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ORIGINAL ARTICLE

Nargis Naz · Riffat Batool · Sana Fatima
Mansoor Hameed · Muhammad Ashraf
Farooq Ahmad · Muhammad Sajid Aqeel Ahmad

Adaptive components of tolerance to salinity in a saline desert grass *Lasiurus scindicus* Henrard

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Abstract Five differently adapted natural populations of the native salt desert grass *Lasiurus scindicus* Henrard from Lesser Cholistan Desert (Pakistan) in South Punjab of east central Pakistan, were evaluated to examine their mechanism of adaptation to saline stress based on some key morpho–anatomical and physiological characteristics. Five ecotypes were collected from one saline site, two moderately saline sites, and two highly saline sites. Anatomical adaptations in each ecotype critically supported the physiological, but the adaptations were of specific nature depending on the type of each site's normal habitat conditions. Higher salinities resulted in increased Na^+, Cl^-, Mg^{2+}, Ca^{2+} and K^+ content in root and shoot. At root level, some specific structural modifications like increased sclerification in cortical and pith regions, endodermal thickness, and number and size of xylem vessels are vital for water conservation under osmotic stress. Several characteristics were promising for increasing the plants ability to deal with osmotic stress, including at the stem level, increased sclerification, stem area, cortical region thickness and vascular bundle area, and at the leaf level, significant structural modifications such as leaf thickness, epidermal thickness, sclerenchymatous area, cortical area, metaxylem area and bulliform cell area were promising. All these may contribute towards water conservation, which ultimately account for ecotype survival under saline–induced physiological droughts.

Keywords Bulliform cell · Desert halophyte · Ion content · Salt excretion · Sclerification · Structural adaptation

Introduction

World agriculture is severely affected by abiotic stresses, the most important are salinity, drought, heat and cold, may reduce yield by more than 50 % or more for many crop plants (Wang et al. 2003; Öztürk et al. 2006; Ashraf et al. 2009, 2012; Mittler and Blumwald 2010; Hakeem et al.2013). Salinization of water and soil is among the environmental hazards that severely reduce the agricultural production all over the world (Maqsood et al. 2013). High soil salt content affects plant growth by imposing physiological drought, ion toxicity and oxidative stress (Chen et al. 2007; Cuin and Shabala 2007) which ultimately modify plant morphology, anatomy and physiology. The plant's response to external stress is complex involving not only morpho-anatomical changes but also internal metabolic adjustments that enable the plant to survive under adverse conditions (Demmig-Adams et al. 2008). The increase in population pressure along with severe climatic changes sternly affects the crop productivity and as a result, the development of tolerant varieties is the first priority of scientist (Newton et al. 2011; Lobell et al. 2011).

Highly complex mechanisms are involved in plant responses to multiple stresses. These mechanisms enable the plant to detect and respond to hazardous environmental constraints in order to minimize their harmful effects (Cramer et al. 2011; Atkinson and Urwin 2012). Determental environmental hazards like water and soil salinization severely reduce the agricultural production all over the world (Jouyban 2012).

The plants inhabiting desert environments subjected to vagaries of nature like water, heat and salt stress. These

N. Naz
Department of Life Sciences, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

R. Batool
Department of Botany, Government College Women University, Faisalabad 38040, Pakistan

S. Fatima · M. Hameed (✉) · F. Ahmad · M. S. A. Ahmad
Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan
E-mail: hameedmansoor@yahoo.com
Tel.: +92-321-6645726
Fax: +92-41-9200764

M. Ashraf
Muhammad Nawaz Shareef University of Agriculture, Multan 60000, Pakistan

plants have developed specific features in their morphology and anatomy which help them to thrive under adverse conditions (Naz et al. 2009; Hameed et al. 2011). *Lasiurus scindicus* is an inhabitant of saline patches in the Cholistan Desert, and a highly nutritive and palatable grass. It dominates the vegetation of sandy habitat that receives low rainfall, however, height and cover may decrease significantly along the increasing salinity gradient (Naz et al. 2010a, 2010b). As this species is native to xero-halophytic conditions by growing there for a long period of time, so must exhibit the tremendous tolerance to abiotic stresses, therefore, the present study was focused on examination of structural and functional aspects of adaptation of this grass to habitats differing in salinity stress.

Materials and methods

Study area

The Cholistan Desert in Pakistan spreads over about 26,000 km^2 in the South Punjab. Huge landmass of the Cholistan desert comprises three distinct ecological zones, inter-dune flats, sand dunes and salt-affected flats. Hard and compact clayey flats (dahars) inter-spread among loose sand dunes. Soil of dahars varies in soil phyco-chemical characteristics, in particular, texture and salinity. Soils of the Cholistan desert are very poor, lacking organic matter <1.0 %. A single dunes may reach to average height of over 100 m or so. The annual rainfall varies from 100 to 200 mm, mean temperature 6.6 °C (December–January) 46 °C (June), with absolute maximum up to 52 °C. The relative humidity varies from 35 to 65 %. Rain water is stored in temporary ponds that evaporates very quickly. Underground water is generally brackish, and relatively deeper about 40 m or so below the soil surface (Arshad et al. 2008).

