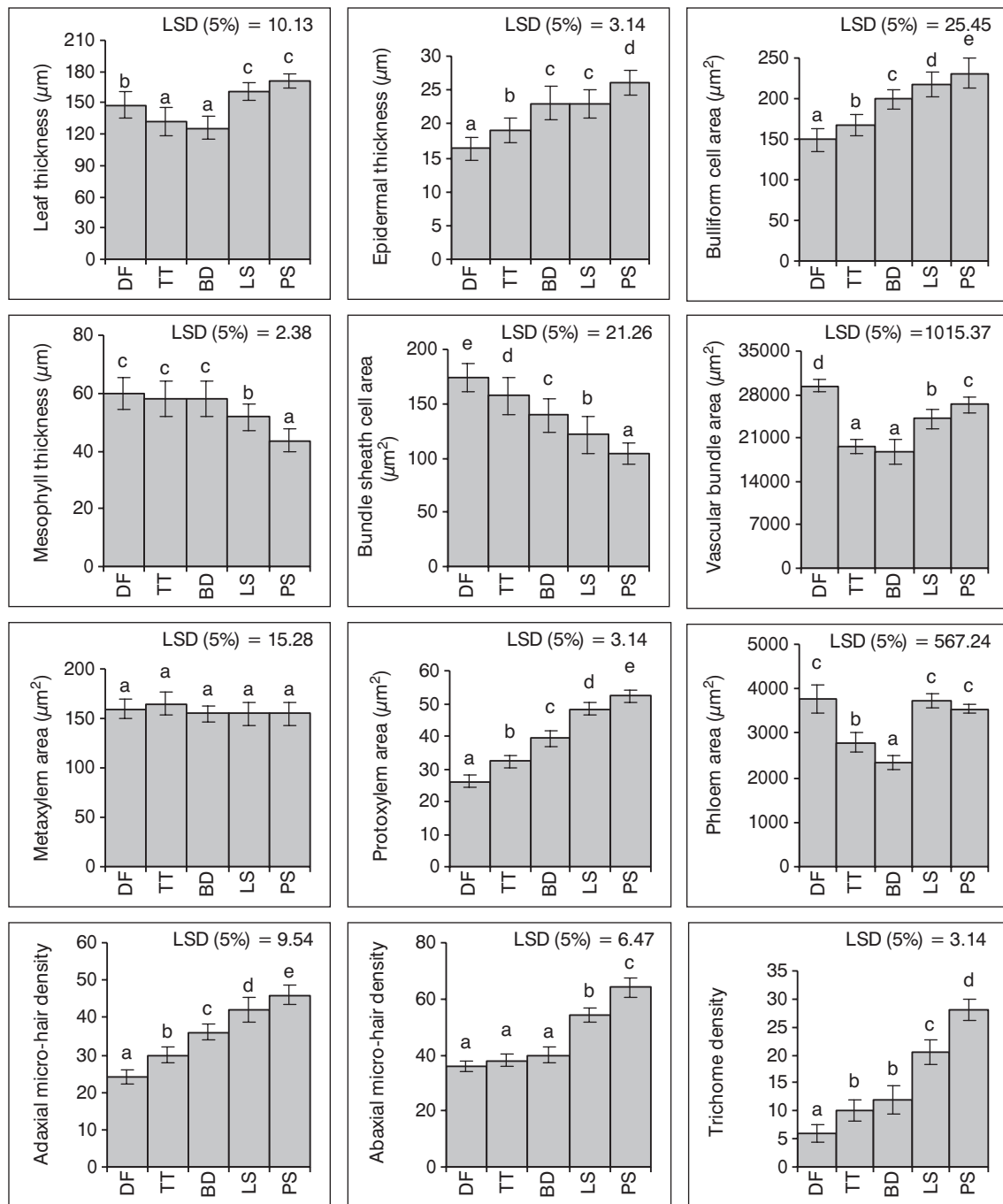


FIG. 6. Leaf anatomical characteristics of *Aeluropus lagopoides* from different habitats in the Cholistan Desert (n = 10, mean values ± SE). Means with same letters are statistically non-significant. DF: Derawar Fort, TT: Traway Wala Toba, BD: Bai Lah Wala Dahar, LS: Ladam Sir, PS: Pati Sir.

