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DEFENSE THROUGH FOLIAR ANATOMICAL ALTERATIONS IN LARGE SPIKE BUFFEL GRASS [*CENCHRUS PRIEURII* (KUNTH) MAIRE] AGAINST MULTIPLE ENVIRONMENTAL STRESSES

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Abstract

Salinity, drought, and temperature stresses are major environmental threats that change the plants' distributional pattern in natural ecosystems. *Cenchrus prieurii* (Kunth) Maire is a typical desert grass species that can colonize different habitats i.e., cool mountains, arid and semiarid regions. It was hypothesized that wide adaptability potential in *Cenchrus prieurii* may be linked to plasticity in morpho-anatomical features. *Cenchrus prieurii* populations were collected from different habitats of the Punjab, Khyber Pakhtoonkhwa and Azad Jammu and Kashmir, which were exposed to multiple environmental stresses, i.e., aridity, salinity, waterlogging and cool temperature and were evaluated for foliar structural modifications. All populations showed morpho-anatomical modifications that were related to water conservation. The Thal Desert populations such as collected from inter-dune flats (DID), marginal area (DMA) and loamy sand (DLS) were superior in midrib, lamina, epidermal and mesophyll thicknesses, and phloem area. The populations facing cold stress like foothill region (CFH) showed thicker lamina, epidermal, thickness, and larger vascular bundles and metaxylem vessels. The population from highest elevation (CHE) collected from the coolest habitats showed the largest parenchymatous cells. The sand dune population (DSD) was collected from the driest area had the thinnest leaves (midrib and lamina), epidermis layer and mesophyll, the smallest parenchymatous cells, bulliform cells, metaxylem vessels and vascular bundles. Another specific feature of SDS population was the formation of large aerenchymatous cavities and numerous microhairs in leaf sheath. Plasticity in anatomical traits was significantly high in all populations, and this might be a strong reason for successful colonization and adaptability of this species to a variety of habitat types, hence contributing significantly to its wide distributional range.

Key words: Cold, Aridity, Salinity, Foliar anatomy, Adaptions.

Introduction

Abiotic stresses like drought, salinity, heat, low temperature, and heavy metals inhibit plant growth and development. Environmental stresses have become a major problem throughout the world due to constant change in the global climatic conditions (Etesami & Maheshwari, 2018). Salinity, drought, and temperature stresses are major environmental threats that change the geographical pattern and plants distributional range in natural ecosystems (Naing & Kim, 2021). Changing climatic pattern is the main cause of the adverse effects of environmental stresses (Khan *et al.*, 2019). Native plant species or populations have capability to survive under the harsh environmental conditions such as, extreme temperature, excessive or less supply of water content, salinity, cold, high wind pressure, and other osmotic stresses (Mariani & Ferrante, 2017). Drought and salinity stress are those environmental stresses that influence soil fertility and alter the land and vegetation structure (Balraj *et al.*, 2017; Ismail *et al.*, 2019).

When grasses are exposed to different environmental stresses, different changes occur in the morpho-anatomical features to survive under harsh environmental conditions (Linder *et al.*, 2018). Morpho-anatomical adaptations in grasses such as thick epidermis, hairiness on abaxial leaf surfaces, small but numerous stomata, lignin deposition in vascular and mechanical tissues are critical for their survival in harsh environmental conditions (Jooste *et al.*, 2016; Abd El-Maboud & Abd Elbar, 2020). Leaf pubescence and stomatal density is linked with prevention of water loss, while leaf pubescence also lowers leaf temperature (Karabourniotis *et al.*, 2020). Plant species growing in different habitats and environmental conditions show great plasticity in leaf trichomes density (Bibi *et al.*, 2021). Anatomical modifications for stress tolerance in grasses are modified and well developed bulliform cells, ribs and furrows on adaxial leaf surface, intensive sclerification and large proportion of storage parenchyma (Ahmad *et al.*, 2016).

Distributional pattern of plant species is primarily controlled by the prevalent environmental conditions that were fixed over prolonged evolutionary period (Khidr *et al.*, 2017). Environmental variation induces certain features in plant species at population level making their survival success in their native habitats. These specific structural, functional modifications are revealed as morpho-anatomical, physiological, and biochemical attributes (Yamori *et al.*, 2014). No work has been reported on *C. prieurii,* especially in relation to anatomical modifications. *Cenchrus* species like *C. biflorus*, *C. ciliaris*, *C. pennisetiformis* and *C. setigerus* can tolerate environmental stresses like aridity, salinity and temperature. A similar response was expected in *C. prieurii* because of its widespread distributional range. It was, therefore, hypothesized that wide adaptability potential in *C. prieurii* may be linked to plasticity in morpho-anatomical features, which enable this species to inhabit multiple stresses. The research questions to be addressed in the study are: 1) which adaptive components in *C. prieurii* are critical for the distributional pattern and survival of this species under multiple environmental stresses? and 2) How specific anatomical modifications vary in differently adapted populations of this grass?

Materials and Methods

Collection of plant material: *Cenchrus prieurii* (Kunth) Maire populations were collected from different habitats of the Punjab, Khyber Pakhtoonkhwa and Azad Jammu and Kashmir, which were exposed to multiple environmental stresses (Fig. 1). Populations from arid regions (Thal Desert) were collected from Sandy desert (DSD), Interdune desert plain (IDS), Marginal area (DMA), and Loamy sand (LS). The cold region populations were collected from Pine Forest (CPF) from Murree region in the Punjab, and Foothills (CFH) from Abbottabad and Highest elevation (CHE) from Thandiani in the Khyber Pakhtoonkhwa. The saline area population was collected from dryland salinity (SDS), moist habitats from the riverbank (MRB) and roadside population from express way Islamabad to Murree (RMW). Six plants of average size from each population keeping a plant-to-plant distance of at least 3 m, and considered as replications. The plants were carefully uprooted with soil auger (20 cm dia.) and sealed in plastic zipper bags. The material was then kept in an icebox and later brought to the laboratory for further analysis. The populations of *C. prieurii* were evaluated for foliar structural modifications under various environmental stresses.

Physiographic and environmental data: Geographic data like altitude, longitude and latitude were recorded by GPS Garmin (eTrex Venture HC, Germany). Meteorological data such as maximum and minimum temperature, annual rainfall and snowfall was collected from the Meteorological Department of Pakistan, Islamabad (https://rmcpunjab.pmd.gov.pk/metData.php).

Soil analysis: Soil from nearby root rhizosphere of each population of the species was taken at the depth of (15-20 cm) to investigate physicochemical features (Table 1). A 200 g of soil sample was taken and dried completely, and then was prepared for measuring ionic content and soil ECe. Saturation percentage was calculated by a formula:

Saturation percentage = Weight of a saturated paste – Dry weight of soil

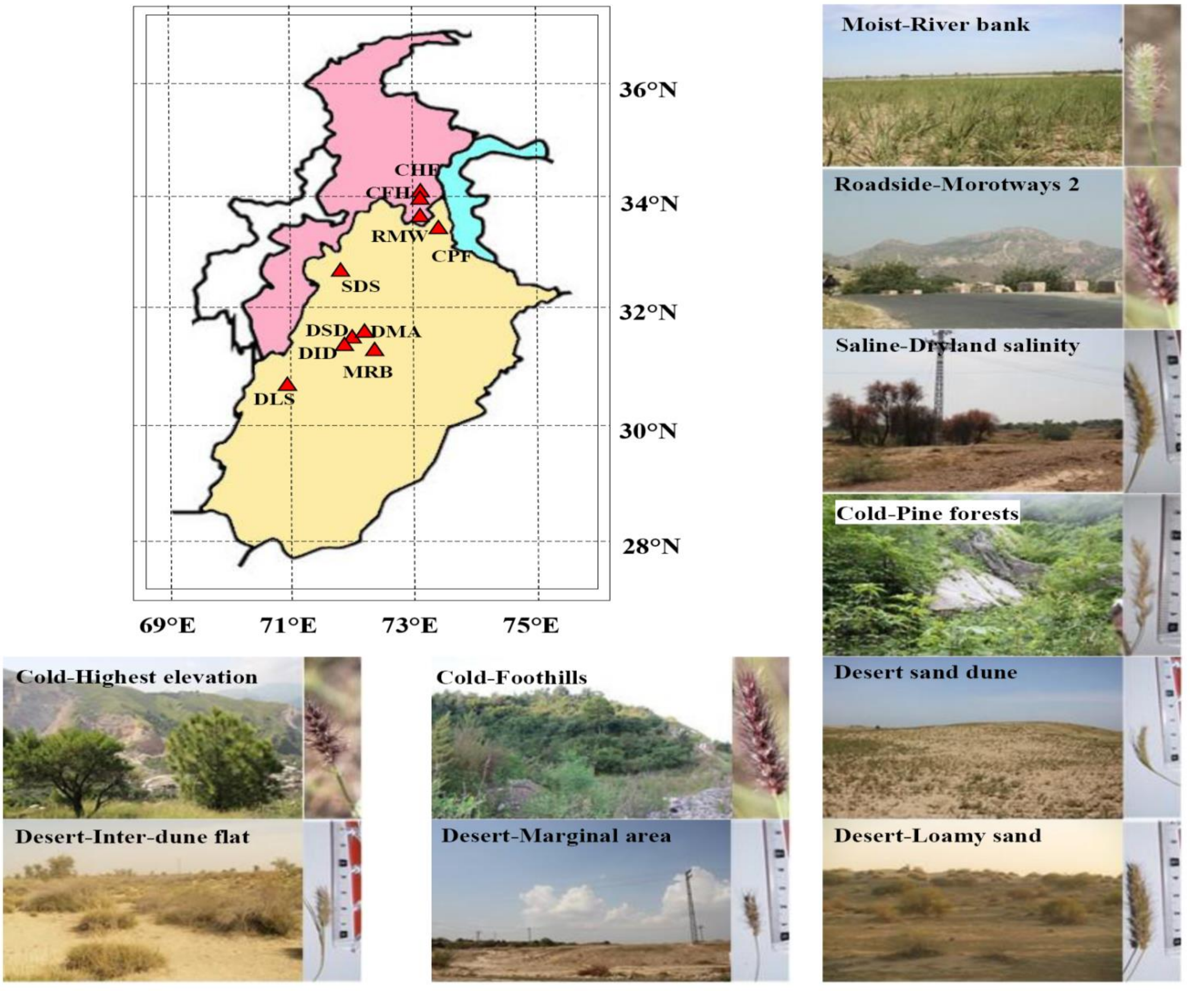


Fig. 1. Map of the Punjab and Khyber Pakhtoonkhwa showing *Cenchrus prieurii* collection sites.

Table 1. Environmental and soil physicochemical characteristics of *Cenchrus prieurii* collection sites exposed to multiple environmental stresses.

Habitats	MxT	MnT	ARF	ASF	Oel	Soil texture	OOM	OSP	OEC	OpH	ONa	OK	OCa	ONO	OPO
MRB	41	6	263	0	148	Sandy loam	1.1	28	2.3	8.1	296	87	67	4.4	4.2
RMW	38	4	772	0	533	Sandy loam	0.83	31	1.2	8.1	126	115	58	3.6	6.2
SDS	40	6	340	0	369	Sandy loam	1.1	25	6.7	8.1	491	61	42	1.8	7.2
CPF	25	-3	1904	1590	2138	Sandy loam	0.92	25	1.01	8.1	146	77	49	2.9	7.1
CHE	32	-1	1127	763	2694	Loam	0.46	34	1.4	7.6	136	52.3	77.5	3.5	7.3
CFH	32	1	1262	210	1220	Loam	0.44	36	1.6	7.7	140	80	52	2.8	9.8
DSD	42	7	136	0	183	Loamy sand	0.83	16	1.6	8.1	235	96	65	4.1	4.6
DID	41	7	145	0	132	Fine sand	0.96	15.1	1.6	8.1	125	69	52	2.7	9.6
DMA	40	5	205	0	181	Loamy sand	0.88	18	3.1	8.1	296	117	93	4.1	5.6
DLS	41	5	185	0	144	Loamy sand	0.88	20	2.9	8	278	141	95	4.1	6

Collection sites: MRB–Moist-river bank, RMW–Roadside-Motorways, SDS–Saline-Dryland salinity, CPF–Cold-Pine forest, CHE–Cold-Highest elevation, CFH–Cold-Foothills, DSD–Desert-Sandy desert, DID–Desert-Interdune desert plain, DMA–Desert-Marginal area, DLS–Desert-Loamy sand.

Environmental traits: MxT – Maximum average temperature (°C), MnT – Minimum average temperature (°C), ARF – Annual rainfall (mm), ASF – Annual snowfall (mm), Oel – Elevation (m a.s.l.).

Soil physicochemical traits: OMO – Organic matter (%), OSP – Saturation percentage (%), OEC – Electric conductivity (dS m^{-1}), OpH – Soil pH, ONa – Soil Na^+ (mg kg^{-1}), OK – Soil K^+ (mg kg^{-1}), OCa – Soil Ca^{2+} (mg kg^{-1}), ONO – Soil $NO3^-$ (mg kg^{-1}), OPO – Soil PO_4^{3-} (mg kg^{-1})

Soil ECe was determined by portable pH/Electrical Conductivity Meter (WTW series InoLab pH/Cond 720, USA) following the methods labeled in Handbook No. 60 (Richards, 1954). Soil Ca^{2+} and K^+ were measured by using flame photometer (Jenway, PFP-7, UK) by running a series of samples (10-100 mg L^{-1}) and standard curves were prepared.

Morphological traits: Six plants (of average size) were randomly taken from each population and considered as replications. After collection of samples, morphological data were taken. Shoot fresh weight was immediately recorded by a portable digital balance (Model: FA2004B, YK Scientific Instrument China). The plant samples were packed in a zipper bag immediately after uprooting and weighing them in the field and preserved in icebox. The number of leaves of each plant was then counted in the Taxonomy Laboratory of Botany Department, while leaf area was measured by the formula devised by Lopes *et al.* (2016). For leaf area, 5 leaves at fixed locations (of each plant) were measured.

$$\text{Area} = \text{Length x Width x Correction factor } 0.75$$

The total leaf area was then calculated by multiplying leaf area with total number of leaves per plant. Dry weight was recorded after completely drying of plant material for 1 week at 65°C.

Anatomical traits: Fresh plant material was collected from the field, and immediately preserved in leak-proof plastic bottles containing formalin acetic alcohol (FAA) solution, which was prepared in a following v/v ratio:

Formaline 5% + Acetic acid 10% + Ethanol 50% + Distilled water 35%

The material was transferred to acetic alcohol solution after 48 h in a following v/v ratio:

Acetic acid 25% + Ethanol 75%

Free-hand sectioning technique was used for the preparation of permanent slides. Plant sections were dehydrated using a series of ethanol grades and stained by biological stains. Safranin dye was used for staining lignified tissues and fast green for parenchymatous tissues with primary walls only. The transverse sections were mounted on a slide using Canada balsam. Digital photographs were taken by a camera-equipped digital compound microscope (Meiji Techno Japan). Micromorphological data (Fig. 3) of the sections were taken by ocular micrometer, which was calibrated with stage micrometer. The area of different cells and tissues was calculated by the following formula, which was derived from the area of a circle:

$$\text{Area} = \frac{\text{Maximum length x Maximum width}}{2} \text{ X } \pi$$

Statistical analysis

The data were subjected to analysis of variance in completely randomized design with six replications, and the means were compared by Duncan's multiple range test. Correlation between soil physicochemical and morpho-anatomical characteristics were calculated using Microsoft Excel workbook (version 16). Multivariate principal component analysis (PCA) was calculated using XLSTAT (V. 2021.1).

Results

Environmental and soil physicochemical traits: The maximum average temperature of arid regions ranged between 40-42°C, while the average minimum temperature was between 5-7°C (Table 1). Areas facing cold stress showed the average maximum temperature from 25-32°C and minimum temperature was usually below 0°C. Drought affected areas showed average maximum temperature between 40-42°C, whereas the average minimum temperature range was between 5-7°C. Moist, saline and road side habitats showed the maximum average temperature between 38-41°C and the average minimum temperature from 4-6°C. Annual precipitation (rainfall + snowfall) was relatively high at high elevation colder areas, especially in the pine region, while arid regions received about 200 mm or even below 150 mm precipitation annually. Soil was generally loamy or sandy loamy in cooler regions while arid regions had fine sand or loamy sand. Organic matter was the highest at roadside and saline areas, while extremely low at high elevations. Soil saturation percentage was below 20% in the arid soils, while at other sites it was between 25-35%. Soil ECe was 6.7 dS m^{-1} (moderately saline) at SDS, while at other sites it was below 4 dS m^{-1}. Soil pH did not vary greatly; the minimum (7.6) was recorded at CHE. Soil Na^+ was the maximum at saline habitat, while soil K^+ and Ca^{2+} was maximum at DMA and DLS respectively. Soil NO_3^- was the maximum at MRB, whereas soil PO_4^{3-} was maximum at CFH.

Morphological traits: Sandy desert conditions suited growth and development of *C. prieurii,* as it produced maximum leaves per plant, and shoot fresh and dry weights (Fig. 2). The largest leaves were observed in the RMA population, while the MRB population showed better shoot fresh and dry weight than all other populations except the DSD population. The number of leaves per plant was the second best in the SDS population. Total leaf area was extremely reduced in the populations from MRB, CHE, DID and DLS.