Plant material

Lasiurus scindicus is a rhizomatous, tussock-forming grass that can reach to 1.5 m in height (Fig. 1). It is native to drier regions of Asia and Africa (Cope 1982), and shows better growth under low rainfall as in the Cholistan Desert (El-Keblawy et al. 2009). This grass is relished by domestic livestock and local wildlife, such as nilgai, chinkara and blackbuck (Assaeed 1997). This species of grass, a major component of the vegetation of the flat plains in the desert, is more palatable than any other native species of the Cholistan Desert, and also stabilizes loose sand dunes (Khan et al. 1999).

Data collection and analysis

Five saline habitats were selected from the Cholistan desert for the present study. The selection was made on the basis of specific halophytic plants inhabiting at that site as well as on specific features of the soil including soil type, soil colour, and moisture content (Table 1). Five ecotypes were collected as follows from: a low saline site, named Derawar Fort (DF); two moderately saline sites, Trawaywala Toba (TT) and Bailahwala Dahar (BD); and two highly saline sites, Ladam Sir (LS) and Pati Sir (PS).

Soil analysis

Soil sampling was conducted at 15 and 24 cm depth in the rhizosphere because these depths have the maximum root mass. 200 g of dried soil was used to measure the saturation percentage, pH and electrical conductivity (ECe). ECe and pH was recorded on pH/EC meter (WTW series InoLab pH/Cond 720, California, USA). Other soil analysis were carried out in lines of the procedure adopted by USDA Laboratory Staff (1954). Soil Na^+, K^+, and Ca^{2+} were estimated with a flame photometer (PFP–7, Jenway, Essex, UK). Serial grades of 10–100 mg L^{-1} standard solutions were prepared for each cation. These standard solutions were used to draw a standard curve for each element. The optical densities of cations were estimated on flame photometer and total concentration of each cation in each soil sample was calculated. Cl^- content was recorded on a digital chloride meter (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

Morpho-anatomical studies

Data for morphological characteristics like root and shoot length, leaf area, leaf hairiness and plant fresh and dry weights were recorded from fully mature plants. For the anatomical studies, a 2 cm piece was taken from fully expanded largest leaf for leaf anatomy, from the 3rd internode of the largest tiller for stem anatomy, and the thickest adventitious root near the root/shoot junction for root anatomy. All adventitious root are almost of equal thickness, so we selected the thickest among them to have a consistency. Formalin–acetic–alcohol solution was used as a fixative that includes v/v 35 % distilled water, 50 % ethanol, 10 % acetic acid and 5 % formalin. The material was then transferred to acetic alcohol preservative solution (v/v 25 % acetic acid and 75 % ethanol). Free-hand sectioning technique was used for the preparation of permanent slides. A serial grades of ethanol were used for dehydration of the material. For staining, safranin was used for lignified tissue and fast green for parenchymatous cells. Anatomical data were recorded on a light microscope (Nikon SE, Anti–Mould, Tokyo, Japan) and ocular and stage micrometers were used for calibration of ocular micrometer. Micrographs of the transverse sections were taken with a digital camera (Nikon FDX-35) on a stereo–microscope (Nikon 104, Japan).

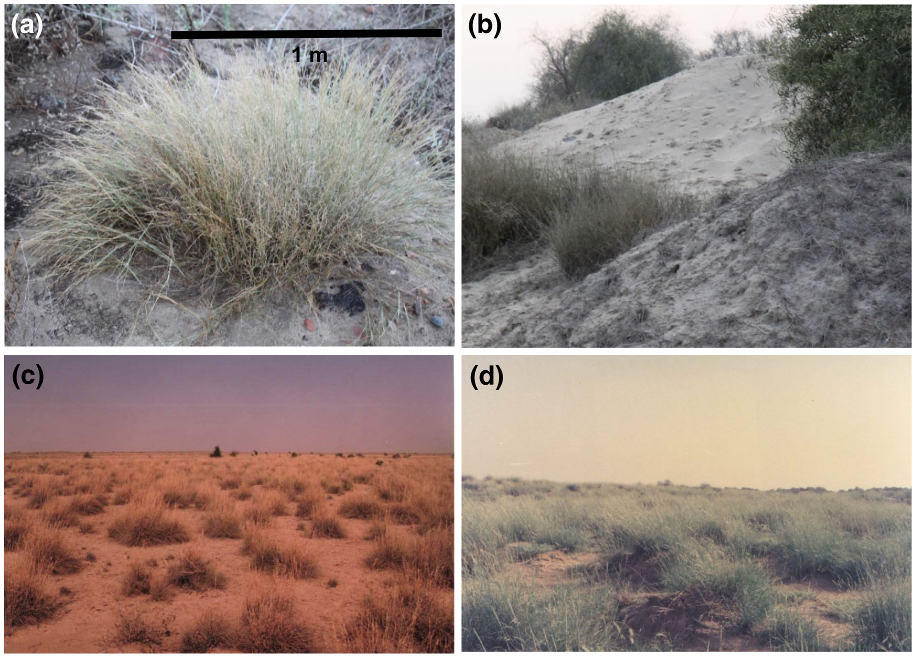

Fig. 1 Habitat ecology of *Lasiurus scindicus* in the Cholistan desert. **a** *Lasiurus scindicus* a highly palatable, nutritious, dominant, drought tolerant grass of the area. It is found almost everywhere, particularly, on sand dunes and interdunal sandy plain areas. **b** Sand dune vegetation dominated by *L. scindicus* along with *Ochthochloa compressa*. **c** Pure lush green community of *L. scindicus* after plenty of monsoon rainfall. In some areas it has pure communities spread over kilometers. **d** Pure community of dry *L. scindicus* in the Cholistan Desert

