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Structural and functional traits mediating the adaptability of creeping signal grass [*Urochloa reptans* (L.) Stapf] across diverse ecozones

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Abstract

Plants of the genus *Urochloa* P. Beauv., popularly known as brachiaria, or marmalade grass, belong to the family Poaceae. *Urochloa reptans* is a member of the genus *Urochloa* and is also known as creeping signal grass. It is widely distributed in tropical and subtropical regions of the world. The present experiment explored the anatomical, structural, functional, and physiological adaptive features of *Urochloa reptans* sampled from sixteen different sites of the Faisalabad region, Pakistan. All the collected populations showed numerous anatomical modifications, and also possess marked physiological characteristics that help them to survive under varying ecological conditions. This leads to their ecological success for existence in heterogeneous environments. The populations of Eden Garden and Canal Road had maximum leaf epidermal thickness, while midrib thickness was higher in the Manawala ecotype. The bulliform cell area was noted to be widened mainly in the Narwala Bangla population. The intensive sclerification of the stem was observed in a sample of Makkuana. The populations from Painsra and Eden Garden possessed larger vascular bundle areas. However, only the Eden Garden population exhibited a larger metaxylem area. The populations native to Canal Road and Sadar Bypass exhibited vigorous root growth in terms of cortical region thickness, endodermal thickness, and pith cell area. Besides this, well-developed aerenchyma cells and metaxylem vessels were observed in the Canal Road and Chicha populations. Overall, *U. reptans* showed much outstanding activity in the context of development, structural, functional, and physiological characteristics in response to the present-day ecological scenario.

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Sites abbreviations: AR: Ayub Research Institute; SD: Samundri; PR: Painsra; CJ: Chak Jhumra; JW: Jaranwala; NG: NIBGE; EG: Eden Garden; MW: Manawala; SB: Sadar bypass; GP: Gobind Pura; CR: Canal Road; KW: Khurianwala; MA: Makkuana; CH: Chicha; ST: Satiana; NB: Narwala Bangla; **Leaf anatomy abbreviations:** LUET: leaf upper epidermal thickness; LLET: leaf lower epidermal thickness; LMT: leaf midrib thickness; LLT: leaf lamina thickness; LVBA: leaf vascular bundle area; LBA: leaf bulliform cell area; UPESTA: upper epidermal stomatal area; UPESTN: upper epidermal stomatal numbers; LWESTA: lower epidermal stomatal area; LWESTN: lower epidermal stomatal numbers; **Stem anatomy abbreviations:** SET: stem epidermis thickness; SST: stem sclerenchyma thickness; SPA: stem phloem area; SCA: stem cortical cell area; SNVB: stem number of vascular

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bundles; SVBA: stem vascular bundle area; SMA: stem metaxylem area; SR: stem radius; **Root anatomy abbreviations:** RET: root epidermal thickness; RCRT: root cortical region thickness; REDT: root endodermal thickness; RPCA: root pith cell area; RMA: root metaxylem area; RACA: root aerenchyma cell area; RCA: root cross-sectional area

Introduction

The Poaceae is recognized as probably the best documented large family of angiosperms and a model group of plants (Hodkinson, 2018; Rzanny et al., 2022), responsible for the majority of human calorie intake as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and sugar cane (*Saccharum officinarum* L.), as well as being architects of grasslands and savannas across the world (Kellogg, 2015). Plants of the genus *Urochloa* P. Beauv., popularly known as brachiaria, or marmalade grass have 18 species registered in Brazil (Shirasuna, 2015), inhabiting the South, Southeast, and Midwest, mainly in soybean and corn fields. The *Urochloa* (Brachiaria) forages, including *Urochloa reptans,* are significant for tropical pastures worldwide and for pastoral livelihoods in sub-Saharan Africa (Vorontsova, 2022). Grasses of the genus *Urochloa*, such as *Urochloa reptans* and *Urochloa ramosa*, can obtain 20% to 40% of their total N in the plant from the atmosphere through the association with N-fixing bacteria (i.e., non-symbiotic N_2 fixation). Furthermore, *U. humidicola* can suppress soil nitrification by releasing inhibitors from roots, which affects $\delta^{15}N$ in plants (Nunez et al., 2018; Villegas et al., 2020). Grasses growing in association with legumes thus benefit from symbiotically fixed N, with decomposing legume roots most likely being the main transfer pathway (Oberson et al., 2013; Villegas et al., 2020; Zhong et al., 2025). However, it is ecologically significant to study grasses of different habitats across the globe to identify their anatomical adaptive components, which help them to thrive in harsh climatic conditions and combat varying ecological factors.

Rainfall, aridity, drought, soil salinity, temperature, and solar irradiance are a few environmental variables that often regulate the abundance and proliferation of plant communities in an environment by affecting their output and development (Li et al., 2019; Bartas, 2024). The two major variables limiting plant development and productivity are salt and drought. They have a significant impact on vital plant biological processes, including respiration and photosynthesis, which eventually result in a significant decline in production (Abdelraheem et al., 2019; Chauhan et al., 2023; Bartas, 2024). To cope with these conditions, plants adapt a plethora of strategies to sort out the prevailing stresses, such as molecular and physio-biochemical adjustments as well as structural modifications (Pompelli et al., 2019; Javaid et al., 2022).

Certain biological and functional modifications allow plants that thrive in challenging conditions to continue growing even in the face of extreme environmental events. The development of water-storing tissues, the formation of excretory structures comprising glands and trichomes, the onset of stem succulence, and an increase in cuticle thickness and deposition of wax are a few examples of these strategies (Iqbal et al., 2022). When combined, these alterations assist with regulating physiological processes, including limiting transpirational loss (Fang and Xiong, 2015; Iqbal et al., 2023), increasing the efficacy of water usage, and controlling turgor by extracting water via extensive root invasion (Blum, 2017). Plants also experience simultaneous physiologic shifts, such as maintaining photosynthetic apparatus efficacy, enhancing stomatal modulation, and elevating enzymatic or non-enzymatic functions (Ozgur et al., 2013; Chauhan et al., 2023), and accumulating solutes that are compatible. In the face of severe strain, all of these morpho-physio-anatomical modifications ensure development and biomass production (Ahmad et al., 2023).

Arid regions are those that experience limited and unpredictable rainfall trends, which result in water scarcity. Plants employ a variety of strategies to thrive in dry environments in order to maximize water intake through deep, thick root networks. This reduces transpiration of water by adjusting osmotic pressure in cells, stomatal closure, leaf surface area reduction, and other mechanisms to continue biological functioning during prolonged dry circumstances (Zhou et al., 2021). Water stress that is disproportionate or lasts for an extended length of time causes diminished growth and premature death of plants (Kidd et al., 2018). As the soil moisture deficits increase, the root length and porosity increase to allow for greater soil penetration and water extraction from greater depths (Kidd et al., 2021; Singh et al., 2023). In addition to undergoing various structural modifications, plants from dry environments adapt their osmoregulatory systems to withstand water scarcity. Plants like these store a variety of substances, including inorganic ions, organic osmolytes, and antioxidative enzymes. They play a role in osmoprotection and ROS scavenging (Kapoor et al., 2020). Free amino acids, proline, and soluble sugars are the organic osmolytes that show a significantly variable accumulation under water shortage. Enzymes are buffered through such solutes when there are excessive electrolytes present. Enzymes are protected by higher cytoplasmic concentrations of these substances as well (Cuizhi et al., 2021; Jomova et al., 2024). A number of anatomic variations, including sclerified leaves, root epidermis and endodermis, leaf pubescence, and root hairs, are also present in these plants thriving in water-deficient conditions (Susetyarini et al., 2020; Iqbal et al., 2023).

Accordingly, it was hypothesized that *Urochloa reptans* populations that thrive in harsh surroundings had evolved certain biological and structural modifications that enable them to remain viable under a variety of climatic stressors. This research sought to ascertain the anatomical modifications in structural, functional, and physiological indices noticed in *U. reptans* in various environments of the Faisalabad region (Pakistan), ascertain the degree to which variations in the environment influence these modifications, and evaluate the importance of these adjustments in supporting the species' invasion and survivability in diverse habitats.

Materials and Methods

Population collection and research strategy

Urochloa reptans was taken from sixteen different locations of the Faisalabad region, Pakistan: Ayub Research Institute, Samundri, Painsra, Chak Jhumra, Jaranwala, NIBGE, Eden Garden, Manawala, Sadar Bypass, Gobind Pura, Canal Road, Khurianwala, Makkuana, Chicha, Satiana, and Narwala Bangla (**Figure 1**). A 50 m transect line was erected at each study site. There were 10 1 m × 1 m quadrats on each transect line, spaced 4 m apart. Within each habitat, three research locations were chosen and considered replications. Three of the ten quadrats were chosen primarily for their physio-biochemical and anatomical characteristics. There was no possibility of excluding *Urochloa reptans* from any particular quadrat, because transect lines were established where a pure colony of this species was present. The following quadrat was decided upon for statistical evaluation if this occurs. Google My Maps has been used to identify each location's coordinate system.

Geographical description of the selected region

Geographically, Faisalabad is located at a latitude of 30°30 N, a longitude of 73°10 E, and an altitude of 213 m. The average climatic conditions of the study areas during sampling were calculated as means, as were day/night RH 33.1/75.1% and day and night temperatures 38.28 ± 4 °C and 22.82 ± 3.6 °C, respectively.

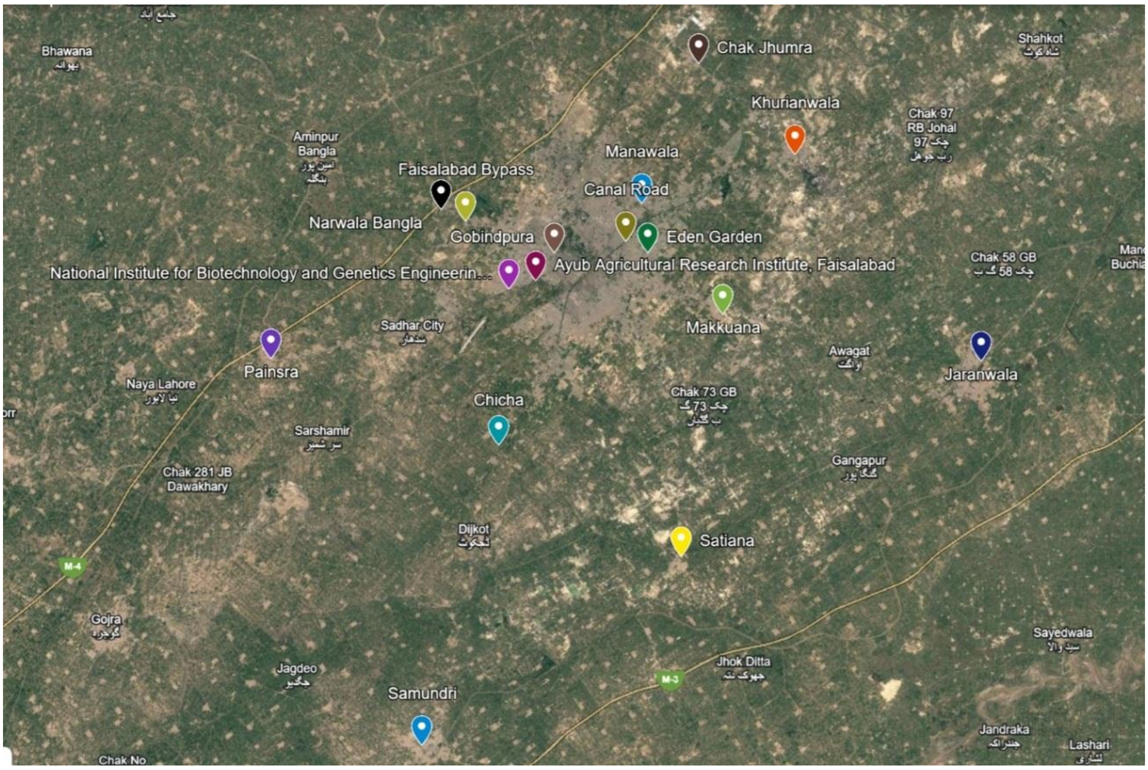


Figure 1: Google My Map of selected sites for *Urochloa reptans* collection

Soil physicochemical parameters

The soil was sandy clay consisting of an average of 65% clay content, 22% sand, and 13% silt. The soil pH ranged within 7.0-8.1, and soil electrical conductivity (ECe) was in the range of 1.8-3.0 dS/m.