cope with environmental conditions in the Cholistan Desert. One of the most prominent changes is the stunted growth of aerial parts (shoot length, leaves per plant, leaf area and fresh and dry weights of shoot), particularly under high salinities, but accompanied by improved development of roots. Restricted growth is an important adaptation for tolerating sali-

ne stress, as plants of short stature may conserve energy essential for vital metabolic processes (Munns & Tester, 2008).

The root parameters, root length and fresh and dry weights generally increase under salinity stress in most halophytic species (Khan *et al.*, 1999), whereas a reverse is true for glycophytic and less salt tolerant

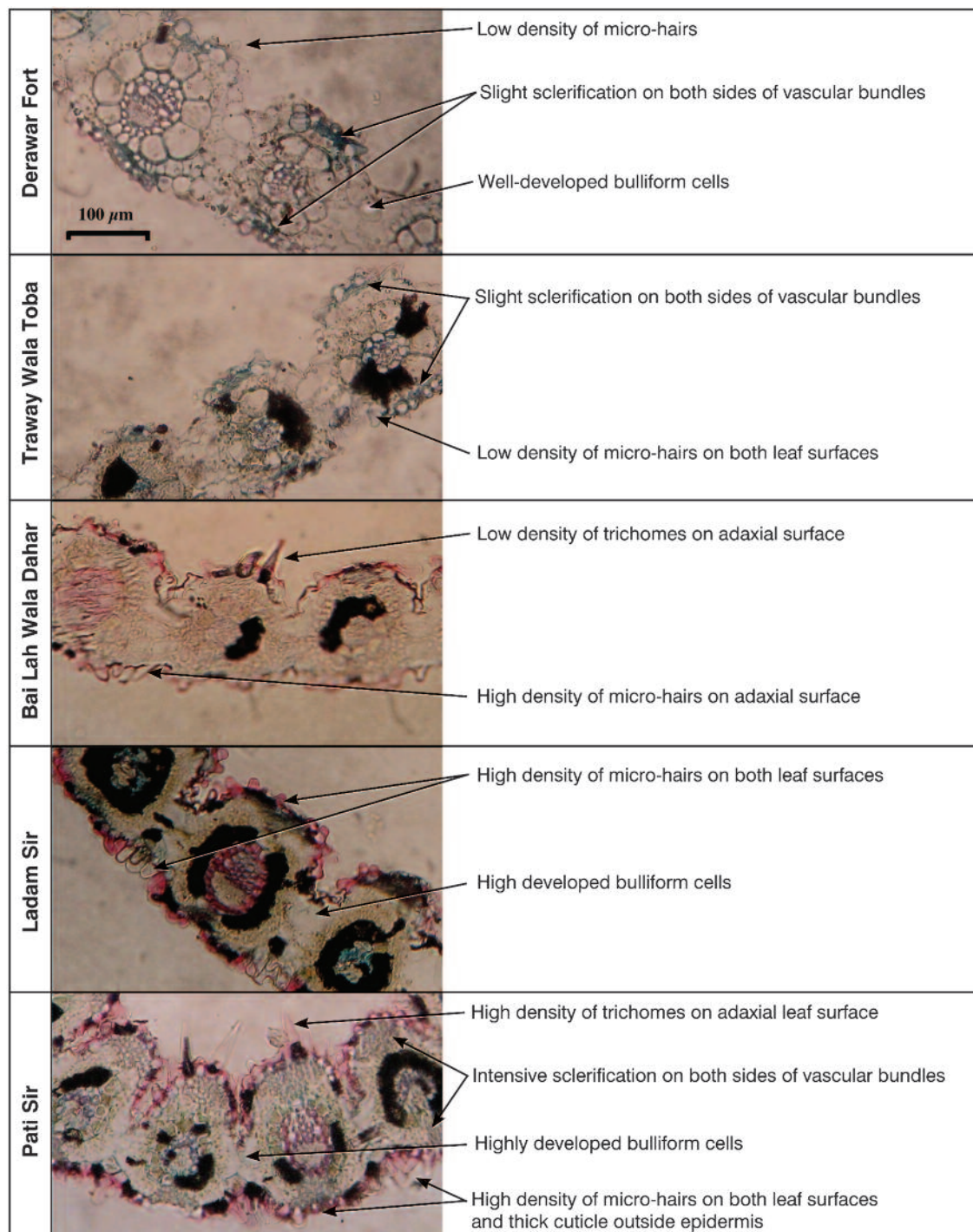


FIG. 7. Cross sections of *Aeluropus lagopoides* leaves from different habitats in the Cholistan Desert.

species (Horie *et al.*, 2012). *Aeluropus lagopoides* is an indicator species of highly saline soils, where it thrives well and utilizes Na<sup>+</sup> for many physiological processes (Pujol *et al.*, 2001; Shabala & Mackay, 2011). Longer and well proliferated roots under high salinities may provide the additional benefit to this species of extracting moisture from deeper layers under physiological drought, a common phenomenon in plants subjected to limited moisture availability (Liu *et al.*, 2004).

At the root level, *A. lagopoides* also displayed specific anatomical modifications to enable its growth and survival under high saline conditions such as sclerification in outer cortical (hypodermis) and pith region, increased endodermal thickness, and aerenchyma formation. Restricted uptake of solutes through the roots may be the first step of salt tolerance mechanisms (Bernstein & Kafkafi, 2002). Roots are directly exposed to saline environments, and enhanced compactness of the epidermal layer along a salinity gradient may play a vital role in solute translocation inside the plant body (Rashid & Ahmed, 2011). However, increased size of root hairs can provide more access by the roots to water (Neumann, 1995; Koyro, 1997), which is important in case of limited water availability under physiological drought.