Table 1 Soil physico-chemical characteristics of habitats of different ecotypes of *Lasiurus scindicus* in the Cholistan Desert

Characteristics	Derawar fort	Trawaywala Toba	Bailahwala Dahar	Ladam Sir	Pati Sir
Coordinates	29º 24′ 32.67″N 71º 27′ 32.34″E	29º 10′ 27.65″N 71º 09′ 21.57″E	29º 38′ 19.43″N 70º 93′ 23.37″E	30º 53′ 26.53″N 72º 64′ 24.62″E	30º 35′ 17.58″ N 72º 63′ 22.45″ E
Associated community	*Sporobolus ioclados -Cymbopogon jwarancusa*	*Sporobolus ioclados -Aeluropus lagopoides*	*Sporobolus ioclados -Haloxylon stocksii*	*Sporobolus ioclados -Ochthochloa compressa*	*Sporobolus ioclados -Suaeda vera*
Relative cover	14.06d	5.89c	5.12c	2.35b	1.21a
Relative frequency	3.16a	6.75d	7.07e	4.37c	3.57b
Relative density	1.05a	4.84e	4.11d	1.79c	1.26b
pH	8.40a	8.34a	8.42a	8.28a	8.31a
ECe	17.60a	24.81b	28.53c	36.36d	46.59e
Na^+ (mg L^{-1})	3801.50a	4381.51b	4501.53c	4920.85d	5221.32e
K^+ (mg L^{-1})	403.64d	388.18c	351.46b	282.17a	286.05a
Ca^{2+} (mg L^{-1})	65.91b	64.97b	63.16b	57.62a	58.88a
Cl^- (mg L^{-1})	1580.28a	2230.54b	2403.37c	2510.28d	2680.33e

Means with same letters in each row are statistically non-significant ($n = 10$)
ECe electric conductivity of an soil extract

Physiological parameters

Analysis of inorganic elements

Concentrated sulphuric acid (H_2SO_4) was added to dried ground material (0.5 g) in digestion tubes (size 10 mL), incubated overnight at room temperature. 35 % hydrogen peroxide (H_2O_2) was then slowly added to digestion tube, and the tubes were ported in a digestion block system (KJELDATHERM basic, C. Gerhardt GmbH & Co. KG, Bonn, Germany) and heated at 350 °C. The digestion tubes were removed from the block after 30 min and then allowed to cool at room temperature. Hydrogen peroxide was then again added the tubes were placed back into the block. The procedure was repeated till the solution turned colourless. The extract was filtered and used for determining K^+, Ca^{2+}, Na^+ Cl^- and Mg^{2+} concentrations. Na^+ and K^+ with recorded a flame photometer (Model 410, Sherwood Scientific Ltd., Cambridge, UK), Ca^{2+} and Mg^{2+} on an atomic absorption spectrophotometer (Model Analyst 3000; Perkin Elmer, Norwalk, CT), and Cl^- with a chloride meter (Model 926; Sherwood Scientific Ltd., Cambridge, UK).

Ion washing

The same leaf (that was used for ionic concentration) was used for the determination of excreted ions, which are deposited on leaf surface via salt hairs and glands. The leaves were immediately washed in 20 ml of deionised distilled water. The excreted ions were recorded from the wash.

Statistical analysis

The experiment was designed in a completely randomized design with ten replications. The data were subjected to analysis of variance, and the mean values were compared by the least significance difference test. Standard error was calculated for each mean value.

Results

Soil analysis

Soil textural classes, saturation percentage, moisture contents, organic matter, soil pH, ECe, and soil Na^+, K^+, Ca^{2+}, and Cl^- contents were determined (Table 1). The degree of salinity of the sites could be ranked as: least (DF) < moderately (TT < BD) < highly salt–affected (LS < PS).

Morpho–physiological parameters

Shoot growth (measured by growth in leagth) of *L. scindicus* was the highest at moderately saline habitats, Trawaywala Toba (TT) and Bailahwala Dahar (BD). Shoot length significantly decreased at highly saline sites Ladam Sir (LS) and Pati Sir (PS); however, the plants inhabiting the least saline Derawar Fort (DF) were smaller in height as compared to those from moderately saline sites. Root length, in contrast, gradually and consistently decreased with increase in salinity level of the habitat, but the difference between the two moderately saline sites TT and BD was not significant (Table 2).

Flag leaf area and total leaf area per plant were considerably high in plants inhabiting moderately saline TT, which were about two-fold greater than those recorded in plants from the other habitats (Table 2). Plants from the highest saline PS showed the minimum value of these two characteristics. Leaf area of the plants from moderately saline BD was relatively higher than that from the least saline DF.

Shoot and root Na^+ content gradually increased in plants as salinity increased to moderately saline BD, but the differences were non-significant (Table 2). However, higher salinities at LS and PS showed a significant increase in this characteristics. The maximum of this parameter was recorded in the plants from highly saline PS. There was a gradual and significant increase in K^+ content of shoot and root with increase in salinity level.

