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Phytoremediation of hypersaline soils by salt excretory C_4 halophytic pan dropseed grass (*Sporobolus ioclados* Nees ex Trin.) through alteration in foliar architecture

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Plants inhabiting saline areas develop specific morpho-anatomical and physiological features to survive. *Sporobolus ioclados* is among the few grass species that dominate highly saline habitats. This is a salt excretory species and can potentially be important for phytoremediation of salt-affected lands. Three ecotypes of *Sporobolus ioclados* (Trin.) Nees (DF-Derawar Fort (LSE), BD-Bailahwala Dahar (MSE), LS-Ladam Sir) from the Cholistan Desert were evaluated to investigate structural and functional modifications for salt tolerance under controlled conditions in hydroponic growth medium using half-strength Hoagland's nutrient solution. Three salinity (NaCl) treatments were provided, namely 0 (control), 150, and 300 mM. All three ecotypes showed different structural and physiological modifications under salinity stress. Structural and functional traits were more developed in the HSE. Modifications. Structural features include intensity of sclerification and thicker leaves. Functional features were high concentration of toxic ions excretion, organic osmolytes accumulation, and maintenance of leaf turgor, photosynthesis and water use efficiency. All these confer it an excellent material for the phytoremediation as well as revegetation of highly saline lands.

Keywords Ion excretion, Microhairs, Phytoremediation, Organic osmolytes, Sclerification

Salinity stress is a major threat to growth and biomass production of plants, particularly those colonizing arid and semiarid regions. Plants develop specific structural and functional features that are critical for survival therein[1]. Salt tolerance is a complex mechanism[2], therefore, salt tolerant (or halophytic) species are the model plants to explore adaptive traits. These traits can be incorporated in glycophytes for the enhancement of salt tolerance traits[3]

Halophytes develop very specific features to handle high salinity. Structural-based mechanisms include well-developed rooting system[4], salt-excreting microhairs[5], increased succulence[6], intensive sclerification[7], salt excretory glands[8], and stomatal size, density and orientation[9]. Functional features are restricted or selective ion uptake[10] and toxic ion excretion or compartmentalization[11,12]. More importantly, turgor maintenance by accumulating organic osmolytes[13]. High concentration of Na^+ and Cl^- in saline soils causes ionic imbalance

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in plants. This affects the uptake by root cells of several other metabolically active ions, which hampers several metabolic activities in different parts of plants i.e. leaf, stem and root[14].

There are about 250 species in the genus *Sporobolus*. In Pakistan, ten species are reported[15]. The genus *Sporobolus* has many species that are known for high degree of salinity tolerance. The examples are *Sporobolus airoides*[16], *S. spicatus* (Vahl) Kunth[17], *S. arabicus*[18], and *S. virginicus*[19]. *Sporobolus ioclados* (pan dropseed) dominates salt-affected inter-dune flats in the Cholistan Desert[20].

Halophytic C4 grasses, including *Sporobolus* species, have developed specific mechanisms to cope salinity stress. Among these, "salt glands" which are bicellular leaf epidermal structures eliminating excess saline ions from shoots by excretion under salinity stress[21]. It is a perennial, stoloniferous grass. This species is widely distributed in coastal areas and desert habitats[22]. It is a highly palatable grass that is often over-grazed and can form a major component of plant communities[23]. Plant features, specifically anatomical characteristics, are strongly influenced by environmental heterogeneity[24]. However, genetically fixed characteristics during the evolutionary history of a plant express themselves under controlled environments[25]. Since hydroponic system provides a uniform and homogeneous growth medium, so it is possible to study evolutionary fixed characteristics of *S. ioclados* ecotypes. This led us to hypothesize that ecotypes of *S. ioclados* might respond independently to salt stress. Mechanisms for tolerating high levels of salts may be different in these ecotypes. The present study was conducted to explore tolerance mechanism at structural and functional levels, to evaluate phytoremediation potential, and to correlate structural and functional features under different salinities. This species was previously evaluated for structural and functional features along salinity gradient from its natural habitats[20]. For the present study, *S, ioclados* was examined under controlled conditions to evaluate genetically fixed traits in differently adapted ecotypes. The present study is a part of same project in which two grasses, *Lasiurus scindicus*[26] and *Aeluropus lagopoides*[27] were evaluated under controlled conditions.

Materials and methods

Collection sites

Three ecotypes of *Sporobolus ioclados* (Trin.) Nees from the Cholistan Desert (Fig. 1) were evaluated to investigate structural and functional modifications. The least saline ecotype (LSE) was collected from Derawar Fort. Moderately saline ecotype (MSE) was from Bailahwala Dahar. The highest saline ecotype (HSE) was from Ladam Sir.

Experimentation

Vegetative buds from naturally growing plants of each ecotype of *S. ioclados* were collected and grown in Faisalabad condition for six months to acclimatize under local conditions. A total of 160 ramets (vegetative buds), each with three tillers of equal size, were detached. The ramets were planted in a hydroponic medium using half-strength Hoagland's solution following Hoagland and Arnon[28]. Plastic containers (capacity 25 L) were used for experimentation. The salt treatments were 0 (control), 150, and 300 mM of NaCl. Salinity levels were maintained gradually by adding 50 mM solution daily to prevent sudden salinity shock. The experiment was conducted for 8 weeks.

Structural and functional traits

Detailed structural and functional methodology has already been presented in Naz et al.[26] and Naz et al.[27].

Morphological traits

Morphological characters were measured at the end of the experiment. The number of leaves per plant was manually counted. Leaf area was calculated by the formula devised by Lopes et al.[29].

Plant fresh weight was recorded by a portable digital balance immediately after uprooting the plants from growth medium.

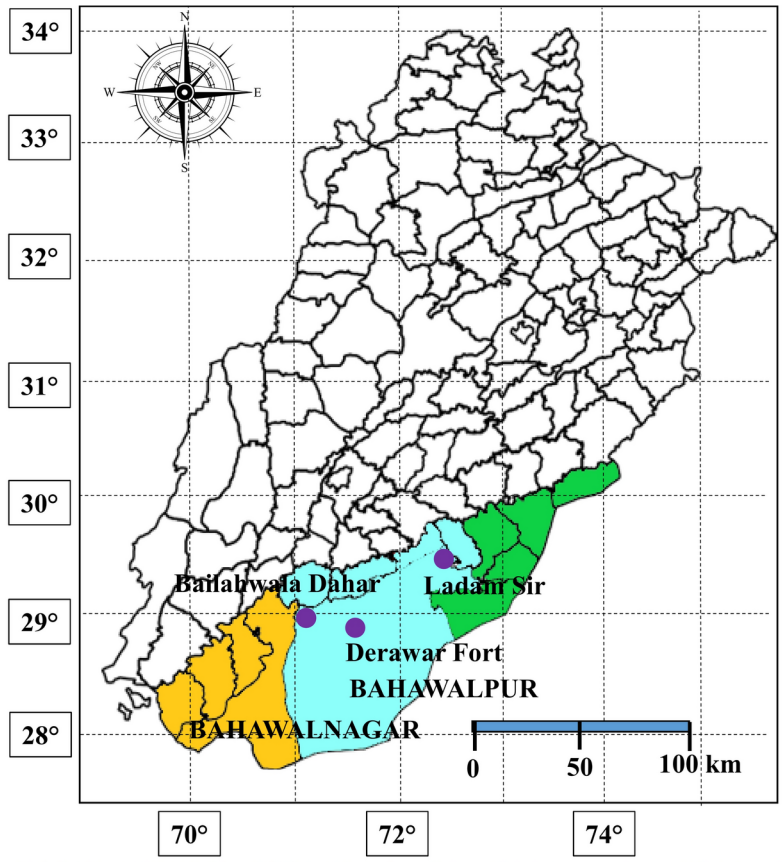
Anatomical traits

Formalin acetic alcohol solution was used for the preservation of plant material for anatomical studies. The material was kept for 24 h and then transferred to acetic alcohol following Ruzin[30]. Sections were cut by a sharp-edge razor blade. Ethyl alcohol grades (30, 50, 70, 90 and 100% in distilled water) were used for dehydration of the sections. Biological stains safranin was used for staining lignified tissue (sclerenchyma and xylem vessels). Fast green will stain primary walls (parenchyma, phloem). Ocular micrometer was calibrated with stage micrometer. Measurements of different tissues and cells were taken with an ocular micrometer. Photographs were taken with a compound microscope (Meiji Techno Japan).

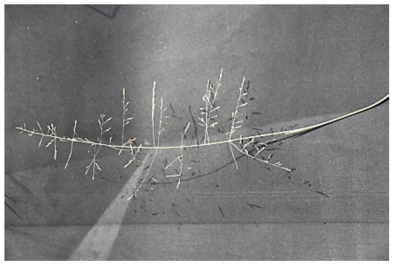
Abaxial stomatal area (μm^2).

Leaf sheath anatomy

- HTh – Leaf sheath thickness (μm)
- HDE – Adaxial epidermal cell area (μm^2)
- HBE – Abaxial epidermal cell area (μm^2)
- HCC – Parenchymatous cell area (μm^2)
- HST – Sclerenchyma thickness (μm)
- HVB – Vascular bundle area (μm^2)
- HMX – Metaxylem area (μm^2)
- HPA – Phloem area (μm^2)



Plant habit



A close-up of inflorescence

A pure *Sporobolus ioclados* community in Lesser Cholistan

Derawar Fort

Bailahwala Dahar

Ladam Sir

Derawar Fort (LSE)- Elevation 113 m; ECe 15 dS m^{-1}; Na^+ 3236 mg kg^{-1}; Cl^- 1493 mg kg^{-1}.

Bailahwala Dahar (MSE)- Elevation 94 m; ECe 28 dS m^{-1}; Na^+ 4266 mg kg^{-1}, Cl^- 1994 mg kg^{-1}.

Ladam Sir (HSE)- Elevation 133 m; ECe 49 dS m^{-1}; Na^+ 5139 mg kg^{-1}; Cl^- 2638 mg kg^{-1}

Fig. 1. Map of the Cholistan Desert and pictorial view of habitats and habit of *Sporobolus ioclados* from the Cholistan Desert.

Leaf blade anatomy

- LTh – Leaf thickness (µm)
- LST – Sclerenchymatous thickness (µm)
- LDE – Adaxial epidermal cell area ($µm^2$)
- LBE – Abaxial epidermal cell area ($µm^2$)
- LBC – Bulliform cell area ($µm^2$)

- LVB – Vascular bundle area (μm^2)
- LMX – Metaxylem area (μm^2)
- LPA – Phloem area (μm^2)

Epidermal appendages

- LDM – Adaxial microhair density
- LBM – Abaxial microhair density
- LTD – Trichome density
- LTL – Trichome length (μm)
- LDS – Adaxial stomatal density
- LBS – Abaxial stomatal density
- LDA – Adaxial stomatal area (μm^2)
- LBA – Abaxial stomatal area (μm^2)

Gas exchange traits

Gas-exchange parameters were recorded by an infrared gas analyzer (LCA-4 ADC, Analytical Development Company, Hoddesdon, England). Specifications are presented in (Table 1). Leaf water potential was attained by a M-615 Scholander chamber (MMM—Mosler Tech Support, UK) from 8:00–10:00 a.m. Osmotic potential was recorded by a Wescor 5500 Vapor Pressure Osmometer (Artisan Technology Group, USA) (Table 2).

Gas exchange parameters

- NAR-Net assimilation rate ($\mu mol\ m^{-2}\ s^{-1}$)
- TrR-Transpiration rate ($mmol\ m^{-2}\ s^{-1}$)
- StC-Stomatal conductance ($mmol\ m^{-2}\ s^{-1}$)
- SCC-Substomatal CO_2 concentration ($\mu mol\ mol^{-1}$)
- WUE-Water use efficiency

Organic osmolytes

Total amino acids were recorded at optical density 570 nm by a UV–Visible spectrophotometer (Hitachi 220, Japan) following Moore and Stein[31]. Total soluble proteins were estimated at optical density 620 nm by a spectrophotometer (Hitachi, 220, Japan) following Lowry et al.[32]. Total soluble sugars were estimated at optical density 620 nm by a spectrophotometer (Hitachi, 220, Japan) in accordance with Yemm and Willis[33]. Proline was recorded according to Bates et al.[34] and absorbance was recorded at 520 nm.

Organic osmolytes

- TFP-Total free amino acids ($\mu g\ g^{-1}$)
- TSP-Total soluble proteins ($\mu g\ g^{-1}$)
- TSS-Total soluble sugars ($mg\ g^{-1}$)
- TPr-Total proline ($\mu g\ g^{-1}$)

Chlorophyll pigments

Chlorophylls *a* and *b* were attained in accordance with Arnon[35]. Carotenoids were estimated by the Wellburn[36] method. Absorbance recorded at 645, 663 and 480 nm using a spectrophotometer (Hitachi-220 Japan).

Photosynthetic pigments

- Cha-Chlorophyll a ($mg\ g^{-1}$)
- Chb-Chlorophyll b ($mg\ g^{-1}$)
- Car-Carotenoids ($mg\ g^{-1}$)

Characteristic	Specification
Time range	9:00 to 11:00 a.m
Molar air flow per unit leaf area	419.5 mmol $m^{-2}s^{-1}$
Atmospheric pressure	97.6 k Pa
Water vapor pressure into chamber	6.5–8.7 mbar
PAR at leaf surface	up to 1719 μmol $m^{-2}\ s^{-1}$
Leaf temperature	27.3 to 32.8°C
Ambient temperature	21.7 to 27.1°C
Ambient CO_2 concentration	357 μmol mol^{-1}

Table 1. Specifications for the IRGA (infrared gas analyzer).