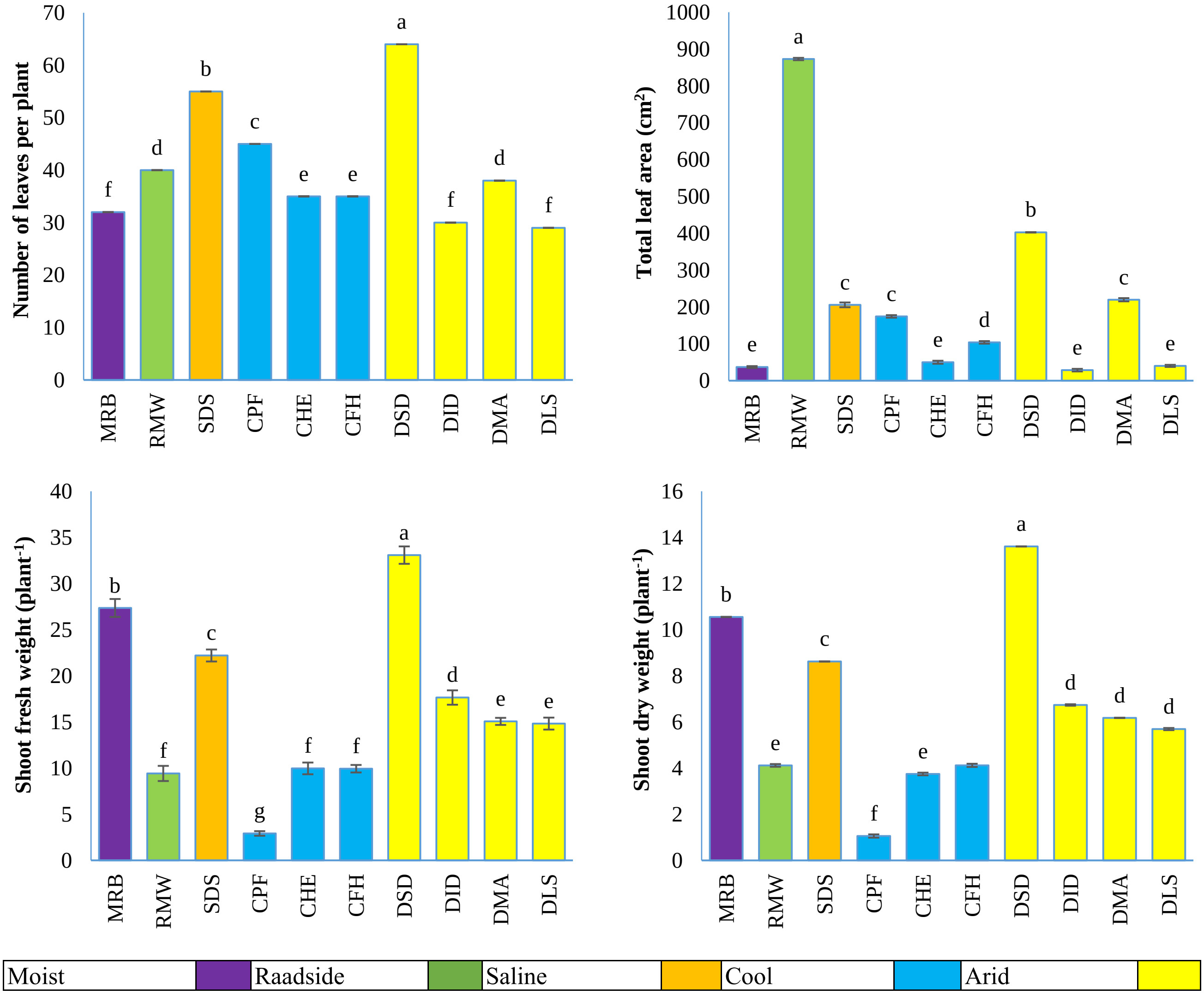


Fig. 2. Morphological characteristics of *Cenchrus prieurii* collected from habitats exposed to multiple environmental stresses.

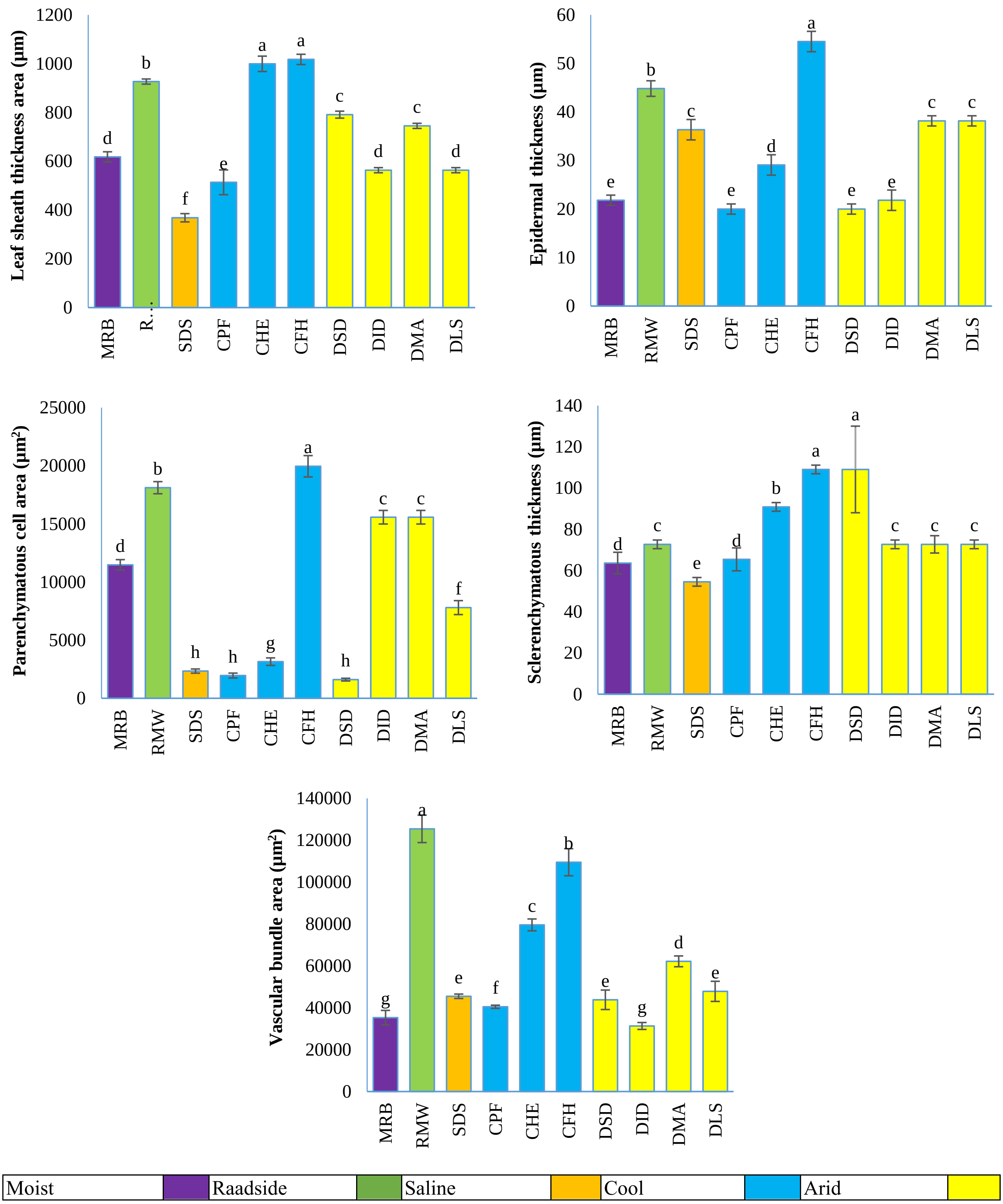


Fig. 3. Leaf sheath anatomical characteristics of *Cenchrus prieurii* collected from habitats exposed to multiple environmental stresses.

Leaf anatomical traits: Midrib thickness was the maximum in the populations from DLS and DMA (Figs. 3 and 4). Lamina thickness was the maximum in the CFH population, which was closely followed by the populations from DID, DLS and DMA. The lamina thickness was extremely reduced in DSD. The epidermis was relatively thicker in populations collected from DID, DMA, CPF, MRB and RMW. The cortical cell area was exceptionally larger in the populations from CHE and DLS. Cortical cells were significantly reduced in the populations from CFH, DID, DSD and DMA.

Mesophyll thickness was significantly higher in the populations from DID, DLS, DMA, CHE and CSH. The thinnest mesophyll layer was recorded in the DSD population (Figs. 3 and 4). Bulliform cell area was the maximum in the RMW population, which was followed by the bulliform cell area of DMA population. Bulliform cells were not distinguishable in CHE and CFH populations, while extremely reduced in the CPF population. Same was the case with DID population, where bulliform cells were not distinct.

The vascular bundle area and metaxylem area was the largest in the CFH population. The metaxylem area was significantly reduced in all other populations. Phloem area was the maximum in the DLS population. Populations from the SDS and CFH population showed larger phloem area than the other populations. The minimum phloem area was recorded in the populations from MRB and CHE.

Leaf sheath anatomical traits: Leaf sheath thickness was significantly higher in the populations from CHE and CFH, which was closely followed by the RMW population. The thinnest leaf sheath was noted in the SDS population (Figs. 5 and 6). Epidermal thickness was the highest in the population from CFH. The thinnest epidermis was recorded in four populations, i.e., from MRB, CPF, SD and DID.