Table 2 Morpho-physiological characteristics of *Lasiurus scindicus* from various habitats in Cholistan desert

Characteristics	Derawar Fort	Trawaywala Toba	Bailahwala Dahar	Ladam Sir	Pati Sir
Morphology					
Shoot length (cm)	76.54b	82.07c	82.03c	78.70b	69.66a
Root length (cm)	139.84d	133.00c	133.93c	126.82b	122.33a
Flag leaf area (cm^2)	4.25a	8.71c	4.91b	4.95b	4.15a
Total leaf area $plant^{-1}$ (cm^2)	48.73b	78.92e	54.57d	51.16c	44.39a
Shoot ionic content					
Shoot Na^+ (mg g^{-1})	5.17a	5.10a	5.57a	6.50b	7.17c
Shoot K^+ (mg g^{-1})	9.67a	16.53b	19.53c	33.97e	24.97d
Shoot Ca^{2+} (mg g^{-1})	0.90b	0.97c	0.93bc	0.87b	0.70a
Shoot Cl^- (mg g^{-1})	8.43a	8.97b	9.53c	9.70c	10.37d
Shoot Mg^{2+} (mg g^{-1})	0.48a	0.50a	0.53a	0.57a	0.51a
Shoot PO_4^{3-} (mg g^{-1})	451.67e	302.33d	251.67c	228.00b	161.33a
Root ionic content					
Root Na^+ (mg g^{-1})	5.13a	5.37a	5.73a	8.77b	14.83c
Root K^+ (mg g^{-1})	5.67a	10.47b	19.40c	28.27e	21.73d
Root Ca^{2+} (mg g^{-1})	0.23a	0.27a	0.40b	0.43b	0.73c
Root Cl^- (mg g^{-1})	8.13a	8.27a	8.77a	10.80b	13.50c
Root Mg^{2+} (mg g^{-1})	0.29a	0.33a	0.39a	0.59b	0.33a
Root PO_4^{3-} (mg g^{-1})	320.33d	503.00e	130.00c	114.00b	98.33a
Excreted ions					
Na^+ (mg L^{-1})	12.47a	17.21b	17.93b	20.22b	28.73c
K^+ (mg L^{-1})	10.34b	11.51c	8.29a	7.83a	7.68a
Ca^{2+} (mg L^{-1})	9.03a	9.82a	15.95b	33.44d	18.96c
Cl^- (mg L^{-1})	6.35a	8.06b	9.72b	14.13c	18.92d
Mg^{2+} (mg L^{-1})	0.34a	0.35a	0.37a	0.38a	0.39a

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

Shoot Ca^{2+} was the maximum in plants from moderately saline TT, but further increase in salinity level of the habitats showed a gradual decrease in this parameter. In contrast, root Ca^{2+} showed a consistent increase with increase in salinity level, the plants from highly saline PS showed a significantly increased accumulation of Ca^{2+} in roots than plants from all other habitats.

Shoot and root Mg^{2+} gradually increased with increasing salinity levels of the habitat, but decreased at the highest level of PS (Table 2). However, the difference among the different habitats with respect to shoot and root Mg^{2+} was not significant. A significant decrease was noticed in shoot with increase in salinity levels, whereas, root was maximum at moderate saline TT, and thereafter significantly decreased with increasing salinity levels of the habitats.

A consistent and significant increase in excreted Na^{+} and Cl^{-} was recorded in *L. sindicus* ecotypes with increasing salinity levels of these habitats (Table 2). Excreted Ca^{2+} also showed a significant increase, but up to high salinity of LS. This parameter decreased significantly at the highest saline site PS. An increase in excreted Mg^{2+} was also recorded, but the differences among different ecotypes were not significant. Excreted K^{+} was the maximum at moderately saline TT, but higher salinity level of the habitats resulted in a gradual but non-significant decrease.

Anatomical parameters

Root anatomy

Root cross-section area was significantly higher at moderately saline TT, but a decrease was recorded along with salinity levels. Tissues outside the endodermis were disintegrated in the ecotype from moderately saline BD and highly saline LS and PS. Length of root hairs was significantly higher at moderately saline TT as compared to that at the least saline DF (Table 3). A similar trend was seen in the case of cortical cell area and thickness of epidermal and sclerenchymatous region, but the difference was not significant in the case of cortical region thickness. All these parameters were not possible to record in the ecotypes from BD, LS, and PS due to disintegration of tissues.

A gradual and significant increase in endodermal cell area was recorded with increasing levels of salinity in the habitats (Table 3). Pericyle thickness, however, increased up to moderately saline BD. A further increase in salinity level of the habitat depicted a significant decrease.

Metaxylem vessel number in ecotypes increased significantly along with salinity level of the habitats, but up to highly saline LS (Table 3). Plants inhabiting the highest saline PS showed a significant decrease in this parameter. Area of metaxylem gradually and significantly increased with increasing salinity levels. Phloem area showed a significant and consistent increase with increase in salinity level of the habitats, but up to high saline LS. A significant decrease in this attribute was noted in plants inhabiting the highest saline PS.

A gradual increase was recorded in pith area as the salinity level of the habitat increased, but the differences among the moderately saline BD and highly saline LS and PS were not significant. Pith cell area increased with increasing salinity level of the habitat up to moderately saline BD. Plants inhabiting the highest saline PS site showed significantly reduced cells of pith parenchyma (Table 3).

Stem anatomy

A significant and consistent increase was recorded in the stem cross-section area as the salinity of various habitats increased (Table 3). The ecotypes inhabiting highly saline sites (LS and PS) showed considerably thicker stem than those from the other sites. Cuticle thickness did not alter at lower saline DF and moderately saline TT and BD, but it increased significantly at higher salinities.

