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# MODIFICATIONS IN ANATOMICAL TRAITS OF TALL FLAT SEDGE *CYPERUS EXALTATUS* RETZ. COLONIZING DIFFERENTLY SALT-AFFECTED WETLANDS

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**Abstract**

Salinity stress is one of the major abiotic stresses that affect plant growth and development worldwide. This study aimed to investigate the anatomical attributes of widely distributed halophytic halophytes which are economically important due to their numerous bioactive compounds of high nutritive value. The members of the family Cyperaceae are of high importance among halophytes due to their ability to grow in a diverse range of environments. The study of anatomical attributes of *C. exaltatus* Retz. in varying salt-affected habitats is a novel approach and has not been reported earlier. For this purpose, different populations of *C. exaltatus* were collected from a diverse range of saline habitats and analyzed for variation in their morpho-anatomical attributes in response to varying degree of salinity. The results showed that *C. exaltatus* can grow under higher salinity levels, but excessive salts of growth medium caused a reduction in overall plant growth. The anatomical modifications along salinity gradient observed in *C. exaltatus* included an increase in the size of vascular bundles, formation of lysigenous aerenchyma cavities, and high intensity of sclerification. These modifications enabled *C. exaltatus* to survive in a saline environment and provide a useful resource for the revegetation of coastal areas and hypersaline -degraded rangelands in Pakistan. Overall, this study provides insight into the anatomical attributes of *C. exaltatus* and its ability to survive in hypersaline environments. The findings of this study may have practical applications for the cultivation of halophytes in saline soils for the purpose of food and feed production, as well as for the restoration of degraded ecosystems.

**Key words:** *Cyperus exaltatus*, Sedge, Anatomical, Salinity, Adaptations.

## Introduction

Salinity is one of the major hazards faced by plants worldwide including Pakistan along with approximately a hundred other countries (Karimi *et al*., 2018, Mumtaz *et al*., 2021a, Angon *et al*., 2022) where more than eight hundred million hectares of land have been affected (Karimi *et al*., 2018). Soil salinity is caused by several primary and secondary factors which may include the geological makeup of an area, waterlogging, problems related to the drainage of water, high rates of evaporation and use of groundwater beyond the limits (Kahlown *et al*., 2003, Qadir *et al*., 2008; Liu *et al*., 2023). Salinity is one of the major abiotic stresses that are faced by vegetation of Pakistan which has affected about 21 million hectares of land. Among the causes of salinity, sodium chloride tops the list with a contribution of about 50% to the damage of agricultural lands (Jafari *et al*., 2009, Shah *et al*., 2011).

Salinity poses serious threats to germination, growth, nutrition, and other physiological activities of the plant world that are evident at structural as well as functional and at molecular levels (Gupta & Huang, 2014, Fahad *et al*., 2015, Matsuda *et al*., 2023). Salinity can inhibit photosynthesis, especially in the presence of sodium chloride which disturbs physiological processes and thus causing a decrease in plant biomass production (Munns & Tester, 2008; Flowers & Colmer, 2015). The ionic imbalance caused by salinity, especially in the form of sodium chloride distorts the ability of plants to uptake the essential nutrients from the soil solution (Zhu, 2002; Rahmani *et al*., 2023). Thus, saline affects the morphology, anatomy, and physiology of plants which results in an overall decrease in plant growth (Athar *et al*., 2009, Chourasia *et al*., 2022).

Halophytes are plant species that are adapted to saline environments, which can survive high salinities salinity and hence, provide an alternative for salinity-sensitive traditional crops (Pirasteh-Anosheh *et al*., 2016; Ibraheem *et al*., 2022). Survival of such species may rely on osmotic adjustment, producing succulence, and developing an antioxidative defense system (Kumari *et al*., 2015; Grigore & Toma, 2021). Such plants can adapt to saline environments of deserts, marshes, and coastal areas by making several modifications for regulating growth and development. Halophytes are also known for having numerous bioactive compounds which are not only helpful in dealing with various types of abiotic stresses but may also have a high nutritive value, and thus can be utilized to feed many cattle (Flowers & Colmer, 2015).

Among halophytes, the members of family Cyperaceae are of high importance. They can grow well in a diverse range of environments that includes dry barren soils to hyper saline wetlands and acidic soils, with courtesy of physiological and anatomical adaptations (Mumtaz *et al*., 2021a, Mumtaz *et al*., 2021b). Genus *Cyprus* is the largest genus from family Cyperaceae and is widely distributed in Pakistan (Mumtaz *et al*., 2021b). Many species of this genus such as *C. arenarius* and *C. laevigatus* have been reported to be suitable for revegetation of coastal areas and sand dunes (Agha *et al*., 2021) along with the salinity degraded rangelands (Mumtaz *et al*., 2021a).