Trait	Eco	Treat	Inter	Trait	Eco	Treat	Inter	Trait	Eco	Treat	Inter
Morphological characteristics											
MLN	0.58^{NS}	0.6^{NS}	0.3^{NS}	MLA	23.97***	5.79*	3.34*	MRF	10.10**	1.04^{NS}	2.23^{NS}
MSF	0.29^{NS}	1.2^{NS}	0.3^{NS}	MLF	0.69^{NS}	0.57^{NS}	0.59^{NS}	MRD	30.42***	0.64^{NS}	2.49^{NS}
MSD	5.34*	0.6^{NS}	0.7^{NS}	MLD	3.25^{NS}	3.83*	1.80^{NS}				
Leaf sheath anatomy											
HTh	54.74***	4.2*	5.1**	HDE	47.85***	8.36**	57.81***	HBE	24.83***	48.60***	14.01***
HCC	162.62***	99.2***	57.6***	HST	46.66***	87.02***	4.71**	HVB	22.68***	31.99***	31.05***
HMX	151.67***	15.9***	19.4***	HPA	178.71***	4.08*	20.11***				
Leaf blade anatomy											
LTh	50.4***	2.4^{NS}	0.1^{NS}	LST	55.01***	42.74***	2.77^{NS}	LDE	374.17***	1.53^{NS}	1.53^{NS}
LBE	0.8^{NS}	47.1***	0.8^{NS}	LBC	181.60***	131.73***	135.54***	LVB	16.66***	1.63^{NS}	3.10*
LMX	32.6***	29.0***	1.9^{NS}	LPA	68.74***	18.99***	17.66***				
Epidermal appendages											
LDM	267.0***	81.3***	36.1***	LBM	472.43***	39.86***	39.86***	LTD	13.63***	101.73***	3.49*
LTL	147.5***	53.8***	5.1**	LDS	4.30*	5.01*	0.40^{NS}	LBS	15.98***	0.49^{NS}	11.88***
LDA	101.5***	1.2^{NS}	0.5^{NS}	LBA	31.50***	3.42^{NS}	0.36^{NS}				
Leaf water relations											
OsP	12.3***	3.7*	0.3^{NS}	WtP	3.80*	5.45*	0.19^{NS}	TuP	87.88***	2.17^{NS}	1.02^{NS}
Organic osmolytes											
TFA	3.1^{NS}	52.3***	0.1^{NS}	TSP	0.83^{NS}	3.77*	0.07^{NS}	TSS	7.95**	4.82*	0.13^{NS}
TPr	15.26***	34.7***	0.3^{NS}								
Gae exchange parameters											
NAR	5.0*	30.4***	0.1^{NS}	TrR	56.28***	41.67***	3.68*	StC	16.05***	7.05**	1.55^{NS}
SCC	5.0*	71.6***	4.3*	WUE	16.18***	45.59***	0.46^{NS}				
Photosynthetic pigments											
Cha	37.1***	6.8**	0.9^{NS}	Chb	15.69***	3.32^{NS}	6.24**	Car	7.37*	5.12*	1.34^{NS}
Ionic content											
RtNa	5.0*	30.4***	0.1^{NS}	RtK	56.28***	41.67***	3.68*	RtCa	16.05***	7.05**	1.55^{NS}
RtCl	5.1*	70.8***	4.2*	StNa	16.18***	45.59***	0.46^{NS}	StK	35.69***	54.81***	0.27^{NS}
StCa	26.8***	7.1**	0.3^{NS}	StCl	15.51***	55.24***	0.54^{NS}	LfNa	131.49***	4.42*	3.05*
LfK	37.8***	7.4**	1.7^{NS}	LfCa	12.96***	14.93***	0.93^{NS}	LfCl	42.45***	13.13***	1.16^{NS}
Excreted ions											
ExNa	28.3***	94.4***	7.2**	ExK	9.75**	73.54***	4.20*	ExCa	9.94**	17.89***	5.29**
ExCl	56.4***	41.9***	3.8*								
Water content											
RWC	12.5***	2.4^{NS}	8.0**	SWC	7.86**	3.85**	1.64^{NS}	LWC	0.93^{NS}	0.46^{NS}	0.77^{NS}
Succulence											
RSc	0.2^{NS}	3.3^{NS}	1.5^{NS}	SSc	33.04***	11.63***	4.78**	LSc	0.98^{NS}	2.88^{NS}	1.04^{NS}
Bioconcentration factor (BCF)											
RBN	6.4**	163.3***	5.2**	RBC	14.76***	50.04***	5.10**	SBN	8.37**	90.35***	5.80**
SBC	15.4***	124.6***	6.3**	LBN	5.03*	278.20***	4.10*	LBC	16.36***	236.80***	3.89*
EBN	38.3***	16.1***	9.6***	EBC	2.75^{NS}	171.63***	3.11*				
Translocation factor											
STN	0.2^{NS}	157.9***	1.6^{NS}	STC	9.49**	52.26***	0.59^{NS}	LTN	51.59***	131.48***	8.71***
LTC	33.6***	18.93***	1.4^{NS}								
Dilution factor											
RDN	12.7***	163.6***	3.6*	RDC	9.46**	52.15***	3.55*	SDN	40.95***	96.44***	4.64**
SDC	0.2^{NS}	155.4***	3.3*	LDN	5.75*	248.82***	2.26^{NS}	LDC	4.50*	220.34***	3.08*

Table 2. Two-way analysis of variance (F-ratio) showing ecotypes × treatment interaction. *NS* not significant, *Significant at $p < 0.05$, **Significant at $p < 0.01$, ***Significant at $p < 0.001$. Abbreviations are given in (Tables 3, Table 4, Table 5, Table 6).

Ionic content

Tissue ionic content was measured in accordance with Wolf[37]. Cations (Na^+, K^+ and Ca^{2+}) were estimated with a flame photometer (Model 410, Sherwood Scientific Ltd., Cambridge, UK). Chloride content was attained by a 926-chloride meter (Sherwood Scientific Ltd., Cambridge, UK).

Excreted ions
Ten fresh leaves were incised from the plant and washed with 100 ml of deionized H_2O. Excreted ions were then estimated from the washed off water. The Na^+ and K^+ were attained with a 410-flame photometer (Sherwood Scientific Ltd., Cambridge, UK). The Ca^{2+} and Mg^{2+} were measured with an Analyst 3000-atomic absorption spectrophotometer (Perkin Elmer, Norwalk, USA). The Cl^- was attained with a 926-chloride meter (Sherwood Scientific Ltd., Cambridge, UK).

Leaf water content and succulence
Leaf water content and leaf succulence were calculated by the formulae:

$$\text{Water content } \left(\text{g plant}^{-1}\right) = \text{Freshweight} - \text{Dry weight}$$

Succulence was calculated in accordance with Tiku[38].

$$\text{Succuence (g water/g plant DW)} = \frac{\text{(Fresh weight)}}{\text{Dry weight}}$$

Phytoremediation traits
Phytoremediation traits were recorded in accordance with of Diwan et al.[39].

$$\text{Root bioconcentration factor} = \frac{\text{Na}^+/\text{Cl}^- \text{ in roots (mg g}^{-1}\text{DW)}}{\text{Na}^+/\text{Cl}^- \text{ in medium (mg L}^{-1})}$$

$$\text{Stem bioconcentration factor} = \frac{\text{Na}^+/\text{Cl}^- \text{ in stem (mg g}^{-1}\text{DW)}}{\text{Na}^+/\text{Cl}^- \text{ in medium (mg g}^{-1})}$$

$$\text{Leaf bioconcentration factor} = \frac{\text{Na}^+/\text{Cl}^- \text{ in leaves (mg g}^{-1}\text{DW)}}{\text{Na}^+/\text{Cl}^- \text{ in medium (mg g}^{-1})}$$

$$\text{Ion Excretion Efficiency} = \frac{\text{Excreted Na}^+/\text{Cl}^-\text{(mg L}^{-1})}{\text{Na}^+/\text{Cl}^- \text{ in leaves (mg g}^{-1})}$$

$$\text{Stem translocation factor} = \frac{\text{Na}^+/\text{Cl}^- \text{ in stem } \left(\text{mg g}^{-1} \text{ DW}\right)}{\text{Na}^+/\text{Cl}^- \text{ in roots } (\text{mg g}^{-1} \text{ DW})}$$

$$\text{Leaf translocation factor} = \frac{\text{Na}^+/\text{Cl}^- \text{ in leaves } \left(\text{mg g}^{-1} \text{ DW}\right)}{\text{Na}^+/\text{Cl}^- \text{ in stem } (\text{mg g}^{-1} \text{ DW})}$$

Dilution factor was calculated in accordance with Abbas et al.[39].

$$\text{Dilution factor } \left(\text{mg g}^{-1} \text{ DW}\right) = \frac{\text{Na}^+\text{or Cl}^- \text{ in leaves, stem or root}}{\text{Na}^+ \text{ or Cl}^- \text{ in medium}}$$

where DW is the dry weight.

Statistical analysis

A two factorial completely randomized design with 4 replications was used for experimentation. The data were analyzed by a two-way analysis of variance using Minitab statistical software (v. 17). Means were compared by a Tukey test. A principal component analysis (PCA) was run to investigate relationship among growth, anatomical, physiological and excreted ions using R Studios (V 1.1.463). Heatmaps were constructed to evaluate the relationship of excreted ions with morpho-anatomical and physiological traits using a customized R code. Estimated response was calculated for excreted ions and different morho-anatomical and physiological traits. Transpiration rate and sub-stomatal CO_2 concentration exhibited a significant variation ($p < 0.05$) of interaction, while interaction in chlorophyll *b* varied significantly at $p < 0.01$. In ionic content, root, shoot and leaf Na^+, K^+, Ca^{2+} and Cl^- showed significant variation of ecotypes and treatment, while interaction varied significantly in root Cl^- and root K^+ ($p < 0.05$). In excreted ions, interaction varied significantly in excreted Na^+ ($p < 0.01$), excreted K^+ ($p < 0.05$), excreted Ca^{2+} ($p < 0.01$) and excreted Cl^- ($p < 0.05$). Root water content depicted a significant variation of interaction ($p < 0.01$), while stem succulence varied significantly at $p < 0.01$. In Bioconcentration factor, ecotypes, treatments and their interaction varied significantly in all traits. In leaf translocation factor, interaction varied significantly at $p < 0.001$. All traits of dilution factor showed significant variation regarding ecotypes, treatments and their interaction.

Results

Leaf area showed significant variation of ecotypes ($p < 0.001$), treatments ($p < 0.05$) and interaction of ecotypes and treatments ($p < 0.05$) as presented in (Table 3). Ecotypes varied significantly in root fresh weight ($p < 0.01$), root dry weight ($p < 0.001$) and shoot dry weight ($p < 0.05$). In all leaf sheath anatomical traits, ecotypes, treatments and their interaction varied significantly. In leaf blade anatomical traits, interaction showed varied significantly in bulliform cell area ($p < 0.001$), vascular bundle area ($p < 0.05$) and phloem area ($p < 0.001$).

In epidermal appendages, interaction revealed significant variation in adaxial and abaxial microhair density ($p < 0.001$), trichome density ($p < 0.05$), trichome length ($p < 0.01$) and abaxial stomatal density ($p < 0.001$).

Morphology

Morphological traits like leaf number and area, and root fresh weight decreased with salinity in the Derawar Fort (LSE), whereas leaf dry weight increased (Table 3). Traits such as stem fresh weight, dry weight and root dry weight increased only at 150 mM. Leaf fresh weight decreased significantly at 150 mM but thereafter increased at 300 mM. The Bailahwala Dahar (MSE) showed a decrease in leaf number and leaf area with salinity levels. Traits such as root and leaf fresh increased with salinity, while stem fresh weight increased only at 300 mM. Stem dry weight decreased only at 300 mM, while an increase was observed in leaf dry weight of Bailahwala Dahar (MSE). The Ladam Sir (HSE) responded differently with increasing salinity levels. Traits such as leaf number, leaf area, root fresh weight, leaf fresh weight and leaf dry weight increased with salinity. Stem fresh weight and root dry weight increased only at 300 mM, while no change was recorded in stem dry weight.

Among habitats, the morphological number of leaves, total leaf area, root fresh weight and stem fresh weight showed a significant decrease in the least saline ecotype (LSE) at highest salinity level (300 mM), while number of leaves and root fresh weight were highest in highly saline ecotype at 300 mM and 150 mM respectively. Stem fresh weight, leaf fresh weight, root dry weight, stem dry weight and leaf dry weight were the maximum in highly saline ecotype (HSE) at 300 mM salinity level.

Leaf sheath anatomy

Leaf sheath anatomical traits such as thicknesses of leaf sheath, adaxial and abaxial epidermis and sclerenchyma, and areas of vascular bundle and metaxylem increased with salinity in the Derawar Fort (LSE), (Table 3, Fig. 2), Phloem area decreased with salinity, while parenchymatous cell area decreased only at 300 mM in this ecotype. In the Bailahwala Dahar (MSE), a consistent increase with salinity was recorded in leaf sheath anatomical traits such as adaxial and abaxial epidermal thickness, parenchymatous cell area, sclerenchymatous thickness and metaxylem area. Leaf sheath thickness, vascular bundle area and phloem area decreased with salinity in the Bailahwala Dahar (MSE). In the Ladam Sir (HSE), leaf sheath anatomical traits such as leaf sheath thickness, adaxial epidermal thickness and metaxylem area decreased with salinity, while all other leaf sheath anatomical traits increased.

Among habitats, leaf sheath thickness and adaxial epidermal cell area were the maximum in highly saline ecotype (HSE) at control level while minimum in moderately saline ecotype (MSE) at 300 mM and 0 mM respectively. Abaxial epidermal cell area and parenchymatous cell area were the highest in least saline ecotype at 150 mM salinity, while the minimum values for these parameters were recorded in moderately saline ecotype at 0 mM salinity level. Sclerenchyma thickness was maximum in least saline ecotype at 300 mM salinity level, while the minimum in moderately saline ecotype at 0 mM salinity level. Vascular bundle area was the highest in highly saline ecotype at 300 mM level, while lowest in moderate saline ecotype at 150 mM level. Metaxylem area was recorded highest in highly saline ecotype at 0 mM level, the lowest in least saline ecotype at 0 mM level. Phloem area was observed the maximum in highly saline ecotype at 300 Mm, while the minimum in moderately saline ecotype at 300 Mm.

Leaf blade anatomy

Leaf blade anatomical traits such as sclerenchymatous thickness, adaxial and abaxial epidermal thicknesses, and metaxylem area increased with salinity in all ecotypes, while leaf thickness and bulliform cell area decreased (Table 3, Fig. 3 and Fig. 4). Vascular bundle area increased only at 150 mM in the Derawar Fort (LSE), decreased with salinity in the Bailahwala Dahar (MSE) and increased with salinity in the Ladam Sir (HSE). The phloem area increased only at 150 mM level, while decreased at 300 mM in the Derawar Fort (LSE). Thuss trait decreased with salinity in the Bailahwala Dahar (MSE) and Ladam Sir (HSE).

Among habitats, leaf thickness, bulliform cell area, vascular bundle area and phloem area were the maximum in moderately saline ecotypes at 0 mM salinity level, while were recorded as the minimum in moderately saline (300 mM), highly saline ecotype (300 mM), least saline ecotype (0 mM) and highly saline ecotype (300 mM) respectively. Sclerenchymatous thickness, adaxial epidermal cell area, abaxial epidermal cell area and metaxylem area were recorded highest in highly saline ecotype at 300 mM salinity level, while the lowest values for the Sclerenchymatous thickness was observed in least saline ecotype at 0 mM, adaxial epidermal cell area, abaxial epidermal cell area and metaxylem area in highly saline ecotype 0 mM salinity level.

Epidermal appendages

Traits like microhair density in abaxial and adaxial leaf surfaces, trichome density and trichome length increased invariably with salinity in all ecotypes, however all these were significantly higher in the Ladam Sir (HSE) (Table 3). Stomatal density on abaxial surface increased with salinity in the Derawar Fort (LSE), while decreased in the Bailahwala Dahar (MSE) and HSE. Adaxial stomatal density and abaxial stomatal area decreased with salinity in the *S. ioclados* ecotypes. The Stomata area decreased with salinity in the Derawar Fort (LSE) and Bailahwala Dahar (MSE), while not altered in the Ladam Sir (HSE).

Among habitats, the maximum adaxial microhair density, abaxial microhair density, trichome density and trichome length were recorded in highly saline ecotype at 300 mM level, while the minimum values for these traits were observed in highly saline ecotype (0 mM), least saline ecotype (0 mM), moderately saline ecotype (0 mM) and moderately saline at (0 mM) respectively. The highest adaxial stomatal density, adaxial stomatal area and abaxial stomatal area were observed in moderately saline ecotype at 0 mM level. The maximum abaxial stomatal density was recorded in least saline ecotype at 300 mM, while minimum in least saline ecotype at 0 mM salinity level.