Epidermal thickness was the maximum in plants inhabiting moderately saline TT, but the differences in this parameter among plants from various habitats were not significant (Table 3). Sclerencyma was not observed in the plants from lower salinities (DF and TT). However, thickness of the sclerenchymatous tissue was significantly higher in the plants from higher salinities (LS and PS) than that recorded in those inhabiting moderately saline BD habitat.

A significant increase in cortical region thickness was observed as the salinity level of the habitat increased (Table 3). However, quite an opposite trend was recorded in the cellular area of cortical cells, wherein a significant decrease along the increasing salinity gradient was recorded.

Vascular bundle area increased significantly, but the phloem area decreased as the salinity level of the habitats increased. Metaxylem vessel area, however, increased up to moderately saline BD, but thereafter a significant decrease was recorded with a further increase in salinity level (Table 3).

Leaf anatomy

An increase in leaf (midrib and lamina) thickness was recorded with increasing salinity levels of the habitats, but the differences among the different ecotypes were not significant, particularly in those inhabiting highly saline habitats (Table 3). A significant increase in epidermal thickness at both leaf surfaces was recorded with increase in salinity gradient of different habitats. A significant increase in sclerenchymatous area and cortical region thickness was also recorded as the salt level increased. A similar trend was recorded in the case of cellular area of cortical parenchyma, but the plants inhabiting the highest saline PS showed a slight but non–significant decrease.

Table 3 Anatomical characteristics of *Lasiurus scindicus* from various habitats in Cholistan desert

Characteristics	Derawar Fort	Trawaywala Toba	Bailahwala Dahar	Ladam Sir	Pati Sir
Root anatomy					
Root cross-sectional area (μm^2)	1.02d	1.28e	0.70c	0.63b	0.55a
Root hair length (μm)	634.07b	1498.71c			
Epidermal thickness (μm)	24.96b	27.73c			
Sclerenchymatous thickness (μm)	31.89b	59.63c			
Cortical thickness (μm)	142.83b	146.99b			
Cortical cell area (μm^2)	979.00b	1890.02c			
Endodermal cell area (μm)	181.30a	217.56b	258.35c	285.54d	421.51e
Pericycle thickness (μm)	8.32a	9.71a	15.25c	13.87bc	12.48b
Metaxylem vessels number	9.63a	12.35b	13.21bc	14.73c	12.98b
Metaxylem area (μm^2)	1803.90a	3984.00b	5674.59c	5389.05d	7614.46e
Phloem area (μm^2)	648.14a	947.28b	1006.20c	1201.09d	979.00bc
Pith area (μm^2)	0.12a	0.18b	0.25c	0.26c	0.28c
Pith cell area (μm^2)	104.63b	144.15c	174.39d	169.76d	87.20a
Stem anatomy					
Stem cross-sectional area (mm^2)	1.25a	1.33b	1.43c	2.23d	2.47e
Cutical thickness (μm)	4.16a	4.16a	4.16a	6.23b	8.32c
Epidermal thickness (μm)	19.41a	21.09a	18.32a	18.32a	18.32a
Sclerenchymatous thickness (μm)	0.00a	0.00a	41.60b	70.72c	72.11c
Cortical thickness (μm)	854.08a	919.36b	935.68b	1191.36c	1370.88d
Cortical cell area (μm^2)	91799.35e	60129.97d	37249.89c	36970.86b	26298.14a
Vascular bundle area (μm^2)	14683.71a	16061.40b	19427.14c	19880.56d	20857.15e
Metaxylem area (μm^2)	534.83c	892.89d	1269.08e	326.33b	281.01a
Phloem area (μm^2)	1477.57e	1096.85d	697.99c	657.20b	607.34a
Leaf anatomy					
Midrib thickness (μm)	149.60a	157.76a	198.56b	195.84b	201.28b
Lamina thickness (μm)	59.84a	97.92b	100.64b	111.52b	114.24b
Lower epidermis thickness (μm)	19.04a	24.48b	27.20c	29.92c	38.08d
Upper epidermis thickness (μm)	27.20a	27.20a	35.36b	35.36b	38.08c
Sclerenchymatous area (μm^2)	209.27a	244.15b	331.34c	470.86d	540.61e
Cortical thickness (μm)	282.88a	307.36a	334.56ab	345.44a	397.12b
Cortical cell area (μm^2)	1761.35a	2336.84ab	3854.04bc	5475.87c	5371.24bc
Mesophyll thickness (μm)	136.00c	92.48b	76.16a	76.16a	73.44a
Bundle sheath thickness (μm)	24.96c	22.19bc	19.41b	19.41b	15.25a
Vascular bundle area (μm^2)	2755.38a	4185.38a	4673.67a	7376.73b	5214.29a
Metaxylem area (μm^2)	575.49a	680.12c	732.44d	749.88e	610.37b
Phloem area (μm^2)	2267.08a	2546.11b	4970.14e	4133.06d	3522.70c
Bulliform cell area (μm^2)	1318.93a	1672.46b	1699.66b	2057.72c	2453.19d
Abaxial stomata number	33.67d	31.67c	19.33b	9.00a	8.33a
Adaxial stomata number	18.33bc	21.00b	31.00c	15.33b	11.33a
Abaxial stomatal area (μm^2)	7.93b	7.43b	9.18c	6.95b	5.06a
Adaxial stomatal area (μm^2)	12.02c	10.33b	8.40ab	7.39a	7.22a
Trichome length (μm)	2354.37e	1995.43d	1158.67c	793.91b	326.84a
Trichome number (mm^{-2})	15.32a	19.47b	21.51b	29.67c	47.44d

Means with same letters in each row are statistically non-significant ($n = 10$)
NR not recorded

A consistent decrease in bundle sheath thickness and mesophyll thickness was observed with increase in salinity level, however, the differences among different ecotypes were not significant with respect to these parameters (Table 3). A significant and sequential increase was recorded in vascular bundle area, metaxylem area, and phloem area with increase in salinity levels, but plants inhabiting the highest saline PS showed a significant decrease in these characteristics. Bulliform cell number significantly increased with increase in salinity gradient of the habitats.