Functional and anatomical characterization

To examine the anatomical and functional attributes, only a small sample of each of the leaf, stem, and root was taken. A formalin-acetic alcohol solution of v/v 5% formalin, 50% ethanol, 10% acetic acid, and 35% distilled water was used for preserving the gathered plant specimens for 24 h. To preserve it for an extended time, the plant components were thereafter added to an acetic alcohol solution that included v/v 75% ethyl alcohol and 25% acetic acid. By the use of a multipurpose razor blade, the plant parts were sliced utilizing a freehand cutting approach. To make the permanent slides, the plant parts were dried using a repeated ethyl alcohol process (Ruzin, 1999). Fast green was employed to stain live and actively functioning tissues, while safranin was utilized to stain lignin tissues. Placing the transverse sections on DPX was followed by covering the material with a coverslip. A digitized compound microscope with a camera (Meiji Techno MT4300-LV-HD) was used to take the permanent slide photographs. Utilizing a visual micrometer, measurements were obtained and calibrated using a stage micrometer.

Assessment of physiological parameters

Photosynthetic pigments

The photosynthetic pigments were measured using the procedure developed by Arnon (1949). Accordingly, fresh leaf samples (each 0.5 g) were mixed in 5 mL of 80% acetone at 0–4 °C. The precipitates were separated from the extract by centrifuging them for 5 min at 10,000 rpm. Lastly, a UV-visible spectrophotometer (Hitachi-220, Japan) was used to determine absorption at 480, 645, and 663 nm.

Total soluble proteins

The Bradford's (1976) method was used to determine the total soluble proteins. In a cooled pestle and mortar, 250 g of fresh leaves were ground with 5 mL of potassium phosphate buffer. Next, the homogenized extract was poured into an Eppendorf tube. After centrifuging the samples for 15 minutes at 12,000 rpm, the supernatant was collected in another Eppendorf tube. Five milliliters of the Bradford reagent and 0.1 mL of the plant sample extract were combined in a test tube. After the samples were vortexed, a spectrophotometer was used to measure the absorbance at 595 nm.

Activities of enzymatic antioxidants

Superoxide dismutase (SOD) was assessed using the Giannopolitis and Ries's (1977) procedure. This procedure comprises evaluating how likely the enzyme is to stop the photochemical decline of nitroblue-tetrazolium (NBT). Chance and Maehly (1955) proposed the technique to measure catalase and peroxidase activity. Following solution preparation, a cuvette was filled with 1.9 mL of cold phosphate buffer, 1 mL of H_2O_2, and 1 mL of plant extract. Following the breakdown of H_2O_2, a spectrophotometer (IRMECO U2020 UV-visible) was used to track the drop in absorbance at 240 nm. The CAT activities were computed using the drop in the rate of absorbance.

To measure the POD activity, a reaction mixture including 50 µL of enzyme extract, 100 µL of hydrogen peroxide, 750 µL of buffer, and 100 µL of guaiacol was poured into a cuvette. H_2O_2 was added to start the reaction, and a spectrophotometer was used to measure the rise in absorbance at 470 to track the production of the oxidized product, tetraguaiacol. The rate at which absorbance changed over time was used to compute the POD activities.

Statistical analysis of data

One-way analysis of variance (ANOVA) was performed on the collected data for statistical computation using Statistix 8.1 and for graphical representation, Microsoft Excel (Version 2021) (Microsoft Corporation, Redmond, WA, USA) software. To assess the impact of different habitats, the R statistical tool (version 4.2.2) was employed. Clustered heat map visualizations were created using a specially written R-studio function to examine the ecological dependence of reported plant features that contribute to the invasion proliferation of *U. reptans* in a variety of environments (R Development Core Team, 2017). Additionally, this data was assessed for a correlation to decipher the association among various anatomical adaptive traits using the Origin Pro (2022) statistical software.

Results

Leaf anatomy

Midrib

A wide range of leaf anatomical modifications was observed in *Urochloa reptans* collected from multiple sites in the Faisalabad region, Pakistan. Mainly understudied leaf adaptive components were upper epidermis thickness, lower epidermis thickness, midrib thickness, lamina thickness, vascular bundle area, and bulliform cell area (**Figures 2, 3, 4 & 7**). Representative leaf transverse sections of the *Urochloa reptans* populations inhabiting different habitats of the Faisalabad region are shown in **Figures 2, 3, 4 & 7.** In our study (plant specimens collected from different habitats), the maximum leaf upper epidermis thickness was recorded in the Eden Garden (21.962 ± 0.606 µm) and Canal Road (24.325 ± 5.915 µm) populations, while the minimum was in the Ayub Research Institute (5.282 ± 0.606 µm) as well as in the Samundri population (4.865 ± 0.501 µm). However, the maximum lower epidermal thickness was observed in the Canal Road (21.962 ± 0.606 µm), whereas the minimum was in the Manawala specimens (5.143 ± 0.501 µm). Midrib thickness was significantly highest in the Manawala (425.34 ± 7.231 µm) and Narwala Bangla populations (423.95 ± 7.363 µm), though the minimum was in the Painsra population (187.65 ± 9.641 µm). The thickest lamina was noted in the Khurianwala (191.82 ± 7.231 µm) sample, while the shortest was in the Eden Garden (109.81 ± 6.066 µm). The vascular bundle area was greater in the Gobind Pura population (8056.442 ± 1143.377 µm^2). On the other hand, smaller values were recorded in the Jaranwala population (3160.639 ± 510.923 µm^2). The widened bulliform cell area was documented in the Narwala Bangla sample (1946.480 ± 7.295 µm^2), whereas the narrow bulliform cell area was recorded in the populations of Sadar bypass (141.591 ± 9.404 µm^2) and Canal Road (159.124 ± 13.892 µm^2) (**Figure 5**).

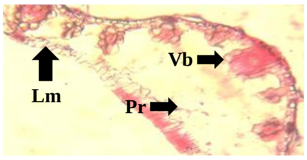
Epidermal studies

Along with epidermal thickness, stomatal area and number of stomata also play a significant role in plants exposed to water-stressed conditions. Our findings demonstrated that a larger stomatal area of adaxial epidermis was noted in the Chicha population (793.98 ± 30.77 µm^2), while a smaller one was recorded in the Samundri ecotype (96.95 ± 7.51 µm^2). However, a large number of stomata were counted in the Narwala Bangla sample (38 ± 1.45), whereas the minimum was counted in the Khurianwala ecotype (15 ± 1.15) (**Table 1**). Abaxial epidermal cross-sectioning studies revealed that the Canal Road sample (933.48 ± 40.22 µm^2) had a larger stomatal area, while a smaller stomatal area was measured in the Jaranwala population (134.44 ± 13.68 µm^2). Besides, the maximum number of stomata was recorded in the sample of the Satiana site (61 ± 1.76), but the minimum was noted in the Chicha ecotype (23 ± 1.45) (**Table 1**; **Figures 2, 3, 4 & 7**).

Table 1: Leaf upper and lower epidermis features of *Urochloa reptans* collected from different sites of the Faisalabad region. Values represent means ± standard error of three replicates. Means sharing the same letters do not differ significantly based on the Tukey test, α = 0.05.

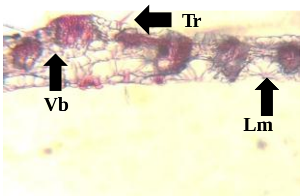
Sites of collection	UPESTA	UPESTN	LWESTA	LWESTN
Ayub Research Institute	478.42±21.63b	24±0.57de	433.60±26.85b	33.66±0.88fgh
Samundri	96.95±7.51i	28±0.57bc	301.99±14.67e	33±1.73gh
Painsra	164.95±14.51gh	15.66±0.88g	202.16±19.44f	35±1.15efg
Chak Jhumra	370.25±21.55c	16.33±0.884g	410.70±16.80bcd	44±1.73c
Jaranwala	320.11±17.18d	40±1.15a	134.44±13.68g	38±1.52de
NIBGE	242.19±20.47ef	21±1.15f	286.46±21.77e	33.66±2.02fgh
Eden Garden	135.12±12.94hi	26±0.57cd	271.52±14.92e	54.66±1.45b
Manawala	362.69±19.68c	28±1.15bc	178.07±20.88fg	38.66±1.76d
Sadar Bypass	237.59±17.16f	27.33±0.88bc	373.81±25.23cd	36.66±1.45def
Gobind Pura	242.51±18.97ef	22.66±1.45ef	448.81±19.54b	30.66±1.45h
Canal Road	188.27±12.18g	13.66±0.88g	933.48±40.22a	32.66±2.02gh
Khurianwala	104.65±11.37i	15±1.15g	429.69±27.48b	34.66±2.02fg
Makuana	280.26±18.85de	26.33±0.88cd	363.01±20.74d	43.33±1.76c
Chicha	793.98±30.77a	28.66±0.88bc	278.85±18.76e	23.66±1.45i
Satiana	231.76±16.92f	29.33±0.88b	420.67±19.08bc	61.33±1.76a
Narwala Bangla	449.77±21.19b	38.66±1.45a	425.22±21.63bc	52.66±1.45b

Abbreviations: Upper epidermal stomatal area (UPESTA; µm^2); Upper epidermal stomatal numbers (UPESTN); Lower epidermal stomatal area (LWESTA; µm^2); Lower epidermal stomatal numbers (LWESTN)



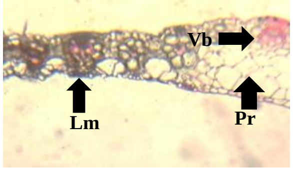
Enlarged, sclerified vascular bundle (Vb), larger parenchyma cell (Pr), broad leaf lamina (Lm).

Ayub Research Institute (AR)



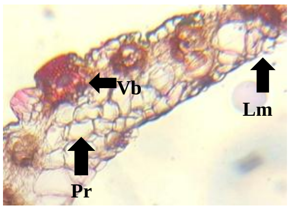
Trichomes (Tr) visible, broad lamina (Lm) and sclerified vascular bundles (Vb).