*Cyprus exaltatus* Retz. can grow widely across the

salinity-affected areas in Pakistan. The highest accumulation of antioxidant like proline at hyper saline sites validated the ability of this species to adapt to hypersaline environments. The highest growth rate along with the higher proportion of photosynthetic pigments was recorded in moderately saline environments. It was evident that *C. exaltatus* can grow under higher salinity, however, the higher levels of salinity caused a reduction in the overall growth of the plant (Imran *et al*., 2022).

Investigating salt tolerant species is currently a hot issue for researchers all over the world. Wetlands, especially salt marshes, are under consistent threat as these areas are degraded at a rapid pace. As a result, species colonizing salt marshes are at the verge of extinction. Therefore, it is urgently required to investigate new species like *C. exaltatus* which has never been explored for salinity tolerance regarding anatomical modification. The study was aimed to investigate structural modifications in *C. exaltatus* in response to varying salt-affected habitats. It was hypothesized that *C. exaltatus* may show different modifications along salinity gradient. The research questions to be addressed were: 1) Is there any difference in anatomical traits along salinity gradient? 2) If yes, then how do these modifications contribute towards salinity tolerance? and 3) which traits is critical for the survival of this species in hypersaline habitats?

## Material and Methods

Halophytes are widely known for their ability to survive different kinds of environments by making changes in their morphological and anatomical structures along with modifications in their physiology. To determine these adaptive components, differently adapted populations of the *C. exaltatus* were collected from areas which ranged from highly salinity-affected sites to lesser saline sites. The site 1 (ECe 38.21 dS $m^{-1}$, $Na^+$ 5522 mg $kg^{-1}$, $Cl^-$ 2978 mg $kg^{-1}$), site 2 (ECe 36.55 dS $m^{-1}$, $Na^+$ 5267 mg $kg^{-1}$, $Cl^-$ 3745 mg $kg^{-1}$) and site 3 (ECe 28.22 dS $m^{-1}$, $Na^+$ 4456 mg $kg^{-1}$, $Cl^-$ 2442 mg $kg^{-1}$) were hypersaline. The site 4 (ECe 21.83 dS $m^{-1}$, $Na^+$ 2578 mg $kg^{-1}$, $Cl^-$ 1634 mg $kg^{-1}$) and site 5 (ECe 1722 dS $m^{-1}$, $Na^+$ 2442 mg $kg^{-1}$, $Cl^-$ 1163 mg $kg^{-1}$) were moderately saline. The site 6 (ECe 2.33 dS $m^{-1}$, $Na^+$ 1132 mg $kg^{-1}$, $Cl^-$ 401 mg $kg^{-1}$) and site 7 (ECe 1.98 dS $m^{-1}$, $Na^+$ 821 mg $kg^{-1}$, $Cl^-$ 267 mg $kg^{-1}$) were non-saline. Afterwards, the plants were analyzed for variation in their morpho-anatomical traits in response to salinity gradient. Field trips were organized for the collection of these populations from different salt-affected wetlands. The soil samples were taken from the collection sites for the soil physicochemical traits. A short description of the collection sites is given in (Table 1).

To study the anatomical parameter, the transverse sections of root, stem and leaf were prepared using the free-hand sectioning technique. For this purpose, the specimens were fixed in formalin acetic alcohol solution having a composition v/v formalin 10%, acetic acid 5%, ethyl alcohol 50% and distilled water 35% for two days. Afterwards it was transferred to acetic alcohol solution for long-term preservation. The protocol of dual staining with dehydration (safranin and fast green) was employed to facilitate the preparation of permanent slides following Ruzin (1999). Quantitative measurements were made using an ocular micrometer mounted on a light microscope. Moreover, the micrographs were captured using a Sony 16 MP digital camera that was fixed to a light microscope.

The anatomical features measured for the stem comprised stem radius, epidermis cell area, sclerenchyma area, parenchyma cell area, vascular bundle area, metaxylem area, and stem aerenchyma area. The anatomical parameters assessed for the leaf included lamina thickness, sclerenchyma area, abaxial and adaxial epidermis cell area, mesophyll cell area, metaxylem area, vascular bundle area, and aerenchyma area. Root anatomical parameters included root radius, epidermis cell area, endodermis thickness, aerenchyma area, parenchyma cell area, vascular bundle thickness, and metaxylem area.