The parenchymatous cell area was the greatest in the population from CFH, followed by the parenchymatous cell area recorded in the population from RMW. The desert populations (DID and DMA) showed significantly larger parenchymatous cells than the other desert populations. Parenchymatous cells were greatly reduced in the populations collected from SDS salinity, CPF, CHE and DSD.

Sclerenchymatous thickness was the maximum in two populations, i.e., CFH and DSD (Figs. 5 and 6). Extensive sclerification was also noted in all other populations, especially in the epidermal region and outside vascular tissue. The largest vascular bundles were recorded in the population collected from RMW, followed by the CFH population. Area of vascular bundles was extremely reduced in the populations collected from MRB, SDS, CPF, DSD and DID.

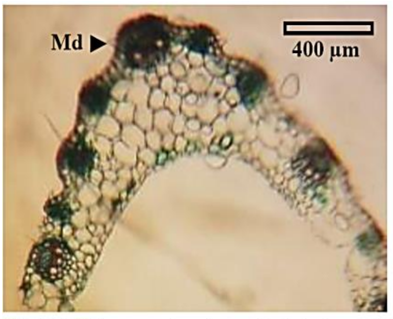
Specific anatomical modifications: Three different types of hairiness were observed in the *C. prieurii* leaves (Fig. 4), which were long hairs with swollen base, vesicular hairs, and sharp-pointed small trichomes (e.g., the CPF population). Bulliform cells were highly developed in many populations, covering almost the entire adaxial surface. Four populations, MRB, MW, CPF and DSD populations, possessed significantly thinner leaves, with large fan shaped bulliform cells. Midrib shape was variable in *C. prieurii* populations as thick conical shape was recorded in the DLS, DMA and DID populations with high proportion of storage parenchyma. Rounded midrib was seen in the populations collected from SDS, CHE and CFH. The most promising feature in leaf sheath of *C. prieurii* was the sclerification outside vascular bundles, or even in the entire outer surface of epidermis (Fig. 6). Extensive sclerification was also recorded in the vascular bundles, or in a few cases on the lower side of vascular bundles.

Relationship between soil physicochemical and morpho-anatomical traits: The PCA biplot between soil physicochemical and foliar morpho-anatomical traits of *C. prieurii* are presented in Fig. 7. Soil Na^+ and ECe showed a close relationship with shoot fresh and dry weights in the populations collected from RMW, DMA and DLS (Fig. 7a). A strong association was recorded between soil pH, total leaf area, and number of leaves per plant in the population collected from RMW and CPF (Fig. 7a). Leaf midrib thickness, mesophyll thickness and phloem area were strongly associated with soil ECe, Ca^{2+}, K^+ and Na^+ in the DLS and DMA populations (Fig. 7b). Leaf epidermal thickness showed strong association with soil pH and organic matter in the populations collected from SDS, RMW, MRB, DID, DSD and CPF (Fig. 7b). A strong association of leaf sheath characteristics like sheath thickness, epidermal thickness, vascular bundle area and sclerenchymatous thickness was observed with soil saturation percentage in the populations collected from CFH, CPF and RMW. The other populations were not associated with leaf sheath anatomical characteristics, while more closely associated with soil physicochemical traits (Fig. 7c).

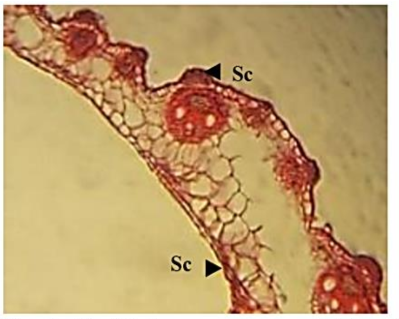
Correlation between soil physicochemical traits with morphological and foliar anatomical traits: Pearson's correlation coefficient (*r*) showed negative correlation ($p<0.05$) of elevation with shoot fresh weight, while no correlation of elevation was recorded with other characteristic either morphological or foliar anatomical traits (Table 2). Soil organic matter positively correlated with leaf epidermis, while negatively correlated with leaf sheath traits like sheath thickness, vascular bundle area and sclerenchymatous thickness. Saturation percentage positively correlated with leaf sheath vascular bundle area. Soil pH positively correlated with leaf epidermal thickness and negatively with leaf sheath thickness. Soil K^+ was positively correlated with leaf bulliform area, while soil Ca^{2+} was positively correlated with midrib thickness.

Discussion

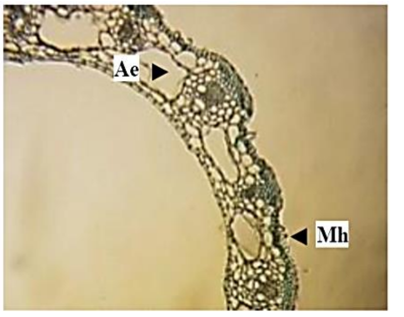
Cenchrus species, i.e., *C. ciliaris, C. pennisetiformis,* and *C. setigerus* are widely distributed and can colonize in different environmental conditions through some specific structural and functional modifications (Hussain *et al.*, 2020). *Cenchrus biflorus* and *C. prieurii* are adapted to hyperarid, arid and semiarid conditions, which is a characteristic of climatic conditions in Pakistan (Tamme *et al.*, 2010; Stein *et al.*, 2014). The differently adapted populations of *C. prieurii* were collected from diverse habitats like roadsides, cool mountains, semiarid regions, moist habitats along riverbank and Thal Desert. Morphological features such as root length, root dry weight, shoot fresh and dry weights and leaves per plant were larger in the desert populations. This showed the perfect adaptation of *C. prieurii* to sandy desert like Thal Desert (Rafay *et al.*, 2013).



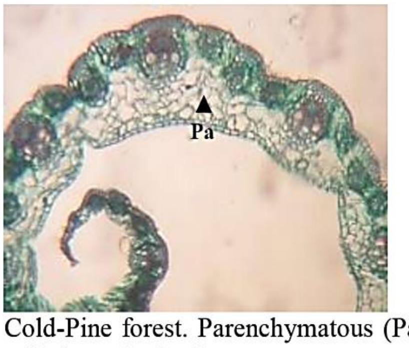
Moist-River bank. Midrib (Md) prominent.



Roadside-Motorways. Extensive sclerification in and outside vascular bundles.

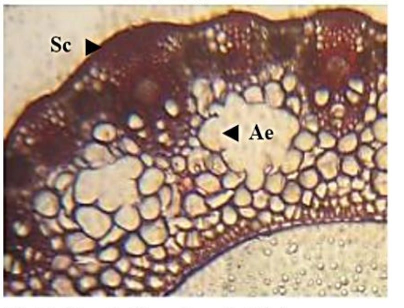


Saline-Dryland salinity. Large aerenchymatous (Ae) cavities in the parenchymatous region; High density of microhairs (Mh) on outer surface.

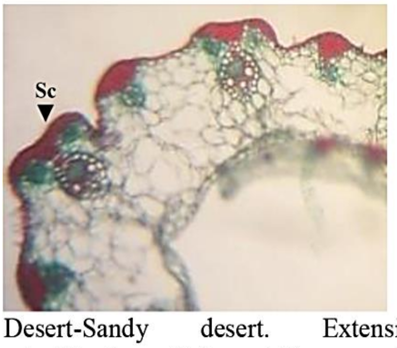


Cold-Pine forest. Parenchymatous (Pa) cells irregular in shape.

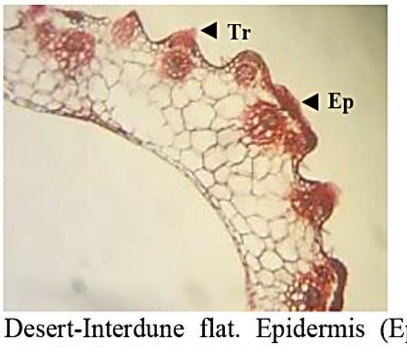
Cold-Highest elevation. Extensive sclerification (Sc) on outer side of vascular bundles; midrib indistinguishable.



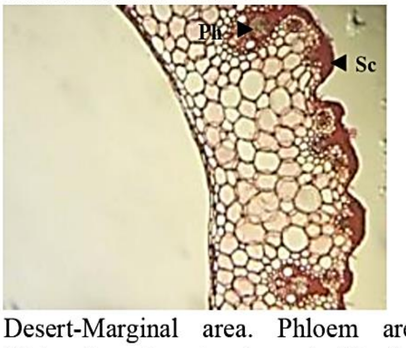
Cold-Foothills. Leaf thickness greatly increased; lysigenous cavities (Ae) in the parenchymatous region; extensive sclerification (Sc) in epidermis and outside vascular bundles.



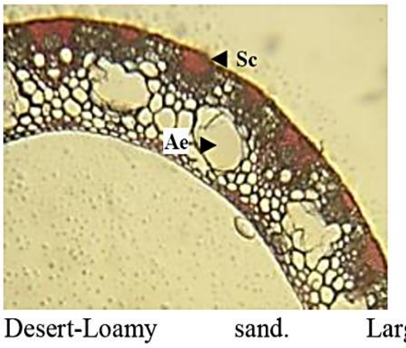
Desert-Sandy desert. Extensive sclerification (Sc) outside vascular bundles; outer surface uneven.