Habitat →	LSE			MSE			HSE		
Salt levels→	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM
Morphological characteristics									
MLN	55.3 ± 3.4aD	46.4 ± 2.1bE	42.4 ± 2.5cG	58.2 ± 3.2aC	55.3 ± 3.4cD	56.2 ± 2.4bD	44.4 ± 2.5cF	60.2 ± 2.0bB	62.2 ± 3.5aA
MLA	115.8 ± 8.8aE	107.4 ± 8.5bF	58.5 ± cG	197.1 ± 12.5aA	189.5 ± 12.4bB	168.5 ± 12.4cC	132.2 ± 8.7cD	164.8 ± 10.2bC	189.4 ± 11.4aB
MRF	12.1 ± 0.5aC	11.5 ± 0.7bD	9.6 ± 0.4cF	10.6 ± 0.7cE	11.4 ± 0.6bD	13.8 ± 0.9aB	10.3 ± 4.7cE	14.7 ± 0.9aA	13.8 ± 0.7bB
MSF	6.6 ± 0.3bD	7.2 ± 0.4aBC	6.5 ± 0.2bD	7.3 ± 0.3bB	6.8 ± 0.3cCD	8.5 ± 0.4aA	7.2 ± 0.3bB	7.6 ± 0.3bB	8.9 ± 0.4aA
MLF	15.8 ± 1.1bE	14.6 ± 0.8cF	17.6 ± 1.1aBC	16.3 ± 1.1cE	17.9 ± 1.1aB	17.4 ± 1.3bC	16.9 ± 0.8bD	18.3 ± 1.1aA	18.0 ± 1.1aA
MRD	3.6 ± 0.1bC	3.9 ± 0.2aB	3.8 ± 0.1abBC	3.8 ± 0.2aBC	3.8 ± 0.1aBC	3.9 ± 0.2aB	3.7 ± 0.1bBC	3.7 ± 0.1bBC	4.2 ± 0.2aA
MSD	1.2 ± 0.01bC	1.5 ± 0.01aA	1.1 ± 0.01bCD	1.3 ± 0.1aBC	1.1 ± 0.01ab	0.9 ± 0.01bD	1.5 ± 0.01aAB	1.6 ± 0.01aA	1.7 ± 0.1aA
MLD	3.5 ± 0.1cE	4.4 ± 0.2bC	4.7 ± 0.2aB	4.1 ± 0.2bD	4.8 ± 0.2aB	4.1 ± 0.1bD	3.9 ± 0.1bD	4.9 ± 0.2aAB	5.1 ± 0.2aA
Leaf sheath anatomy									
HTh	187.4 ± 12.4cE	325.1 ± 15.5aB	266.1 ± 18.6bD	177.7 ± 13.8aE	158.4 ± 10.6b	154.8 ± 9.8bF	373.5 ± 16.7aA	314.1 ± 22.5bB	289.6 ± 18.7cC
HDE	77.6 ± 4.4cE	177.5 ± 12.4bB	309.6 ± 19.4aA	38.6 ± 1.9cF	116.8 ± 8.7b	155.5 ± 10.7aC	309.7 ± 22.7aA	114.7 ± 8.8bD	78.1 ± 3.6cE
HBE	154.6 ± 10.6cE	461.8 ± 24.7aA	290.5 ± 17.6bD	74.3 ± 5.3cG	135.4 ± 9.8bF	309.8 ± 19.9aD	154.3 ± 10.2cE	348.5 ± 22.6bC	405.3 ± 24.6aB
HCC	929.9 ± 77.8bC	3795.5 ± 167.6aA	834.7 ± 50.7cD	159.8 ± 10.5cG	278.4 ± 18.5aE	234.6 ± 15.4bF	185.3 ± 15.6cG	1233.3 ± 98.8aB	948.5 ± 56.7bC
HST	10.4 ± 0.9cG	49.7 ± 2.2bB	58.1 ± 2.4aA	9.3 ± 0.4cG	21.4 ± 1.4bF	29.2 ± 1.7aE	9.3 ± 0.5c	36.8 ± b1.5D	42.6 ± 2.4aC
HVB	5421.3 ± 234.8cG	8364.8 ± 524.9aD	7617.7 ± 565.4bE	9349.4 ± 773.6aC	4879.9 ± 398.6bG	3484.6 ± 279.6cH	6971.1 ± 455.6cF	11,075.8 ± 897.6bB	13,016.7 ± 998.5aA
HMX	38.5 ± 1.9bF	387.9 ± 14.7aE	396.9 ± 21.8aE	382.5 ± 19.9cE	789.6 ± 55.7bD	1540.7 ± 100.5aA	1546.6 ± 101.7aA	1363.8 ± 101.8bB	1248.5 ± 99.7cC
HPA	1626.4 ± 110.5aC	1557.3 ± 100.9bD	1425.7 ± 95.9cE	929.6 ± 85.3aG	463.1 ± 25.7bH	308.4 ± 18.8cI	1236.9 ± 101.5cF	2163.8 ± 176.4bB	2891.5 ± 178.6aA
Leaf blade anatomy									
LTh	236.4 ± 17.4aC	217.7 ± 15.6bD	187.1 ± 12.5cF	296.3 ± 21.4aA	253.8 ± 19.4bB	215.3 ± 15.6cE	291.7 ± 25.1aA	221.4 ± 14.5bD	215.7 ± 15.6bD
LST	10.2 ± 0.8cF	21.3 ± 1.4bE	29.5 ± 1.5aD	30.6 ± 2.4cD	39.2 ± 1.5bC	42.5 ± 2.2aB	19.7 ± 1.1cE	38.6 ± 1.9bC	44.6 ± 2.8aA
LDE	38.1 ± 1.9cD	59.3 ± 3.5bC	77.8 ± 4.5aB	37.4 ± 2.4cD	59.4 ± 3.4bC	77.0 ± 4.4aB	36.3 ± 2.1cD	74.6 ± 5.2bB	85.2 ± 6.1aA
LBE	84.6 ± cE	95.1 ± 6.4bD	157.4 ± 10.6aAB	37.3 ± 1.8cG	135.7 ± 9.8bC	152.9 ± 12.8aB	19.5 ± 1.1cH	71.6 ± 5.4bF	158.3 ± 12.6aA
LBC	372.3 ± 30.8aE	150.6 ± 10.4bF	93.4 ± 7.2cG	2205.8 ± 199.8aA	1106.6 ± 86.4b	1069.0 ± 95.4cC	432.6 ± 33.5aD	91.4 ± 6.4bG	67.5 ± 5.3cH
LVB	1549.3 ± 143.5bI	1592.7 ± 135.6aH	1239.9 ± 115.4cG	11,628.0 ± 886.4aA	8514.2 ± 756.7bB	6970.2 ± 577.4cC	3253.7 ± 255.6cF	4137.5 ± 345.8bE	4957.6 ± 373.5aD
LMX	65.34 ± 4.3cDE	123.6 ± 7.6bB	158.8 ± 12.6aA	61.4 ± 3.5cE	67.4 ± 4.4bD	73.0 ± 5.7aD	62.8 ± 4.8cE	117.5 ± 8.3bC	161.6 ± 13.4aA
LPA	876.4 ± 65.7bE	1013.8 ± 88.7aD	781.6 ± 70.7cF	1947.9 ± 166.3aA	1116.6 ± 97..5bB	1097.6 ± 99.4cC	1083.7 ± 95.4aC	659.6 ± 55.9bG	537.7 ± 35.6cH
Epidermal appendages									
LDM	34.2 ± 2.2cE	43.6 ± 2.2bE	48.3 ± 2.3aC	32.4 ± 2.1cF	46.3 ± 2.5bD	48.2 ± 2.3aC	29.3 ± 1.6cG	53.5 ± 2.1bB	69.3 ± 3.3aA
LBM	19.3 ± 0.9cH	58.3 ± 3.8bE	61.4 ± 0.3aD	37.5 ± 2.5cG	59.2 ± 2.7bE	63.7 ± 3.4aC	43.9 ± 1.9cF	68.54 ± 4.3bB	76.5 ± 5.4aA
LTD	3.5 ± 0.1cE	3.9 ± 0.1bE	4.2 ± 0.2aDE	2.6 ± 1.0cF	3.6 ± 0.1bE	4.7 ± 1.8aD	4.0 ± 0.1cC	8.1 ± 0.5bB	14.6 ± 1.1aA
LTL	15.2 ± 1.1cG	65.6 ± 4.3bE	143.3 ± 11.4aC	18.5 ± 1.0cG	53.3 ± 2.7bF	65.4 ± 3.2aE	121.1 ± 9.4cD	169.7 ± 11.8bB	208.5 ± 15.6aA
LDS	28.5 ± 1.6aD	24.6 ± 1.5bE	23.9 ± 1.5cF	33.3 ± 1.4aA	29.3 ± 1.5bC	28.5 ± 1.3cD	32.7 ± 1.5aB	29.4 ± 1.6bC	28.2 ± 1.4cD
LBS	25.4 ± 1.2cE	37.5 ± 2.3bC	49.2 ± 3.2aA	36.4 ± 1.2aC	33.2 ± 1.7bD	25.4 ± 1.1cE	39.1 ± 2.3aB	37.2 ± 2.3bC	34.6 ± 1.9cD
Continued									

Habitat →	LSE			MSE			HSE		
Salt levels→	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM
LDA	345.5 ± 25.6aBC	317.6 ± 19.8bD	314.4 ± 24.3bD	382.1 ± 20.7aA	365.3 ± 2.1bB	338.3 ± 20.4cC	128.3 ± 0.9aE	137.2 ± 1.1aE	134.3 ± 9.8aE
LBA	314.6 ± 20.7aD	283.7 ± 22.5bE	271.3 ± 22.1cF	370.2 ± 20.4aA	351.5 ± 19.4b	343.6 ± 21.7cC	276.3a ± 20.7F	212.4 ± 15.8bG	194.9 ± 12.6cH

Table 3. Leaf anatomical characteristics of differently adapted ecotypes of *Sporobolus ioclados* Nees ex Trin. from the Cholistan Desert under salt stress. Means with similar letters (a, b or c) for each habitat are statistically non- significant at $p \leq 0.05$. Small letter indicate comparison within treatments, while capital letters indicate comparison of overall means. Ecotypes: LSE – Least saline ecotype, MSE – Moderately saline ecotype, HSE – Highest saline ecotype. Morphological characteristics: MLN – Number of leaves (plant^{-1}). MLA – Total leaf area (cm^2), MRF – Root fresh weight (g plant^{-1}), MSF – Stem fresh weight (g plant^{-1}), MLF – Leaf fresh weight (g plant^{-1}), MRD – Root dry weight (g plant^{-1}), MSD – Stem dry weight (g plant^{-1}), MLD – Leaf dry weight (g plant^{-1}). Leaf sheath anatomy: HTh – Leaf sheath thickness (µm), HDE – Adaxial epidermal cell area (µm^2), HBE – Abaxial epidermal cell area (µm^2), HCC – Parenchymatous cell area (µm^2), HST – Sclerenchyma thickness (µm), HVB – Vascular bundle area (µm^2), HMX – Metaxylem area (µm^2), HPA – Phloem area (µm^2). Leaf blade anatomy: LTh – Leaf thickness (µm), LST – Sclerenchymatous thickness (µm), LDE – Adaxial epidermal cell area (µm^2), LBE – Abaxial epidermal cell area (µm^2), LBC – Bulliform cell area (µm^2), LVB – Vascular bundle area (µm^2), LMX – Metaxylem area (µm^2), LPA – Phloem area (µm^2). Epidermal appendages: LDM – Adaxial microhair density, LBM – Abaxial microhair density, LTD – Trichome density, LTL – Trichome length (µm), LDS – Adaxial stomatal density, LBS – Abaxial stomatal density, LDA – Adaxial stomatal area (µm^2), LBA – Abaxial stomatal area (µm^2).

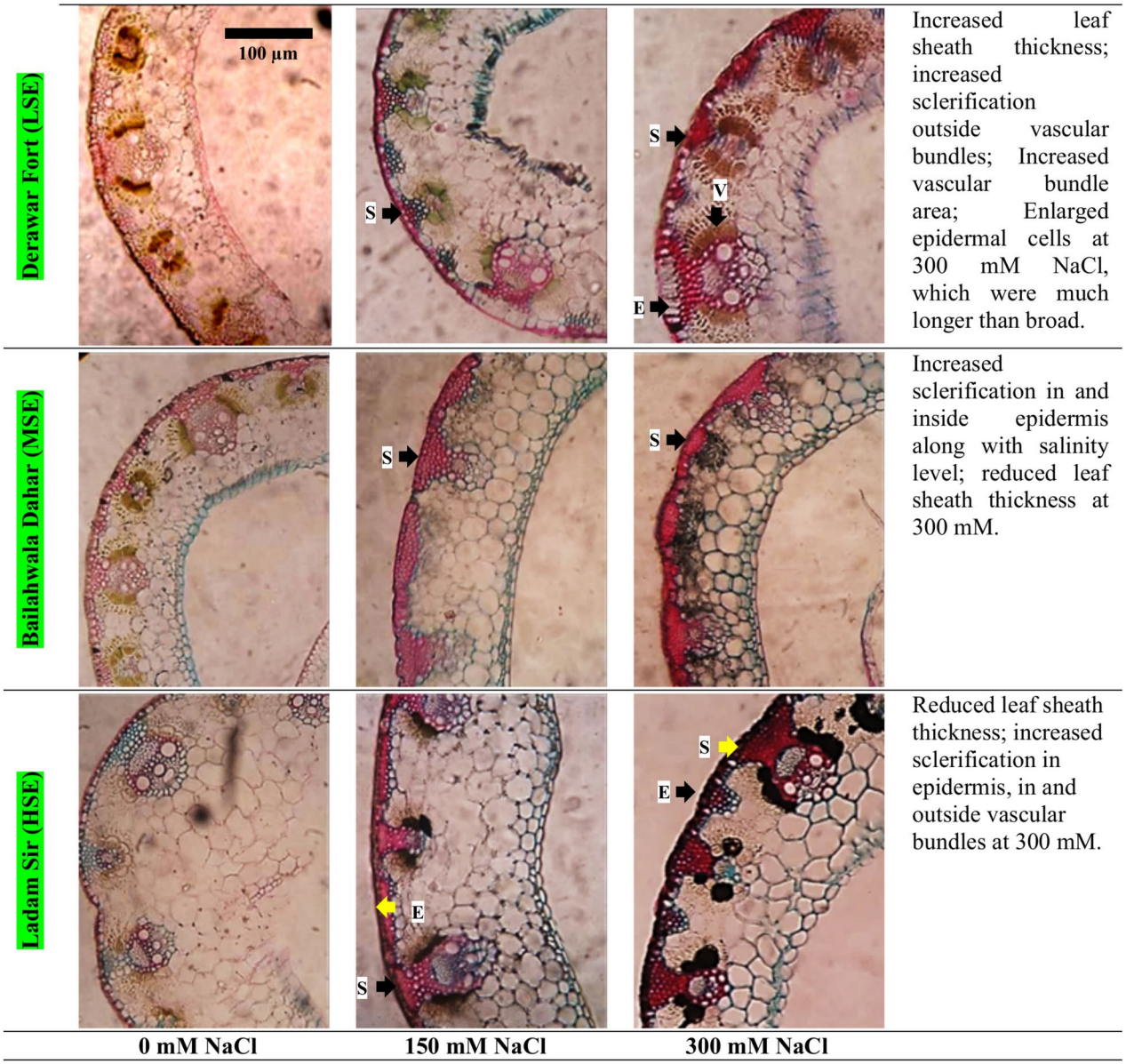


Fig. 2. Leaf sheath anatomy of *Sporobolus ioclados* from the Cholistan Desert grown hydroponically under different levels of salt.

Physiological parameters

Leaf water relations

The leaf osmotic potential and water potential invariably became more negative with salinity. Leaf turgor potential increased significantly with salinity in the Ladam Sir (HSE). It decreased in the Bailahwala Dahar (MSE) as salinity level increased. In the Bailahwala Dahar (MSE), turgor potential was not affected at lower salt levels, but it decreased at 300 mM (Table 4).

Among habitats, the highest osmotic potential was observed in highly saline ecotype at 300 mM salinity, while the minimum osmotic potential was recorded in least and highly saline ecotypes at 0 mM level. Water potential was observed maximum in least saline ecotype at 300 mM salinity level, while was the minimum in highly saline ecotype at 0 mM. The maximum turgor potential was observed in moderately saline ecotype at 0 and 150 mM salinity levels.