Number of stomata on abaxial leaf surface significantly decreased with increase in salinity level of the habitats, but the differences between the ecotypes from the highly saline LS and PS were not significant (Table 3). In contrast, stomatal number on adaxial leaf surface increased up to moderately saline BD, but higher levels resulted in a significant decrease in this attribute. Stomatal area on the abaxial leaf surface, however, was the maximum at moderately saline BD, but it decreased significantly at higher salinities (LS and PS). Stomatal area on adaxial surface showed a consistent decrease along salinity gradient, but the differences among different ecotypes did not decrease significantly. However, the number of stomata increased as salinity level of the habitats increased. Plants inhabiting the highest saline PS had extremely reduced trichome size, but exceedingly high in density per mm^2 (Table 3).

Discussion

Differentially adapted populations of *L. scindicus* responded very specifically under habitat with varying salt levels in relation to structural and functional features. Since this species is moderately tolerant to salt stress (Naz et al. 2010a, 2010b), reduced growth, and particularly under higher salinities is beneficial for its survival (Table 2), because successful survival is more important than their vigorous growth (Hameed and Ashraf 2008).

Increasing salinity gradient of the habitats imparted specific morphological and anatomical changes, which indicates adaptability potential of this grass to cope with environmental hazards in the Cholistan desert (Hameed et al. 2013). Increasing salinity of the habitats hampered root growth in this species (Table 2), which is a characteristic feature of most plant species that have relatively less tolerance against salinity stress. A number of similar observations have earlier been reported by many researchers (Monteverdi et al. 2008; Munns and Tester 2008). Moderate salinities at Trawaywala Toba (TT) were more suitable for leaf development, as both flag leaf area and total leaf area per plant were the maximum in plants from this site. However, higher salinities had a negative impact on leaf area, as well as overall growth in