Painsra (PR)



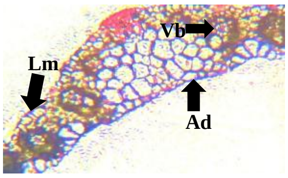
Greatly enlarged leaf parenchyma cells (Pr), seclerified, small vascular bundles (Vb) and broad seclerified leaf lamina (Lm).

Jaranwala (JW)



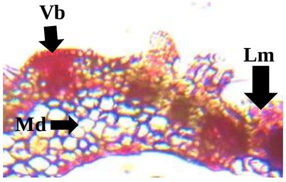
Enlarged leaf lamina (Lm), sclerified vascular bundles (Vb) and large sized parenchyma cells (Pr).

Canal Road (CR)



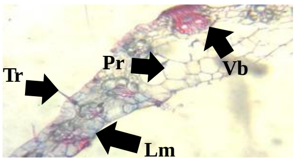
Small leaf lamina (Lm), sclerified vascular bundle (Vb), smaller cortical cell towards the adaxial surface.

Makkuana (MA)



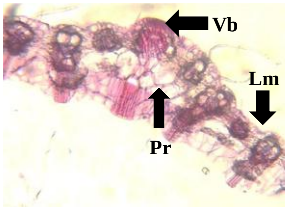
Sclerified vascular bundles (Vb), broadest midrib (Md) thickness, small secleriified lamina (Lm) .

Satiana (ST)



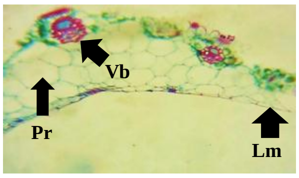
Greatly enlarged parenchyma cells (Pr), large sized trichomes (Tr) and vascular bundles (Vb).

Eden Garden (EG)



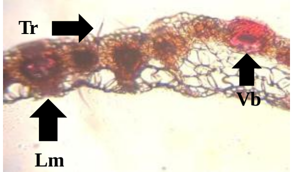
Enlarged sclerified vascular bundles (Vb), large parenchyma cells (Pr), broad leaf lamina (Lm).

Sadar Bypass (SB)



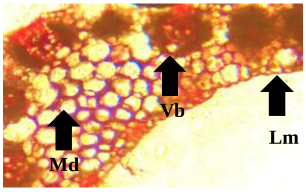
Enlarged parenchyma (Pr), sclerified vascular bundles (Vb) reducued leaf lamina (Lm)

Samundri (SD)



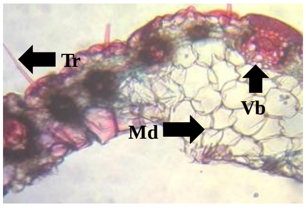
Visible trichomes (Tr), large leaf lamina (Lm) thickness, sclerified vascular bundles (Vb).

Chak Jhumra (CJ)



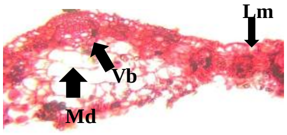
Broadest leaf midrib (Md) thickness, small sclerified vascular bundles (Vb) and large lamina (Lm).

NIBGE (NG)



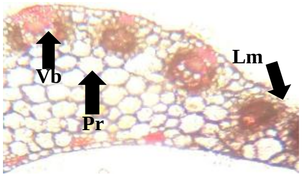
Enlarged and sclerified vascular bundle (Vb), large size trichomes (Tr) and broadest midrib (Md) thickness

Khurianwala (KW)



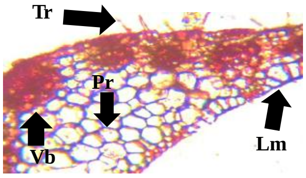
Chicha (CH)

Sclerified vascular bundles (Vb), broadest midrib (Md) and reduced, seclerified lamina (Lm), small aerenchymatous cavities.



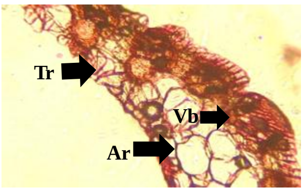
Midrib (Md) thickness enlarged, larged size parenchyma cells (Pr) and sclerified vascular bundles (Vb).

Narwala Bangla (NB)



Sclerified vascular bundles (Vb), large sized parenchyma cells (Pr), visible trichomes (Tr) broad lamina .

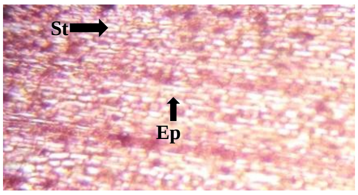
Manawala (MW)



Enlarged and sclerified vascular bundles (Vb) on Abaxial side, prominent parenchyma cells (Pr), large aerenchymatous (Ar) cavities.

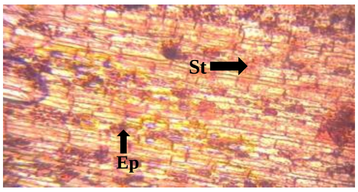
Gobind Pura (GP)

Figure 2: Leaf transverse sections of *Urochloa reptans*



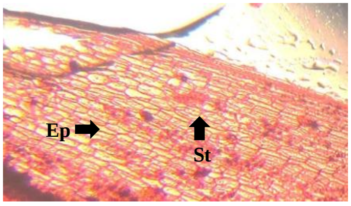
Small epidermal cells (Ep), stomata (St) elliptic and small.

Ayub Research Institute (AR)



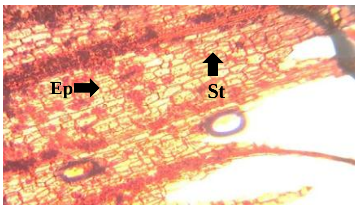
Stomata (St) elliptic and small, Small epidermal cells (Ep),

Samundri (SD)



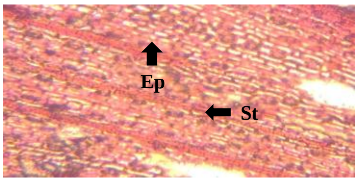
Small epidermal cells (Ep), Stomata (St) elliptic and numerous.

Painsra (PR)



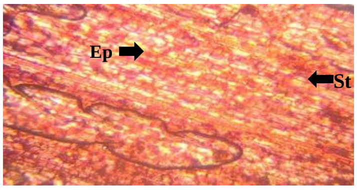
Stomata (St) elliptic and numerous, Small epidermal cells (Ep),

Chak Jhumra (CJ)



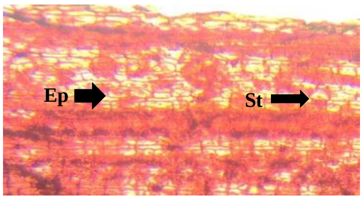
Small epidermal cells (Ep), stomata (St) elliptic and numerous.

Jaranwala (JW)



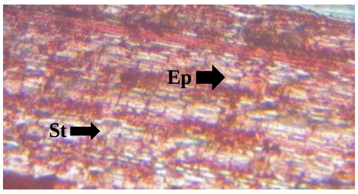
Stomata (St) few and elliptic in shape, thick epidermal cells (Ep)

NIBGE (NG)



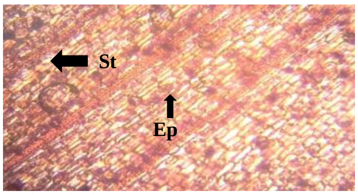
Large epidermal cells (Ep), stomata (St) elliptic and numerous.

Canal Road (CR)



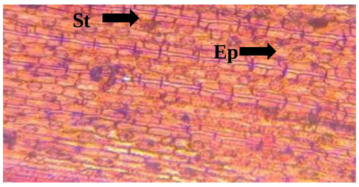
Stomata (St) few and elliptic in shape, large epidermal cells (Ep)

Khurianwala (KW)



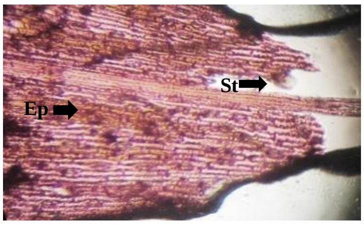
Small epidermal (Ep) cells, stomata (St) elliptic and small.

Satiana (ST)



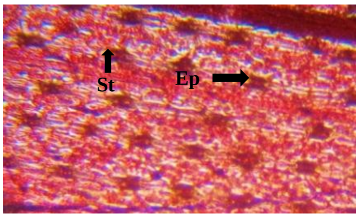
Stomata (St) elliptic and small and large epidermal cells (Ep)

Narwala Bangla (NB)



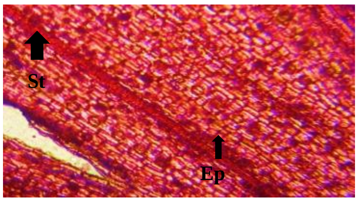
Small epidermal (Ep) cells, large elliptical stomata (St).

Eden Garden (EG)



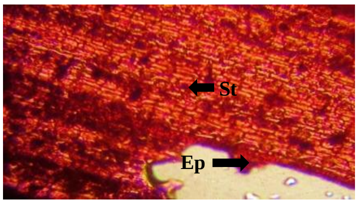
Stomata (St) elliptic and numerous and large, thin epidermis (Ep).

Manawala (MW)



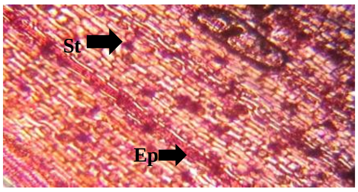
Small epidermal cells (Ep), and large stomata (St)

Sadar Bypass (SB)



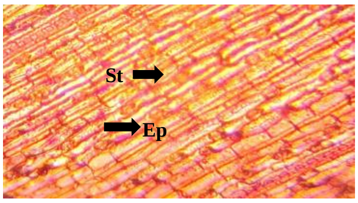
Stomata (St) few and elliptic in shape and small and thin epidermal (Ep) cells

Gobind Pura (GP)



Large epidermal cells (Ep), elliptical stomata (St)

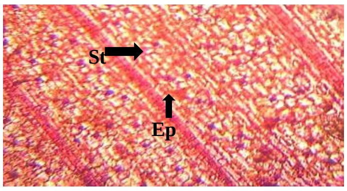
Makkuana (MA)



Stomata (St) numerous and elliptic Large and thin epidermal cells. (Ep).

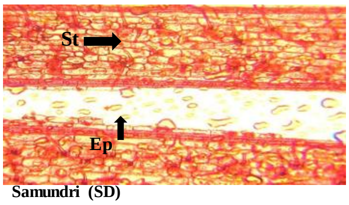
Chicha (CH)

Figure 3: Leaf upper epidermal transverse sections of *Urochloa reptans*



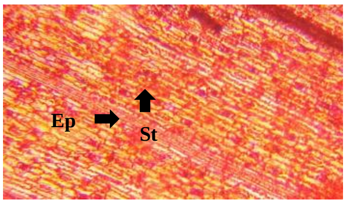
Ayub Research Institute (AR)

Small epidermal cells (Ep), stomata (St) elliptic and small.