Six plants were randomly selected for anatomical studies from each study site and considered as replications. The data were analyzed by one-way analysis of variance to investigate significant differences among various anatomical traits. The means were tested by least significant difference test. Moreover, the relationship between differently salt-affected habitats and plant anatomical traits were evaluated through multivariate principal component analysis.

## Results

**Stem anatomical traits:** Plants growing at hypersaline Site 1 revealed the lowest stem radius (803.3 µm) and metaxylem area (310.0 $\mu m^2$). The highest sclerenchyma area (506.7 $\mu m^2$) and parenchyma area (2623.3 $\mu m^2$) was recorded at hypersaline Site 1 (Fig. 1). Plants at hypersaline Site 2 exhibited the highest epidermis cell area (360.0 $\mu m^2$), vascular bindle area (4063.3 $\mu m^2$) and aerenchyma area (193.3 $\mu m^2$). The highest sclerenchyma area (506.7 $\mu m^2$) was observed in plants from hypersaline Site 3. Plants colonizing non-saline Site 6 depicted the thickest stem radius (1503.3 µm). The lowest epidermal cell area (213.3 $\mu m^2$), sclerenchyma area (296.7 $\mu m^2$), parenchyma area (2096.7 $\mu m^2$), vascular bundle area (3790.0 $\mu m^2$) and aerenchyma area (70.0 $\mu m^2$) were recorded in plants inhabiting non-saline Site 7. The broadest metaxylem vessels (413.3 $\mu m^2$) were noted at non-saline Site 7 (Table 2).

**Table 1. Habitat description of *Cyperus exaltatus* collection sites from the Punjab and Kyber Pakhtoonkhwa.**

| Sites | Sites | Salinity level | Habitat description |
|---|---|---|---|
| Site 1 | Chiniot Road Sahianwala | Hypersaline | Hypersaline saltmarsh near Sahianwala, Faisalabad |
| Site 2 | RBC Sahianwala | Hypersaline | Saline waterlogged area near Sahianwala, Faisalabad |
| Site 3 | 109 SB Kirana | Hypersaline | Hypersaline dryland near Kirana Hills, Sargodha |
| Site 4 | Old Civil Lines Sargodha | Moderately Saline | Disposal water channel, Sargodha |
| Site 5 | 112 SB Kirana | Moderately Saline | Moderately salt-affected area at Kirana Hills, Sargodha |
| Site 6 | Upper Dir | Non-saline | Foothill region of Kohistan mountains, Upper Dir |
| Site 7 | 111 SB Kirana | Non-saline | Foothills of Kirana Hills, Sargodha |