Desert-Interdune flat. Epidermis (Ep) sclerified; Pointed trichomes (Tr) on adaxial surface.



Desert-Marginal area. Phloem area (Ph) enlarged; extensive sclerification (Sc) in epidermis and outside vascular bundles.



Desert-Loamy sand. Large aerenchymatous (Ae) cavities in the parenchymatous region; epidermis, hypodermis, and vascular bundles extensively sclerified (Sc).

Fig. 4. Leaf sheath transverse sections of *Cenchrus prieurii* exposed to multiple environmental stresses in the Punjab and Khyber Pakhtoonkhwa.

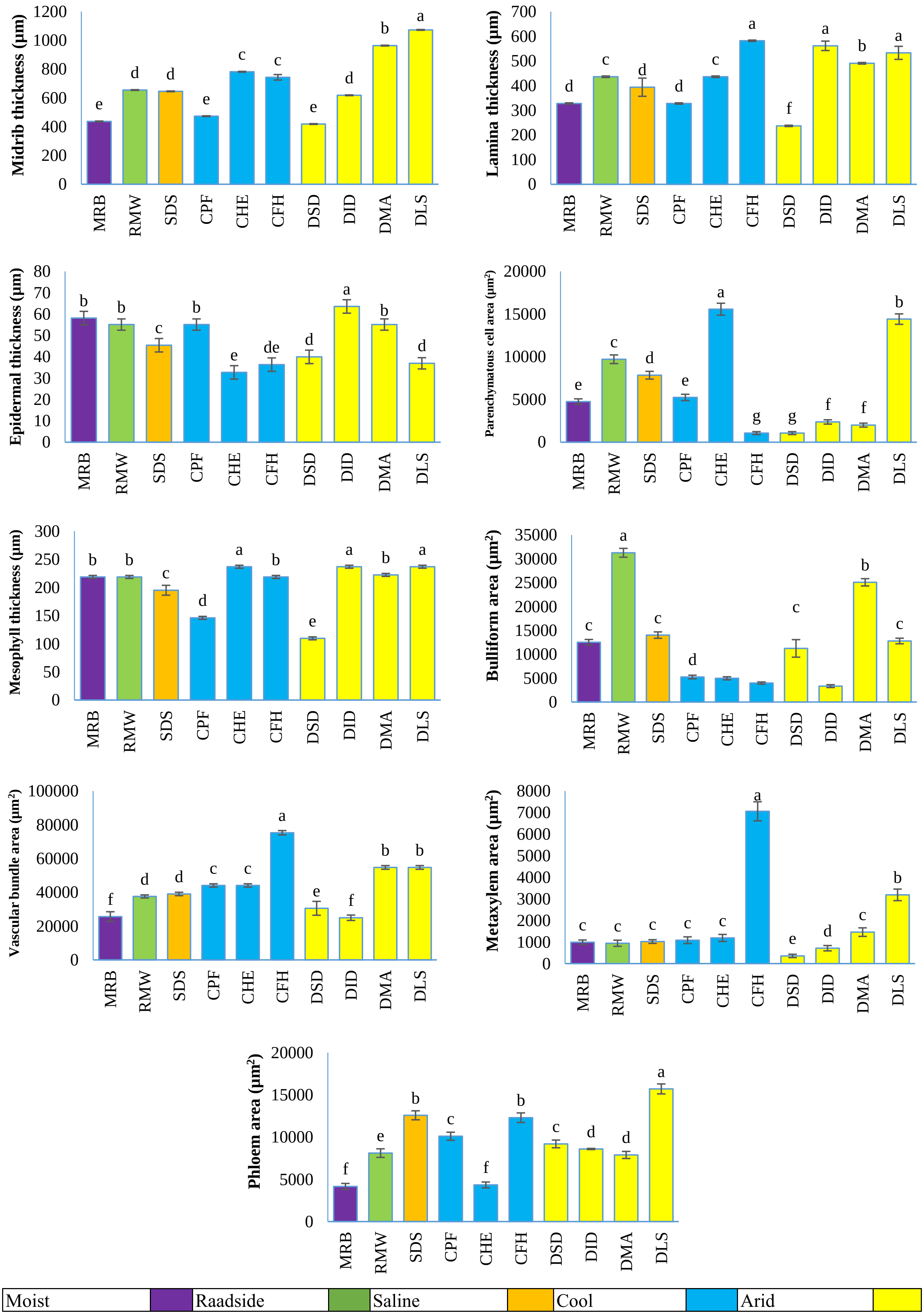
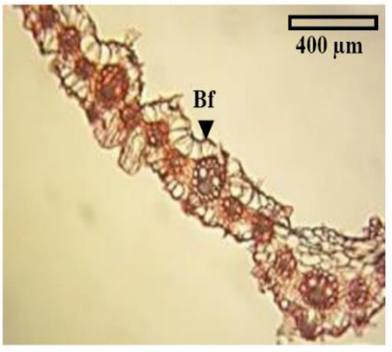
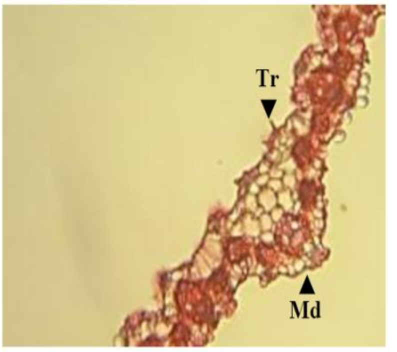


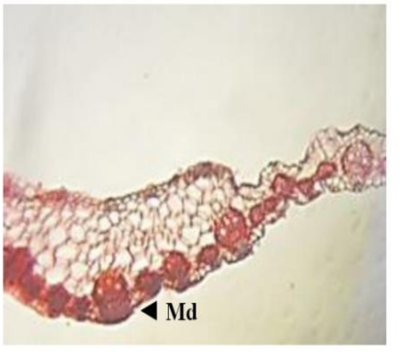
Fig. 5. Leaf anatomical characteristics of *Cenchrus prieurii* collected from habitats exposed to multiple environmental stresses.



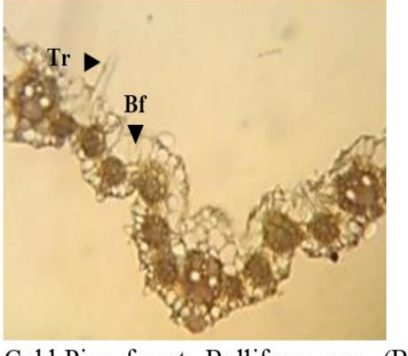
Moist-River bank. Bulliform (Bf) well developed, fan shaped.



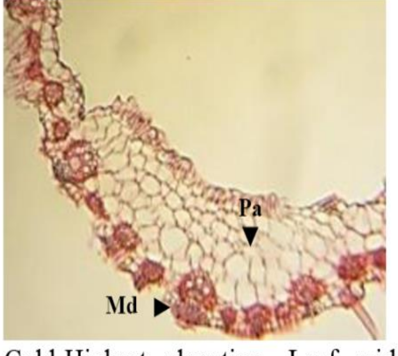
Roadside-Motorways. Leaf midrib (Md) conical; small, pointed trichomes (Tr) on both leaf surfaces.



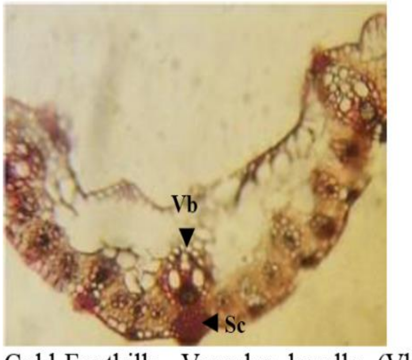
Saline-Dryland salinity. Leaf midrib (Md) rounded.



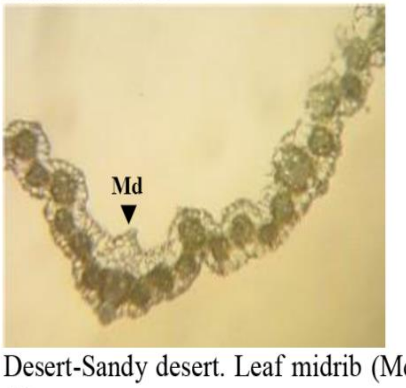
Cold-Pine forest. Bulliform area (Bf) increased; long trichomes (Tr) on adaxial surface.



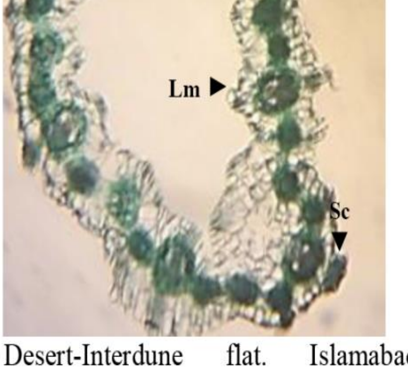
Cold-Highest elevation. Leaf midrib (Md) rounded; parenchyma (Pa) enlarged.