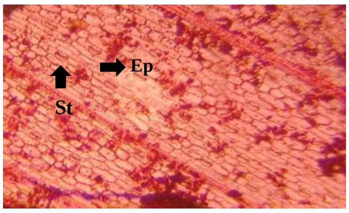
Samundri (SD)

Stomata (St) elliptic and small, Small epidermal cells (Ep),



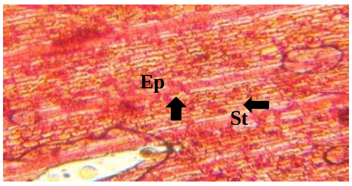
Painsra (PR)

Small epidermal cells (Ep), Stomata (St) elliptic and numerous.



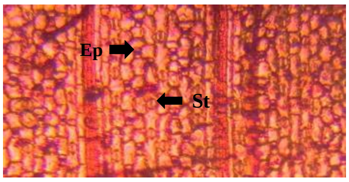
Chak Jhumra (CJ)

Stomata (St) elliptic and numerous, Small epidermal cells (Ep),



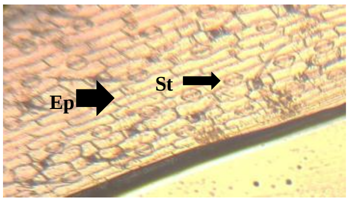
Jaranwala (JW)

Small epidermal cells (Ep), stomata (St) elliptic and numerous.



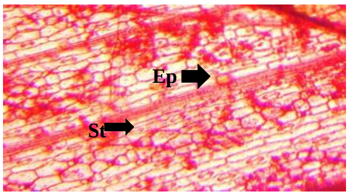
NIBGE (NG)

Stomata (St) few and elliptic in shape, thick epidermal cells (Ep)



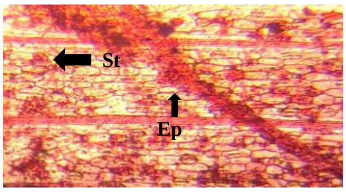
Canal Road (CR)

Large epidermal cells (Ep), stomata (St) elliptic and numerous.



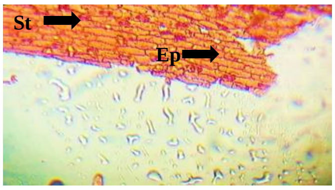
Khurianwala (KW)

Stomata (St) few and elliptic in shape, large epidermal cells (Ep)



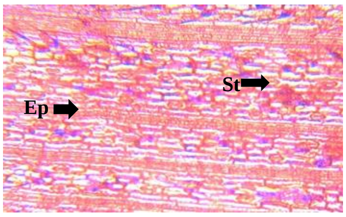
Makkuana (MA)

Small epidermal (Ep) cells, stomata (St) elliptic and small.



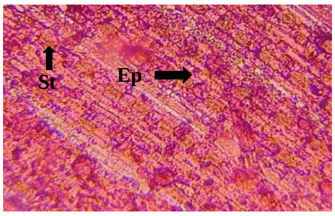
Chicha (CH)

Stomata (St) elliptic and small and large epidermal cells (Ep)



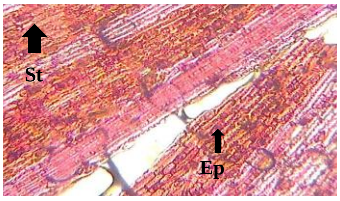
Satiana (ST)

Small epidermal (Ep) cells, large elliptical stomata (St).



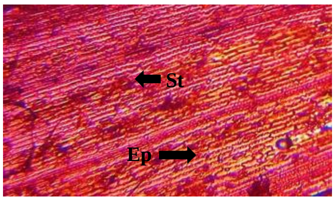
Narwala Bangla (NB)

Stomata (St) elliptic and numerous and large, thin epidermis (Ep).



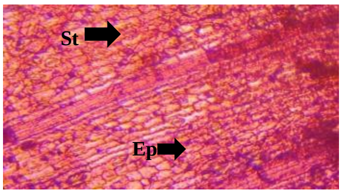
Eden Garden (EG)

Small epidermal cells (Ep), and large stomata (St)



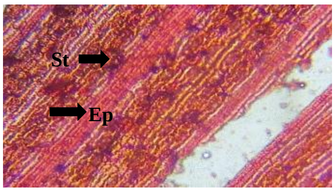
Manawala (MW)

Stomata (St) few and elliptic in shape and small and thin epidermal (Ep) cells



Sadar Bypass (SB)

Large epidermal cells (Ep), elliptical stomata (St)



Gobind Pura (GP)

Stomata (St) numerous and elliptic Large and thin epidermal cells. (Ep).

Figure 4: Leaf lower epidermal transverse sections of *Urochloa reptans*

Stem anatomy

Representative stem transverse sections of the *Urochloa reptans* populations inhabiting different habitats of the Faisalabad region are shown in **Figures 5 & 8**. In our collected specimens from various sites of the Faisalabad region, it was found that the maximum thickness of epidermis was recorded in the Manawala (17.653 ± 0.501 µm), while the minimum was in the Jaranwala and Makkuana populations (4.865 ± 0.368 µm). Furthermore, stem sclerification was maximum measured in the sample of Makkuana (84.79 ± 6.066 µm), whereas the minimum was in the Eden Garden specimen (34.333 ± 0.501 µm). It is revealed from this anatomical adaptive component exploration study that a large number of vascular bundles were found in the Sadar bypass plant sample (17.666 ± 1.454 /mm²), while the minimum was noted in the Chicha population (8.333 ± 1.454 /mm²). The widened cortical cell area was seen in the Manawala sample (6940.655 ± 1124.446 µm²), though the minimum was measured in the Ayub Research Institute sample (3160.639 ± 515.783 µm²). Vascular bundle area and phloem area anatomical measurements showed that the Painsra and Eden Garden populations had the maximum (14186.444 ± 1363.513; 1248.771 ± 218.503 µm²), meanwhile the Canal Road and Khurianwala specimens had the shortest (1302.511 ± 148.240; 198.610 ± 18.345 µm²). The population collected from Eden Garden showed the maximum metaxylem area (1187.744 ± 148.518 µm²). In contrast, the samples from Gobind Pura and Sadar Bypass had the minimum recorded metaxylem area (312.192 ± 22.748; 323.305 ± 27.062 µm²). In the present research, the maximum stem radius was measured in a sample of Painsra (157121.132 ± 4440.210 µm), but the minimum was recorded in the Khurianwala population (90720.375 ± 3636.427 µm) (**Figures 5 & 8**).

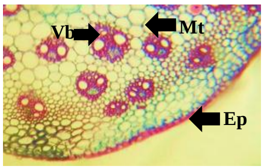
Root anatomy

Representative root transverse sections of the *Urochloa reptans* populations inhabiting different habitats of the Faisalabad region are shown in **Figures 6 & 9**. Anatomical modifications are considered as one of the adaptive components that help plants to thrive in areas of acute water shortage, along with various other important roles, particularly under abiotic stress conditions. In our findings, maximum epidermal thickness was noted in the Canal Road sample (34.055 ± 0.501 µm), while the minimum was in the populations of the Ayub Research Institute and NIBGE (4.726 ± 0.368 µm). The thickest cortical region was observed in the Canal Road specimen (227.96 ± 8.465 µm). However, the minimum thickness of the cortical region was found in the Gobind Pura and Samundri samples (30.58 ± 6.066; 34.472 ± 0.606 µm). Endodermal thickness was recorded as highest in the samples of Sadar Bypass and Khurianwala sites (26.549 ± 0.846 µm), while the minimum was recorded in the Satiana site (9.174 ± 0.482 µm). The thickest root in terms of pith cell area and aerenchyma cell area was noted in the Canal Road and Narwala Bangla samples (230.079 ± 13.447; 4258.210 ± 778.790 µm²), though narrow pith and aerenchyma cell area were found in the populations of Makkuana and Chicha (20.858 ± 3.609; 1062.047 ± 44.635 µm²). In contrast, cells were absent in the samples from Satiana, NIBGE, Gobind Pura, Canal Road, Khurianwala, and Makkuana. The metaxylem area and root cross-sectional area were the maximum in the Chicha and Canal Road samples (2627.794 ± 639.958; 222629.258 ± 7194.658 µm²), whereas the narrow metaxylem area and root cross-sectional area were noted in the Khurianwala and Makkuana populations (445.49 ± 17.849; 70608.870 ± 4353.589 µm²) (**Figures 6 & 9**).

Photosynthetic pigments

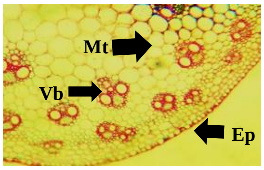
Data showed that photosynthetic pigments (**Table 2**) were reduced under water shortage and salt-affected areas. In our study, photosynthetic pigments (Chl. a, Chl. b, total chlorophyll, Chl. a/b ratio, and carotenoids) were found to be in varying concentrations in different populations. The maximum concentration of Chl. a was documented (1.69 ± 0.003 mg g^{-1}) in the Canal Road population, which was followed by (1.54 ± 0.005 mg g^{-1}) that of Manawala, and Sadar bypass (1.54 ± 0.001 mg g^{-1}; 1.28±0.002 mg g^{-1}), while the minimum value of Chl. a was observed (0.06 ± 0.004 mg g^{-1}) in the Satiana population. However, the maximum concentration of Chl. b (3.29 ± 0.004 mg g^{-1}) was observed in the Khurianwala population, which was followed by the Painsra (3.15 ± 0.038 mg g^{-1}) and Ayub Research Institute (2.94 ± 0.007 mg g^{-1}). In contrast, the minimum values were observed (0.45 ± 0.004 mg g^{-1}) in the Makuana population, followed by that in the Manawala population (0.31±0.041 mg g^{-1}). The highest concentration of total chlorophyll (3.72 ±0.101 mg g^{-1}) was recorded in the Khurianwala population, which was followed by that in the Painsra (3.70 ± 0.033 mg g^{-1}), and Sadar bypass (3.30 ± 0.003 mg g^{-1}) populations, while the minimum values were observed (1.73 ± 0.004 mg g^{-1}) in the Makuana ecotype followed by that of Gobind Pura (1.57 ± 0.006 mg g^{-1}) **(Table**

2).



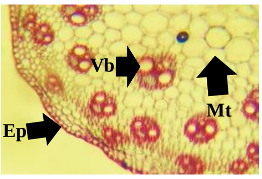
Ayub Research Institute (AR)

Sclerification in epidermis (Ep) and vascular bundle (Vb), enlarged metaxylem (Mt) area.



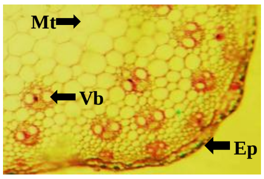
Samundri (SD)

Thin sclerified epidermis, round, large number of metaxylem, extensively sclerified vascular bundles (Vb).



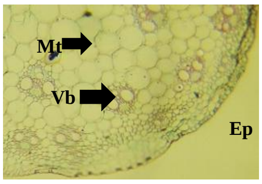
Painsra (PR)

Sclerified thin epidermis (Ep), large Vascular bundle (Vb), enlarged metaxylem (Mt) area.