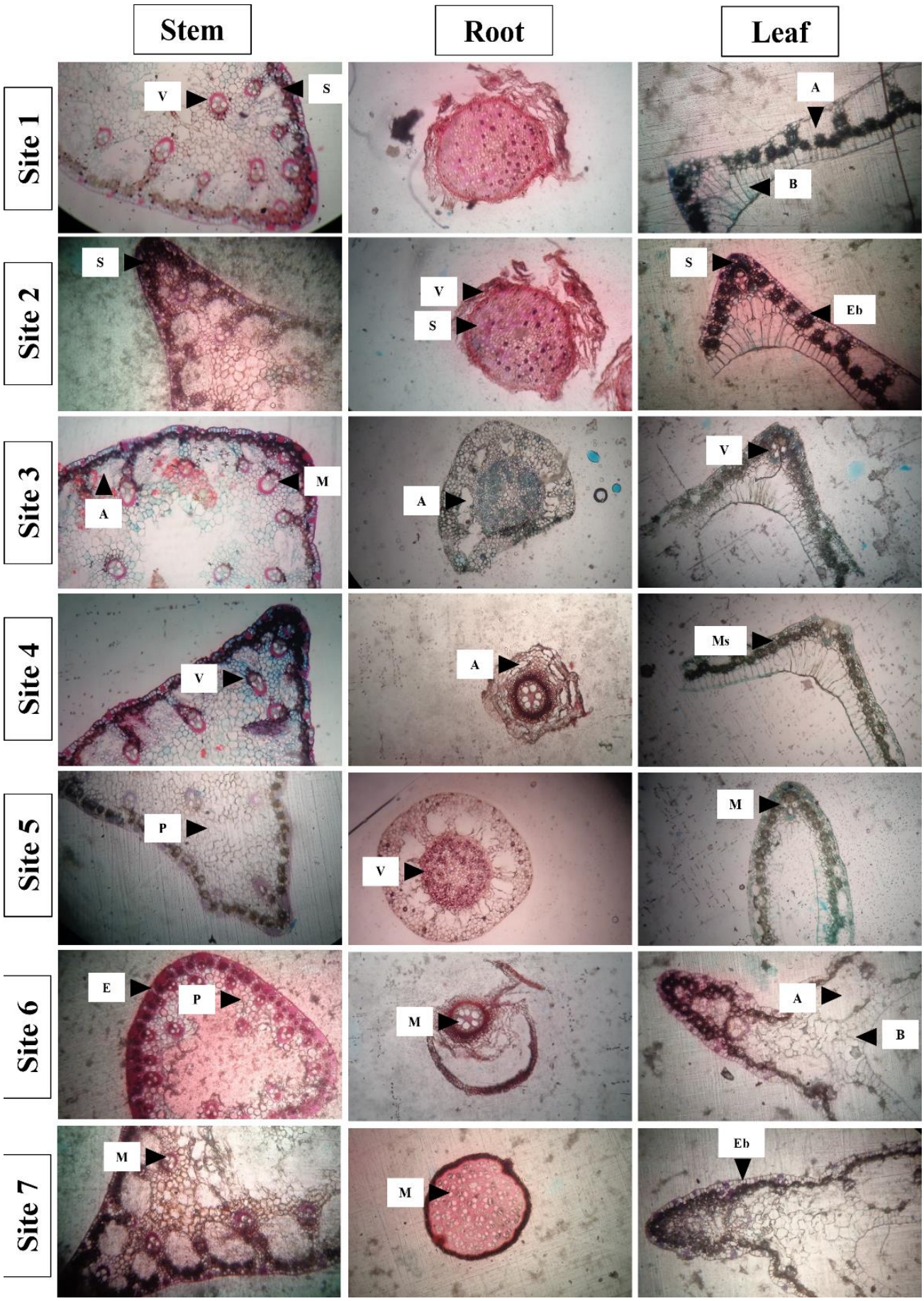


Fig. 1. Root, stem and leaf anatomical modifications in differently adapted ecotypes of *Cyperus exaltatus* along salinity gradient.

**Table 2. Stem anatomical traits of *Cyperus exaltatus* collected from differently salt-affected habitats in the Punjab and Khyber Pakhtoonkhwa (Means ± SE).**

| Salinity | Sites | Stem radius (µm) | Epidermis cell area (µm²) | Sclerenchyma area (µm²) | Parenchyma area (µm²) | Vascular bundle area (µm²) | Metaxylem area (µm²) | Aerenchyma area (µm²) |
|---|---|---|---|---|---|---|---|---|
| HS | Site 1 | 803.3 ± 3.3f | 353.3 ± 3.3a | 506.7 ± 6.7a | 2623.3 ± 14.5a | 4026.7 ± 36.7ab | 310.0 ± 0.0e | 186.7 ± 3.3ab |
| | Site 2 | 820.0 ± 5.8f | 360.0 ± 0.0a | 500.0 ± 0.0a | 2446.7 ± 3.3b | 4063.3 ± 36.7a | 343.3 ± 3.3c | 193.3 ± 3.3a |
| | Site 3 | 920.0 ± 0.0d | 350.0 ± 0.0a | 506.7 ± 3.3a | 2600.0 ± 0.0a | 3993.3 ± 3.3abc | 330.0 ± 0.0d | 180.0 ± 5.8b |
| MS | Site 4 | 893.3 ± 6.7e | 300.0 ± 5.8b | 456.7 ± 6.7b | 2396.7 ± 8.8c | 3943.3 ± 8.8c | 340.0 ± 0.0c | 100.0 ± 0.0c |
| | Site 5 | 1030.0 ± 11.5c | 300.0 ± 5.8b | 436.7 ± 8.8c | 2390.0 ± 5.8c | 3980.0 ± 5.8bc | 346.7 ± 3.3c | 103.3 ± 3.3c |
| NS | Site 6 | 1503.3 ± 3.3a | 220.0 ± 0.0c | 310.0 ± 5.8d | 2210.0 ± 20.8d | 3736.7 ± 27.3d | 390.0 ± 5.8b | 83.3 ± 3.3d |
| | Site 7 | 1420.0 ± 11.5b | 213.3 ± 3.3c | 296.7 ± 3.3d | 2096.7 ± 17.6e | 3790.0 ± 37.9d | 413.3 ± 3.3a | 70.0 ± 5.8e |
| | LSD $_{(0.05)}$ | 21.953 | 10.809 | 17.09 | 37.637 | 80.975 | 9.3606 | 12.085 |
| | Mean squares | 248886*** | 11427.0*** | 24793.7*** | 110043*** | 45355.6*** | 3844.44*** | 8552.38*** |
| | Error mean squares | 157 | 38.1 | 95.2 | 462 | 2138.1 | 28.57 | 47.62 |