Organic osmolytes

Organic osmolytes increased with salinity (Table 4). The HSE accumulated significantly higher concentration of free amino acids, soluble proteins, soluble than Derawar Fort (LSE) or Bailahwala Dahar (MSE). Concentration of all these were significantly lower in the Derawar Fort (LSE). Among habitats, the maximum total free amino acids, total soluble proteins, total soluble sugars and proline were recorded in highly saline ecotype at 300 mM salt level, while the minimum in all these parameters in least saline ecotype at 0 mM level.

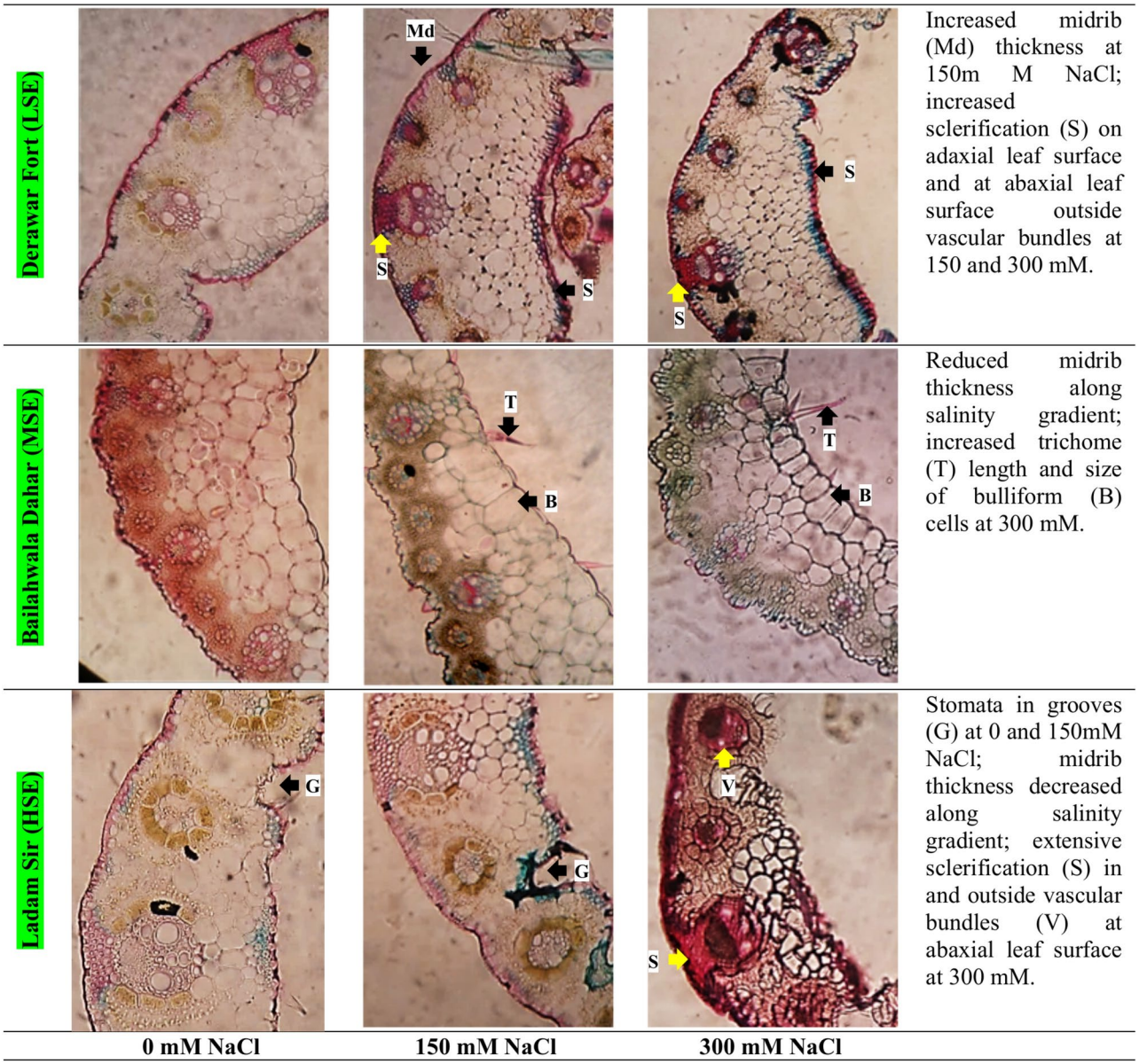


Fig. 3. Leaf blade (midrib) anatomy of *Sporobolus ioclados* from the Cholistan Desert grown hydroponically under different levels of salt.

Gas exchange traits

Water use efficiency (WUE) increased invariably under stress conditions (Table 4). Transpiration rate (*E*), stomatal conductance (*g*) and sub-stomatal CO_2 concentration (C*i*) consistently decreased with salinity. In particular, *g* and C*i* were significantly higher in the Ladam Sir (HSE) under 300 mM. Net assimilation rate (P*n*) decreased significantly in the Derawar Fort (LSE) with salinity. This trait increased only at 150 mM in the Bailahwala Dahar (MSE), while increased significantly with salinity in the Ladam Sir (HSE).

Among habitats, the highest transpiration rate, stomatal conductance and substomatal CO_2 concentration were recorded in least saline ecotype at 0 mM, while the lowest value for these traits were observed in least saline ecotype at 300 mM salinity level.

Photosynthetic pigments

Chlorophyll *a* and *b* invariably decrease with salinity (Table 4). Carotenoids increased in the Derawar Fort (LSE) at 150 mM, while not change in the Bailahwala Dahar (MSE). In the Ladam Sir (HSE), carotenoids increased significantly with salinity. Among habitats, the maximum chlorophyll a and chlorophyll b were recorded in highly saline ecotype at 0 mM and, carotenoids in least saline at 1500 mM level, while all these traits were the minimum in least saline ecotypes at 300 mM salinity level.

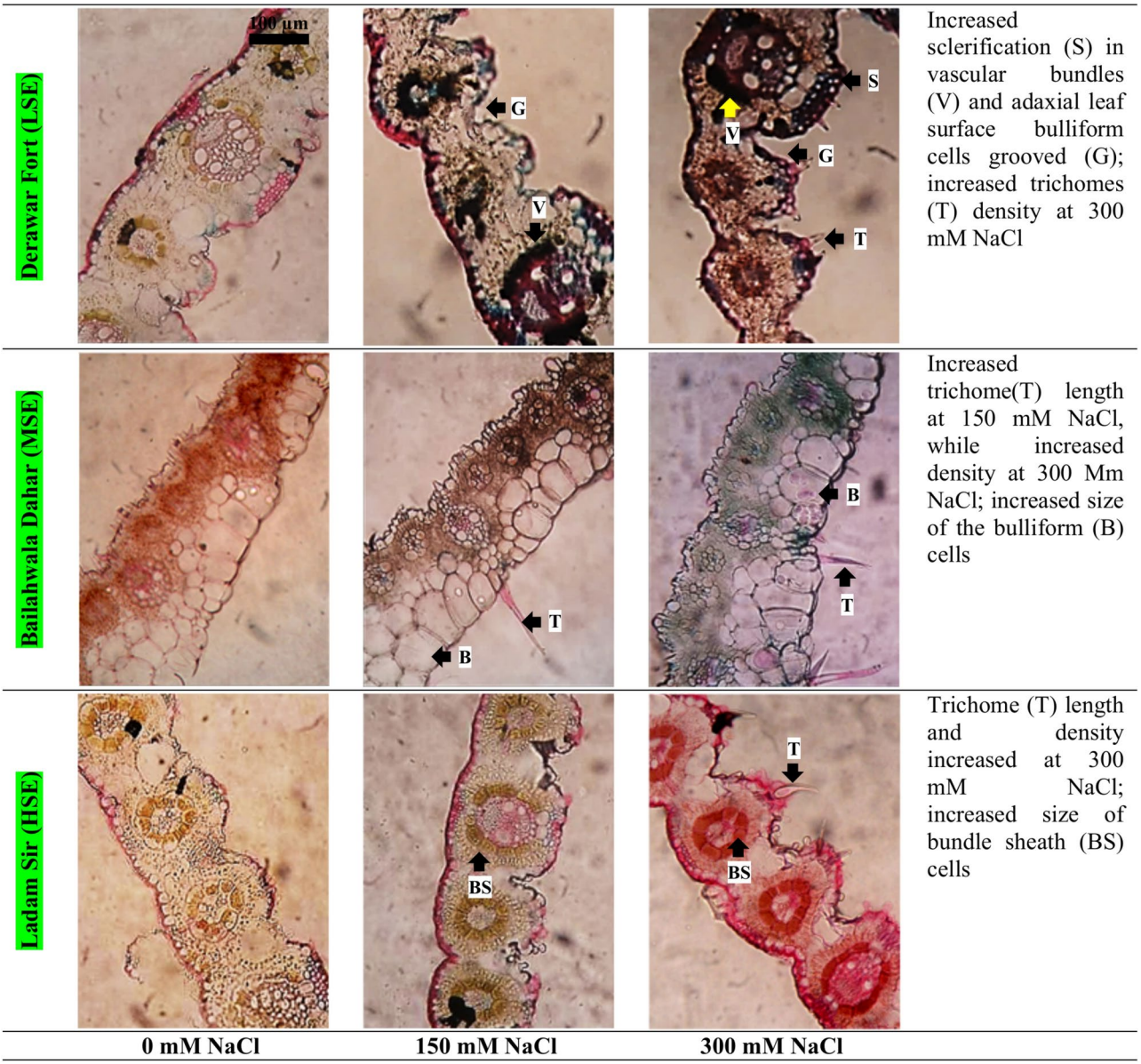


Fig. 4. Leaf blade (lamina) anatomy of *Sporobolus ioclados* from the Cholistan Desert grown hydroponically under different levels of salt.

Tissue ionic content

The root, stem and leaf Na^+ and Cl^- significantly increased in all cases (Table 5). The Derawar Fort (LSE) accumulated significantly higher concentration of Cl^- in roots and Na^+ in the leaves than its counterparts. The Bailahwala Dahar (MSE) accumulated more Cl^- in stem. The stem Ca^{2+} increased up to 300 mM in all three ecotypes, but the Ladam Sir (HSE) accumulated more Ca^{2+} than its counterparts. Leaf Ca^{2+}, however, increased invariably in response to increasing salt stress. Concentration of K^+ in root, stem and leaf of Derawar Fort (LSE) and Bailahwala Dahar (MSE) decreased with salinity. In the Ladam Sir (HSE), stem K^+ decreased with salinity, whereas root and leaf K^+ increased (Table 5).

Leaf excreted ions

In the HSE, concentration of excreted ions (Na^+ and Cl^-) than its counterparts (Table 5). The Derawar Fort (LSE) and Bailahwala Dahar (MSE) excreted mire K^+ and Ca^{2+} than Ladam Sir (HSE). A significant decrease in excreted K^+ and Ca^{2+} was noted at the highest salt level in this ecotype.

Water content

Root water content decreased consistently in the Derawar Fort (LSE) with salinity, while it increased in the Bailahwala Dahar (MSE). In the highest saline ecotype Ladam Sir (HSE), root water content increased at 150 mM salinity level, while decreased significantly at the highest level (Table 6). Stem water content increased only at

Habitats→	Derawar Fort (LSE)			Bailahwala Dahar (MSE)			Ladam Sir (HSE)		
Salt levels→	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM
Leaf water relations									
OsP	1.38 ± 0.1cE	1.56 ± 0.1bD	1.66 ± 0.1aC	1.50 ± 0.01cD	1.69 ± 0.1bC	1.80 ± 0.1aB	1.34 ± 0.1cE	1.57 ± 0.1bD	1.89 ± 0.1aA
WtP	0.56 ± 0.01cG	0.81 ± 0.02bD	1.20 ± 0.1aA	0.47 ± 0.01cH	0.68 ± 0.01bE	1.12 ± 0.1aB	0.43 ± 0.01cH	0.62 ± 0.02bF	0.93 ± 0.02aC
TuP	0.83 ± 0.02aD	0.71 ± 0.01bF	0.46 ± 0.01cG	1.03 ± 0.1aA	1.01 ± 0.1aA	0.87 ± 0.02bC	0.78 ± 0.02bE	0.94 ± 0.03aB	0.92 ± 0.02aB
Organic osmolytes									
TFA	1162.3 ± 98.6cD	1328.7 ± 101.5bC	1556.7 ± 123.6aA	1173.6 ± 101.8cD	1350.6 ± 10.8bC	1593.3 ± 136.7aA	1189.7 ± 95.7cD	1382.0 ± 110.4bC	1645.6 ± 127.6aA
TSP	733.5 ± 57.9bE	857.6 ± 70.8aD	859.6 ± 60.8aD	880.1 ± 74.4cD	1021.9 ± 83.3bC	1129.7 ± 88.6aB	1026.9 ± 87.8cC	1192.2 ± 94.5bB	1301.4 ± 98.3aA
TSS	20.7 ± 1.0cG	25.8 ± 1.4bE	26.9 ± 1.4aD	22.7 ± 1.1cF	28.4 ± 1.6bC	29.6 ± 1.6aB	23.1 ± 1.0cF	30.7 ± 1.5bB	33.6 ± 2.1aA
TPr	131.6 ± 9.8cF	222.4 ± 14.7bE	252.4 ± 15.6aDE	171.7 ± 11.8cF	290.1 ± 18.5bD	329.2 ± 17.5aC	256.8 ± 19.7cDE	442.2 ± 31.9bB	485.91 ± 34.9aA
Gas exchange parameters									
NAR	14.8 ± 1.1aB	12.9 ± 0.1bC	9.3 ± 0.6cE	12.6 ± 1.0bC	13.3 ± 0.1aC	12.6 ± 0.7bC	12.1 ± 0.1cD	15.1 ± 0.8aA	14.0 ± 0.07bB
TrR	3.12 ± 0.1aA	1.50 ± 0.1bC	0.87 ± cE	3.15 ± 0.1aA	1.37 ± 0.01bC	1.07 ± 0.1cD	3.24 ± 0.1aA	2.36 ± 0.1bB	1.17 ± 0.04cD
StC	339.8 ± 21.8aA	147.4 ± 9.9bE	98.3 ± 0.7cF	229.2 ± 15.8aAC	180.1 ± 10.7bD	147.4 ± 10.8cE	300.3 ± 24.8aB	246.1 ± 19.8bC	187.1 ± 56.4cD
SCC	279.7 ± 20.8aA	218.9 ± 15.7bD	132.4 ± 8.8cE	272.4 ± 19.8aA	223.0 ± 15.8bC	195.8 ± 11.9cD	275.3 ± 20.8aA	259.5 ± 18.8bB	240.6 ± 63.1cC
WUE	4.7 ± 0.1cF	8.6 ± 0.3bD	10.6 ± 5.4aB	4.0 ± 0.1cG	9.7 ± 0.4bC	11.8a ± 0.1A	3.7 ± 0.1cG	6.4 ± 0.3bE	11.9 ± 40.8aA
Photosynthetic pigments									
Cha	1.63 ± 0.1aCD	1.32 ± 0.1bE	1.18 ± 0.1cE	1.91 ± 0.1aB	1.55 ± 0.1bCD	1.44 ± 0.1cD	2.23 ± 0.1aA	1.81 ± 0.1bB	1.68 ± 0.008cC
Chb	0.57 ± 0.01aC	0.46 ± 0.01bD	0.33 ± 0.01cE	0.86 ± 0.02aA	0.69 ± 0.02bB	0.49 ± 0.01cD	0.88 ± 0.03aA	0.65 ± 0.02bBC	0.55 ± 0.3bC
Car	0.076 ± 0.003bD	0.133 ± 0.01aA	0.041 ± 0.001cG	0.098 ± 0.002aB	0.060 ± 0.002bE	0.061 ± 0.002bE	0.095 ± 0.003aC	0.055 ± 0.002bF	0.054 ± 0.003b

Table 4. Leaf physiological characteristics of differently adapted ecotypes of *Sporobolus ioclados* Nees ex Trin. from the Cholistan Desert under salt stress. Means with similar letters (a, b or c) for each habitat are statistically non- significant at $p \leq 0.05$. Small letter indicate comparison within treatments, while capital letters indicate comparison of overall means. Ecotypes: LSE – Least saline ecotype, MSE – Moderately saline ecotype, HSE – Highest saline ecotype. Leaf water relations: OsP – Osmotic potential (– MPa), WtP – Water potential (– MPa), TuP – Turgor potential (MPa). Organic osmolytes: TFA – Total free amino acids (μg g^{-1}), TSP – Total soluble proteins (μg g^{-1}), TSS – Total soluble sugars (mg g^{-1}), TPr – proline (μg g^{-1}). Gas exchange parameters: NAR – Net assimilation rate (μmol m^{-2} s^{-1}), TrR – Transpiration rate (mmol m^{-2} s^{-1}), StC – Stomatal conductance (mmol m^{-2} s^{-1}), SCC – Substomatal CO_2 concentration (μmol mol^{-1}), WUE – Water use efficiency. Photosynthetic pigments: Cha – Chlorophyll *a* (mg g^{-1}), Chb – Chlorophyll *b* (mg g^{-1}), Car – Carotenoids (mg g^{-1}).