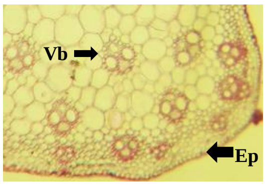
Chak Jhumra (CJ)

Large metaxylem, sclerified scattered vascular bundles, thick wavy epidermis



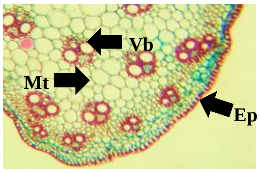
Jaranwala (JW)

Thin epidermis (Ep) and large, non-sclerified vascular bundle, enlarged metaxylem vessels.



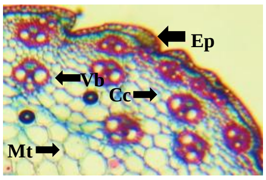
NIBGE (NG)

Thin sclerified epidermis (Ep), extensive sclerification in and outside vascular bundles



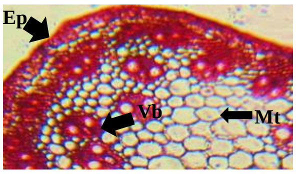
Canal Road (CR)

Large number of vascular bundle (Vb) metaxylem (Mt) cells tightly packed; sclerified epidermis (Ep).



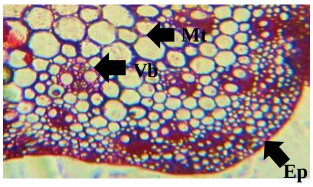
Khurianwala (KW)

Thick irregular epidermis (Ep), small sclerified vascular bundles, tightly packed metaxylem and large cortical cell.



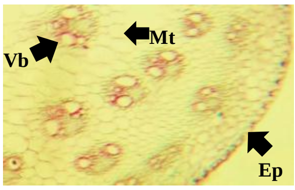
Satiana (ST)

Extensive sclerification in epidermis and Vascular bundle, enlarged metaxylem area.



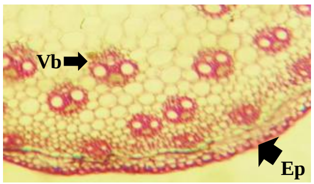
Narwala Bangla (NB)

Enlarged metaxylem, less number of seclerified vascular bundle, thin sclerified epidermis



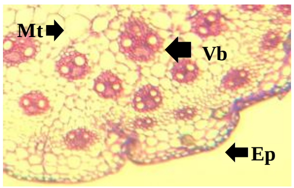
Eden Garden (EG)

Thin epidermis, Scattered and large number of vascular bundle , Small metaxylem area.



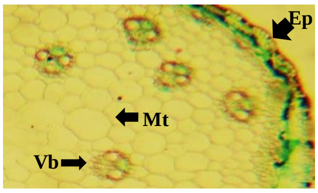
Manawala (MW)

Thick seclerified epidermis, sclerified vascular bundles



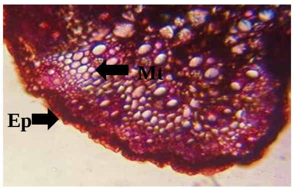
Sadar Bypass (SB)

Thick epidermis, large number of sclerified vascular bundle, enlarged metaxylem vessels.



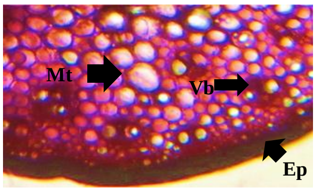
Gobind Pura (GP)

Thick epidermis, enlarged metaxylem cell area, small sized vascular bundles



Makkuana (MA)

Thin sclerified epidermis, condensed, narrow metaxylem vessels.



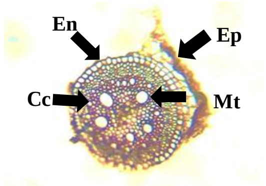
Chicha (CH)

Thick sclerified epidermis, seclerified vascular bundles, enlarged metaxylem

Figure 5: Stem transverse sections of *Urochloa reptans*

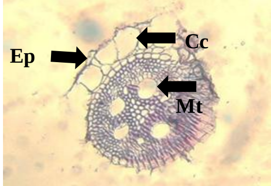
Ayub Research Institute (AR)

Thick sclerified epidermis (Ep) and two cell thick endodermis (En) , enlarged metaxylem (Mt) area.



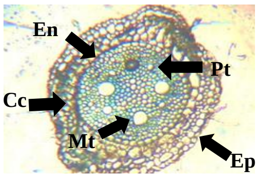
Samundri (SD)

Epidermis(Ep) thick, seclerified, small seized, less number of metaxylem (Mt)), less number of seclerified cortical cells (Cc), extensively thick endodermis (En) sclerified epidermis



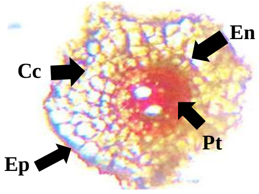
Painsra (PR)

Thin epidermis (Ep), Scattered and large size metaxylem (Mt) cells and very large size cortical cells (Cc) and thick endodermis (En).



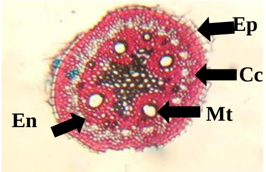
Chak Jhumra (CJ)

One cell thick seclerified epidermis(Ep), small cortical cell area (Cc), less number of metaxylem (Mt) cells sclerified pith cells (Pt)



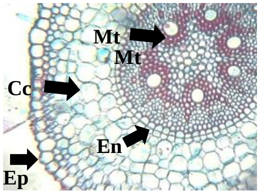
Jaranwala (JW

Thick seclerified epidermis (Ep), large sclerified Pith (Pt), and extensive cortical cell area (Cc) and very thick endodermis (En).



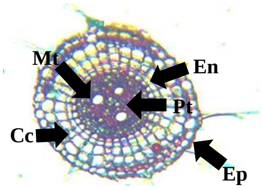
NIBGE (NG)

Thick epidermis(Ep), large metaxylem(Mt) cell area, tick endodermis(En), star shaped pith(Pt), and higly seclerified cortex (Cc).



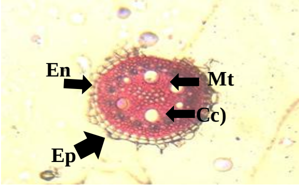
Canal Road (CR)

Thick sclerified epidermis (Ep), large cortical cell (Cc) area, two cell thick endodermis (En) large number of narrow metaxylem (Mt) vessels.



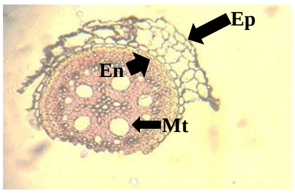
Khurianwala (KW)

Thick epidermis(Ep), thin endodermis(En), seclerified pith(Pt), small sized metaxylem(Mt), cortical cells (Cc) area



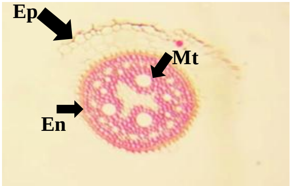
Makkuana (MA)

Extensive sclerification in cortical cells (Cc) and pith(Pt), thin epidermis(Ep) and seclerified endodermis(En), enlarged metaxylem area.



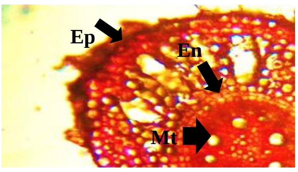
Chicha (CH)

Enlarged metaxylem(Mt), thick irregular epidermis(Ep), thin endodermis(En).



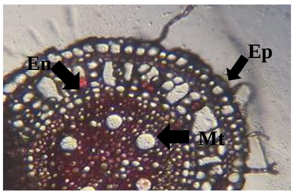
Satiana (ST)

Thick wavevy epidermis (Ep), Scattered and large number of metaxylem cells(Mt) and seclerified pith(Pt).



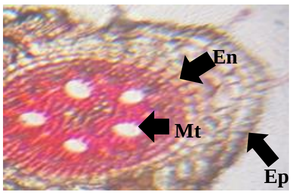
Narwala Bangla (NB)

Thick seclerified epidermis(Ep), sclerified thick endodermis(En), and large number metaxylem (Mt).



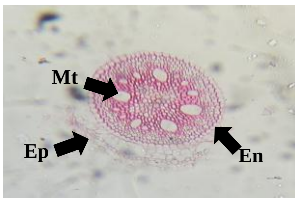
Sadar Bypass (SB)

Thin epidermis(Ep), large, metaxylem (Mt) area and thick endodermis(En).



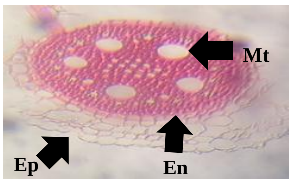
Gobind Pura (GP)

Thick epidermis(Ep) and endodermis(En), large metaxylem (Mt) area.



Manawala (MW)

Thick epidermis(Ep), Small seclerified metaxylem(Mt) cells and thick endodermis.



Eden Garden (EG)

Thick epidermis(Ep), large metaxylem(Mt) area and thick seclerified endodermis(En).

Figure 6: Root transverse sections of *Urochloa reptans*

Table 2: Mean values of photosynthetic pigments, total soluble proteins (TSP), and enzymatic antioxidants of *Urochloa reptans* collected from different sites in the Faisalabad region. Values represent means ± standard error of three replicates. Means sharing the same letters do not differ significantly based on the Tukey test, α = 0.05.

Sites of Collection	Chl. a (mg g^{-1}FW)	Chl. b (mg g^{-1}FW)	Total Chl. (mg g^{-1}FW)	Chl. a/b ratio	Carotenoids (mg g^{-1}FW)
Ayub Research Institute	0.39±0.009i	2.94±0.007ab	3.34±0.004c	0.13±0.004ef	0.084±0.000e
Samundri	0.24±0.023j	2.67±0.010bc	2.92±0.015d	0.09±0.009ef	0.09±0.000e
Painsra	0.54±0.006h	3.15±0.038a	3.70±0.033ab	0.17±0.004def	0.11±0.002d
Chak Jhumra	0.93±0.002f	0.87±0.004i	1.81±0.005hi	1.06±0.006c	0.22±0.002a
Jaranwala	1.15±0.003cd	1.07±0.042hi	2.23±0.039fg	1.07±0.043c	0.17±0.001b
NIBGE	0.20±0.003j	2.34±0.004cd	2.54±0.001ef	0.08±0.001ef	0.11±0.000d
Eden Garden	0.99±0.029ef	1.39±0.254gh	2.39±0.225efg	0.78±0.199cdef	0.08±0.007e
Manawala	1.54±0.005b	0.31±0.041j	1.86±0.037hi	5.02±0.596a	0.09±0.001e
Sadar Bypass	1.54±0.001b	1.76±0.002ef	3.30±0.003c	0.87±0.000cde	0.04±0.002f
Gobind Pura	0.70±0.003g	0.86±0.003i	1.57±0.006i	0.80±0.000cdef	0.18±0.000b
Canal Road	1.69±0.003a	1.66±0.003fg	3.36±0.004bc	1.01±0.002c	0.13±0.002c
Khurianwala	0.42±0.099hi	3.29±0.004a	3.72±0.101a	0.13±0.029ef	0.08±0.007e
Makuana	1.28±0.002c	0.45±0.004j	1.73±0.004i	2.86±0.024b	0.13±0.002c
Chicha	1.13±0.001de	1.15±0.005hi	2.28±0.004fg	0.98±0.005cd	0.18±0.000b
Satiana	0.06±0.004k	2.06±0.003de	2.13±0.004gh	0.031±0.002f	0.04±0.000f
Narwala Bangla	0.87±0.002f	1.84±0.003ef	2.72±0.005de	0.47±0.001cdef	0.17±0.000b