Means sharing similar letters are statistically not significant at $p<0.05$

HS-Hypersaline, MS-Moderately saline, NS-Non-saline

The degree of freedom for site 6 and error 14

***-significant at $p<0.001$

**Table 3. Root anatomical traits of *Cyperus exaltatus* collected from differently salt-affected habitats in the Punjab and Khyber Pakhtoonkhwa (Means ± SE).**

| Salinity | Sites | Root radius (µm) | Epidermis cell area (µm²) | Endodermis thickness (µm) | Aerenchyma area (µm²) | Parenchyma cell area (µm²) | Vascular bundle thickness (µm) | Metaxylem area (µm²) |
|---|---|---|---|---|---|---|---|---|
| HS | Site 1 | 513.3 ± 3.3d | 416.7 ± 3.3a | 10.0 ± 0.0a | 37866.7 ± 66.7a | 590.0 ± 5.8f | 150.0 ± 5.8a | 883.3 ± 3.3d |
| | Site 2 | 493.3 ± 6.7e | 420.0 ± 0.0a | 10.0 ± 0.0a | 37600.0 ± 100.0a | 606.7 ± 6.7e | 150.0 ± 15.3a | 920.0 ± 5.8cd |
| | Site 3 | 506.7 ± 3.3de | 413.3 ± 3.3ab | 10.0 ± 0.0a | 38000.0 ± 0.0a | 720.0 ± 5.8c | 130.0 ± 0.0b | 943.3 ± 3.3c |
| MS | Site 4 | 643.3 ± 8.8c | 396.7 ± 3.3bc | 8.7 ± 0.7b | 35833.3 ± 166.7b | 653.3 ± 3.3d | 80.0 ± 0.0c | 960.0 ± 10.0c |
| | Site 5 | 666.7 ± 8.8b | 390.0 ± 5.8c | 8.0 ± 0.0b | 34000.0 ± 0.0c | 646.7 ± 3.3d | 85.3 ± 2.9c | 1023.3 ± 8.8b |
| NS | Site 6 | 833.3 ± 8.8a | 356.7 ± 3.3d | 6.0 ± 0.0c | 31666.7 ± 333.3d | 876.7 ± 3.3b | 76.0 ± 3.1c | 1256.7 ± 31.8a |
| | Site 7 | 850.0 ± 0.0a | 336.7 ± 13.3e | 6.0 ± 0.0c | 30500.0 ± 500.0e | 946.7 ± 3.3a | 80.0 ± 0.0c | 1246.7 ± 3.3a |
| | LSD $_{(0.05)}$ | 19.86 | 18.33 | 0.7643 | 728.09 | 14.30 | 19.34 | 40.62 |
| | Mean squares | 68715.9*** | 3077.78*** | 9.71429*** | 2.850E+07*** | 57744.4*** | 3557.33*** | 72155.6*** |
| | Error mean squares | 128.6 | 109.52 | 0.19048 | 172857 | 66.7 | 121.9 | 538.1 |

Means sharing similar letters are statistically not significant at $p<0.05$

HS-Hypersaline, MS-Moderately saline, NS-Non-saline

The degree of freedom for site 6 and error 14

***-significant at $p<0.001$

**Table 4. Leaf anatomical traits of *Cyperus exaltatus* collected from differently salt-affected habitats in the Punjab and Khyber Pakhtoonkhwa (Means ± SE).**