The second important step of salt tolerance mechanisms is the resistance to radial flow of water in roots from epidermis to vascular tissue. Water and solutes adopt the radial pathway for their translocation through cortex to xylem (Miyamoto *et al.*, 2001; Horie *et al.*, 2012). Formation and development of sclerenchyma in the outer cortex, and outside the endodermis is critical not only for controlling movement of water and solutes, but also water loss through roots (Hose *et al.*, 2001), thus conserving water, avoiding desiccation and providing mechanical strength under extreme aridity (Balsamo *et al.*, 2006). This increased sclerification has been reported to be a characteristic feature of most salinity tolerant species including *A. lagopoides* (Schreiber *et al.*, 1999; Hameed *et al.*, 2012). Aerenchyma may serve as an additional barrier to water and solute movement, because it can create cavitation in the cortical region, and hence, an interruption in the connection between different tissue systems (Ranathunge *et al.*, 2003). Well-developed endodermis under high salinities may also be critical for radial flow of water and nutrients (Peng *et al.*, 2004).

Increased root cross-section area, which mainly resulted from increased cortical thickness in this stu-

dy, may provide the root with additional capacity to store water which is vital under limited moisture environments (Abdel & Al-Rawi, 2011). Reduced metaxylem cross-section area, particularly under high salinities, is another critical adaptation, as narrower vessels are less prone to damage caused by embolism (Kondoh *et al.*, 2006).

The major impact of salts on the stem was the disintegration of the epidermis and outer cortical region. Increased sclerification in the outermost region with increasing salinity of the habitat may be critical to avoid water loss from the stem, particularly under salinity-induced physiological drought. Increased sclerification in stems under salt stress has earlier been reported in *Spartina alterniflora* (Walsh, 1990), *Prosopis strombulifera* (Reinoso *et al.*, 2004), and *Imperata cylindrica* (Hameed *et al.*, 2009).