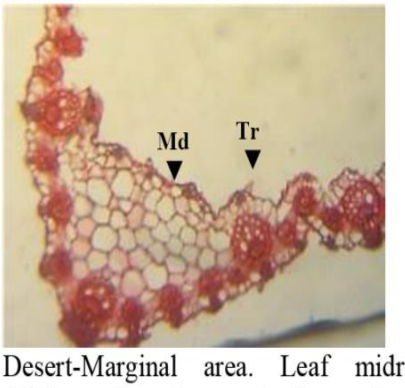
Cold-Foothills. Vascular bundle (Vb) large; extensive sclerification (Sc) on abaxial side of vascular bundles.



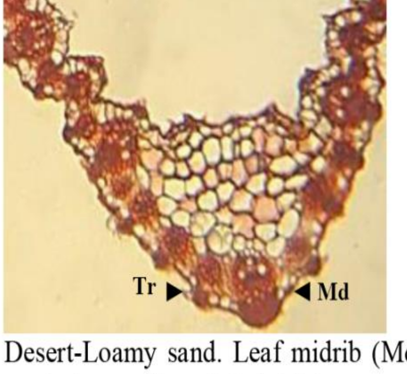
Desert-Sandy desert. Leaf midrib (Md) thin.



Desert-Interdune flat. Islamabad. Lamina (Lm) thick; extensive sclerification (Sc) on abaxial surface.



Desert-Marginal area. Leaf midrib (Md) enlarged, conical; pointed trichomes (Tr) on adaxial surface.



Desert-Loamy sand. Leaf midrib (Md) conical; small, pointed trichomes on adaxial surface.

Fig. 6. Leaf blade transverse sections of *Cenchrus prieurii* exposed to multiple environmental stresses in the Punjab and Khyber Pakhtoonkhwa.

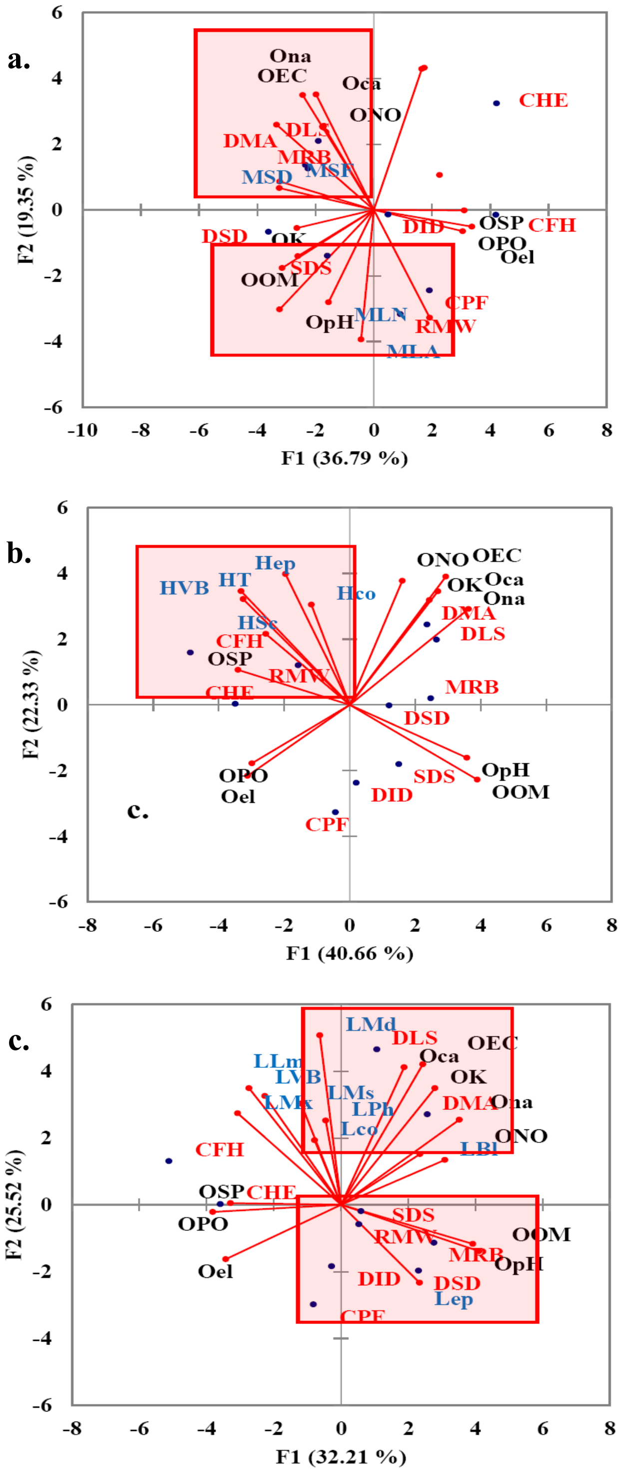


Fig. 7. Relationship between soil physicochemical traits with a) morphological traits, b) leaf blade anatomical traits, and c) leaf sheath anatomical traits of *Cenchrus prieurii* exposed to multiple environmental stresses.

Collection sites: MRB–Moist-river bank, RMW–Roadside-Motorways, SDS–Saline-Dryland salinity, CPF–Cold-Pine forest, CHE–Cold-Highest elevation, CFH–Cold-Foothills, DSD–Desert-Sandy desert, DID–Desert-Interdune desert plain, DMA–Desert-Marginal area, DLS–Desert-Loamy sand.
Soil physicochemical traits: Oel – Elevation (m a.s.l.), OOM – Organic matter (%), OSP – Saturation percentage (%), OEC – Electric conductivity (dS m^{-1}), Ona – Soil Na^{+} (mg kg^{-1}), OK – Soil K^{+} (mg kg^{-1}), Oca – Soil Ca^{2+} (mg kg^{-1}), ONO – Soil NO_3^{-} (mg kg^{-1}), OPO – Soil PO_4^{3-} (mg kg^{-1}).

Morphological traits: MSF-Shoot fresh weight, MSD-Shoot dry weight MLA-Total leaf area, MLN-Number of leaves per plant.
Leaf sheath anatomical traits: HT-Leaf sheath thickness, Hep-Epidermal thickness, HVB-Vascular bundle area, HSc-Sclerenchymatous thickness, Hco-Cortical cell area.
Leaf anatomical traits: Lep-Epidermal thickness, Lco-Cortical cell area, LMs-Mesophyll thickness, LMx-Metaxylem area, LPh-Phloem area, LVB-Vascular bundle area, LMd-Midrib thickness, LLm-Lamina thickness, LBl-Bulliform thickness

Leaf sheath tissues like sheath thickness, epidermis thickness, cortical cell area and sclerenchyma thickness were greatly developed in the population inhabiting cool mountainous region. These variations increase rigidity of *C. prieurii* populations colonizing under cool climates (Fatima *et al.*, 2018). These adaptations are associated to ecological success of this species in cool mountainous region by increasing water storing capacity, inhibiting water loss (Yang *et al.*, 2015), and hence better adjustment to mountainous region (El-Keblawy, 2017). The roadside population was collected along Islamabad-Murree Expressway, from a mountainous foothill. This population showed thicker leaf sheath than the other population, which was covered by thick epidermis, large parenchymatous cells and vascular bundles. This will improve water storage capacity (Fatima *et al.*, 2018), water and nutrients conduction and minimize water loss from leaf sheath surface (Wasim & Naz, 2020). Sclerenchymatous thickness was the maximum outside vascular bundles in the sandy desert population of *C. prieurii.* This population was exposed to hyperarid environmental conditions, and the dramatically increased sclerification was critically important for the survival in environmental adversaries (Parvez *et al.*, 2022). It provides mechanical strength to metabolically active tissues (Ahmad *et al.*, 2016) and prevents flow of water outside the plant body (Al-maskri *et al.*, 2014). The population collected from dryland saline area was least developed in terms of leaf sheath thickness, parenchymatous cell area, sclerenchyma thickness and vascular bundle area. It indicates its low tolerance to saline conditions compared to arid/hyperarid environments.

Leaf anatomical traits like midrib and lamina thickness, epidermal thickness, mesophyll thickness, and phloem area were the maximum in *C. prieurii* populations collected from desert areas (DID, DMA and DLS). Leaf characteristics are generally more responsive to environmental conditions than other plant organs (Wang *et al.*, 2021). Thicker leaves (lamina and midrib) were primarily due to parenchymatous tissues (storage parenchyma and mesophyll), and hence better in water conservation and photosynthetic activity (Riaz *et al.*, 2022). Thicker epidermis layer is vital for desert populations as it can prevent water loss through leaf surface.

The CFH population performed better in terms of lamina thickness, epidermal thickness, mesophyll thickness, vascular bundle area and metaxylem area. Thicker lamina, epidermal layer and mesophyll are linked to water conservation through additional storage capacity (Sarwar *et al.*, 2022) and prevention of water loss through leaf surface (López *et al.*, 2021). The CHE population was collected from the coolest habitats, which showed the largest parenchymatous cells. Larger parenchymatous cells can store more water due to larger vacuoles (Iqbal *et* *al.*, 2022), hence contribute to survival success in harsh water limited environmental conditions of high elevations. The RMW population possessed the largest bulliform cells, which are not only crucial for leaf rolling (Jang *et al.*, 2021) but also for storing additional water (Hameed *et al.*, 2022).