| Salinity | Sites | Lamina thickness (µm) | Sclerenchyma area (µm²) | Abaxial epidermis cell area (µm²) | Adaxial epidermis cell area (µm²) | Mesophyll area (µm²) | Metaxylem area (µm²) | Vascular bundle area (µm²) | Aerenchyma area (µm²) |
|---|---|---|---|---|---|---|---|---|---|
| HS | Site 1 | 343.3 ± 3.3ab | 800 ± 0.0a | 476.7 ± 12.0c | 446.7 ± 3.3a | 720 ± 5.8c | 516.7 ± 8.8a | 1473.3 ± 3.3a | 22333.3 ± 166.7a |
| | Site 2 | 350 ± 0.0a | 786.7 ± 3.3a | 470 ± 10.0c | 420 ± 11.5b | 716.7 ± 3.3c | 500.0 ± 0.0b | 980 ± 420.0b | 21133.3 ± 133.3b |
| | Site 3 | 333.3 ± 12.0b | 790 ± 5.8a | 463.3 ± 8.8c | 396.7 ± 3.3c | 716.7 ± 3.3c | 491.7 ± 3.3c | 1303.3 ± 3.3ab | 21100 ± 208.2b |
| MS | Site 4 | 190 ± 0.0cd | 686.7 ± 6.7b | 516.7 ± 8.8b | 310 ± 5.8d | 806.7 ± 6.7ab | 483.4 ± 3.3d | 1363.3 ± 13.3ab | 20866.7 ± 66.7bc |
| | Site 5 | 183.3 ± 3.3d | 656.7 ± 6.7c | 530 ± 10.0b | 303.3 ± 3.3d | 816.7 ± 6.7a | 476.7 ± 3.3e | 1333.3 ± 3.3ab | 20500 ± 0.0c |
| LS | Site 6 | 203.3 ± 3.3c | 603.3 ± 3.3d | 583.3 ± 12.0a | 250 ± 0.0e | 820 ± 0.0a | 453.4 ± 3.3f | 1260 ± 10.0ab | 19600 ± 100.0d |
| | Site 7 | 190 ± 5.8cd | 596.7 ± 3.3d | 576.7 ± 8.8a | 216.7 ± 6.7f | 796.7 ± 3.3b | 451.7 ± 3.3f | 1186.7 ± 6.7ab | 19666.7 ± 176.4d |
| | LSD (0.05) | 16.657 | 14.299 | 30.81 | 17.924 | 14.299 | 6.62 | 481.99 | 420.36 |
| | Mean squares | 19604.8*** | 23815.9*** | 7422.22*** | 23176.2*** | 7460.32*** | 6782.54*** | 73154.0ns | 267412*** |
| | Error mean squares | 90.5 | 66.7 | 309.52 | 104.8 | 66.67 | 57.14 | 75752.4 | 57619 |

Means sharing similar letters are statistically not significant at p<0.05
HS-Hypersaline, MS-Moderately saline, NS-Non-saline
The degree of freedom for site 6 and error 14
***-significant at p<0.001, ns-not significant

**Root anatomical traits:** Plants from the highest saline Site 1 revealed the thickest endodermis (10.0 µm) and vascular bundles (150.0 µm), while the smallest parenchyma cell area (µm²) and narrowest metaxylem vessels (883.3 µm²) were recorded in plants collected from Site 1 (Fig. 1, Table 3). The thickest epidermis (420.0 µm), endodermis (10.0 µm) and vascular bundles (150.0 µm) were found in plants from highly saline Site 2, whereas the root radius was the lowest (493.3 µm). Plants colonizing highly saline Site 3 depicted the highest endodermis thickness (10.0 µm) and aerenchyma area (38000.0 µm²). Non saline Site 6 plants showed the endodermis (6.0 µm) and vascular bundles (76.0 µm), but the broadest metaxylem vessels (1256.7 µm²). Plants growing at the least saline Site 7 exhibited thickest root radius (850.0 µm) and the largest parenchyma cells (946.7 µm²). The thinnest epidermis (336.7 µm), endodermis (6.0 µm) and smallest aerenchyma cavities (30500.0 µm²) were seen in the plants from least saline Site 7.

**Leaf anatomical traits:** The highest saline Site 1 plants showed the highest sclerenchyma area (800.0 µm²), adaxial epidermis cell area (446.7 µm²), metaxylem area (516.7 µm²), vascular bundle area (1473.3 µm²), and aerenchyma area (2223.3 µm²). The thickest lamina (350.0 µm) and the smallest mesophyll cells (716.7 µm²) and vascular bundles (980.0 µm²) were found in plants from highly saline Site 2 (Fig. 1, Table 4). Plants inhabiting highly saline Site 3 revealed the smallest abaxial epidermis (463.3 µm²) and mesophyll cells (716.7 µm²). The moderately saline Site 4 plants exhibited the thinnest leaf midrib (183.3 µm). Plants collected from non-saline Site 6 had the largest abaxial epidermis cells (583.3 µm²) and mesophyll cells (820.0 µm²), while the smallest aerenchyma cavities (19600 µm) were recorded at this site. The smallest sclerenchyma area (596.7 µm²) and adaxial epidermal cells (216.7 µm²), and the narrowest metaxylem vessels (451.7 µm²) were seen in plants collected from the least saline Site 7.