	TSP (mg g^{-1}FW)	SOD (U mg^{-1} protein)	POD (U mg^{-1} protein)	CAT (U mg^{-1} protein)
Ayub Research Institute	3.10±0.012h	20.60±0.156de	15.03±0.151e	4.75±0.037d
Samundri	2.93±0.005ij	19.76±0.125fg	16.76±0.207bc	4.89±0.015cd
Painsra	3.57±0.016g	21.11±0.129d	13.27±0.101fg	4.19±0.0322e
Chak Jhumra	2.79±0.002jk	19.38±0.078gh	16.27±0.041bcd	5.05±0.028c
Jaranwala	3.89±0.009e	18.97±0.104hi	12.94±0.124fg	3.95±0.018f
NIBGE	4.65±0.038c	21.77±0.096c	11.42±0.209h	2.99±0.034i
Eden Garden	4.94±0.036b	20.29±0.084ef	11.44±0.083h	3.20±0.020h
Manawala	3.73±0.031f	18.79±0.061hij	15.79±0.257cde	4.32±0.044e
Sadar Bypass	3.11±0.024h	18.45±0.059ij	15.33±0.182de	5.35±0.037b
Gobind Pura	2.75±0.033k	23.75±0.130a	18.30±0.511a	5.56±0.079a
Canal Road	4.37±0.023d	17.65±0.092k	8.79±0.057i	3.35±0.031gh
Khurianwala	3.05±0.031hi	20.60±0.099de	17.08±0.062b	5.37±0.028b
Makuana	5.09±0.045a	18.24±0.088j	12.29±0.223gh	3.39±0.028gh
Chicha	2.58±0.0163l	22.88±0.167b	19.31±0.175a	5.40±0.024ab
Satiana	2.87±0.022jk	19.67±0.102g	16.29±0.133bcd	5.07±0.028c
Narwala Bangla	4.67±0.020c	19.30±0.077gh	13.53±0.172f	3.49±0.029g

The highest concentration of carotenoids (0.22 ± 0.002 mg g^{-1}) was documented in the population from Chak Jhumra, followed by that of Chicha (0.18±0.000 mg g^{-1}), of Jaranwala (0.17 ± 0.001 mg g^{-1}), and Makuana (0.13 ± 0.002 mg g^{-1}), while the minimum values were observed in the Khurianwala population (0.08 ± 0.007 mg g^{-1}), followed by that of Satiana (0.04 ± 0.000 mg g^{-1}) (**Table 2**).

Total soluble proteins (TSP)

Statistical analysis of the data revealed significant variation in the TSP of plants collected from different areas. In our findings, the maximum concentration of TSP was observed in the samples collected from Makuana and Eden Garden, while the minimum was in the population from Chicha (**Table 2**). However, the minimum TSP was found in the Gobind Pura and Chak Jhumra samples. Moreover, the NIBGE and Canal Road samples showed maximum values of total soluble proteins.

Enzymatic antioxidants

The maximum activity of superoxide dismutase (SOD) was observed in the samples collected from Gobind Pura and Chicha, while the minimum SOD activity was found in the populations of Canal Road and Makuana (**Table 2**). The peroxidase activity was found to be maximum in the samples from Chicha and Gobind Pura, while the minimum was noted in the Canal Road, NIBGE, and Eden Garden populations, as well as in those from Makuana and Jaranwala. Analysis of variance of the data revealed a significant variation in the activities of the catalase (CAT) enzyme in plants from various areas. The maximum CAT activity was observed in the samples collected from Gobind Pura and Chicha, while the minimum CAT activity was found in the populations from NIBGE and Eden Garden.

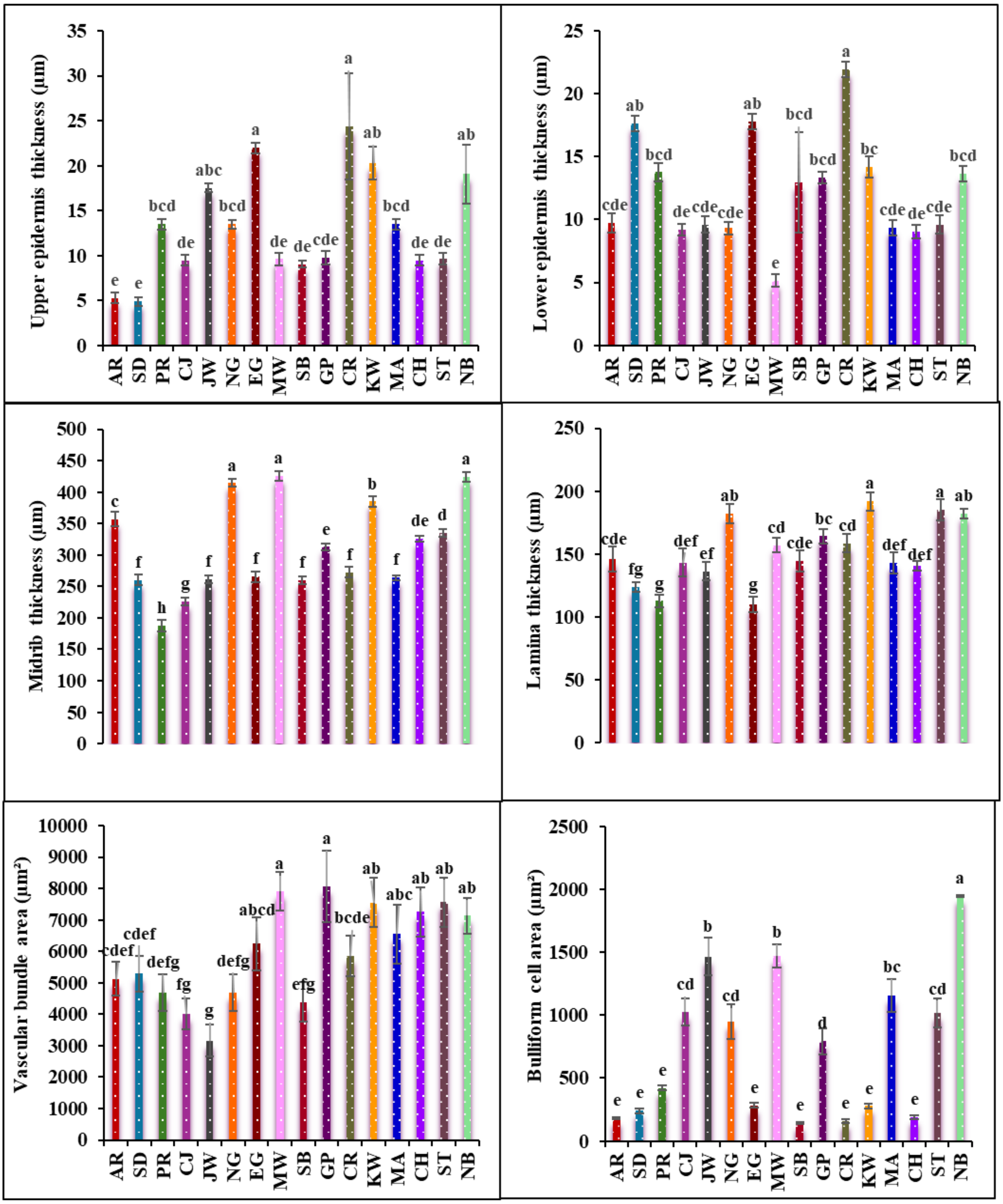


Figure 7: Leaf adaptive components in *Urochloa reptans* collected from various sites

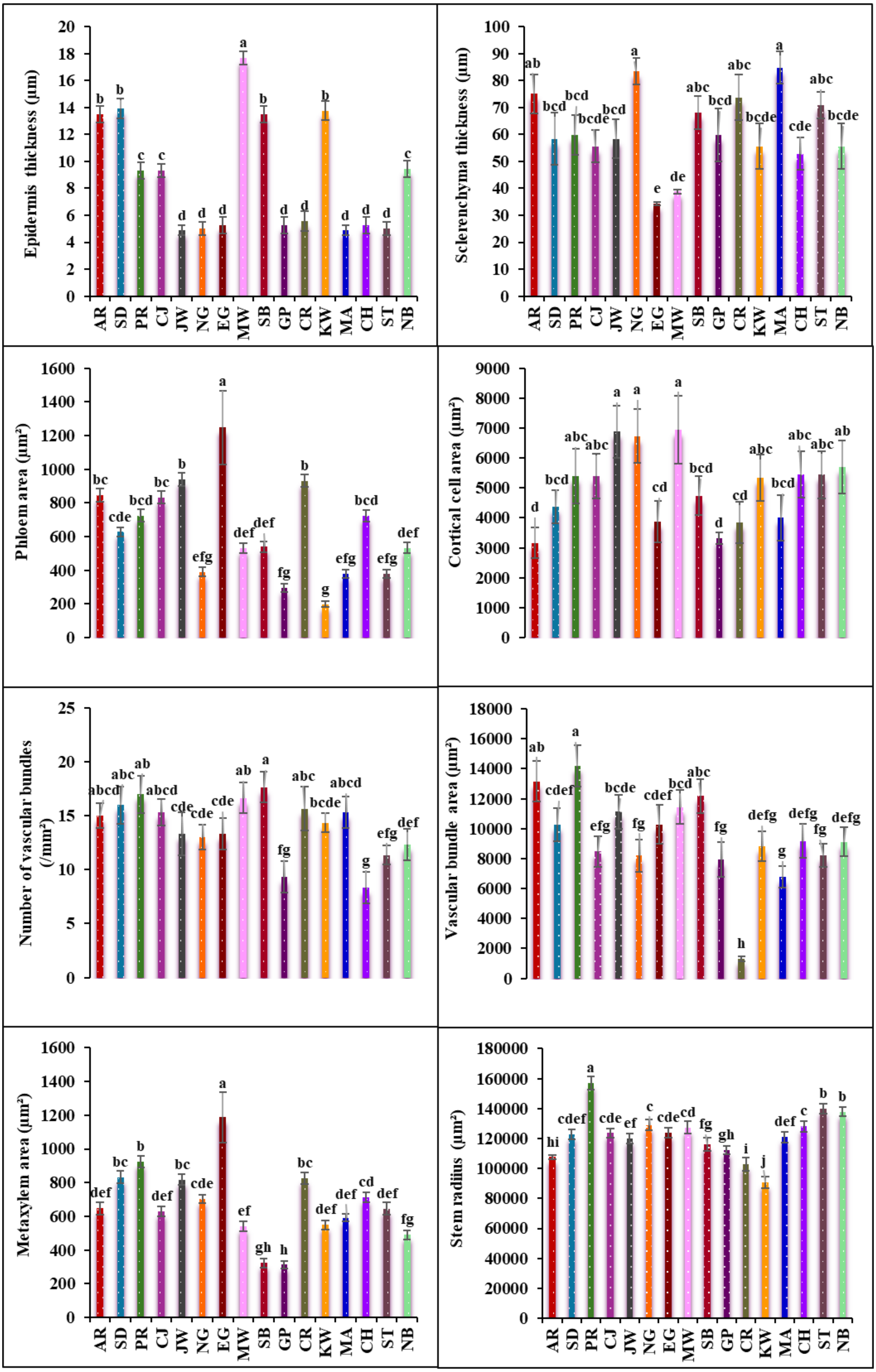


Figure 8: Stem adaptive components in *Urochloa reptans* collected from various sites