Habitats→	Derawar Fort (LSE)			Bailahwala Dahar (MSE)			Ladam Sir (HSE)		
Salt levels→	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM
Root ionic content									
RtNa	21.1 ± 1.4cI	34.3 ± 2.3bF	59.7 ± 3.5aC	24.9 ± 1.2cHI	40.4 ± 2.8bE	70.5 ± 0.4aB	29.8 ± 1.6cG	48.5 ± 2.3bD	84.5 ± 5.8aA
RtK	11.8 ± 0.8aB	8.6 ± 0.5bDE	7.5 ± 0.4cF	13.0 ± 1.1aA	9.6 ± 0.5bCD	8.3 ± 0.4cEF	9.7 ± 0.5cC	13.7 ± 0.10aA	11.4 ± 0.8bB
RtCa	7.0 ± 0.2bG	8.1 ± 0.5aE	7.1 ± 0.4bG	8.1 ± 0.5cE	9.3 ± 0.5aB	9.1 ± 0.5bC	7.7 ± 0.4cF	8.9 ± 0.4bD	10.4 ± 0.5aA
RtCl	13.0 ± 0.9cF	43.1 ± 2.3bCD	65.6 ± 4.6aA	12.2 ± 0.9cF	36.4 ± 2.1bDE	55.4 ± 0.2aB	10.7 ± 0.5cF	32.1 ± 1.8bE	48.8 ± 2.4aBC
Stem ionic content									
StNa	8.3 ± 0.4cE	22.5 ± 1.1bC	36.7 ± 2.4aA	6.2 ± 0.3cE	19.2 ± bD	27.5 ± 1.5aB	8.7 ± 0.4cE	23.6 ± 1.2bC	28.7 ± 1.5aB
StK	14.9 ± 1.1aB	6.9 ± 0.2bD	5.7 ± 0.2bE	15.4 ± 1.1aAB	7.1 ± 0.4bCD	6.6 ± 0.2cDE	16.2 ± 1.2aA	7.5 ± 0.3bC	6.2 ± 0.2cDE
StCa	14.0 ± 1.1bD	22.7 ± 1.4aB	22.2 ± 1.3aBC	12.0 ± 0.8cE	19.2 ± 1.3aC	15.7 ± 1.1bD	15.6 ± 1.1bD	25.0 ± 1.5aA	24.4 ± 1.5aA
StCl	8.6 ± 0.4cFG	18.3 ± 1.1bD	26.2 ± 1.7aC	9.4 ± 0.5cF	20.2 ± 1.4bCD	28.8 ± 1.7aA	7.1 ± 0.3cG	15.1 ± 1.1bE	21.6 ± 1.1aC
Leaf ionic content									
LfNa	12.8 ± 0.8bD	14.9 ± 0.10aB	14.8 ± 0.10aB	9.5 ± 0.5bF	11.5 ± 0.7aE	10.4 ± 0.7bEF	11.4 ± 0.7cE	16.2 ± 1.1aA	13.5 ± 0.9bC
LfK	11.8 ± 0.7aA	8.6 ± 0.4bD	7.9 ± 0.4cE	9.5 ± 0.5aC	7.1 ± 0.3bF	6.4 ± 0.2cG	7.0 ± 0.3bF	10.0 ± 0.5aB	6.4 ± 0.2cG
LfCa	8.4 ± 0.4cG	11.3 ± 0.8bE	12.7 ± 0.8aD	9.1 ± 0.5cFG	12.3 ± 0.8bDE	14.6 ± 1.1aB	9.7 ± 0.5cF	13.1 ± 0.9bC	15.6 ± 1.1aA
LfCl	7.6 ± 0.3cD	9.6 ± 0.5bC	11.6 ± 0.7aA	6.3 ± 0.2cEF	7.9 ± 0.3bD	10.2 ± 0.5aB	5.4 ± 0.2cF	7.2 ± 0.3bDE	9.4 ± 0.4aC
Excreted ions									
ExNa	27.6 ± 1.6cF	132.2 ± 9.8bE	192.4 ± 14.6aD	28.6 ± 1.7cF	138.1 ± 11.4bE	292.1 ± 18.9aB	39.5 ± 2.6cF	245.7 ± 19.6bC	390.7 ± 24.6aA
ExK	10.9 ± 0.9cH	17.2 ± 1.3bG	18.2 ± 1.3aF	17.8 ± 1.3cFG	19.4 ± 1.2bE	22.5 ± 1.6aC	22.7 ± 1.2cB	27.9 ± 1.6aA	21.0 ± 1.5bD
ExCa	7.0 ± 0.2cH	10.8 ± 0.5bD	11.0 ± 0.9aC	8.1 ± 0.4cF	13.8 ± 1.1bB	14.8 ± 0.10aA	7.7 ± 0.3cG	9.0 ± 0.4bE	6.2 ± 0.2aI
ExCl	17.6 ± 1.3cE	37.9 ± 1.9bD	40.7 ± 2.8aBC	15.8 ± 1.1cE	33.8bD	48.8 ± 2.2aB	20.7 ± 1.2cE	38.6 ± 2.4bCD	55.7 ± 0.2aA

Table 5. Leaf ionic content and excreted ions of differently adapted ecotypes of *Sporobolus ioclados* Nees ex Trin. from the Cholistan Desert under salt stress. Means with similar letters (a, b or c) for each habitat are statistically non- significant at $p \leq 0.05$. Small letter indicate comparison within treatments, while capital letters indicate comparison of overall means. Ecotypes: LSE – Least saline ecotype, MSE – Moderately saline ecotype, HSE – Highest saline ecotype. Root ionic content: RtNa – Root Na^+ (mg g^{-1}), RtK – Root K + (mg g^{-1}), RtCa – Root Ca^{2+} (mg g^{-1}), RtCl – Root Cl^- (mg g^{-1}). Stem ionic content: StNa – Stem Na^+ (mg g^{-1}), StK – Stem K^+ (mg g^{-1}), StCa – Stem Ca^{2+} (mg g^{-1}), StCl – Stem Cl^- (mg g^{-1}). Leaf ionic content: LfNa – Leaf Na^+ (mg g^{-1}), LfK – Leaf K^+ (mg g^{-1}), LfCa – Leaf Ca^{2+} (mg g^{-1}), LfCl – Leaf Cl^- (mg g^{-1}). Excreted ions: ExNa – Na^+ (mg L^{-1}), ExK – K^+ (mg L^{-1}), ExCa – Ca^{2+} (mg L^{-1}), ExCl – Cl^- (mg L^{-1}).

300 mM in the Bailahwala Dahar (MSE) and Ladam Sir (HSE), while remained unaffected in the Derawar Fort (LSE) under salinity stress. Leaf water content was the highest at 300 mM level in the Derawar Fort (LSE), while it was maximum at 300 mM salinity in the Bailahwala Dahar (MSE) and at 150 mM in the Ladam Sir (HSE).

Root succulence was the highest at 0 mM in the Derawar Fort (LSE), while it was maximum at 300 mM level in the MSE and at 150 mM level in the Ladam Sir (HSE) (Table 6). Stem succulence was the highest at 300 mM NaCl in all ecotypes, the maximum was recorded in the Bailahwala Dahar (MSE). Leaf succulence was the greatest at 0 mM NaCl in the Derawar Fort (LSE) and Ladam Sir (HSE), while it remained unchanged in the Bailahwala Dahar (MSE).

Phytoremediation

Root, stem and leaf Na^+ and Cl^- bioconcentration factor decreased consistently with increasing salinity levels in all ecotypes of *S. ioclados* (Table 6). Excreted Na^+ bioconcentration factor was the maximum at 150 mM salinity in the Bailahwala Dahar (MSE) and Ladam Sir (HSE), while it was the highest in the Derawar Fort (LSE) at 0 mM NaCl. The excreted Cl^- bioconcentration factor, however, decreased with salinity in all three ecotypes.

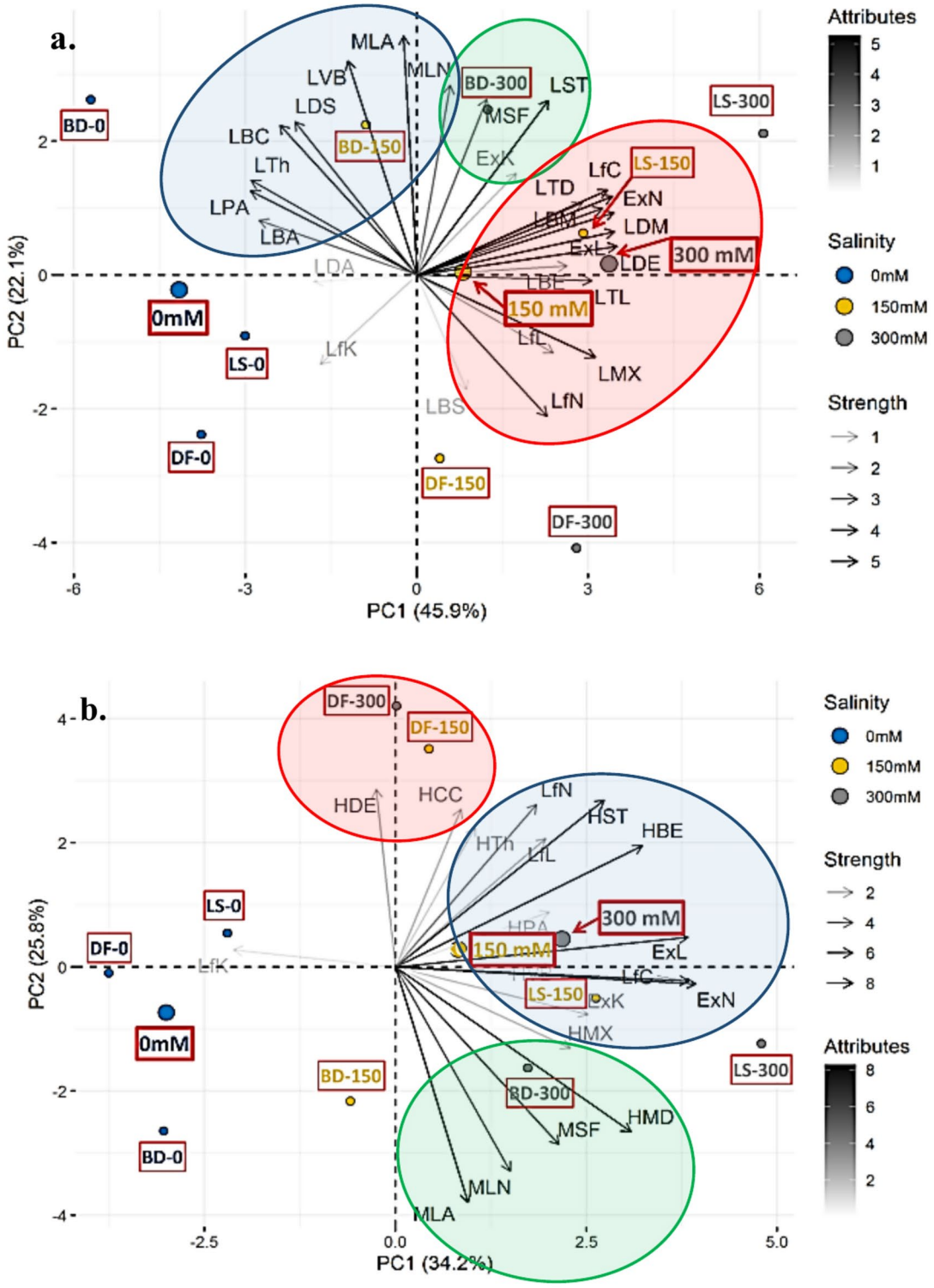
Stem Na^+ (STN) and Stem Cl^- (STC) translocation factor was the greatest in all three ecotypes at 0 mM NaCl (Table 6). Leaf Na^+ (LTN) translocation factor increased significantly as salinity levels increased in all three ecotypes. Leaf Cl^- (LTC) translocation factor was the maximum at 300 mM NaCl in the Bailahwala Dahar (MSE) and Ladam Sir (HSE), while at 150 mM in the Derawar Fort (LSE). Root, stem and leaf Na^+ and Cl^- dilution factor decreased consistently with salinity increasing levels in all three ecotypes.