The DSD population from driest area showed the thinnest leaves (midrib and lamina), epidermis layer and mesophyll, the smallest parenchymatous cells, bulliform area, metaxylem area and vascular bundle area. Reduction in leaf anatomical traits is vital for survival under extremely hot arid conditions like sandy deserts. It will minimize transpiration rate by reducing leaf area and thickness by making leaves tougher and fibrous (Ahmad *et al.*, 2022). More importantly, the narrow metaxylem vessels are ecologically critical because of their resistance to collapse under extreme aridity (Ahmad *et al.*, 2022).

Specific features of the SDS population are the large aerenchymatous cavities and numerous microhairs on the outer leaf sheath surface. Lysigenous air cavities have earlier been reported by Rahat *et al.*, (2022) in a halophytic grass *Diplachne fusca,* which were related to bulk salt conduction under high salinities. Microhairs are vesicle-like structures which accumulate salt and then burst releasing salts outside plant body as reported by Naz *et al.* (2018) in *Aeluropus lagopoides,* and Fatima *et al.* (2021) in *Cymbopogon jwarancusa*.

Various soil physicochemical traits were associated with morphological and foliar anatomical characteristics. Soil Ca^{2+} was linked to shoot fresh and dry weights of *C. prieurii.* Growth enhancement in different *Cenchrus* species has been reported by improving soil Ca^{2+} by several researchers, e.g., Neves *et al.*, (2020) in *C. purpureus,* Pedroza-Parga *et al.*, (2022) in *C. ciliaris,* Ojo *et al.*, (2020) in *C. americanus,* Ali *et al.*, (2022) in *C. pennisetiformis* and Zhou *et al.*, (2021) in *C. pauciflorus.* Leaf number and area showed association with soil organic matter and pH. All leaf sheath anatomical traits were related to soil saturation percentage. Leaf traits like midrib and mesophyll thicknesses, phloem area and bulliform area were influenced by soil ionic content. Ionic contents are directly associated with growth and tissue development (EL Sabagh *et al.*, 2021). Leaf epidermis thickness showed association with soil organic matter and pH.

The *C. prieurii* populations were collected from diverse habitats, ranging from hypersaline to extreme aridity, cool mountainous regions, riverbanks, and roadsides. These populations were exposed to abiotic stress like aridity, salinity, waterlogging and cool temperature. All showed modifications in structural and functional attributes that were related to water conservation. The most important were epidermal thickness, intensive sclerification especially in and outside vascular bundles, formation of aerenchymatous cavities, large bulliform cells, and high proportion of storage parenchyma. Plasticity in morphological and anatomical features was extremely high, which enabled the *C. prieurii* populations to adapt to a variety of habitats, hence contributed significantly to its wide distributional range.

Table 2. Pearson's correlation coefficients soil physicochemical traits with morphological and foliar anatomical traits.

Variables	Oel	OOM	OSP	OEC	OpH	Ona	OK	Oca	ONO	OPO
MSF	-0.714	0.442	-0.463	0.289	0.374	0.549	0.141	0.210	0.279	-0.515
MSD	-0.715	0.415	-0.461	0.276	0.378	0.541	0.162	0.197	0.295	-0.526
MLA	-0.114	0.033	0.098	-0.336	0.324	-0.230	0.345	-0.194	0.094	-0.267
MLN	-0.013	0.179	-0.225	-0.336	0.306	-0.001	0.034	-0.061	-0.204	-0.307
Lep	-0.282	0.664	-0.371	-0.007	0.720	0.018	-0.009	-0.334	-0.021	-0.058
Lco	0.230	-0.166	0.327	0.010	-0.358	-0.086	0.134	0.451	0.076	-0.106
LMs	-0.154	-0.168	0.285	0.375	-0.352	-0.032	0.050	0.303	0.007	0.338
LMx	0.195	-0.590	0.478	0.122	-0.572	-0.106	0.059	-0.089	-0.142	0.507
LPh	-0.128	0.054	-0.201	0.174	0.104	0.031	0.558	0.185	-0.351	0.281
LVB	0.337	-0.628	0.415	0.221	-0.555	-0.033	0.197	0.180	-0.100	0.377
LMd	-0.134	-0.293	-0.017	0.637	-0.311	0.233	0.496	0.701	0.133	0.154
LLm	-0.074	-0.361	0.127	0.282	-0.375	-0.226	0.095	0.107	-0.232	0.695
LBl	-0.461	0.387	-0.132	0.325	0.550	0.377	0.733	0.363	0.354	-0.585
HT	0.284	-0.856	0.555	-0.192	-0.673	-0.294	-0.241	-0.185	0.316	0.126
Hep	-0.016	-0.458	0.507	0.160	-0.382	-0.135	0.375	0.163	-0.170	0.345
HVB	0.259	-0.652	0.697	-0.268	-0.476	-0.440	0.058	-0.172	-0.019	0.247
HSc	0.169	-0.775	0.176	-0.180	-0.569	-0.182	-0.253	-0.240	0.209	0.174
Hco	-0.285	-0.190	0.191	0.202	-0.047	-0.099	0.151	-0.218	0.098	0.339

Significant and positive at $p<0.05$ Significant and negative at $p<0.05$

Soil physicochemical traits: Oel – Elevation (m a.s.l.), OOM – Organic matter (%), OSP – Saturation percentage (%), OEC – Electric conductivity (dS m^{-1}), Ona – Soil Na^{+} (mg kg^{-1}), OK – Soil K^{+} (mg kg^{-1}), Oca – Soil Ca^{2+} (mg kg^{-1}), ONO – Soil NO_3^{-} (mg kg^{-1}), OPO – Soil PO_4^{3-} (mg kg^{-1}).

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Leaf anatomical traits: Lep-Epidermal thickness, Lco-Cortical cell area, LMs-Mesophyll thickness, LMx-Metaxylem area, LPh-Phloem area, LVB-Vascular bundle area, LMd-Midrib thickness, LLm-Lamina thickness, LBl-Bulliform thickness.

Conclusion

Cenchrus prieurii populations are perfectly adapted to multiple environmental stresses. The biomass production and foliar anatomical traits varied greatly along habitat types. This species apparently more adapted to sandy hyperarid conditions of the Thal Desert. Sclerification in leaf sheath outside vascular bundles was the maximum in this ecotype. Among leaf blade anatomical characteristics, leaf thickness, epidermal and mesophyll thickness, parenchymatous cell area and phloem area were the highest in desert populations. Plasticity in anatomical traits were significantly high, and this might be a strong reason for successful colonization of this species to a variety of habitat types.

References

Abd El-Maboud, M.M. and O.H. Abd Elbar. 2020. Adaptive responses of *Limoniastrum monopetalum* (L.) Boiss. growing naturally at different habitats. *Plant Physiol. Rep.*, 25: 325-334.

Ahmad, K.S., M. Hameed, S. Fatima, M. Ashraf, F. Ahmad, M. Naseer and N. Akhtar. 2016. Morpho-anatomical and physiological adaptations to high altitude in some Aveneae grasses from Neelum Valley, Western Himalayan Kashmir. *Acta Physiol. Plant.*, 38: 1-14.

Ahmad, K.S., A. Javaid, M. Hameed, S. Fatima, F. Ahmad, M. Ashraf and I. Ahmad. 2022. Survival strategies in two high altitude *Sorghum* species from western Himalayas. *Acta Physiol. Plant.*, 44: 1-13.

Ali, B., M. Umar, M. Azeem, Z. Uddin and Z.S. Siddiqui. 2022. Salt tolerance screening of a newly developed wheat variety (AZRC-DK-84) in saline environment using halophytic grass (*Cenchrus penisettiformis*) as a test model. *Acta Physiol. Plant.*, 44: 1-16.

Al-maskri, A., M. Hameed, M. Ashraf, M.M. Khan, S. Fatima, T. Nawaz and R. Batool. 2014. Structural features of some wheat (*Triticum* spp.) landraces/cultivars under drought and salt stress. *Arid Land Res. Manag.*, 28: 355-370.

Balraj, B., N. Senthilkumar, C. Siva, R. Krithikadevi, A. Julie, I. V. Potheher and M. Arulmozhi. 2017. Synthesis and characterization of zinc oxide nanoparticles using marine *Streptomyces* sp. with its investigations on anticancer and antibacterial activity. *Res. Chem. Intermed*., 43: 2367-2376.

Bibi, S., M.S.A. Ahmad and M. Hameed. 2021. Role of leaf micro-structural and topographical traits in ecological success of some arid zone grasses. *Pak. J. Bot.*, 53: 691-700.

El-Keblawy, A.A. and A.H.A. Khedr. 2017. Population structure and ecological role of *Moringa peregrina* (Forssk.) Fiori. at its northwestern range edge in the Hajar Mountains. *Plant Biol.*, 151: 29-38.