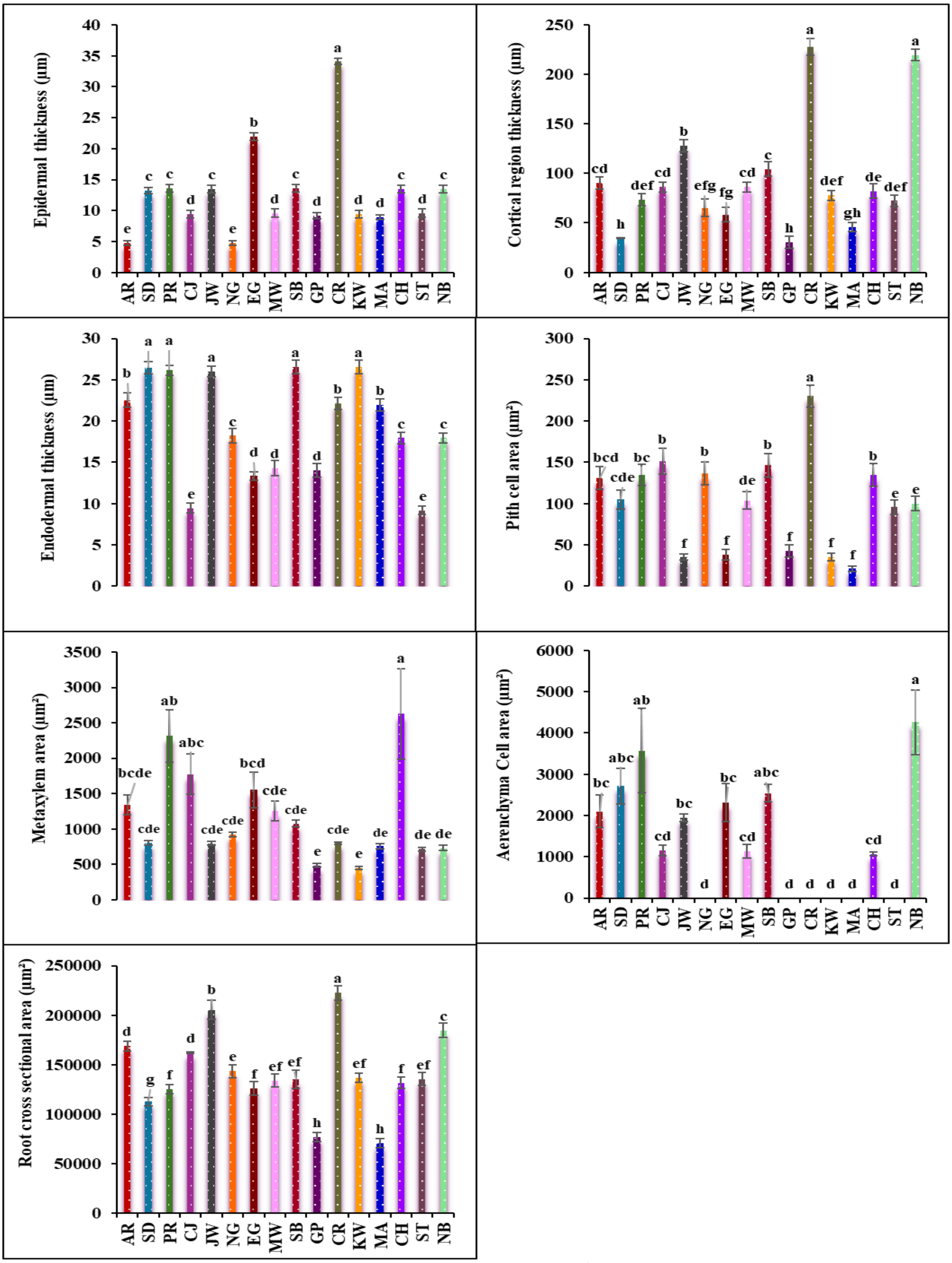


Figure 9: Root adaptive components in *Urochloa reptans* collected from various sites

Heatmap analysis

A two-way heatmap with a dendrogram was sketched out to observe the ecological impact of different collection sites on various observations of *Urochloa reptans* (**Figure 10**). The observations were divided into groups according to how similar they were under different environmental conditions, and the relationships between the groups were shown by colored squares. The yellow color exhibited a strong positive association, while the dark blue color exhibited a strong negative correlation for various anatomical observations, impacted by a heterogeneous environment. The Heatmap was clustered into four groups. In the first group, SST, RPCA, RCRT, and RCA were clustered. These parameters are strongly positively correlated with the samples of Canal Road and Narwala Bangla populations and weakly correlated with Chak Jhumra and Ayub Research Institute ecotypes. With a wide range of environmental heterogeneity of the collection sites, such as Khurianwala, Satiana, Manawala, and Painsra, the above-mentioned attributes showed a weak correlation, while a strongly negative correlation with the populations of Gobind Pura and Makkuana. This group demonstrated that the heterogeneous habitats had a significant role in developing the adaptive components, such as SST, RPCA, RCRT, and RCA of *Urochloa reptans*, which might have helped them in their survival under varying environmental conditions. The second group included SPA, SMA, LUET, LLET, and RET, which were strongly positively correlated with the samples from Canal Road and Eden Garden, whereas a weak correlation was observed with the Narwala Bangla and Jaranwala ecotypes, and a strong negative correlation was observed with Gobind Pura. The third group contained mainly the understudied anatomical attributes of *Urochloa reptans,* except for leaf anatomical characteristics. These attributes were strongly positively correlated with the ecotypes of Painsra and Narwala Bangla, while showing a negative correlation with the specimens of Canal Road and Khurianwala. In the fourth group, LBA, SCA, LVBA, LMT, and LLT were clustered. These parameters were strongly positively correlated with the Manawala, Narwala Bangla, and Khurianwala populations, and weakly correlated with the samples of Chicha, Satiana, and Makkuana, while strongly negatively correlated with the specimens from Painsra and Samundri, and weakly negatively correlated at the collection sites of Sadar Bypass, Chak Jhumra, and Gobind Pura.

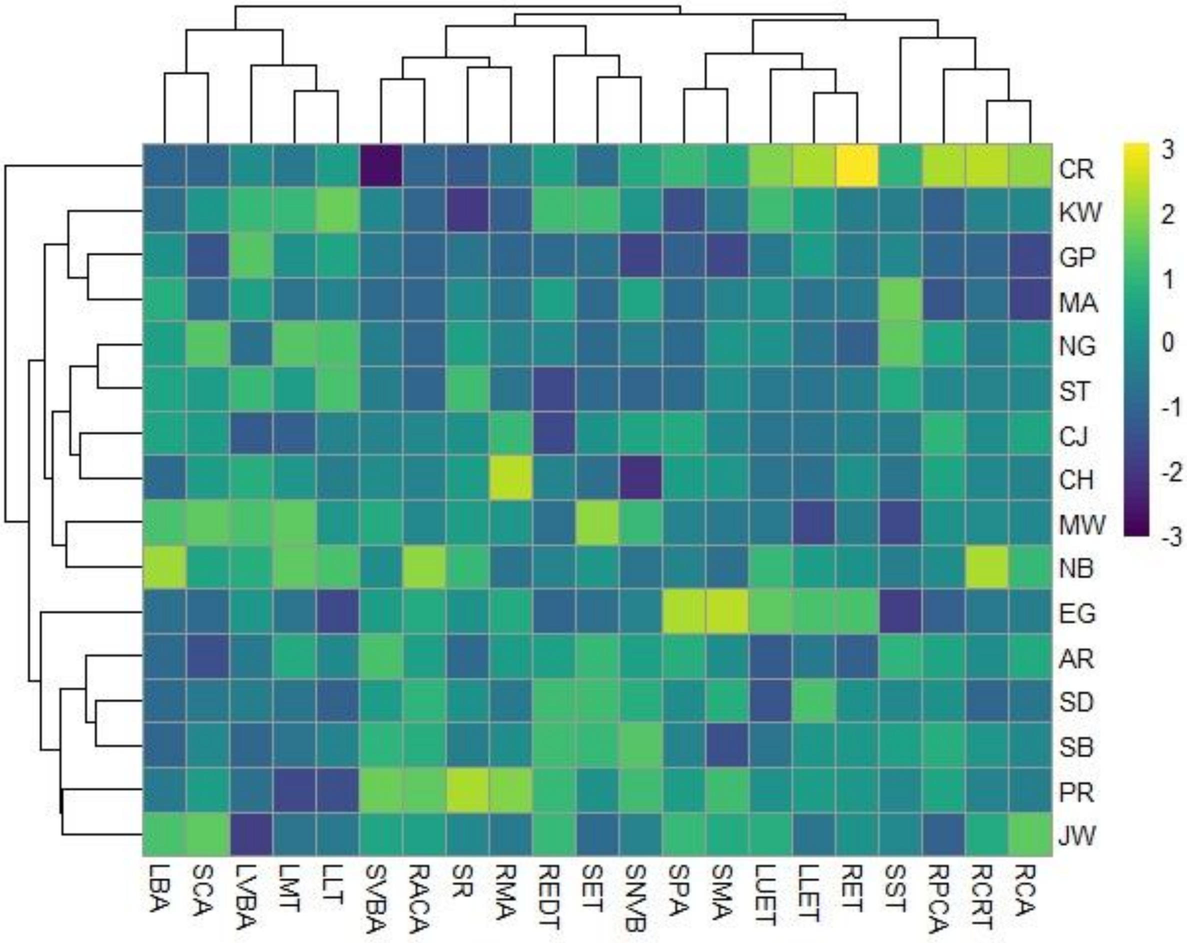
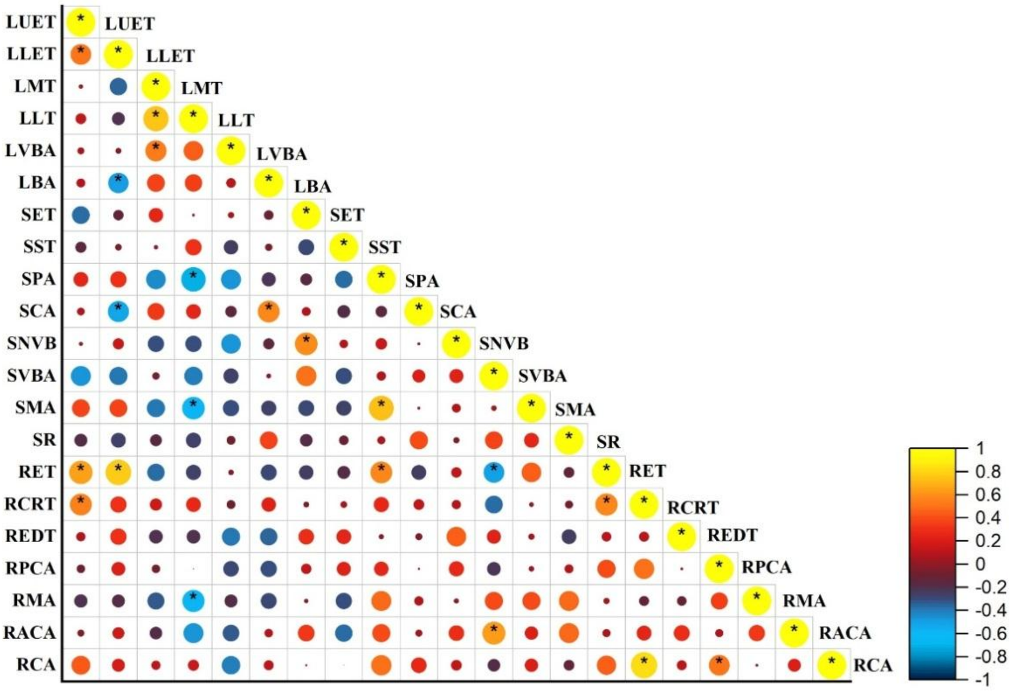