Relationship between structural and functional traits in Sporobolus ioclados

Principle component analysis between growth and leaf anatomical traits showed three distinct clusters (Fig. 5a). Leaf metaxylem area (LMX) and trichome length (LTL) showed close association with growth attributes leaf Na^+ (LfN) and Cl^- (LfL) in the Derawar Fort (LSE) at 150 mM. The second cluster showed strong association between adaxial epidermal thickness with excretory Na^+ (ExN) in the Ladam Sir (HSE) at 300 mM, while adaxial microhairs density (LDM), abaxial microhairs density (LBM), trichome density (LTD) showed strong association with growth attributes like excretory Cl^- (ExL), and leaf Ca^+ (ExC) in the Ladam Sir (HSE) at 150 mM. The third cluster showed a strong relationship of excretory K^+ (ExK) with shoot fresh weight (MSF) and sclerenchymatous thickness (LST) in the Bailahwala Dahar (MSE) at 300 mM. The anatomical attributes like abaxial stomatal area

Habitats→	Derawar fort (LSE)			Bailahwala dahar (MSE)			Ladam sir (HSE)		
Salt levels→	0 mM	150 Mm	300 mM	0 mM	150 Mm	300 mM	0 mM	150 Mm	300 mM
Water content									
RWC	8.5 ± 0.4aC	7.6 ± 0.4bD	5.8 ± 0.2cF	6.8 ± 0.2cE	7.6 ± 0.3bD	9.9 ± 0.6aB	6.6 ± 0.3cE	11.0 ± 0.9aA	9.6 ± 0.5bB
SWC	5.4 ± 0.2aC	5.7 ± 0.2aBC	5.4 ± 0.2aC	6.0 ± 0.2bB	5.7 ± 0.2bBC	7.6 ± 0.5aA	5.7 ± 0.3bBC	6.0 ± 0.4bB	7.2 ± 0.3aA
LWC	12.3 ± 0.8bB	10.2 ± cC	12.9 ± 0.9aA	12.2 ± 0.9bB	13.1 ± 1.1aA	13.3 ± 1.1aA	13.0 ± 1.1aA	13.4 ± 0.9aA	12.9 ± 0.9aA
Succulence									
RSc	1.8 ± 0.4aB	1.6 ± 0.1bD	1.5 ± 0.1cE	1.5 ± 0.1bE	1.7 ± 0.1bC	1.6 ± 0.1aC	1.4 ± 0.1cF	1.9 ± 0.1aA	1.6 ± 0.1bD
SSc	5.5 ± 0.1bCD	4.8 ± 0.2cE	5.9 ± 0.2aB	5.6 ± 0.2cC	6.2 ± 0.3bB	9.4 ± 0.4aA	4.8 ± 0.2bE	4.8 ± 02bE	5.2 ± 0.2aD
LSc	4.5 ± 0.1aA	3.3 ± 0.1cF	3.7 ± 1.1bCED	4.0 ± 0.2abBC	3.7 ± 0.1bCDE	4.2 ± 0.2aAB	4.3 ± 0.2aAB	3.7 ± 0.2bCDE	3.5 ± 0.1bEF
Bioconcentration factor (BCF)									
RBN	0.037 ± 0.001aC	0.010 ± 0.001bF	0.013 ± 0.001bEF	0.043 ± 0.001aB	0.018 ± 0.001bE	0.010 ± 0.001cF	0.052 ± 0.002aA	0.021 ± 0.001bD	0.012 ± 0.001c
RBC	0.015 ± 0.001aA	0.008 ± 0.0004bD	0.009 ± 0.0004bCD	0.014 ± 0.001aA	0.010 ± 0.001bC	0.005 ± 0.0002cE	0.012 ± 0.001aB	0.009 ± 0.0004aCD	0.005 ± 0.0002bE
SBN	0.014 ± 0.001aA	0.007 ± 0.0003bC	0.008 ± 0.0004bC	0.011 ± 0.001aB	0.008 ± 0.0004aC	0.004 ± 0.0002bD	0.015 ± 0.001aA	0.010 ± 0.001bB	0.004 ± 0.0002cD
SBC	0.010 ± 0.001aA	0.003 ± 0.0001bDE	0.004 ± 0.0002bD	0.011 ± 0.001aA	0.006 ± 0.00031bC	0.003 ± 0.0001bDE	0.008 ± 0.0004aB	0.004 ± 0.0002bD	0.002 ± 0.0001bE
LBN	0.022 ± 0.001aA	0.004 ± 0.0002bEF	0.003 ± 0.0002bFG	0.016 ± 0.001aC	0.005 ± 0.00025bE	0.002 ± 0.0001bG	0.020 ± 0.002aB	0.007 ± 0.0003bD	0.002 ± 0.0001cG
LBC	0.009 ± 0.0003aA	0.002 ± 0.0001bDE	0.002 ± 0.0001bDE	0.007 ± 0.0004aBC	0.002 ± 0.0001bDE	0.001 ± 0.0001bE	0.006 ± 0.0003aC	0.002 ± 0.0001bDE	0.001 ± 0.0001bE
EBN	0.048 ± 0.002aE	0.038 ± 0.0001Gc	0.042 ± 0.0002bF	0.050 ± 0.002bE	0.060 ± 0.0025aC	0.042 ± 0.002cF	0.069 ± 0.003bB	0.107 ± 0.01aA	0.057 ± 0.003cD
EBC	0.020 ± 0.001aB	0.007 ± 0.0003bE	0.006 ± 0.0003bEF	0.018 ± 0.001aC	0.010 ± 0.001bD	0.005 ± 0.0002cF	0.023 ± 0.001aA	0.011 ± 0.001bD	0.005 ± 0.0003c
Translocation factor (TF)									
STN	1.55 ± 0.1aA	0.66 ± 0.03bC	0.40 ± 0.02cF	1.52 ± 0.1aA	0.60 ± 0.03bD	0.38 ± 0.01cF	1.31 ± 0.1aB	0.68 ± 0.03bC	0.47 ± 0.2cE
STC	0.89 ± 0.4aA	0.52 ± 0.03bC	0.44 ± 0.02bCD	0.67 ± 0.03aB	0.39 ± 0.02bDE	0.35 ± 0.01bEF	0.77 ± 0.3aB	0.48 ± 0.02bCD	0.43 ± 0.2bF
LTN	2.15 ± 1.3cE	8.89 ± 0.4bD	13.04 ± 0.9aBC	3.02 ± 0.2cE	11.97 ± 0.1bC	28.11 ± 1.9aA	3.46 ± 0.1cE	15.20 ± 1.2bB	29.01 ± 1.9aA
LTC	2.30 ± 1.5aE	3.96 ± 0.2aD	3.49 ± 0.1aD	2.51 ± 0.1bE	4.28 ± 0.2aC	4.81 ± 0.2aB	3.82 ± 0.1bD	5.37 ± 0.3aAB	5.95 ± 0.3aA
Dilution factor (DF)									
RDN	13.20 ± 1.1aC	3.87 ± 0.1bD	4.93 ± 0.2bD	16.43 ± 1.2aB	6.68 ± 0.4bBC	3.98 ± 0.2cD	19.20 ± 1.4aA	7.80 ± 0.4bB	5.15 ± 0.3cCD
RDC	5.28 ± 0.3aA	3.16 ± 0.1bBCD	3.51 ± 0.1bBC	5.20 ± 0.3aA	3.90 ± 0.1aB	2.03 ± 0.1bC	4.46 ± 0.2aAB	3.34 ± 0.1abBCD	1.92 ± 1.1bD
SDN	1.73 ± 0.1aAB	0.98 ± 0.04aCD	0.88 ± 0.3aD	1.41 ± 0.1aBC	0.92 ± 0.4abCDE	0.36 ± 0.1bE	2.27 ± 0.1aA	1.64 ± 0.1bD	0.71 ± 0.3cD
SDC	1.16 ± 0.1aB	0.52 ± 0.02bCD	0.41 ± 0.1bDE	1.38 ± aA	0.63 ± 0.3bC	0.24 ± 0.1cF	1.19 ± 0.1aAB	0.68 ± 0.3bC	0.35 ± 0.01cEF
LDN	7.81 ± 0.4aA	1.90 ± 0.1bE	1.51 ± 0.1bEF	6.75 ± 0.3aB	2.41 ± 1.8bD	0.62 ± 0.3cG	7.75 ± 0.3aA	3.44 ± 0.2bC	1.00 ± 0.05cFG
LDC	3.01 ± 0.1aA	0.79 ± 0.03bF	0.77 ± 0.3bF	2.91 ± 2.4aB	1.07 ± 0.1bD	0.39 ± 0.1cH	2.39 ± 0.1aC	0.99 ± 0.5bE	0.45 ± 0.02cG

Table 6. Succulence and phytoremediation traits in differently adapted ecotypes of *Sporobolus ioclados* Nees ex Trin. from the Cholistan Desert under salt stress. Means with similar letters (a, b or c) for each habitat are statistically non- significant at $p \leq 0.05$. Small letter indicate comparison within treatments, while capital letters indicate comparison of overall means. Ecotypes: *LSE* least saline ecotype, *MSE* moderately saline ecotype, *HSE* highest saline ecotype. Water content: *RWC* root water content, *SWC* stem water content, *LWC* leaf water content. Succulence: *RSc* Root succulence, *SSc* stem succulence, *LSc* leaf succulence. Bioconcentration factor: RBC – Root BCF, SBC – Stem BCF, LBC – Leaf blade BCF, EBC – Excreted ions BCF. Translocation factor: *STF* root to stem TF, *LTF* stem to leaf TF, Dilution factor *RDF* root DF, *SDF* Stem DF, *LDF* Leaf DF.



(LBS), phloem area (LPA), leaf thickness (LTh), bulliform cell area (LBC), adaxial stomatal density (LDS) and vascular bundle (LVB) were strongly associated with morphological traits, leaf area (MLA) and leaves per plant (Number of leaves) in the Bailahwala Dahar (MSE) at 150 mM (Fig. 5a).

Growth and leaf sheath anatomical traits presented three isolated groups (Fig. 5b). Morphological attributes like plant leaf area (MLA), leaves per plant (MLN), shoot fresh weight (MSF) showed strong relationship with microhair density (HMD) in the Bailahwala Dahar (MSE) at 300 mM. The second cluster showed strong association among morphological characteristics and leaf sheath metaxylem area (HMX) with excretory K^+ (ExK), excretory Na^+ (ExN) and leaf Ca^{2+} (LfC) in the Ladam Sir (HSE) at 150 mM. Leaf Na^+ (LfN), leaf Cl^- (LfL) and excretory Cl^- (ExL) showed strong association with leaf sheath thickness (HTh), sclerenchymatous thickness (HST), phloem area (HPA) and abaxial epidermal thickness (HBE) in the Ladam Sir (HSE) at both

◀**Fig. 5**. Principal component analysis showing relationship among structural and functional traits in *Sporobolus ioclados* from the Cholistan Desert grown hydroponically under different levels of salt. (**a**) Relationship between growth, leaf anatomy, ionic content and excretory ions, (**b**) Relationship between growth, leaf sheath anatomy, ionic content and excretory ions. Ecotypes: DF-Derawar Fort (LSE), BD-Bailahwala Dahar (MSE), LS-Ladam Sir (HSE); Morphological characteristics: MLN – Number of leaves (plant^{-1}). MLA – Total leaf area (cm^2), MRF – Root fresh weight (g plant^{-1}), MSF – Stem fresh weight (g plant^{-1}), MLF – Leaf fresh weight (g plant^{-1}), MRD – Root dry weight (g plant^{-1}), MSD – Stem dry weight (g plant^{-1}), MLD – Leaf dry weight (g plant^{-1}). Leaf blade anatomy: LTh – Leaf thickness (µm), LST – Sclerenchymatous thickness (µm), LDE – Adaxial epidermal cell area (µm^2), LBE – Abaxial epidermal cell area (µm^2), LBC – Bulliform cell area (µm^2), LVB – Vascular bundle area (µm^2), LMX – Metaxylem area (µm^2), LPA – Phloem area (µm^2). Epidermal appendages: LDM – Adaxial microhair density, LBM – Abaxial microhair density, LTD – Trichome density, LTL – Trichome length (µm), LDS – Adaxial stomatal density, LBS – Abaxial stomatal density, LDA – Adaxial stomatal area (µm^2), LBA – Abaxial stomatal area (µm^2). Leaf sheath anatomy: HTh – Leaf sheath thickness (µm), HDE – Adaxial epidermal cell area (µm^2), HBE – Abaxial epidermal cell area (µm^2), HCC – Parenchymatous cell area (µm^2), HST – Sclerenchyma thickness (µm), HVB – Vascular bundle area (µm^2), HMX – Metaxylem area (µm^2), HPA – Phloem area (µm^2).

150- and 300-mM levels. The third cluster showed a strong association between parenchymatous cell area (HCC) and adaxial epidermal thickness (HDE).

Chlorophyll pigments (chlorophyll *a* (Cha) and chlorophyll *b* (Chb) closely grouped with o mM levels of all three ecotypes (Fig. 6a), as were the gas exchange traits (transpiration rate (TrR), stomatal conductance (StC) and sub-stomatal CO_2 concentration (SCC). Water use efficiency (WUE) was strongly associated with the Bailahwala Dahar (MSE) at the highest salt level (300 mM). Among leaf water (Fig. 6b), Leaf water potential (WtP) and osmotic potential (OsP) were linked to 300 mM salt level, while turgor potential (TuP) was associated with the Ladam Sir (HSE) and Bailahwala Dahar (MSE) at 150 mM. Organic osmolytes such as total soluble proteins (TSP), total soluble sugars (TSS) and proline content (TPr) were clustered with the Ladam Sir (HSE) at 0 and 150 mM, and MSD at 150 mM.

Heatmaps showing relationship of structural and functional traits

Among leaf sheath anatomical traits, leaf area was negatively correlated with leaf sheath metaxylem area and excretory K in the Derawar Fort (LSE) at 0 mM, (Fig. 7a). A negative correlation of leaf number and leaf area was observed with microhair density and metaxylem area in the Derawar Fort (LSE) at 300 mM. Leaf Na^+ negatively influenced leaf sheath anatomical traits such as abaxial epidermal thickness, leaf sheath thickness, vascular bundle area and phloem area. A positive correlation of leaf Na^+ was recorded with parenchymatous cell area, abaxial epidermal thickness, sclerenchymatous thickness and leaf sheath thickness in the Derawar Fort (LSE)at 150 mM. Leaf blade anatomical traits like leaf thickness, adaxial stomatal density and vascular bundle area showed positive correlation with leaf area in the Bailahwala Dahar (MSE) at 0 mM. A closely associated cluster was noticed among excretory ions (Na^+, Cl^- and K^+) with leaf anatomical traits such as adaxial epidermal thickness, adaxial microhair density, sclerenchymatous thickness, trichome length and trichome density, and morphological traits such as leaf number and stem fresh weight (Fig. 7b).

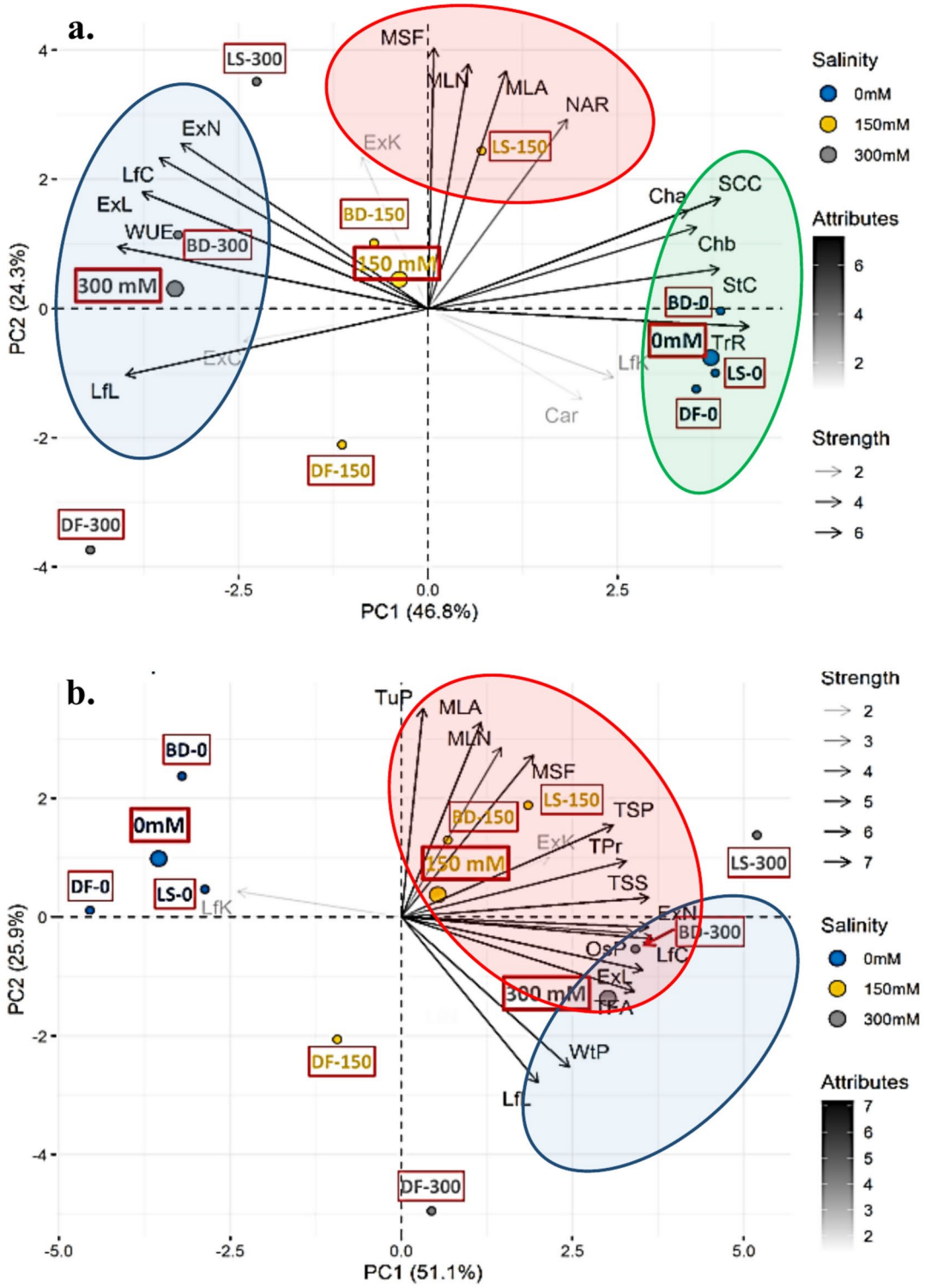
Excretory ions such as Na^+, Cl^- and Ca^{2+} were positively correlated with leaf Cl^-, leaf Ca^{2+} and water use efficiency in Ladam Sir (HSE) and Bailahwala Dahar (MSE) at 300 mM (Fig. 7c). Net CO_2 assimilation rate showed positive correlation with leaf number, leaf area and stem fresh weight in the HSE at 300 mM, while negatively correlated with the Derawar Fort (LSE) at 300 mM. Organic osmolytes such as soluble protein, soluble sugars and proline positively correlated with excretory K^+ in the HSE at 150 and 300 mM, whereas negatively in the Derawar Fort (LSE) at 0 mM (Fig. 7d). Free amino acids and osmotic potential positively correlated with excretory Na^+ and Cl^- and leaf Ca^{2+} in the Ladam Sir (HSE) at 300 mM.