**Relationship amino anatomical characteristics of *C. exaltatus* along salinity gradient:** The principal component analysis (PCA) of stem anatomical attributes indicated a clear association of stem radius and metaxylem area with lesser saline sites as they show a decrease in response to salinity (Fig. 2). The PCA of root anatomical attributes revealed a close association of root radius, root metaxylem and parenchyma cell area with lesser saline sites, indicating a negative influence of salinity on these traits. The PCA of leaf anatomical attributes indicated a negative influence of salinity on mesophyll and lower epidermis cell area by plotting them alongside the lesser saline sites while the other attributes showed an incline towards the hypersaline sites (Fig. 2).

## Discussion

The anatomical modifications in plant species may serve as markers for plants identification and tools to evaluate tolerance potential in species against numerous environmental stresses (Naskar and Palit, 2015). *Cyperus exaltatus* growing in a diverse range of salinity levels is affected by high salinity (Imran *et al*., 2022). In this article,

we focused the study of anatomical attributes of *C. exaltatus* which corroborated the results of our previous study by showing several parameters being affected negatively under high salinity. However, a few modifications were also observed which may have enabled the species to get adapted to highly saline environments.

Among the anatomical attributes of stem, stem radius and metaxylem area were affected negatively under salinity. A reduction in stem radius and vascular bundles thickness with a rise in salinity is reported in different plants (Pimmongkol *et al*., 2002; Castillo-Campohermoso *et al*., 2020). Epidermal cell area, sclerenchyma area, parenchyma cell area, vascular bundles area and stem aerenchyma area were larger among populations at hypersaline sites, thereafter, decreased at moderately saline sites whereas these parameters showed the least values at less saline sites. However, less variation among the population was observed in the case of parenchyma cell area. The increase in such parameters under salt stress may be regarded as an adaptive strategy to tolerate salinity. Grigore & Toma (2007) reported enlargement of parenchyma in cortex of halophytes of Chenopodiaceae under salinity. An increase in size of parenchyma tissue for water storage has been reported in various studies in response to salinity (Hasanuzzaman *et al*., 2018, Mumtaz *et al*., 2021a). Similarly increase in size of sclerenchyma cells is regarded as a protective strategy in plants that are grown in saline conditions (Driesen *et al*., 2021, Waldron & Selvendran, 1990). It also provides mechanical support to plants (Hameed *et al*., 2009, Vasellati *et al*., 2001). Salt stress which results from increasing salinity leads to morpho-anatomical changes in trichomes, root, sclerenchyma, aerenchyma, area of cortex, metaxylem area, bulliform cells, the conductance of stomata and water use efficacy (Ahmad *et al*., 2016). Our study is also in line with Naz *et al*., (2013) who reported aerenchyma area, and improved sclerification in both external hypodermis and inward multi-layered sclerenchyma over the endodermis cortical layers in *Aeluropus lagopoides* to overcome the salinity impacts.

Reduction in root radius, parenchyma cell area and metaxylem area was observed among the populations at hypersaline sites C. However, root radius depicted more variation among populations. These parameters decreased at hypersaline sites as these sites were strongly associated with presences $Na^+$, $Cl^-$, EC and a higher pH. The values of these parameters at less saline sites Upper Dir and 112 SB Sargodha were higher, as these sites showed strong association with organic matter, soil saturation and inorganic ions $K^+$, $Ca^{2+}$, and $PO_4^{3-}$. The $Na^+$ and $Cl^-$ ions in higher concentration cause ionic imbalance which results in injury to root membranes and reduction in the uptake of potassium (Islam *et al*., 2011). Hussain *et al*., (2012) stated a decrease in $K^+$ and $PO_4^{3-}$ concentrations and elevated levels of $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ under applied salinity. It results in an increase in osmotic pressure of plants helping in osmotic balance and salinity tolerance. Adriana *et al*., (2015) studied *Eragrostis plana* and found an increase in root area, metaxylem area under saline environments. The root cortex becomes larger in response to salinity (Akram *et al*., 2002) while Uddin *et al*., (2012) observed damaged cortex in turf grasses which were salinity sensitive.

Endodermis cell area, endodermis thickness, vascular bundle thickness, and aerenchyma cell area, all these parameters showed higher values among populations at hypersaline sites. Endodermis cell area and aerenchyma cell area decreased gradually at moderately and less saline sites respectively, however, aerenchyma cell area showed narrow variation among the populations. Endodermis thickness and vascular bundle thickness decreased discontinuously among populations, however, vascular bundle thickness depicted little variation among moderately and hypersaline sites. Increased endodermis thickness offers resistance against salinity (Mansoor *et al*., 2015). It blocks the inward flow of water and ions like $Na^+$ and $Cl^-$, which deposited on the margins of endodermal cells during their symplastic transport because of Casparian strips (Møller *et al*., 2009).