EL-Sabagh, A., M.S. Islam, M. Skalicky, M. Ali Raza, K. Singh, M. Anwar Hossain and A. Arshad. 2021. Salinity stress in wheat (*Triticum aestivum* L.) in the changing climate: Adaptation and management strategies. *Front. Agron.*, 3: 661932.

Etesami, H. and D.K. Maheshwari. 2018. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicol. Environ. Saf.*, 156: 225-246.

Fatima, S., M. Hameed, F. Ahmad, M. Ashraf and R. Ahmad. 2018. Structural and functional modifications in a typical arid zone species *Aristida adscensionis* L., along altitudinal gradient. *Flora*, 249: 172-182.

Fatima, S., M. Hameed, N. Naz, S.M.R. Shah, M. Naseer, M.S.A. Ahmad, M. Ashraf, F. Ahmad, S. Khalil and I. Ahmad. 2021. Survival strategies in khavi grass [*Cymbopogon jwarancusa* (Jones) Schult.] colonizing hot hypersaline and arid environments. *Water Air Soil Pollut.*, 232: 1-17.

Hameed, M., S. Fatima, F. Ahmad, S.N. Zafar, M.S.A. Ahmad, U. Iqbal and I. Ahmad. 2022. Anatomical modifications in some *Bambusa* Schreb. species to combat ecological constraints. *Int. J. Appl. Exp. Biol*., 1: 21-31.

Hussain, M., A.R. Butt, F. Uzma, R. Ahmed, S. Irshad, A. Rehman and B. Yousaf. 2020. A comprehensive review of climate change impacts, adaptation, and mitigation on environmental and natural calamities in Pakistan. *Environ. Monit. Assess.*, 192: 1-20.

Iqbal, U., M. Hameed, F. Ahmad, M.S.A. Ahmad, M. Ashraf. M. Kaleem and M. Irshad. 2022. Contribution of structural and functional modifications to wide distribution of Bermuda grass *Cynodon dactylon* (L.) Pers. *Flora*, 286: 151973.

Ismail, A.H., C.C. Lim and W.M.W. Omar. 2019 Evaluation of spatial and temporal variations in zooplankton community structure with reference to water quality in Teluk Bahang Reservoir, Malaysia. *Trop. Ecol*., 60: 186-198.

Jang, S., S. Shim, Y.K. Lee, D. Lee and H.J. Koh. 2021. Major QTLs, qARO1 and qARO9, additively regulate adaxial leaf rolling in rice. *Front. Plant Sci.*, 12: 626523.

Jooste, M., L.L. Dreyer and K.C. Oberlander. 2016. The phylogenetic significance of leaf anatomical traits of southern African *Oxalis*. *Evol. Biol*., 16: 1-19.

Karabourniotis, G., G. Liakopoulos, D. Nikolopoulos and P. Bresta. 2020. Protective and defensive roles of non-glandular trichomes against multiple stresses: Structure–function coordination. *J. For. Res.*, 31: 1-12.

Khan, A., M. Ali, A.M. Khattak, W.X. Gai, H.X. Zhang, A.M. Wei and Z.H. Gong. 2019. Heat shock proteins: dynamic biomolecules to counter plant biotic and abiotic stresses. *Int. J. Mol. Sci*., 20: 5321.

Khidr, Z.A., F.A. Ebad and H.A. El–Khawaga. 2017. Osmoregulation and antimicrobial activity of two Egyptian true xerophytes; *Launaea spinosa* (Forssk.) and *Leptadenia pyrotechnica* (Forssk.). *Egyp. J. Desert Res*., 67: 331-349.

Linder, H.P., C.E. Lehmann, S. Archibald, C.P. Osborne and D.M. Richardson. 2018. Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. *Biol. Rev*., 93: 1125-1144.

Lopes, D.M., N. Walford, H. Viana and C.R. Sette Junior. 2016. A proposed methodology for the correction of the leaf area index measured with a ceptometer for *Pinus* and *Eucalyptus* forests. *Revista Arvore*, 40: 845-854.

López, R., F.J. Cano, N.K. Martin-StPaul, H. Cochard and B. Choat. 2021. Coordination of stem and leaf traits define different strategies to regulate water loss and tolerance ranges to aridity. *New Phytol.*, 230: 497-509.

Mariani, L. and A. Ferrante. 2017. Agronomic management for enhancing plant tolerance to abiotic stresses-drought, salinity, hypoxia, and lodging. *Horticulture,* 3: 52.

Naing, A.H. and C.K. Kim. 2021. Abiotic stress-induced anthocyanins in plants: Their role in tolerance to abiotic stresses. *Physiol. Plant.*, 172: 1711-1723.

Naz, N., S. Fatima, M. Hameed, M. Ashraf, M. Naseer, F. Ahmad and A. Zahoor. 2018. Structural and functional aspects of salt tolerance in differently adapted ecotypes of *Aeluropus lagopoides* from saline desert habitats. *Int. J. Agric. Biol.*, 20: 41-51.

Neves, T.I., R. Abrahão, C.A. Uyeda, C. de Souza Silva and A.S. de Deus. 2020. Assessment of the physicochemical characteristics of soil planted with elephant grass-*Cenchrus purpureus* (Schumach.) Morrone-following application of biosolid and chemical fertilization. *Environ. Prog. Sust. Energy*, 39: 13274.

Ojo, V.O.A., F.T. Adeshina, G.A. Adetokunbo, S.O. Jimoh, T.A. Adeyemi, J.L. Njie and O.S. Onifade. 2020. Effects of swine manure application and row spacing on growth of pearl millet (*Cenchrus americanus*) during the establishment period and quality of silage produced in Southwest Nigeria. *Trop. Grasslands*, 8: 115-124.

Parvez, I., I. Ahmad, M. Hameed and M.S.A. Ahmad. 2022. Plasticity in anatomical traits of *Cyperus niveus* Retz. along elevational gradient of dry sub-Himalayan mountains. *Arid Land Res. Manag.*, 1-25. https://doi.org/10.1080/15324982.2022.2138632

Pedroza-Parga, E.H., A. Pedroza-Sandoval, M.A. Velásquez-Valle, I. Sánchez-Cohen, R. Trejo-Calzada and J.A. Samaniego-Gaxiola. 2022. Effect of soil cover on the growth and productivity of buffel grass (*Cenchrus ciliaris* L.) in degraded soils of arid zones. *Rev. Mexicana de Ciencias Pecuarias*, 13: 866-878.

Rafay, M., R.A. Khan, S. Yaqoob and M. Ahmad. 2013. Nutritional evaluation of major range grasses from Cholistan Desert. *Pak. J. Nutr.,* 12: 23-29.

Rahat, Q.U.A., M. Hameed, S. Fatima, M.S.A. Ahmad, M. Ashraf, F. Ahmad and A. Younis. 2022. Structural determinants of phytoremediation capacity in saltmarsh halophyte *Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult. subsp. *fusca*. *Int. J. Phytorem.*, 1-16. https://doi.org/10.1080/15226514.2022.2098251

Riaz, S., S. Basharat, F. Ahmad, M. Hameed, S. Fatima, M.S.A. Ahmad, S.M.R. Shah, A. Asghar, M.A. El-Sheikh and P. Kaushik. 2022. *Dactyloctenium aegyptium* (L.) Willd. (Poaceae) differentially responds to pre-and post-emergence herbicides through micro-structural alterations. *Agriculture,* 12: 1831.

Richards, L.A. 1954. Improvement and management of soils in arid and semi-arid regions in relation to salinity and alkalinity. Diagnosis and improvement of saline and alkali soils. *USDA Handbook* 60: 34-54.

Sarwar, Y., A. Asghar, M. Hameed, S. Fatima, F. Ahmad, M.S.A. Ahmad and I. Ahmad. 2022. Structural responses of differentially adapted *Cenchrus setigerus* Vahl ecotypes to water deficit. *Environ. Exp. Bot.*, 194: 104746.

Wang, R., N. He, S. Li, L. Xu and M. Li. 2021. Spatial variation and mechanisms of leaf water content in grassland plants at the biome scale: evidence from three comparative transects. *Sci. Rep.*, 11: 1-12.

Wasim, M.A. and N. Naz. 2020. Anatomical adaptations of tolerance to salt stress in *Cenchrus ciliaris* L., a saline desert grass. *J. Animal Plant Sci.*, 30: 1548-1566.

Yamori, W., K. Hikosaka and D.A. Way. 2014. Temperature response of photosynthesis in C_3, C_4, and CAM plants: temperature acclimation and temperature adaptation. *Photosynth. Res*., 119: 101-117.

Yang, Y., C. Dong, S. Yang, X. Li, X. Sun and Y. Yang. 2015. Physiological and proteomic adaptation of the alpine grass *Stipa purpurea* to a drought gradient *PLoS ONE*, 10: 0117475.

Zhou, L., X. Tian, B. Cui and A. Hussain. 2021. Physiological and biochemical responses of invasive species *Cenchrus pauciflorus* Benth. to drought stress. *Sustainability*, 13: 5976.

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