Estimated response curves

Response curves among excretory ions and morphological traits showed high variability, particularly in the Derawar Fort (LSE) (Fig. 8a). The influence of excreted ions in leaf sheath anatomical traits was relatively linear, however slight variation was recorded in the Derawar Fort (LSE) (Fig. 8b). Response of excreted ions on leaf blade anatomical traits showed variability, especially in the Ladam Sir (HSE). Variability was relatively low in the Derawar Fort (LSE) and Bailahwala Dahar (MSE) (Fig. 8c). Variability was the maximum regarding influence of excretory ions on epidermal appendages, the Derawar Fort (LSE) showed greater variability (Fig. 8d). Response of excreted ions to leaf water relations and organic osmolytes was linear (Fig. 8e), while high deviation was seen in gas exchange traits and chlorophyll pigments in response to excretory ions (Fig. 8f). Leaf ionic content deviated strongly in response to excreted ions, the Derawar Fort (LSE) was relatively more deviated (Fig. 8g).

Discussion

Plant adaptations develop in species of even in ecotypes when exposed to a specific set of environments for longer periods[40]. Several researchers reported structural and functional variations in ecotypic of various plant species of the family Poaceae. The examples are Qian et al.[41] in Kentucky bluegrass (*Poa pratensis*), Horie et al.[42] in rice (*Oryza sativa*), Hameed et al.[18] in *Sporobolus arabicus*, Hameed et al.[43] in *Imperata cylindrica,* Rahat et al.[44] in *Diplanchne fusca,* Naz et al.[45] in *Suaeda vera* and Fatima et al.[46] in *Aristida adscensionis*. Ecotypes of *Sporobolus ioclados* were collected at a distance at least 50 km from each other. Structural and functional mechanisms were different in these ecotypes suggesting the independent evolution within the Cholistan Desert.



Leaf number and area significantly reduced by salinity in the Derawar Fort (LSE) and Bailahwala Dahar (MSE). A decrease in leaf number and area is an important modification that significantly reduces transpiration rate. Leaf fresh and dry weights increased in Derawar Fort (LSE) under stress, whereas root, stem and leaf fresh weight increased in Bailahwala Dahar (MSE) and Ladam Sir (HSE). Dry weights of underground and above ground organs generally increased in Ladam Sir (HSE), which is a clear sign of high salinity tolerance[47,48]. The HSE maintained growth and development under high salinities, as was reported in halophytic species[17,49]. The Derawar Fort (LSE) showed better growth at 150 mM salt level, but growth parameters reduced at higher salinities.

Leaf anatomical traits responded similarly in all three ecotypes with few exceptions. Leaf traits like sclerenchymatous thickness, epidermal cell area on both leaf surfaces, and metaxylem area increased with salinity levels. Sclerification is linked to mechanical strength of soft tissue[45]. It also plays a role in prevention of water

◀**Fig. 6**. Principal component analysis showing relationship among structural and functional traits in *Sporobolus ioclados* from the Cholistan Desert grown hydroponically under different levels of salt. (**a**) Relationship between growth, physiology, ionic content and excretory ions, (**b**) Relationship between growth, leaf water relations, ionic content and excretory ions. Ecotypes: DF-Derawar Fort (LSE), BD-Bailahwala Dahar (MSE), LS-Ladam Sir (HSE). Morphological characteristics: MLN – Number of leaves (plant^{-1}). MLA – Total leaf area (cm^2), MRF – Root fresh weight (g plant^{-1}), MSF – Stem fresh weight (g plant^{-1}), MLF – Leaf fresh weight (g plant^{-1}), MRD – Root dry weight (g plant^{-1}), MSD – Stem dry weight (g plant^{-1}), MLD – Leaf dry weight (g plant^{-1}). Leaf water relations: OsP – Osmotic potential (– MPa), WtP – Water potential (– MPa), TuP – Turgor potential (MPa). Organic osmolytes: TFA – Total free amino acids (µg g^{-1}), TSP – Total soluble proteins (µg g^{-1}), TSS – Total soluble sugars (mg g^{-1}), TPr – Total proline (µg g^{-1}). Gas exchange parameters: NAR – Net assimilation rate (µmol m^{-2} s^{-1}), TrR – Transpiration rate (mmol m^{-2} s^{-1}), StC – Stomatal conductance (mmol m^{-2} s^{-1}), SCC – Substomatal CO_2 concentration (µmol mol^{-1}), WUE – Water use efficiency. Photosynthetic pigments: Cha – Chlorophyll a (mg g^{-1}), Chb – Chlorophyll b (mg g^{-1}), Car – Carotenoids (mg g^{-1}). Root ionic content: RtN – Root Na^+ (mg g^{-1}), RtK – Root K + (mg g^{-1}), RtC – Root Ca^{2+} (mg g^{-1}), RtL – Root Cl^- (mg g^{-1}). Stem ionic content: StN – Stem Na^+ (mg g^{-1}), StK – Stem K^+ (mg g^{-1}), StV – Stem Ca^{2+} (mg g^{-1}), StL – Stem Cl^- (mg g^{-1}). Leaf ionic content: LfN – Leaf Na^+ (mg g^{-1}), LfK – Leaf K^+ (mg g^{-1}), LfC – Leaf Ca^{2+} (mg g^{-1}), LfL – Leaf Cl^- (mg g^{-1}). Excreted ions: ExN – Na^+ (mg L^{-1}), ExK – K^+ (mg L^{-1}), ExC – Ca^{2+} (mg L^{-1}), ExL – Cl^- (mg L^{-1}).

loss especially when in and outside vascular tissue[50]. Intensity of sclerification, however, was significantly higher in Ladam Sir (HSE) than its counterparts. Thick epidermis on leaf surface is a characteristic of desert species, a critical modification for water conservation in harsh arid, saline and hot conditions[23]. Epidermis at both leaf surfaces was significantly thicker in HSE than Derawar Fort (LSE) or Bailahwala Dahar (MSE), which indicates a higher degree of salt tolerance in this ecotype. Metaxylem vessel diameter increased significantly under salinity. Broader vessels are associated with better conduction of water and nutrients[7], and under high salinities this modification contributes towards nutrient translocation for various metabolic processes[51]. Leaf vascular bundle area increased with salinity stress only on the Ladam Sir (HSE). This ecotype maintained normal development of vascular tissue under high salinities, hence more tolerant than other ecotypes. Leaf thickness significantly decreased under salinity stress in all ecotypes, as was the thickness of bulliform cells. Grooved bulliform cells in the Ladam Sir (HSE) and Derawar Fort (LSE), in addition to thinner leaves is a critical modification for easier leaf rolling[44]. Such condition controls transpiration rate by protecting stomata and adaxial leaf surface from direct contact with external environment[52]. Large parenchymatous bulliform cells on entire adaxial side of midrib in the Bailahwala Dahar (MSE) may not involve in leaf rolling but immensely important in storing additional water[53].

Epidermal traits like microhairs density on both leaf surfaces, trichome length and density increased invariably in all ecotypes under salinity. Microhairs are small appendages specifically associated with salt excretion by bursting and releasing salts outside plant body[45]. Trichomes, in contrast, is a characteristic of desert species. Trichomes lower the leaf temperature[54] break wind intensity and reflect solar radiation, that results in lowering of transpiration rate and critically important to colonize plants in saline desert environments[55]. Density of microhairs and trichomes and trichome length were significantly larger in the Ladam Sir (HSE) than in the other two ecotypes, indicating its better adaptation for high salinities. Size of bundle sheath cells increased only in the Derawar Fort (LSE) under salinity, which is associated with C_4 photosynthesis[56].

Thicker leaf sheaths were observed in the Derawar Fort (LSE) under salinity stress, which was primarily due to storage parenchyma proportion. This feature is helpful under longer periods of drought (or physiological drought) by storing more water in parenchymatous tissue[9]. Abaxial epidermal thickness increased under salinity in all ecotypes, a characteristic of desiccation tolerant desert species[44]. Sclerification on outer surface of leaf sheath, especially outside vascular bundles also increased significantly under salinity. This is not only associated with mechanical strength to metabolically active tissues[54], but also prevents water loss[57]. Other leaf sheath anatomical traits like adaxial epidermal thickness, and areas of parenchymatous cell, vascular bundle, metaxylem and phloem responded invariably to salinity stress, i.e., increased in some ecotypes while decreased in others under salinity stress.

The Ladam Sir (HSE) showed distinct behaviour in terms of gas exchange parameter (especially water use efficiency), accumulation of organic osmolytes and concentration of chlorophyll pigments. All these were significantly higher in this ecotype at the highest salt level. This is a clear-cut indication of a higher degree of tolerance than its counterparts[53]. The Ladam Sir (HSE) accumulated all osmolytes in greater amounts, which confers its better adaptation[58]. Another unique feature of Ladam Sir (HSE) was the increased net assimilation rate under salinity stress, which is directly linked to water use efficiency. This is important for survival under high salinities[59].

Ionic content like Na^+, Ca^{2+} and Cl^- increased under salinity in all ecotypes, while K^+ content decreased in all ecotypes. In *S. ioclados,* K^+ may not be involved in neutralizing Na^+ toxicity, instead Ca^{2+} uptake is more important. Uptake of Ca^{2+} among with Na^+ has earlier been reported by Ievinsh et al.[60] and Kapadia et al.[61] in tomato; Naz et al.[26] in sewan grass (*Lasiurus scindicus* Henr.), Tadayyon et al.[62] in castor oil plant (*Ricinus communis* L.) plant and Hassan et al.[63] in in barley (*Hordeum vulgare* L.). Effective excretion of toxic salts out of the plant body is a major adaptation of many halophytic species[64,65]. Increased density of salt-excretory microhairs under salinity was noticed in all ecotypes. In the Ladam Sir (HSE), density of microhairs on both leaf surfaces was exceedingly high, particularly at higher salt levels. This may justify the higher rate of Na^+ and

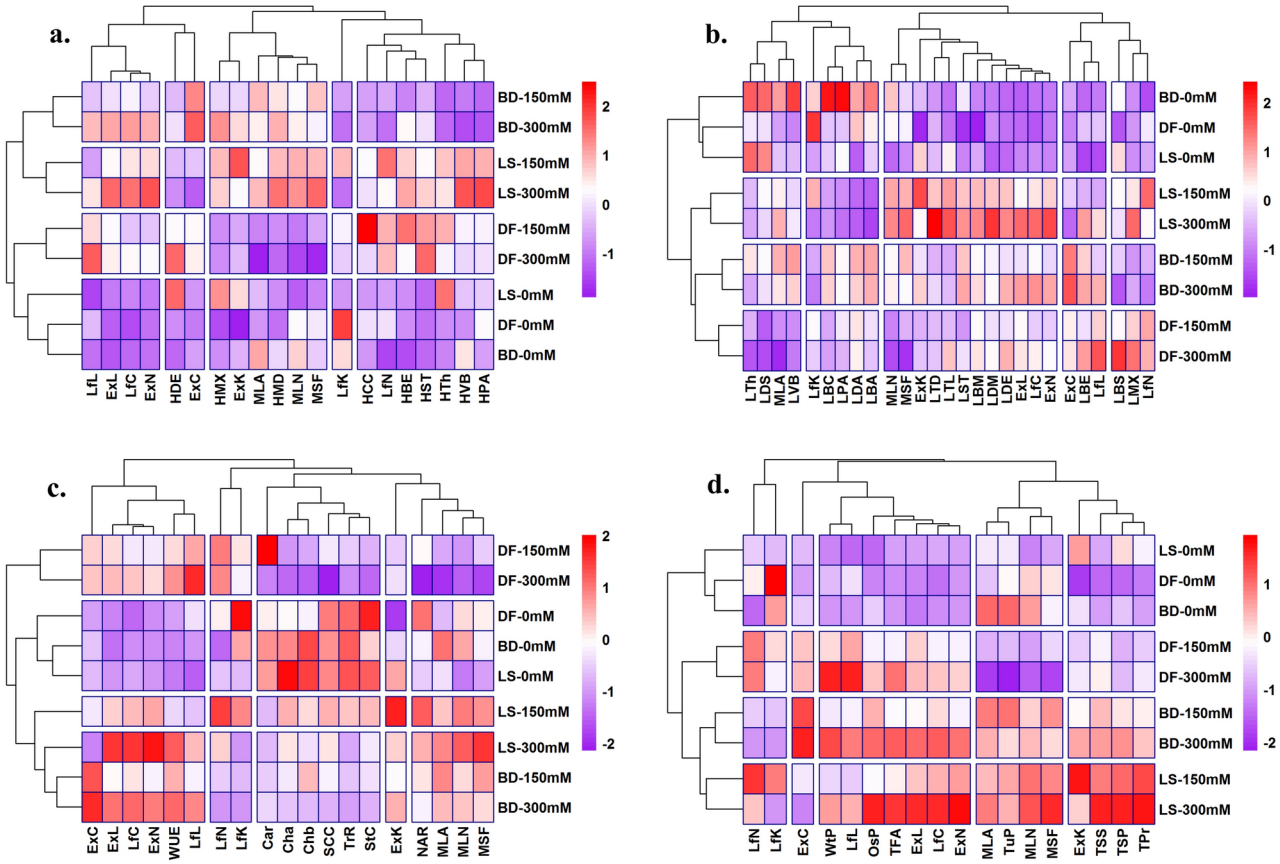
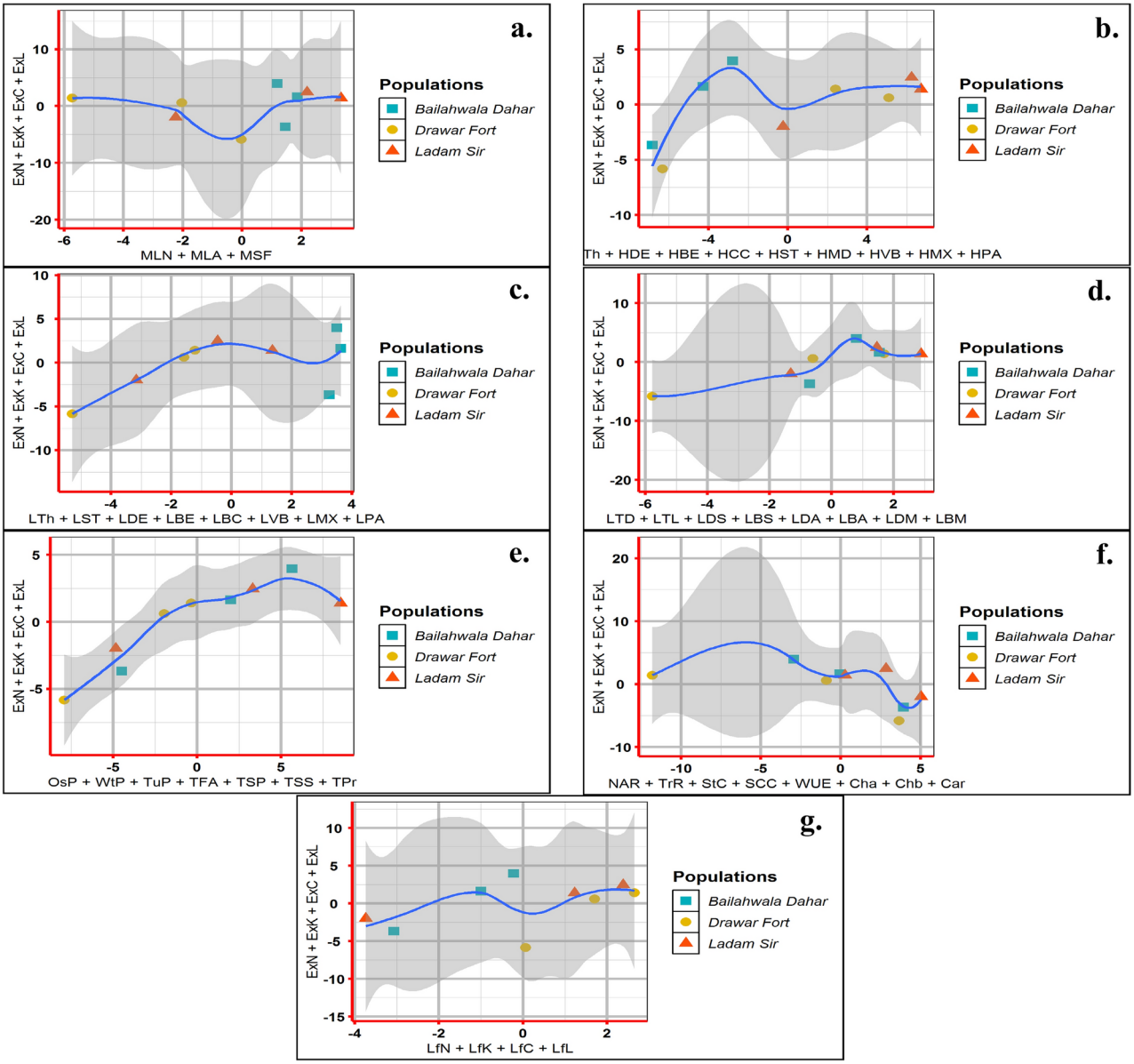