Halophytes show anatomical and morphological adaptations like salt bladders, salt glands (for a particular rejection or amassing of ions), or succulence (dilution of ion concentration) in the plant tissue (Gupta & Huang 2014, Kumari *et al*., 2015). In the roots of *Sporobolus ioclados,* an increase was seen in the thickness of root tissues at higher salinity (Naz *et al*., 2016). Increment in the thickness of cell walls and the expansion of various cells is caused by salinity (Minic & Jouanin, 2006).

Most of the leaf anatomical parameters leaf lamina thickness, sclerenchyma cell area, upper epidermis cell area, metaxylem cell area, aerenchyma cell area and vascular bundle area of *Cyperus exaltatus* showed higher values at hypersaline sites. Thereafter, values decreased at moderate saline sites and less saline sites, however, each parameter varied differently among the sites. Sclerenchyma cell area and metaxylem cell area depicted less variation among the populations at all sites.

Salinity affected the anatomical parameters mesophyll cell area and lower epidermal cell area at hypersaline sites, areas at these sites were smaller and thereafter increased at other sites. The mesophyll cell area was larger at both moderately as well as less saline sites whereas lower epidermal cell area was maximum and statistically par at less saline sites. Jakovljević *et al*., (2013) reported a larger area of upper epidermal cells more frequent than lower epidermis in sedges of Cyperaceae. Less saline sites showed a strong association with organic matter, soil saturation and inorganic ions $K^+$, $Ca^{2+}$, and $PO_4^{3-}$. Various specific anatomical alternation has been recorded under the stress of salinity in sedges such alternations included sclerification of cells, enlargement in bulliform and production of much more aerenchymatous cavities (Lillebø *et al*., 2003). Thicker epidermis enables plants to overcome the problem of water loss for survival under high salinity (Nawazish *et al*., 2006). Succulence in halophytes is enhanced by an increase in the mass of aerenchyma cells as well as lamina and midrib thickness. That is effective for water storage in saline soils (Colmer *et al*., 2009).

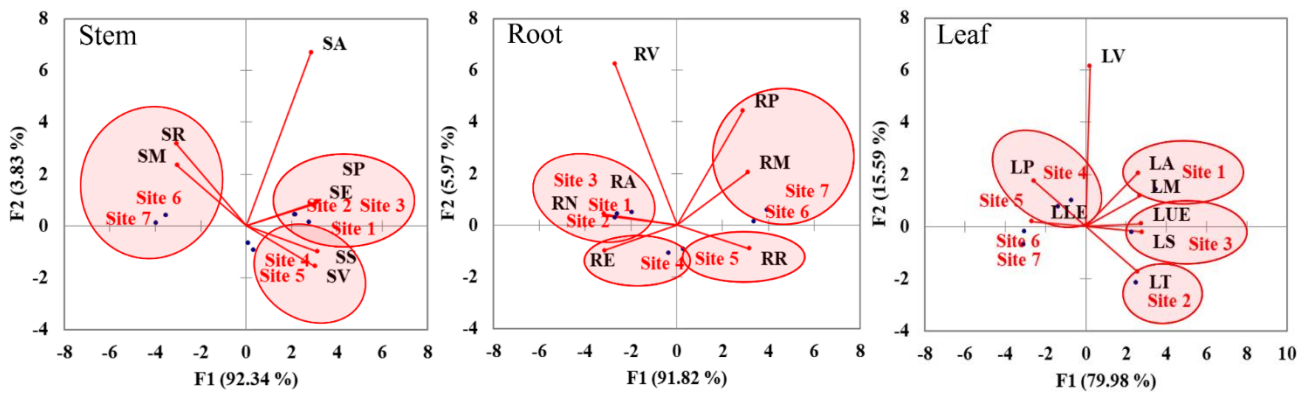


Fig. 2. Principal component analysis showing relationship among root, stem and leaf anatomical traits of *Cyperus exaltatus* along salinity gradient.

## Conclusion

It was concluded that there was a significant variation in root, stem and leaf anatomical traits of *C. exaltatus* populations collected from differently salt-affected habitats. The anatomical traits that contributed to salinity tolerance were thick epidermis, extensive sclerification especially outside vascular bundles, high proportion of storage parenchyma, aerenchyma formation in parenchymatous region, thick endodermis, and enlarged vascular bundles. These modifications may guarantee the successful survival of *C. exaltatus* populations to cope with oxidative stress caused by water scarcity because of high salinities. These findings suggest that *C. exaltatus* has developed adaptive strategies to tolerate saline environments. The anatomical modifications may serve as a tool to evaluate the tolerance potential of plant species against environmental stresses.

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