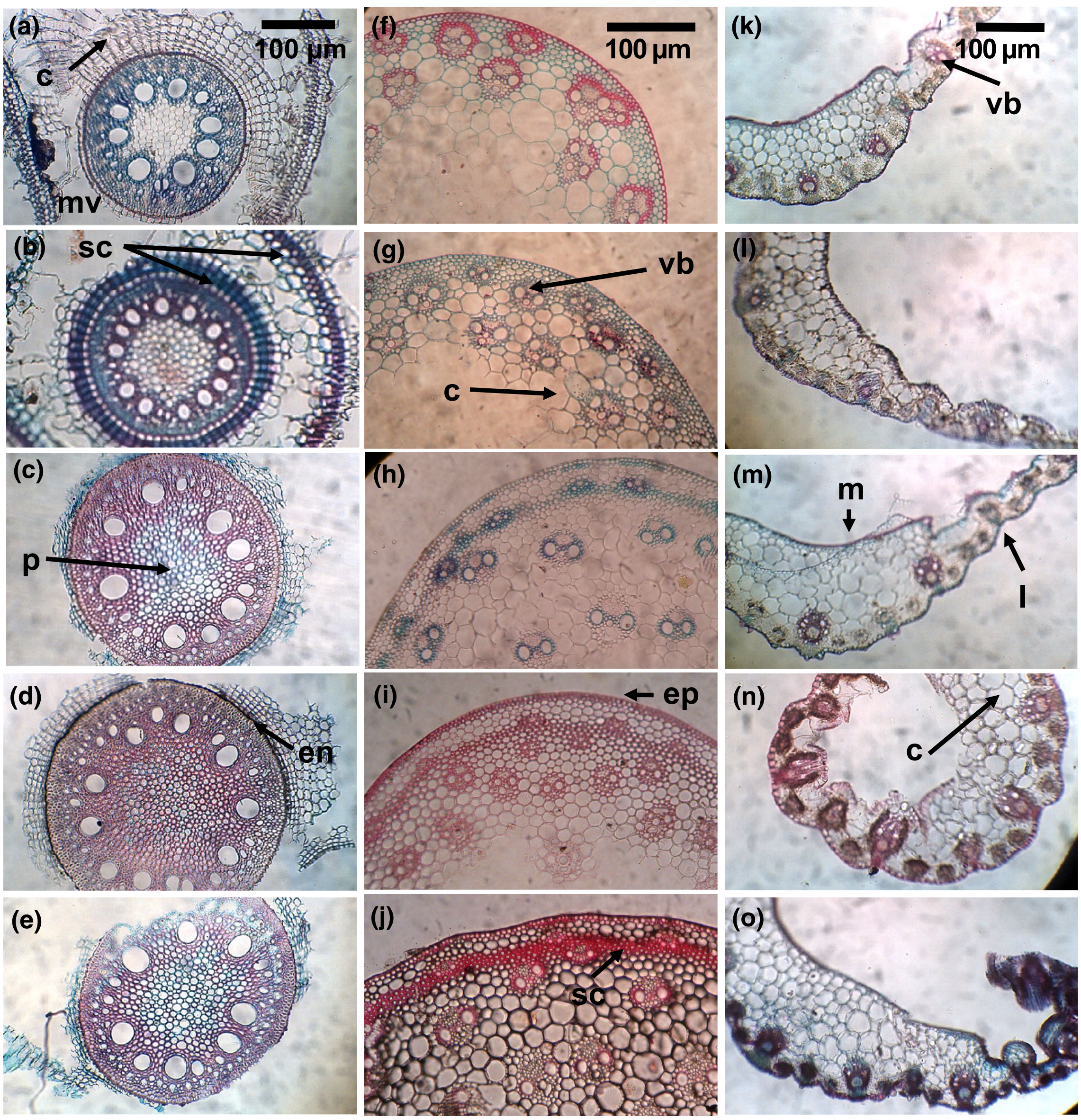

Fig. 2 Anatomical studies of Lasiurus scindicus ecotypeas from the Cholistan Desert. Root transverse sections. **a** Derawar Fort, **b** Trawaywala Toba, **c** Bailahwala Dahar, **d** Ladam Sir, and **e** Pati Sir, stem, **f** Derawar Fort, **g** Trawaywala Toba, **h** Bailahwala Dahar, **i** Ladam Sir, and **j** Pati Sir, and leaf, **k** Derawar Fort, **l** Trawaywala Toba, **m** Bailahwala Dahar, **n** Ladam Sir, and **o** Pati Sir (*c* cortex, *en* endodermis, *ep* epidermis, *l* leaf lamina, *m* leaf midrib, *mv* metaxylem vessels, *p* pith, *sc* sclerenchyma, *vb* vascular bundle)

L. scindicus. Alem et al. (2002) reported a similar decrease in leaf area in durum wheat in response to increased salinity.

As it is expected, concentration of toxic ions like Na^+ and Cl^- in roots and shoots increased as the salinity level of the habitat increased (Table 2), but at the same time, increased concentration of beneficial ions, in particular, that of K^+ and Ca^{2+}, may certainly contribute to high degree of salt tolerance in this species. Ashraf (2004) also reported high K^+:Na^+ and Ca^{2+}:Na^+ ration in salt tolerant grasses. At the same time, there was no visible effect of increasing salinity gradient on uptake of Mg^{2+}, but the concentration significantly decreased. Qian et al. (2001) in Kentucky bluegrass and Grieve et al. (2004) in wheatgrass also reported similar findings.

At the root level, *L. sindicus* showed some specific structural modifications like sclerification in cortical and pith region, which may enhance mechanical strength of root, critical for preventing root damage under harsh climates (Fig. 2). Root of *L. scondicus* are exposed to extreme unfavourable environmental conditions in the Cholistan Desert, where the soils is compact saline soil or saline-sodic. Parenchymatous tissues, in particular cortex and epidermis disintegrated as a result in three ecotypes from higher salinities. Increased endodermal thickness may control radial movement of water in roots (Table 3), and increased number and size of xylem vessels is vital for efficient conduction and translocation of water and other nutrients, and it may again contribute to its survival under harsh environments (Rashid and Ahmed 2011). The major impact of salts on roots was the disintegration of epidermis and outer cortical region, particularly at higher salinities. The survival of this species in such circumstances may depend on increased sclerification and endodermal thickness, which is critical for minimizing water loss through roots (Ahmad 1996).

At stem level, increased sclerification was one of the most prominent features in *L. scindicus* ecotypes (Fig. 2). Moreover, a significant increase in stem area, cortical region thickness and vascular bundle area along a salinity gradient may increase its potential for water conservation in terms of water loss from plant surface and increased storage ability (Table 3), both vital for the survival under limited moisture availability caused by osmotic stress. Increased sclerification in stem also been observed by several authors in the stem of different plant species, such as *Aeluropus lagopoides* (Hameed et al. 2013) and *Prosopis strombulifera* (Reinoso et al. 2004).

At leaf level, significant structural modifications in *L. sindicus* ecotypes included increased leaf thickness, epi-