Fig. 7. Heatmaps showing relationship among structural and functional traits in *Sporobolus ioclados* from the Cholistan Desert grown hydroponically under different levels of salt. (**a**) Relationship between growth, leaf sheath anatomy and excretory ions, b. Relationship between growth, leaf anatomy and excretory ions, c. Relationship between growth, physiology and excretory ions, d. Relationship between growth, leaf water relations and excretory ions. Ecotypes: DF-Derawar Fort (LSE), BD-Bailahwala Dahar (MSE), LS-Ladam Sir (HSE). Morphological characteristics: MLN – Number of leaves (plant^{-1}). MLA – Total leaf area (cm^2), MSF – Stem fresh weight (g plant^{-1}). Leaf sheath anatomy: HTh – Leaf sheath thickness (µm), HDE – Adaxial epidermal cell area (µm^2), HBE – Abaxial epidermal cell area (µm^2), HCC – Parenchymatous cell area (µm^2), HST – Sclerenchyma thickness (µm), HVB – Vascular bundle area (µm^2), HMX – Metaxylem area (µm^2), HPA – Phloem area (µm^2). Leaf blade anatomy: LTh – Leaf thickness (µm), LST – Sclerenchymatous thickness (µm), LDE – Adaxial epidermal cell area (µm^2), LBE – Abaxial epidermal cell area (µm^2), LBC – Bulliform cell area (µm^2), LVB – Vascular bundle area (µm^2), LMX – Metaxylem area (µm^2), LPA – Phloem area (µm^2). Epidermal appendages: LDM – Adaxial microhair density, LBM – Abaxial microhair density, LTD – Trichome density, LTL – Trichome length (µm), LDS – Adaxial stomatal density, LBS – Abaxial stomatal density, LDA – Adaxial stomatal area (µm^2), LBA – Abaxial stomatal area (µm^2). Leaf water relations: OsP – Osmotic potential (– MPa), WtP – Water potential (– MPa), TuP – Turgor potential (MPa). Organic osmolytes: TFA – Total free amino acids (µg g^{-1}), TSP – Total soluble proteins (µg g^{-1}), TSS – Total soluble sugars (mg g^{-1}), TPr – Total proline (µg g^{-1}). Gas exchange parameters: NAR – Net assimilation rate (µmol m^{-2} s^{-1}), TrR – Transpiration rate (mmol m^{-2} s^{-1}), StC – Stomatal conductance (mmol m^{-2} s^{-1}), SCC – Substomatal CO_2 concentration (µmol mol^{-1}), WUE – Water use efficiency. Photosynthetic pigments: Cha – Chlorophyll a (mg g^{-1}), Chb – Chlorophyll b (mg g^{-1}), Car – Carotenoids (mg g^{-1}). Root ionic content: RtN – Root Na^+ (mg g^{-1}), RtK – Root K + (mg g^{-1}), RtC – Root Ca^{2+} (mg g^{-1}), RtL – Root Cl^- (mg g^{-1}). Stem ionic content: StN – Stem Na^+ (mg g^{-1}), StK – Stem K^+ (mg g^{-1}), StV – Stem Ca^{2+} (mg g^{-1}), StL – Stem Cl^- (mg g^{-1}). Leaf ionic content: LfN – Leaf Na^+ (mg g^{-1}), LfK – Leaf K^+ (mg g^{-1}), LfC – Leaf Ca^{2+} (mg g^{-1}), LfL – Leaf Cl^- (mg g^{-1}). Excreted ions: ExN – Na^+ (mg L^{-1}), ExK – K^+ (mg L^{-1}), ExC – Ca^{2+} (mg L^{-1}), ExL – Cl^- (mg L^{-1}).

Cl^- excretion in this ecotype. Single-celled trichomes were also seen in the Ladam Sir (HSE), which were absent in other ecotypes. This may provide additional protection in minimizing water loss from leaf surface[44].

Tissue water content and succulence generally increased in the Bailahwala Dahar (MSE) and Ladam Sir (HSE) under salinity, which is a critical adaptation in these ecotypes. This will increase their capability of surviving in prolonged periods of drought[49]. The bioconcentration factor and dilution factor of Na^+ and Cl^- invariably decreased with salinity levels in all ecotypes. The major salt tolerance mechanism might not the salt compartmentalization, rather this species relied on salt excretion. This was confirmed by leaf translocation factor of Na^+ and Cl^-, which increased in the Bailahwala Dahar (MSE) and HSE under salinity[66].



Response to salinity stress of all three ecotypes was different under controlled conditions, which is an indication of their independent evolution while growing in differently salt-affected habitats[54]. The Derawar Fort (LSE) showed better growth at a lower salt level. Specific features for salinity tolerance were thick leaves, larger vascular bundles and phloem area in the leaf blade. Additionally, thick epidermis equipped with dense microhairs is associated with salinity tolerance. The major physiological traits that caused reduction in biomass production were poorly maintained turgor and photosynthesis rate. The Bailahwala Dahar (MSE) depended on proportion of parenchymatous cells and increased photosynthesis rate, for salinity tolerance. Furthermore, high concentration of Ca^{2+} and K^{+} in leaves can neutralize the Na^{+} or Cl^{-} toxicity.

Salinity tolerance in the Ladam Sir (HSE) relied on several structural and functional features for high degree of salinity tolerance. Structural features were high proportion of sclerenchyma, thick epidermis, high density of salt-excretory microhairs and trichomes, thicker leaves, large vascular bundles and greater proportion of phloem. Functional features were better maintenance of turgor and high concentration of osmolytes. In addition, better maintenance of photosynthesis (and water use efficiency) and better excretion of toxic ions indicated high salinity tolerance of the Ladam Sir (HSE).

Conclusion

All three ecotypes showed different structural and functional modifications to cope with saline stress of the growth medium. Overall, the mechanism based on morpho-anatomical and physiological features is very much developed in the Ladam Sir (HSE). This ecotype can safely be concluded as an excellent genotype for phytoremediation and revegetation of saline lands. More importantly, features such as proportion of sclerenchyma, density of trichomes and microhairs, salt excretion, leaf succulence, photosynthesis rate and accumulation of organic osmolytes are the yardstick to judge degree of salinity tolerance in *S. ioclados* ecotypes.

◀**Fig. 8**. Estimated response of structural and functional traits in *Sporobolus ioclados* from the Cholistan Desert grown hydroponically under different levels of salt. (**a**) Response of excretory ions to growth parameters, (**b**) Response of excretory ions to leaf sheath anatomical parameters, (**c**) Response of excretory ions to leaf anatomical parameters, (**d**) Response of excretory ions to leaf epidermal appendages, (**e**) Response of excretory ions to leaf water relations and osmoprotectants, (**f**) Response of excretory ions to gas exchange parameters and chlorophyll pigments, (**g**) Response of excretory ions to leaf ionic content. Ecotypes: DF-Derawar Fort (LSE), BD-Bailahwala Dahar (MSE), LS-Ladam Sir (HSE). Morphological characteristics: MLN – Number of leaves (plant^{-1}). MLA – Total leaf area (cm^2), MSF – Stem fresh weight (g plant^{-1}). Leaf sheath anatomy: HTh – Leaf sheath thickness (µm), HDE – Adaxial epidermal cell area (µm^2), HBE – Abaxial epidermal cell area (µm^2), HCC – Parenchymatous cell area (µm^2), HST – Sclerenchyma thickness (µm), HVB – Vascular bundle area (µm^2), HMX – Metaxylem area (µm^2), HPA – Phloem area (µm^2). Leaf blade anatomy: LTh – Leaf thickness (µm), LST – Sclerenchymatous thickness (µm), LDE – Adaxial epidermal cell area (µm^2), LBE – Abaxial epidermal cell area (µm^2), LBC – Bulliform cell area (µm^2), LVB – Vascular bundle area (µm^2), LMX – Metaxylem area (µm^2), LPA – Phloem area (µm^2). Epidermal appendages: LDM – Adaxial microhair density, LBM – Abaxial microhair density, LTD – Trichome density, LTL – Trichome length (µm), LDS – Adaxial stomatal density, LBS – Abaxial stomatal density, LDA – Adaxial stomatal area (µm^2), LBA – Abaxial stomatal area (µm^2). Leaf water relations: OsP – Osmotic potential (– MPa), WtP – Water potential (– MPa), TuP – Turgor potential (MPa). Organic osmolytes: TFA – Total free amino acids (µg g^{-1}), TSP – Total soluble proteins (µg g^{-1}), TSS – Total soluble sugars (mg g^{-1}), TPr – Total proline (µg g^{-1}). Gas exchange parameters: NAR – Net assimilation rate (µmol m^{-2} s^{-1}), TrR – Transpiration rate (mmol m^{-2} s^{-1}), StC – Stomatal conductance (mmol m^{-2} s^{-1}), SCC – Substomatal CO_2 concentration (µmol mol^{-1}), WUE – Water use efficiency. Photosynthetic pigments: Cha – Chlorophyll a (mg g^{-1}), Chb – Chlorophyll b (mg g^{-1}), Car – Carotenoids (mg g^{-1}). Root ionic content: RtN – Root Na^+ (mg g^{-1}), RtK – Root K + (mg g^{-1}), RtC – Root Ca^{2+} (mg g^{-1}), RtL – Root Cl^- (mg g^{-1}). Stem ionic content: StN – Stem Na^+ (mg g^{-1}), StK – Stem K^+ (mg g^{-1}), StV – Stem Ca^{2+} (mg g^{-1}), StL – Stem Cl^- (mg g^{-1}). Leaf ionic content: LfN – Leaf Na^+ (mg g^{-1}), LfK – Leaf K^+ (mg g^{-1}), LfC – Leaf Ca^{2+} (mg g^{-1}), LfL – Leaf Cl^- (mg g^{-1}). Excreted ions: ExN – Na^+ (mg L^{-1}), ExK – K^+ (mg L^{-1}), ExC – Ca^{2+} (mg L^{-1}), ExL – Cl^- (mg L^{-1}).

Data availability

Raw data can be accessed by Corresponding author Syed Mohsan Raza Shah, Assistant Professor Department of Botany, University of Education Dera Ghazi Khan Campus, Pakistan). The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions
N.N: Planned and carried out the experimental work; M.H: Supervisor who planned the research work; F.A.: Supervised research planning, collection and data analysis; M.S.A.A., K.S.A.: Biostatistician; data visualization, modeling and interpretation; S.M.R.S., S.B., S.F., A.A., Z.A.: Research execution, biochemical analysis, anatomical photography and data collection; K.F.A., G.D.A.Q., A.H. and E.F.A.: writing, reviewing and editing; A.H. and E.F.A: funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
The study does not include any animal or human subjects and no specific ethical approval is needed. Other necessary guidelines set by University of Agriculture, Faisalabad for handling of plant material during conduction of laboratory work were followed. All samplings were done with the least possible disturbances to plant communities and environment. After completion of study, all experimental materials were properly discarded/incinerated in a controlled environment to avoid bio-contamination.

Plant ethics statement
The voucher specimens used for plant identification are deposited to the herbarium facility of the Department of Botany, University of Agriculture, Faisalabad, and are available for verification on request. Voucher number were 19-1-2018 (Moderately saline Bailahwala Dahar), 19-2-2018 (least saline Derawar Fort), and 19-3-2018 (highly saline Ladam Sir). The specimens were verified from Dr. Mansoor Hameed, Professor and Plant Taxonomist, Department of Botany, University of Agriculture Faisalabad, Pakistan and Dr. Farooq Ahmad, Associate Professor and expert in Grass Systematics, Department of Botany, University of Agriculture Faisalabad, Pakistan. Specimens can be accessed by Corresponding author Syed Mohsan Raza Shah, Assistant Professor Department of Botany, University of Education Dera Ghazi Khan Campus, Pakistan). Anatomical slides, photographs and raw data calculated from these photographs are available can be requested if needed can be accessed by Syed Mohsan Raza Shah, Assistant Professor Department of Botany, University of Education Dera Ghazi Khan Campus, Pakistan.

Publication ethics statements
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All authors agree to publish and there is no conflict for publication of this manuscript for publication in this Journal.

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