Furthermore, a well developed endodermis-like layer near the stem periphery in plants growing at high salinity may be an important adaptation. Endodermis is a feature of root anatomy that not only provides mechanical strength to the root, but also prevents radial flow of water and nutrients through the root tissue (Enstone *et al.*, 2003). This layer in the stem may be a unique feature, which has not been previously reported in any other grass species. This layer may also protect the stem from other environmental hazards as the outer layers (epidermis and cortex) were found to have disintegrated in *A. lagopoides* plants from all habitats.

Another crucial modification of stem under high salinity was increased vascular bundle area in different ecotypes of *A. lagopoides*. Larger vascular tissue (metaxylem, protoxylem, phloem and sieve areas), particularly under limited water availability, is certainly responsible for better transport of water and nutrients as well as photosynthesis (Awasthi & Pathak, 1999).

There was a negative impact of high salinity on stem cross-section area. Salinity stress may retard tissue development at early growth stages, and ultimately the reduction in stem cross-section area is very much understandable (Boughalleb *et al.*, 2009). Cortical cell area, on the other hand, was higher at moderate salinities than in the less saline habitats. Parenchymatous cells comprises the main storage tissue in stems (Scholz *et al.*, 2007), and the larger cells can store water more efficiently, which is critically important for plants to thrive well under harsh dry habitats.

In leaf anatomy, increase in epidermis thickness (along with thick cuticle) under high salinities may be

a significant adaptation, especially under physiological drought condition (Barhoumi et al., 2007a). This is a characteristic feature of most desert plants (Jianjing et al., 2012). In addition, increased development of bulliform cells in *A. lagopoides* can play a significant role in water conservation by reducing water loss through leaf surfaces because these cells facilitate considerable leaf rolling under physiological drought (Alvarez et al., 2008). Such modification is believed to minimize transpirational water loss through the leaf surface (Vasellati et al., 2001). Thick epidermis with a dense cuticle is a characteristic feature of most desert plants (Jianjing et al., 2012), and an epidermis with large bulliform cells can be considered as an important adaptation to salinity-induced physiological drought.

Metaxylem area was not affected by high salinity in this grass but protoxylem area increased significantly, which might have promoted water and solute conduction through the leaves. This way, excess salts can move to the salt excretory structures (see below) and ultimately extrude out of the plant body (Barhoumi et al., 2007b; Labidi et al., 2010). *Aeluropus lagopoides* has specific salt hairs (micro-hairs) which consist of two cells; the outer excretes salts out of the plant body (Salama et al., 1999). The density of micro-hairs increased enormously on both adaxial and abaxial leaf surfaces with increase in salinity level. Since this species relies greatly on exclusion of toxic salts, increased micro-hairs at high salinities may thus play a crucial role in its adaptability and successful survival under harsh environments (Naz et al., 2009). Moreover, increased density of trichomes (non-excretory epidermal appendages) is also critical for checking excessive water loss through the leaf surface (Dolatabadian et al., 2011), which is especially important under hot arid and saline desert environments, as the trichomes are involved in the control of radiation, thereby maintaining leaf temperature. Moreover, they protect leaves from dry hot environments by developing a humid air layer between the leaf surface and the atmosphere (Khokhar et al., 2012).

Overall, the plants of this species growing at the highest salinities showed very specific structural adaptations, which are important not only for reducing water loss from the plant surface, but also for restoring water under extreme physiological drought. At the root level, increased sclerification near the root periphery (exodermis) and just above the endodermis is critical in preventing water loss. At the stem level, development of an endodermis-like layer in addition

to increased sclerification might be critical, as this species has to face multiple stresses including high salinity, temperature, and drought. At the leaf level, development of bulliform cells, extensive formation of micro-hairs and trichomes, and intensive sclerification are vital for salt excretion and water conservation in this species. Prevention of water loss under such conditions might explain the successful growth and survival of this ecotype under such harsh conditions. In conclusion, *A. lagopoides* has developed important structural modifications for salinity and/or drought tolerance during its evolution. These modifications are ecotype-specific and enable the respective ecotypes to thrive under a variety of salt-prone habitats.

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