Figure 10: Heatmap representing the association of root, stem, and leaf anatomy

Correlation Analysis:

Pearson's correlation analysis manifested a strong association between the leaf, stem, and root anatomical modifications **(Figure 11)**. In this statistical analysis, the sign of each studied parameter is represented by a level of significance (*Significant at $P \leq 0.05\%$). A few of the leaf anatomical parameters, including LUET, LLET, LLT, and LVBA, along with RCRT, showed a strong association with the majority of indicators. On the other hand, SNVB, SVBA, SR, and RMA revealed a strong negative correlation with most of the indices. Furthermore, LMT, LBA, SET, SPA, and SCA exhibited a weak negative correlation. However, SST, RET, REDT, RPCA, RACA, and RCA did not show considerable correlations with most of the indices.



* p<=0.05

Figure 11: Correlation matrix between root, leaf, and stem anatomy of *Urochloa reptans*

Discussion

Based on our research, different populations of *Urochloa reptans* with a variety of adaptable traits demonstrated remarkable behavior through different growth patterns, microstructural arrangements, and physiological modifications. The research presented here advances our knowledge of how *Urochloa* populations that exist naturally preserve their habitat and adjust to altering ecological circumstances. Plants grown in Canal Road, Eden Garden, Manawala, Jaranwala, and Samundri sites had large epidermal thickness in both leaf and stem, along with enlarged cortical cell area. Plants grown in contaminated areas frequently produce thicker leaves in terms of extensive epidermal thickness of upper and lower sides, and larger epidermal cell areas to prevent the loss of water, and shield them from hazardous contaminants (Yang et al., 2021). To take up additional nutrients and water from the soil, plants can grow larger networks of roots. This adaptation allows them to compensate for the negative effects of pollution and dirt on the above-ground parts (Li et al., 2019). Our cross-sectional study of roots revealed that the samples from Canal Road, Jaranwala, and Narwala Bangla possessed a large root cross-sectional area, facilitating the development of an extensive root system. This study of root anatomical modification assists plant species in thriving under varying environmental conditions.

The thicker leaf lamina is one of the important anatomical alterations for providing additional space for carbon sequestration and retention of water; therefore, it might be beneficial for plants to thrive in a climate with water scarcity (Bibi et al., 2021). Other studies also identified changes at the physiological and structural levels caused by a variety of environmental contaminants (Uka et al., 2017; Wu et al., 2023). Multiple foliar epidermal features, such as stomatal area and number of stomata, have been investigated by many authors as an indicator for the appraisal of environmental quality (Wang et al., 2024). In our cross-sectional studies of leaf epidermis, we found that the maximum number of stomata was in the Satiana population on the lower epidermis as compared to the upper epidermis. Additionally, a larger stomatal area was measured in the Chicha sample on the adaxial side of the leaf in comparison to the abaxial epidermis. Moreover, the palisade and light parenchyma had black phenolic deposits (Gostin, 2009). Research on the effects of plant micromorphology and leaf epidermal components revealed that leaves in the areas of contamination exhibit reductions in length and width, as well as a greater number of trichomes and thicker epidermal cells per leaf area (Saadabi, 2011; Toth et al., 2024).

Neverova et al. (2013) studied the effects of pollutants from industry on the anatomical traits of normal leaves of birch (*Betula pendula*). The most evident negative consequences were the increase in the cells in the lower epidermis and the decrease in cuticle thickness. The thickness of a lamina, the top layer of epidermal tissue, and the crucial rise in the thickness of the midrib were identified as adaptive modifications in our study.

By how long and how severe the drought stress is, plants can undergo a variety of morphological, physiological, biochemical, and molecular alterations (Aboughadareh et al., 2017; Yoo et al., 2024). Drought results in a reduction in plant height, biomass, productivity, and leaf area index. Grain productivity can be impacted by the growth stage at which water scarcity occurs (Samarah, 2016). The effects of drought are more severe during reproductive development phases, particularly before or during blooming. By scavenging reactive oxygen species, modulating their osmotic pressure (OA), stomatal closure, and producing triggered proteins, plants may withstand stress caused by drought (Ashraf and Foolad, 2007; Li et al., 2023). Additionally, plant resilience to drought depends on anatomical adaptive components that are generally associated with enhancing water uptake and storage by roots, reducing loss of water from leaves during water shortages, and automatically reinforcing tissues to stop withering that could cause permanent collapse and damage to cells (Nour et al., 2024).

Sclerification of the epidermal and exterior cortex region prevents the disintegration of fragile cortex tissue (Quintana-Pulido et al., 2018). The understudy *Urochloa reptans* ecotypes, mainly from Makkuana and NIBGE sites, showed intensive stem sclerification, which is considered one of the adaptive strategies to shield stem tissues from collapsing. Additionally, sclerification is very advantageous because it protects the inner actively metabolizing tissue from drying up and thawing (Ahmad et al., 2016; Bi et al., 2017). Furthermore, plants growing at Manawala, Eden Garden, Sadar bypass, and Painsra sites showed increased cortical region, metaxylem, phloem cell areas, maximum number of vascular bundles, and enlarged vascular bundle areas under soil moisture deficit, as shown in **Figure 11**. Moreover, Painsra ecotype ranked the best in having a large stem area, which could be due to an increase in the size of epidermal cells (extensive thickness).

The Chicha, Narwala Bangla, and Canal Road ecotypes exhibited increased root cellular region in terms of pith cell area, aerenchyma cell area, and metaxylem cell area; it may have been due to more development of internal tissues like cortex and its cell size and phloem region for storage of extra water (Nawaz et al., 2013; Peña-Rojas et al., 2024). Plants can withstand a shortage of water because their structural components are more adapted to their surroundings. In arid conditions, a thicker root cortex area (storing tissue) assures retention of water. Under water deficit conditions, an abundance of sclerified cells adjacent to the root's capillary takes place, and cortex regions continue root development, and trigger taking in water more easily. In the context of water scarcity, the root's cortical region is essential for preserving water, and the root epidermis cells (together with the overall thickness of the endodermis) can hold up to soil compaction, as indicated in **Figure 9.** Increased cell-level storing capability of parenchymatous tissues can retain more water, imparting to them a rigidity in opposition to soil and wind pressure and fortifying the softened, actively metabolizing tissues, so as to prevent them from collapsing (Sarwar et al., 2022). Plants that are stressed by water scarcity and dry conditions need to reduce water loss, conserve water, and use available water effectively. Extensive root networks are often developed by plants that are drought-resistant to access water from deeper layers of the soil, where the availability of water may be higher. To draw in water from a larger region, these roots might grow out extensively (Kalra et al., 2024). Many strategies are employed by plants to survive and thrive in dry and water-deficient environments, including numerous anatomical variations. Concerning the conditions, different plant species might manifest distinct combinations of these adjustments. In addition to alterations in morphology, certain plants may exhibit behavioral and physiological modifications to improve their overall ability to withstand drought (Maurel et al., 2016; Haghpanah et al., 2024).

The survival of various grass species in their naturally occurring habitats is influenced by physiological adaptations, including photosynthetic efficacy and the antioxidant defense system, along with their anatomical adaptive markers. Various studies have described how different abiotic stress levels cause a reduction in chlorophyll contents, and hence a decrease in plant photosynthetic activity (Hameed et al., 2009; Acosta-Motos et al., 2017; Yang et al., 2021). However, it is elaborated that in the present investigation, Chl. a, Chl. b, total Chl., Chl. a/b ratio, and carotenoid contents were noted in higher concentrations in Khurianwala, Manawala, and Chak Jhumra ecotypes (**Table 2**). Such changes in photosynthetic pigments offer plants an opportunity to optimize their physiological performance in water-deficient habitats (Habibi and Ajory, 2015; Shah and Satti, 2023). Plants typically generate antioxidants, but when adverse conditions arise, their level of activity rises, allowing plants to withstand climatic impediments (Eraslan et al., 2016; Rao et al., 2025). It is elucidated that the *Urochloa reptans* populations from different habitats possessed higher antioxidant potential, which makes their ecological survival successful in heterogeneous climatic and soil conditions. Higher concentrations of TSP and enhanced activities of enzymatic antioxidants such as SOD, POD, and CAT were noted in the samples from Makkuana, Gobind Pura, and Chicha (**Table 2**). Enhanced antioxidant enzyme activity of a population is again vital for survival in a stressed habitat, as it reduces ROS production and assemblage in different organelles that enable an organism or a genotype to thrive in an environment of oxidative stress (Srivastava et al., 2014; Rao et al., 2025). Numerous studies have earlier shown that plants under physiologically drought and saline conditions first respond by developing more sclerenchymatous tissue in their leaves along with other plant organs (Hameed et al., 2014; Nawaz et al., 2013; Al-Maskri et al., 2014; Yang et al., 2021; Kalra et al., 2024). It assists in reducing the loss of water and tissue disintegration in plants through evaporation from the plant body.

Conclusion

It has been shown that *Urochloa reptans* has phenotypic versatility across morphological and functional aspects, enabling the species to colonize heterogeneous contextual scenarios. As a result of certain substantial modifications in development, the structural and functional traits, this species survived and thrived in a dry to semi-desert habitat. Some of these adaptive modifications are a deeper root network, higher biomass productivity, chlorophyll content, total soluble proteins, total antioxidant activity, leaf thickness, growth of sclerenchyma bundles in cortex, a large number of vascular tissues, epidermal thickness, broadening of metaxylem vessels, and expansion of storage parenchymatous tissues (cortex and pith). The ecological viability of the species under a variety of climatic circumstances, such as salinity, dryness, and high temperatures in dry and drought-prone regions, is greatly influenced by all of the aforementioned characteristics.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors

Research planning: HR; Research supervision: FA and MH; Manuscript writing and proofreading: JS, EAW, AH, SY, TR and AR

Permissions and ethical compliance

This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material

No supplementary material is included with this manuscript.

Conflict of interest

The authors declare no conflict of interest.

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent

All authors have critically read this manuscript and agreed to publish in IJAaEB.

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