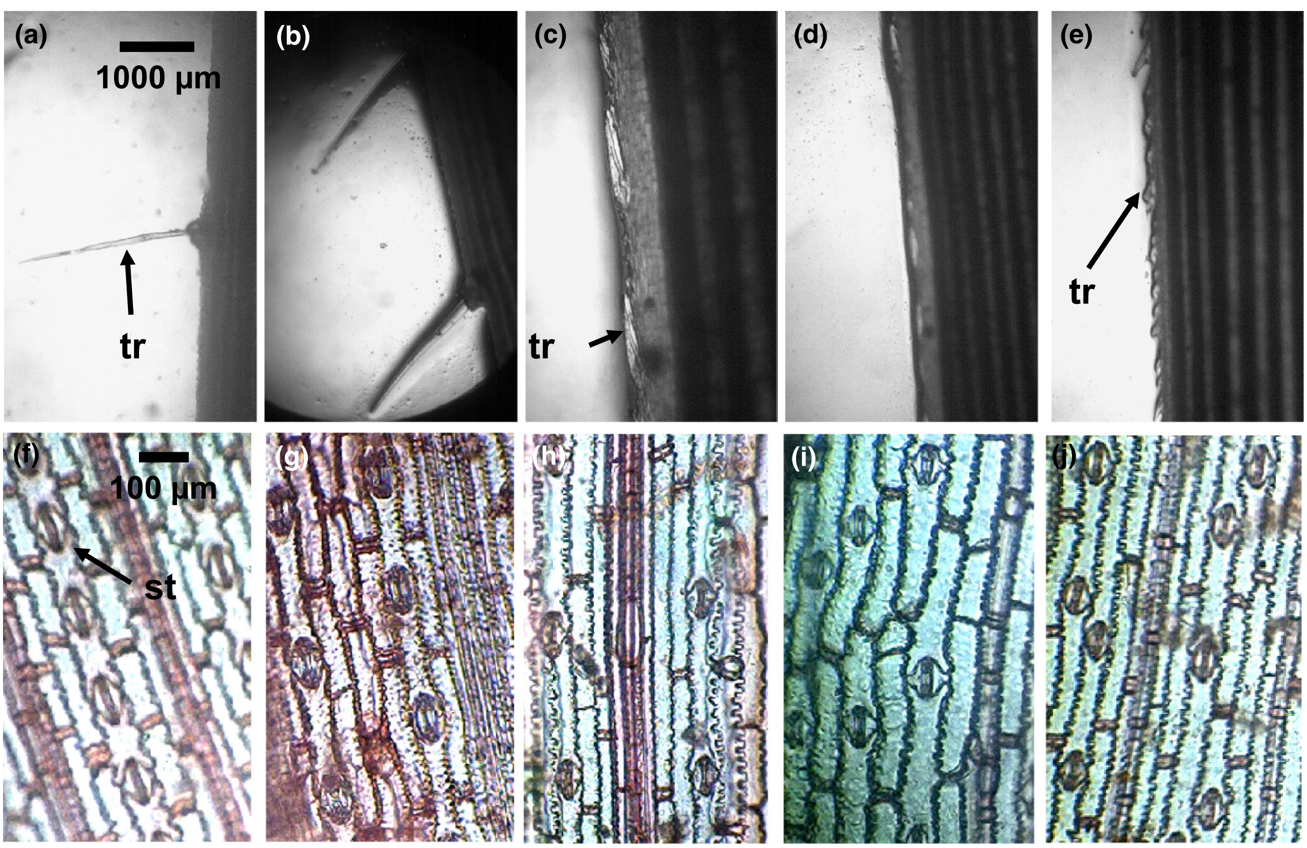

Fig. 3 Anatomical studies of Lasiurus scindicus ecotypeas from the Cholistan Desert. Leaf margin, **a** Derawar Fort, **b** Trawaywala Toba, **c** Bailahwala Dahar, **d** Ladam Sir, and **e** Pati Sir, and leaf surface view, **f** Derawar Fort, **g** Trawaywala Toba, **h** Bailahwala Dahar, **i** Ladam Sir (*tr* trichomes, *st* stomata)

dermal thickness, sclerenchymatous area, cortical area, metaxylem area and bulliform cell area (Fig. 2). All these may contribute to water conservation, which ultimately accounts for species survival under physiological droughts like salinity (Balsamo et al. 2006). Another distinctive feature is the reduction in stomatal density and area (Table 3), which can significantly control transpirational water loss from leaf surface (Fig. 3). This was further supported by increased trichome density on leaf margins (Abdel and Al-Rawi 2011).

Lasiurus scindicus is a salt excretory grass, which is capable of excreting excess of toxic ions. It can also accumulate toxic ions such as Na^+ and Cl^- in the shoot tissue to some extent (Gulzar et al. 2003; Arndt et al. 2004). At higher salinities, mechanism of salinity tolerance seemed to be shifted towards excretion, rather than dumping off the toxic ions in cell organelles and tissues, as also reported by Naz et al. (2009). The predominant ions secreted by plants are generally Na^+ and Cl^- with K^+, Ca^{2+} and Mg^{2+} in minor quantities (Munns 2002), as was also recprded in *L. scindicus* ecotypes. However, for Mg^{2+} and K^+ ions, excretion patterns at the studied sites were inconsistent. A decrease in Cl^- content under higher levels of salinity indicates the involvement of some other mechanisms to prevent the plant from excreting K^+ that hampers its growth under higher levels of salinity. A sharp increase in root and shoot K^+ and root Ca^{2+} seems to be involved in its survival under saline conditions.

The Na^+ is used in the metabolic processes in a number of halophytic species (Munns and Tester 2008). *Lasiurus scindicus* not only excreted excessive ions, but also accumulated them in the leaves independent of their habitats (Table 3). Retention of the physiologically advantageous ions like Ca^{2+} and K^+ in shoot is certainly useful for osmotic balance regulation, and this is critically important for the successful survival (Ashraf 2004).

Anatomical adaptations investigated in this grass critically support the physiological adaptations confirming its successful survival in extreme desert saline conditions. The highly saline LS population, showed increased sclerification in the vascular region, inside endodermis and in outer cortical region. Moreover, intensive sclerification was recorded in stem and midrib region of the leaf and leaf sheath. Highly developed bulliform cells were prominent in the leaf lamina. These modifications may give rigidity to root tissues and also significantly reduce water loss through plant surfaces (Alvarez et al. 2008). Salt accumulation is the prominent feature of this species to cope with high salinities.

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